THE BIOLOGICAL DIVERSITY AND AQUACULTURE OF CLARIID AND PANGASIID CATFISHES IN SOUTH-EAST ASIA

Proceedings of the mid-term workshop of the
"Catfish Asia Project"
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FOREWORD

The mid-term workshop of the "Catfish Asia" project (full title: "*Characterisation, utilisation and maintenance of biological diversity for the diversification and sustainability of catfish culture in South East Asia*", DG XII, INCO-DC, contract IC18-CT96-0043) was held at the Can Tho University, Can Tho, Vietnam, from May 11 to 15, 1998.

The objectives of the workshop were to realise a synthesis of the studies carried out during the first half of this project and, from this basis, define more accurately research lines for the second part of the programme.

The workshop brought together 32 scientists belonging to the Belgium, French, Indonesian and Vietnamese institutions associated in the realisation of the project. Representatives of the production sector involved in catfish culture in the Mekong Delta also attended the meeting. A visit of the amazing site of Chau Doc, located on the upper part of the Mekong Delta, was organised and provided to the participants a view of the extremely dynamic *Pangasius* floating-cage culture activity in this area and of its associated fish processing plants.

A total of 37 communications on research topics related to the biodiversity and culture of catfishes in SE Asia have been presented. Thirty one of them are compiled in the present volume, which is organised in three main parts: I) Contexts and research goals, II) Biological diversity and, III) Diversification and optimisation in aquaculture production. As the research is still going on, some of these communications refer to preliminary work while others can be already considered as achieved contributions.

We hope that these proceedings will contribute to a first dissemination of the basic and applied results obtained in this fruitful Asian-European collaborative research project.

The editors wish to thank the Can Tho University, the staff of its College of Agriculture and more particularly Dr Nguyen Thanh Phuong and Ms Huynh Thi Tu, for the excellent work they did in the practical organisation of this meeting. They are also grateful to the European Commission (DG XII) for its essential support in the realisation of the "Catfish Asia" project.

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THE "CATFISH ASIA" PROJECT: BACKGROUNDS, AIMS AND PROSPECTS

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Abstract

Catfishes, and in particular Clariidae and Pangasiidae, are important aquatic resources in Asia, where their culture represented an annual production of about 124,000 t in 1993. In the clariids, this production results mostly from the use of F1 hybrids between the introduced African catfish (*Clarias gariepinus*) and various local clariid species. In the pangasiids, various culturing techniques were empirically developed for some native species, whose juveniles are most often captured in the wild. Further development of this catfish culture industry faces serious problems related to the poorly known systematic of these fish groups, the scarce knowledge of the biology and aquaculture potential of autochthonous species, the limitation of seed supply and the declining performances reported in some cultivated stocks.

In this context, the "Catfish Asia" project which deals with the two catfish families, Clariidae and Pangasiidae, has the main following goals:

- To acquire a stronger knowledge of the biological diversity of SE Asian catfishes and to enhance its utilisation through a correct identification and characterisation of valuable species, populations and strains of aquaculture interest. This approach represents an important precondition to the sustainable management of cultivated and natural stocks and to guide conservation efforts of these economically important resources. It will also contribute to a better knowledge of their phylogeographic relationships.
- To acquire sound biological bases for the development of catfish culture in the SE Asian region. The evaluation of the aquaculture potential of the autochthonous species (diversification) and the optimisation of their rearing cycle (artificial propagation) through technologies adapted to the local conditions are essential elements for a better production in the future.

The research work associates six institutes and laboratories from Indonesia, Vietnam, France and Belgium. The specific objectives, general methodologies, first results and prospects of the project are presented.

INTRODUCTION

Among the freshwater fish, the Siluriformes (including both autochthonous and exotic species) represent an important group in Asia. Several species are actively exploited by fisheries and in a variety of aquaculture production systems. Although ranking beyond carps and tilapias, the total volume of cultured catfishes in Asia has shown fast increase during the last 20 years and was estimated around 124,000 t in 1993 (Csavas, 1994). In the Lower Mekong Basin, catfishes of the clariid and pangasiid families are of particular significance for aquaculture. In 1993, they represented an estimated annual production of about 21,000 t in Vietnam, 36,000 t in Thailand and 6,000 t (75 % of the total freshwater cultivated fish production) in Cambodia. In Indonesia, clariids are the main cultivated catfishes (4,000 t in 1992) but pangasiids present also a high potential for aquaculture, particularly in Sumatra and Kalimantan (Sudarto and Sumastri, 1994).

Indigenous culture techniques were developed for native species that are generally preferred by local consumers. However, in clariids, the actual trend is to cultivate F1 hybrids between the introduced African catfish (*Clarias gariepinus*) and various local species (*C. macrocephalus* or *C. fuscus* in Thailand or Vietnam, *C. meladerma* in Indonesia). These hybrids appear to combine the estimated flesh quality of local species and the faster growth rate and disease resistance of the introduced one. Because of the presence of a variety of pangasiid species and their omnivorous

nature, culture techniques can be adapted to the local conditions. Pangasiids are used both in small-scale or industrial production systems and can be reared in high-density cage culture, low input polyculture systems, integrated livestock/fish farming or with human waste utilisation (Peignen, 1993; Cacot, 1994; Csavas, 1994). The ability of some of these catfishes to undergo aerial respiration allows their use for a valorisation of poorly oxygenated aquatic environments.

However, major constraints for further development and sustainable management of cultivated and natural catfish resources still remain. A part of the encountered problems is listed below, as they were identified in 1996.

- Aquaculture has often been based on the utilisation of introduced species while the knowledge on the biology and the potential of autochthonous species remains scarce. As an example, the African catfish *Clarias gariepinus* has been spread all over SE Asia where it is cultured either as such or after hybridisation with local *Clarias* species. In Indonesia, although more than 10 pangasiid species were listed from the ichthyofauna, the only *Pangasius* cultured in this country remained *Pangasius hypophthalmus*, which was initially introduced from Thailand.
- Diversification of the cultivated species is required both for a better response of fish culturists to market demands, and for a better fit with the diversity of habitats and consumer preference. However, main limitations are the followings.
  - The systematic of Siluriformes remains poorly known in this region and information on their genetic structure (species, populations) is very limited. In the pangasiid family, despite a systematic revision of the group (Roberts & Vidthayanon, 1991), numerous discrepancies were found in recent descriptions of the fish fauna. This was particularly the case for the Mekong delta where the available information relative to taxonomy and even the number of represented species was still inconsistent (Khoa & Huong, 1993; Lenormand, 1996). In Indonesia, the only local pangasiid species tested for aquaculture was misidentified as *P. pangasius* and remained to be correctly named. For SE Asian clariids, the situation was even more confusing as the most recent revision was made by David in 1935. In the absence of reliable identification keys, cultured species are often misidentified. This situation impairs a comprehensive view of the culture potential of these fishes and a correct interpretation of the information published on their biology and culture.
  - In most cases seed supply is impaired by the absence of reproductive control in captivity and by fluctuating or limited natural wild juvenile resources (Csavas, 1994; Cacot, 1994).
- Declining performances in cultured fishes have been reported in several areas in SE Asia (Main and Reynolds, 1993).
- Introductions of exotic species for pure culture or hybridisation with native species could induce diseases due to parasites (Welcomme, 1988; Kottelat, 1990) and genetic impacts on native gene pools (Hindar *et al.*, 1991).

Therefore, the precise description and characterisation of species, populations and strains in these fish groups represent a condition *sine qua non* to the sustainable management of their cultivated and natural stocks and to guide conservation efforts of these economically important resources. They should also contribute to a better knowledge of their phylogeographic relationships.

The sound evaluation of the aquaculture potential of the autochthonous species (diversification) and the optimisation of their rearing cycle (particularly artificial propagation) through technologies adapted to the local conditions appears as essential elements for a better production in the future.

These topics were retained has the main goals of the "Catfish Asia" project, which focuses on the two main catfish families of economic importance, the Clariidae and Pangasiidae. The genesis, specific objectives, general methodologies, first results and expected outcomes of the project are presented in the present paper.

GENESIS OF THE PROJECT AND PARTNERSHIP

The first contact between the European and Asian partners today associated in the "Catfish Asia" project took place in 1992 during a prospective mission of two of us in the Southeast Asian region (Lazard & Legendre, 1993). This first

contact allowed the identification of research fields related to fish biology and culture, and partner institutions to develop collaborative programmes. The cooperation was initially engaged by exchange of scientists and students between France, on one side, and Vietnam and Indonesia, on the other side.

In Vietnam, inquiries were made on catfish production systems (Peignen, 1993; Bazir, 1994; Cacot, 1994) and a preliminary study on the systematic, biology and aquaculture potential of pangasiid species from the Mekong Delta was carried out (Lenormand, 1996). Starting from 1994, the French Ministry of Foreign Affairs supported a collaborative programme on the control of reproduction of *Pangasius bocourti*, associating two French (CIRAD and IRD) and three Vietnamese institutions (the Can Tho University (CTU), the University of Agronomy and Forestry (UAF) and the AGIFISH Company). This programme led to the very first spawn of this species in captivity, obtained in May 1995. It represented an important success as, until this date, the millions of *P. bocourti* juveniles necessary to sustain the 15,000 tonnes of annual aquaculture production of this species in the Mekong delta were entirely dependent on captures from the wild.

The study of populations genetic of SE Asian *Clarias* species was also started in 1995 in a cooperation between IRD, the Central Research Institute for Fisheries (CRIFI-RIFF) based in Jakarta, Indonesia, and the University Montpellier II.

These different activities and their results provided a solid basis and motivated the preparation of a more ambitious collaborative research programme on the biodiversity and aquaculture of catfishes in SE Asia. Since November 1996, this programme, abbreviated as "Catfish Asia" [1], is coordinated by IRD and supported by the European Commission. It associates 6 research institutions, from France (IRD and CIRAD), Belgium (Musée Royal de l'Afrique Centrale and Katholieke Universiteit Leuven), Indonesia (CRIFI-RIFF) and Vietnam (CTU) (Fig. 1).

A part of the research is also conducted in close cooperation with the AGIFISH Company in Vietnam and the services of the Directorate General for Fisheries in Indonesia, allowing real possibilities for a rapid and efficient transfer of results from research to the production sector and fish farmers.

OBJECTIVES OF THE "CATFISH ASIA" PROJECT

In order to enhance the **utilisation of the biological diversity** of the local freshwater ichthyofauna, acquire **sound biological bases** for the development of aquaculture, provide an appraisal of the present situation in order **to guide sustainable management** of cultivated and natural fish resources, and strengthen North-South-South cooperation between the European Union, Indonesia and Vietnam by the **transfer and exchange of technology**, the project aims at the following specific objectives:

- To characterise species, populations and strains of autochthonous Clariidae and Pangasiidae catfishes for:
  - A thorough knowledge of their taxonomy and appraisal of their phylogeny and zoogeography.
  - A general inventory of available resources that could be used for culture.
- To contribute to the knowledge of their life history.
- To implement monitoring tools that could be used for the analysis of population microstructuration and monitoring of genetic diversity in cultivated fish stocks (i.e. development of DNA microsatellite loci).
- To assess and compare the aquaculture potential of species, populations and hybrids in the Pangasiidae and Clariidae.
- To develop artificial propagation and culture techniques adapted to local conditions for some target species for which captive broodstock can be available:
  - Identification of the environmental requirements to attain full sexual maturity under rearing conditions and optimisation of induced breeding and artificial fertilisation procedures.
  - Assessment of some nutritional, behavioural and environmental requirements of larval and juveniles stages and optimisation of larval rearing methods.

[1] *Full title of the project : Characterisation, utilisation and maintenance of biological diversity for the diversification and sustainability of catfish culture in South-East Asia.*

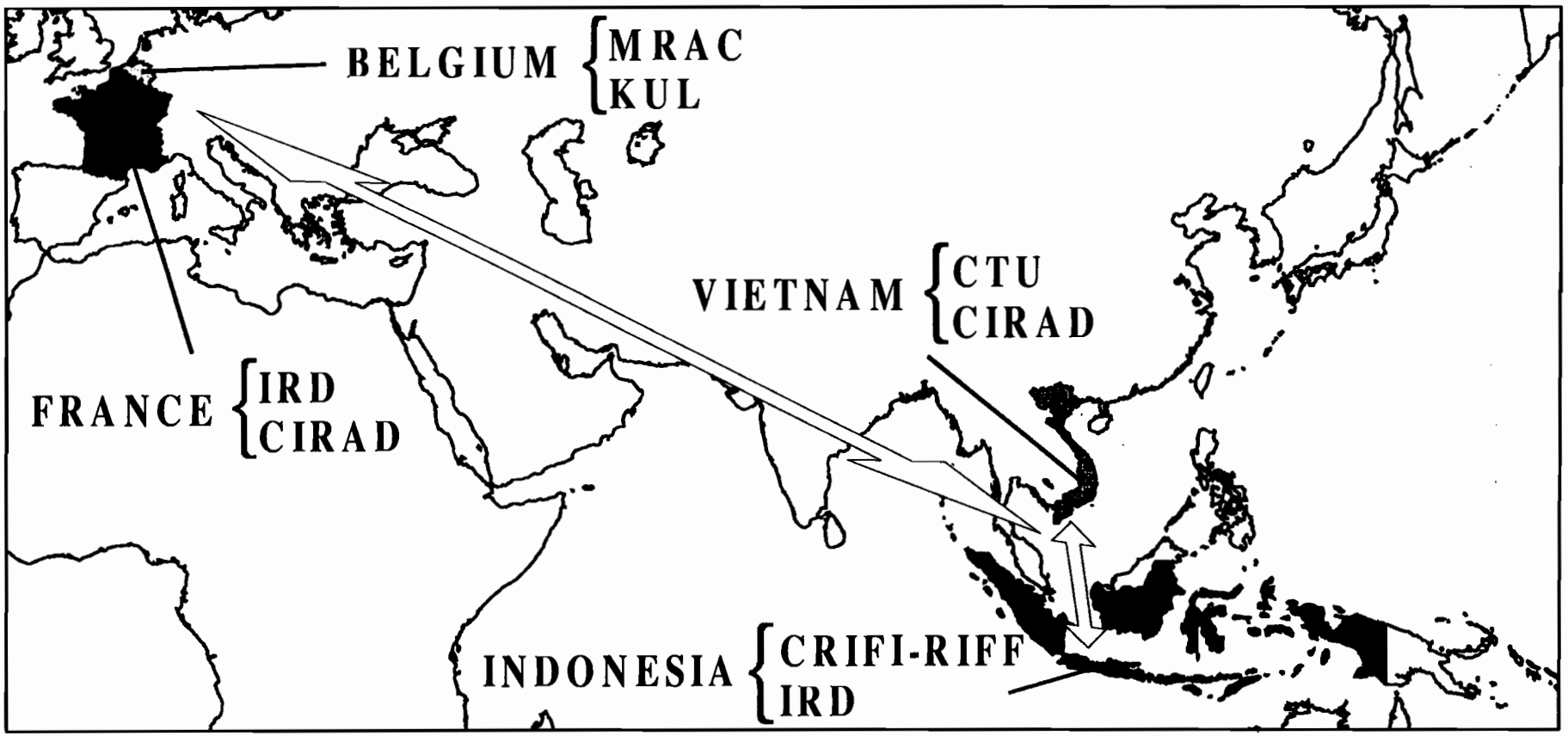

Figure 1 : Institutions involved in the Catfish Asia project and their localisation.

IRD : Institut de Recherche pour le Developpement (Coordinating Institute)
CIRAD : Centre de Coopération Internationale en Recherche Agronomique pour le Developpement
RIFF : Research Institute for Freshwater Fisheries
CTU : Can Tho University
MRAC : Musée Royal de l'Afrique Centrale
KUL : Katholieke Universiteit Leuven

The research work is divided into two major parts:

The **first part** aims at the identification and characterisation of species and populations of actual or potential interest for aquaculture. Three complementary approaches are used: 1) morphometric analysis, 2) estimation of genetic variation, and 3) characterisation of gill parasite communities.

- *The morphometric analysis* consists in a number of measurements, meristic counts and special morphological observations taken on representative samples of species or populations. This part of the work should result in correct and detailed descriptions of the different species, populations or hybrids.
- *The estimation of genetic variation* includes different techniques adapted to the research goals: protein electrophoresis, mitochondrial DNA analysis and microsatellite DNA analysis.
  - ➢ Protein electrophoresis is the most suited to examine evolutionary relationships within a great number of species belonging to the same or different genera. It gives rapidly accurate data in systematic investigations. However, in closely related taxa, differences in variation in allelic frequencies are often indicative but not sufficient to assign a single fish to a particular stock.
  - ➢ The restriction endonuclease analysis of mitochondrial DNA (mt-DNA) is a more accurate approach for population analysis at the intraspecific level. As the differences in mt-DNA base sequences (generated by mutation) are transmitted maternally without recombination, the mt-DNA analysis provides a strong support for stock identification and zoogeography.
  - ➢ The microsatellites DNA analysis allows much greater stock discrimination and is used as a complementary approach to screen wild populations for overall genetic variation.

The investigation of intraspecific differentiation may allow the identification of differentiated populations of potential interest for future use in aquaculture. In addition, the development of DNA microsatellite loci provides highly discriminating tools which could be used subsequently for the characterisation and monitoring of genetic diversity in cultivated fish stocks.

- *The characterisation of gill parasite communities (Monogenea)* completes the genetic and morphometric analyses and provide complementary elements for the study of host's phylogeography. Monogenea have, toward their hosts, a specificity which is generally oioxenous (one species of parasite is present on only one host species). When the specificity is larger (stenoxenous) it is either due to lateral transfer or to genetic relatedness of the hosts. Parasites are identified by morphological studies (optic microscopy) of their sclerotized parts (genital and haptoral apparatus).

For these studies, representative fish samples from wild or culture origin are collected at the regional scale. The morphology, genetics and parasitology of this material are studied concurrently. During the sampling campaigns, observations are also made on the biological traits (reproductive strategies, feeding habits...) of the collected species.

The **second part** of the research is oriented toward a diversification of the cultivated species, the identification of the best performing ones (including hybrids) and an optimisation of the culture practices (particularly artificial propagation).

- *The global and comparative evaluation of the aquaculture potential of pangasiid species* is carried out following a two-stage procedure (Legendre, 1992; Lenormand, 1996):
  - ➢ On the most objective biological and economical criteria, a preliminary screening, the preselection stage, aim to retain those species that, among the entire group, present the greatest potential for a given type of aquaculture.
  - ➢ Then, in the selection stage, an evaluation of the aquaculture performances of the preselected species is made in culture trials.

The choice of an aquaculture candidate depends directly on biological characteristics of the species and on the environment, as well as on the economic and cultural context of the areas or countries concerned (e.g. pangasiids are more appreciated in Sumatra and Kalimantan than in Java). The preselection is based on information obtained from field sampling, market studies and literature analysis. In this view, the maximal size of species represents a useful biological criterion, both in regards to specific market demand for fish size and as a rapid estimator of growth rate (Legendre & Albaret, 1991).

The culture trials are carried out in experimental aquaculture stations from juveniles caught in the wild. Three main characteristics are studied: survival, growth and sexual maturation. The fish are generally tagged for individual identification.

In the clariids, the research work is mostly oriented toward a *comparative evaluation of zootechnical performances of different hybrids and of their parental species*. The following criteria are generally considered: survival, growth, age/size at first sexual maturity, gonadal development, viability of the gametes and possibilities of obtaining viable F2 or back cross fry. Despite an increasing use of *Clarias* hybrids in SE Asian aquaculture, there is a lack of reliable and detailed data on their biological traits and zootechnical performances in comparison to parental species. Similarly, preliminary investigations on hybridisation have been started in the pangasiids.

The *establishment of reliable artificial propagation techniques* is sought in pangasiids actually identified for their interest in aquaculture (particularly *P. djambal* in Indonesia, *P. bocourti* in Vietnam and *P. hypophthalmus* in both countries). This task includes three complementary research actions.

- The *identification of the environmental requirements to attain full sexual maturation* under rearing conditions is done through investigations on the gonadal development of tagged brooders kept in earthen ponds and/or floating cages. The sexual maturity of the females is regularly followed using especially the oocyte diameter and the position of their germinal vesicles determined after intraovarian biopsy. In males, spermiation, volume of collected semen and motility of spermatozoa are used as the main maturity criteria.
- The *optimisation of the induced breeding procedures* involves the following steps: a) defining selection criteria for receptive brooders, b) establishing efficient hormonal treatments to induce oocyte maturation and ovulation, c) determining the optimal latency period between injection and stripping of ova and, d) proposing appropriate standardised techniques of artificial fertilisation and egg incubation.
- The *optimisation of larval rearing methods* requires precise knowledge on larval biology and the development of specifically adapted larval rearing systems. Adequate preys, feeding requirements, behavioural particularities (e.g. occurrence or not of cannibalism) and appropriate weaning time are characteristics particularly considered.

Finally for some *Pangasius* species already used in aquaculture, two supplementary topics were added to the initial objectives of the project: the evaluation of the nutritional requirements of juveniles and a survey of the main pathological problems encountered in various culture systems.

FIRST RESULTS AND PROSPECTS

In 1998, at its mid-term, the Catfish Asia project has already led to many significant results, in terms of both basic and applied research. These results are presented into details all along the contributions compiled in this volume. Some of the main aspects and their implications in terms of further research and development are commented hereafter.

- Representative fish samples from wild or culture origin have been collected from the following areas: Java, Sumatra, Kalimantan, the Lower Mekong Basin (particularly Mekong Delta) and the Chao Phraya River Basin. These campaigns helped to precise the zoogeography of several taxa. Supplementary sampling campaigns remain to be done from other areas in order to complete the collection of species and improve the assessment of their phylogeography.
- In the pangasiids, 18 of the 21 nominal species described by Roberts and Vidthayanon (1991) were already collected and genetically analysed. Considering the observed genetic distances, the results suggest that several groups previously recognised as possible subgenus of *Pangasius* should be elevated at the genus level. Two possible new species, *Pangasius* sp1 and sp2 were also identified. As no specimens of *P. pangasius* could be collected yet, the exact status of *Pangasius* sp1 remains unclear. Up to now, it was however misidentified as *P. djambal*. A detailed morphometric analysis of all specimens collected remains to be done in this family. The fact that *Pangasius* sp1 was collected mostly in the estuarine part of the rivers (in the Mekong Delta, Sumatra and Kalimantan) confer to this fish a special interest, as it might represent a candidate for brackish water aquaculture both in Vietnam and

Indonesia.

In the clariids, the morphometric analyses and genetic investigations carried out (not all presented in this volume) confirm the needs for a systematic revision of this group, as far as the Asian species are concerned.

The establishment of reliable identification keys in these two families should be a major objective of the second half of the project.

- The parasitic analysis of pangasiid species showed the occurrence of more than fifty species of gill monogenea, of which 3 or 4 only were already described. An important work of description of the newly discovered monogenean species is currently carried out. As about 80% of these monogenea are host specific, the analysis of monogenean parasite communities at the host species or populations levels will represent a useful complement to the morphometric and genetic studies for the inference of hosts phylogeny.
- Several functional new microsatellite markers have been developed in both *C. batrachus* and *P. hypophthalmus*. Some markers previously developed for *C. gariepinus* were also operational in these two species. In a short term, these markers will be used for the molecular identification of wild caught *Clarias* species in Sumatra and the study of their small-scale genetic structure. Samples of cultured *C. gariepinus* from various Asian localities will be also analysed to identify their level of inbreeding.
- Among the 21 recognised species of Pangasiids (now probably 23), only 2 of them (*P. bocourti* and *P. hypophthalmus*) are actually cultured on a large scale in SE Asia. Investigations were also performed on *P. gigas* in Thailand, initially for a conservation purpose.

  Assuming the validity of the two new species discovered during the project, 13 pangasiids are present in Indochina (Mekong Basin) and 13 in the Indo-Malay Archipelago (5 endemic to the Borneo Island); 5 species are common to both geographical areas. Due to their relatively small size, 5 species do not seem to present much interest for culture in comparison to the others pangasiids: *P. macronema*, *P. micronema*, *P. pleurotaenia*, *P. polyuranodon* and *Helicophagus waandersii*. Among the species reaching larger sizes, captive stocks of 5 of them have been constituted during the first half of the project in order to evaluate their aquaculture potential: *P. conchophilus*, *Pangasius* sp1 and *P. larnaudii* in Vietnam, and *P. nasutus* and *P. djambal* in Indonesia. The latter species already showed a much faster growth rate than *P. hypophthalmus*. Besides *Pangasius* sp1, another species, *P. krempfi*, found in the estuaries of the Mekong Delta, may present a particular interest for brackish water culture and should be investigated.
- An important part of the work has been oriented towards an optimisation of induced breeding and larval rearing procedures of species already reproduced in captivity (*P. bocourti* and *P. hypophthalmus*). Significant progresses were obtained for both species and reliable methodologies were established, taking into account the seasonal variations of reproduction, the hormonal treatments applied and the viability of gametes and embryos. In *P. hypophthalmus*, the possibility of an all year round production of fry was demonstrated, and the survival and growth of larvae in controlled environment were strongly improved by using appropriate prophylactic treatments and feeding strategies. The transfer of these optimised artificial propagation methods to fish farmers already started in Vietnam and in Indonesia.
- Besides these two species, the reproduction in captivity of *P. conchophilus* and *Pangasius* sp1 in Vietnam and of *P. djambal* in Indonesia was also obtained for the first time from wild specimens acclimatised to culture conditions. Although artificial propagation techniques still needs to be specifically adapted for those fishes, these first successes now open strong opportunities for the development of their use in aquaculture.
- The availability of juveniles in some species also permitted to start investigations on their feeding strategies and nutritional requirements. The clear tendency of *P. bocourti* juveniles to start fat deposition even when fed at a low feeding rate and the apparent inverse relationship between fat deposition and gonad maturation in adult females of this species are aspects requesting further studies.
- Hybridisation is a manipulation permitted by the control of reproduction in captivity. Several crosses were tested in the clariids and the reciprocal hybrids between *P. bocourti* and *P. hypophthalmus* also proved to be viable in Vietnam. However, although hybrids may present several valuable qualities for

aquaculture and are always fascinating to produce, evaluation of their performances should be reserved to research station with closed facilities. Uncontrolled hybrid production trials on fish farms have been made both in Vietnam and Indonesia between various pangasiid species. The risk that individuals could escape from fish farms is high and may have serious impacts on the native gene pools. Therefore the production of hybrids in aquaculture should be considered only after a full evaluation of their performances in comparison to those of their parental species and an assessment of their possible fertility.

The possibility or not of making hybrids between two species is also a good indication of their genome compatibility. The fact that the hybridisation between *C. gariepinus* and *C. batrachus* was successful in Bangladesh and neither in Indonesia nor in Vietnam, suggests that the nominal species *C. batrachus* corresponds to a species complex. It should be noticed that high genetic divergences were also observed between the low and highlands *C. batrachus* populations of Sumatra. Therefore the actual status of this important species for aquaculture clearly requests further investigations.

The final workshop of the "Catfish Asia" project is planned in May 2000 and will be organised in Bogor, Indonesia. A full synthesis of the results obtained during the 4 years of this collaborative programme will be presented at this occasion.

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INTEREST OF BASIC AND APPLIED RESEARCH ON *PANGASIUS* SPP. FOR AQUACULTURE IN THE MEKONG DELTA: SITUATION AND PROSPECTS

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Abstract

Aquaculture of the Mekong catfish, *Pangasius* spp., is of major economic interest in the Mekong Delta in Vietnam. The culture of this fish has always been depending on collecting fry and fingerling in the wild, mainly upwards the Mekong River in Cambodia. A collaborative research programme has led, for the first time, to the artificial reproduction of *Pangasius bocourti* and the biotechnical process has been extended to other species of *Pangasius*. This work opens many doors both in terms of research and of development which are described in this paper.

WHY WORKING ON PANGASIUS?

A few words of history

The first glance at *Pangasius* aquaculture in Vietnam took place in November 1992 when the promoters of the "Catfish Asia" Project (Lazard & Legendre, 1993) came on mission to Ho Chi Minh City where they met the scientists of the University of Agriculture and Forestry (UAF) of Thu Duc, to Can Tho City at University of Can Tho and to Chau Doc where they met the staff of AFIEX (now, AGIFISH).

They felt totally amazed facing such a level of development in *Pangasius* floating cage and pond production. The first step was to propose to the Vietnamese partners from UAF to carry out diagnostic studies on both these *Pangasius* production systems (Cacot, 1994; Peignen, 1993) with an additional one on aquaculture in man-made lakes (Bazir, 1994). All these studies took place in 1993 and were conducted by teams mixing Vietnamese and French graduating students.

This was the real starting point of the "Catfish Asia Project", as far as the Vietnamese component is concerned. The assessment on the *Pangasius* culture sector pointed out clearly that the main bottleneck for *Pangasius* culture large scale development was the lack of artificial reproduction/controlled propagation of this fish for supplying the Mekong catfish farmers.

Situation of Pangasius *fry and fingerlings supply for aquaculture*

- In the Mekong Delta, the aquaculture production of *Pangasius* exceeds significantly the production from capture fisheries, showing the economical importance of aquaculture in the global fisheries sector.
- Fry and fingerlings of both cultured *Pangasius* species (*P. bocourti* and *P. hypophthalmus*) come totally from the wild (estimation: 80% from Cambodia and 20% from Vietnam for *Pangasius bocourti*). About 20 to 25 millions of fingerlings pieces of each species are estimated to be required for culture purposes, both in cages and in ponds.
- The expenses for cage production show that more 50% (even much more, depending on the year) is due to fingerlings costs.

MOTIVATIONS OF RESEARCH WORK ON *PANGASIUS*

First of all, the main motivation for carrying out research work on *Pangasius* spp. in the Mekong Delta aims at studying biological bases on autochthonous fish, then enhancing the use of local biodiversity.

Entering more into details, motivations of this work are, among others, as follows.

Generally speaking, it can be assessed that only **a few basic and applied researches** has been conducted on *Pangasius* spp and particularly in Vietnam where, nevertheless, Mekong Catfish is of major economic value. In Vietnam, only *Pangasius sutchi* (now *P. hypophthalmus*) has been studied and only 5 references have been reported through the various bibliographic databases as to July 1997 (Table 1). The main research works carried out on *Pangasius* reproduction and genetics are summarised briefly in Table 2.

- **Taxonomy** among the *Pangasius* genus was until recently a mess. As an example, when the French-Vietnamese collaborative research programme on artificial propagation started in 1994, *Pangasius bocourti* was still denominated *P. pangasius* and *P. hypophthalmus* was known as *P. micronema* (Roberts & Vidthayanon, 1991). And most probably, there remains a lot to be done in this field.
- As **no artificial reproduction** techniques were available on these species cultured in Vietnam, the demand for basic and applied research in this field was very strong, both from the scientists (University of Can Tho) and the farmers (AGIFISH).
- **Species diversification** is one of the main requests from the fish farmers. The fish farmers are probably, among the population of farmers around the world, those who are the most in the process of seeking diversification of cultured species for better income and benefits. This explains probably why all the fish farmers are more or less somewhat scientists and why research work in this field is, in the same time, so difficult and motivating. Diversification of species appears to be a priority for *Pangasius* farmers in Vietnam.

REPRODUCTION
- **induced spawning (mainly on *P. sutchi*)**
- **sperm preservation**
- **incubation methods/techniques**
- **embryonic development**
- **morphological study of gonads**

GENETICS
- **caryotype study**
- **heritability of some morphological characters**
- **hybridisation**
  - ***P. sutchi* x *C. batrachus*** } → **hybrids**
    x *C. macrocephalus* }
  - ***C. macrocephalus* x irradiated sperm *P. sutchi*** → **gynogenetic *C. macrocephalus***

Table 2: Main research works carried out on *Pangasius* reproduction and genetics.

STATUS OF RESEARCH ON *PANGASIUS* IN THE MEKONG DELTA IN 1998

The research programme which is carried out since about 4 years already led to very significant results which can be summarised as follows. These

Countries	Systematic	Wild populations and fishing	Biology	Reproduction	Genetics	Culture techniques	Nutrition	Pathology	Total
Bangladesh	1	3	3						**9**
China	2		1	1		3			**5**
India		2	1		1	1			**4**
Indonesia	1	1	2	1				1	**8**
Laos		1				2			**1**
Malaysia	1	1		3			5	2	**12**
Thailand	1	1	5	12	6	8	4	5	**42**
Vietnam				1		2		2	**5**
Indochina Peninsula	1					1			**2**
South-east Asia						1			**1**
World	1					1			**2**
Undetermined	1		6					1	**8**
Total	**9**	**9**	**17**	**18**	**7**	**19**	**9**	**11**	**99**

Table 1: Status of scientific and technical references on *Pangasius* from the various databases: CAB, AGRIS, BIOSIS, ASFA, PASCAL, AGRICOLA (15.07.1997).

significant results are due to the fact that the opportunity was given to conduct experimentation both in ponds at Can Tho University and in floating cages in Chau Doc.

Reproduction in captivity

This work started in 1994 and the first artificial reproduction of *P. bocourti* in the world took place in Can Tho University in May 1995. The bio-technical process developed on this species was extended to *P. hypophthalmus* and to inter-specific hybrids (Cacot *et al.*, in preparation).

AGIFISH company, which closely participated to this experimental work, was able to produce several millions of fry of *Pangasius* spp. in 1997 within its new built hatchery in Chau Doc: 3 000 000 pieces of *P. hypophthalmus*, 1 000 000 pieces of hybrid *P. hypophthalmus* female x *P. bocourti* male and 400 000 pieces of *P. bocourti*.

Fry and fingerling nursing

The work on fry and fingerling was only made possible due to availability of fry in large quantities and started in 1996. If the main problems related to *P. bocourti* larval rearing seem to be solved with satisfying survival rates (Hung *et al.*, in press), it is still not the case for *P. hypophthalmus* which requires additional research work on feeding related to its cannibalistic behaviour and its need for feeding on live preys.

Taxonomy

Even if now, things seem to be approximately clear about the taxonomy of *Pangasius* species and particularly in the Mekong Delta, investigations in this field are still required in order to answer remaining questions about the occurrence of questionable species (*P. djambal* for example).

"New" species for aquaculture

There is no doubt that, in the future, other species of *Pangasius* will be cultured by fish farmers and this trend towards a species diversification will be boosted thanks to the artificial reproduction control.

Table 3 gives the main characteristics of the *Pangasius* species of potential interest for aquaculture found in the Mekong Delta.

Special emphasis should be put on hybrids which can be considered as "new" species.

Following the successful reproduction in captivity and the reliable technique developed, *Pangasius* hybrids have started to be produced on quite a large scale in Vietnam in the AGIFISH Chau Doc hatchery. Many fish hybrids are already used on a large scale for aquaculture purposes around the world (Table 4). Nevertheless, the potential of pure species has to be carefully assessed before starting hybridisation programmes

Scientific name	Vietnamese name(s)	Growth (scale from 1 to 5)	Robustness (scale from 1 to 4)	Fat (scale from 1 to 3)	Market Value (scale from 1 to 3) ( ) : interest for processing +fat ; ++non fat	REMARKS
P. bocourti	ca ba sa	3	2	3	2 (++)	
P. conchophilus	ca hu	2	3	2	1 (+)	
P. djambal (?)	ca bong lau* ca tra ban	3	3	1	2 (+)	
P. hypophthalmus	ca tra	5	4	1	1 (+)	not a good reputation (latrine ponds)
P. larnaudii	ca vo dem	4	1	3	2 (++)	strong pathologic problems in culture
P. macronema	ca xac soc	1	no data	1	1	NO INTEREST FOR AQUACULTURE low growth rate, low price, no potential for processing
P. micronema	?	1	no data	?	no data	
P. polyuranodon	ca dua	1	no data	1	1	
P. sanitwongsei	ca vo co	5	no data	2	no data (+)	ggressiveness in captivity
P. krempfi	ca bong lau*	NO CULTURE IN CAGES MAINLY BECAUSE NO GOOD SURVIVAL WHEN MANIPULATIONS/HANDLING			3 (+)	THE BEST FOR BRACKISH WATER CULTURE

* commercial name (used on markets and Long Xuyen factory).

Table 3: Main characteristics of *Pangasius* species of Mekong Delta for aquaculture (from Lenormand, 1996).

because the large scale artificial production of hybrids is not without risk for the environment and, moreover, could have very harmful impacts on natural populations (Table 5).

PROSPECTS FOR RESEARCH AND DEVELOPMENT IN *PANGASIUS* AQUACULTURE IN THE MEKONG DELTA

All the work already done on *Pangasius* aquaculture in the Mekong Delta in a very short time (4 years) opens very numerous gates for the future, both in terms of research and in terms of development.

Research

The main research topics in the field of *Pangasius* aquaculture in the next future appear to be the following ones.

Species hybridised	Effect/Advantage and Comments	Reference
Ctenopharyngodon idella x *Aristichthys nobilis*	Sterile – Natural triploïds	Allen & Wallendorf, 1987
Misgurnus mizolepis x *M. anguillicandatus*	High hatch and survival Probably fertile	Kim *et al.*, 1995
Hypophthalmichthys molitrix x *Aristichthys nobilis*	Fertile + positive heterosis for growth rate Food and feeding strategies intermediate to parents	Krasnai, 1987
Cyprinus carpio x *Labeo rohita* x *Cirrhinus mrigala* x *Catla catla*	Sterile, good growth in monoculture and survival, good seinability Tetraploid carps x diploid cyprinids → triploids Many deformities and high juvenile and larval mortality	Khan *et al.*, 1990
Oreochromis spp. crosses	- all male offspring - cold tolerance - salinity tolerance - colour (red tilapia)	many authors !!
Colossoma macropomum x *Piaractus mesopotamicus* x *P. brachypoma*	Good growth rate and good early survival probably fertile	FAO, unpublished Senhorini *et al.* 1988
Clarias gariepinus x *Clarias macrocephalus*	Superior flesh and growth characters Artificial spawning induction/fertilisation required	Suresh, 1991
Clarias gariepinus x *Heterobranchus longifilis*	Fertile F1 and F2 hybrids and back crosses	Nwadukwe, 1995
C. gariepinus x *Heterobranchus bidorsalis*	Positive heterosis for growth rate and size	Salami *et al.*, 1993

Table 4: Some examples of fish hybrids used in aquaculture as reported by FAO 1997).

Pangasius hypophthalmus ♀ x *Pangasius bocourti* ♂ (1995)

Fecundity: >> *P. bocourti*

- Quality of flesh: good for processing (Agifish) > *P. bocourti* ?
- Growth rate ⎫ to be assessed accurately and compared
- Food conversion ratio ⎭ with the pure parental strains
- Fertility of hybrids: ?
- If fertile: F_1, F_2... F_n
  backcrosses with parents
  selection breeding programme possible
  DANGER: crosses of escaped hybrids in the wild
  if crosses with natural populations possible

MAXIMUM CARE OF CULTURE CONDITIONS (hatchery, ponds, cages) HAS TO BE TAKEN

Table 5: Some considerations on *Pangasius* hybrid(s).

- *Optimisation of reproduction in captivity*
  - broodstock management aiming at increasing the quality of gametes, eggs and larvae;
  - ovulation treatments diversification and fertilisation and eggs incubation practices optimisation;
  - fecundity of *P. bocourti* increase by several means: broodstock management (particularly by improving the nutrition practices in terms of quality and quantity) and increasing the number of spawning around the year ;
  - extending upwards and downwards in the time the reproduction period.
- *Fry and fingerling production*
  - larvae and fry management for reducing cannibalism and mortality rate in *P. hypophthalmus* (including antibiotic treatment trials);
  - larvae and fry feeding optimisation (live and artificial feed) in different rearing environments and systems (tanks, aquaria, ponds, hapas,...).
- *Feeding practices for market size* Pangasius *production*
  - improving the traditional feeding practices of *Pangasius* spp cultured in floating cages.
- *Pathology*
  - considering globally the pathologic aspects of *Pangasius* culture the approach in this field should include several items from culture practices (eco-pathology) to chemical treatment trials.

Development

The results obtained in the framework of the "Catfish Asia" Research Project should be transferred to the producers at two levels:

- Large-scale level: this transfer is already going on with AGIFISH, one among the main producers of *Pangasius* in floating cages in Chau Doc and the main processing factories manager (Long Xuyen).
- Small-scale level: the transfer of artificial reproduction of *Pangasius* and mass fry production could be conducted, after a careful pilot scale technology verifying step, by institutions such as Can Tho University.

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MARKETING OF PANGASIID CATFISHES IN JAVA AND SUMATRA, INDONESIA

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Abstract

One major obstacle for the development of fisheries is related to marketing. The objectives of the presented study were to analyse the market system of Pangasiid in selected areas in Java and Sumatra, Indonesia. The main conclusions were as follows: pangasiid marketing in Java and Sumatra is a profitable activity - total demand for *Pangasius hypophthalmus* seed in South Sumatra per se was around 2 000 000 seeds.month^{-1} - supply of *Pangasius hypophthalmus* is seasonal - larval survival rate is very low - people in Sumatra like *Pangasius djambal* more than *P. hypophthalmus* even though its price is higher - *Pangasius djambal* production still rely on capture in the river even if first success in induced-spawning of this species in captivity offers promising perspectives. Information on the marketing of *Pangasius* can be used by the policy maker as a reference for the development of an appropriate culture system as well as its future planning and strategy.

INTRODUCTION

Pangasiid catfishes are gaining in popularity as well as in importance among several other important fishes in Indonesia. This fact is due to the better performance of the fish, reasonable price, high fecundity, high growth rate, adaptability to a wide range of environment and good quality of the meat. In Indonesia there are two kinds of pangasiid, namely *Pangasius hypophthalmus*, which was introduced from Thailand in 1972 and called "Jambal Siam", and the others, which are indigenous species locally called "Patin". Among these, *Pangasius djambal*, mostly distributed in Sumatra and Kalimantan, is particularly appreciated by consumers. The availability of Patin relies only on the catch from the natural bodies of water, due to the difficulties in breeding the fish in captivity. In contrast, *Pangasius hypophthalmus* can be spawned in captivity and is therefore gaining in popularity for grow out. This paper presents information and discussion on the production, price trend and marketing of pangasiids. The data are based on literature and on studies conducted in the fish markets in Bogor, Sukabumi, Bekasi (West Java) and Palembang (South Sumatra).

IMPORTANCE OF THE STUDY

One major obstacle for the development of fisheries in Indonesia is related to marketing. Data as well as information on fish markets are still scarce. The information on performance of the business of pangasiid in Java and Sumatra can be used by the policy maker as a reference for the development of a system for the domestication of the fish as well as its future planning and strategy.

OBJECTIVES

The objectives of the study were to analyse the market system of Pangasiid in selected areas in Java and Sumatra, specifically :

- To estimate the marketing cost and profit margin at various market levels.
- To describe the marketing channel of the fish.
- To identify the problems in marketing pangasiids faced by the farmers and the traders.

METHODS

Source of data

The primary data were collected from the 9 farmers producing seed in Bogor, Sukabumi and Bekasi (Java), and 20 fish farmers, 10 fishermen, 5 brokers, 6 wholesalers and 8 retailers in Sumatra. The data were collected during the period of March-April 1998.

Method of data collection

Three primary data collection were carried out by

questioners to the breeders, farmers, fishermen and traders. The questioners for the breeders and farmers concerned breeding techniques as well as growing techniques.

Analysis

Data were analysed for the statistics frequency, average and percentage. The data were used to predict the status of marketing margin, marketing practice and problems.

RESULTS AND DISCUSSION

Seed production

Location

The majority of the seed production activities of *Pangasius hypophthalmus* are located in West Java (Bekasi, Bogor and Sukabumi) and Jakarta. Some of the breeder are also found in Sumatra. The reason for the existence of the majority of seed production activities in West Java is that the farmers in Sumatra have not yet mastered the breeding technique. The farm breeders usually keep the fish in nursery until 1 inch (2.5 cm) in length. At this size the transportation is still easy. At bigger fish size the pectoral spine becomes stiffer and can easily break the plastic bags used for transportation resulting in increased fish mortality.

Fish seed production technique

The fish seed production is generally carried out by the following steps:

- The male and female brooders are reared in separate ponds.
- The mating period corresponds to the wet season, around 4-6 month/year (October to March), during which hormonal induced breeding is performed.
- The selection for the parent stock is based on the colour of the papillae and the softness of the belly.
- The female are fasted one day prior to hormonal induction.
- After one day of fasting the fish receive injections of Ovaprim at a total dose of 0.9 ml.kg^{-1} body weight. A first injection (1/3 of the dosage) is followed six hour later by a second injection (2/3 of the dosage).
- Six hour after the second injection, the fish is usually ready for ovulation.
- The eggs of one female are fertilised using the sperm from two males.
- Generally one out of three females fails to produce eggs of good quality.
- The fertilised eggs are incubated in aquarium.
- Larvae of 2-3 days of age are fed *Artemia* nauplii until 8 days old.
- At 8-12 days of age larvae are fed with *Moina*.
- At 12-30 days of age larvae are fed with fresh tubifex.
- The fry are kept in aquarium for about 30 days, after reaching 1 inch long they are ready to be sold.

Growing of the fish for consumption

Areas for growing

In Sumatra, the farmers mainly grow pangasiids in the provinces of South Sumatra, Jambi and Riau where the demand for these fish is very high, whereas in other provinces in Sumatra it remains moderate. The high preference of the fish in the three provinces was due to the limited availability of the indigenous *Pangasius* in those areas. The shortage of *Pangasius djambal* is due to the high rate of consumption and insufficient production making the demand shifting to *Pangasius hypophthalmus* as a substitute.

Grow out technique

The grow out of *Pangasius hypophthalmus* is done in wooden or bamboo cages, each with a size of 2.5 x 1.5 x 1 m. The cages are placed along the edge of a river. One cage is stocked with 500-1000 fish of 2 inch size. The growing period is 4-6 months, during which fish are fed with commercial feed. The production reaches up to 250-500 kg/cage. The growing in captivity takes place in the wet season when the river is at its maximum water depth. In the dry season the supply of pangasiid drives to the catch of indigenous fish *Pangasius djambal*. When the water level in the river is not high, the fish become easier to catch.

Flow of the marketing

Seed marketing channel

In *Pangasius hypophthalmus* seed marketing, the producers are fish seed farmers and the consumers are growout farmers. A seed marketing channel system traces the flow of the product from the producer to the final consumer through seed marketing intermediaries. *Pangasius hypophthalmus* seed can take several routes before reaching the ultimate consumer. In Java and Sumatra islands, the identified alternative channels were as follows:

1 Producer → Broker → Wholesaler → Retailer → Fish farmer

2 Producer → Broker → Wholesaler/Retailer → Fish farmer

3 Producer → Broker → Retailer → Fish farmer

The shortest channel was the third one where the producer sold the fish directly to the retailer and eventually to the consumer. The trade route was short in markets which were relatively near the source of supply.

Consumption and marketing channel of Pangasius hypophthalmus

The producers are farmers who grow *Pangasius hypophthalmus* in wooden or bamboo cages until the fish reach 500 gram in individual weight whereas the consumers are people and restaurants.

Three fish marketing lines have been identified:

1 Producer → Broker → Wholesaler → Retailer → Consumer

2 Producer → Broker → Retailer → Consumer

3 Producer → Retailer → Consumer

Among the three lines, the last one is the most efficient with fish going directly from the producer to the retailer thus bypassing broker and wholesaler.

Marketing Channel of Patin or Pangasius djambal

Two Patin marketing lines have been identified which are as follows:

1 Fisherman → Wholesaler → Retailer → Consumer

2 Fisherman → Wholesaler/Retailer → Consumer

The marketing channels above show that the Patin marketing is efficient.

The Patin marketing channel from the fisherman (as producer) to the consumer goes through a wholesaler and a retailer. It indicates that the marketing cost is not high, therefore the consumer prices do no significantly differ from the fisherman prices.

Market size

Seed Production

Seed production in West Java was conducted by 30 farmers with a production level ranging from 100 000 to 1 000 000 seed.month^{-1}.

The production period is 4-6 months, the capacity of seed production by 27 farmers ranged from 100 000 to 200 000 seed.month^{-1}, that of 2 farmers 500 000 seed.month^{-1} and one farmer produces 1 million seed.month^{-1}.

The production capacity depends on capital availability, number of available broodfish and farmer technical knowledge.

Brokers in Bogor and Palembang collect the seed mainly from the farmers. The number of seed collected are limited, depending on the ability of supply. From the broker, the seeds usually go to the wholesaler, as is the case in Palembang, South Sumatra. The wholesaler in South Sumatra also acts as a retailer. The total demand of *Pangasius hypophthalmus* seed in South Sumatra is around two million seed.month^{-1}.

Fish Grower

The average ownership of cages by a fish farmer in South Sumatra is around 2-6 cages. The duration of the fish growing is around 4-6 months. Fish for consumption are normally sold alive and the fish broker could collect up to two tons of fish to channel to wholesaler. Every wholesaler has the ability to sell 500 kg of fish to the retailer in the market. Every retailer is capable of selling 50 kg of fish per day.

Price, cost and margin

Price

From the interviews with the farmers the average price of a 1 inch long *Pangasius hypophthalmus* was around Rp. 100. The price fluctuation between 1997 and 1998 is presented in Figure 1. The data show that the highest price of the seed occurs at the peak of the demand or in the raining season.

The price fluctuation of *Pangasius hypophthalmus* of body weight greater than 500 gram is given in Figure 2.

Figure 1 and 2 show that, the highest price of the seed and consumption fish size occur in the wet season whereas the lowest in the dry season. As previously indicated, in the dry season the Patin or *Pangasius djambal* become available for capture and therefore people will go back to the preferable taste.

The price fluctuation of *Pangasius djambal* can be seen in Figure 3.

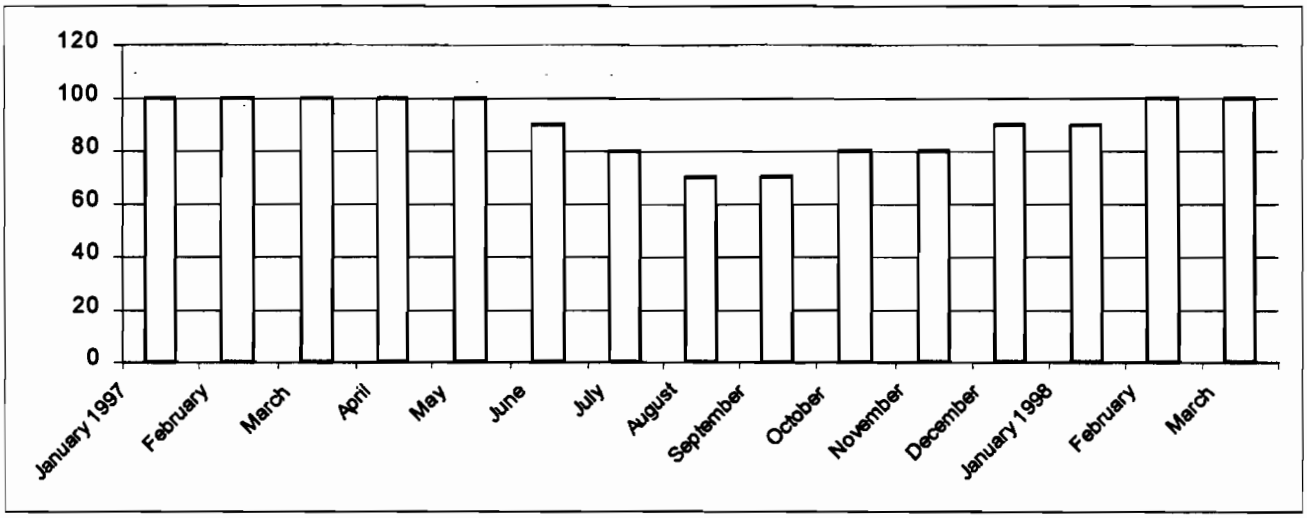

Figure 1: Average price (Ind. Rp.) Fluctuation of *Pangasius hypophthalmus* seed at farmer's level in West Java during 1997-1988.

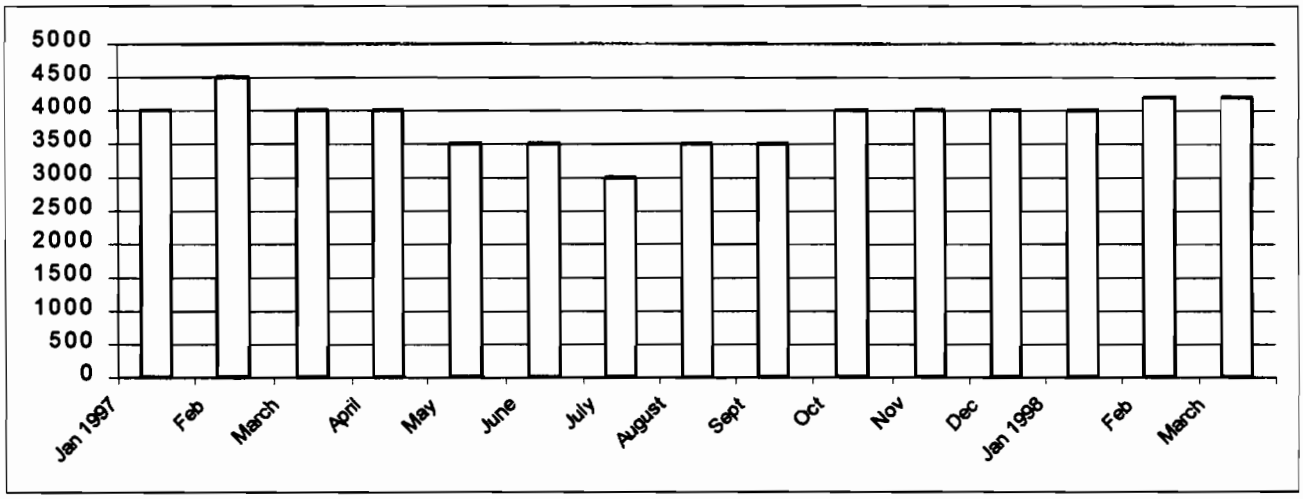

Figure 2: Average price (Ind. Rp.) fluctuation of consumption size *Pangasius hypophthalmus* in South Sumatra at farmers level during 1997–1998.

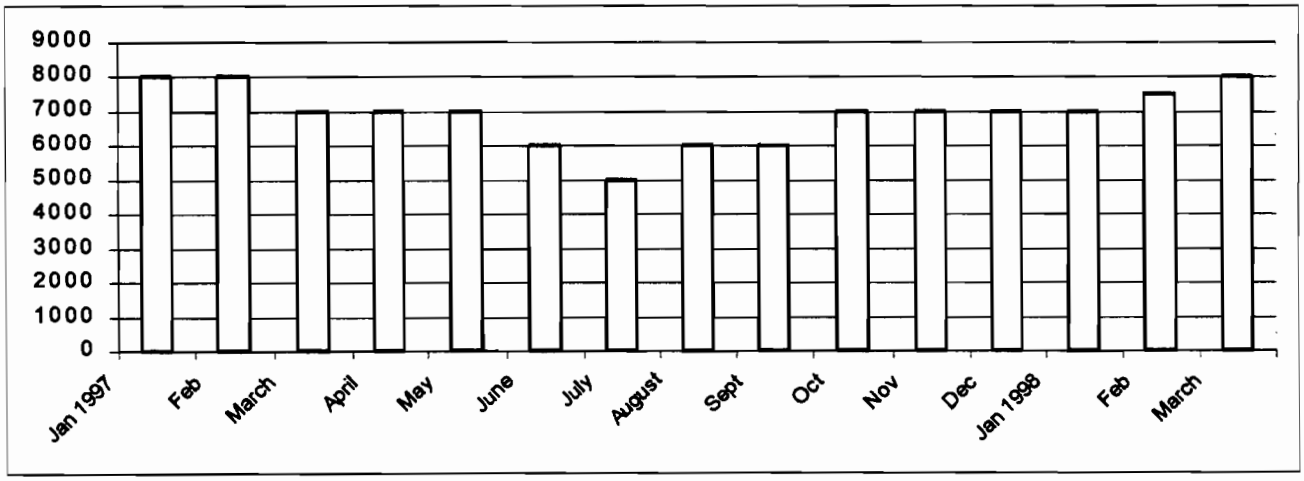

Figure 3: Average price (Ind. Rp.) fluctuation of Patin or *Pangasius djambal* at fisherman's level in South Sumatra during 1997–1998.

The price of Patin or *Pangasius djambal* was twice higher than that of *Pangasius hypophthalmus*, indicating the consumer preference for Patin in comparison to the other species. The price of Patin considered here was the price at fisherman's level (the producer level). The Patin price at consumer level was 75-100% higher than that of the price at fisherman level.

Cost and Margin

The price, cost and profit of seed fish within the marketing channel are presented in Table 1.

The data show that the broker collect the 1-inch fish from the farmer at the price of Rp. 100/seed. This seed were then reared to 2-inch size. The broker sold the 2-inch fish at Rp. 220/seed. Thus broker got the highest profit.

The price, cost and profit of consumption size *Pangasius hypophthalmus* is presented in Table 2.

The highest profit as indicated in the table is again received by the broker, whereas the retailer spent more selling cost. The consumer paid Rp. 5000/kg.

The price, cost and profit in the marketing of Patin or *Pangasius djambal* can be seen in Table 3.

In general, the broker (wholesaler), received the highest profit margin but also faced the highest risk factors.

Production and Marketing Problems

In Indonesia *Pangasius hypophthalmus* (seed or fingerling) are used as seed for growing out to consumption size for domestic markets, as export commodity and as ornamental fish. However the demand of the seed fluctuates, all around the year. The supply of the fish is seasonal because spawning occurs mainly in rainy season. Moreover the larval survival rate is very low about 10-20%.

Patin (*Pangasius djambal*) production is still dependent on catching in the river as it is only recently that successful induced-breeding of this fish could be achieved in captivity (Legendre *et al.*, 1999). Significant supply of Patin occurs only in the dry season when the river water level is low and permit an easy capture of the fish.

	Farmer	Broker	Wholesaler	Retailer
Price (Ind. Rp./seed)	100	220	230	240
Cost (Ind. Rp./seed)		79	6	7
Profit (Ind. Rp./seed)		41	4	3

Table 1: Average price, cost and profit of seed along the marketing chain of *Pangasius hypophthalmus* in West Java in 1998.

	Farmer	Broker	Wholesaler	Retailer
Price (Ind. Rp./Kg)	4200	4450	4700	5000
Cost (Ind. Rp./Kg)		72	110	125
Profit (Ind. Rp./Kg)		178	140	175

Table 2: Average price, cost and profit of *Pangasius hypophthalmus* at consumption size within the marketing channel in South Sumatra in 1998.

	Fisherman	Wholesaler	Retailer
Price (Ind. Rp./kg)	8000	9500	12000
Cost (Ind. Rp./Kg)		600	1100
Profit (Ind. Rp./Kg)		900	1400

Table 3: Average price, cost and profit within the marketing channel of Patin in South Sumatra in 1998.

The people in Sumatra like Patin or *Pangasius djambal* very much, even though the price is much higher than that of *Pangasius hypophthalmus*.

In the market, the price is around Rp. 5 000 per kg for *Pangasius hypophthalmus*, while for *Pangasius djambal* it reaches Rp. 11 000 per kg in the dry season and Rp. 18 000 per kg in the rainy season when the fish is difficult to catch.

The estimated demand for "Patin" in South Sumatra per se is 126 000 kg per month. This figure is obtained from the mean of fish sold by the wholesalers in the area.

From the information above it can be seen that marketing prospect of Patin is promising. Research to produce the technology to culture the Patin to fulfil the demand all around the year has to be carried out.

CONCLUSIONS

- The pangasiid marketing in Java and Sumatra is a profitable venture.
- The marketing channels of pangasiid are simple channels, which mean that the consumer level prices do not significantly differ from those at the producer's level.
- The total demand for *Pangasius hypophthalmus* seed in South Sumatra per se was around 2 000 000 seeds.month^{-1}.
- The supply of *Pangasius hypophthalmus* is seasonal because the spawning take place only in rainy season and the larval survival rate is very low about 10-20%.
- The people in Sumatra like Patin or *Pangasius djambal* very much, even though the price is much higher than that of *Pangasius hypophthalmus*.
- The Patin or *Pangasius djambal* production still rely on capture in the river, but a first success in induced-spawning of this species in captivity offers promising perspectives.
- Research to formulate culture technique of local *Pangasius* has to be continued.

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PRELIMINARY RESULTS ON THE MORPHOLOGICAL CHARACTERISATION OF NATURAL POPULATIONS AND CULTURED STRAINS OF *CLARIAS* SPECIES (SILURIFORMES, CLARIIDAE) FROM VIETNAM

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Abstract

A morphometric analysis of wild and cultured *Clarias* specimens originating from Vietnam indicated the presence of three species : *C. batrachus*, *C. macrocephalus* and *C. gariepinus*. The latter is an African species that has been introduced for fish culture purposes. The status of a fourth species, *C. fuscus*, previously reported from the northern part of the country, could not be clarified yet as no material could be examined. This is the subject of forthcoming research.

INTRODUCTION

Three *Clarias* species have been reported in literature as naturally occurring in Vietnam: *C. batrachus* (Linnaeus, 1758), *C. macrocephalus* Günther, 1864 and *C. fuscus* (Lacépède, 1803) (Ha-Dinh-Duc, 1982). The former two species are widespread, while the latter has only been reported from the North of the country. A fourth species, *C. gariepinus* (Burchell, 1822) naturally occurs in Africa and has been introduced to Vietnam for fish culture purposes, in particular hybridisation with *C. macrocephalus*. Identification of these species in the field is sometimes problematic and there is some doubt on the correctness of their specific identification.

As part of an overall systematic revision of the south-east Asian *Clarias* species, this paper presents preliminary results of the morphometric analysis of *Clarias* specimens collected in Vietnam.

MATERIAL AND METHODS

Hundred and eight specimens originating from Vietnam have been examined. They were tentatively identified when collected and included the following species: *Clarias batrachus*, *C. macrocephalus* and *C. gariepinus* and the hybrid between *C. gariepinus* x *C. macrocephalus*. All specimens of *C. batrachus* (N=34) and *C. macrocephalus* (N=35) were collected from the wild. For each species, about half of them were bought at Can Tho market and the others at Thu Duc and Binh Chanh markets (Ho Chi Minh City area). The *C. gariepinus* (N=24) and hybrid (N=15) specimens were obtained respectively from three different fish farms. In total 73 fish were correctly preserved and were used for a detailed morphometric analysis. The material is deposited in the collection of the Musée Royal de l'Afrique Centrale, Tervuren, Belgium. The four syntypes of *Clarias macrocephalus* housed in the collections of the British Museum (Natural History) London, have also been examined.

On each specimen 30 point-to-point measurements were taken using dial calliper. Measurements follow Teugels (1986). They include (Figure 1): 1) Total length (TL); 2) Standard length (SL); 3) Maximum body depth (MBD); 4) Caudal peduncle depth (CPD); 5) Head length (HL); 6) Head width (HW); 7) Snout Length (SNL); 8) Interorbital distance (IOW); 9) Eye diameter (ED); 10) Nasal barbel length (NBL); 11) Maxillary barbel length (MBL); 12) Inner mandibular barbel length (IMBL); 13) Outer mandibular barbel length (OMBL); 14) Occipital

process length (OPL); 15) Occipital process width (OPW); 16) Frontal fontanel length (FFL); 17) Frontal fontanel width (FFW); 18) Premaxillary toothplate width (PMW); 19) Vomerine toothplate width (VMW); 20) Predorsal distance (PDL); 21) Preanal distance (PAL); 22) Prepelvic distance (PPL); 23) Prepectoral distance (PPEL); 24) Dorsal fin length (DFL); 25) Distance between occipital process and dorsal fin origin (OP-DF); 26) Pectoral spine length (PESL); 27) Pectoral fin length (PEFL); 28) Pelvic fin length (PFL); 29) Anal fin length (AFL); 30) Caudal fin length (CFL). For each specimen, the number of gill rakers on the first branchial arch has been counted. Using radiographs the following six meristic counts were made on each specimen: 1) Number of dorsal fin rays; 2) Number of anal fin rays; 3) Number of vertebrae; 4) Number of abdominal vertebrae; 5) Number of caudal vertebrae. Finally a number of special morphological observations were noted on each specimen: shape of the occipital process; shape of the frontal fontanel; serrations on the pectoral spine.

The data obtained were introduced in a database for subsequent factor analysis. Principal component analysis (PCA) was done using the STATISTICA (StatSoft Inc.) package (versions 3.1 for analysis and 4.5 for graphs). Measurements are log transformed before the PCA was run on the covariance matrix. An independent PCA was run on the correlation matrix for the untransformed meristic count data.

RESULTS

A comparison between natural populations originating from the Can Tho and Ho Chi Minh City areas for both *Clarias batrachus* and *C. macrocephalus* did not enable to distinguish them. Therefore, all specimens of each species were subsequently considered as one group for further analysis.

It should be noted however that in a PCA of all the *C. macrocephalus* specimens examined, the

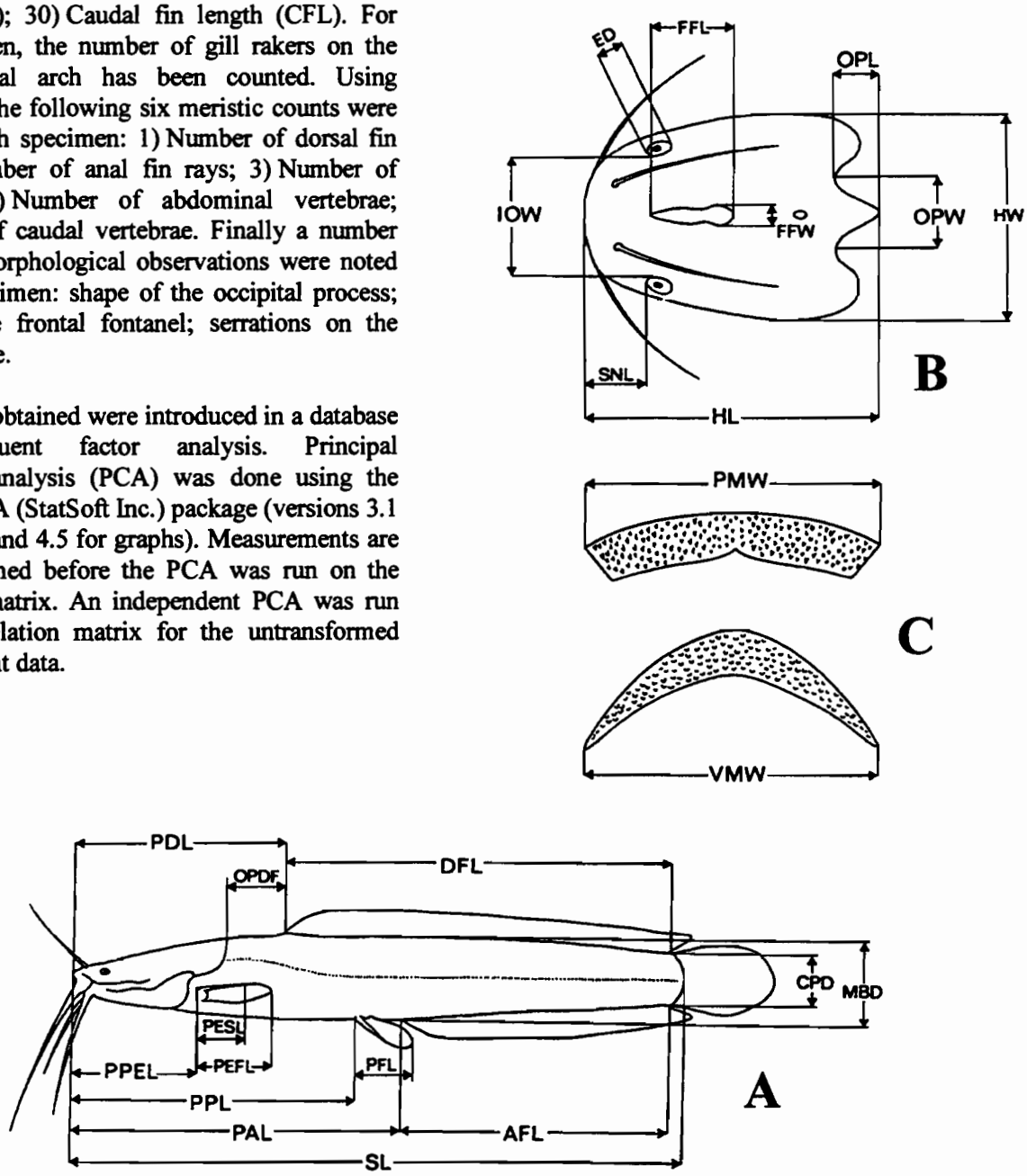

Figure 1: Measurement taken on the body (A), the head (B) and the toothplates (C)
For abbreviations see text.

type material, originally described from Thailand, was, at least in part, distantly set from the other specimens. Two of the types are small-sized (168-174 mm Standard Length) and have a reduced (18) number of gill rakers on the first branchial arch, while the others are large-sized (266-267 mm SL) and show 32-33 gill rakers on the first arch. The former has a pointed occipital process while in the latter it is extremely rounded. Comparison between the type material and equally sized specimens indicated that the small-sized *C. macrocephalus* types differ significantly. Therefore it is most likely that the type material of *C. macrocephalus* in fact includes two different species. Ongoing research on *Clarias* specimens from Thailand, the type locality of *C. macrocephalus* will clarify this.

Figure 2 illustrates the plot of a PCA for 23 log-transformed metric variables (excluding total length, standard length, nasal, maxillary, inner and outer mandibular barbel length and caudal fin length) for all specimens examined of *C. batrachus* and *C. macrocephalus*.

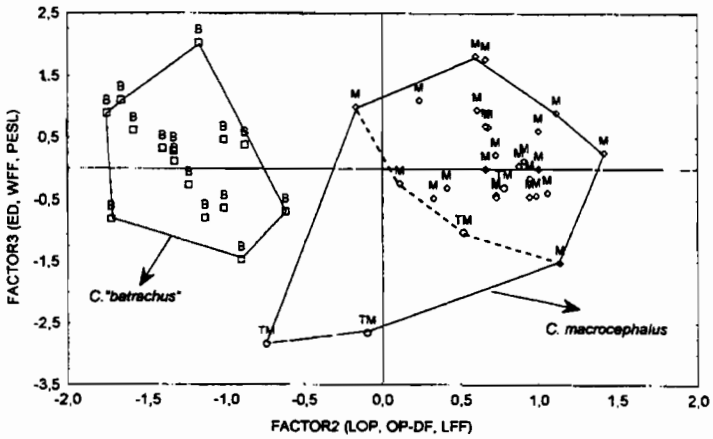

Figure 2: Plot of a Principal Component Analysis using 23 log transformed metric variables for *Clarias* species occurring in Vietnam. B = *C. batrachus*, M = *C. macrocephalus*, TM = Syntypes of *C. macrocephalus*. Stippled line: excluding the two aberrant syntypes of *C. macrocephalus*.

All specimens of *C. batrachus* are situated on the negative part of the second factor while all but three (only one if the aberrant type specimens are excluded) *C. macrocephalus* are located on the positive part of the second factor. The second factor is merely defined by (in decreasing importance) the length of the occipital process, the distance between the occipital process and the dorsal fin origin and the length of the frontal fontanel. These characters easily enable to distinguish both species (Fig. 3).

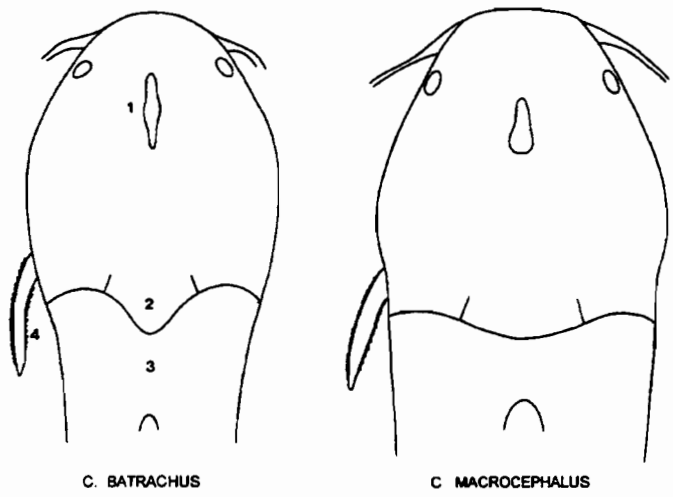

Figure 3: Most striking external morphological differences between *Clarias batrachus* and *C. macrocephalus*. 1. Frontal fontanel shape; 2. Occipital process shape; 3. Distance between occipital process and dorsal fin origin; 4. Inner pectoral spine serrations.

Figure 4 illustrates the number of gill rakers in function of the standard length for the different *Clarias* species found in Vietnam as well as for the hybrid between *C. gariepinus* x *C. macrocephalus*. *Clarias gariepinus* is distinguished from all the others by its numerous gill rakers. The hybrid *C. gariepinus* x *C. macrocephalus* has an intermediate number of gill rakers between that of the two parental species. *Clarias batrachus* has the lowest gill raker number.

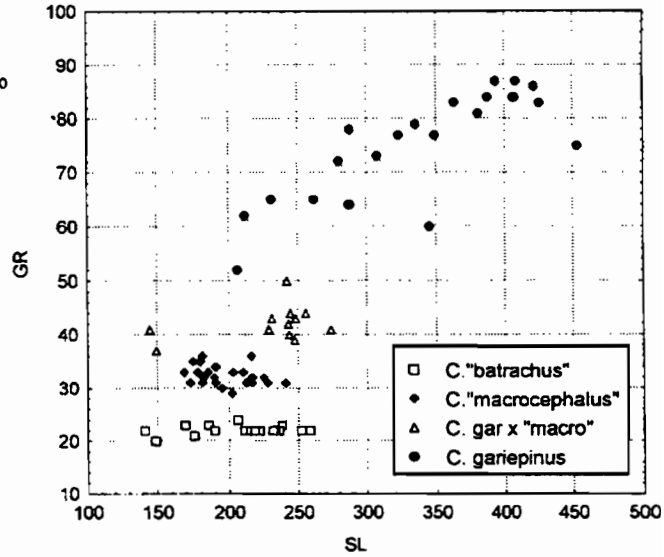

Figure 4: Number of gill rakers on the first branchial arch in function of the standard length (mm) for the different *Clarias* species from Vietnam and the hybrid between *C. gariepinus* x *C. macrocephalus*.

Figure 5 shows the plot of a PCA for 23 log-transformed metric variables (excluding total length, standard length, nasal, maxillary, inner and outer mandibular barbel length and caudal fin length) for all specimens examined from Vietnam.

Clarias batrachus and *C. macrocephalus* are distantly set (*cf. supra*). *Clarias gariepinus* and *C. macrocephalus* partly overlap and their hybrids are superposed with the two parental species. Note that there is hardly any overlap between *C. gariepinus* and *C. batrachus*, two species for which the artificial hybridisation was unsuccessful.

DISCUSSION

The results obtained so far are still preliminary and incomplete. No specimens of *Clarias fuscus* have been examined so far, but a shipment is expected in the near future. A recently sent collection has not been examined so far.

Nevertheless the results show interesting data. The type material of *Clarias macrocephalus* apparently includes two species. The two small specimens do not correspond to the currently accepted definition of this species (rounded occipital process; relatively high number of gill rakers; ...).

The real status of these specimens is presently being examined.

No morphometrical differences have been observed between natural populations of both *Clarias batrachus* and *C. macrocephalus* from the two locations studied in the South of Vietnam. No striking differences were found between *C. gariepinus* cultured in Vietnam and *C. gariepinus* naturally occurring in Africa. Finally, the hybrid between *C. gariepinus* x *C. macrocephalus* shows an external morphology which is intermediate between that of the parental species.

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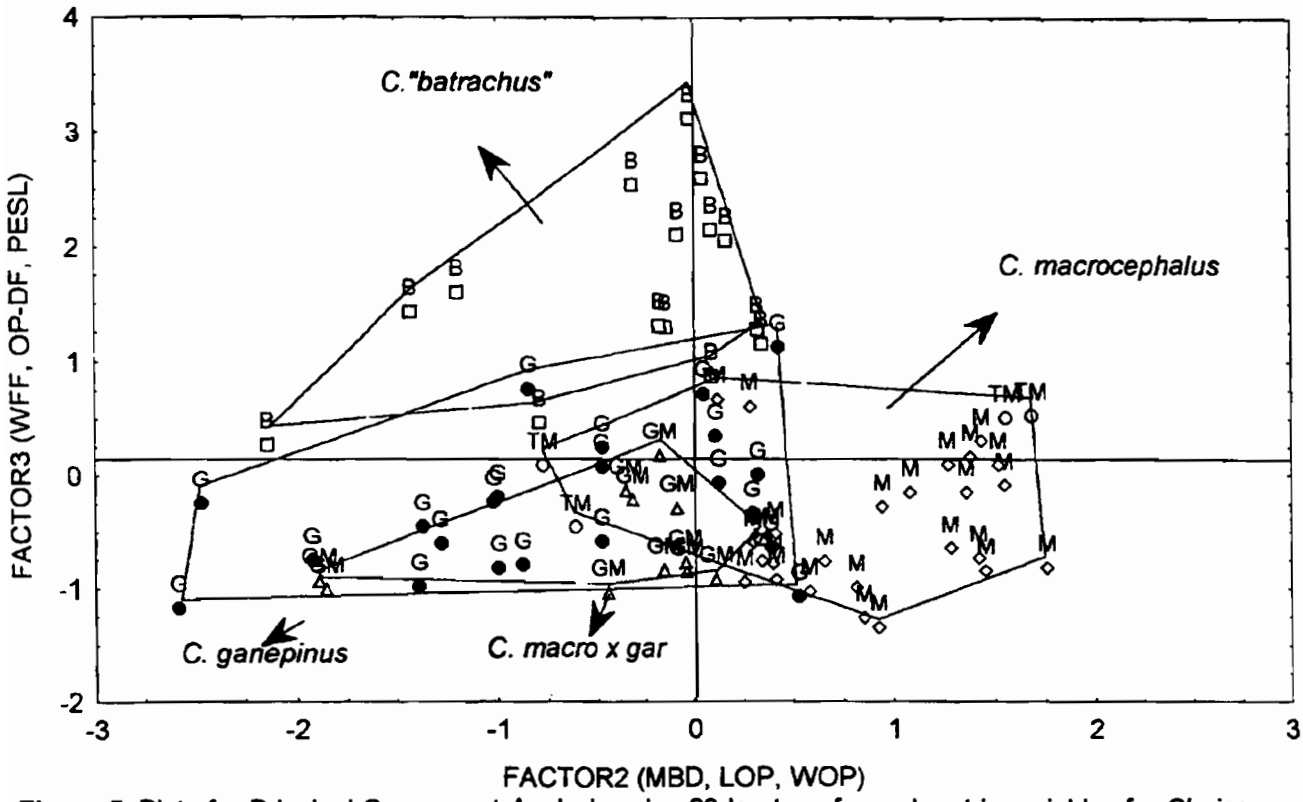

Figure 5: Plot of a Principal Component Analysis using 23 log transformed metric variables for *Clarias* species occurring in Vietnam. B = *C. batrachus*, M = *C. macrocephalus*, TM = Syntypes of *C. macrocephalus*, G = *C. gariepinus*, GM = *C. gariepinus* x *C. macrocephalus*.

PRELIMINARY RESULTS ON THE MORPHOLOGICAL CHARACTERISATION OF NATURAL POPULATIONS AND CULTURED STRAINS OF *CLARIAS* SPECIES (SILURIFORMES, CLARIIDAE) FROM INDONESIA

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Abstract

As part of an ongoing systematic revision of the south-east Asian *Clarias* species, this paper presents the results of a morphometric study of 317 specimens from Indonesia: 255 of them were collected from the wild in Sumatra and Kalimantan and 62 came from fish culture stations in Java and Sumatra.

The results obtained indicated the presence of five or six species : *Clarias batrachus*, *C. macrocephalus* (?), *C. meladerma*, *C. leiacanthus*, *C. teijsmanni* and *C. nieuhofii*. Preliminary identification characters are given.

INTRODUCTION

Fourteen nominal *Clarias* species have been reported in literature as naturally occurring in Indonesia: *C. batrachus* (Linnaeus, 1758), *C. leiacanthus* Bleeker, 1851; *C. macrocephalus* Günther, 1864; *C. magur* (Hamilton Buchanon, 1822); *C. melasoma* Bleeker, 1852 (with its unjustified emendation *C. melanosoma*); *C. meladerma* Bleeker, 1846 (with its unjustified emendation *C. melanoderma*); *C. olivaceus* Fowler, 1904; *C. nieuhofii* Valenciennes, 1840; *C. pentapterus* Bleeker, 1851; *C. pulcher* Popta, 1904; *C. punctatus* Valenciennes, 1840; *C. teijsmanni* Bleeker, 1857; *C. thienemanni* Ahl, 1934 and *C. cataractus* (Fowler, 1939). The original description of nine of them was based on specimens originally collected in this country: *C. leiacanthus*, *C. melasoma* (in part), *C. pentapterus* an *C. pulcher* were originally described from "Borneo" (present Kalimantan); *C. meladerma*, *C. punctatus* and *C. teijsmanni* were originally described from Java and *C. melasoma* (in part), *C. olivaceus* and *C. thienemanni* were originally described from Sumatra.

The systematic status of some of these nominal species has already been studied by previous authors. Hora (1936) considered *Clarias magur* as a junior synonym of *C. batrachus*. Bleeker (1858) synonymised *C. melasoma* with *C. meladerma*, although Fowler (1941) considered both species as junior synonyms of *C. dussumieri* Valenciennes, 1840. *Clarias olivaceus* was synonymised with *C. batrachus* by Fowler (1941). Bleeker (1857) considered *C. pentapterus* as a junior synonym of *C. nieuhofii*. Weber & De Beaufort (1913) supposed that *C. pulcher* is a junior synonym of *C. teijsmanni*. Bleeker (1858) considered *C. punctatus* as a junior synonym of *C. batrachus*. Although these synonymies have to be checked, the *Clarias* species presently reported from Indonesia mainly refer to *C. batrachus*, *C. leiacanthus*, *C. macrocephalus*, *C. meladerma*, *C. nieuhofii*, *C. teijsmanni* and *C. thienemanni*.

The identification of these species is problematic as no detailed species descriptions nor diagnostic keys are available. As part of an overall revision of the systematic of the south-east Asian *Clarias* species, this paper presents preliminary results on the morphological characterisation of *Clarias* species from Indonesia.

MATERIAL AND METHODS

Three hundred and seventeen specimens collected during the "Catfish Asia" project have so far been examined. Of these 255 originated from the wild and were sampled in Sumatra and Kalimantan. They were tentatively identified by the collectors as *Clarias batrachus*, *C. meladerma*, *C.* "lembat" (vernacular name) and *C.* "bacot" (vernacular name). The remainder consists of cultured specimens (*C. batrachus*) originating from fish culture stations in Java and Sumatra. The specimens were deposited in the collection of the Musée Royal de l'Afrique Centrale, Tervuren, Belgium. The following type material has also been examined: the four syntypes of *Clarias macrocephalus* and the holotype of *C. leiacanthus* housed in the British Museum (Natural History) London; the holotype of *C. nieuhofii* housed in the Muséum National d'Histoire Naturelle, Paris; the two syntypes of *C. meladerma* and the holotype of *C. tejsmanni* housed in the Rijksmuseum voor Natuurlijke Historie, Leiden; the holotype and three paratypes of *C. olivaceus* housed in the Academy of Natural Sciences in Philadelphia; and the four syntypes of *C. thienemanni*, housed in the Zoölogisches Museum der Humboldt Universität, Berlin.

On each specimen 30 point-to-point measurements were taken using dial calipers. Measurements follow Teugels (1986). They include (Figure 1): 1) Total length (TL); 2) Standard length (SL); 3) Maximum body depth (MBD); 4) Caudal peduncle depth (CPD); 5) Head length (HL); 6) Head width (HW); 7) Snout Length (SNL); 8) Inter-orbital distance (IOW); 9) Eye diameter (ED); 10) Nasal barbel length (NBL); 11) Maxillary barbel length (MBL); 12) Inner mandibular barbel length (IMBL); 13) Outer mandibular barbel length (OMBL); 14) Occipital process length (OPL); 15) Occipital process width (OPW); 16) Frontal fontanel length (FFL); 17) Frontal fontanel width (FFW); 18) Premaxillary toothplate width (PMW); 19) Vomerine toothplate width (VMW); 20) Predorsal distance (PDL); 21) Preanal distance (PAL); 22) Prepelvic distance (PPL); 23) Prepectoral distance (PPEL); 24) Dorsal fin length (DFL); 25) Distance between occipital

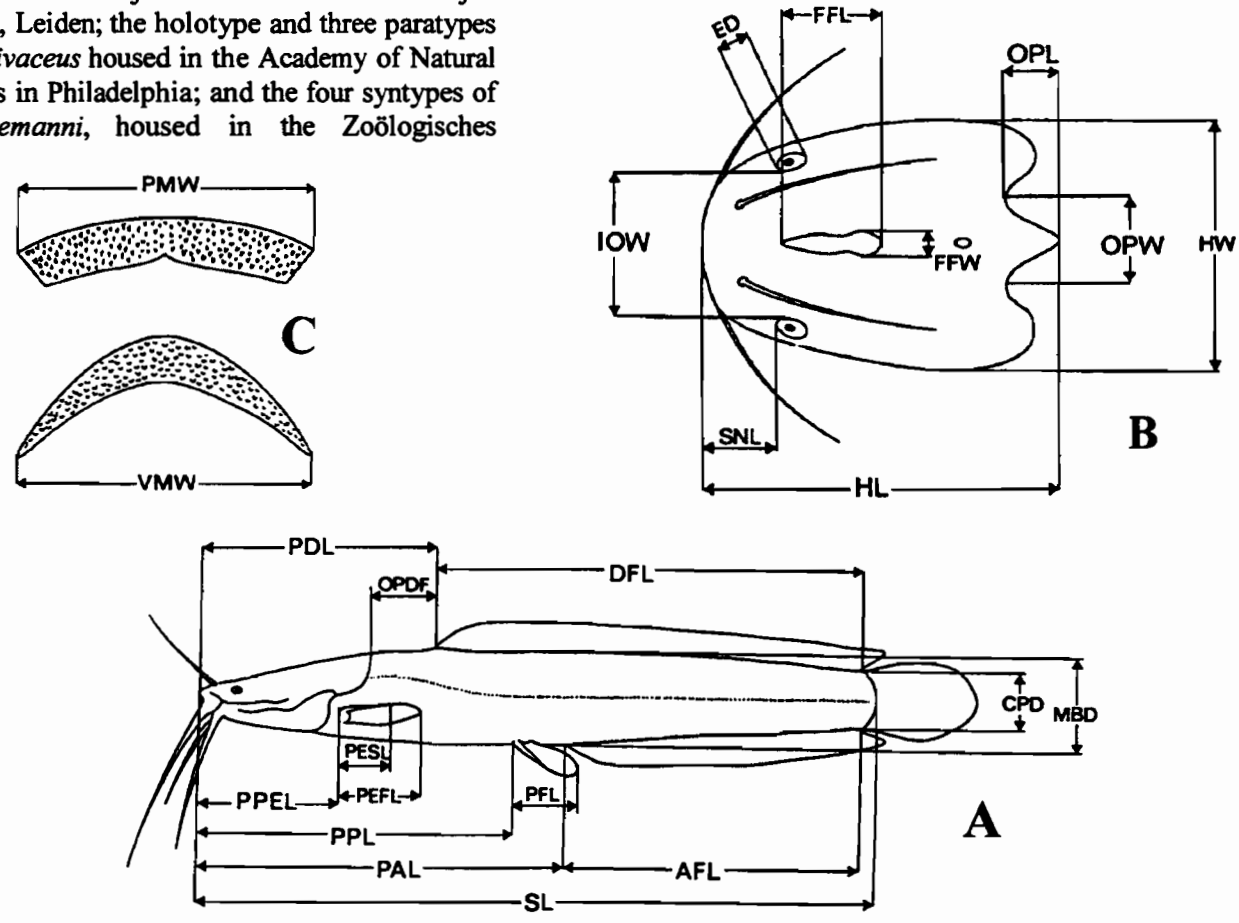


Figure 1: Measurement taken on the body (A), the head (B) and the toothplates (C)
For abbreviations see text.

process and dorsal fin origin (OP-DF); 26) Pectoral spine length (PESL); 27) Pectoral fin length (PEFL); 28) Pelvic fin length (PFL); 29) Anal fin length (AFL); 30) Caudal fin length (CFL). For each specimen, the number of gill rakers on the first branchial arch has been counted. Using radiographs the following six meristic counts were made on each specimen : 1) Number of dorsal fin rays; 2) Number of anal fin rays; 3) Number of vertebrae; 4) Number of abdominal vertebrae; 5) Number of caudal vertebrae. Finally a number of special morphological observations were noted on each specimen: shape of the occipital process; shape of the frontal fontanel; serrations on the pectoral spine.

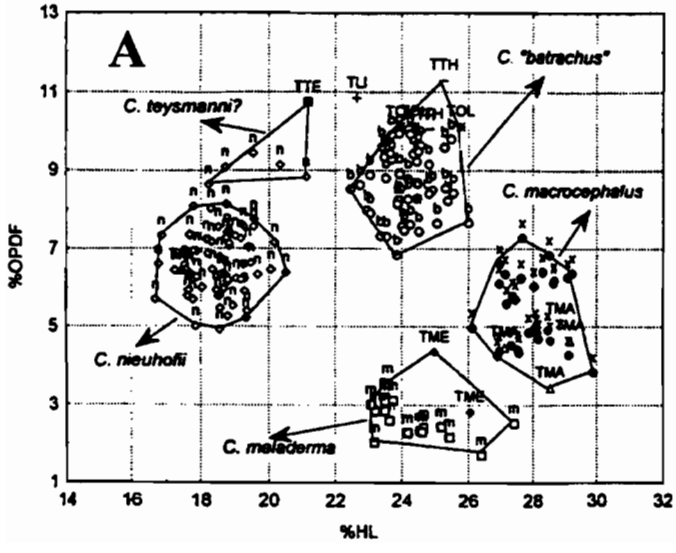
The data obtained were introduced in a database for subsequent factor analysis. Principal component analysis (PCA) was done using the STATISTICA (StatSoft Inc.) package (versions 3.1 for analysis and 4.5 for graphs). Measurements are log transformed before the PCA was run on the covariance matrix. An independent PCA was run on the correlation matrix for the untransformed meristic count data.

RESULTS

It should be noted that the results presented below are preliminary. Work is continuing on additional material.

Sumatra

We first compared the variation of the individual metric variables for all specimens examined.



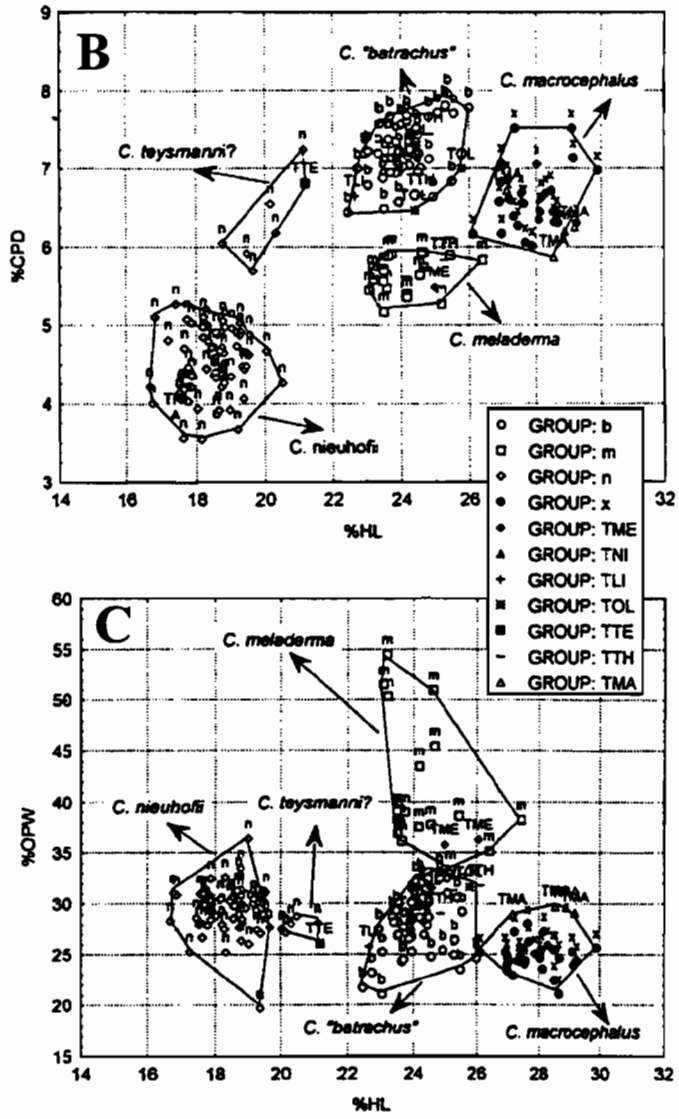


Figure 2: **A.** Distance between occipital process and dorsal fin origin (in percentage of standard length) in function of the head length (in percentage of standard length); **B.** Caudal peduncle depth (in percentage of the standard length) in function of the head length (in percentage of the standard length); **C.** Occipital process width (in percentage of standard length) in function of the head length (in percentage of standard length) for the *Clarias* specimens from Sumatra. TME = types of *C. meladerma*; TNI = type of *C. nieuhofii*; TLI = type of *C. leiacanthus*; TOL = types of *C. olivaceus*; TTE = type of *C. teijsmanni*; TTH = types of *C. thienemanni*; TMA = types of *C. macrocephalus*.

Figure 2 illustrates the results for the occipital process width, the distance between the occipital process and the dorsal fin length and the caudal peduncle depth. For each of these variables a number of groups can be distinguished. When we

look at the position of the type material, some groups can tentatively be identified: *C. nieuhofii* (= *C.* "lembat") and *C. teijsmanni* (= *C.* "bacot") are clearly separated, although some of the specimens originally identified as *C. nieuhofii* are located close to the *C. teijsmanni* type. In the group originally identified as *C. batrachus*, two groups are present: the first one may correspond to *C. batrachus* (the type specimen of this species is lost, see Teugels & Roberts, 1987); it includes the type series of *C. olivaceus* and *C. thienemanni* and also the type of *C. leiacanthus* is usually close to this group. The second group includes the types of *C. macrocephalus*. However as reported by Teugels *et al.* (1999), the syntypes of this species apparently include two species and ongoing research has to clarify their status.

Figure 3 illustrates the plot of a PCA of 24 log transformed metric variables (excluding total length, standard length, nasal, maxillary, inner and outer mandibular barbel length and caudal fin length) for 46 *Clarias* specimens from Sumatra. Only those specimens for which a complete data set is available are included in the analysis.

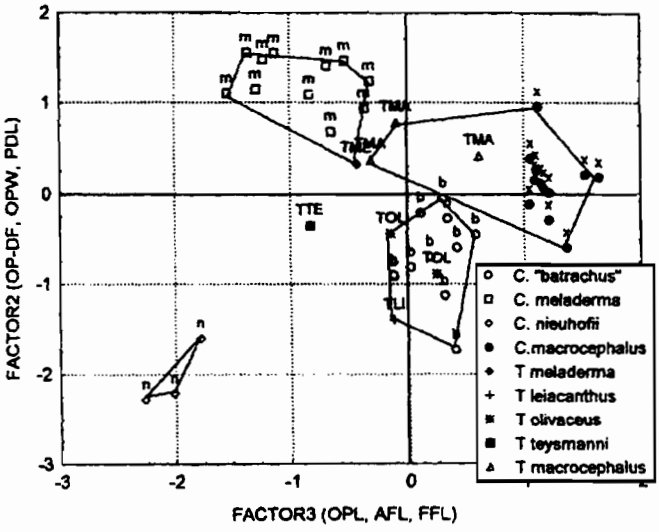


Figure 3: Plot of a principal component analyses using 24 log transformed metric variables taken on 46 *Clarias* specimens from Sumatra. T refers to types.

The *C. nieuhofii* specimens are distantly set on both the second and the third factor. The *C. meladerma* polygon is distantly set from the others on the third factor, while the *"C. batrachus"* group and the *"C. macrocephalus"* (? see above) group are distantly set on the second factor. Note the isolated position of the type of *C. teijsmanni*. The second factor in this analysis is merely defined by the distance between the occipital process and the dorsal fin origin, the occipital process width and the predorsal length. The third factor is defined by the occipital process length, the anal fin length and the frontal fontanel length.

Figure 4 illustrates the plot of a PCA of 21 log transformed metric variables (excluding total length, standard length, nasal, maxillary, inner and outer mandibular barbel length, caudal fin length, dorsal fin length, distance between dorsal and caudal fin and anal fin length) for 116 *Clarias*

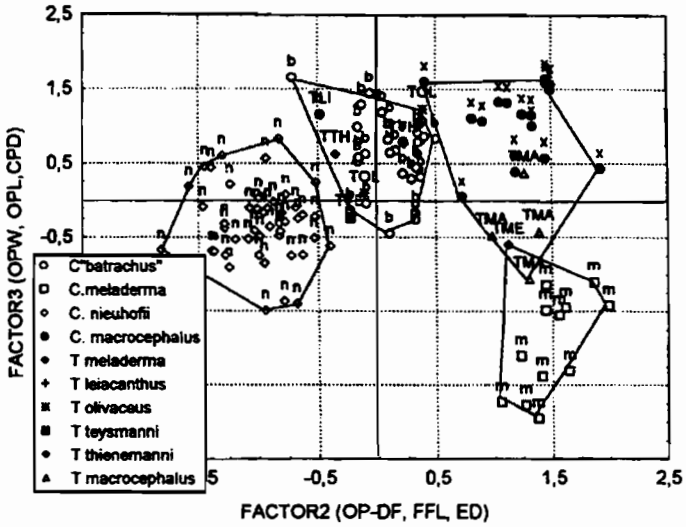


Figure 4: Plot of a principal component analyses using 21 log transformed metric variables taken on 116 *Clarias* specimens from Sumatra, Java and Kalimantan. T refers to types.

specimens from Sumatra. Only those specimens for which a complete data set is available are included in the analysis. Four groups are tentatively recognised: three of them can be separated on the second factor, which is merely defined by the distance between the occipital process and the dorsal fin origin, the frontal fontanel length and the eye diameter; the fourth group can largely be separated from the others on the third factor, which is merely defined by the occipital process length and width and the caudal peduncle depth. Naming the groups however is still problematic: the *"C. batrachus"* group includes the types of *C. olivaceus*, *C. thienemanni*, *C. teijsmanni* and *C. leiacanthus*; the *C. meladerma* group contains a syntype of *C. meladerma*, but also one syntype of *C. macrocephalus* and two other syntypes are closely set to this group. Finally the *C. nieuhofii* group appears as one complex.

Sumatra, Java and Kalimantan

Figure 5 illustrates the plot of a PCA of 24 log transformed metric variables (excluding total length, standard length, nasal, maxillary, inner and outer mandibular barbel length and caudal fin length) for *Clarias* specimens from Sumatra, Java and Kalimantan. The position of the *C. teijsmanni* specimens, close to the *C. nieuhofii* polygon, confirms what is mentioned above. Remarkably, the type series of *C. olivaceus* do not fit in the "*C. batrachus*" polygon. Also the *C. leiacanthus* type is distantly set from *C. batrachus*. Other results confirm those obtained in figure 3.

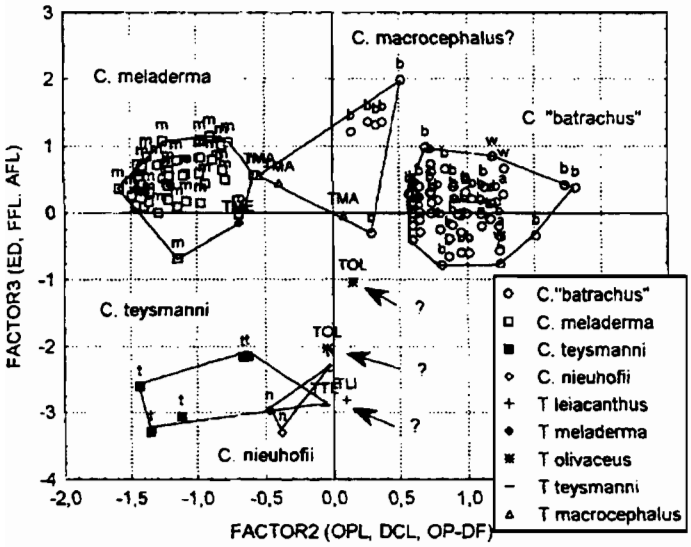


Figure 5: Plot of a principal component analyses using 24 log transformed metric variables taken on *Clarias* specimens from Sumatra, Java and Kalimantan. T refers to types.

***Comparison between wild and cultured specimens from* C. batrachus**

Figure 6 illustrates the plot of a PCA of 24 log transformed metric variables (excluding total length, standard length, nasal, maxillary, inner and outer mandibular barbel length and caudal fin length) taken on wild and cultured specimens from *Clarias batrachus* from Sumatra, Java and Kalimantan. Neither on the second nor on the third factor the wild specimens can be distinguished from the cultured strains. Also the specimens from the different islands cannot be distinguished morphometrically.

DISCUSSION

The preliminary results obtained on the morphometric characterisation of *Clarias*

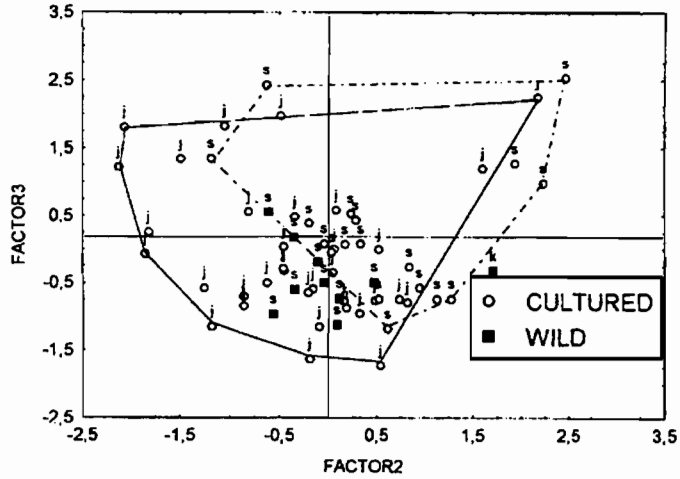


Figure 6: Plot of a principal component analyses using 24 log transformed metric variables taken on wild and cultured *Clarias batrachus* specimens from Sumatra, Java and Kalimantan.

populations from Indonesia, indicate the presence of probably five or six species: *C. batrachus*, *C. macrocephalus* (?), *C. meladerma*, *C. leiacanthus*, *C. teijsmanni* and *C. nieuhofii*.

Clarias batrachus is recognised amongst others by the pointed occipital process, the short distance between the occipital process and the dorsal fin origin, the reduced number of gill rakers (20-25), the head length and the small toothplates; *C. macrocephalus* (?) has a rounded occipital process, its pectoral spine shows numerous serrations (up to 70) on both sides and it has a high number of gill rakers on the first arch (up to 35); *C. meladerma* has a very rounded occipital process, the inner side of its pectoral spine has no serrations and there are 20-25 gill rakers on the first arch; *C. leiacanthus* seems close to, and may be identical to *C. batrachus*; *C. teijsmanni* is recognised by a very long distance between the occipital process and the dorsal fin origin; *C. nieuhofii* differs by a very short head, an anguilliform body and a confluency between dorsal, caudal and anal fins.

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FIRST RESULTS ON THE DIVERSITY OF GILL PARASITES OF SOME CATFISHES HOST SPECIES IN SOUTH-EAST ASIA

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Abstract

First results on the diversity of monogenetic parasites from Catfishes in south-east Asia are presented.

Fifty five different species belonging to two already know and one new genera were found among which 3 or 4 only were previously described.

The general host parasitic species richness and parasitic specificity are summarised. Implications of these two descriptors at host species and population levels are discussed.

INTRODUCTION

Monogenean gill parasites are known to be probably the most specific parasites toward their fish host (Poulin, 1992), and then may be used as "biological tags" in systematic and phylogenetic studies of hosts. This have been recently demonstrated on Cichlids from West African freshwaters (Pariselle, 1996).

The first step in such study is to described the parasitic species found on the different host species sampled, especially since in tropical area the studies related this topic remain scarce.

We present here the first results on the diversity of catfishes gill parasites in south-east Asia.

MATERIAL AND METHODS

The fish were dissected on site immediately after capture, the left branchial arches, separated by dorsal and ventral section, were frozen in liquid Nitrogen, then preserved in a deep freezer at the laboratory until examination. To verify the specific identity of host fishes, the carcasses were numbered, fixed and preserved in formalin. After thawing, the parasites were detached from the gill, using a strong water current, and transferred individually with a mounted needle directly into a drop of ammonium picrate-glycerine mixture on a slide, according to Malmberg (1957).

The preparation was then covered with a round cover slip and after several hours, necessary for the proper impregnation by the mounting medium, the cover slip was sealed. From these preparations, drawings were made of the sclerotised pieces of the haptor and of the copulatory complex (stained with the ammonium picrate) using a camera lucida.

RESULTS

Eighteen fish species belonging to the genera *Pangasius* Valenciennes, 1840, two from *Helicophagus* Bleeker, 1858, three from *Clarias* Scopoli, 1777, one from *Pseudeutropius* (Schilbeidae) and one from *Laides*, sampled in Vietnam, Thailand and Indonesia, were studied for their monogenean gill parasites (Table 1). Fifty five species of monogenean gill parasites were found, of which 3 or 4 only were already described (see: Lim, 1996).

Only three host species seems to have no

Pangasius bocourti Sauvage, 1880	*Pangasius conchophilus* Roberts & Vidthayanon, 1991	*Pangasius djambal* Bleeker, 1846	*Pangasius humeralis* Roberts, 1989
sp1	sp1	sp9	sp29
sp9	**sp3**	sp23	**sp41**
sp23	**sp4**	sp29	sp42
	sp5	**sp40**	**sp43**
		sp44	

Pangasius hypophthalmus (Sauvage, 1878)	*Pangasius krempfi* Fang & Chaux, 1949	*Pangasius larnaudii* Bocourt, 1866	*Pangasius macronema* Bleeker, 1851
sp1	sp2	sp1	**sp13**
sp6		**sp7**	**sp14**
		sp8	sp15
		sp9	**sp16**
		sp10	**sp17**
		sp11	sp18
		sp12	

Pangasius micronema Bleeker, 1847	*Pangasius nasutus* (Bleeker, 1862)	*Pangasius nieuwenhuisii* (Popta, 1904)	*Pangasius pleurotaenia* Sauvage, 1878
sp30	**sp24**	**sp62**	sp42
sp33	**sp25**		**sp45**
sp34	**sp26**		**sp46**
sp35	**sp27**		
sp36	**sp28**		
sp37			
sp38			
sp39			

Pangasius polyuranodon Bleeker, 1852	*Pangasius sanitwongsei* Smith, 1931	*Pangasius n. sp 1*	*Helicophagus typus* Bleeker, 1858
sp1	sp1	sp1	sp1
sp15	sp9	sp2	sp9
sp18	**sp22**	sp42	sp30
sp19		**sp47**	sp31
sp20		**sp48**	sp32
sp21		**sp49**	

Helicophagus waandersii Bleeker, 1858	*Clarias bathrachus* L.	*Clarias meladerma* Bleeker, 1846	*Clarias nieuofi* Valenciennes, 1840
sp1	spC1	spC1	spC1
sp9	**spC2**		
sp31			
sp32			

Laides sp	*Pseudeutropius sp*
sp51	**sp50**
	n. g.1

Table 1: Monogenetic species from Catfishes in south-east Asia
(parasitic species found on one host only are in bold and underlined)

monogenetic parasites (*P. gigas* Chevey, 1930, *P. lithostoma* Roberts, 1989 and *Pangasius* n. sp 2).

The parasites found on hosts of the genera *Pangasius* and *Helicophagus* (49 species) were all belonging to the genus *Thaparocleidus* Jain, 1952.

On *Pangasius* on the 47 species recovered (of which 44 are specific of this genus), 38 were host specific (in bold and underlined in Table 1), 1 was founded on one host species but also on *Helicophagus*, 4 were found on two host species, 2 on 3 hosts species 1 one four and 1 one 7 host species.

Five parasite species were found on *Helicophagus* catfishes, two of them were genus specific and are shared by the 2 species of *Helicophagus* (sp31 and 32).

The parasites from *Clarias* hosts (two species) belonged to the genus *Quadriacanthus* Paperna, 1961.

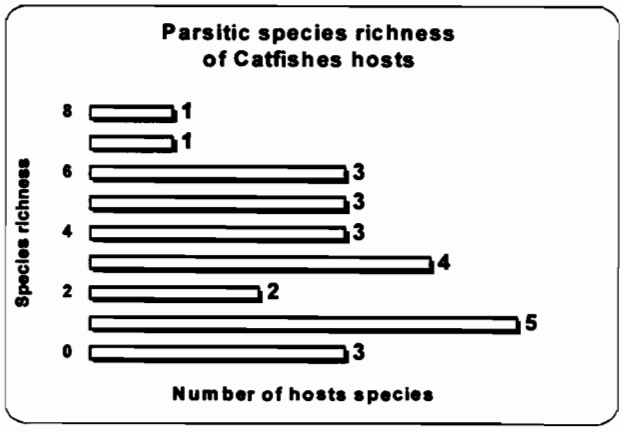
On *Pseudeutropius* and *Laides* there was, on each host, one species of *Thaparocleidus* Monogenea which were both species specific. There were on *Pseudeutropius* two species belonging to a new, and extraordinary genus.

DISCUSSION

I. General scale

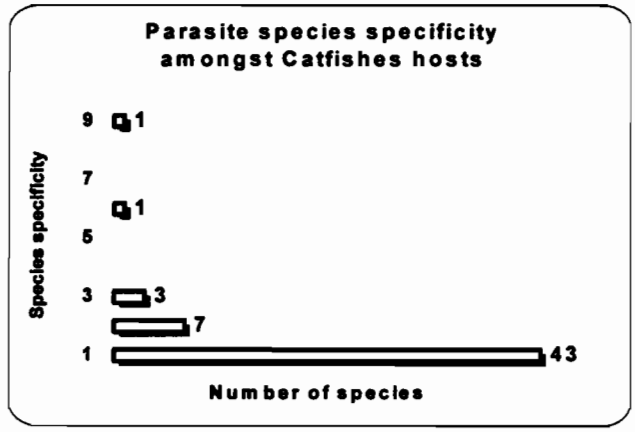
Species richness

For East Asian catfishes, as in other models studied before (West African cyprinids or cichlids, Guégan, 1990, El Gharbi, 1993, Pariselle, 1996), the parasitic species richness amongst the hosts (which is the number of parasite species found on one hosts species) is variable with a random distribution:



Parasite specificity

The parasite species specificity (which is the number of host species on which we may found a parasite species) is also variable: from 1 up to 9. But, as in African fishes, the distribution looks like a negative binomial one:



For these two parameters, the south-east Asian host/parasite system is very similar to other systems all over the world.

II. Host species level

Pangasius sp1 founded in Sumatra, Kalimantan and Vietnam and previously confused with *P. djambal* seems, for the Monogenea to be different from *P. djambal* (no shared parasitic species). So for the parasites (and for the genetic), *Pangasius* sp. 1 is a good species:

Pangasius djambal	***Pangasius* sp. 1**
sp9	sp1
sp23	sp2
sp29	sp42
sp40	sp47
sp44	sp48
	sp49

Phylogenetic studies (see Pouyaud *et al.*, 1999) showed that the genus *Laides* should be placed now within the family Pangasiidae and not in Schilbeidae with the genus *Pseudeutropius*, as noticed in the literature (Kottelat & Whitten, 1993). Then, we have studied the monogenetic fauna from these two genera, to see if parasite confirm or not the genetic results. As showed previously (Table 1) *Laides* and *Pseudeutropius* both have one parasite belonging to the genus *Thaparocleidus*. But *Pseudeutropius* had also two

species from a new genus. This fact led us to think that, as demonstrated by genetic studies, *Pseudeutropius* is farther from *Pangasius* than *Laides*. The new genus is very different from those described on the African species of the family Schilbeidae (five genera), belonging to *Schilbetrema* and *Schilbetrematoides*, only genera parasitic on schilbeid hosts in this continent.

III. Population level

The study of individuals of *Pangasius djambal* coming from different locations shows an "in common" species (sp9), while two (sp40 and sp44) are location specific. As sp9 is also founded on other pangasiid hosts (i. e. *P. bocourti*, *P. sanitwongsei* or *Helicophagus* species) nothing, for the moment could be conclude:

	P. djambal	
Kalimantan	Java	Sumatra
sp9	**sp9**	**sp9**
	sp29	sp23
		sp40
		sp44

On the other hand, on *Pangasius* sp. 1 the parasitic fauna are completely different between Vietnam and Indonesia populations. Within Indonesian populations, all the species found in Sumatra are also present in Kalimantan.:

	Pangasius sp. 1	
Kalimantan	Sumatra	Vietnam
sp42	sp47	sp1
sp47	sp49	sp2
sp48		
sp49		

So, for the parasites, as seen previously, *P. djambal* is different from *Pangasius* sp. 1, but *P.* sp. 1 shows two different populations, that could be due to:

- geographical specificity of the parasites, but sp1 is founded in Sumatra and Kalimantan on other hosts species, and then is not geographically specific,
- difference in the environment: Vietnamese population came from estuarine areas, when Indonesian one came from freshwaters,
- systematic difference between Vietnamese (from one side) and Indonesian populations from the other one, but they are not so different with genetic studies.

As *Pangasius* sp. 1 could be in fact *Pangasius pangasius* and as we don't know the parasitic fauna of this species, the question remains.

CONCLUSION

Even if these first results have to be completed by new samples and by the formal description of the numerous new species and genus; they show that such study on the monogenetic fauna may contribute to the comprehension of hosts phylogeny and represent a useful complement to morphometric and genetic studies for the characterisation of species and populations.

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MITOCHONDRIAL DNA DIFFERENTIATION OF POPULATIONS OF *CLARIAS BATRACHUS* FROM SOUTH-EAST ASIA

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Abstract

RFLP analysis of mitochondrial DNA (mtDNA) was used to study variation within 13 populations of *Clarias batrachus*, sampled respectively in Vietnam, Thailand and in the Indonesian Archipelago.

In this study an amplified region corresponding approximately to 2.3 kilobases of the Cytochrome-b and D-loop genes was digested using 8 restriction enzymes (HinfI, Hin6I, MvaI, MspI, HaeIII, BamHI, NdeII, DraI). 12 mtDNA haplotypes were found in 40 specimens.

Each sampling location was characterised by one haplotype, except Palembang (Sumatra, Indonesia) and Samarinda (Kalimantan, Indonesia) where 2 and 4 haplotypes were found respectively. The consensus tree calculated from 15 more parsimonious networks showed that mtDNA haplotypes are geographically distributed. Three well differentiated clusters were identified. The first cluster is composed by populations from both Thailand and Vietnam, the second cluster by two populations from West Sumatra and the last cluster by all other populations. High genetic divergence were observed in Sumatra between populations which come from highlands (altitude more than 1000 meters in Bukittingui, and 300 meters in Nias Island) and populations located in lowlands.

The significant genetic relatedness observed between populations from Sumatra (Jambi, Palembang, Muara Tebo, Teluk Kuantan) and populations from Java indicate a possible common origin which is probably in Kalimantan. This result is supported by the high diversity of haplotypes revealed in Samarinda (Kalimantan) and their intermediate position in the genetic network. The populations from highlands in West Sumatra which share a common haplotype are genetically more related to populations from Thailand and Vietnam than to populations from the rest of Indonesia. These populations seems to be relict populations from a first colonisation event which arise from the continental part of Asia. The fact that the highland haplotype is not observed in lowlands populations of Sumatra suggests that the colonisation way from Vietnam and Thailand is certainly ancient and that a strong bottleneck effect occurred in lowland area, probably due to a marine transgression. During a more recent marine regression, the present lowlands of Sumatra could have been newly colonised by flounders coming from Kalimantan.

INTRODUCTION

The Clariidae is a large family of the Siluriformes, with about 15 genera inhabiting freshwaters from Africa eastward to India, south-east Asia and south-eastern East Asia (Menon, 1951; Nelson, 1976). The genus *Clarias* is the largest genus of the family with approximately 45 species (Garcia-Franco, 1993). *Clarias batrachus*, *C. macrocephalus*, *C. fuscus* and *C. gariepinus* are still the main species used in aquaculture. These species exhibit considerable intra-specific geographic variations in morphological and chromosomal characters, leading to overlaps and species misidentification (Garcia-Franco, 1993). This author demonstrated with a study on caryotypes that *C. batrachus* from India are closer to the species *C. fuscus* than to populations of *C. batrachus* from south-east Asia. As a consequence, the evolutionary history of these species remains to be clarified, as well as their specific status which remains unclear. In that way, a RFLP analysis of

mitochondrial DNA (mtDNA) was used in order to study genetic variation within populations of *Clarias batrachus* sampled respectively in Vietnam, in Thailand and in the Indonesian Archipelago.

MATERIAL AND METHODS

Sampling

Fish were collected in 13 locations (Fig. 1) respectively in Can Tho (Vietnam), So Phisat (Thailand), Samarinda (Kalimantan), Nias Island, Bukittingui, Teluk Kuantan, Muara Tebo, Jambi, Palembang, Tegineneng (Sumatra), Sukamandi, Solo, Brantas (Java).

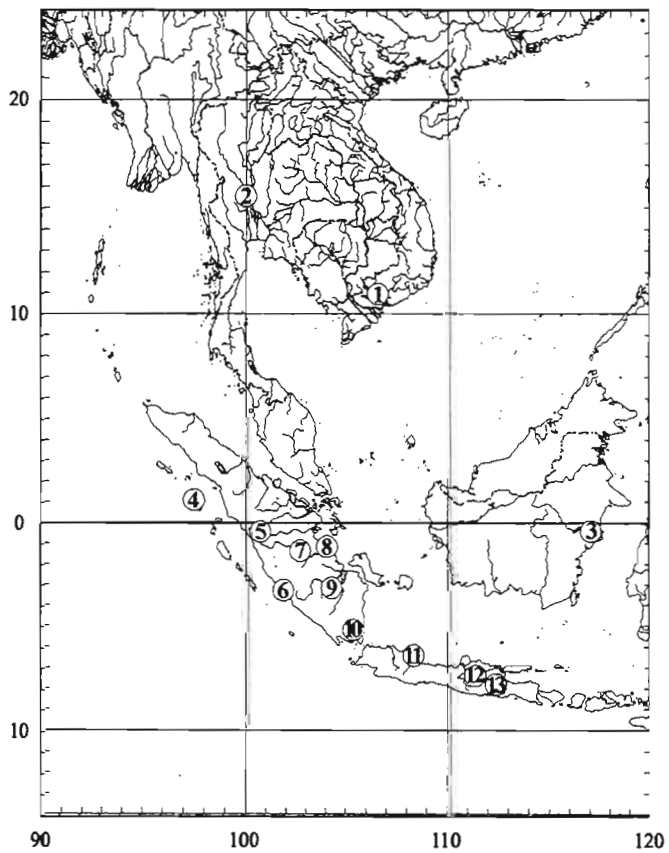

Figure 1: *Clarias batrachus* samples locations.

DNA extraction

A piece of muscle (60 mg) from each specimen preserved at –20°c was extracted using a standard Phenol-Chloroform protocol.

Amplification of the Cyt-b-D-loop region of mitochondrial DNA

An amplified fragment of approximately 2300 bases was obtained using the following primers V-GLU (5' GACTTGAAGAACCACCGTTG) and HN20 (5' GTGTTATGCTTTAGTTAAGC) identified by Bernatchez (comm. pers.). The amplification conditions were as follow : 3mM MgCl2, 0.5 mM of each dNTP's, 0.2 µM of each primer, 2 units of taq polymerase (Promega), 1x of taq Polymerase buffer and 5 µl of DNA solution in a final volume of 50µl. The amplification program was : 95°C for 1 min, 91°C for 1 min, 50°C for 1 min, 72°C for 2 min 30 sec. The last three steps were repeated 35 times. A final elongation was performed at 72°C for 10 min.

Collection of restriction enzymes

Eight 4-base recognition restriction endonucleases were used to digest the amplified mtDNA.

Digestion of the amplified products

Five to seven µl of the PCR-amplified region was digested by 5 units of one of the restriction enzymes in a final volume of 20 µl containing the appropriate buffer. Digestion were then done at 37°C overnight. The digestion products were separated and visualised in 2% horizontal ethidium bromide stained TBE agarose gel. Patterns of restriction digest products for enzyme *Hin*fI are presented in Figure 2.

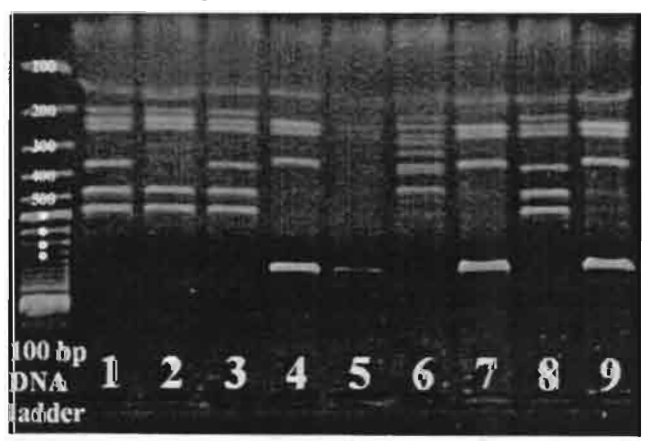

Figure 2: Patterns of restriction digest product for enzyme HinfI to compose a haplotype definition of *Clarias batrachus* population.

Each column represent a digested sample. The column in left side is the 100 bp DNA ladder (mix of DNA markers from 100 bp to 2000 bp). On this picture specimen 1, 3, 8 share the same pattern of restriction. The specimen number 2 has a private haplotype which differs from previous haplotype by the acquisition of a new restriction site leading to the disappearance of a band in the middle part of the pattern (approx. 360bp) and apparition of new bands below 180 bp). Specimen 4, 7, 9 share an

other kind of pattern. The specimen with number 6 corresponds to an other species, *Clarias meladerma*.

Data analyses

Each restriction enzyme give a particular pattern of digested restriction fragments for one specimen. A collection of different patterns can be obtained with the same enzyme for different specimen if the number of restriction sites is variable. In that case each pattern is defined by a particular phenotype with a code letter.

For one specimen, the set of phenotypes observed for the different restriction enzymes used is referred as an haplotype. A matrix of presence-absence of each restriction pattern could be assessed for each sample and used in order to implement the phylogenetic relationships among these samples. We used in this study the parsimony of Wagner (program MIX) and the bootstrapping re-sampling technique (program SEQBOOT). These programs are available in the PHYLIP software package (Felsenstein, v. 3.5).

RESULTS

All the enzymes used cleaved the PCR mtDNA product. An identical multibanded phenotype among all samples of *C. batrachus* was observed with the enzyme *Msp*I. Because this enzyme provide different patterns with the other species belonging to the *Clarias* genus, it was useful for assuming a good identification of *Clarias batrachus*.

The haplotypes collection is given in Table 1 for each analysed population. One haplotype was found in each population, except in Palembang and Samarinda where 2 and 4 haplotypes were revealed respectively.

The corresponding genetic network calculated from the 15 more parsimonious trees is presented in Figure 3. MtDNA haplotypes are geographically distributed. Bootstrap up to 50% indicate the existence of three well differentiated clusters. The first one is composed by populations of Can Tho and Sophisat, the second one by populations of Bukittinggi and Nias island and the third cluster by all other populations. Populations which come from Sumatra are not monophyletic and do not share a common origin. The populations which come from highlands (mountains areas up to 1000 meters at Bukittinggi, up to 300 meters in Nias Island) in Sumatra are genetically related to populations from the continental part of Asia (Vietnam and Thailand). By contrast, all other populations in Sumatra which are located in lowlands (altitude between 10 and 200 meters) are genetically related to populations from Java and Kalimantan Islands.

DISCUSSION

The distribution of species or populations and their genetic differentiation depend on biological, environmental and historical factors. Numerous works have shown that the genetic structure of freshwater fish populations was fashioned by fluctuations of sea level and alternative flowing and drying seasons during the past (see Hamilton, 1976 ; Maley, 1987, 1991, Maley et al., 1990 for more details). The last marine regression (-110 meters below actual sea level) which is dated

	HinfI	HingI	MvaI	MspI	HaeIII	BamHI	Nde2	DraI
Tegineneng	A	B	C	A	C	B	C	B
Jambi	B	A	C	A	C	A	A	B
SoPhisat	C	A	A	A	A	A	A	A
Bukittingui	D	B	B	A	B	A	A	B
Nias	D	B	B	A	B	A	B	B
Telukkuantan	E	A	C	A	C	A	A	B
Palembang 1	B	A	C	A	C	A	A	B
Palembang 2	F	A	C	A	C	A	A	B
Samarinda 1	B	A	C	A	D	A	A	C
Samarinda 2	B	A	C	A	D	A	A	B
Samarinda 3	E	A	C	A	D	A	A	C
Samarinda 4	E	B	C	A	D	B	A	B
Can Tho	G	A	D	A	A	A	A	B
Java	E	A	C	A	C	A	A	B
MuaraTebo	E	A	C	A	C	A	A	B

Table 1: Composite mtDNA haplotype definitions for *Clarias batrachus*. Letters refer to restriction fragment pattern that occur in these populations.

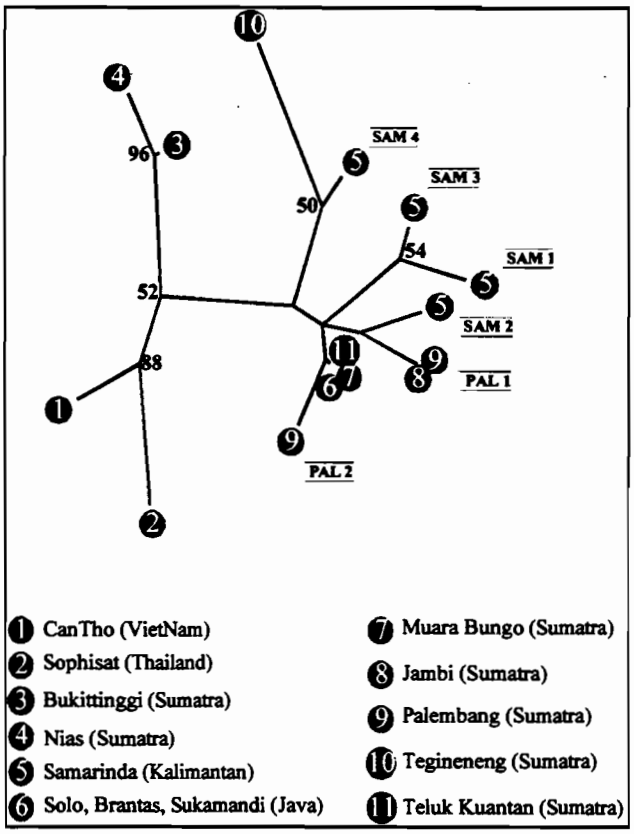

Figure 3: Genetic network calculation from 15 parcimonious trees of *Clarias batrachus* based on haplotype pattern.

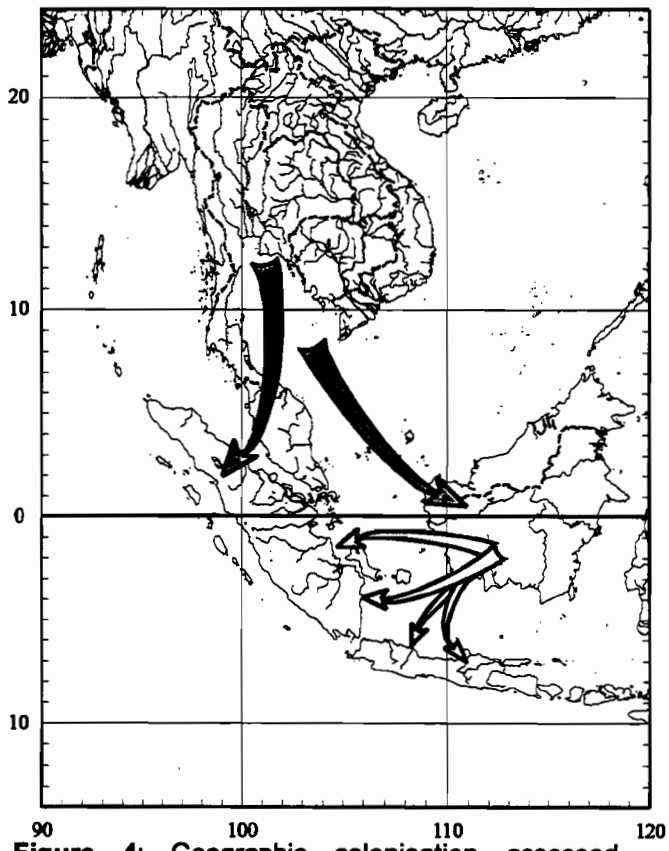

Figure 4: Geographic colonisation assessed scenario of *Clarias batrachus* based on haplotype pattern.

20,000 years before present led to the disappearance of the South China sea and Java sea, meaning possible connections between river drainage which are presently independent and a possible dissemination of freshwater ichthyofauna. By contrast, the last marine transgression (6 meters above actual sea level) was responsible of the disappearance of many lowland areas and the decreasing of many freshwater populations, excepting in refuge areas as large river systems and highlands.

Considering on the one hand that Menon (1951), on the basis of evidences provided by paleontology and distribution records, stated that the Clariidae family originated from somewhere in South China during the early Pliocene period and, on the other hand that Garcia-Franco (1993) suggested that *Clarias batrachus* originated from North Burma, it is possible to reconstitute some of the past events that prevailed upon the current distribution of *C. batrachus* populations (Fig. 4). In these conditions, Kalimantan and Sumatra could have been colonised during a marine regression by populations coming from the continental part of Asia. Because colonisation events come with bottleneck effects due to the small size of populations that generally go from one basin to another, the flounder populations which entered in Sumatra highlands had conserved only one haplotype. This haplotype is currently shared by populations of Nias and Bukittinggi. By contrast in Kalimantan, the bottleneck effects were not so high as in Sumatra probably because the colonised area was more accessible. In that way an important genetic diversity is observed (4 haplotypes). The fact that the haplotype from highlands in Sumatra is never observed in lowlands suggest a very poor gene flow between upstream and downstream in the same river basin (case of Batang Hari River) and that populations of *Clarias batrachus* were probably extinct in lowlands with marine transgression. The current populations sampled in low altitude areas in Sumatra (Jambi, TelukKuantan, Muara Tebo, Palembang, Tegineneng) show close relationships with population from Kalimantan. This is also the case for populations of Java which are characterised by only one haplotype also observed in Telukkuantan and genetically related with haplotypes of Kalimantan. These results suggest that Java Island

and Sumatra lowlands were colonised more recently from Kalimantan, possibly during the last marine regression that occurred 20,000 years ago.

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PHYLOGENETIC RELATIONSHIPS AMONG PANGASIID CATFISH SPECIES (SILURIFORMES, PANGASIIDAE) AND NEW INSIGHTS ON THEIR ZOOGEOGRAPHY

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Abstract

With the aim of detecting phylogenetic relationships among Pangasiidae catfishes, 23 enzyme loci were studied on 18 nominal species of the genera *Pangasius* and *Helicophagus* (Roberts & Vidthayanon, 1991). In order to assess the taxonomic position of *Laides* genus in the Schilbeidae or in the Pangasiidae, 2 species belonging to the genus *Laides* and 1 species of the genus *Pseudeutropius* (Siluriformes, Schilbeidae) were added in the study.

The results indicate that the species *Laides hexanema* and *Laides sinensis* appear to be genetically related with the Pangasiidae. The phylogenetic tree obtained shows a clustering of species which validate the genus *Helicophagus* but indicates that the genus *Pangasius* is polyphyletic.

The genus *Pangasius* is composed of 3 genetic differentiated groups. Group 1 is composed by 3 species belonging to two different morphological entities validated by Roberts and Vidthayanon (1991) as possible subgenus. *Pangasius hypophthalmus* and *P. gigas* which share common genetic characters validate the subgenus *Pangasianodon*. The possession of many private alleles by *P. pleurotaenia* confirms the morphological originality of this species which was therefore considered to belong to the monotypic subgenus *Pteropangasius*. The second group consists of *P. micronema*, *P. macronema*, *P. lithostoma* and *P. polyuranodon* and the last group represents all the other species of the genus *Pangasius*. By contrast *Neopangasius* considered as a possible subgenus by Roberts and Vidthayanon (1991) is polyphyletic. Although relative genetic similarities were found between *P. humeralis* and *P. nieuwenhuisii* which are located in group 3, the species *P. lithostoma* appears to be more closely related to species belonging to the group 2. By reference to the *Helicophagus* genus, the genetic distances estimated between these genetic entities suggest that *Pangasianodon* and *Pteropangasius* could be elevated to the genus level. Low genetic distance between groups 2 and 3 lead us to maintain species of both groups in the genus *Pangasius*. In the same way the genus *Laides* could be placed in Pangasiidae as proposed by Roberts (1989). The results also confirm the nominal species revision proposed by Roberts and Vidthayanon (1991).

New insights are given in this paper, like the presence of *Pangasius djambal* in all major basins of Sumatra and the fact that, contrarily to previous statements, this species was never utilised for aquaculture in Indonesia so far. *Helicophagus typus* is not extinct, three specimens were caught in the Batang Hari river (Sumatra) in February 1997 and two specimens in the Kapuas river (West Kalimantan) in June 1997. Two possible new species were discovered, the first one (ref. sp1) occurring in Sumatra (Musi, Batang Hari and Indragiri rivers), in Kalimantan (Mahakam River) and in Vietnam (Mekong delta), the second one (ref. sp2) was observed in East Kalimantan (Berau River). The taxon sp1 shares several characteristics with *Pangasius pangasius*. However this latter species is not supposed to be represented in the Mekong River nor in Indonesian waters.

INTRODUCTION

The tropical Asian catfish family Pangasiidae is characterised by a noticeable ecological and morphological diversity. In term of distribution, some species are endemic like *P. gigas* in the Mekong river, other have disjunctive distribution like *P. macronema* which occurs in mainland Asia

only in the Mekong and Chao Phraya basins, while in the Indonesian archipelago, it is known only from southern Kalimantan and Java. Finally many species have a wide distribution like *P. micronema* and *P. polyuranodon* which are reported from most of the basins of south-east Asia. The Pangasiidae are freshwater fishes, with the exceptions of *P. pangasius*, *P. polyuranodon* and *P. krempfi* which can enter in saline waters. Concerning maximum size and growth rate, the situation is also contrasted. Some species, as *P. macronema* never grow longer than 200 mm SL while *P. gigas* can reach over 3 m for more than 300 kg body weight. These species exhibit a wide range of feeding behaviours.

With these considerations, it is noteworthy that phylogenetic relationships among populations of Pangasiidae remain problematic. Large distribution which leads in many cases to significant population differentiation and difficulty to access to comparative material in foreigner museums were responsible of many misidentifications. Before the systematic revision of Roberts & Vidthayanon (1991), a particular confusion prevailed. These authors studied type specimens and other material of Pangasiidae on 39 nominal species or subspecies, resulting to the recognition of 18 previously described species as valid. Three new species were also described bringing to 21 the total number of species recognised in Pangasiidae. They subdivided Pangasiidae in two genera, *Pangasius* Valencienne, 1840 and *Helicophagus* Bleeker, 1858. *Neopangasius* Popta, 1904, *Pangasianodon* Chevey, 1930 and *Pteropangasius* Fowler, 1937 were recognised by Roberts & Vidthayanon (1991) as possible subgenus of *Pangasius*. Two species have been placed in the genus *Laides* Jordan, 1919 and their position in Schilbeidae or in Pangasiidae is subject to controversy.

On the basis of the systematic revision of Roberts & Vidthayanon (1991) and in order to assess the phylogenetic relationships among Pangasiid catfish species, 23 enzyme loci were studied on 18 species of the genera *Pangasius* and *Helicophagus*. One species of the genus *Pseudeutropius* (Siluriformes, Schilbeidae) and two species of the genus *Laides* were also characterised at 19 enzyme loci in order to precise the taxonomic position of the genus *Laides*.

MATERIALS AND METHODS

216 individuals belonging to 62 populations of four genera (*Pangasius*, *Helicophagus*, *Laides*, *Pseudeutropius*) were analysed. The geographic origin (river system and location) and size of the samples are indicated in Figure 1 and table 1.

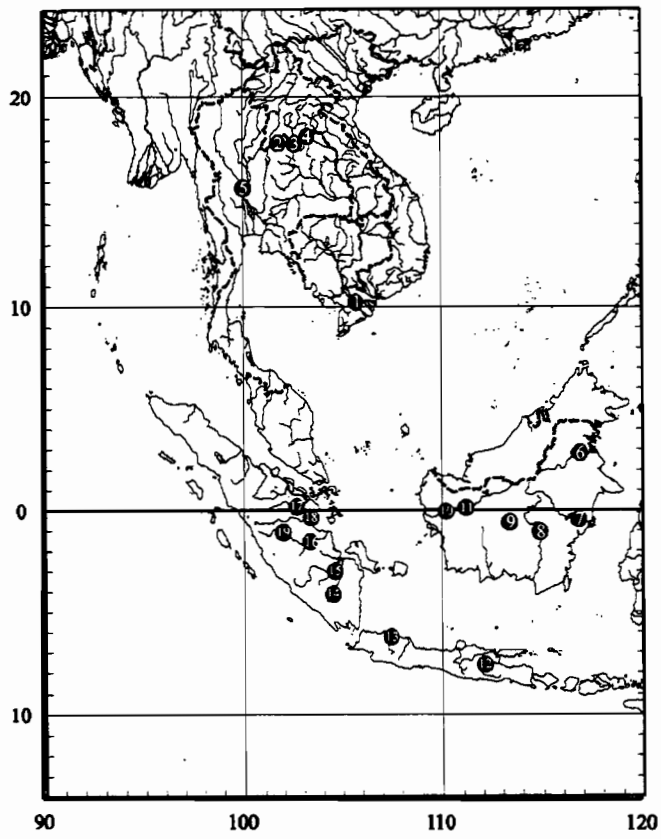

Figure 1: Sampling locations of studied specimens.

Captured specimens were dissected and the tissue samples were stored in liquid nitrogen for transfer to the laboratory. They were then stored at -20° C for several months pending analysis. Two eyes and 2 cm^3 of muscle and liver were removed from each individual. To obtain optimal results, the samples were homogenised several hours before analysis. Each specimen was identified with key available in Roberts & Vidthayanon (1991) and stored in formalin for further examination.

The methods of starch gel electrophoresis were adapted from Pouyaud & Agnèse (1995). Table 2 shows the enzyme systems and the buffers used as well as the organs in which the different loci were expressed. The nomenclature is that proposed by Shaklee *et al.* (1990).

Species	CL	Sampling origin	Abbrev.	N	(*Hobs*)	(P95)
Pangasius Hypophthalmus	5	Tchao Praya Nakhom Sawan Thailand	HYP THA 2	3	0.0725	0.1739
Pangasius Hypophthalmus	1	Mekong Can Tho Viet Nam	HYP VIE 1	6	0.0870	0.3478
Pangasius Hypophthalmus	2	Mekong Thabo Thailand	HYP THA 1	6	0.0290	0.1304
Pangasius Hypophthalmus	13	CRIFFI Strain Sukamandi Indonesia	HYP JAV 2	3	0.0725	0.1739
Pangasius macronema	5	Tchao Praya Nakhom Sawan Thailand	MAC THA 2	5	0.1826	0.3478
Pangasius macronema	3	Mekong Nong Khai Thailand	MAC THA 1	4	0.1196	0.2174
Pangasius macronema	1	Mekong Can Tho Viet Nam	MAC VIE 1	5	0.0696	0.1739
Pangasius pleurotaenia	5	Tchao Praya Nakhom Sawan Thailand	PLE THA 2	5	0.0348	0.1304
Pangasius pleurotaenia	4	Mekong Bung Kan Thailand	PLE THA 1	1	0.0435	0.0435
Pangasius pleurotaenia	1	Mekong Can Tho Viet Nam	PLE VIE 1	1	0.0000	0.0000
Pangasius larnaudii	5	Tchao Praya Nakhom Sawan Thailand	LAR THA 2	4	0.0978	0.2174
Pangasius larnaudii	3	Mekong Nong Khai Thailand	LAR THA 1	1	0.0000	0.0000
Pangasius larnaudii	1	Mekong Can Tho Viet Nam	LAR VIE 1	1	0.0000	0.0000
Pangasius conchophilus	1	Mekong Can Tho Viet Nam	CON VIE 1	5	0.0609	0.1304
Pangasius conchophilus	2	Mekong Thabo Thailand	CON THA 1	3	0.0580	0.0870
Pangasius conchophilus	5	Tchao Praya Nakhom Sawan Thailand	CON THA 2	4	0.0326	0.0435
Pangasius sanitwongsei	4	Mekong Bung Kan Thailand	SAN THA 1	3	0.0000	0.0000
Pangasius krempfi	1	Mekong Can Tho Viet Nam	KRE VIE 1	3	0.0290	0.0870
Pangasius gigas	3	Mekong Nong Khai Thailand	GIG THA 1	2	0.0652	0.1304
Helicophagus waandersii	4	Mekong Bung Kan Thailand	WAN THA 1	4	0.0326	0.1739
Helicophagus waandersii	16	Batang Hari Jambi Sumatra Indonesia	WAN SUM 3	2	0.0000	0.0000
Helicophagus waandersii	5	Tchao Praya Nakhom Sawan Thailand	WAN THA 2	3	0.0000	0.0000
Helicophagus typus	16	Batang Hari Jambi Sumatra Indonesia	TYP SUM 3	3	0.0290	0.0870
Pangasius djambal	16	Batang Hari Jambi Sumatra Indonesia	DJA SUM 3	7	0.0311	0.1739
Pangasius djambal	15	Musi Palembang Sumatra Indonesia	DJA SUM 2	4	0.0435	0.0870
Pangasius djambal	12	Brantas Jombang Java Indonesia	DJA JAV 1	10	0.0739	0.2609
Pangasius djambal	8	Barito Muara Tewe Kalimantan Indonesia	DJA KAL 3	1	0.0000	0.0000
Pangasius djambal	17	Indragiri Rengat Sumatra Indonesia	DJA SUM 4	7	0.0435	0.1304
Pangasius bocourti	1	Mekong Can Tho Viet Nam	BOC VIE 1	7	0.0807	0.3043
Pangasius bocourti	4	Mekong Bung Kan Thailand	BOC THA 1	4	0.0471	0.1739
Pangasius sp1	15	Musi Palembang Sumatra Indonesia	SP1 SUM 2	5	0.0435	0.0435
Pangasius sp1	7	Mahakam Samarinda Kalimantan Indonesia	SP1 KAL 2	4	0.0543	0.2174
Pangasius sp1	1	Mekong Can Tho Viet Nam	SP1 VIE 1	3	0.0870	0.1304
Pangasius micronema	17	Indragiri Rengat Sumatra Indonesia	MIC SUM 4	5	0.0783	0.2174
Pangasius micronema	10	Kapuas Sanggau Kalimantan Indonesia	MIC KAL 5	4	0.1196	0.2174
Pangasius micronema	12	Brantas Dam Karet Java Indonesia	MIC JAV 1	5	0.0783	0.2609
Pangasius micronema	18	Indragiri Teluk Kuantan Sumatra Indonesia	MIC SUM 5	3	0.1014	0.2174
Pangasius micronema	14	Tulang Bawang Kotabumi Sumatra Indonesia	MIC SUM 1	3	0.0725	0.1739
Pangasius micronema	8	Barito Muara Tewe Kalimantan Indonesia	MIC KAL 3	2	0.1522	0.3043
Pangasius sp2	6	Berau Tanjung Redeb Kalimantan Indonesia	SP2 KAL 1	3	0.0145	0.0435
Pangasius nasutus	9	Kayanan Palangkaraya Kalimantan Indonesia	NAS KAL 4	3	0.0290	0.0870
Pangasius nasutus	10	Kapuas Sanggau Kalimantan Indonesia	NAS KAL 5	2	0.0435	0.0435
Pangasius nasutus	8	Barito Muara Tewe Kalimantan Indonesia	NAS KAL 3	1	0.0435	0.0435
Pangasius nasutus	16	Batang Hari Jambi Sumatra Indonesia	NAS SUM 3	3	0.0000	0.0435
Pangasius nasutus	15	Musi Palembang Sumatra Indonesia	NAS SUM 2	3	0.0000	0.0435
Pangasius nasutus	17	Indragiri Rengat Sumatra Indonesia	NAS SUM 4	1	0.0435	0.0435
Pangasius nieuwenhuisii	7	Mahakam Samarinda Kalimantan Indonesia	NIE KAL 2	4	0.0761	0.1739
Pangasius lithostoma	11	Kapuas Sintang Kalimantan Indonesia	LIT KAL 6	1	0.0000	0.0000
Pangasius humeralis	11	Kapuas Sintang Kalimantan Indonesia	HUM KAL 6	1	0.0000	0.0000
Pangasius polyuranodon	17	Indragiri Rengat Sumatra Indonesia	POL SUM 4	8	0.1087	0.2609
Pangasius polyuranodon	8	Barito Muara Tewe Kalimantan Indonesia	POL KAL 3	4	0.0435	0.1304
Pangasius polyuranodon	1	Mekong Can Tho Viet Nam	POL VIE 1	6	0.0870	0.2174
Pangasius polyuranodon	5	Tchao Praya Ayuttaya Thailand	POL THA 2	1	0.1304	0.1304
Pangasius polyuranodon	10	Kapuas Sanggau Kalimantan Indonesia	POL KAL 5	1	0.0870	0.0870
Pangasius polyuranodon	15	Musi Palembang Sumatra Indonesia	POL SUM 2	3	0.1739	0.2609
Pangasius polyuranodon	16	Batang Hari Jambi Sumatra Indonesia	POL SUM 3	3	0.1159	0.1739
Pangasius polyuranodon	14	Tulang Bawang Kotabumi Sumatra Indonesia	POL SUM 1	3	0.0725	0.1739
Laides hexanema	5	Tchao Praya Nackom Sawan Thailand	HEX THA 2	2	0.0263	0.0526
Laides hexanema	19	Batang Hari Muara Bungo Sumatra Indonesia	HEX SUM 6	3	0.0175	0.0530
Laides hexanema	4	Mekong Bung Kan Thailand	HEX THA 1	2	0.0263	0.0526
Laides sinensis	4	Mekong Bung Kan Thailand	SIN THA 1	2	0.0000	0.0000
Pseudeutropius brachypoptes	19	Batang Hari Muara Bungo Sumatra Indonesia	BRA SUM 6	3	0.0000	0.0000

Table 1: Species identification, code of sampling origin (CL, *cf.* Fig. 1), sampling origin, species abbreviations (Abbrev.), size of samples (N), observed heterozygosity (H_{obs}) and polymorphic loci indices (P^{95}).

Mean observed heterozygosities ($H_{obs.}$) and polymorphic (P^{95}) indices were computed using the GENETIX package (Belkhir *et al.*, 1996). Standard genetic distances were estimated using the Nei's formula (1978). Phenograms were generated from distance matrix by Fitch cluster analysis using the PHYLIP package (Felsenstein, 1989). The robustness of the data set was tested by a resampling method such as bootstrapping (SEQBOOT in PHYLIP). This method is well developed by Felsenstein (1985) and involves the creation of new data set by sampling *N* characters randomly with replacement, so that the resulting data set has the same size as the original, but some characters have been left out and others are duplicated. The random variation of the results from analysing these bootstrapped data sets can be shown statistically to be typical of the variation that we would get from collecting new data sets. Bootstrap values were computed over 500 replications of resampled distances matrix.

A multivariate analysis (factor analysis of correspondence) was performed using the BIOMECO program (Lebreton *et al.*, 1990) from a matrix of alleles coded in presence or absence for each individual and each locus from raw electrophoresis data. This statistical analysis method was used with the goal to assess the genetic relatedness of species belonging to genus *Laides*.

RESULTS

The study concerned 23 loci (Table 2) excepting for species belonging to *Laides* and *Pseudeutropius* genera for which loci Sod-3, Sod-4, Adh and Sdh were not considered due to difficulty of interpretation. Analysis of the zymograms showed considerable polymorphism at the loci studied with a total of 199 alleles evidenced for all species. Only the locus Ldh-1 was monomorphic for the same allele in all the samples. The average rate of observed heterozygosity ($H_{obs.}$) for all loci per population was between 0.000 and 0.1826 and the average rate of polymorphism ($P^{95\%}$) between 0.000 and 0.3478.

A phylogenetic tree (Fig. 2) was obtained from the matrix of pairwises Nei's genetic distances between all pair of taxa. The results confirm all nominal species proposed by Roberts and Vidthayanon (1991). Bootstrap tests validated four genetic differentiated groups. Within these groups, 7 significant clusters were observed, in which occurrence probability was above 0.70. However internal topology of the genetic network was not

Enzyme system	Abbreviation	Locus	Tissue source	Electrode buffer
Aspartate aminotransferase	AAT	*Aat*	Liver	TEB
Alcohol dehydrogenase	ADH	*Adh*	Liver	POULIK ½
Creatine kinase	CK	*Ck-1*	Eyes	MC 2
		Ck-2	Eyes	MC 2
Fructose biphosphatase	FBP	*Fbp*	Liver	MC 2
Glucosephosphate isomerase	GPI	*Gpi-1*	Muscle Eyes	RW
		Gpi-2	Muscle Eyes	RW
Isocitrate dehydrogenase	IDHP	*Idhp-1*	Muscle	MC 2
		Idhp-2	Liver	MC 2
Lactate dehydrogenase	LDH	*Ldh-1*	Eyes	MC 2
		Ldh-2	Eyes	MC 2
Malate dehydrogenase	MDH	*Mdh-1*	Eyes	MC 2
		Mdh-2	Eyes	MC 2
Mannose phosphate isomerase	MPI	*Mpi*	Liver	POULIK ½ TEB
Phosphoglucomutase	PGM	*Pgm*	Muscle	RW
6-Phosphogluconate dehydrogenase	6PGD	*6Pgd*	Liver	MC 2
Protein Total	PT	*Prot-1*	Muscle	MC 2
		Prot-2	Muscle	MC 2
Superoxide dismutase	SOD	*Sod-1*	Liver	POULIK ½
		Sod-2	Liver	POULIK ½
		Sod-3	Liver	MC 2
		Sod-4	Liver	MC 2
Sorbitol dehydrogenase	SDH	*Sdh*	Liver	POULIK ½

Table 2: Enzymes systems, buffers, locus, tissue specificity and electrode buffer investigated in the study. MC 2, Morpholine citrate, pH 6.2; RW, Ridgeway, Lithium hydroxide-borate, pH 8.3; POULIK ½, boric acid-sodium hydroxide, pH 8.2; TEB, Tris-borate-EDTA, pH 8.6.

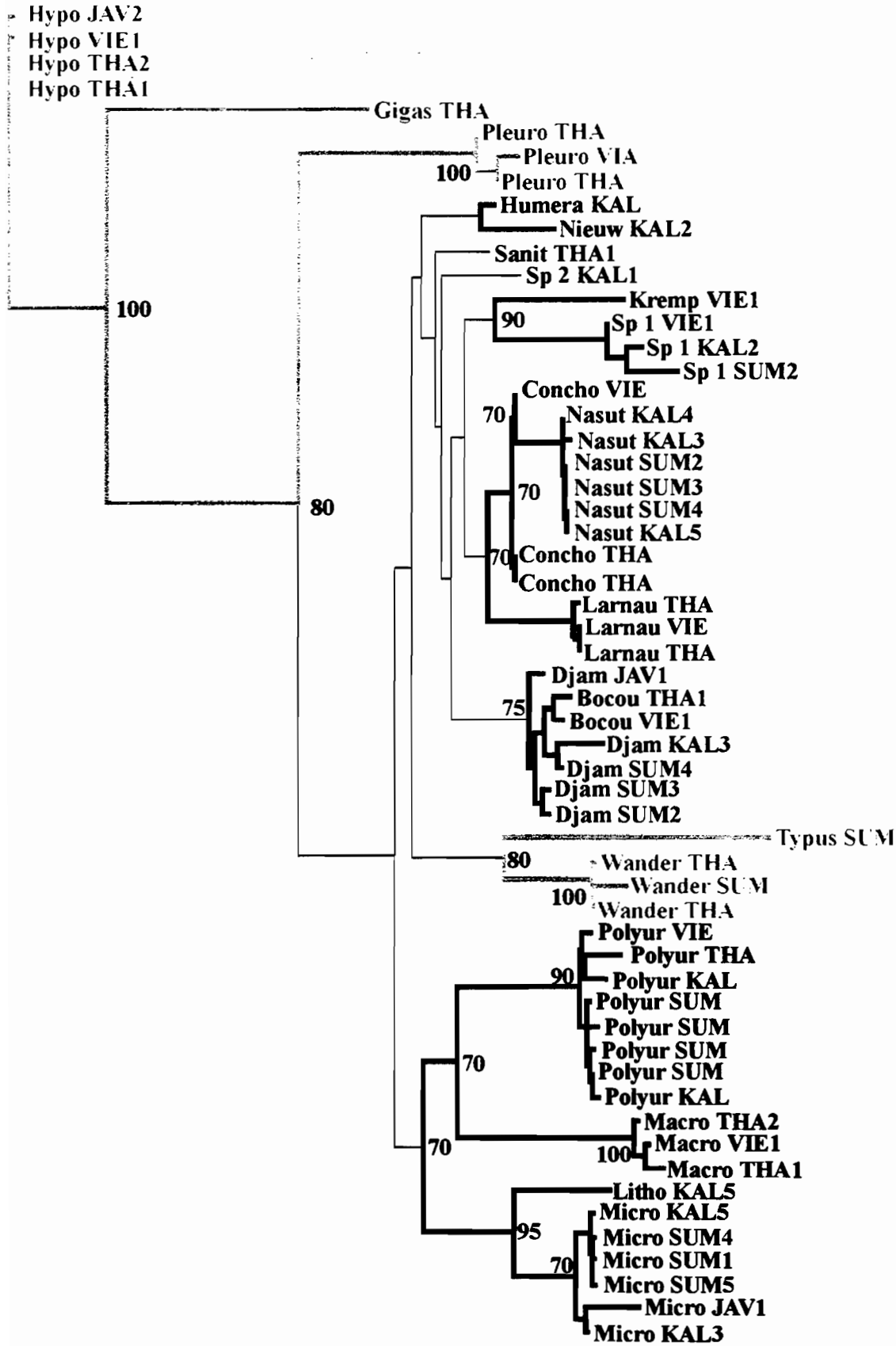

Figure 2: Phenogram produced by Phylip on 20 species of Pangasiidae. This tree was generated from distance matrix by Fitch Cluster Analysis. Bootstrap values were computed over 500 replications of resampled distance matrix.

significant. The cluster 1 is composed of *P. hypophthalmus*, *P. gigas* and *P. pleurotaenia*, the cluster 2 is defined by *H. waandersii* and *H. typus*, the cluster 3 by *P. conchophilus*, *P. nasutus* and *P. larnaudii*, the cluster 4 by *Pangasius* sp1 and *P. Krempfi*, the cluster 5 by *P. djambal* and *P. bocourti*, the cluster 6 by *P. nieuwenhuisii* and *P. humeralis* and finally the cluster 7 comprises *P. micronema* and *P. lithostoma*, *P. polyuranodon* and *P. macronema*. *Pangasius sanitwongsei* and *Pangasius* sp2 constitute a particular situation because they are characterised by the possession of many alleles shared with species belonging to clusters 3, 4 and 5. *Pangasius* sp1 and *Pangasius* sp2 mentioned above are probably new species because they were not identified with the specific keys proposed by Roberts & Vidthayanon (1991). *Pangasius* sp1 was caught both in Indonesia (Kalimantan at Samarinda on Mahakam River; Sumatra at Palembang on Musi River, Jambi on Batang Hari River and Rengat on Indragiri River) and in Vietnam (Binh Dai and Can Tho in Mekong delta). *Pangasius* sp1 is genetically related to *P. krempfi*, nevertheless they are reproductively isolated because no intermediate genotype where observed in sympatric condition as in the Mekong Delta. *Pangasius* sp2 was observed only in East Kalimantan at Tanjung Redeb on Berau River.

The multivariate analysis projections of individuals referring to their genotype multilocus relatedness is presented on Fig. 3. On the first projection (plan formed by axis 1 and 2) the axis 1 which is the most informative clearly show a huge genetic divergence between Schilbeidae and Pangasiidae families. Axis 1 also reveals a genetic differentiation between *P. pleurotaenia* and all other pangasiid species. On the second projection (plan formed by axis 2 and 3), the species belonging to genus *Laides*, *P. hypophthalmus* and *P. gigas* form three genetically independent entities, formally separated on axis 2 from the group constituted by other pangasiids. *Helicophagus waandersii* and *H. typus* are differentiated from the other species on axis 3.

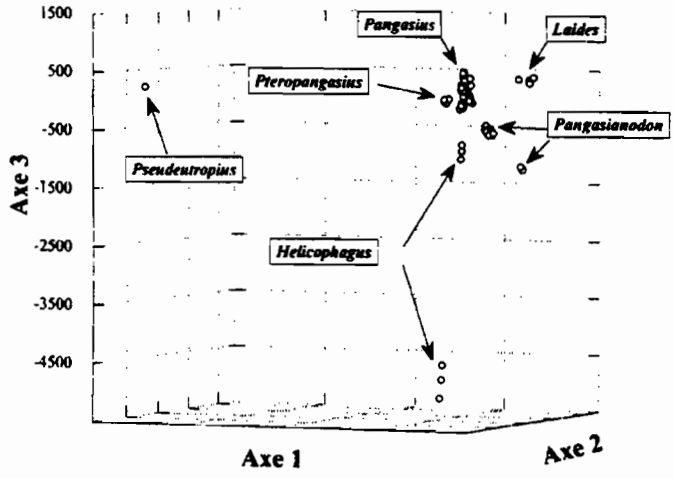

Figure 3: Multivariate analysis projections of individuals referring o their multilocus relatedness.

DISCUSSION

Speciation and evolutionary processes

The tropical Asian catfish family Pangasiidae displays a strong genetic differentiation with 199 alleles evidenced on 23 loci. For comparison the phylogenetic relationships assessed by Pouyaud & Agnèse (1995) among species belonging to genera *Oreochromis*, *Sarotherodon*, *Tilapia*, *Chilochromis*, *Pelmatochromis*, *Tylochromis*, *Hemichromis* and *Chromidotilapia* (Cichlidae) revealed 95 alleles on 24 loci. Fossils record of the family Pangasiidae (Sanders, 1934 in Roberts & Vidthayanon, 1991) from Tertiary deposits (during period between 65 and 1 million years before present) in central Sumatra indicate that occurrence of this family in south-east Asia is ancient. Under these conditions, cumulative fluctuations of sea water levels during this period have undoubtedly fashioned the pangasiid group leading to the notable ecological and morphological diversity observed actually. Large river basins like Mekong, Tchao Phraya and Kapuas have probably played a major role in term of refuge zone during the past. These rivers possess *P. nasutus* (Malay peninsula, Indonesia); *P. bocourti* (Vietnam and Thailand) and *P. djambal* (Indonesia). The genetic distances between both couple of species (d = 0.109 between samples of *P. nasutus* from Muara Tewe in Kalimantan and *P. conchophilus* from Mekong in Vietnam; d = 0.158 between samples of *P. bocourti* from Thailand and *P. djambal* from Java in Indonesia) are comparable with average distances occurring within the species *P. polyuranodon* (d = 0.106 between population of Muara Tewe in Kalimantan and population caught in Tchao Phraya) or *P. micronema* (d = 0.145 between population of Teluk Kuantan in Sumatra and population of Solo in Java).

Systematic implications

The multivariate analysis clearly indicate that *Laides hexanema* and *Laides sinensis* are genetically related with the pangasiid species. Nei's genetic distances (Table 3) confirm that *Laides* species are genetically closer to pangasiid species than to *Pseudeutropius* species. Therefore the genus *Laides* should be placed in Pangasiidae as firstly proposed by Roberts (1989).

	Pseudeutropius (Schilbeidae)	*Laides*
Laides	1.19	
Pangasiidae	1.05	0.52

Table 3: Nei's genetic distances between Pangasiidae, *Laides* and Schilbeidae.

The phylogenetic tree obtained shows a clustering of species which validate the genus *Helicophagus* but cannot agree the actual composition of the genus *Pangasius* which is polyphyletic. *Neopangasius*, *Pangasianodon* and *Pteropangasius* are the three subgenus recognised by Roberts & Vidthayanon (1991).

Our study confirms the species relatedness described within these three groups except in the subgenus *Neopangasius* in which *P. lithostoma* could not be inserted. This species is genetically closer to *P. micronema*. Considering the genus *Helicophagus* as a reference, average genetic distances between each group (Table 4) suggest that *Pangasianodon* and *Pteropangasius* should be elevated to the genus level. The genus *Pangasianodon* would then be composed by *P. hypophthalmus* and *P. gigas* and the genus *Pteropangasius* by only one species *P. pleurotaenia*. *Neopangasius* may remain valid with *P. nieuwenhuisii* and *P. humeralis* as a subgenus of *Pangasius*. *Pangasius lithostoma*. *P. micronema*, *P. macronema*, *P. polyuranodon*, *P. lithostoma*, *P. sanitwongsei*, *P. krempfi*, *P. larnaudii*, *P. nasutus*, *P. conchophilus*, *P. djambal*, *P. bocourti* could be maintained in the genus *Pangasius*. Additional genetic analyses using other markers like ribosomal sequences may provide evidence of other subgenus in the *Pangasius* genus.

Possible finding of two new species

Pangasius sp1 was caught in Sumatra (Palembang, Musi River; Rengat, Indragiri River), in Kalimantan (Samarinda, Mahakam River) and in Vietnam (Mekong River). In these localities this species was misidentified as *P. djambal*. This species shares many characteristics with *Pangasius pangasius* like gill raker counts, its occurrence in saline waters (reported by fishermen both in Indonesia and Vietnam) and a relatively similar palatal dentition. As in *P. pangasius*, the palatal dentition is curved and constituted by related close-sets. Referring to the dentition picture available in Roberts & Vidthayanon (1991) for *P. pangasius*, the *Pangasius* sp1 dentition presents some differences. The two vomerine tooth plates are always linked like an horizontal number 8. On each side the palatine tooth plates are small, round and separated. As no samples of *Pangasius pangasius* could be obtained at this stage from India or Bangladesh for comparison, the status of *Pangasius* sp1 remains unclear. A detail morphological examination of the specimens collected during this study will be carried out in a near future. They may represent a new pangasiid species, but at the present state the possibility that these specimens belong to the species *P. pangasius* cannot be discarded. In this latter hypothesis, *P. pangasius* would become the pangasiid species with the largest geographic distribution, from India to Vietnam and Indonesian archipelago.

Pangasius sp2 was caught in East Kalimantan (Tanjung Redeb, Berau river). In this location the species was misidentified as *P. polyuranodon*. This species share genetic characteristics with species such as *P. djambal* and *P. bocouti*. The internal position of this fish in the phylogeny and its isolated biogeographic area (basin regarding to the Macassar strait along the Wallace line) may indicate that this species has colonised this area since a very ancient period. Further analysis will permit to precise if this species has conserved

	Pangasianodon	*Pteropangasius*	*Helicophagus*	*Pangasius 1*
Pteropangasius	0.656			
Helicophagus	0.701	0.675		
Pangasius 1	0.568	0.538	0.230	
Pangasius 2	0.607	0.530	0.180	0.080

Table 4 : Nei's genetic distances between each possible genus and subgenus in pangasiids.

primitive characters. The two vomerine form a single large median tooth plate like in *P. polyuranodon*, but the palatines tooth plates are very elongate and are parallel with the lateral sides of the vomerine plate. Up to now this shape of palatal dentition was never reported in the pangasiids.

Some new data on zoogeography

Helicophagus typus was considered as probably extinct in Sumatra because the last specimen was collected in Palembang in 1908 (Roberts & Vidthayanon, 1991). We can assume that *H. typus* still occurs in the Batang Hari River (Sumatra, Jambi) where 3 specimens (around 600 mm) were collected the 27 February 1997 and two supplementary specimens (621 and 342 mm SL) were found in the Kapuas River on the 27 June 1997 (Sanggau and Sintang, West Kalimantan).

Until now, *Pangasius djambal* was reported only from Java and Kalimantan, we can assume however that this species occurs in all major basins of Sumatra as Musi, Batang Hari and Indragiri Rivers. The species is abundant on the markets in these locations. Nevertheless, this species has a restricted distribution in Java where it seems to be present only in Solo and Brantas Rivers. Contrarily to the situation stated by Roberts and Vidthayanon (1991), *Pangasius djambal* was never utilised in aquaculture in Java until now, as anywhere else in Indonesia. The only pangasiid species cultured in Indonesia is *Pangasius hypophthalmus* which was introduced from Thailand in 1972.

Because of human activities located on river banks, organic and chemical pollution, dams construction, over-fishing, many endemic species are threatened with extinction as is the case of *Pangasius gigas* in the Mekong. In Indonesia, such a situation is observed in Java where *P. djambal* and *P. micronema* could be found only in the Brantas and Solo Rivers in central Java. Local information strongly suggests that these species have now disappeared from anywhere else on the island. By contrast, *Pangasius hypophthalmus* which has been found in the Citarum River (West Java) and Batang Hari River tributaries (Sumatra) could now be considered as part of the Indonesian ichthyofauna.

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PRELIMINARY DATA ON GENETIC VARIATION IN THE GENUS *CLARIAS* AND *PANGASIUS* ON THE BASIS OF DNA MICROSATELLITE LOCI

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Abstract

Fourteen dinucleotide DNA microsatellite loci were screened in the catfishes *Clarias gariepinus*, *C. batrachus* and *Pangasius hypophthalmus*. Six loci showed allelic variation in *Clarias batrachus*, 6 in *C. gariepinus* and 3 in *P. hypophthalmus*. They will be used for the screening of wild and cultured populations.

INTRODUCTION

Catfishes (Siluriformes) constitute a major taxon among the bony fishes. They are especially widespread in the neotropics and constitute a major component of the highly diverse fauna of south-east Asia, including the Indonesian archipelago and the Mekong rivershed. They are of growing interest in aquaculture; currently more than 100,000 tons of clariids and pangasiids are produced in SE Asia. Part of this aquaculture is supported by the exotic species *Clarias gariepinus* and its hybrids (Na-Nakorn *et al.*, 1993). Despite their significance in biological evolution and aquaculture, the genetics of indigenous species has been studied fragmentarily. The review by Volckaert and Agnèse (1996) includes few references on this topic apart from Daud *et al.* (1989), Ismail *et al.* (1989) and Na-Nakorn *et al.* (1997). The Catfish Asia Project aims at filling in some of the missing information.

In this report we detail on an interspecific comparison of in total 14 DNA microsatellite dinucleotide primersets in 3 catfish species (*Clarias gariepinus*, *C. batrachus* and *Pangasius hypophthalmus*).

MATERIALS AND METHODS

Two strategies have been envisaged to obtain microsatellite DNA primers specific to SE Asian catfishes of the family Clariidae and Pangasiidae: (1) testing of DNA microsatellite primers developed for *Clarias gariepinus*, in the related species *C. batrachus* and *Pangasius hypophthalmus* and (2) development of completely new DNA dinucleotide microsatellite primers for *C. batrachus* and *P. hypophthalmus*.

The testing of a selected number of primers developed for *C. gariepinus* has been performed on 2% agarose gels with the microsatellite DNA primers and according to the PCR directions of Galbusera *et al.* (1996).

The second strategy, namely development of microsatellite markers specific to *Pangasius hypophthalmus* and *Clarias batrachus*, has been done according to a modified version of the biotin capture method of Kandpal *et al.* (1994). It includes the following steps:

Selection of oligonucleotides:

The following dinucleotide oligonucleotides (Eurogentech, Belgium) were ordered:

- CA repeat: $(CA)_{15}$
- *MboI* adaptor:

5'-ATCGCAGAATTCGCACGAGTACTACC
GTCTTAAGCGTGCTCATGATGC-5'

Construction of a standard gDNA library:

Genomic DNA from the Asian Catfishes, *Clarias batrachus* and *Pangasius hypophthalmus*, was completely digested with *MboI*. The digested DNA (5µg) was ligated to the *MboI* adapter (15µg) in a 300µl reaction volume with 5µl T4-ligase (BRL). After overnight incubation at 22°C, the free linkers were separated from the DNA-fragments on a 1.2% agarose gel. The ligated DNA was purified from the gel with the Jetsorb extraction kit (Imtech). The purified fragments were amplified in a PCR reaction with one of the *MboI* adapter oligonucleotides as a primer. The fragments were cloned into the TA-cloning vector from the TA-cloning kit from Invitrogen and transformed into *E. coli* cells.

Construction of an enriched gDNA library:

Because of the low yield of positive clones with the traditional protocol, we used a modified version of the biotin capture method of Kandpal *et al.*, 1994. The protocol is identical up to the first PCR reaction with the *MboI* primer. The enrichment procedure is as follows. The amplified DNA was denatured in a boiling water bath for 10 min and hybridised to the biotinylated CA repeat. The hybridisation was carried out overnight in a 100µl volume containing 2 to 3 µg amplified DNA, 1 µg repeat, 0.5% SDS and 0.5M sodium phosphate (pH 7.4) at 50°C. The hybridisation mixture was incubated with 0.15ml streptavidin coated magnetic beads (Promega) for 30 min at ambient temperature. The beads were precoated with 100µg/ml sonicated salmon sperm DNA. The supernatant was removed by centrifugation after incubation. The beads were washed 3 times with 1ml 1xTris buffer (100mM Tris pH 7.5, 150mM NaCl) at ambient temperature, 50°C and 65°C, once with 0.1xTris at 65°C and once with distilled water at 65°C. The eluents were concentrated with centricontubes (Amicon) to a volume of 100µl. An aliquot of the concentrate was used to start the same PCR reaction as above. These fragments were cloned into the TA-cloning vector from the TA-cloning kit from Invitrogen and transformed into *E. coli* cells.

Screening of the library:

Two strategies were used. First we hybridised the colonies directly with the CA repeat probe after a colony lift, although occasionally problems were encountered to locate the positive clones. Alternatively we isolated individual colonies and performed a PCR on each colony. We spotted 5µl of this reaction on a dot blot, which was hybridised with the CA repeat probe. From each positive clone, we prepared a miniprep to sequence the insert (Amersham).

RESULTS

***Testing of DNA microsatellite primersets of* Clarias gariepinus**

Five primersets (Cga04, Cga06, Cga09, Cga11 and Cga14) developed specifically for *Clarias gariepinus* were tested on a wild population of the same species (Lake Mweru, Zambia), *C. batrachus* (collected at the fish market of Can Tho) and *Pangasius hypophthalmus* (collected at the fish market of Can Tho and from Can Tho University aquaculture station). The "Cga" primersets amplified highly repetitive dinucleotide loci in *C. batrachus* (3 cases) and *P. hypophthalmus* (2 cases) (Table 1). Since research is in progress, several combinations still have to be verified.

***Testing of DNA dinucleotide microsatellite primersets of* Clarias batrachus**

Five primersets (Cba02, Cba04, Cba06, Cba09 and Cba10) developed specifically for *Clarias batrachus* were tested on a wild population of the same species (collected at the fish market of Can Tho), *C. gariepinus* (Lake Mweru, Zambia) and *Pangasius hypophthalmus* (collected at the fish market of Can Tho and from Can Tho University aquaculture station). The "Cba" primersets amplified highly repetitive dinucleotide DNA sequences in *C. batrachus* (2 cases;1 primerset is polymorphic), *C. gariepinus* (1 case) and *P. hypophthalmus* (in progress) (Table 2). Since research is in progress, several combinations still have to be checked.

	Cga04-KUL	Cga06-KUL	Cga09-KUL	Cga11-KUL	Cga14-KUL
C. gariepinus (n = 10) Lake Mweru	124/128/132/134/ 136/140/150 (7)	141/143/145/ 147/151 (5)	186/190/192/ 195/202/203 (6)	181/183/185 (3)	179/195/197/199/ 201/205/207/217 (8)
C. batrachus (n = 10) Can Tho	negative	negative	182/184/186/188/ 194/196/198/200/ 202/204/210 (11)	197/199/211/ 205/215/223/ 243 (7)	178/180/184 (3)
P. hypophthalmus (n = 10) Can Tho	negative	negative	178/182/192 (3) (n = 5)	183 (1)	negative

Table 1: Interspecific comparison of *C. gariepinus* DNA microsatellite primersets on *C. batrachus* and *P. hypophthalmus*. The alleles observed as well as the number of alleles observed is given in brackets. I.P.: in progress

	Cba02-KUL	Cba04-KUL	Cba06-KUL	Cba09-KUL	Cba10-KUL
C. batrachus (n = 10) Can Tho	I.P.	184 (1)	221/247/251 (n = 8) (3)	208/210 (2)	I.P.
C. gariepinus (n = 10) Lake Mweru	I.P.	I.P.	249/251 (n = 7) (2)	I.P.	negative
P. hypophthalmus (n = 10) Can Tho	negative	I.P.	I.P.	I.P.	negative

Table 2: Interspecific comparison of *C. batrachus* DNA microsatellite primersets on *C. gariepinus* and *P. hypophthalmus*. The alleles observed as well as the number of alleles observed is given in brackets.

	Phy05-KUL	Phy07-KUL	Phy09-KUL	Phy12-KUL
P. hypophthalmus (n = 10) Can Tho	202/204/206/ 208/210 (5)	214/215/216/ 260/270 (?) (5)	positive (8)	I.P.
C. gariepinus (n = 10) Lake Mweru	negative	negative	negative	negative
C. batrachus (n = 10) Can Tho	negative	306/308/336/338 (4)	188 (1)	negative

Table 3: Interspecific comparison of *P. hypophthalmus* DNA microsatellite primersets on *C. gariepinus* and *C. batrachus*. The alleles observed as well as the number of alleles observed is given in brackets.

***Testing of DNA dinucleotide microsatellite primersets of* Pangasius hypophthalmus**

Four primersets (Phy05, Phy07, Phy09 and Phy12) developed specifically for *Pangasius hypophthalmus* were tested on a population of the same species (collected at the fish market of Can Tho and from the CTU aquaculture station), *C. gariepinus* (Lake Mweru, Zambia) and *C. batrachus* (collected at the fish market of Can Tho, Vietnam). The "Phy" primersets amplified

highly repetitive dinucleotide DNA sequences in *P. hypophthalmus* (3 cases), *C. gariepinus* (1 case) and *C. batrachus* (1 case; 1 monomorphic locus) (Table 3). Since research is in progress, several combinations still have to be checked.

DISCUSSION

The African catfish loci have been successfully tested on 2 Asian species. The markers developed specifically for the Asian species are currently being tested on a siluriforme "zoo-pannel". So far, six loci showed allelic variation in *Clarias batrachus*, 6 in *C. gariepinus* and 3 in *P. hypophthalmus*. It is still too early to make conclusions about the genetic structure of wild populations (to be studied particularly in *Clarias nieuhofii*) and cultured populations (*C. batrachus*, *C. gariepinus* and *Pangasius hypophthalmus*). Such information should become available in the near future.

In general our data confirm that the more evolutionary distant a taxon, the lower the chance of successful amplification and the lower the genetic variability observed by heterologous primer sets. The loci Cga09 and Cga11 primers show major allelic variation in *C. batrachus*, a species which belongs to the Clariidae but is suspected from molecular data not to fit in the *Clarias* genus (Arndt, pers. comm.).

In addition, tetranucleotide microsatellite loci are being developed by our laboratory. It has become clear that the lower allelic variation of tetranucleotide DNA repeats suits better our goals of parental and population genetic characterisation (Jarne & Lagoda, 1996).

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PRELIMINARY DATA ON SPECIES COMPOSITION AND DISTRIBUTION OF PANGASIID CATFISHES (SILURIFORMES, PANGASIIDAE) IN THE LOWER MEKONG RIVER BASIN

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Abstract

The composition and distribution of Pangasiidae in the lower Mekong River basin, southern Vietnam, was studied through four periods, from April 1997 to April 1998. The fish were mainly collected by trawl net, but some were also collected by long-line, hand-line, gill net along Tien River and Hau River.

The investigation showed the presence of 9 species of *Pangasius* and 1 species of *Helicophagus*, in which *Pangasius bocourti* (ca Ba Sa), *Pangasius conchophilus* (ca Hu), *Pangasius hypophthalmus* (ca Tra), *Pangasius larnaudii* (ca Vo dem), *Pangasius krempfi* (ca Bong lao) and *Pangasius* sp1 present a high commercial value. Almost all pangasiid species are distributed in fresh water-bodies, but *Pangasius krempfi*, and *Pangasius* sp1 (ca Tra nghe or ca Tra ban respectively) were found mainly in brackish-water. Pangasiidae are distributed mainly from Vinh Xuong to Hong Ngu in Tien River, but at the Vam Nao confluence, in Hau River, they were particularly abundant, where water current is rapid and water depth important. The size and weight distributions of collected species changed in every investigated period. From July 1997 to December 1997, young fish were predominant. On the other hand, *Pangasius macronema* was always found in abundance during all sampling campaigns. Many kinds of fishing gears have been used to catch Pangasiidae in the Mekong delta, such as trawl net, long-line, hand line, gill net, seine net and stow net. Some of them represent a threat for the fish. Increased control and management from local government is needed for sustainable fishery resource development and protection.

INTRODUCTION

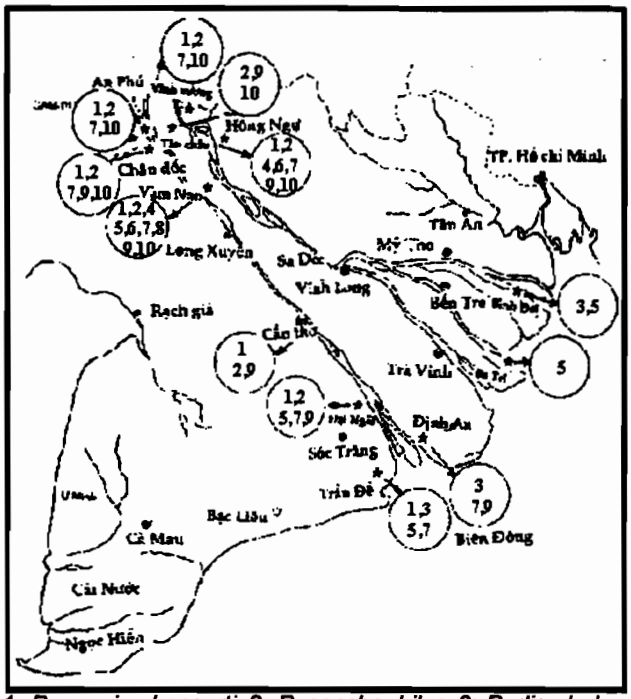
Pangasiidae is a family of fish, commonly known as catfish, belonging to Sub-Order Siluriformes, Order Siluriformes. Many species in this family are of high economic value and are important commodity for exportation. Most of the species distribute widely in the Mekong Basin. They are found in Thailand, Burma, Laos, Cambodia, Malaysia and Vietnam.

According to Tyson and Vidthayanon (1991), family *Pangasiidae* consists of two genera: *Pangasius* (Valenciennes, 1840) and *Helicophagus* (Bleeker, 1858). *Pangasius* consists of 19 species and *Helicophagus* has only two species. Most of the species in this family are freshwater fish, only two species (*Pangasius krempfi* and *Pangasius djambal*) are found in marine habitat and one species (*Pangasius polyuranodon)* is found in brackish-water habitat. Considering geographical distribution, eleven species have been found in Thailand, ten species in Indonesia and three in Malaysia. According to Lenormand (1996), eleven pangasiid species have been identified in the Lower Mekong Basin in Vietnam, among which ten belong to *Pangasius* and one to *Helicophagus*. Of the ten species in genus *Pangasius*, one species was found only in the floating fish-raising cages and could not be found in natural habitat.

This research, supported by the Catfish Asia project, is planned to be conducted from April 1997 to April 1999 to investigate the distribution patterns of catfish in the Lower Mekong Basin and to study the ecology of several species of high economic value. The results of this study will later contribute to the elaboration of plans for conservation of resource and promotion of sustainable catfish culture.

MATERIALS AND METHODS

The major sampling sites were chosen along the two main branches of the Mekong River (Tien and Hau Rivers) from the Cambodian border down to the river's mouths (Fig. 1).



1: *Pangasius bocourti*; 2: *P. conchophilus*, 3: *P. djambal*; 4: P. *hypophthalmus*, 5: *P. krempfi*; 6: *P. larnaudii*; 7: *P. macronema*; 8: *P. pleurotaenia*; 9: *P. polyuranodon*; 10: *Helicophagus waandersii*

Figure 1: Distribution map of Pangasiid catfishes in the Mekong basin (Vietnam).

Local fishing gears such as trawl net, gill net and some others were used for collecting fish samples. Samples were collected four times a year: two in the dry season and two in the wet season. Water temperature, pH, transparency, salinity, dissolved oxygen concentration (DO) and chemical oxygen demand (COD) were measured at each sampling area. Other environmental parameters - water depth, water current's velocity, bottom substrate and natural food source - were also noted. During the campaigns, local fishermen were interviewed on the distribution patterns of catfish, the harvest season and the harvest sizes of catfish. The fish samples were preserved in 10% formalin and transported to the laboratory of the Department of Environment and Natural Resources Management for examination and measurements.

RESULTS AND DISCUSSIONS

Environmental parameters

In the dry season: Table 1 shows that the water temperatures were high in the dry season, ranging between 29.8°C to 32.4°C. Salinity was low at the river's mouths (12-14 $g.L^{-1}$at Tien River's mouth; 4-9 $g.L^{-1}$ at Hau River's mouth). Values of pH were stable because they were less affected by water drained from inland fields. Water transparency was high due to low sediment content in the dry season. Water depths varied greatly between the sites, ranging from 5m to 30m. The sites with great water depths included Vinh Xuong, Tan Chau, Hong Ngu, Long Khanh, Vam Nao, Chau Doc. Many species of Pangasiidae were found at these sites, a large variety of fishing gears such as trawl net, gill net and fishing line, being used to catch them.

In the wet season: during the wet season the water temperature was lower than during the dry season, usually below 30°C (Table 2). Values of pH were low because they were affected by rainwater drained out from inland fields. Water transparency was low due to high sediment content in floodwater. Water salinity in the wet season was also low (1.0-2.1 $g.L^{-1}$ at Hau river's estuary; 1.9-2.3 $g.L^{-1}$ at Tien river's estuary) because the water was diluted by upstream water during rainy season.

Species composition of Pangasiidae at the sites (Table 3)

Among a total of 1400 specimens sampled, 10 species of *Pangasiidae* were found, nine of them belonging to the genus *Pangasius* and only one to the genus *Helicophagus*. At this stage, the specific identification of one of these species, *Pangasius* sp1, remains uncertain. This species, which is commonly found at the river's estuaries, was initially identified as *Pangasius djambal* by Lenormand (1996), but this identification could not be confirmed from both morphological observations and genetic analyses recently carried out (see Pouyaud *et al.*, 1999). In general, all the species found appeared to be common at the studied sites except *P. pleurotaenia* which was rarely observed in the natural habitat.

These results were in agreement with previous observations made by Lenormand (1996) in the same area. Three species (*P. gigas, P. micronema* and *P. sanitwongsei*), categorised as Indochina and Thailand species by Tyson and Vidthayanon (1991), were not found during our survey.

Distribution of Pangasiidae in the Lower Mekong River Basin, Vietnam

The results of the distribution of pangasiid catfishes in the main stream and estuarine areas of the Tien and Hau Rivers are summarised in Table 4. The Tables 5 and 6 indicate the pangasiid

Sites	Parameters						
	Temp. (°C)	pH	DO (ppm)	COD (ppm)	Salinity ($g.L^{-1}$)	Transparency (cm)	Depth ** (m)
Tien River							
Vinh Xuong	31.7	7.1	7.7	5.1	0	73	20-30
Tan Chau	31.1	6.8	8.4	5.3	0	97	25-30
Hong Ngu	29.8	6.8	-	-	0	-	10-20
Long Khanh	30.3	7.5	8.2	6.4	0	112	10-15
Ham Luong *	31.0	7.5	8.8	6.8	12	40	5-10
Cua Âai *	31.0	7.5	7.2	7.2	14	25	5-10
Hau River							
An Phu	32.1	6.8	7.5	4.6	0	96	< 10
Chau Doc	30.2	6.8	7.7	5.4	0	62	15-20
Vam Nao	32.5	7.1	8.8	4.8	0	32	20-30
Can Tho	31.2	7.4	7.8	6.5	0	78	10-15
Dai Ngai	32.3	6.9-7.2	7.4	4.7	0.1	12	10-15
Long Phu	30.8	6.8	7.0	4.5	2.5	-	10-15
Âinh An *	31.7	6.9	7.0	5.1	4.0	20	10-15
Tran De *	32.4	7.3-6.8	7.5	8.4	9.0	17	8-10

*: near river's mouths **: from interviews with local fishermen

Table 1: Measurements of aquatic environmental parameters in the dry season.

Sites	Parameters					
	Temperature (°C)	pH	DO (ppm)	COD (ppm)	Salinity ($g.L^{-1}$)	Transparency (cm)
Tien River						
Vinh Xuong	28.5	6.0	7.8	4.4	0	12
Tan Chau	28.5	6.5	8.9	5.1	0	14
Long Khanh	28.0	6.5	8.1	6.4	0	16
Ham Luong *	30.1	6.8	7.7	8.8	2.3	25
Cua Dai *	29.3	6.7	9.2	5.2	1.9	8
Hau River						
An Phu	28.5	6.5	7.7	3.8	0	14
Cháu Doc	28.0	6.5	8.3	4.2	0	14
Vam Nao	28.5	6.5	7.6	6.0	0	14
Can Tho	28.5	6.7	8.2	7.2	0	21
Âai Ngai	27.5	-	7.2	8.1	0	18
Âinh An *	27.5	6.8	8.0	7.6	1.0	16
Tran De *	28.0	6.8	9.4	8.0	2.1	8

*: near river's mouths

Table 2: Measurements of aquatic environmental parameters in the wet season.

	Species composition	Local name
1	*Pangasius bocourti* Sauvage, 1880	Ca Ba sa
2	*Pangasius conchophilus* Roberts and Vidthayanon, 1991	Ca Hu
3	*Pangasius hypophthalmus* Sauvage, 1878	Ca Tra nuoi
4	*Pangasius krempfi* Fang and Chaux, 1949	Ca Bong lao
5	*Pangasius larnaudii* Bocourt , 1866	Ca Vo dem
6	*Pangasius macronema* Bleeker,1851	Ca Xac soc
7	*Pangasius pleurotaenia* Sauvage, 1878	Ca Xac bau
8	*Pangasius polyuranodon* Bleeker, 1852	Ca Dua
9	*Pangasius* sp1 *(?)*	Ca Tra Nghe, Tra Ban
10	*Helicophagus waandersii* Bleeker, 1858	Ca Tra chuot

Table 3: Species composition of Pangasiidae samples collected on Tien and Hau River (from 04/97 to 05/98).

specific richness at the different localities studied and for different sampling periods on the Tien River and Hau River, respectively.

Results from Tien River's sites

On the Tien River, the sampling sites at which the highest of pangasiid species were observed were Long Khanh, Hong Ngu and Vam Nao (Table 5). The most common species observed were: *H. waandersii, P. conchophilus, P. macronema* and *P. polyuranodon*. The two species *Pangasius spl* and *P. krempfi* were found only in the estuarine brackish-water areas. However, local fishermen, during the interviews, stated that these species could be also found in the freshwater area of Vam Nao far upstream from the estuaries.

The number of species found at Vinh Xuong and Tan Chau was low. These areas had great water depths and large current's velocities. The types of local fishing gears used in the investigation may not have been appropriate for deep-water areas with high current's velocities. Particularly, the trawling speed may have been too slow to catch this kind of fish. Further investigation with improved methods and equipment need to be done in these deep-water areas to obtain more reliable data.

Results from Hau River's sites

The results from the investigation at the sites on the Hau River showed that the numbers of species were the highest at the following sites: An Phu, Chau Doc, Can Tho and Dai Ngai (Table 6). The number of species found in the vicinity of Chau Doc appeared to be the more stable over time. This may be due to the facts that there are many floating fish-raising cages in this area and that the water current was not very strong which may be suitable for many species. The species which are common in freshwater habitat, such as *Pangasius bocourti, Pangasius conchophilus, Pangasius macronema, Pangasius polyuranodon*, were found at similar frequencies through the sites from An Phu to Dai Ngai (near the river's estuary). The two species *Pangasius krempfi* and *Pangasius* spl also appeared with equal frequencies through the estuarine sites such as Dai Ngai, Bai Gia, Tran De and Dinh An.

No.	Species composition	Tien River		Hau River	
		River	Estuary	River	Estuary
1	*Pangasius bocourti*	+	-	+	+
2	*Pangasius conchophilus*	+	-	+	-
3	*Pangasius hypophthalmus*	+	-	-	-
4	*Pangasius krempfi*	-	+	+	+
5	*Pangasius larnaudii*	+	-	-	-
6	*Pangasius macronema*	+	-	+	+
7	*Pangasius pleurotaenia*	+	-	-	-
8	*Pangasius polyuranodon*	+	-	+	+
9	*Pangasius* spl	-	+	-	+
10	*Helicophagus waandersii*	+	-	-	-

Table 4: Distribution patterns of Pangasiidae species.

No	Sampling location	Surveying time			
		I (Avril.97)	II (July.97)	III (Dec.97)	IV (Avril.98)
1	Vinh Xuong	0	4	0	2
2	So Thuong	0	3	0	0
3	Tan Chau	0	3	0	3
4	Long Khanh	3	4	7	7
5	Hong Ngu	6	0	6	2
6	Vam Nao	3	4	7	4
7	Binh Dai (estuaries)	1	2	1	1
8	Ba Tri (estuaries)	0	0	1	0

Table 5: Variation of the number of pangasiid species at the different sampling sites over time on the Tien River (from April 1997 to April 1998).

Several authors have conducted surveys and published data on species composition and distribution patterns of pangasiid catfishes in the Mekong River. The compiled results are presented in Table 7.

Table 7 indicates that most of the species in *Pangasius* family distribute widely in the Indochina region. These species include: *bocourti, conchophilus, hypophthalmus, krempfi, macronema, polyuranodon* and *pleurotaenia.* From the present study, ten species have been found in the Mekong Delta so far, among which nine species belong to genus *Pangasius* and one species belongs to genus *Helicophagus*, while Lenormand (1996) found only 8 species in natural habitat (including *H. waandersii*) and two species in floating fish-raising cages (*P. micronema, P. sanitwongsei*). Species composition in Cambodia is richer than other areas in the region. However, It should be noted that the effective presence of some Pangasiid species in the Mekong River is still controversial (see below). The two species *P. gigas* and *P. sanitwongsei* are found only upstream from the Vietnamese part of the Mekong (Rainboth, 1996; Roberts & Vidthayanon, 1991). However, according to the interviews with local fishermen, these two species were previously present in Vietnam, at Long Khanh and Hong Ngu (on the Tien River). The *Pangasius gigas* fish that were caught by Vietnamese fishermen before could reach individual weight up to 250 Kg.

Rainboth (1996) stated that a specimen of *Pangasius pangasius* was collected at My Tho market in 1974. According to this author, this

No.	Sampling location	Surveying time			
		I (Avril 97)	II (July 97)	III (Dec. 97)	IV (April 98)
1	An Phu	0	4	0	1
2	Kinh Xang	2	3	0	0
3	Chau Doc	2	3	3	3
4	Can Tho	1	3	0	5
5	Cai Con	1	2	0	5
6	Dai Ngai	3	0	5	1
7	Bai Gia	3	1	0	1
8	Tran De (estuary)	0	1	1	0
9	Dinh An (estuary)	0	3	0	0

Table 6: Variation of the number of pangasiid species at the different sampling sites over time on the Hau River (from April 1997 to April 1998).

Species composition	Mekong Delta	Laos	Cambodia	Thailand
Pangasius bocourti	6 ; 8	3	1 ; 7	2
Pangasius conchophilus	6 ; 8	3 ; 4 ; 5	7	2
Pangasius djambal	-	-	1 ; 7	2
Pangasius gigas	-	3	1	-
Pangasius hypophthalmus	6 ; 8	2 ; 3	1	2
Pangasius krempfi	6 ; 8	3 ; 5	1 ; 7	2
Pangasius larnaudii	6 ; 8	-	1 ; 7	2
Pangasius macronema	6 ; 8	3 ; 5	1 ; 7	2
Pangasius micronema	6	-	1 ; 2 ; 7	2
Pangasius pangasius	-	-	1(?) ; 7(?)	-
Pangasius pleurotaenia	8	4 ; 5	7	2
Pangasius polyuranodon	2 ; 6 ; 8	3 ; 5	1 ; 7	2
Pangasius sanitwongsei	2 ; 6	3 ; 5	1 ; 7	2
Pangasius sp1	8	-	-	-
Helicophagus waandersii	6 ; 8	4 ; 5	1 ; 7	2

Sources: (1): Kottelat, 1985; (2): Roberts & Vidthayanon, 1991; (3): Roberts, 1993; (4): Warren, April 1994; (5): Roberts & Baird, 1995; (6): Lenormand, 1996; (7): Rainboth, 1996; (8): Thuong *et al.*,1997.

Table 7: Pangasiid species listed from the Mekong Delta and other neighbouring areas in the region.

species distribute from India to Vietnam in estuarine brackish-water habitat, while Roberts and Vidthayanon (1991) considered that this species is absent from the Mekong.

Pangasius micronema is not a common species in the Lower Mekong Basin. It distributes mainly from Indonesia to Thailand (Rainboth, 1996). In Vietnam, Lenormand (1996) found this species in a floating fish-raising cage but the fish were brought from Cambodia originally. There has been no clear information indicating the presence of this species in the Mekong Delta either.

The species *Pangasius spl* appears mostly limited to the estuarine habitat. This species, which was tentatively identified as *P. djambal* by Lenormand (1996), is reported for the first time in Vietnam from the Tien and Hau rivers' estuaries.

The species *Helicophagus waandersii* which distributes widely in Indochina region is also found in Vietnam.

Size variation of Pangasiidae fish collected in natural habitat in the Lower Mekong Basin:

Indication of the size structure of the pangasiids sampled in this study is summarised in Table 8.

- *Pangasius bocourti* only appeared at the inland rivers. Sizes varied greatly between sampling times. Small-size fish appeared mostly in the samples of sampling 2 and 3 from July to December 1997. This is the breeding season of most of the fish species. *P. bocourti* was more particularly abundant at Vam Nao and Tan Chau on the Tien River.
- *Pangasius conchophilus* only appeared in the freshwater part of the rivers. Small-size fish appeared mostly in fieldtrip 2 in July 1997. Fish size ranged from 3 cm to 4 cm at Can Tho. The largest fish were 21 cm collected in fieldtrip 4 in December 1997 at Vam Nao on the Tien River. The sites in which this species was found in abundance were Tan Chau, Long Khanh, Hong Ngu, Vam Nao on Tien River; and Chau Doc, Can Tho, Cai Con on Hau River.
- *Pangasius hypophthalmus* only appeared on Tien River on the third field trip (Dec/1997). The fish size ranges from 16.0-30.8cm. This species was not found to be abundant. One the reason may be that the trawling net was not an appropriate way to catch this species. The better way to collect sample of this species would be using fishing lines and gillnet.
- *Pangasius larnaudii* appeared only on Tien River in samples collected at Tan Chau, Hong Ngu and Vam Nao, ranging in size from 10 cm to 25 cm.
- *Pangasius macronema* was mostly represented in the samples at Long Khanh, Hong Ngu, and Vam Nao on the Tien River and also at the river's estuary in the wet season. The smallest fish sizes observed were 3 cm at Dai Ngai on the Hau River and 4 cm at Long Khanh in July. This species is a widely distributing one with small-sized individuals that can be caught easily by trawl net..
- *Pangasius pleurotaenia* seems to be rare in the Mekong Delta. During the investigation, only one individual, 19-cm in size, was caught at Vam Nao on Tien River in December 1997.
- *Pangasius polyuranodon* appeared mainly in freshwater areas. In the wet season, they were also found in the river's estuaries. The sites where this fish was the most abundant in the samples were Tan Chau, Long Khanh, Hong Ngu, Vam Nao on the Tien River; and Chau Doc, Cai Con and Dai Ngai on the Hau River. Most of the fish collected were small (5 cm at Tan Chau in July and at Dinh An (Hau River's estuary) in December 1997). Like *P. macronema*, this species represented an important part of fish collected in the samples.
- *Pangasius krempfi* distributed mostly at the rivers' estuaries. It was found only once at Vam Nao on Tien River in December 1997. The average size was 64 cm raging from 32 cm to 77 cm on Hau River and from 42 cm to 58 cm on the Tien River.
- *Pangasius* spl was collected at the river's estuaries using gill net and trawl net. One fish of 72 cm in size was also collected at Vam Nao using trawl net. The size of the specimens collected at the river's estuaries varied greatly ranging from 5 cm in July to 34 cm in October and to 12 cm in December (caught by trawl net at Hau River's estuary). The specimens collected at Tien River's estuaries were larger in size, ranging from 31 to 72 cm.
- *Helicophagus waandersii* was generally small in size. Fish size remained stable over the sites; from 10 cm to 23 cm on the Hau River and 11 cm to 14 cm on the Tien River. Most of the specimens were collected at Vinh Xuong, Tan Chau, Long Khanh and Hong Ngu on the Tien River, and Chau Doc on the Hau River. None was found at the river's estuaries.

Species	Field trip	Tien River		Hau River	
		River	Estuaries	River	Estuaries
Pangasius bocourti (*Ca Ba Sa*)	1	15.3-32.5	-	-	-
	2	7.8-13.3	-	6.6 -14.5	-
	3	7.4-19.9	-	6.0-16.2	8.7-16.0
	4	27.2-28.5	-	-	-
Pangasius conchophilus (*Ca Hu*)	1	9.8-29.6	-	-	-
	2	2.7-12.8	-	3.3-13.5	-
	3	2.6-21.8	-	2.5-23.7	-
	4	16.8-25.3	-	4.5-7.6	-
Pangasius hypophthalmus (*Ca Tra nuoi*)	1	-	-	-	-
	2	-	-	-	-
	3	16.0-30.8	-	-	-
	4	-	-	-	-
Pangasius larnaudii (*Ca Vo dem*)	1	22.1-26.0	-	-	-
	2	-	-	-	-
	3	17.9-27.3	-	-	-
	4	-	-	-	-
Pangasius macronema (*Ca Xac soc*)	1	1.8-14.5	-	3.1-7.3	-
	2	3.0-21.4	-	3.0-4.6	-
	3	7.4-18.9	-	2.0-11.5	-
	4	6.7-18.3	-	5.4-9.4	-
Pangasius pleurotaenia (*Ca Xac bau*)	1	-	-	-	-
	2	-	-	-	-
	3	19.0	-	-	-
	4	-	-	-	-
Pangasius polyuranodon (*Ca Dua*)	1	6.0-19.0	-	3.2-14.3	-
	2	3.9-13.8	-	4.2-8.2	5.5
	3	8.2-21.0	-	5.1-15.9	8.6
	4	8.8-24.8	-	6.7-11.2	-
Pangasius krempfi (*Ca Bong lao*)	1	-	-	25.2-37.0	-
	2	-	30.5-56.0	-	-
	3	64.0	17.5-72.0	49.5	28.2-49.5
	4	-	55.5-61.0	76.5	-
Pangasius sp1 (*Ca Tra ban, Tra nghe*)	1	-	27.6-33.2	-	-
	2	-	50.0	-	4.4-5.6
	3	72.0	-	-	7.2-20.5
	4	-	-	-	-
Helicophagus waandersii (*Ca Tra Chuot*)	1	6.7-15.4	-	-	
	2	7.2-25.7	-	8.0-16.2	
	3	13.8-26.7	-	-	
	4	10.0-11.7	-	12.9-16.0	

Table 8: Size range of *Pangasiidae* fish collected in natural water bodies in the Lower Mekong basin, Vietnam (Total length: cm).

Current situation of exploitation and proposed solution for sustainable fishery of Pangasiidae in the lower Mekong river of Vietnam.

Fishery of *Pangasiidae* in the lower Mekong River basin had been established for a long time. Based on characteristics of fishing ground and habitat and biology of Pangasiidae several styles of fishing gear have been developed such as encircling net, seine net, trawl net, gill net, hand line, long line, stow net and scrub traps. These are the main fishing gears contributing to the total fishery production of pangasiids. However, resource reservation could be hampered by the improper use of these fishing gears.

Hand line and long line are popular gears. Hand

lines are used seasonally (mainly from June to November) to exploit small sized fish in species including *P. bocourti, P. conchophilus* and *P. macronema*. It is suitable for water depth of 10-20 m, with an average daily production of 10-20 fish. Long lines are used widely along the Tien and Hau Rivers, particularly at Vam Nao (from January to April) and in the estuaries. The long line gears have a total length of 500-1000 m (or 400-500 hooks). At Vam Nao, they was used mainly for exploiting *P. polyuranodon, P. bocourti* and *P. macronema* weighing from 50-200 g/fish, or up to 1-4 kg/fish in some cases. In Vam Nao site, the highest catch were observed in October-November for *P. conchophilus* and *P. polyuranodon* and February-March for *P. krempfi*, which could be up to 15-20 kg/day. In the estuaries, Long lines are mainly used to catch *Pangasius* spl at the confluence or along the riversides where there are important quantities of "Ban trees" (*Sonneriatia casceolaria*) living.

Gill nets are used all along the Tien and Hau Rivers. They are made of nylon with a mesh size of 2a=140 mm, length of 500-700 m and width of 8-10 m. They are set either floating with water current or fixing across the river at about 1-3 m above the river bottom. Gill net mainly caught large size fish of 3-5 kg such as *P. krempfi, P. bocourti* and *P. larnaudii*. Average catch of gill net varied from 6 to 9 kg/day, the main catching season for Pangasiids being from January to April at Vam nao. However, at the estuaries, it could be operated all year-round for catching *P. krempfi* mainly. Fishing-ground for *P. krempfi* is from seashore up to 10 km far offshore, where the water depth is about 3-10 m. During the main fishing season, from February to July, catches of one net unit reach about 50-600kg/day.

Stow net for *P. hypophthalmus* larvae collection were seasonally operated. They were used during the period from June to August (or May to July lunar year), and operated mainly in the area from Vinh Xuong, Thuong thoi Hau to Phu Thaun in the rivers. However, in the estuarine areas, stow net (Day hang khoi) mainly caught big size *P.* sp 1 in March-May with a yield of about 200kg/day and small *P.* sp 1 in October-November with 200-300 kg/day.

Trawl net consisted of two kinds: beam trawl net and bottom otter trawl net. The design of beam trawl net is 5-7 m wide; 0.4-0.7 m high; 10-20 m long and 2a=8-16 mm mesh size and is operated by a boat equipped with a 10-20 HP engine. The bottom otter trawl net are of 10-12 m wide; 1-1.5 m high; 15-20 m long, mesh size at cod-end of 2a=8-20 mm and boat engine of 20-30 HP. The trawl net operated all year round in estuaries, while some trawl net could operate in the main rivers (e.g. in Long Xuyen, Chau Doc and Dong Thap) excepting during the flood season due to strong water current. The best fishing season of trawl net was from April-July mostly for small size *P. conchophilus* and *P. polyuranodon* of 4-6 cm body length and from September-January for larger sized fish of 0.2-1 kg/fish. However, *P. macronema* could be caught all year long with the highest production reaching 30-100 kg/day.

Pull cash net were used in any location along Tien and Hau Rivers. Diameter of pull cash net mouth is 20-30 m. This was operated all around the year and could caught all kind of pangasiids.

There are also many other fishing gears (encircling net, seine net, scrub trap ...) operated in the surveyed areas to catch pangasiid catfishes along main and secondary river systems.

Some recommendations for sustainable fishery and protection of Pangasiidae

For sustainable fishery of Pangasiidae, efforts should be made to (i) limit over-fishing; (ii) forbid and control strictly the use of electrical fishing gears (such as electrical beam trawl net, electrical shock fishing gear); (iii) prevent the use of fishing gears with very small mesh size of barrier net and of cod-end of trawl net ("mesh size" should be >20 mm); and (iv) prohibit the use of stow net along the rivers, except for fry collection of *P. hypophthalmus* at Thuong Phuoc and Thuong Thoi Hau from June to August

CONCLUSIONS

The results obtained through the 4 surveys of the Pangasiidae resources realised on the Tien and Hau Rivers, indicate that :

- 10 species of Pangasiidae were found, they include 9 species belonging to *Pangasius* genus and one species belonging to *Helicophagus* genus. Almost all of them are large sized species which are suitable for culture and fishing in the waterbeds of Mekong delta.
- Three species, *P. gigas, P. sanitwongsei* and *P. micronema*, previously reported in this area, were not found in the Mekong basin during our surveys and sampling.

- Most of catfish species which belong to Pangasiidae live in freshwater and are widely distributed. Two species, *Pangasius krempfi* (Ca bong lao) and *Pangasius* spl (Ca tra nghe), were mainly found in estuaries. However, these two latter species were also widely distributed in freshwater in the area of Vam Nao (Tien River).
- Most Pangasiidae were found at Long Khanh, Hong Ngu, and Vam Nao areas. Further studies should be done on the influence of hydrographic conditions on the distribution of pangasiids.
- Due to recent over-fishing of Pangasiidae and the threat that it represents for some of these species, there is a need that the local government and fishery resource protection agencies increase their control and management on fishing gears along to rivers effectively.

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DESCRIPTION OF THE SEXUAL CYCLE RELATED TO THE ENVIRONMENT AND SET UP OF THE ARTIFICIAL PROPAGATION IN *PANGASIUS BOCOURTI* (SAUVAGE, 1880) AND *PANGASIUS HYPOPHTHALMUS* (SAUVAGE, 1878), REARED IN FLOATING CAGES AND IN PONDS IN THE MEKONG DELTA

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Abstract

First artificial propagation of *Pangasius hypophthalmus* was reported in Vietnam in 1980, but results did not allow extension for large scale production of fry. Otherwise, for *P. bocourti*, no reproduction was yet reported in 1994. Then, investigations were carried out on the reproduction of the two species in two rearing conditions, floating cages and ponds.

Climate in South Vietnam is characterised by two alternate seasons, dry (December-April) and rainy (May-November). Development of oocyte shows an annual cycle in the two species. In *P. bocourti*, maturity of females peaks before the rainy season, while in *P. hypophthalmus* maturity period is a bit delayed, as it occurs at the end of dry season and the beginning of the rainy season. For the two species, development of oocyte do not differ between the two environments, leading to conclusion that rise of water temperature and/or photoperiod probably induce the oocyte development. In males, mature fish produce milt when stripped. In the two species, mature male are observed at the same time as the females. However, in *P. bocourti*, some males can produce milt all year round. In the two species, sexual maturity peaks before, or at the early beginning, of the water flow in the Mekong River.

As no female was observed with ovulated oocyte in captivity, oocyte maturation was induced with hCG treatment. Females of *P. bocourti* require a long preliminary treatment with 3 to 10 injections at a low dose (530 UI.kg^{-1}). Afterwards oocyte are larger and more sensitive to the resolving treatment, including one or two injections (2020 or 1460–2070 UI.kg^{-1}). By contrast, females of *P. hypophthalmus* do not require a preliminary treatment, as they have naturally large oocyte sensitive to the resolving treatment, including one (2530 UI.kg^{-1}), two (2530–2520 UI.kg^{-1}) or three injections (490–1000–1500 UI.kg^{-1}). Application of such treatments led to ovulation and ova collection by stripping in 59% and 88% of the treated females, in *P. bocourti* and *P. hypophthalmus* respectively. Ova collected are smaller in *P. hypophthalmus* than in *P. bocourti*, 1.0 and 1.9 mm diameter respectively. One gram of ova in *P. bocourti* and *P. hypophthalmus* contains respectively 251 and 1437 ova.g^{-1}. The relative fecundity is lower in *P. bocourti* than in *P. hypophthalmus*, respectively 4.7 10^3 and 48.8 10^3 ova per kg of body weight. Influence of the rearing conditions and the fish morphology and fattening are discussed. Ovulation was usually induced once a year but preliminary data showed that 30% of females of the two species can be induced twice per year.

In males, compared to the natural spermiation per kilogram of body weight, a single injection of hCG (2000 UI.kg^{-1}) allows to collect 10 and 5 times more milt respectively in *P. bocourti* and *P. hypophthalmus*. In *P. bocourti*, a single injection of LHRHa (30 µg.kg^{-1}) associated with domperidone (3 mg.kg^{-1}) induces also a rise of milt collected, but lower than hCG. Generally, volumes of milt collected from *P. bocourti* (5–495 µl.kg^{-1}) are lower than in *P. hypophthalmus* (11–2092 µl.kg^{-1}). The same figure is observed about the spermatozoa concentration, lower in *P. bocourti* (2.4 10^9–36.5 10^9 spz.ml^{-1}) than in *P. hypophthalmus* (25.7 10^9–63.4 10^9 spz.ml^{-1}).

Milt of both species, diluted twice in 155 mM NaCl solution (9 g.l^{-1}) buffered at pH 7, can be stored during 24 h without change of its fertility. Motility of spermatozoa is brief and stops before one minute in tap water. So better fertilisation is obtained for *P. hypophthalmus* with a 34 mM NaCl activation solution (2 g.l^{-1}). The optimal dilution of milt in the activation solution is 10^2-10^4 and 10^2 times for *P. bocourti* and *P. hypophthalmus* respectively.

INTRODUCTION

Pangasius bocourti and *P. hypophthalmus* are catfishes of the Pangasiidae family belonging to the *Pangasius* genus. Both originated from the Mekong River and the Chao Phraya River in Thailand (Roberts & Vidthayanon, 1991). *Pangasius hypophthalmus* was sprayed to other area such as Malaysia, Indonesia and China (Pan & Zheng, 1983). *Pangasius hypophthalmus* is able to breath air through the swim bladder which allows the fish to stand in water with low level of dissolved oxygen (Browman & Kramer, 1985). *Pangasius bocourti* shows also the same characteristic. Moreover, fast growth and omnivorous regime make these species interesting for aquaculture (Lenormand, 1996). *Pangasius bocourti* has been reared in floating cages on the Mekong River since 1960, whereas this fish is never found in pond (Cacot, 1993). Now its amount of production in Vietnam reaches 15000 tons per year, which represents 75% of the production in floating cages. Most of this production is destined to the frozen filet processing and exportation, for an annual income of 22 billions USD. On the opposite side, *P. hypophthalmus* has been reared in the Mekong delta in ponds probably for centuries (Peignen, 1993). Now its amount of production is estimated to 20000 tons per year which are locally consumed, fresh or dried.

In 1994, the rearing of the two species was based on the catch of juveniles from the Mekong River, mostly in the Cambodian area. At least 20 billions of fingerlings of each species were needed per year in Vietnam. Drop of the catch and increasing of the demand since 1990 led to an important increase of the juvenile price for the Vietnamese fish aquaculturist (0.6 USD per 100 g juvenile). Faced to this bottle neck, production of fry by artificial propagation was necessary. The first reproduction of *P. hypophthalmus* was reported in Thailand in 1959 by Boonbrahm and later in Indonesia (Hardjamulia *et al.*, 1981) and in Malaysia (Thalathiah *et al.*, 1983). Now in these countries, the supply of fry for production is provided only by the artificial propagation and nursing. In Vietnam, the first artificial propagation was obtained in 1981 (My Anh *et al.*, 1981), but results were not sufficient to allow a large scale production of fry. For *P. bocourti*, no artificial propagation was reported in the world in 1994.

The presented research started in early 1994 in the frame of a PhD course of the author. Investigations have covered several fields of experiments that will be reported in a complete thesis report. Based on this work, the present paper aims to summarise the main results obtained on the reproductive cycle, induced breeding, gamete production and management for both *P. bocourti* and *P hypophthalmus* reared in floating cages or ponds in the Mekong delta.

MATERIAL AND METHODS (Figure 1)

Broodstocks

Since the systematic revision of Pangasiidae by Roberts and Vidthayanon (1991), the *Pangasius* species previously called *Pangasius pangasius* in Vietnam, should now be called *Pangasius bocourti* (Sauvage, 1880). The *Pangasius* species previously called *Pangasius micronema* in Vietnam and *Pangasius sutchi* (Fowler, 1937) in Thailand, Indonesia and Malaysia, should now be called *Pangasius hypophthalmus* (Sauvage, 1878).

In Vietnam, *P. bocourti* and *P. hypophthalmus* are locally called respectively "Ca ba sa" and "Ca tra". "Ca ba sa" means fish with three layers of fat, and experimental results on fish growth tend to confirm such appellation (see Le Thanh Hung *et al.*, 1999). "Ca tra" is in fact the genus name given locally to the pangasiid species in the Mekong delta, among which *P. hypophthalmus* is the most abundant species.

In 1994, the AGIFISH company was rearing large fishes in floating cages on the Mekong River in order to perform artificial propagation. These fishes had an estimated age of 3 and 6 years respectively in *P. bocourti* and *P. hypophthalmus*. Some large *P. hypophthalmus* were also available in a pond at the Can-Tho University. All these fishes are wild fish as they were caught at the juvenile stage in the Mekong River. The two species do not show any sexual dimorphism, as already mentioned by Roberts (1991).

However, in the broodfish used, body weight and fork length are respectively 30 and 8% bigger in females than in males.

Rearing conditions

Part of broodstocks available in 1994 were transferred into the experimental floating cages and ponds. The use of two rearing conditions allowed to assess the effect of the environment on the sexual cycle in the two species. In the two

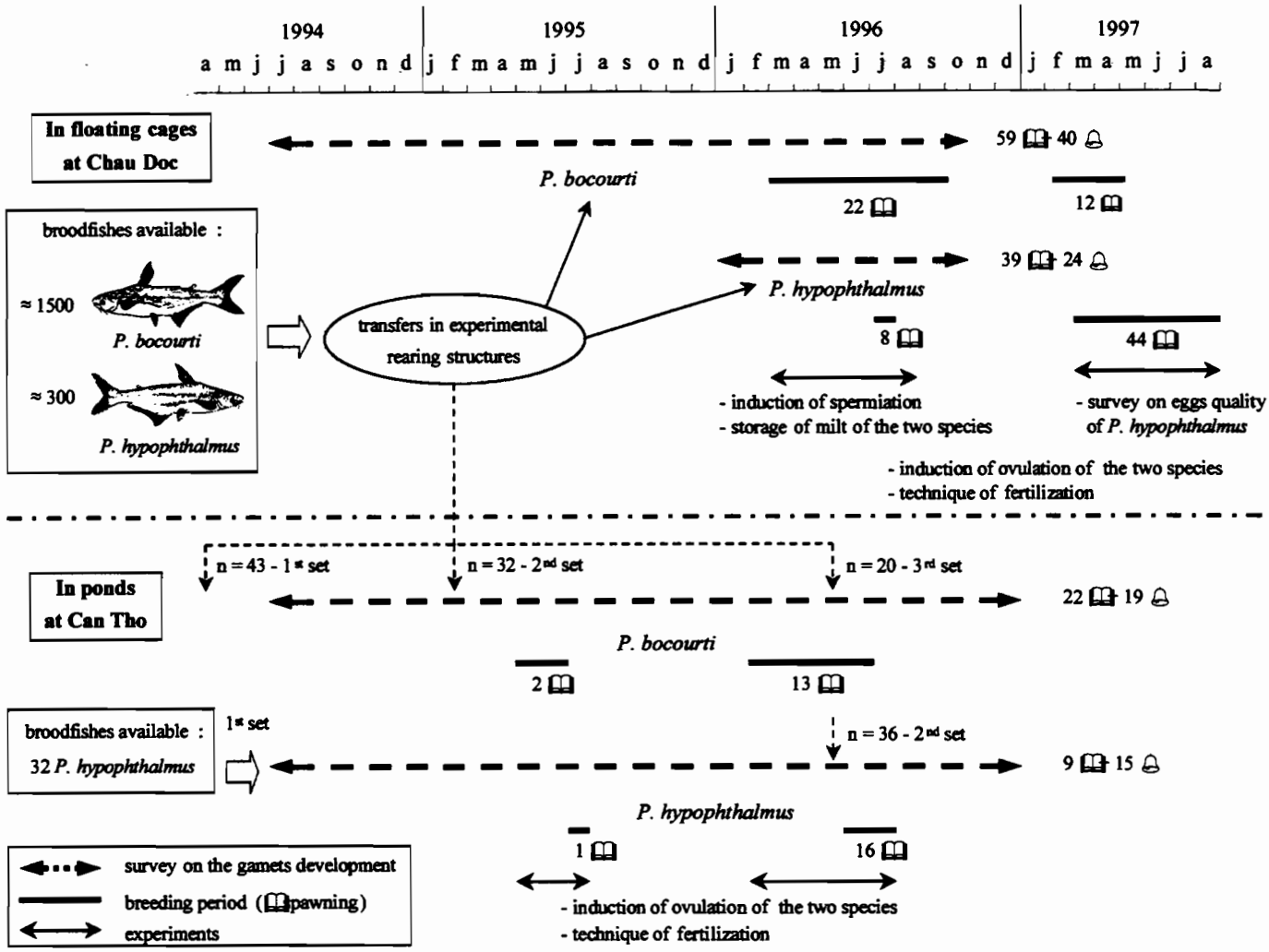


Figure 1: Distribution of broodstocks and experiments.

rearing structures, fish density is low in regard to those in growing conditions (Table 1). So broodfish in ponds could feed the natural food such as snail. Broodfish were also fed artificial feed given as moist or dry pellet. Each food was made with 2 to 6 raw materials. Twelve raw materials were used to prepare the pellet, considered as protein source (fish meal, cow liver, blood four), carbohydrate source (broken rice, corn), together protein and carbohydrate sources (soybean cake, rice bran, industrial food named PROCONCO C50) , and vitamin and mineral source (soybean and rice germs, industrial premix). Raw materials with high carbohydrate content were considered as source of energy as well as pellet binder. In moist pellet, leaves of the kapok tree *Ceiba pantandra* was also used specifically as pellet binder. The main ingredient was the local fish meal, despite its poor quality (44.6 and 37.4% of protein and ash on the dry matter). The average protein content of the food was 38.3 and 47.0% for the fish reared in cages and in ponds (Table 2). The fish were fed twice a day, morning and afternoon, at a feeding rate higher for *P. bocourti* than for *P. hypophthalmus* (Tableau 3). This difference was due first to the higher feed intake in *P. hypophthalmus* and to the higher fat content in *P. bocourti.*

Consequently, the quantity of protein provided was higher for *P. hypophthalmus* than for *P. bocourti*, respectively of 3.3–3.4 and 5.5–4.7 grams per kilo and per day (in floating cages–in ponds). In both species, carbohydrates were considered as energy source, as the study of fish in nature showed that fish have an omnivorous regime (Lenormand, 1996).

Survey on the development of gametes

In order to get information about the development of gonads, broodfishes of *P. bocourti* and *P. hypophthalmus* were caught regularly, respectively every 28–66 and 22–69 days (in ponds

	P. bocourti	*P. hypophthalmus*
In floating cages :		
-broodfishes per cage	50	50
-volume in m^3	45 and 54	27 and 54
-fish per m^3	1.1	1.6
-fish biomass $kg.m^3$	6.4	8.1
In ponds :		
-broodfishes per pond	36	31
-surface in m2	658 and 725	416 and 600
-surface (m2) per fish	19.5	15.2
-fish biomass in $g.m^2$	304.7	455.1

Tableau 1: Rearing structures (average values).

		In floating cages	In ponds
Moister (% TM) :			
- in moist pellet		34.5	43.7
- in dry pellet		12.9	10.2
Proteins	%DM	47.0	38.3
Lipids		6.9	7.5
Carbohydrates		18.5	16.0
Cellulose		4.4	3.4
Other (*)		-	10.7
Ash		23.2	24.0

* : carbohydrate and cellulose when the two components are not analysed.

Tableau 2: Composition of feed (average values).

		In floating cages		In ponds	
		P. bocourti	*P. hypophthalmus*	*P. bocourti*	*P. hypophthalmus*
Feeding rate (% biomass per day)		1.0	1.5	1.2	1.5
Proteins	$g.kg^{-1}.day^{-1}$	3.4	5.6	3.5	4.7
Lipids		0.5	0.9	0.8	1.0
Carbohydrates		1.2	2.2	1.2	1.6

Tableau 3: Nutrients provided by the feed (average values).

–in floating cages). In females, oocytes were sampled by ovarian biopsy with a flexible pipe ("Pipelle de Cornier"). Then oocyte diameter was measured under binocular lens, the modal diameter being used as the main criterion of oocyte development. Such method was applied to study the seasonal development of oocyte in *Heterobranchus longifilis* in Ivory Coast (Legendre, 1986). In males, presence of milt was checked by stripping of the fish. For both males and females, these observations were made on anaesthetised fish, by standing in solution of 0,25 $ml.l^{-1}$ phenoxy-2-ethanol. Fishes were tagged with blue spots on the skin of the belly, with saturated alcian solution injected using a "dermojet". Fishes were also tagged using intra-muscular electronic pit tag. So the gamete development was individually assessed.

Artificial propagation

Artificial propagation was conducted on selected mature fish. Mature females can respond to hormonal induction of ovulation. This stage is reached when mode of the oocyte diameter is 1.3 and 1.0 mm respectively in *P. bocourti* and *P. hypophthalmus*. Mature males produce milt when stripped. Mature broodfish were stood in tanks (3.5 m^3) with water exchange and water aeration during the hormonal treatment.

Three kinds of hormones were used :

- pituitary extracts of local common carp and Clariidae,
- human Chorionic Gonadotropin (hCG) produced by ORGANON,
- analogue of LHRH produced by BACHEM: (Des-Gly^{10}, D-Arg^6, Trp^7, Leu^8, Pro-$NHRt^9$) – LHRH, associated with two kinds of dopamine antagonists: pimozide or domperidone.

In females, first ovulation was obtained with hCG and then the use of this hormone was assessed during the following experiments. Use of hCG to induce oocyte maturation is common in other species of tropical catfishes such as *Heterobranchus longifilis* (Legendre, 1986), and *Clarias macrocephalus* (Carreon *et al.*, 1976, in Donaldson & Hunter, 1983). In *P. hypophthalmus*, use of hCG was reported in Vietnam (Huy *et al.*, 1990) and in Malaysia (Thalathiah *et al.*, 1988). In both cases, this hormone was associated with pituitary extracts of common carp, and, only in Indonesia, with homoplastic pituitary extract. In

Indonesia, use of hCG associated with pituitary extracts of common carp was also reported to induce oocyte maturation in the locally named "Ikan Patin", *Pangasius pangasius* (Meenakarn, 1986).

Hormonal treatments were also performed in males in order to enhance the milt production, with hCG in *P. bocourti* and *P. hypophthalmus*, and with LHRHa associated with domperidone in *P. bocourti* only.

Assessment of the quality of gametes

Quality of milt and ova collected was assessed by fertilisation trials made with about 300 ova set in half litre box. Incubation took place in these boxes in standing or current water. Motility of spermatozoa was checked before use of sperm, using criterion established by Sanchez–Rodriguez (Sanchez-Rodriguez & Billard, 1977). Fertilisation trials were made in standard conditions, with hundred times diluted milt in the activation solution (6 or 10 ml). Fertilisation and hatching rates were measured during incubation and percentages of abnormal larvae after hatching.

RESULTS

Reproductive cycle related to the environment (Figure 2)

The Mekong delta is a tropical area located at 10° North latitude. This area has a monsoon climate, with well marked dry and rainy seasons. Photoperiod has a range of 1 h 15. Air temperature ranges from 21.0 to 35.5°C, with an average value of 26.6 ± 1.4°C. The Mekong River is a typical flood plain river (Welcomme, 1979) with a low and high water time during the year. During the low water time, the flow is affected by the tide in the South Chinese Sea – named "East Sea" in Vietnam – causing balance of the flow alternatively from upstream and downstream four times per day. During the high water time, the flow runs only from upstream. These conditions are observed in both Chau-Doc and Can-Tho, where the water has nil salinity throughout the year.

In the two different rearing environments, sexual maturation of the two species shows a yearly marked cycle.

In *P. bocourti*

Oocyte development occurs when temperature increases from 26 to 31°C, respectively in January and April. Afterwards, proportion of mature females decreases with the temperature. This evolution is similar in both ponds and floating cages, whereas water transparency shows different patterns in the two environments. Indeed, in ponds and in floating cages, sexual maturation reaches a pick when transparency is respectively low and high. Water flow is also different between the two environments, as it is nearly nil in ponds whereas it ranges from 0 to 37.5 $m.mn^{-1}$ in the river in April, at the sexual maturation pick. Whatever the environment, sexual maturation reaches a pick during the dry season, before the rainy season. So comparison between ponds and cages leads to conclusion that temperature is probably the main parameter inducing the gonad development. However, photoperiod can also be involved as the oocyte development occurs also when the daytime increases.

In *P. hypophthalmus*

Oocyte development is delayed in regard to *P. bocourti* as it starts since March or April, when the water temperature is already high (30–31 °C). Mature females were observed in floating cages in various environmental conditions: in the dry and rainy season, when water transparency was high or low, during the low or high water periods. Sexual cycle shows also a similar schedule in both ponds and floating cages. However, in this species, more accurate information is needed especially by checking broodfish within shorter intervals (monthly).

In both species, males show the same periods of sexual maturation as the females in the two rearing conditions. Nevertheless in *P. bocourti* some males can produce milt throughout the year.

***Maturation in female* P. bocourti** (Figure 3)

Oocyte development was studied individually in 11 females. Out of the reproduction season, only small oocytes are observed, with very few oocytes larger than 0.6 mm. About 2 months later, oocytes are larger and show a single and marked mode. However, several trials failed to induce ovulation at such maturity stage. In fact, only large oocytes – from 1.60 mm of diameter – are sensitive to an hCG treatment. These called "sub-mature" females required a preliminary treatment involving several injections of hCG at low a dose, once per day.

Number of injections ranges from 3 to 10 and depends on the initial oocyte size, as females with well developed oocytes need less injections than

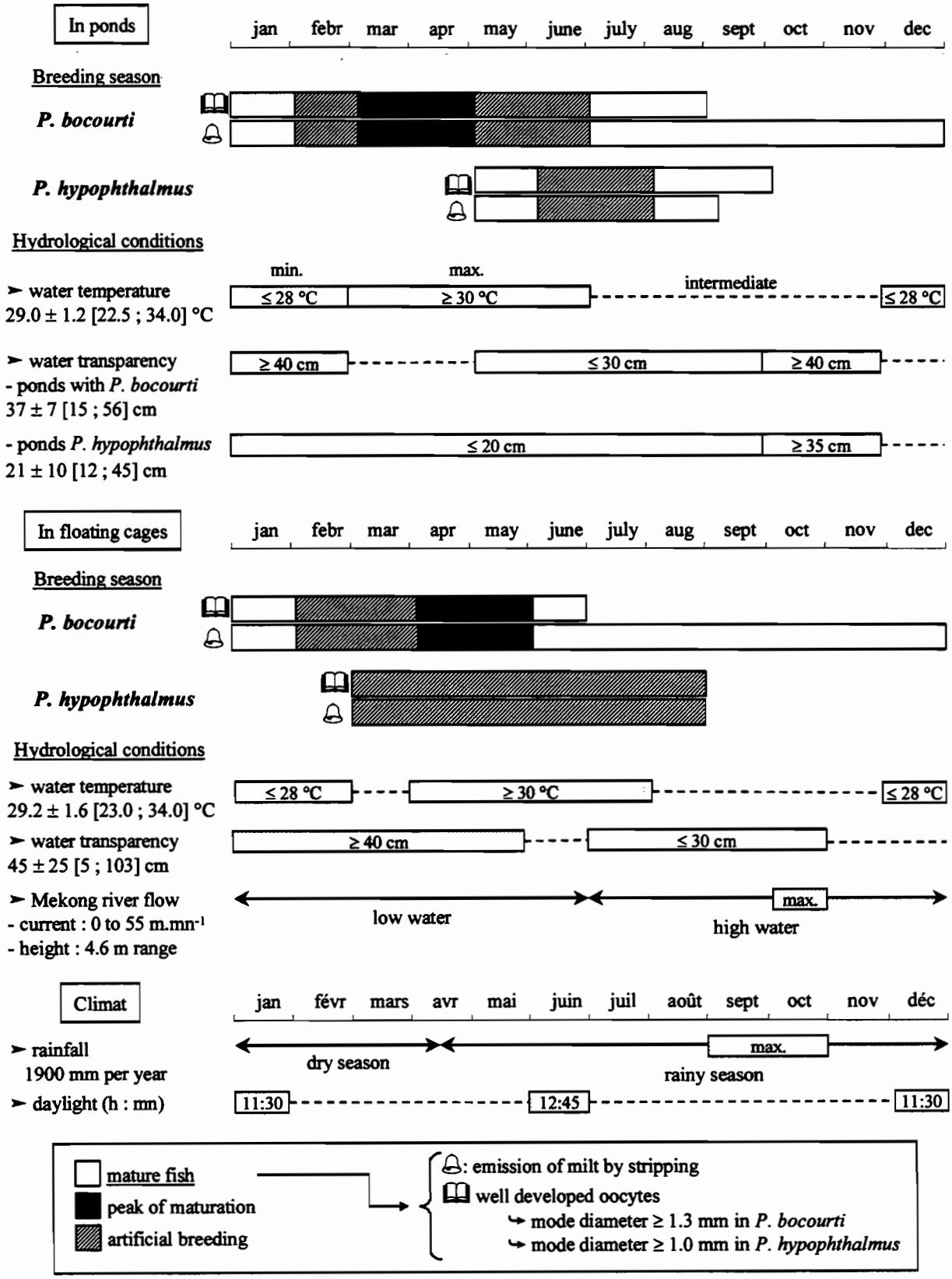


Figure 2: Reproductive cycle related to the environment.

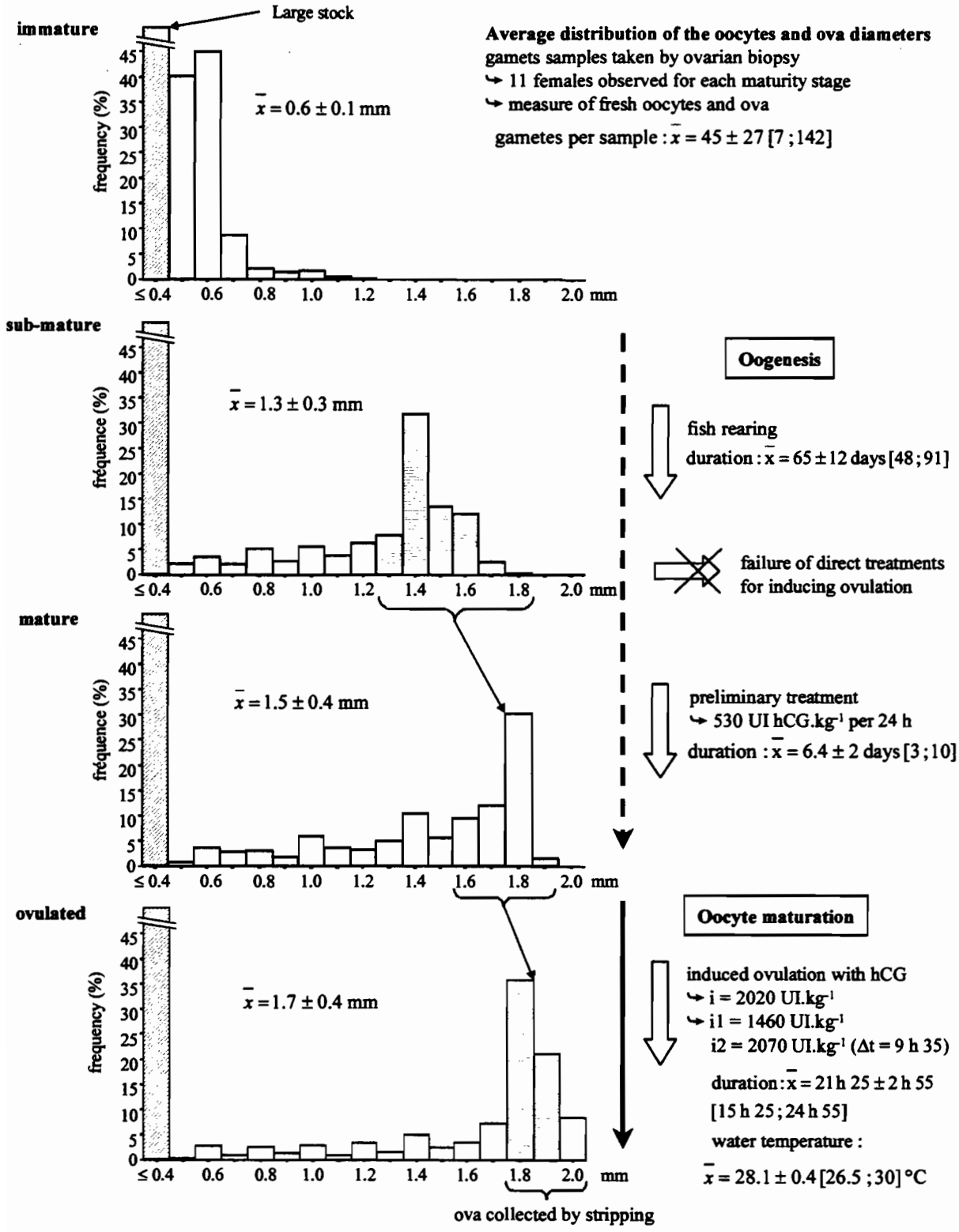


Figure 3: Oocyte development in females *P. bocourti* reared in ponds.

those having smaller oocytes. This treatment leads to an oocytes size increasing, and the proportion of oocytes from 1.60 mm to above increases from 14 to 53%. At this stage, females are considered as mature or sensitive to the second step of the treatment which will induce the oocyte maturation followed by ovulation. It involves one or two injections of hCG, at a higher doses and shorter interval between the two injections than for the preliminary treatment.

***Maturation in female* P. hypophthalmus** (Figure 4)

As in *P. bocourti*, oocyte development was studied individually in 17 females. Only 5 females were observed at the resting stage as the oocytes sampling is usually uneasy at this stage, due to the small size of the ovary. At this stage, out of the reproduction period, only small oocytes are observed, with very few oocytes larger than 0.5 mm. About 3 months later, oocytes are larger and show a single and marked mode. However, this duration has to be assessed with more precision, by checking fish within shorter interval (monthly). At this stage, females are sexually mature as they respond positively to an hormonal treatment inducing oocyte maturation followed by ovulation. In this species, oocytes are sensitive to the hormonal treatment from 1.0 mm diameter. At the mature stage such large oocytes represent 63% of the oocytes samples in average. The treatment applied involves one, two or three injections of hCG. An optional and short preliminary treatment can be applied before, with one or two injections of hCG at a lower dose. Even if it leads to an increasing of the oocyte size, this preliminary treatment does not seem to be necessary.

Process of oocyte maturation (Figure 5)

In both *P. bocourti* and *P. hypophthalmus* mature females, oocytes sampled by biopsy are surrounded by their follicular envelopes which can be seen by the presence of small capillary blood vessels (Figure 4). Also the germinal vesicle stands at the central position in all oocytes. The oocyte maturation induced by the hCG treatment is characterised by the migration of the germinal vesicle at the oocyte periphery, followed by the germinal vesicle breakdown. Considering all the females induced, reared in ponds and in floating cages, the success rate is 59% (n = 76) in *P. bocourti* and 88% (n = 67) in *P. hypophthalmus*.

Fecundity of females (Figure 6)

Cases of first spawning and single spawning

One gram of ova contains 5 times more ova in *P. hypophthalmus* than in *P. bocourti*. This difference is related to the smaller size of ova in *P. hypophthalmus*. After the first treatment of the year, the relative fecundity is twice higher in *P. hypophthalmus* than in *P. bocourti* when considering the weight of ova collected, and 10 times higher when considering the amount of ova. However in both species the relative fecundity is widely variable, as the coefficient of variation is 68% in both *P. bocourti* and *P. hypophthalmus*. In *P. bocourti*, the relative fecundity is two times higher in fishes reared in ponds than in floating cages. This lower fecundity in floating cages is associated with a preliminary treatment shorter and a higher stoutness (condition factor) than in broodfish reared in ponds. Otherwise, in broodfishes reared in floating cages, the relative fecundity is negatively related to the stoutness and the fattening (fat content in the belly).

On the opposite side, in *P. hypophthalmus* reared in floating cages, the relative fecundity is positively related to the stoutness. In this species, the fattening is much lower than in *P. bocourti*, however there is not enough data to assessed any relationship between fattening and the fish fecundity. In both species, into each rearing conditions, there is no relationship between the absolute or the relative fecundity and the fork length and the body weight.

Case of multiple spawning

Preliminary data show that some of the broodfish in both species are able to spawn twice a year. A second spawning occurred in the *P. bocourti* females getting high fecundity at the first spawning, compared to the single spawning females. In *P. hypophthalmus*, a second spawning occurred in half of the females in which the first spawning occurred during the first two months of the breeding season. In *P. bocourti*, fecundity at the second spawning is 6 times lower than at the first spawning. By contrast, in *P. hypophthalmus*, the second spawning fecundity is 75% higher than the first one. In both species, cumulated fecundity of the two successive spawns is higher than fecundity in the case of a single spawning, of 2.4 and 3.1 times in *P. hypophthalmus* and *P. bocourti* respectively. Finally, in one female *P. hypophthalmus* three spawning were obtained at 50 and 66 days of interval.

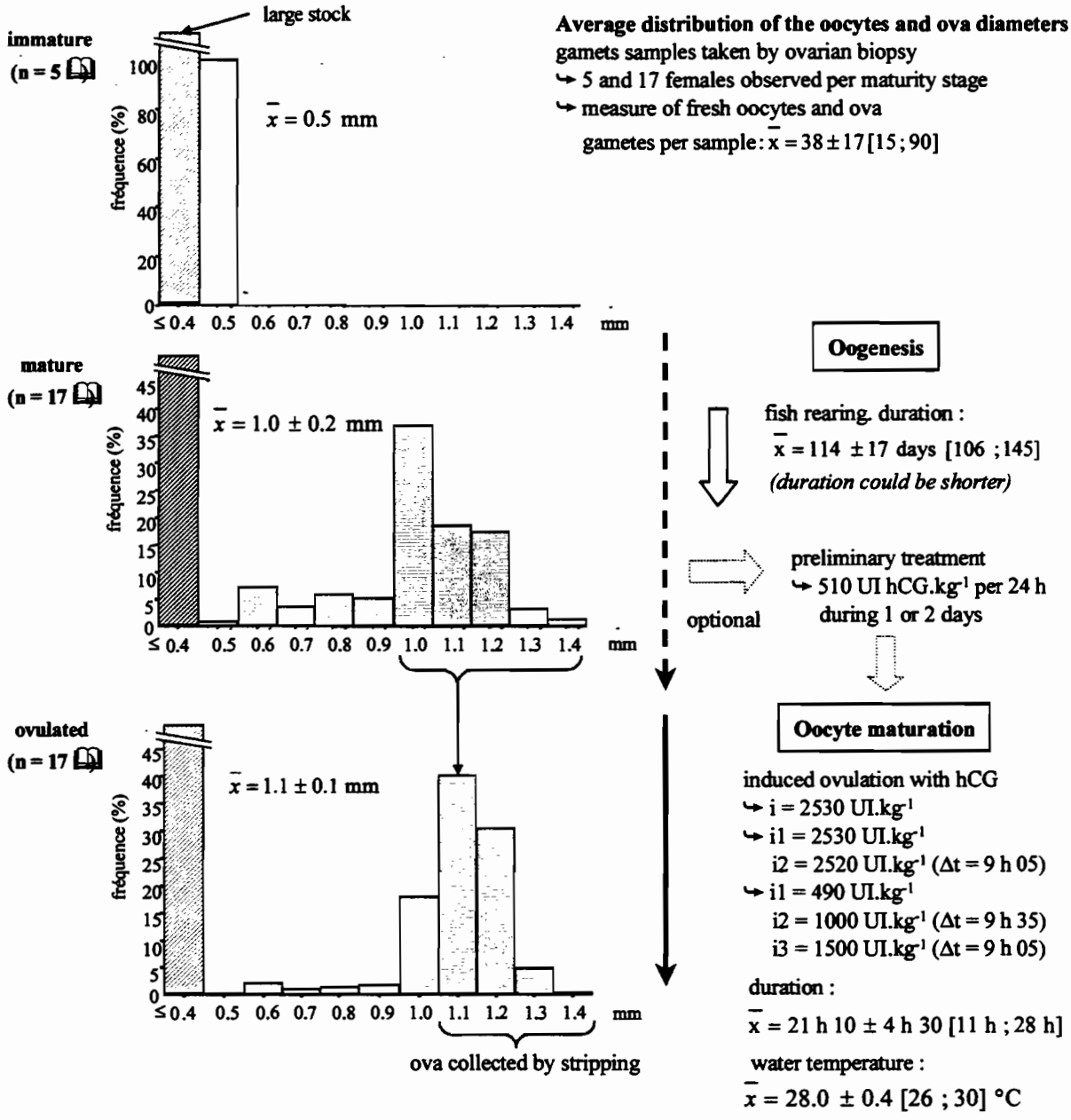


Figure 4: Oocyte development in females *P. hypophthalmus* reared in ponds.

Characteristics of milt (Figure 7)

Mature males produce naturally milt when stripped. Volume of milt per kilo of body weight and spermatozoa concentration are respectively 4.5 and 3.6 higher in *P. hypophthalmus* than in *P. bocourti*. Milt production can be enhanced by hCG injection. Then the volume of milt collected is 10.4 and 5.4 higher than naturally, respectively in *P. bocourti* and *P. hypophthalmus*. In *P. bocourti*, injection of LHRHa associated with domperidone induces also, but at a lower level than hCG, a production of milt which is 5.6 times higher than naturally. In the case of induction with hCG, the volume of milt collected increase rapidly to a pick reached 12 h after injection, and then drops. On the opposite side, LHRHa plus domperidone induces a progressive increase of the volume until 48 h to maybe more.

In both species, the duration of spermatozoa motility is short in pure water. However, with sperm of *P. bocourti*, motility of spermatozoa is twice longer into 2 $g.l^{-1}$ salt water than in pure water.

Management of gametes and fertilised eggs

All the procedures used for the collection of gametes, artificial fertilisation and egg incubation are presented in Figure 8.

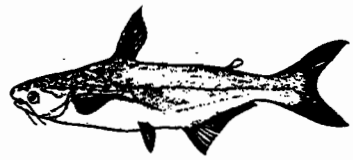
	P. bocourti	*P. hypophthalmus*
Body weight : average value ± SD [min. ; max]	7,0 ± 1,1 kg [4,8 ; 9,7] per 🕮	7,3 ± 1,6 kg [5,0 ; 13,3] per 🕮
Ova per gram of ova :	251 ova [222 ; 280] .g^{-1}	1437 ± 90 ova [1316 ; 1524] .g^{-1}

from 1995 to 1997, with broodfishes reared in floating cages and in ponds

induced females (hCG)	76 🕮		67 🕮
	⊕ ? priming		
First & single spawning	n = 45 🕮(59 %)	⊖ stoutness	⊕ n = 59 🕮(88 %)
10^3 ova.kg^{-1}	**4,7 ± 3,2 [0,4 ; 16,7]**	⊖	**48,8 ± 33,6 [5,5 ; 145,7]**
grams ova.kg^{-1}	*18,6 ± 13,3 [1,4 ; 66,4]*	fattening	*34,0 ± 23,4 [3,8 ; 101,4]*

	fishes in ponds in 1996	fishes in floating cages in 1997
induced females (with hCG)	10 🕮	33 🕮
Single spawning	n = 7 🕮(70 %)	n = 24 🕮(73 %)
10^3 ova.kg^{-1}	**4,9 ± 1,7 [2,3 ; 6,6]**	**52,8 ± 32,4 [10,1 ; 134,9]**
grams ova.kg^{-1}	*19,5 ± 6,6 [9,0 ; 26,2]*	*36,7 ± 22,6 [7,0 ; 93,8]*
1st spawning	n = 3 🕮(30 %)	n = 9 🕮(27 %)
10^3 ova.kg^{-1}	**13,3 ± 3,1 [10,4 ; 16,7]**	**47,3 ± 33,0 [24,0 ; 127,3]**
g ova.kg^{-1}	*52,9 ± 12,5 [41,6 ; 66,4]*	*32,9 ± 22,9 [16,7 ; 88,6]*
Characteristics	🕮with the highest fecundity	🕮with early spawning
2nd spawning	n = 3 🕮	n = 9 🕮
interval	53 ± 25 days [25 ; 75]	73 ± 26 days [40 ; 117]
10^3 ova.kg^{-1}	**2,2 ± 1,2 [1,0 ; 3,4]**	**82,9 ± 40,7 [37,2 ; 170,2]**
grams ova.kg^{-1}	*8,7 ± 4,8 [3,9 ; 13,6]*	*57,7 ± 28,3 [25,9 ; 118,4]*
1st + 2nd spawnings	n = 3 🕮	n = 9 🕮
10^3 ova.kg^{-1}	**15,5 ± 4,0 [12,6 ; 20,1]**	**130,2 ± 69,3 [73,6 ; 297,5]**
grams ova.kg^{-1}	*61,6 ± 16,0 [50,1 ; 79,9]*	*90,6 ± 48,2 [51,2 ; 207,0]*

Figure 5: Compared fecundity of females *P. bocourti* and *P. hypophthalmus* related to the rank of spawning.



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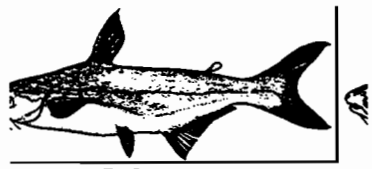
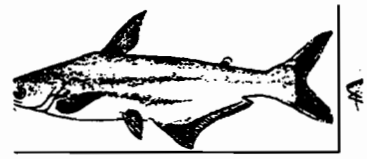
Single spawning	n = 7 🕮(70 %)	n = 24 🕮(73 %)
10^3 ova.kg^{-1}	**4.9 ± 1.7 [2.3 ; 6.6]**	**52.8 ± 32.4 [10.1 ; 134.9]**
grams ova.kg^{-1}	*19.5 ± 6.6 [9.0 ; 26.2]*	*36.7 ± 22.6 [7.0 ; 93.8]*
1st spawning	n = 3 🕮(30 %)	n = 9 🕮(27 %)
10^3 ova.kg^{-1}	**13.3 ± 3.1 [10.4 ; 16.7]**	**47.3 ± 33.0 [24.0 ; 127.3]**
g ova.kg^{-1}	*52.9 ± 12.5 [41.6 ; 66.4]*	*32.9 ± 22.9 [16.7 ; 88.6]*
Characteristics	🕮with the highest fecundity	🕮with early spawning
2nd spawning	n = 3 🕮	n = 9 🕮
interval	53 ± 25 days [25 ; 75]	73 ± 26 days [40 ; 117]
10^3 ova.kg^{-1}	**2.2 ± 1.2 [1.0 ; 3.4]**	**82.9 ± 40.7 [37.2 ; 170.2]**
grams ova.kg^{-1}	*8.7 ± 4.8 [3.9 ; 13.6]*	*57.7 ± 28.3 [25.9 ; 118.4]*
1st + 2nd spawnings	n = 3 🕮	n = 9 🕮
10^3 ova.kg^{-1}	**15.5 ± 4.0 [12.6 ; 20.1]**	**130.2 ± 69.3 [73.6 ; 297.5]**
grams ova.kg^{-1}	*61.6 ± 16.0 [50.1 ; 79.9]*	*90.6 ± 48.2 [51.2 ; 207.0]*

Figure 6: Relative fecundity in females *P. bocourti* and *P. hypophthalmus*.



P. bocourti



P. hypophthalmus

	P. bocourti	*P. hypophthalmus*
Body weight: average value ± SD [min. ; max.]	5.2 ± 1.0 kg [3.3 ; 6.9] per ♂	5.5 ± 1.1 kg [3.9 ; 6.7] per ♂

broofishes reared in floating cages

Volume & concentration of semen

Natural spermiation :	n = 8 ♂	n = 4 ♂
volume (µl.kg⁻¹)	**33 ± 32 [5 ; 90]**	**151 ± 258 [11 ; 537]**
concentration (10⁹ spz.ml⁻¹)	*13.3 ± 10 [2.4 ; 27.3]*	*48.0 ± 18.6 [25.7 ; 65.5]*
Induced spermiation :		
➢ 2000 UI hCG.kg⁻¹	n = 4 ♂	n = 4 ♂
optimal period of latency	12 h	24 h
volume (µl.kg⁻¹)	**345 ± 125 [237 ; 495]**	**823 ± 850 [338 ; 2092]**
concentration (10⁹ spz.ml⁻¹)	*26.9 ± 13.4 [7.0 ; 36.2]*	*57.2 ± 6.8 [47.5 ; 63.4]*
➢ 30 µg LHRHa + 3 mg domperidone kg⁻¹	n = 4 ♂	-
optimal period of latency	(48 h)	-
volume (µl.kg⁻¹)	**187 ± 147 [75 ; 385]**	-
concentration (10⁹ spz.ml⁻¹)	*15.8 ± 6.3 [9.3 ; 24.0]*	-
Overall volume & concentration of semen are	independent	positively related
Water temperature	[28.1 ; 30.4]°C	[27.8 ; 29.5]°C

Motility of spermatozoa

In two activating solutions :		
➢ pure water	57 ± 12 seconds [40 ; 98]	45 ± 9 seconds [30 ; 69]
➢ 2 g.l⁻¹ NaCl	112 ± 41 seconds [39 ; 210]	-

( ) : data which have to be confirmed

- : lacking data

Figure 7: Characteristics of the semen collected in males *P. bocourti* and *P. hypophthalmus*.

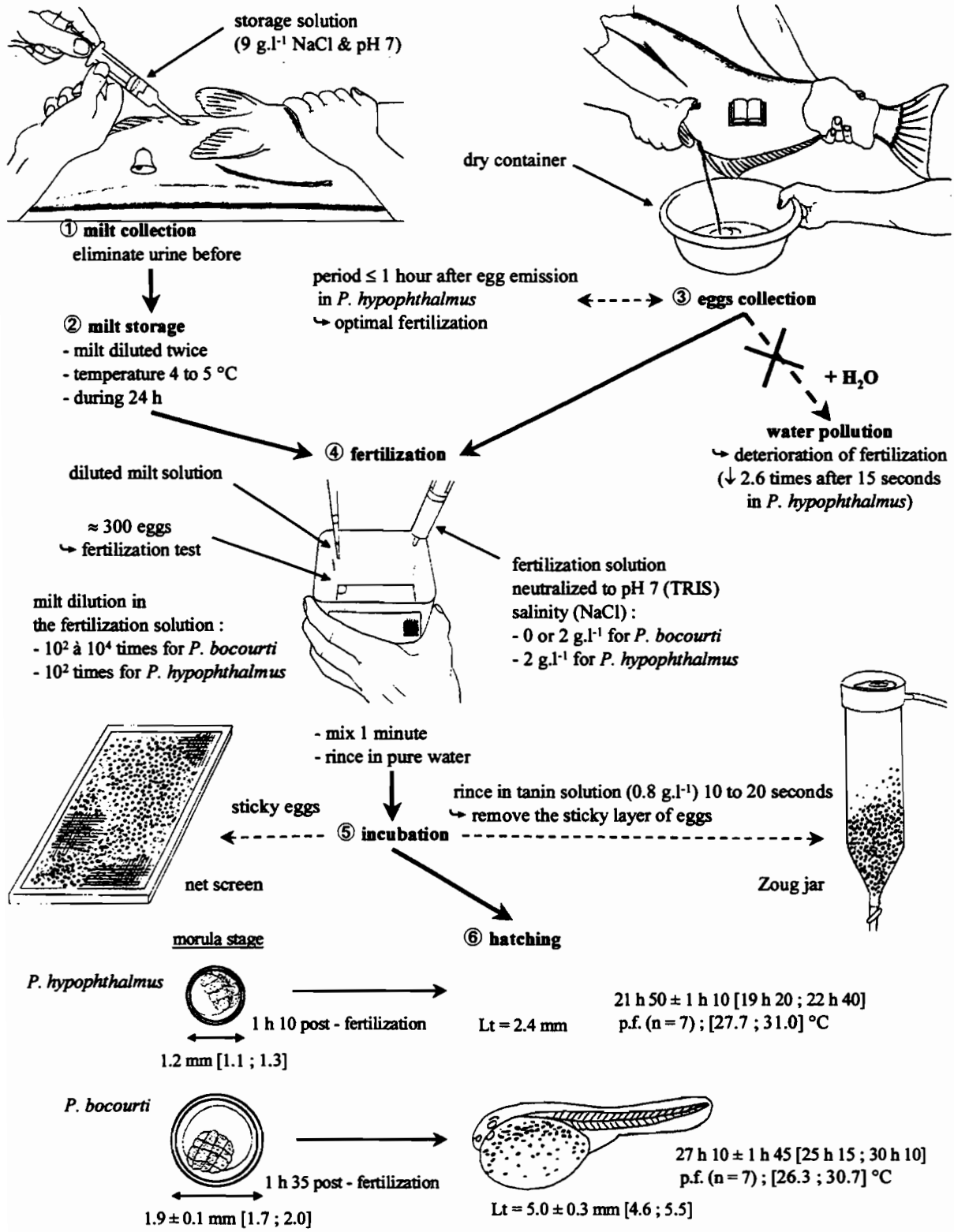


Figure 8: Management of gametes, from collection of semen and ova to the hatching of eggs.

DISCUSSION

Type of oocyte development and spawning frequency

Out of the breeding season, females of both species have only one group of small oocytes. Later, during the breeding season, oocytes distribution still shows an important group of small oocytes but also a new and single marked group of large oocytes. Large oocytes are spawned during one spawning season, whereas the stock of small oocytes will be available for the following breeding seasons. However, in some cases and especially in *P. hypophthalmus*, a new recruitment of the small oocytes can occur during the same breeding season, leading to a second spawning. Such pattern indicates that females of the two species are group–synchrone maturation fish. This pattern is intermediate to the synchronous and asynchronous way of oocyte development (Vlaming, 1983). In synchronous species, such as European Eel *Anguilla anguilla* and Sockeye Salmon *Onchorynchus nerka*, there is only a single group of oocytes in the ovary whatever the stage of oogenesis. In such species, spawning usually occurs only once a life. In asynchronous species, such as Tilapia and *Blennius pholis*, there is at the same time oocytes at different stages of oogenesis in ovary, without any marked groups.

In both *P. bocourti* and *P. hypophthalmus*, hCG treatment induces a single spawning of all the large oocytes group. In cases of partial ovulation of the large oocytes, ova produced cannot be collected by stripping. Otherwise, in *P. hypophthalmus*, the quality of ova drops quickly after ovulation, showing that ova has to be collected rapidly – 1 hour after emission of ova – to guaranty a good fertility (Campet, 1997). So, in nature and in the two species, oocyte maturation is probably a synchronous process concerning all the large oocytes in the ovary. Then, at least in *P. hypophthalmus*, it is rapidly followed by a single – or several near time – spawning.

Sexual cycle related to the environment

In both *P. bocourti* and *P. hypophthalmus*, gametes development show a yearly cycle which is related to the evolution of environment. Role played by the water temperature and/or the photoperiod fluctuations are probably the main parameter(s) involved in this process. On the opposite side, fluctuations of water flow and water transparency did not appear to be preponderant in regard to the development of gametes. Otherwise, gamete development in *P. hypophthalmus* occur two months after *P. bocourti*, suggesting that *P. hypophthalmus* requires higher temperature and/or longer daytime than *P. bocourti* to start sexual maturation.

Contrasting with the situation observed in Vietnam, in *P. hypophthalmus* reared in ponds in Indonesia, the oocyte development does not show such yearly evolution as the average oocyte diameter stands high throughout the year (see Legendre *et al.*, 1999). Moreover, in these conditions females are able to reproduce three to four times per year. The environment in this sub-equatorial area is relatively stable as the temperature stands high, ranging from 28 to 31°C, the photoperiod range is only half an hour and the rainy season is little marked. Such observations tend to confirm the role of the environmental fluctuation in the yearly reproduction cycle in *P. hypophthalmus* in the South Vietnam area.

In the presented study and in the two rearing conditions, spawning were obtained in *P. bocourti* mainly during the low water time in the Mekong River. In *P. hypophthalmus*, it occurred a bit later, mainly at the beginning of the water flow period. Roberts (1983) has reported migration for reproduction in several species of Pangasiidae in the Mekong River, among which *P. bocourti* and *P. hypophthalmus*. Migration occurs from May to July at the Khone waterfalls in Southern Laos, upstream the Mekong delta. This period corresponds to the beginning of the water flow in the river. So the spawning in both captivity and nature probably occur at the same time in *P. hypophthalmus*. However, in *P. bocourti*, the spawning in captivity is probably advanced in regard to the spawning in nature. This delay could be due to the preliminary treatment applied in this fish to achieved the oocyte development. According to this hypothesis, several days of treatments in captivity could replace several weeks in nature to reach the optimal stage of maturity.

Spawning before flow was reported by Welcomme (1979) to be a common behaviour in fish living in the flood plain river such as the Mekong. This strategy provides good conditions for the larvae as they develop in the floodplain, where food is abundant and predation risk is low. Indeed, fingerlings of both *P. bocourti* and *P. hypophthalmus* spray in the Mekong delta from June to September, when the water flow increases (Lenormand, 1996).

***Differences of reproduction performances between* P. bocourti *and* P. hypophthalmus**

In both ponds and floating cages, females of *P. bocourti* do not achieved the oogenesis, as they require a preliminary treatment before inducing ovulation. Such treatment was reported in female Orinnoco cachama (*Colossoma oculus*), in order to advance the gonadal development up to the pre-ovulation stage (Woynarovitch & Horvath, 1980). In this case, the preliminary treatment involved 5 injections of pituitary extracts, at 5–10% of the decisive dose, with 24 hours between two injections. Afterwards the decisive dose is given within two injections, at respectively 40 and 60% of the dose.

On the opposite side, females *P. hypophthalmus* held in culture conditions reach naturally an advanced stage of oogenesis which allows a simple treatment to induce the oocyte maturation. That is probably why artificial propagation provides better results in *P. hypophthalmus* than in *P. bocourti*, both in terms of success rate and relative fecundity. The two species differ also in term of lipid storage as the broodfish of *P. bocourti* can develop an important fat tissue in the abdominal cavity, whereas *P. hypophthalmus* seems to be a lean fish. Such phenomenon was also observed in 10 g fingerlings of *P. bocourti* by Le Thanh Hung (personal communication), in case of excess feeding with a 60% of dry matter protein feed. In female broodfish of *P. bocourti*, the fat tissue is probably used during oogenesis for the ovary growth, as there is a negative relationship between the development of the two tissues.

Two hypothesis can be advanced to explain this poor gonad development observed in *P. bocourti* compared to the one in *P. hypophthalmus*.

Firstly, the environment may be not fully suitable in the rearing conditions, as it could lack some stimuli involved in the gonad development, such as fluctuation of the water flow. According to Roberts (1993), spawning probably occurs in a waterfall area, with a strong water flow and then hydrological conditions differing a lot from those in ponds or in floating cages. In some other species living in floodplain river, role of the hydrological fluctuations in reproduction was demonstrated. In the catfish *Pimelodus maculatus*, an increasing of both water temperature and water flow are necessary to induce the gonad development in the Jaguari River (Basile Martins, 1975, in Welcomme, 1979). In the Parana River, *Prochilodus platensis* isolated fish in the flood plain cannot mature, whereas fish in the main channel of the river can achieved their sexual maturation (Bonetto, 1975, in Welcomme, 1979).

Secondly, for most of the species living in floodplain river, feeding mainly occur during the high water time, when food is abundant, whereas the low water time is a starvation period (Chevey & Le Poulain, 1940; Welcomme, 1979). In *Clarias gariepinus* living in the Shire river, the feeding intake is three times higher during the high water time than during the low water time (Willoughby & Walker, 1977, in Welcomme, 1979). So in such rivers, fish are able to constitute and to mobilise alternatively their body reserve, such as lipid, during the high and low water time. In *Alestes leuciscus* living in the delta of Niger, the growth in weight is reversible as the condition factor can decrease of 30% within one year (Daget, 1957, in Welcomme, 1979). In the Mekong River, Pangasiidae probably follow such ecological figure, which could be accentuated in broodfish, as they have to migrate and to constitute their gonads during the starvation period. However, in rearing conditions, broodfish were fed continuously throughout the year, without alternative periods of feeding and starvation. Consequently, fish probably store more reserve than they can use for growth and reproduction. Finally, the fat tissue probably interferes with the development of gonad in the abdominal cavity because of congestion. This hypothesis is reinforced by the fact that in floating cages from 1994 to 1996, the reducing of feeding rate led to a reducing of the fat content in the body cavity of broodfish, from 12.1 to 6.4% of body weight, whereas the gonado-somatic index increased from 0.5 to 3% in the same time. Finally, negative effects of both constraints, environments and feeding regime, can be cumulated in the rearing conditions.

Pangasius hypophthalmus appears as a more tolerant species concerning its environment in regard to the reproduction performances. Such hypothesis is reinforced by the presence of *P. hypophthalmus* in small canals in the Mekong Delta, where water quality is poor, whereas the presence of *P. bocourti* is restricted to the main river channel (Lenormand, 1996). It could be a reason why fecundity in *P. hypophthalmus* does not differ between the two rearing conditions, whereas fecundity in *P. bocourti* is twice higher in ponds than in cages. About the feeding regime related to reproduction, *P. hypophthalmus* could also show a different figure than *P. bocourti*. This

species probably feed throughout the year, or with a shorter period of starvation than in *P. bocourti*. According to this hypothesis, fish do not need large reserve to go on the starvation time. Otherwise, broodfish of *P. hypophthalmus* probably spawn in the river not as far as *P. bocourti*, as for the former species very small fry are collected in the Mekong delta (Lenormand, 1996), then fewer energy has to be spent for migration.

Difference of fecundity between the two species could also depend on the genetic aspect. The relative fecundity is ten times higher in *P. hypophthalmus* than in *P. bocourti*, respectively 48800 and 4700 ova per kilo of body weight. However the survival rate of larvae in conditions of mass production at the AGIFISH station is about 1 and 75% respectively in *P. hypophthalmus* and *P. bocourti* one month after hatching (Vo Phuoc Hung, AGIFISH Co, pers. comm.). Thus, considering the fact that fertilisation rate is about the same in the two species, the number of one month old fingerlings per kilo of female broodfish is 7 times higher in *P. bocourti* than in *P. hypophthalmus*. Even if results are different in natural conditions, larvae of *P. hypophthalmus* seem to be weak in comparison to *P. bocourti*. Consequently, in *P. hypophthalmus*, emission of high number of small ova could compensate the low survival rate of larvae.

Two other aspects related to the fish physiology could also affect the fecundity in *P. bocourti*. First, broodfish of both species have been reared in floating cages from the fingerling stage (50 g and several months old). During the first two years, those fish were reared at a high density – up to 200 $kg.m^{-3}$ – and fed at satiety with moist pellet containing mostly carbohydrates from rice bran. Such conditions led to very fat fish in *P. bocourti* (Cacot, 1993). It can also affect the formation of gonad and then the future fecundity of fish. It was shown in Plaice fish that biotic and abiotic factors experienced during the beginning of fish life determine later the fish fecundity (Simpson, 1951, in Kartas & Quignard, 1984). Secondly, fecundity could be affected by the age of fish if too old, as broodfish of *P. bocourti* were 6 to 9 years old when the present study was carried out. Such negative effect of age was reported in Sturgeon, common Carp and Pike (Nikolsky, 1969, in Kartas & Quignard, 1984). However the age at the first maturity remains unknown in *P. bocourti*.

High variability of the fecundity in the two species

In both species fecundity is highly variable. Such variability can have genetic cause as broodfish used are wild fish, initially caught in the Mekong River. In *P. hypophthalmus*, important migration for reproduction were reported, from the Great Lake "Ton-Le-Sap" to upstream, below or even above the Khone waterfall (Bardach, 1959) and (Sao Leang & Saveum, 1955, in Welcomme, 1979). On the opposite side, very small fry of *P. hypophthalmus* are caught on the bank of the Ton-Le-Sap (Bazir, SAMADHI, pers. comm.), suggesting that reproduction takes place on the flooded banks. Consequently, it could exist migratory and sedentary strains in *P. hypophthalmus*, characterised by two different levels of fecundity. Such figure was shown in the Malma trout living in Alaska (*Salvelinus malma*), where the migratory strain has higher fecundity than the sedentary strain (Blackett, 1973, in Kartas & Quignard, 1984). Otherwise, some strains may be more or less adapted to the rearing conditions in regard to their reproduction performances.

Concentration of milt

In both species, hormonal treatments induce an increasing of the volume of milt collected, whereas the spermatozoa concentration in milt does not decrease, or even tends to increase. Such response suggests that hormones stimulate the production of both seminal liquid and spermatozoa. Usually the hormonal treatment induces an increase of the volume collected, together with a reduced spermatozoa concentration resulting from dilution in the seminal liquid, as reported in common Carp (Saad & Billard, 1987).

Otherwise, concentration of spermatozoa in milt of *P. bocourti* and *P. hypophthalmus* are high compared to those in other species (Tableau 4).

CONCLUSION

Research carried out since 1994 on *P. bocourti* and *P. hypophthalmus* reproduction have led to the development of artificial propagation techniques in the two species. Extension of results has been carried out and allowed a mass production of fingerlings in Chau-Doc, where 30 000, 350 000 and 1 000 000 of fingerlings where produced respectively in 1995, 1996 and 1997. However,

	Concentration of milt (10^9 spermatozoa per ml)	References and authors
Ca ba sa: *P. bocourti*	13 – 26.9	(Eeckhoutte, 1996)
Ca tra: *P. hypophthalmus*	48 – 70.1	(Eeckhoutte, 1996)
Silure: *Silurus glanis*	7.18 ± 1.3	(Saad & Billard, 1995)
Tilapia: *Oreochromis niloticus*, *O. aureus, O. mossambicus*	2.8	(Rana & Mc Andrew, 1989)
Carpe: *Cyprinus carpio*	24.0 ± 3.5	(Saad & Billard, 1987)
African catfish: *Heterobranchus longifilis*	2.94 [1.26 – 4.26]	(Legendre *et al.*, 1992)
African catfish: *Clarias gariepinus*	4.00 [2.32 – 7.02]	(Legendre *et al.*, 1992)

Tableau 4: Concentration of spermatozoa in milt of some species.

more extension has still to be done to reach the 50 millions of fingerlings required in South Vietnam.

Production of juveniles faced now to two main bottle necks in each species. First, the availability of broodfish in the two species, restricted because fish probably reach sexual maturity after several years. So it involves to rear fish during a longer time than in other species artificially reproduced in the Mekong Delta, like Cyprinidae. But yet we do not know the age at the first maturity and if it is affected by the rearing conditions. In Indonesia, *P. hypophthalmus* reared in ponds can reach sexual maturity within the second year (Legendre, pers. comm.). However, in other places, broodfish of *P. hypophthalmus* were at least three years old. The second problem in *P. bocourti* is the low fecundity associated to the high fat content in broodfish. In *P. hypophthalmus*, the major constraint is the high mortality of larvae occurring from 3 to 6 days after hatching.

The two species studied can artificially crossbreed. Then hybrids are usually produced for rearing in floating cages since 1995, using ova of *P. hypophthalmus* fertilised with sperm of *P. bocourti*. Production of this hybrid allows the production of numerous offspring, due to both the high fecundity of *P. hypophthalmus* and the high survival rate of *P. bocourti*. Hybrid has also a white flesh inherited from *P. bocourti*, which is appreciated by the export market. However, clear data about the zootechnical performances and the fertility of the hybrid are still lacking.

PERSPECTIVES

In order to get more knowledge on the reproduction performances of both *P. bocourti* and *P. hypophthalmus*, further experiments will focus on the effects of broodfish feeding related to the fecundity and the quality of ova. Also a preliminary survey will start on the hybrid between the two species, in order to evaluate its growth potential and reproduction characteristics. These experiments will be carried out in floating cages on the Mekong River, from 1998 to 2000.

Beside the study in rearing conditions, survey aiming to precise the fish ecology in the Mekong River is also planned. This second approach will provide better understanding of the fish physiology and reproduction strategy in nature.

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ABSENCE OF MARKED SEASONAL VARIATIONS IN SEXUAL MATURITY OF *PANGASIUS HYPOPHTHALMUS* BROODERS HELD IN PONDS AT THE SUKAMANDI STATION (JAVA, INDONESIA).

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Abstract

An evaluation of the seasonal variations of sexual activity of *P. hypophthalmus* broodstock held in 50 m^2 ponds was carried out between April 1997 and April 1998 at the Sukamandi station (West Java, 6°S of latitude). These variations were assessed through the evolution of oocyte diameter of individual females, the proportion of fluent males and the success of hormonal-induced breeding trials.

High proportions of fluent males and mature females with post-vitellogenic oocytes of modal diameter superior to 1.00 mm were found at anytime. As a consequence, successful induced breeding of this species using hormonal treatments could be performed regardless of season and allowed an all year long production of fry. This continuous sexual maturity of *P. hypophthalmus* broodstock, during both the dry and rainy seasons, may have resulted to a large extent from rather stable conditions, with a monthly mean water temperature constantly high (28-31°C) and a low variation of day length during the year. Ovulation was successfully induced at 3, 2 and 4 months interval in a same *P. hypophthalmus* female. This indicated a quick recovery of oogenesis in females reared in the ponds.

INTRODUCTION

Pangasius hypophthalmus (Sauvage, 1878) (senior synonym of *P. sutchi*, Roberts and Vidthayanon, 1991) is an important economic freshwater species in South East Asia. The complete breeding cycle in captivity of this pangasiid catfish originating from the Mekong and Chao Phraya River Basins, was first obtained in Thailand about 30 years ago (Potaros & Sitasit, 1976). Since that time, the fish has been introduced to other areas where its use in aquaculture has progressively gained in importance. In Indonesia, the species was successfully induced to breed for the first time at the beginning of the eighties, after it was introduced in this country from Thailand in 1972 (Hardjamulia *et al.*, 1981).

Despite the economic interest of *P. hypophthalmus*, published data related to its biology and culture are still scarce. In particular, it was rather surprising that detailed study of the reproductive cycle of this catfish could not be found in the literature, either for wild or captive stocks. Nevertheless, Potaros and Sitasit (1976) mentioned that the spawning season of *P. hypophthalmus* was between June and September in the Thai climatic conditions, while Saidin and Othman (1986) reported broodstock to be mature from June to December-January in Malaysian ponds. In Indonesia, Hardjamulia *et al.* (1981) also reported that *P. hypophthalmus* females from a broodstock held in 18 m^2 tanks were sexually mature during a limited period of the year, corresponding mainly to the rainy season, from October to April. However, we observed recently the occurrence of mature *P. hypophthalmus* of both sexes even during the dry season in the ponds of the Sukamandi station located in West Java.

It was therefore decided to start an evaluation of the seasonal variations of sexual activity of *P. hypophthalmus* broodstock held in pond conditions. These variations were assessed through

the evolution of oocyte diameter of individual females, the proportion of fluent males and the success of hormonal-induced breeding trials. The demand for *Pangasius* seeds is high in Indonesia and it would be of major importance to be able to produce them all year round.

MATERIAL AND METHODS

The ponds experiments were carried out at the Sukamandi Station of the Research Institute for Freshwater Fisheries (Indonesia). Sukamandi is located in the Western part of the Java Island, at 6° S of latitude. In this area, two climatic seasons are generally distinguished: a rainy season from October to April and a dry season from May to September. The range of yearly variation of day length is about 30 minutes.

Fish origin and maintenance

The *P. hypophthalmus* brooders used in this study descended from the fish initially introduced from Thailand in 1972 and were 3-4-years-old and 2 to 5 kg individual body weight. Seasonal variations of sexual activity were followed up on broodstock of equilibrated sex ratio (1:1) reared at a stocking density of 0.3 fish.m^{-2} in two replicated 50 m^2 ponds. The fish were fed two times per day, 6 days a week, with a 35% crude protein pelleted feed distributed at a daily rate of 1-1.5% of total biomass. All fish were tagged using PIT tags (passive inductive transponder, Fish Eagle©) in order to allow their individual identification. Supplementary groups of *P. hypophthalmus* brooders were also maintained in other ponds of the station with equivalent rearing conditions, except for a higher stocking density (0.6 fish.m^{-2}). Fish from these latter groups were used for a part in the induced breeding trials.

Seasonal changes in sexual maturity

The sexual state of broodfish was checked monthly between April 1997 and April 1998. In order to follow the vitellogenesis stage without killing the females, fish were anaesthetised in a 0.3 mL.L^{-1} phenoxy-2-ethanol solution and a sample of oocytes was taken by intraovarian biopsy using a polypropylene tube of 3.5 mm external diameter (Pipelle de Cornier™). The diameters of 30 to 50 oocytes per sampled fish were measured with a micrometer using a binocular microscope (x25). The modal oocyte diameter was used as the main criterion of female sexual maturity (Legendre, 1986). The sexual stage of males was determined by the possibility of obtaining semen after gentle stripping.

All along the study, the daily fluctuations of pond water temperature were registered continuously with electronic thermometers (Stowaway Optic Prosensor©). Rainfalls were measured daily with a pluviometer implemented nearby the ponds.

Induced oocyte maturation and ovulation

Between December 1996 and April 1998, 54 *P. hypophthalmus* females were treated at different period of the year with Ovaprim© (n=50) or human chorionic gonadotropin (hCG; n=4) for inducement of oocyte maturation and ovulation. The treated females were chosen after intraovarian biopsy on the basis of a diameter of their oocytes greater than 1.0 mm. They received two successive hormone injections at 8 h interval with corresponding doses of 0.3 ml.kg^{-1} then 0.6 ml.kg^{-1} female BW with Ovaprim, and 500 IU.kg^{-1} then 2,000 IU.kg^{-1} with hCG (Legendre *et al.*, 1999; Cacot, 1999). Selected males were producing milt at stripping, they received a single Ovaprim injection of 0.3-0.4 ml.kg^{-1} applied at the moment of first injection of females.

When ovulation was detected, ova were collected by stripping, weighed and immediately fertilised. A sample of ova was also weighed to the nearest mg and fixed in 5% formalin for subsequent counting and total fecundity estimates. The sperm was collected by stripping directly in a syringe containing a 0.9% NaCl solution (dilution rate of 1/5), then preserved at 5°C for a maximum period of 3 hours before being used for fertilisation.

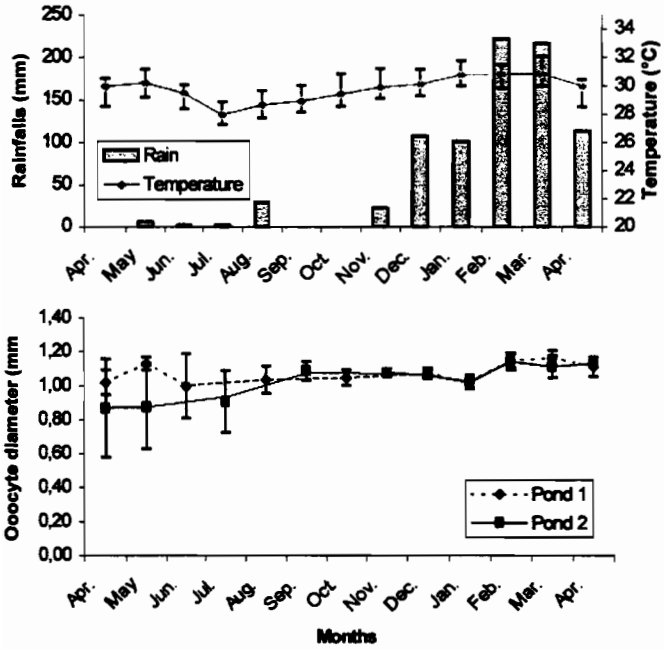
For each female the quality of ova was assessed by hatching rates obtained in replicated batches of 200-300 eggs fertilised with 0.2 ml of diluted sperm. Spermatozoa activation was obtained by addition of 10 ml freshwater. After 1min of gentle stirring, eggs were rinsed to remove excess milt and transferred for incubation in a plastic recipient containing 300 ml of standing water at ambient temperature (27-30°C). Hatching ended after 26-29 h of incubation, and the hatching rates were evaluated 35-40 h after fertilisation.

RESULTS

Seasonal changes in sexual maturity

The follow up carried out between April 1997 and April 1998 clearly showed that sexually mature *P. hypophthalmus* could be found all year round in the ponds of the Sukamandi station.

The yearly range of variation of mean oocyte diameters were between 1.00 - 1.16 mm, and 0.89 - 1.14 mm, in females of the two fish populations reared in 50 m^2 ponds (Fig. 1). A greater variability of oocyte diameter was observed between individuals in fish from the two ponds in June-July. The monthly proportion of females with post-vitellogenic oocytes of diameter greater than 1.00 mm was comprised between 57 and 100%, and 75 and 100%, in the two ponds respectively. The lowest values (57 and 75%) were observed in June-July. This period corresponded to a slight lowering of water temperature (reaching a minimal mean value of 27.9°C in July) and very low rainfalls (Fig. 1). It is to be noted that despite a total absence of rain, the proportion of mature females (oocyte diameter greater than 1.00 mm) was more than 85% in September-October 1997.



(*): no data.
Monthly mean temperature ± mean of daily minimum and maximum.

Figure 1: Seasonal evolution of rainfalls and mean water temperature, and mean (± sd) oocyte diameters of two groups of *P. hypophthalmus* females reared in ponds at the Sukamandi station between April 1997 and April 1998.

Similarly to females, fluent males were observed at anytime. The proportion of mature males (giving milt at stripping) was greater than 78% at all sampling dates, except in June-July. During these latter months, the proportion of fluent males dropped down to minimal values of 38 and 50% in the two ponds, respectively. However, several individuals were constantly observed at a fluent stage at all sampling performed.

Induced oocyte maturation and ovulation

In total, 54 *P. hypophthalmus* females were used for induced breeding trials between December 1996 and April 1998. The ovulation rate, quantity of egg collected by stripping and hatching percentages obtained for these fishes are presented in Table 1 as a function of the month at which trials were performed.

Successful induced ovulation could be obtained all year long and, among the 54 fish treated, only 7 did not respond to the hormonal treatment. The monthly means of fecundity and hatching rates varied between 63,000 and 226,000 eggs per kg body weight and between 47 and 97%, respectively. Despite the low number of fish examined in some months, it appeared clearly that ova and sperm of good quality could be obtained at all period of the year, as indicated by observation of individual hatching rates greater than 70% at anytime (Table 1).

Moreover, several females of the *P. hypophthalmus* broodstock were successfully induced to ovulate 2 to 4 times successively at a few months interval. As an example, ovulation was induced 4 times successively in the same female at 2-4 months interval between July 1997 and March 1998. No detrimental effect was found of such successive induced breeding treatments on the quantity and quality of eggs produced (Table 2).

DISCUSSION

The present study demonstrated that high proportions of fluent males and mature females with post-vitellogenic oocytes of modal diameter superior to 1.00 mm can be found at anytime in the *P. hypophthalmus* broodstock held in ponds at Sukamandi. As a consequence, successful induced breeding of this species using hormonal treatments could be performed regardless of season and

	January	February	March	April	May	June	July	August	September	October	November	December
Nb of female treated	6	2	8	8	2	2	2	0	2	3	2	17
Female BW (kg)	3.6 [3.5-3.7]	3.2 [3.1-3.3]	2.7 [2.3-4.0]	3.4 [2.3-4.2]	3.2 [3.0-3.4]	3.4 [3.2-3.5]	3.1 [2.6-3.6]	-	3.3 [3.1-3.5]	3.6 [3.4-3.8]	4.5 [3.3-5.8]	3.1 [2.2-4.5]
Oocyte diameter (mm)	1.15 [1.12-1.16]	1.14 [1.12-1.16]	1.19 [1.16-1.20]	1.15 [1.12-1.20]	1.17 [1.16-1.18]	1.18 [1.16-1.20]	1.16 [1.16-1.16]	-	1.14 [1.12-1.16]	1.12 [1.12-1.12]	1.08 [1.08-1.08]	1.08 [1.00-1.16]
Ovulation (%)	50	100	87.5	62.5	100	100	100	-	100	100	100 (*)	100 (*)
Relative fecundity (egg.kg-1) x 1000	201 [126-295] (3)	ND	180 [62-269] (6)	225 [125-322] (5)	95 (1)	ND	130 [108-153] (2)	-	226 [180-272] (2)	184 [148-231] (3)	63 (1)	87 [28-204] (9)
Hatching (%)	56.8 [30.2-83.3] (2)	ND	51.5 [21.3-81.6] (2)	67.2 [25.4-90.3] (3)	46.9 [21.6-72.2] (2)	95.5 (1)	96.9 [95.5-98.2] (2)	-	87.0 [83.0-91.0] (2)	79.7 (1)	74.0 (1)	88.0 [0.0-95.0] (8)

[ ] : Extreme values
( ) : Number of observations
(*) : Ovulation was partial in 1 of 2 females in November and in 5 of 17 females in December; data obtained for these 6 females are not included in analyses of fecundity and hatching rate.

Table 1: Monthly repartition of number of *P. hypophthalmus* female induced to ovulate by hormonal treatment (between Decembre 1996 and April 1998) at the Sukamandi station; with corresponding mean female body weight, mean oocyte diameter before treatment, percentage of ovulation, mean relative number of egg collected and mean hatching rate obtained.

Female n°	Date	Hormonal treatment	Body weight (g)	Initial oocyte diameter (mm)	Relative fecundity ($egg.kg^{-1}$) x 1000	Hatching rate (%)	fraction of deformed larvae (%)
1	01/07/97	Ovaprim	3600	1.16	153	98.2	3.7
1	04/10/97	Ovaprim	3750	1.12	175	3.0	-
1	02/12/97	hCG	3500	1.16	37	72.0	15.3
1	27/03/98	Ovaprim	4024	1.20	269	81.6	6.4
2	02/12/97	hCG	2600	1.08	204	84.0	9.5
2	14/04/98	Ovaprim	3160	1.18	322	90.3	3.2

Table 2: Number of egg collected, hatching rate and fraction of deformed larvae (deformed larvae/total hatched) after repeated induced ovulation in two *Pangasius hypophthalmus* females reared in pond at the Sukamandi station and individually identified by their PIT tag.

allowed an all year long production of fry.

It is the first time that a continuous sexual activity is reported in this species, either in wild or captive conditions. All previous reports on the breeding cycle of *P. hypophthalmus* in Thailand, Malaysia or Indonesia, concluded to a reproductive season limited to 4-8 months in the year (Potaros & Sitasit, 1976; Saidin & Othman, 1986; Hardjamulia *et al.*, 1981). Unfortunately the range of variation of environmental factors, such as temperature, was not given in these papers. Recently, a study carried out on broodstock cultivated in ponds or floating cages in Vietnam also showed a clearly marked spawning period, restricted to March-September for both males and females (see Cacot, 1999). In Vietnam (Mekong Delta), this period corresponded to the end of the dry season and the beginning of the rainy season, and was associated to the highest water temperature (28-31°C) and longest day length in the year.

In the present study, the continuous sexual maturity of *P. hypophthalmus* broodstock, during both the dry and rainy seasons, may have resulted to a large extent from rather stable conditions with a monthly mean water temperature constantly high (28-31°C) and a low variation of day length during the year. The range of variation of day length is narrower in Java Island (half an hour) than in the Mekong Delta (1h15).

The fact that ovulation could be successfully induced at 3, 2 and 4 months interval in the same *P. hypophthalmus* female, also shown a quick recovery of oogenesis of fish reared in the ponds. The absence of negative effects of such repeated treatments on fecundity or hatching rates indicated the completeness of oogenesis during these short periods. However, the minimal period of time between two successive induced ovulations remains to be assessed in this species. In the African catfish *Clarias gariepinus*, a same female can be stripped every 6-8 weeks when maintained at 25°C (Hogendoorn & Vismans, 1980). In *Heterobranchus longifilis*, an other African clariid, Nunez-Rodriguez *et al.* (1995) reported that a new reproductive cycle can be achieved in less than one month after an hCG-induced ovulation.

Although mature *P. hypophthalmus* males and females were found all year long at the Sukamandi station, the sexual maturity tended to be reduced in June-July. This was seen at the population level by a decrease in the proportion of fluent males and mature females, as well as a wider variation of oocyte diameters between individuals. It should be noted that the months of June-July corresponded to a slight drop down of water temperature and to the period of shortest day length in the year. However, some individuals were systematically at an advanced stage of sexual maturity (fluent males and females with post-vitellogenic oocytes) at each time they were observed during the monthly checking. No clear trend of cyclic changes in fish fecundity or hatching rates were observed in relation to season. Nevertheless, due to the low number of fish induced to spawn in certain months, supplementary observations remain necessary before a conclusion could be drawn for these traits. A continuous reproduction together with seasonal variations of fecundity were already reported for *H. longifilis* cultivated in lagoon enclosures (Legendre, 1986).

Complementary investigations are currently carried out at the Sukamandi station in order to assess more precisely the seasonal variations in the intensity of the sexual activity of *P. hypophthalmus* brooders.

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FIRST RESULTS ON GROWTH AND ARTIFICIAL PROPAGATION OF *PANGASIUS DJAMBAL* (SILURIFORMES, PANGASIIDAE) IN INDONESIA

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Abstract

Observations on growth rate and occurrence of first sexual maturity were done on a population of 75 *Pangasius djambal* originally caught in the wild, individually tagged, and reared in 200 m^2 ponds at a stocking density of 0.4-0.5 fish.m^{-2} at the Sukamandi research centre (Java). These fish, weighing initially between 190 and 1100 g were fed with a 35-40% crude protein pelleted feed and followed up during a period of 16 months. Two trials of induced ovulation were also carried out on females from a small stock of a dozen adult fish reared in floating cages at the Danau Teluk fish culture station (Sumatra).

Between June 1997 and October 1998, the mean body weight of fish increased from 555 g to 4162 g, which corresponded to a daily weight gain of 7.4 g.d^{-1}. The highest individual growth rate observed between two successive sampling dates was of 16.5 g.d^{-1}. The mean growth rate of *P. djambal* females (8.9 ± 2.2 g.d^{-1}) was significantly higher than that of males (6.3 ± 1.9 g.d^{-1}) and first sexual maturity occurred several months earlier in males than in females.

Oocyte maturation and ovulation were induced with two successive injections of Ovaprim at 8 h interval, corresponding to a total dose of 0.9 mL.kg^{-1}. Among four females treated, three ovulated and could be stripped. Mean hatching rates obtained after artificial fertilisation of eggs varied between 8 and 31%. After 27 days of age, the survival rate of larvae fed live *Artemia* nauplii then dried feed was 61%. No cannibalism was observed during the larval rearing which did not appear as a critical phase of the breeding cycle.

Although preliminary, these results confirm the great potential interest of using *P. djambal* for aquaculture. The induced breeding and larval rearing carried out for the first time in this species represent a breakthrough in the control of its biological cycle in captivity.

INTRODUCTION

Catfishes of the family Pangasiidae are of great economic importance in Indonesia. Although 13 pangasiid species were reported to belong to the local ichthyofauna (see Roberts & Vidthayanon, 1991 and Pouyaud *et al.*, 1999), their biology and potential for aquaculture remain largely unknown. Nowadays, *Pangasius hypophthalmus* Sauvage, 1878, initially introduced to Indonesia from Thailand in 1972, remains the only pangasiid catfish produced in aquaculture in this country.

Among the local pangasiids, *Pangasius djambal* Bleeker, 1846 is one of the fish species most appreciated by consumers in Sumatra and other Indonesian areas. It reaches large size with individual body weight of more than 20 kg (unpublished data). However, up to now its culture has not been possible due to the lack of fry. In this context, the control of its reproduction in captivity represents a strategic goal. Contrarily to the statement of Roberts and Vidthayanon (1991, p. 98), *P. djambal* has never been utilised in aquaculture so far. This confusion may result from the fact that "jambal" is a common name given in Indonesian language to several *Pangasius*.

As a part of a programme of evaluation of the potential of autochthonous pangasiid species for aquaculture, this paper presents a preliminary assessment of growth performance and sexual maturation of *P. djambal* in culture conditions. The first success of hormonal induced ovulation, artificial fertilisation and larval rearing is also reported for this species.

MATERIAL AND METHODS

Origin of fish

Between March and May 1997, a captive stock of *P. djambal* has been constituted. The wild fish, captured by fishermen in the Indra Giri River (Riau province, Sumatra), were firstly stocked in floating cages in the river area of capture and were then transferred by car (about 8 h transportation) to the Sungai Gelam station (DGF-Loka) in Jambi (Sumatra) where they were adapted to pond environment during 2 to 4 weeks. In June 1997, a part of these fish remained at the Sungai Gelam station, while 75 individuals weighing between 190 g and 1100 g (mean body weight of 555 g) were transferred by plane and car to the Sukamandi research centre of RIFF (Java Island) (about 15 h transportation) to serve as future experimental broodstock. Fish transportation was carried out in plastic bags, under oxygen atmosphere, using a specifically adapted packing technique avoiding bags to be cut by the sharp pectoral and dorsal spines of fish (Pouyaud & Sudarto, in prep.). This technique was fully satisfactory and 100% of the fish remained alive after transportation. Based on growth rate observed subsequently in culture conditions they were estimated to be 0.5-1.5 years old.

Besides the fish stocks constituted at Sukamandi and Sungai Gelam, a dozen of older *P. djambal* caught from the wild 4 years ago were held at the Danau Teluk fish culture station (Dinas Perikanan, UPPPU) in Jambi. By courtesy of Dinas Perikanan Provinsi Jambi, these adult fish of 2-5 kg body weight and about 5 years of age, never reproduced so far, were used for induced spawning trials. The species identification of these fish was confirmed after genetic analysis (isoenzymes polymorphism) of their descendants obtained from the artificial propagation reported here (Pouyaud, pers. comm.).

Rearing conditions and sampling

At Sukamandi, between June 1997 and July 1998, the fishes were placed in two 200 m^2 ponds in mixed culture with *Pangasius nasutus*, at a total stocking density of 0.4-0.5 fish.m^{-2}. In July 1998, the 75 *P. djambal* were grouped in a same 200 m^2 pond and reared in monoculture. They were fed during the whole period with a 35-40% crude protein pelleted feed, distributed two time per day and six days a week at a daily ration decreased progressively from 2% to 1% of fish biomass.

In January 1998, each fish was implanted with a P.I.T. tag (Fish Eagle ©) in order to allow individual identification. From this moment and then every three months, all individuals were anaesthetised in a bath of 0.3 $mL.L^{-1}$ phenoxy-2-ethanol, measured for their standard length, weighed using an electronic balance (± 1 g) and examined for their sexual maturity.

No external characteristics allowed for distinction of sexes. Males were identified only when sexually mature by emission of sperm upon hand-pressure onto the abdomen and females when oocytes could be sampled by intra-ovarian biopsy. Measurements of oocyte diameter were carried out using binocular microscope (x 25) equipped with a micrometer.

At Danau Teluk, the broodfish were reared together with brooders of *P. hypophthalmus* in floating cages implanted in a lake connected to the Batang Hari River system. They were fed with various commercial pelleted feeds containing 25 to 40% crude protein. The site is characterised by important seasonal changes in water depth (± 8 m) and water quality, with periods of low oxygen concentration (Rusli Yulidar, pers. comm.).

Artificial propagation

Two trials of induced spawning were carried out at Danau Teluk in November 1997 and February 1998. Three females in the first case and one in the second, found with oocytes at an advanced stage of vitellogenesis after intra-ovarian biopsy, received hormonal treatment to induce ovulation. In November, the females were treated with two successive injections of Ovaprim done at 8 h interval and respective doses of 0.3 and 0.6 $mL.kg^{-1}$. In February, the female received two priming injections of hCG (500 $IU.kg^{-1}$) at 24 h interval and, 24 h after the last hCG injection, the same treatment with Ovaprim as in November was applied. At each injection time and then every 2-3 h after the second Ovaprim injection (in November fish were checked only 8 h after second injection), a sample of oocytes was taken by intra-ovarian biopsy to follow the evolution of oocyte diameter and maturation. The position and state of germinal vesicle was assessed after fixation of a sub-sample of oocytes in Serra's solution (60% ethanol, 30% formalin, 10% acetic acid). The males received a single Ovaprim injection (0.4 $mL.kg^{-1}$) given at the same time as the second Ovaprim injection of females. The sperm collected by stripping was diluted in a 9 $g.l^{-1}$ NaCl solution

in order to prevent its activation due to possible mix with urine. After ovulation, the stripped eggs were fertilised with sperm mixed from 3 males in November and sperm from one male in February. The procedures of artificial fertilisation, egg incubation and estimation of hatching percentages were the same as those previously described by Legendre (1986) for the African catfish, *Heterobranchus longifilis*.

On the day of hatching, a group of 350 larvae was transferred from Danau Teluk to the Sukamandi research center. During the first 3 weeks, they were reared in two 40 L aquarium in stagnant water changed every day by 50%, and fed in excess with live *Artemia* nauplii. They were then transferred to 80 L aquarium and progressively weaned to a 40% crude protein dried feed distributed ad libitum. Every week the fry were totally counted and twenty fish individually weighed (± 0.1 mg).

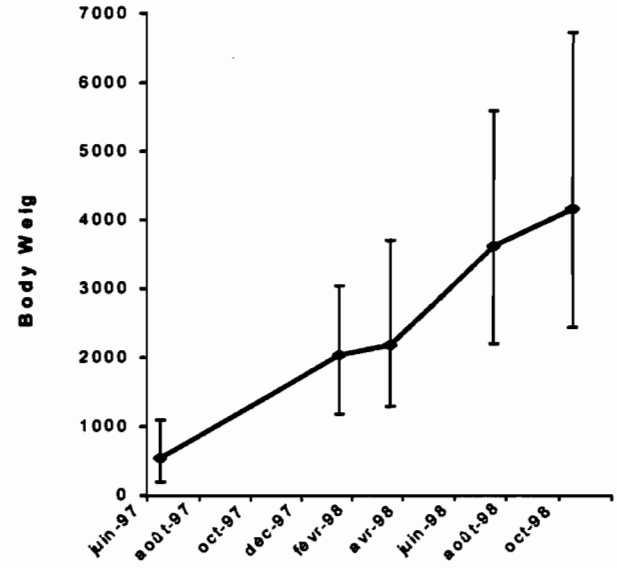
RESULTS

Survival and growth rates

The survival rate at the end of the whole period of observation was still of 100%.

Between each sampling dates, the observed growth rates were equivalent for the *P. djambal* held in the two ponds, therefore the data were pooled for presentation. The corresponding growth curve for the 16-months period of observation is given in Figure 1. During this period, from June 1997 to October 1998, the mean body weight increased from 555 g to 4162 g which corresponded to a daily weight gain of 7.4 $g.d^{-1}$. A mean growth rate of 6.3 $g.d^{-1}$ was also observed over a 5 months period for *P. djambal* in the ponds of the DGF-Loka station in Jambi (Maskur, pers. comm.). It should be noticed for comparison that the mean growth rate of *Pangasius hypophthalmus* observed in similar culture conditions at the Sukamandi station is generally around 5 $g.d^{-1}$ (unpublished data).

Electronic tagging of all fishes allowed to estimate growth variations between individuals. The minimal and maximal individual growth rates observed during the 9 months period from January to October 1998 were of 2.6 $g.d^{-1}$ and 12.8 $g.d^{-1}$, respectively. The highest individual growth rates found between two successive sampling dates were 14.9 $g.d^{-1}$ in male and 16.5 $g.d^{-1}$ in female fish.



Vertical bars indicate range between the smallest and biggest individuals within the population of 75 fish.

Figure 1: Growth of *Pangasius djambal* cultivated in pond at a stocking density of 0.4 $fish.m^{-2}$ at the Sukamandi station..

These results illustrate the remarkable growth potential of this species.

Individual identification of all fishes also permitted to test for a possible growth difference related to sex. Individual daily weight gain calculated between January and October 1998 indicated a significantly faster growth in females (8.9 ± 2.2 $g.d^{-1}$) than in males (6.3 ± 1.9 $g.d^{-1}$) (Table 1).

First sexual maturity

In October 1998, a total of 38 males and 19 females could be identified in the population of 75 fish, while sex of 18 individuals remained undetermined.

Sixteen fluent males were found as early as January 1998 in the population at an estimated age of about 1-2 years. Supplementary males that could be identified by sperm emission were found at each sampling examination: 3 in March, 12 in July and 7 in October. On this latter month, 36 males (95%) showed abundant sperm production while only 2 were not fluent, indicating an increased sexual activity at this period. By contrast the number of females that could be identified for the first time by intra-ovarian biopsy showed a slower evolution: 4 in January, 4 in March, 4 in July and 7 in October. It is only during this latter sampling that some females (21%) were found with developing oocytes of 0.68-1.28 mm maximum diameter. In

	MALE (n = 38)	FEMALE (n = 19)
Body weight at tagging (g) (08/01/1998)	2148 ± 428 [a] [1420 – 2972]	2159 ± 465 [a] [1535 – 3040]
Final body weight (g) (23/10/1998)	3929 ± 760 [a] [2436 – 5758]	4622 ± 983 [b] [2862 – 6720]
Daily weight gain ($g.d^{-1}$) (between January and October 1998)	6.3 ± 1.9 [a] [2.6 – 10.4]	8.9 ± 2.2 [b] [3.2 – 12.8]

Figures with same superscripts in the same line are not significantly different ($p<0.05$).
Mean ± sd ; [-] : extreme values

Table 1: Growth of males and females of *Pangasius djambal* in pond.

all other situations, oocyte diameter never exceeded 0.2-0.3 mm.

These results clearly indicate that sexual maturity occurs several months earlier in males than in females. If the development of gonads is at the expense of somatic tissue, then the early maturity of male could be responsible, at least in part, for their lower growth rate in comparison to female. An earlier sexual maturity of males was also observed in cultured stocks of *Pangasius hypophthalmus*. In this species, males were already fully mature (presence of intra-testicular sperm) at the age of 10 months and a mean body weight of 472 ± 78 g, while the first females with ovaries containing post-vitellogenic oocytes were observed at an age of 19 months and a mean body weight of 2249 ± 279 g (unpublished data).

Artificial propagation

From the broodfish stocked in floating cages at Danau Teluk, Three females in November 1997 and one in February 1998 were found with oocytes at an advanced stage of vitellogenesis after intra-ovarian biopsy. The mean oocyte diameter before hormonal treatment is given for each female in Table 2. After Ovaprim injections, three of these females ovulated and could be stripped. Mean ova diameter was of 1.8, 1.9 and 1.8 mm for the three females, respectively. Examination of oocytes sampled by intra-ovarian biopsy and fixed in Serra's solution after the second Ovaprim injection, indicated that only oocytes of size equal or superior to 1.55-1.60 mm reached the stage of germinal vesicle breakdown and ovulated. As also reported for *Pangasius bocourti* (Cacot, 1999), oocytes smaller than this threshold did not respond to hormonal treatment in *P. djambal*. As a matter of fact, the smallest diameter of ova found within the population of ova collected by stripping was of 1.64, 1.68 and 1.64 mm for the three females, respectively. Oocytes of the female that did not ovulate were the smallest compared to other females used (Table 2) and probably not fully achieved their vitellogenesis at the moment of experiment.

The weight of eggs collected could not be determined due to absence of appropriate balance, it was estimated to approximately 20 g, 200 g and 6 g for the three females, respectively. Mean hatching rates obtained after artificial fertilisation varied between 8 and 31% (Table 2). Depending on females and experiments, this rather low egg quality and quantity could be attributed either to inappropriate latency time between injection and egg collection, incomplete gonad development or unsuitable rearing conditions of broodstocks. Obviously, further investigations have to be done to define seasonal variations of sexual activity, and optimal conditions for broodstock management and induced breeding in this species.

Fry produced in November 1997 were reared at the Danau Teluk station but all died after 3-6 weeks of age as a result of disease outbreak due to *Ichthyophthirius* infection (Rusli Yulidar, pers. comm.). The larvae obtained in February 1998 were reared in aquarium after transfer at the Sukamandi station where first observations on their development (Table 3) and behaviour could be performed. After 27 days of age, the survival rate of fry was of 61%. It was still of 60% after two months of rearing. In contrast to the situation prevailing in *P. hypophthalmus* (Subagja *et al.*, 1999) and similarly to what is known from *P. bocourti* (Hung *et al.* 1999), no cannibalism was observed in *P. djambal* during the larval rearing which did not appear as a critical phase of the breeding cycle.

Several other biological characteristics of *P. djambal*, such as size of ova and larvae (Table 3), appear to be very similar to those of *P. bocourti* (see Cacot, 1999; Hung *et al.*, 1999). These two species are genetically closely related (Pouyaud *et al.*, 1999) and similar morphologically, differing mostly by the number of rakers on first gill arch

Date	Water temperature (°C)	Female body weight (g)	Initial oocyte diameter (mm)	Latency time after 2nd Ovaprim injection	Number of egg collected	Hatching rate (%)
November 97	30-31	4300	1.58	No ovulation		
November 97	30-31	4200	1.68	12	-	22.0
November 97	30-31	4100	1.84	8	-	7.8
February 98	30-32	1900	1.66	6	5200	31.0

Table 2: Ovulation success, number of egg collected and hatching rate obtained during the first trials of induced-ovulation of *Pangasius djambal*.

Mean ova diameter before fertilisation	1.8 ± 0.1 mm (a)
Mean weight of ova	2.95 mg (b)
Range of incubation duration at 27-30°C	29-36 hours
Mean total length of larvae at hatching	4.7 ± 0.2 mm (a)
Duration of yolk sac absorption at 28-29°C	3 days
Mean total length of larvae at first feeding	8.6 ± 0.3 mm (a)
Mean body weight of larvae at first feeding	4.1 mg (b)
Mean body weight of fry at 27 days of age	607 ± 304 mg (a)
Survival rate after 27 days from hatching	61%

(a) Mean ± sd ; (b) Mean from global weight of 100 eggs and 10 larvae

Table 3: Size of ova, duration of egg incubation and growth and survival of *Pangasius djambal* fry obtained from induced-breeding trial of February 1998.

(Roberts & Vidthayanon, 1991). Both of them possess the biggest eggs and larvae reported so far among pangasiid species. Induced breeding of a local pangasiid, called "*Pangasius pangasius*", was previously reported in Sumatra (Indonesia) by Meenakarn, 1986 and Arifin, 1987. From the results of the Catfish Asia project (Pouyaud *et al.*, 1999, and unpublished data), it is now confirmed that this fish was misidentified and could not be *P. pangasius*. However, as the specimens used by these authors or descendants of these fishes could not be found for identification, it is impossible to precise the correct name of the species they used. It seems clear, however, that it could not be *P. djambal* when looking at the biological data given in these two papers.

Meenakarn (1986) reported fecundity of 100,000-130,000 egg per female kg and an incubation period of 40-44 h at 27-30°C. Such a fecundity with the eggs of *P. djambal* (mean egg weight of about 3 mg) would represent an unrealistic gonado-somatic index of 30-40%. Also, the duration of egg incubation observed in *P. djambal* was shorter at a same temperature (Table 3). Arifin (1987) reported that the size of ova of "*P. pangasius*" was 1.4-1.6 mm for a weight of 2.0-2.2 mg and that cannibalism displayed by the larvae could explain their low survival rate. However, from our data, *Pangasius djambal* presents bigger ova (by 30% in weight) and the larvae do not show cannibalistic behaviour.

CONCLUSION

The good adaptation of *Pangasius djambal* to pond environment, as well as its resistance to handling, high growth rate and ability to become sexually mature in captivity, confirm the great potential interest of this species for aquaculture. So far, the culture of this catfish which is among the *Pangasius* most appreciated by consumers in Indonesia, has not been possible due to lack of fry.

The feasibility of fry production from captive broodstock has been demonstrated in this study. Although preliminary, the present results represent an important breakthrough: it is the first time that induced ovulation, artificial fertilisation and larval rearing of *P. djambal* are performed successfully. The limited number of mature broodfish available

on fish farms is currently the main constraint for the start of its production in aquaculture.

ACKNOWLEDGEMENT

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PRELIMINARY RESULTS ON THE INDUCED SPAWNING OF TWO CATFISH SPECIES, *PANGASIUS CONCHOPHILUS* AND *PANGASIUS* SP1, IN THE MEKONG DELTA, VIETNAM

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Abstract

Pangasius conchophilus and *Pangasius* sp1 can reach full sexual maturity in the confined conditions of pond culture in the Mekong Delta. Three females of each species were artificially induced to spawn using three successive injections of human chorionic gonadotropin (hCG). After the collection of ova by stripping and their artificial fertilisation, the fertilisation rates were of 82 to 94% in *P. conchophilus* and 13 to 88% in *Pangasius* sp1. Except in one case, more than 90% of fertilised egg hatched in both species. These preliminary results give promising prospects for a future mass production of fry of these two catfishes and for their culture in the Mekong Delta.

INTRODUCTION

Pangasius conchophilus and *Pangasius* sp1 (the specific identification of this fish is still uncertain, see Pouyaud *et al.*, 1999) are fish species of high commercial value that are raised in floating cages in the Mekong River in Vietnam. They have really delicious taste in comparison to other catfish. At present, the aquaculture production of these two species is still low in comparison to other pangasiids, such as *Pangasius hypophthalmus* and *Pangasius bocourti*, since the quantity of seeds collected from the nature is insufficient. Therefore, the objectives of the present study are: (i) identifying appropriate hormonal-induced breeding treatments for these species and (ii) determining an appropriate methodology of fingerling nursing in order to supply gradually fish seed to meet the catfish fingerling demand of the farmers.

MATERIALS AND METHOD

Broodfish culture

Broodfishes were reared in a pond of 560 m^2 and 1.2 m deep, in which the water was exchanged two times per month during the high tide. Water quality was determined weekly including temperature: 26-33°C; pH: 6.5-7.5; transparency: 28-52 cm and DO: 1.6-8.5 $mg.L^{-1}$.

Broodfishes were estimated to be about two years old with an individual body weight of 0.4-1.5 kg (*P. conchophilus*) and 0.7-1.9 kg (*P.* sp1). They were collected from the nature and previously cultured in cage before being transferred in pond at the Can Tho University on August 1st 1997. Both species, including males and females, were reared in the same pond with a proportion of 60% of *P.* sp1 and 40% of *P. conchophilus*. The stocking density was about 1 fish for 3 m^2.

Broodfishes were fed artificial feed containing 90% of fishmeal and 10% of cassava starch with a weekly supplementation of 3% soybean oil and 0.5% vitamin premix (Vimevit). The daily feeding rate was about 2-3% of total fish biomass. The feed was passed through a mincer to form small pellets and distributed to the fish twice a day. The chemical composition of the diet was the following:

- Crude protein 41.16%
- Crude lipid 3.43%
- Mineral 34.86%
- Moisture 12.31%

Spawning

Broodfish were weighed monthly to adjust the feeding rate. Oocyte samples were taken monthly to measure the diameter. Only broodfishes with an

advanced stage of maturity were selected for the artificial spawning: females with large oocytes and males with milt.

Fish received injections of a human chorionic hormone diluted in 9‰ NaCl solution. The fertilised eggs were spread on substrates which were then poured in an incubation tank with slight water current. The fertilisation rate was determined at the gastrula stage of embryo.

RESULTS

The mean oocyte diameter of *P. conchophilus* (Fig. 1) increased from April and reaches its maximal value in July. However, there was a small proportion of females having oocytes ready for spawning in May, indicating that the reproduction time for the species started in May.

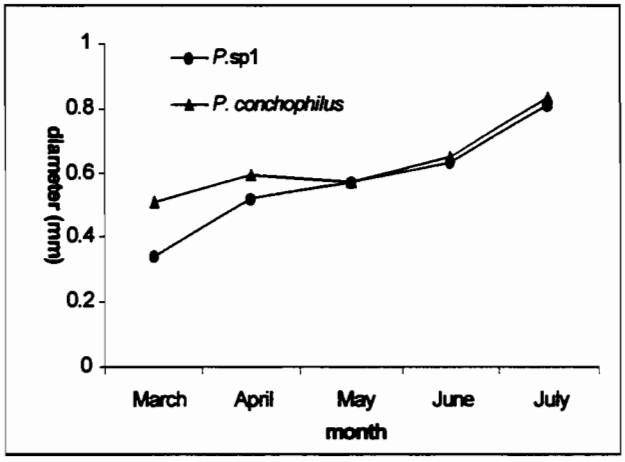

Figure 1: Evolution of oocyte diameter of *Pangasius conchophilus* and *P.* p1 from March to July 1998.

The ratio of matured fish, determined in May, was 60% (male) and 18.7% (female). In June and July, the ratios were higher (Table 1). That indicated that June and July are the two main spawning months in *P. conchophilus*.

The evolution of oocytes diameter of *Pangasius* sp1 had the same trend as in *P. conchophilus* (Fig. 1). The spawning season of the species started in May. The ratio of matured fish determined in May, June and July has a trend to increase from May to July (Table 1).

Therefore, both species have the same trend of maturation and spawning time, which are similar to that observed in *Pangasius hypophthalmus* but differ from *P. bocourti*, from March to June.

	March	April	May	June	July
P. conchophilus					
Female	0.00	22.2	18.7	26.7	65.0
Male	4.30	46.2	60.0	64.3	84.6
P. sp1					
Female	16.7	13.3	33.3	47.1	60.0
Male	16.0	62.9	52.0	50.0	36.8

Table 1: The maturation ratio (%) of *Pangasius conchophilus* and *P.* sp1 from March to July 1998.

The sexual maturation of female was indicated by external criteria such as soft swollen abdomen and a protuberant genital papilla. However, for the inducement of spawning in female, multi-injections of hormone with the interval of 8-24 hours are used. The preliminary dosage of 500 UI hCG.kg^{-1} of female body weigh is used, 2-3 times successively at 24 hours interval. Then another dosage is applied to induce ovulation, a first time with 1000-1500 UI hCG and a second time with 2500 UI hCG.kg^{-1} of body weight within 8-10 hours after the first one (see Cacot, 1999). Before having the final and decisive hormonal injection, at least seventy percent of oocytes had a diameter of 1.12-1.20 mm in *Pangasius* sp1 and 0.96-1.04 mm in *P. conchophilus*. For the male fish, milt was stripped by a soft press on the abdomen near the genital pore. A single dosage of 2,000 UI HCG.kg^{-1} of male body weigh is injected at the same time as the decisive ovulatory injection for the female.

Oocytes were ovulated after 8-10 hours since the decisive dosage injection (*Pangasius conchophilus*) and 11-12 hours (*Pangasius* sp1) at the temperature of 28-30°C. The dry fertilisation was applied for both species. The results of induced spawning are shown in Table 2. For *P. conchophilus*, all of the three inducements of spawning gave quite high fertilisation rates, ranging from 81% to 94% and the hatching rates, ranging from 92 to 95%. It is clear that the induced spawning of *P. conchophilus* is realistic. The spawning technique is similar to that used in *P. hypophthalmus*.

For *Pangasius* sp1, the first induced spawning gave a low fertilisation rate (13%) and no hatched larvae. However, in the second and third induced spawning the fertilisation and hatching rates were quite high (Table 2). It is possible that in the first spawning, oocytes were not yet completely matured at the stripping time. Thus, fecundity was

Fish species	Date	Temp. (°C)	Body weight (kg)	Total length (cm)	Number of egg collected	Fecundity (egg.kg^{-1})	Fertilisation rate (%)	Hatching rate (%)
P. conchophilus	30/04/98	29-31	1.6	49.5	42,000	26,250	93.9 ± 1.1	92.9 ± 0.6
	25/05/98	29-31	0.85	39.0	28,800	32,882	85.3 ± 2.2	92.6 ± 2.5
	25/05/98	29-31	0.7	37.0	18,000	25,714	81.7 ± 1.1	96.5 ± 2.0
P. sp1	19/03/98	29-31	1.7	48.5	14,400	8,470	13.3	-
	25/05/98	29-31	2.0	52.0	119,040	59,520	88.0 ± 0.1	91.5 ± 1.2
	25/05/98	29-31	1.15	46.5	16,740	14,556	86.5 ± 1.8	90.5 ± 1.3

Table 2: Ovulation success, number of egg collected and hatching rate after the first trials of induced-ovulation of *Pangasius conchophilus* and *P.* sp1.

very low in comparison to the second and third spawning.

CONCLUSION

In conclusion, the two species have the same breeding season from May to July. The technique to induce spawning of the two species studied is similar to that used for *P. hypophthalmus*.

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EFFECT OF EGGS INCUBATION TECHNIQUE ON HATCHING RATE, HATCHING KINETIC AND SURVIVAL OF LARVAE IN THE ASIAN CATFISH *PANGASIUS HYPOPHTHALMUS* (SILURIFORMES, PANGASIIDAE)

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Abstract

The influence of incubation system on embryos development and subsequent survival of larvae was evaluated on eggs from two *P. hypophthalmus* females. All tests were performed at a same temperature of 28.5-29.5°C, given by the water of a re-circulating system to which all incubation devices were connected. The following situations were tested: 1- Happa (adhesive eggs); 2- Floating screen net (adhesive eggs); 3- McDonald type incubator (stickiness of eggs being suppressed with a clay suspension); 4- Plastic basket with water from the re-circulating system (stickiness of eggs being suppressed with a clay suspension); 5- Plastic basket with water from the re-circulating system (adhesive eggs); 6- Plastic basket with mineral water (adhesive eggs). No significant difference was found in hatching rates whatever the conditions of egg incubation. The development time and hatching kinetic were very similar in all systems; at 28.5-29.5°C, the first hatched larvae were observed between 19 and 21h post-fertilisation and the duration of the hatching period lasted 6 to 8 h. However, hatching tended to occur slightly earlier in the McDonald type incubator, probably as a consequence of mechanical agitation of eggs. Up to the age of 4 days, no significant difference was found in the survival of larvae as a function of the type of incubator from which they were issued. All together, the results indicate that, when properly managed, the incubation methods can hardly be responsible for the variability of hatching rates or survival of larvae often observed in different reproduction trials or from farm to farm.

INTRODUCTION

Pangasius hypophthalmus (Sauvage, 1878) (senior synonym of *P. sutchi*; Roberts & Vidthayanon, 1991) was introduced to Indonesia in 1972. Its hormonal induced-breeding was reported for the first time in this country by Hardjamulia *et al.* (1981). Since then the fry production has been developed by fish farmers.

In fish, the incubation techniques developed depend on the specific characteristics and requirement of eggs (adhesive or not, buoyant or not) (Woynarovitch & Horvath, 1980; Legendre *et al.*, 1996). The eggs of *P. hypophthalmus* are negatively buoyant, spherical or slightly oval in shape and become sticky after contact with water. They adhere to each other or to any substrate via a sticky mucous coating covering all their surface. Due to these characteristics, several incubation systems were used for this species by fish farmers in Indonesia: egg were incubated in monolayer in stagnant or running water, or in funnels (McDonald or Zuger jars) after suppression of stickiness by covering their surface with clay particles. McDonald type incubators are round-bottomed containers in which a downward water flow allows the eggs to rotate gently in the water column. The procedures of egg incubation vary largely from one farm to another. In some production systems, using large-sized McDonald or Zuger Jars, the duration of incubation tended apparently to be shorter than in stagnant water incubation systems. However no clear conclusions could be made from these observations because they concerned eggs from different individuals incubated in different conditions of water temperature. The question of the influence of an earlier hatching time on subsequent survival of

larvae was also raised.

The aim of this study was to compare the duration of the incubation period, hatching kinetic, hatching rate and early survival of larvae after incubation of the same *P. hypophthalmus* eggs in various conditions and systems.

MATERIAL AND METHODS

Experiments on incubation of *P. hypophthalmus* eggs were carried out at the Sukamandi Station of the Research Institute for Freshwater Fisheries (West Java, Indonesia). The *P. hypophthalmus* brooders used in these experiments were 4-years-old and 3.0 to 4.0 kg individual body weight. The procedures of induced breeding, gametes management and artificial fertilisation of eggs corresponded to those described by Legendre *et al.* (1999). Oocyte maturation and ovulation were induced with two successive Ovaprim® injections of 0.3 $ml.kg^{-1}$ female BW and 0.6 $ml.kg^{-1}$ given at 8 h interval. The males received a single Ovaprim® injection of 0.3-0.4 $ml.kg^{-1}$ applied at the moment of first injection of females.

In a **first experiment**, ova from two different females were stripped after induced ovulation and immediately fertilised with sperm pooled from 4 different males. The sperm was diluted directly in a 0.9% NaCl solution (dilution rate of 1/5) at stripping, then preserved at 5°C before use. For each female, batches of 200-300 eggs were fertilised with 0.2 ml of diluted sperm. Spermatozoa activation was obtained by addition of 10 ml freshwater. After 1 min of gentle stirring, eggs were rinsed to remove excess milt and transferred for incubation in the different situations tested. In some treatments, clay was used to suppress egg stickiness. In this case, after 1 min of gametes contact as indicated above, one spoon of clay suspension was added to the fertilisation medium for one supplementary minute of gentle stirring, before rinsing and transferring eggs to incubators.

Six incubation treatments, involving mini-incubators of 0.3-0.5 L each, were tested (3 replications per treatment) on eggs from the two different females. As all incubators were connected, immersed or placed to float in a same re-circulating water system, the temperature during incubation was strictly equivalent in all treatments (28.5-29.5°C). The incubation methods tested were the followings:

1. Happa made of fine mesh net placed in the tanks of the re-circulating system (adhesive eggs, no agitation of eggs, unrestricted water exchange).
2. Floating screen net placed in the tanks of the re-circulating system (adhesive eggs, no agitation of eggs, water exchange restricted to bottom).
3. McDonald type incubator connected to the re-circulating water system (stickiness of eggs suppressed with a clay suspension, agitation of eggs, unrestricted water exchange).
4. Plastic box filled with water from the re-circulating system and floating on it (stickiness of eggs suppressed with a clay suspension, no agitation of eggs, absence of water exchange).
5. Plastic box filled with water from the re-circulating system and floating on it (adhesive eggs, no agitation of eggs, absence of water exchange).
6. Plastic box filled with spring water and placed to float on water of the re-circulating system (adhesive eggs, no agitation of eggs, absence of water exchange).

All together, 36 groups of eggs were followed (6 treatments x 3 replicates x 2 females). The water quality in the different incubation systems was followed regularly during the experiment, pH varied between 7.1 to 8.5 and dissolved oxygen was in all cases higher than 5 $mg.L^{-1}$. Ammonia and nitrite concentrations were determined using Aquaquant® kits (Merck 14423, 14424) and ranged between 0,2 to 0,4 $mg.L^{-1}$, and between 0,012 to 0,05 $mg.L^{-1}$, respectively.

Hatching kinetics were followed up on two batches of eggs per female and per incubation treatment. In each situation, between the moment at which the first hatching was observed and the end of the hatching period, new hatched larvae were counted every hour, removed from the incubator and placed in a separate recipient.

After hatching has been completed in each group of eggs, all hatched larvae were counted and hatching percentages were determined from the initial number of ova used. For each female, the weight of one ova was determined by weighing about 0.5 g of ova collected after stripping and counting them after fixation in formalin 5% (two replications per female). Individual weight of ova was 0.64 mg in one female and 0.69 mg in the other. From these data, the number of ova used in each incubation trial was estimated by weighing them ($p \pm 0.1$ mg) before fertilisation.

In order to test for a possible effect of incubation method on subsequent survival of larvae, three replicated groups of 30 larvae issued from the incubation treatments n°1, 2, 3, and 5 were followed up to 4-days of age. Each group of 30 larvae was reared in spring water in a 300 ml plastic container and fed in excess with *Artemia* nauplii starting from 36 hours after hatching. The feeding frequency was of 8 meals per day at 09:00, 12:00, 15:00, 18:00, 21:00, 24:00, 03:00 and 06:00. Water of each plastic container was changed four times per day and dead larvae were removed and counted at the same time. On the last day of experiment (day 4), all the remaining larvae were individually counted for calculation of actual survival rate.

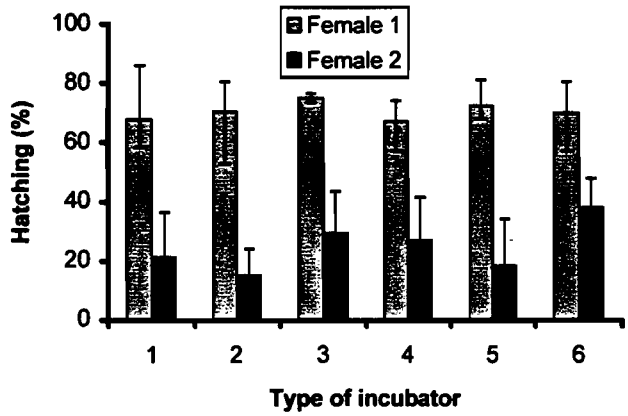
The hatching percentages and the survival rates of 4-days-old larvae obtained as a function of the method used for egg incubation were compared using two way ANOVA (incubation treatment x female). When necessary, angular transformation of data was carried out in order to stabilise the residual variance.

A **complementary experiment** was carried out to in conditions closer to those generally observed in *Pangasius* hatcheries. It aimed also to assess more accurately the possible effect of egg agitation on the duration of the incubation period. For this, using the eggs of two supplementary females, three different situations were compared: incubation in happas (60 x 60 x 80 cm, no egg agitation), incubation in mini-McDonald type incubators (0.5 L in volume, egg maintained in movement with a water flow of 15 $mL.s^{-1}$ as in first experiment) and incubation in bigger McDonald type incubators (10 L in volume, egg maintained in movement with a water flow of 45 $mL.s^{-1}$). About 200-300 eggs were placed in the mini-McDonald incubators while the number of eggs was about 50 times greater in the happas and big McDonald incubators. Each treatment was tested with 3 replications per female. The procedures of induced breeding and egg fertilisation were equivalent to those described for the first experiment. For incubation carried out in McDonald type incubators, clay was used to suppress egg stickiness as previously indicated. All the incubators were implemented or connected in a same re-circulating water system, allowing a same water temperature (28.5–29.5°C) in all situations.

RESULTS AND DISCUSSION

Hatching rates

The hatching rates obtained as a function of the different situations and systems of egg incubation tested are presented in Figure 1 for the two females used in the first experiment. A clear difference was found in the egg quality of the two females, mean hatching rates ranging from 67 to 75% and from 15 to 38% in female 1 and 2, respectively. By contrast, hatching rates did not significantly differ between treatments. Thus limitation of water exchange, treatment with clay to avoid stickiness or gentle agitation of eggs did not affect embryos survival in the conditions applied in this study.



Vertical bars indicate range between replicates.

Figure 1: Effects of the type of incubator used on the hatching rate of eggs from two *P. hypophthalmus* females. The conditions of egg incubation were the followings : 1- Happa (adhesive eggs); 2- Floating screen net (adhesive eggs); 3- McDonald type (stickiness of eggs being suppressed with a clay suspension); 4- Plastic box with water from the re-circulating system (stickiness of eggs being suppressed with a clay suspension); 5- Plastic box with water from the re-circulating system (adhesive eggs); 6- Plastic box with mineral water (adhesive eggs).

In the supplementary experiment, in which larger quantities of eggs were involved, fungus (*Saprolegnia*) started to develop on the eggs a few hours before hatching. However this fungal attack was much stronger on the eggs incubated in happas than on the ones covered with clay particles and slightly agitated in the McDonald type incubators. Fungus development remained very limited in all situations tested in the first experiment.

Development time and hatching kinetic

The development time of fish eggs is temperature dependent (Woynarovitch & Horvath, 1980; Mac Intosh & Little, 1995). It was therefore of prime importance to compare the development time of *P. hypophthalmus* eggs placed in the different incubation systems at a same temperature. This was done by implementing or connecting all the incubation systems tested in a same re-circulating water system.

The hatching kinetic was followed on two replicated groups of egg from two different females, in each of the various incubation situations tested (see Fig. 2 for examples). From these observations, the time lapse between fertilisation and first hatching or 50% hatching, and the total duration of hatching (between the first and last eggs to hatch) were determined. These data are presented in Table 1 for the eggs of the two females used in the first experiment. In all cases, the development time of *P. hypophthalmus* eggs was very similar, the curves of hatching kinetic being superposed. At 28.5-29.5°C, the first hatched larvae were observed between 19 and 21 h post-fertilisation and the duration of the hatching period lasted 6 to 8 h. The suppression of egg stickiness by covering them with clay particles did not affect embryos development nor development time. The only detected difference was for the eggs incubated in the McDonald type incubator which hatched 1 or 2 h earlier than in other incubation systems, in one female but not in the other (Fig. 2, Table 1).

In the complementary experiment carried with the eggs of two other females, the development time was also faster by about 2 h in the McDonald type incubators than in happas. This earlier hatching in

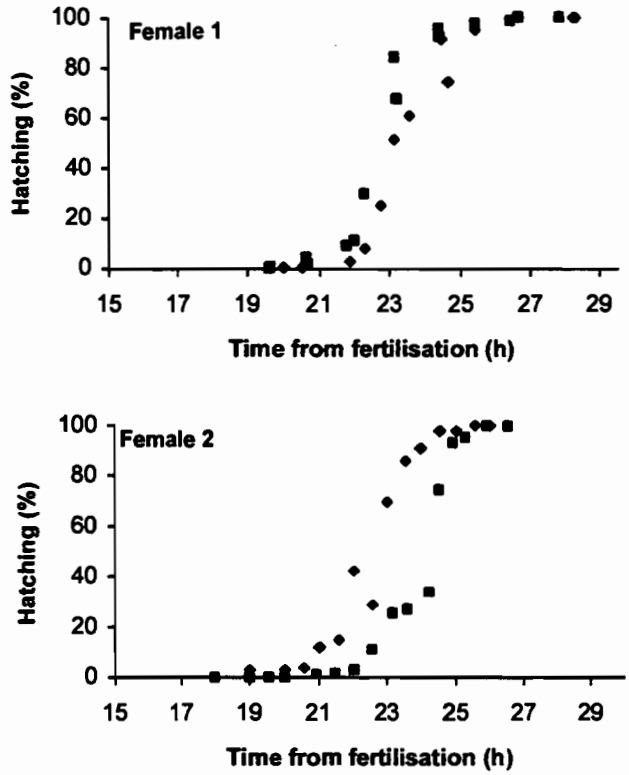


Figure 2: Hatching kinetic of eggs from two *P. hypophthalmus* females incubated in McDonald type incubator (♦) or in plastic box with stagnant water (■) (temperature: 28.5-29.5°C). Observations plotted from two replicates per treatment and female.

funnels was assumed to be a consequence of the slight mechanical agitation of eggs. By contrast, no difference was found between the "mini" and the bigger McDonald incubators. Therefore the mini system could be considered as representative of the bigger incubators generally used in hatcheries. Rana (1986) reported large variations in the embryos

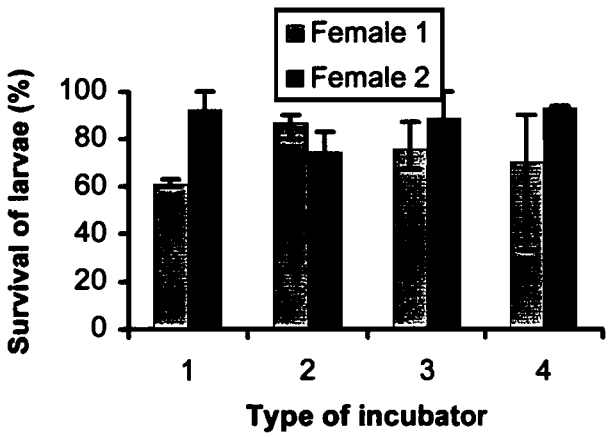
Type of incubator	Female 1			Female 2		
	First hatching (h post-fertilisation)	50% hatching (h post-fertilisation)	Total duration of hatching period (h)	First hatching (h post-fertilisation)	50% hatching (h post-fertilisation)	Total duration of hatching period (h)
1	20	24	7	21	24	6
2	20	24	8	20	24	8
3	20	23	8	19	23	7
4	20	24	8	21	25	8
5	20	23	6	21	24	6
6	20	23	8	21	24	6

Table 1: Development time and duration of hatching period in eggs of two *P. hypophthalmus* females incubated in the following conditions and systems: 1- Happa (adhesive eggs); 2- Floating screen net (adhesive eggs); 3- McDonald type (stickiness of eggs being suppressed with a clay suspension); 4- Plastic box with water from the re-circulating system (stickiness of eggs being suppressed with a clay suspension); 5- Plastic box with water from the re-circulating system (adhesive eggs); 6- Plastic box with mineral water (adhesive eggs). (Observations from two replicates per treatment and female).

survival and development time of *Oreochromis niloticus* and *O. mossambicus* eggs as a function of the shape of the incubator used. The development time of these species was slower in round-bottomed containers than in conical ones (90-102 h compared with 48-72 h).

Survival rate of larvae

After 4 days of rearing, the mean survival rate of *P. hypophthalmus* larvae issued from the different incubation systems tested in the first experiment varied between 61 and 87% in one female and between 74 and 93% in the other (Figure 3).



Vertical bars indicate range between replicates.

Figure 3: Survival rate of *P. hypophthalmus* larvae obtained from eggs of two different females incubated in the following conditions : 1- Happa (adhesive eggs); 2- Floating screen net (adhesive eggs); 3- McDonald type (stickiness of eggs being suppressed with a clay suspension); 4- Plastic box with water from the re-circulating system (adhesive eggs).

No significant effect of incubation techniques was found on the subsequent survival of larvae up to 4-days of age.

CONCLUSION

This study confirmed that, at a same water temperature, hatching of *P. hypophthalmus* eggs tented to occur slightly earlier (1 to 2 hours) in the McDonald type incubators than in all other incubation systems without mechanical agitation of the eggs. However, this slight shortening of the incubation period was not associated to a lowering in hatching rates or subsequent survival of larvae. All together, the results indicate that, when properly managed, the incubation methods can hardly be responsible for the variability of hatching rates or survival of larvae often observed in different reproduction trials or from farm to farm.

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EGG QUALITY OF AN ASIAN CATFISH OF THE MEKONG RIVER (*PANGASIUS HYPOPHTHALMUS*) DURING THE PROCESS OF MATURATION INDUCED BY HCG INJECTIONS

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Abstract

In captive *Pangasius hypophthalmus*, oocyte maturation and ovulation are induced by hormonal injection following a given protocol: preparatory injections (500 UI.kg^{-1}) and decisive injection (2000 UI.kg^{-1}).

The aim of this work is to specify the timing of ovulation and the effects of varying latency period on the quality of ova. Oocytes and ova were collected by intra-ovarian biopsy and hand stripping. Ova quality was estimated by fertilisation rate, hatching rate and proportion of deformed larvae. Ovulation occurred as a synchronous process 8h30 to 9h30 after the last hormonal injection. The first ova obtained (8h30 after injection) were of good quality (85% hatching). At this time, the ovulation rate was 100%.

Three hours after ovulation, ageing of ova started to occur: the proportion of deformed larvae increased (24%) and hatching rate collapsed (35%).

In *P. hypophthalmus*, the optimised latency period was found to be 8h30 and corresponded approximately to the completion of ovulation.

INTRODUCTION

Pangasius hypophthalmus, an Asian catfish from *Pangasiidae* family, is widely cultivated in South Vietnam (Peignen, 1993). A method for artificial propagation of this specie has been recently set up.

The latency period defined as the delay between the last hormonal injection and ova collection is an essential matter in reference to ova quality (Bromage, 1995). Ovulated eggs of oviparous Teleost become overripe if retained in the body cavity and these eggs show a progressive reduction in viability for many species. Early or late collection of gametes can lead to low hatching rate and large proportion of deformed larvae caused by low rate of ovulated oocytes or overripe ova (Legendre & Otémé, 1995). After ovulation the latency period leading to eggs of optimal quality is specific to each specie. This period vary from one hour to 4-6 days (Table 1).

In captive females of *Pangasius hypophthalmus*, final oocyte maturation and ovulation do not occur spontaneously and are hormonally induced with human chorionic gonadotropin (hCG).

The aim of this investigation was to assess the latency period to obtain the best egg quality in terms of hatching rate and proportion of normal larvae.

MATERIALS AND METHODS

P. hypophthalmus were fished in the wild (0.5-200 g) and reared in floating cages during 7 years in order to provide brood fishes for artificial propagation. Brood stock, described in Table 2, consisting of 78 females and 22 males was held in floating cages (50 m^3) on the Mekong River at density of one fish per m^3 (t°= 24-34°C).

The brood-stock was fed once a day with a 40-45% protein (dry matter) dry pelleted feed distributed at a rate of 1.5% of fish biomass (Table 3). Intra-ovarian canulation and binocular lens measure of the oocytes diameter was used to assess the maturity stage of females. Fishes showing more than 60% of oocytes with a diameter of at least 0.9 mm were selected and individually transfer to 1.5 m^3 tank with 10.4 l.mn^{-1} water exchange.

Oocyte maturation and ovulation were induced with intra-muscular hCG injections as it is described in Figure 1.

Males received a single intra-muscular injection of 2000 UI.kg^{-1} of body weight (Eeckhoutte, 1996). The sperm was obtained by stripping and kept in a refrigerator at 2–5 °C after dilution (dilution rate 1:2) in 9 g.l^{-1} NaCl solution adjusted at pH 7 with basic TRIS buffer. The sperm was pooled from three males for fertilising ova. Sperm motility was assessed every hour (Sanchez-Rodriguez & Billard, 1977) to insure optimal conditions.

Oocytes maturation and quality was assessed during a period of 9 hours which starts five hours after the last hormonal injection. Two sampling method were used on this purpose.

Species	Time (h.)	References
Oreochromis niloticus	1h	Rana (Unpublished data)
Prochilodus platensis	1h	Fortuny *et al.* (1988)
Roccus saxatalis	1h	Stevens (1966)
Carassius auratus	2-3h	Formacion & Lam in Formacion (1991)
Macculochella peeli	2-3h	Rowland (1988)
Misgurnus anguilicaudatus	3-8h	Suzuki (1975)
Hippoglossus hippoglossus	4-6h	Kjorsvik *et al.* (1990), Bromage *et al.* (1994), Holmefjord (1991), Norberg *et al.* (1991)
Rhamdia sapo	5-9h	Espinach Ros *et al.* (1984)
Gadus morhua	9-12h	Kjorsvik & Lonning (1983)
Clarias macrocephalus	10h	Mollah & Tan (1983)
Scophthalmus maximus	10-20h	McEvoy (1984), Howell & Scott (1989)
Plecoglossus altivelis	1-2 days	Hirose *et al.* (1979)
Limanda yokahamae	2-3 days	Hirose *et al.* (1979)
Salvelinus alpinus	5 days	Gillet (1991)
Oncorhynchus mykiss	4-6 days	Springate *et al.* (1984)
Clupea harengus	14 days	Hay (1986)
Oncorhynchus kisutch	20 days	Fitpatrick *et al.* (1987)

Table 1: Latency period for different species (from Bromage, 1995).

	Stocking density in cage (kg.m^{-3})	Feeding rate (%)	Weight (kg)	Length (cm)	Body condition index
Male	7.2	1.5	5.8 ± 1.2 [4.0-7.1]	70.9 ± 4.1 [66.0-77.0]	1.3 ± 0.1 [1.2-1.5]
Female			5.5 ± 0.9 [4.5-6.9]	71.4 ± 3.4 [68.5-76.5]	1.5 ± 0.1 [1.4-1.8]

Table 2: Brood fishes characteristics.

Raw material		Nutritional value [a]	
Blood meal	17.6%	Moisture	10.9%
Soya oil	3.0%	Protein	50.7%
Vitamin	0.8%	Lipid	7.2%
Broken rice	9.8%	Carbohydrates	22.6%
Fish meal	35.4%	Fibre	2.7%
Soya meal	33.4%	Hash	16.8%

[a] percent are expressed from dry material except for moisture which is expresses from total material.

Table 3: Composition of the diet and nutritional value.

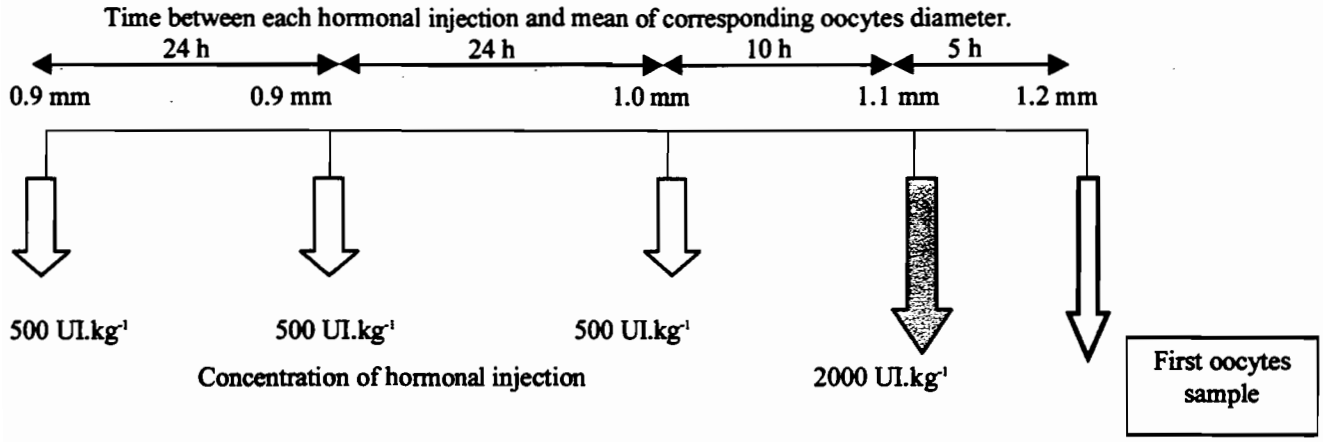


Figure 1: Schematic representation of the hormonal treatment for *P. hypophthalmus* females.

Manual removing (stripping) of eggs which is only efficient once ovulation has occurred and gonadal biopsie which can be used at any time.

Samples of 40 gametes were fixed in Serra's solution (60% ethanol, 30% formalin, and 10% acetic acid, in volume) and were observed using a binocular lens to determine the position of the germinal vesicle (GV). The diameter of 30-50 gametes was also measured under binocular lens. Ovulation rate was evaluated by determining the proportion of ova sticking to their support after been wet with mineral water.

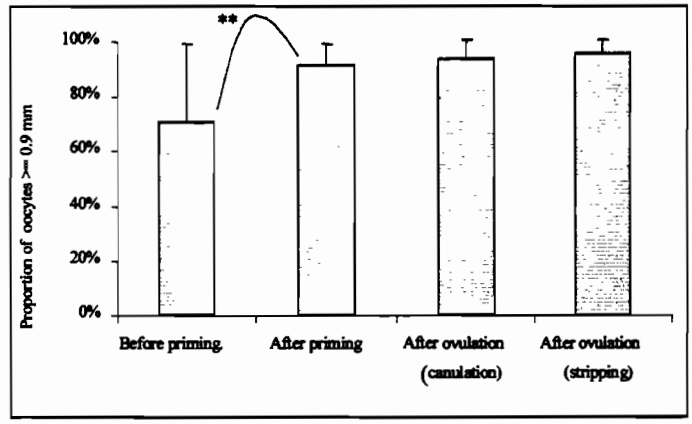
Egg quality assessment was based on fertilisation rate, hatching rate and proportion of deformed larvae obtained from batches of 200-300 eggs. Eggs were fertilised with diluted sperm (1%) in plastic box leading to a ratio of 2 10^6 spermatozoa for one ova. Activation was obtained by addition of 6 ml of fresh water. After 1 mn of gentle stirring, eggs were washed with clean water and placed in glass tanks (t° = 29-30°C) with closed water system for incubation (19–22h).

Fertilisation and hatching rate were respectively assessed 8h and 24h after fertilisation. Proportion of deformed larvae was evaluated when 100% hatching occurred.

RESULTS

The hormonal treatment is composed of two mains steps. First the preliminary treatment composed of two injections of 500 UI.kg^{-1}at 24h interval. Those injections induce the growth of oocyte diameter (Fig. 2). Thus the average oocyte diameter increase from 0.9 mm to 1 mm. This increase concerns oocytes from 0.5 mm and allowed them to be receptive to the ovulation treatment. The second step of the treatment (500 and 2000 UI.kg^{-1} with an interval of 10h) induces the last phenomena of maturation and oocytes diameter reach the size of mature ova (1.2 mm). The germinal vesicle is in central position for 100% of oocytes observed at the time of the last hormonal injection and migrate towards the periphery before breaking down (GVBD). Five hours after the last hormonal injection the GVBD has occurred for 90% of the case.

The ovulation process is achieved 11h after the last hormonal injection.

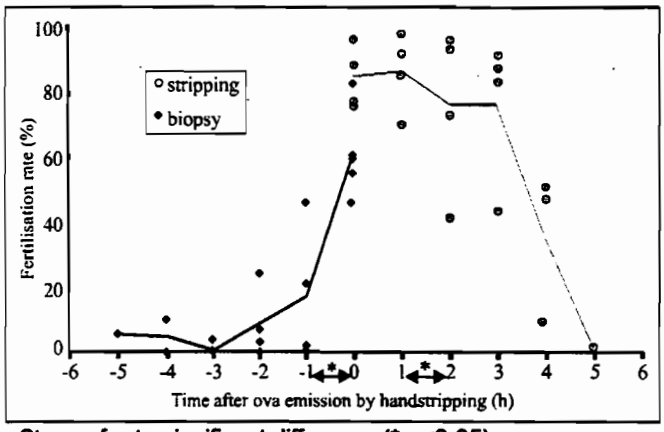


Stars refer to significant difference (** p<0,01).

Figure 2: Influence of hormonal treatment on oocyte diameter.

All the females hormonally induced had a positive response and spawn under artificial conditions.

The optimal quality of ova is obtained as soon as ovulation has occurred, fertilisation rate remains greater than 90% and proportion of deformed larvae lower than 10% for two hours (Fig. 3). The absolute hatching rate (hatching eggs/total eggs) exceeds 80% from 8h30 to 9h30 after the last hormonal injection which underlines the good embryonic development.

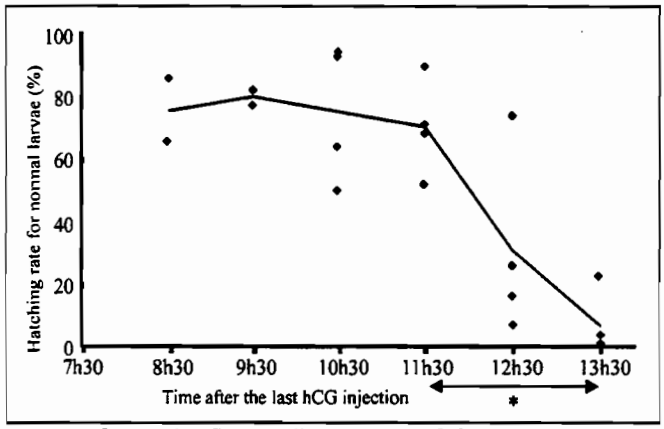


Stars refer to significant difference (* p<0,05).

Figure 3: Influence of the latency period on the fertilisation rate of *Pangasius hypophthalmus*.

After a latency period of 11h30 the viability of eggs start to be affected. Hatching rate and proportion of normal larvae are the main indicator of this decline. Thus the hatching rate of normal larvae dropped down to 30% 12h30 after the last hormonal injection (Fig. 4). One hour later 50% of the larvae hatched are deformed (Fig. 5).

Quality of ova obtained from stripping was always higher than for ova collected by gonadal biopsy (Fig. 6) however the evolution followed the same kinetic for the two sampling methods.

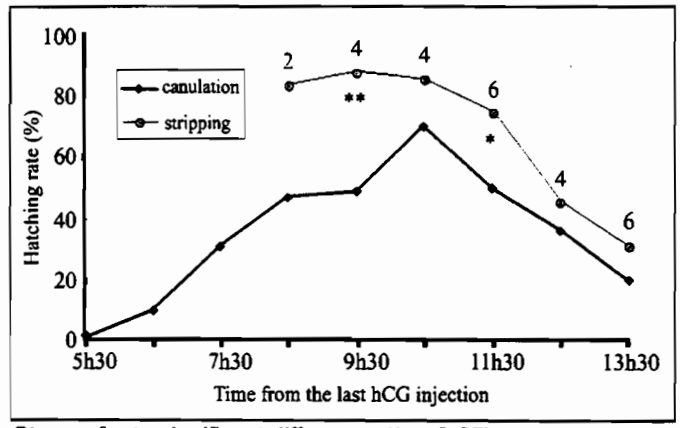


Stars refer to significant difference (* p<0,05).

Figure 4: Influence of the latency period on the production of normal larvae.

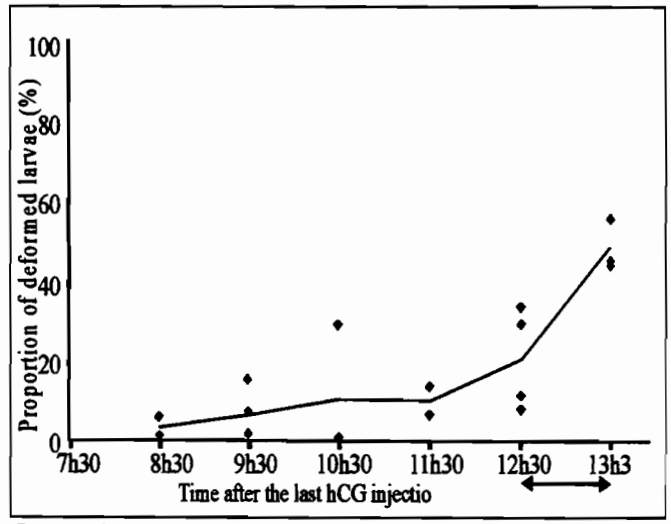
CONCLUSION

In summary the results of this investigation show that ovulation takes place from 8h30 to 12h30 hours after the last hormonal injection. This process is rapidly followed by emission of ova characterised by high hatching rate (hatching rate of 82% between 8h30 to 12h30 of latency) and low proportion of deformed larvae. Then the quality of ova decrease with an increased of latency period



Stars refer to significant difference (* p<0,05)..

Figure 5: Influence of the latency period on the proportion of deformed larvae.



Stars refer to significant difference (* p<0,05, ** P<0,01). Canulation was used on a group of 12 females each hours. The number of females sampled by stripping is mentioned above each point.

Figure 6: Influence of the sampling method on the absolute hatching rate. Ova are collected by intraovarian canulation or stripping.

and fertility declines drastically 12h30 after the last hormonal injection. Therefore eggs should be stripped as soon as ovulation has occurred which in this study correspond to a latency period ranging from 8h30 to 12h30. Further study should be conducted to determine origin of this variation as ovulation appears as a major factor to produce eggs of good quality.

Gonadal biopsy has always been providing lower quality of eggs than stripping. However this sampling method remains the only way to assess the sexual maturity level of fish as long as ovulation has not occurred. Thus results from this method should be considered with special attention.

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EFFECTS OF VARYING LATENCY PERIOD ON THE *IN VIVO* SURVIVAL OF OVA AFTER OVAPRIM- AND HCG-INDUCED OVULATION IN THE ASIAN CATFISH *PANGASIUS HYPOPHTHALMUS* (SILURIFORMES, PANGASIIDAE).

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Abstract

Over a 2-years period at the Sukamandi station (West Java, Indonesia), 87 *P. hypophthalmus* females selected on the basis of a modal oocyte diameter greater than 1.0 mm were treated with Ovaprim (n=77) or hCG (n=10) to induce oocyte maturation and ovulation. The two hormonal treatment led to similar results in terms of ovulation percentage (86 and 90%), hatching rates (69 ± 28 and 82 ± 11 %) and relative fecundity (167,000 ± 18,000 and 128,000 ± 60,000 eggs.kg^{-1}, with Ovaprim and hCG respectively).

The latency period between the last hormone injection and ovulation was negatively correlated to water temperature but showed important variations at a same temperature depending on individual females (e.g. between 5-11 h at 28-29°C). The ovulation time was therefore difficult to predict accurately in this species.

The assessment of survival time of ova maintained *in ovario* after ovulation showed that the process of ageing (overripening of ova) occurs rapidly in *P. hypophthalmus*. The overall quality of ova begun to decrease as early as 2 hours after ovulation and, after 3 hours, hatching rate dropped down and fraction of deformed larvae increased significantly in comparison to those observed at the moment of ovulation. In some individual females this evolution was even more rapid, with a sharp decrease in hatching rates between 1 and 2 hours post-ovulation. The duration of ova survival did not appear to depend on the type of hormonal treatment used (Ovaprim or hCG).

For optimised gamete management in hatcheries, it is therefore recommended to check carefully the females for the occurrence of ovulation (between 3 to 11 h after the last hormone injection, depending on water temperature) and to collect and fertilise the eggs within less than two hours after this moment.

INTRODUCTION

Pangasius hypophthalmus (Sauvage, 1878) (senior synonym of *P. sutchi*; Roberts & Vidthayanon, 1991) is the most common cultured pangasiid catfish all over Southeast Asia. Its aquaculture production, reaching several thousand tons annually, is still dependent on captures of wild fry or fingerlings in some areas (e.g. in Cambodia) but rely increasingly on artificial propagation techniques (Csavas, 1994). The species was successfully induced to breed for the first time in captivity in Thailand after treatment with catfish pituitary gland suspension (Boonbrahm *et al.*, 1966; Potaros & Sitasit, 1976). It was then introduced to Indonesia from Thailand in 1972, where its hormonal induced-breeding was reported for the first time by Hardjamulia *et al.* (1981). Since that period, the culture of *P. hypophthalmus* has developed in this country both for food and ornamental purposes. However, despite the economic importance of this catfish, published data related to its biology and culture are still scarce and several problems remain to be solved before its rearing practices could be fully optimised. Poor egg quality and low hatching rates are amongst the difficulties most often reported by fish farmers.

The latency period, defined as the delay between hormonal injection and ova collection, is a key factor in the success of reproduction techniques involving hormonal induced-ovulation and artificial fertilisation in fish (Harvey & Caroldsfeld, 1993; Bromage & Roberts, 1995).

Delayed collection of gametes after ovulation leads to ageing phenomenon which can result in low fertilisation rates, increase in the number of deformed embryos, or increased mortality rates for embryos and larvae (Sakai *et al.*, 1975; Springate *et al.*, 1984). After ovulation, the *in vivo* survival of ova - estimated by the time lapse between ovulation and the moment at which the initial quality of ova begin to drop - varies according to species. This time lapse range from 6-30 days in the rainbow trout (Bry, 1981, Springate *et al.*, 1984) to a few hours in the majority of teleosts studied, including several catfish species (Woynarovitch & Horvath, 1980; Legendre *et al.*, 1996).

Therefore, the aim of this study was to assess the timing of ovulation (latency period) after hormonal treatment and the survival duration of ova maintained *in vivo* after ovulation in *P. hypophthalmus*. In order to test for possible difference related to the type of hormonal preparation used, survival of ova was evaluated after induced ovulation with either a mix of GnRH and Domperidone (Ovaprim®) or human chorionic gonadotropin (hCG). The ovulation rates, and the quantity and quality of ova obtained with these two treatments were also compared. The temporal evolution of ova quality was estimated by hatching rates and proportions of normal and deformed larvae obtained after artificial fertilisation.

MATERIAL AND METHODS

Fish origin and maintenance

The *P. hypophthalmus* brooders used descended from fish initially introduced from Thailand in 1972 and were 3-5-years-old and 2.4 to 5.8 kg individual body weight. They were held at a stocking density of 0.3-0.6 fish.m^{-2} in 50 m^2 ponds at the RIFF Sukamandi station (West Java, Indonesia). The broodstock was fed two times per day, 6 days a week, with a 35% crude protein pelleted feed distributed at a daily rate of 1% of fish biomass.

Latency period and ovulation rate

Over a period of two years, a total of 77 *P. hypophthalmus* females were induced to breed with Ovaprim and 10 others with hCG at the Sukamandi station. Although Ovaprim is generally used in Indonesian hatcheries (Sadili, 1999), responses were also tested with hCG for comparison.

The mature females were chosen after intra-ovarian biopsy on the basis of a modal diameter of oocytes greater than 1.0 mm (1.13 ± 0.05 mm, on average). Selected males were producing milt at stripping. Oocyte maturation and ovulation were induced with two successive Ovaprim[1] injections of 0.3 ml.kg^{-1} female BW and 0.6 ml.kg^{-1} given at 8 h interval. The same procedure was applied with hCG (Organon, France) except for doses, fixed at 500 IU.kg^{-1} and 2,000 IU.kg^{-1} for the first and second injections respectively (Campet, 1997). In all cases, males received a single Ovaprim injection of 0.3-0.4 ml.kg^{-1} applied at the moment of first injection of females. During the treatment the brooders were held in hapas installed in ponds or in large concrete tanks. The mean maintenance temperature of brooders ranged between 27.1 and 31.7°C during the latency period. Within a same trial, the amplitude of thermal variation was generally less than 2°C.

In order to detect the moment of ovulation, gentle stripping trials were generally performed every hour starting from 3 to 7 h after second injection. Nevertheless, ovulation had already occurred in some females at the moment of first stripping trial; in such cases the latency period could not be known precisely. When ovulation was observed, ova were collected by complete stripping, weighed and immediately fertilised. A sample of ova was also weighed to the nearest mg and fixed in 5% formalin for subsequent counting and total fecundity estimates.

The sperm was collected by stripping directly in a syringe containing a 0.9% NaCl solution (dilution rate of 1/5) to prevent spermatozoa activation by dilution with urine, then preserved at 5°C for subsequent fertilisations. The sperm of *P. hypophthalmus* remains generally viable for 24 h at least when preserved and used in these conditions (Eeckhoutte, 1996).

The quality of ova was evaluated from hatching rates obtained with replicated batches of 200-300 eggs fertilised with 0.2 ml of diluted sperm. This corresponded approximately to 6.10^6 spz per ova. Spermatozoa activation was obtained by addition of 10 ml freshwater. After 1min of gentle stirring, eggs were rinsed to remove excess milt and

[1] One ml of Ovaprim (Syndel Laboratories, Canada) contains 20 µg of GnRH [D-Arg6, Trp7, Leu8, Pro9, NEt] and 10 mg Domperidone.

transferred for incubation in a plastic box containing 300 ml of standing water at ambient temperature (27-30°C). Hatching ended after 26-29 h of incubation, and the hatching rates were evaluated 35-40 h after fertilisation.

In vivo *survival of ova after ovulation*

The period of *in vivo* survival of ova after ovulation was studied between May and December 1997 in 13 females treated with Ovaprim and 3 others treated with hCG. The schedule of injections and doses used were the same as those indicated above. The mean maintenance temperature of brooders ranged between 27.1 and 28.8°C during the latency period.

From 5 hours after the second injection, females were checked every hour to follow the process of oocyte maturation on samples collected by intra-ovarian biopsy and fixed in Serra's solution (60% ethanol, 30% formalin, 10% acetic acid, by volume). For females treated with Ovaprim and starting when the first oocytes at a stage of germinal vesicle breakdown (GVBD) were found, part of the gametes collected by intra-ovarian biopsy was also used for fertilisation trials until ovulation has occurred. The moment at which the first ova could be obtained by stripping was considered as the ovulation time (t_0) and served as a reference. At ovulation and then every hour until 7 h post-ovulation, a partial collection of ova (approximately 10 g per stripping) was carried out for each female. For each individual stripping, ova quality was evaluated from three sub-samples of 200-300 eggs, fertilised and incubated following the general procedure presented above. In each case, the sperm from two males was pooled for fertilisation. Two stripping of the males were done in order to prevent possible effects of a lowering in spermatozoa fertilising ability. The sperm collected at the first stripping served to fertilised ova collected up to 3 hours after ovulation, then males were stripped again for subsequent fertilisation tests. During experiment, the motility of spermatozoa was regularly checked using a microscope. After hatching, the proportions of normal and deformed larvae were determined for each batch of eggs by observations with a binocular and counting over an illuminated table.

Statistical analysis

Hatching percentages and fraction of deformed larvae were compared using one way ANOVA followed by Duncan's multiple range test to determine significant differences among means at $p<0.05$. When necessary, angular transformation of data was carried out in order to stabilise the residual variance.

RESULTS

Latency period and ovulation rate

The two hormonal treatments led to similar success in inducing oocyte maturation and ovulation of *P. hypophthalmus* (Table 1 and 2). The percentages of ovulated females were of 86% and 90% with Ovaprim and hCG, respectively. It should be noted however that incomplete ovulation was observed in 10% of the females treated with Ovaprim. These partial responses corresponded to females in which only small quantity of ova could be collected, while important remaining ovarian masses in the abdomen could be felt by hand even after repeated stripping made at a few hours interval. The eggs obtained from such females were systematically of poor quality (6.4 ± 5.1%). By contrast, the quantity and quality of ova collected in other ovulated females were generally high, and similar for fish treated with Ovaprim or hCG (Table 1 and 2). No relationship was found between the initial modal oocyte diameter of the treated females (range 1.04-1.20 mm) and the quantity or quality of ova collected.

The ovulation time was assessed precisely in 43 of the 77 females treated with Ovaprim and in 8 of the 10 females treated with hCG. The latency period between the second hormone injection and ovulation ranged from 3 to 11 hours at a mean temperature of brooders maintenance varying between 27.1 and 31.7°C (Fig. 1). For fish treated with Ovaprim, a significant inverse relationship between the latency period and water temperature was found [$R = 0.486$, $F(1,41) = 12.710$, $p<0.001$]. Nevertheless, a high variability was observed in the latency response of the different females even at a similar temperature (e.g., 5-11 hours at 28.0-29.0°C). With hCG, the latency period (8-11 h) tended to be less variable than with Ovaprim and ranged amongst the highest values observed with this latter hormone (Fig. 1). At a same temperature, the latency response was not related to the initial oocyte diameter of the different females used.

In vivo *survival of ova after ovulation*

In the ova survival study, the latency period to ovulation ranged between 5 to 11 h depending on

	No ovulation	Partial ovulation	Full ovulation
N° and % of females treated	**n = 11; 14%**	n = 8; 10%	n = 58; 76%
		n = 66; 86%	
Relative fecundity ($egg.kg^{-1}$) x 1000	-	**6.4 ± 5.1** [1 – 14] (7)	**167 ± 78** [33 – 317] (49)
Hatching rate (%) *	-	**13 ± 17** [0 – 36] (7)	**69 ± 28** [3 - 99] (42)

*: Only hatching rates estimated within 1 hour after ovulation are considered.
Mean ± sd, [ ]: extreme values, ( ): N° of observations

Table 1: Ovulation percentage, mean relative fecundity and mean hatching rate for 77 *P. hypophthalmus* females treated with Ovaprim at the Sukamandi station.

	No ovulation	Full ovulation
N° and % of females treated	**n = 1; 10%**	**n = 9; 90%**
Relative fecundity ($egg.kg^{-1}$) x 1000	-	**128 ± 60** [35 – 210] (9)
Hatching rate (%) *	-	**82 ± 11** [59 - 95] (9)

*: Hatching rates are estimated within 1 hour after ovulation.
Mean ± sd, [ ]: extreme values, ( ): N° of observations

Table 2: Ovulation percentage, mean relative fecundity and mean hatching rate for 10 *P. hypophthalmus* females treated with hCG at the Sukamandi station.

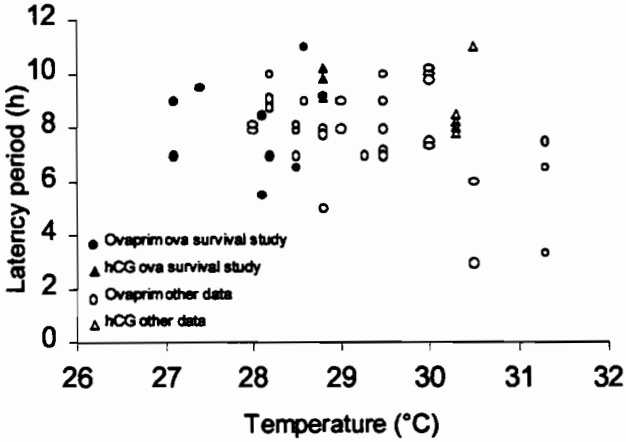

Figure 1: Latency period to ovulation, after the second Ovaprim or hCG injection, as a function of temperature in *P. hypophthalmus* females.

the females (at 27.1-28.8°C). The eggs obtained at the ovulation time (t_o) were generally of good quality with hatching rates ranging between 64 and 96% for all females, except for three individuals induced with Ovaprim (below 40%). Data from these three latter fish were withdrawn from the analysis.

Two hours before ova could be collected by stripping, the majority of oocytes obtained by intra-ovarian biopsy were GVBD, but did not fully achieved their maturation as indicated by very low hatching rate obtained after fertilisation (Fig. 2).

The evolution of hatching rates and fraction of deformed larvae obtained after partial stripping as a function of time from ovulation are given in Figures 2 and 3 for females treated with Ovaprim and hCG, respectively. Responses observed with the two hormonal treatments were very similar. In both cases, a clear inverse evolution was observed between hatching percentages and fraction of deformed larvae. The highest hatching percentages and lowest proportions of deformed larvae were observed at the ovulation time (t_0) and one hour after. As early as 2 hours after ovulation a noticeable decrease in hatching rates was observed, and after 3 hours hatching rates dropped down and fraction of deformed larvae increased significantly in comparison to those observed at t_0 and t_0+1h. When ova were stripped 5 hours or more after ovulation, mean hatching rates did not exceeded 15% and almost all of hatched larvae were strongly deformed.

Nevertheless, individual variations were observed. In the two extreme situations with Ovaprim, high hatching rates (superior to 80 %) were maintained for more than 4 hours in one female while, in another one, hatching percentages dropped from 74% to 9% between 1 and 2 h post ovulation and no hatching occurred at longer latency periods (Fig. 4). The fact that high hatching percentages were obtained for a long period of

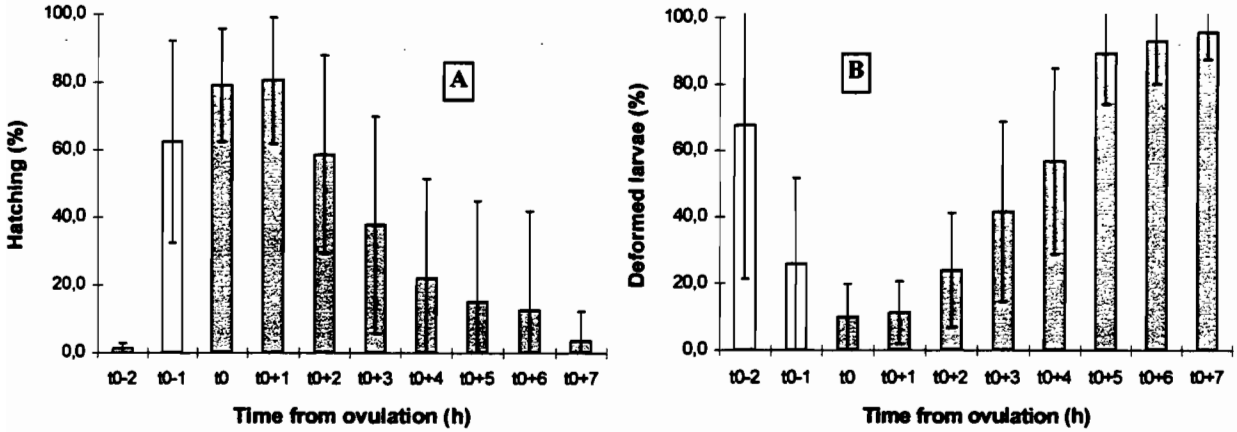

Figure 2: Evolution of mean hatching rate (A) and fraction of deformed larvae (B) as a function of time before (bars in white) and after (bars in grey) ovulation. Means for 10 *P. hypophthalmus* females treated with Ovaprim (water temperature: 27.1-28.6 °C). The moment of ovulation (t_0) is considered here as the first time at which ova can be obtained by stripping. Before ovulation, the egg quality was assessed on samples taken by intra-ovarian biopsy. Vertical bars refer to standard deviation.

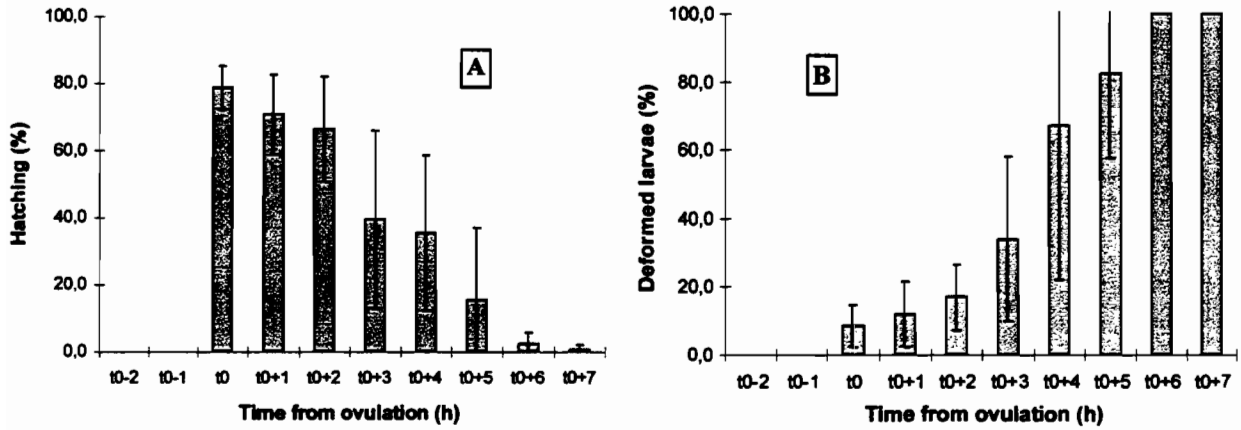

Figure 3: Evolution of mean hatching rate (A) and fraction of deformed larvae (B) as a function of time after ovulation. Means for 3 *P. hypophthalmus* females treated with hCG (water temperature: 28.8 °C). The moment of ovulation (t_0) is considered here as the first time at which ova can be obtained by stripping. Vertical bars refer to standard deviation.

time in one female attested that the rapid drop in egg quality observed in most females corresponded effectively to ageing of ova and not to a lowering of sperm fertilising ability.

DISCUSSION

In this study, both Ovaprim and hCG proved to be efficient in inducing oocyte maturation and ovulation in *P. hypophthalmus*, and led to the collection of ova of an overall good quality. The percentage of ovulated females were high and reached similar values with these two hormonal preparations (86 and 90 %, respectively). These results are equivalent to those of Cacot (1999) who reported ovulation in 88 % of 67 *P. hypophthalmus* females treated with hCG in Vietnam. Saidin *et al.* (1988) obtained 33-100% ovulation after treatment with LHRHa administered alone, i.e. not combined with anti-dopamine antagonists.

A high variability was observed in the quantity of egg collected (from 33,000 to 317,000 egg.kg^{-1}) after induced breeding, either with Ovaprim or hCG. After treatment with hCG, Cacot (1999) observed a similar range of relative fecundity in *P. hypophthalmus* females cultured in ponds or floating cages in Vietnam (from 10,100 to 297,500 egg.kg^{-1}). So far, the origin of such high individual variability, which could not be related to fish size or age, remains poorly understood and requests further investigations.

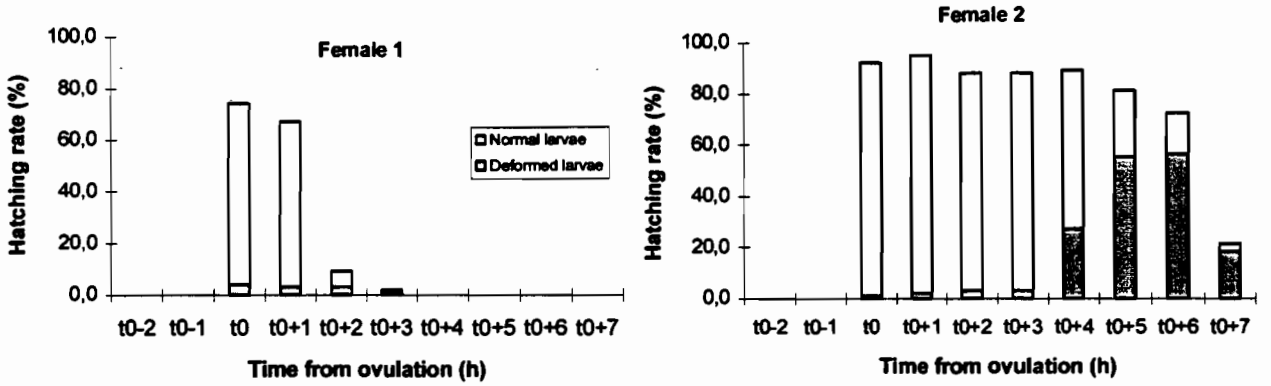

Figure 4: Extreme situations observed in the evolution of hatching rate and proportion of deformed larvae as a function of time after ovulation in two *P. hypophthalmus* females treated with Ovaprim (water temperature: 28.0-28.5°C).

As in other fish species (Woynarovitch & Horvath, 1980; Bromage & Roberts, 1995), a negative relationship was observed in *P. hypophthalmus* between the latency period and water temperature. However, at a same water temperature, the latency period between the second Ovaprim injection and ovulation showed a relatively high range of variation between individuals (5-11 h at 28-29°C). This suggested that gonads of the different females selected on the basis of their oocyte size, were not exactly at a same physiological state. Therefore, more accurate indicators than oocyte diameter alone should be identified to evaluate sexual stage before hormonal treatment in order to reduce variance in latency period.

The assessment of survival time of ova maintained *in ovario* after ovulation showed that the process of ageing (overripening of ova) occurs rapidly in *P. hypophthalmus*. The overall quality of ova begun to decrease as early as 2 hours after ovulation and, after 3 hours, hatching rates dropped down and fraction of deformed larvae increased significantly in comparison to those observed at the moment of ovulation. In some individual females this evolution was even more rapid, with a sharp decrease in hatching rates between 1 and 2 hours post-ovulation (Fig. 4). The evolution of hatching rates and fraction of deformed larvae as a function of time after ovulation were very similar with Ovaprim or hCG and did not appear to depend on the hormonal treatment used to induced ovulation. After hCG-induced ovulation in four *P. hypophthalmus* females in Vietnam, Campet (1997) observed that the initial mean quality of ova drop significantly after 3 hours from ovulation, but the ageing process occurred earlier (less than 2 hours after ovulation) in one individual female. In other catfish species studied, the reported duration of ova survival is generally longer, varying between 2-4 h in *Heterobranchus longifilis* and 10-12 h in *Clarias macrocephalus* (Legendre *et al.*, 1996). Therefore the correct timing of ovulation and moment of ova collection are particularly crucial for further egg development in *P. hypophthalmus* and insufficient checking may explain to a large extent the poor egg quality often reported on fish farms.

In Indonesia, after induced breeding of *P. hypophthalmus*, fish farmers generally apply a standard procedure consisting in the checking of females for ovulation 8-9 hours after the last hormonal injection; ovulated fish are then stripped and ova fertilised while non ovulated females are returned directly to the ponds (unpublished inquiries on fish farms). From the present results showing the effects of water temperature and individual variability on latency period, on one hand, and the short survival duration of ova, on the other hand, it appears clearly that such practice may lead either to discard fish still in the course of oocyte maturation or to collect ova already engaged in the process of overrippening.

In practice, it is therefore recommended to check carefully the females for the occurrence of ovulation (between 3 to 11 h after the last hormone injection, depending on water temperature) and to collect and fertilise the eggs within less than two hours after this moment.

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LARVAL REARING OF THE ASIAN CATFISH, *PANGASIUS BOCOURTI* (SILURIFORMES, PANGASIIDAE): *ARTEMIA* ALTERNATIVE FEEDING AND WEANING TIME.

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Abstract

Three experiments were carried out to evaluate larval rearing in *Pangasius bocourti*. The survival rates of larvae fed *Artemia* nauplii, *Moina* sp. (Cladocera) or tubifex (red blood worms) were not significantly different, being comprised between 91 and 93%. *Artemia* nauplii and tubifex led to similar growth rates (35-36%.d^{-1}), while *Moina* sp. gave an inferior growth rate in comparison to the two former feeds. Commercial trout starter feed gave the lowest survival rate (67%) and growth rate (20%.d^{-1}). However, the use of a dried feed based on yeast improved the survival in comparison to the trout starter diet. Larvae fed dried diets or decapsulated *Artemia* cysts had the same survival rate but a lower growth rate than those fed *Artemia* nauplii. The stomach content analysis showed that the reduced growth in larvae fed decapsulated cysts in comparison to live *Artemia* may reflect more a difference in feed ingestion and preference than a difference in nutritional quality of the feed.

Pangasius bocourti larvae requires 3 days of *Artemia* feeding before being shifted to trout starter feed without negative effects on growth performance. The stomach attains its functional and physiological achievement 3 days after the first feeding. It is obvious that digestive tract development of *P. bocourti* larvae is still going on during the initial feeding.

INTRODUCTION

The Asian catfish, *Pangasius bocourti* Sauvage, 1880, is a major indigenous fish cultured in cages in the Mekong Delta, Vietnam. The annual production reaches about 13 400 tons (Cacot, 1994). The availability of seed has been dependent on fry catching in natural water bodies. Therefore, artificial reproduction and larval rearing represent actually a bottleneck for the production and cage culture development of the species in Vietnam. Induced spawning of the species was successfully carried out for the first time in the Mekong Delta in 1995 (Cacot, 1999). So far, information on the larval rearing of this species is lacking.

Brine shrimp nauplii (*Artemia* sp.) often proved to be an excellent start feed for freshwater and marine fish species (Léger *et al.*, 1986). However, *Artemia* use may not be appropriate in developing countries since *Artemia* cyst price is quite high and it requires some specialised facilities to produce. Successful rearing of fish larvae using live zooplankton was reported for several species (Watanabe *et al.* 1983, Dabrowski, 1984). Among various species of zooplankton, the genus *Moina* (Cladocera) is known to be suitable as initial feed for *Chanos chanos* (Villegas, 1990) and *Clarias macroceplalus* (Fermin & Bolivar, 1991). Tubifex worms have been also used successfully for European catfish (*Silurus glanis)* larval rearing (Ronyai & Ruttkay, 1990). The use of decapsulated *Artemia* cysts, rather than live *Artemia* nauplii, presents several advantages: eliminating the inconvenience of producing live food (Pector *et al.*, 1994), decapsulated cysts are disinfected and have a higher dry weight and energy content (Vanhaecke *et al.*, 1983). They do

not leach nutrient in water like formulated feed and its particle size is appropriate for most fish species (Verreth *et al.*, 1987). As a result, they have been considered as a reference diet for nutritional study of *Clarias gariepinus* (Verreth *et al.*, 1987). It was also reported that some freshwater fish species can be exclusively reared on artificial diets from exogenous feeding such as *Clarias gariepinus*, *Coregonus sp.*, *Cyprinus carpio* and *Heterobranchus longifilis* (Appelbaum *et al.*, 1988; Bergot *et al.*, 1986; Charlon *et al.*, 1986; Legendre *et al.*, 1995).

In hatchery operation and research activity, shifting from live to artificial feed was done as soon as possible when it does not affect anymore growth performance and survival of larvae. It seems that after onset of exogenous feeding, fish larvae require a certain time to develop their ability to adapt to dry feed. Freshwater fish are fairly large at hatching, and thus can accept dry diet at earlier time than marine species. The time depends on the quality of dry feed as well as the physiological and functional development of the digestive tract at the larval stage.

So, the aim of the present study is to evaluate different types of live feed, artificial diet or decapsulated *Artemia* cysts on the acceptability, growth and survival rate of *Pangasius bocourti* larvae. The work also aims at studying the digestive tube development in order to find a relationship between the weaning time and the development of digestive organs.

MATERIAL AND METHODS

P. bocourti broodfish were cultured in ponds at the University of Can Tho, Vietnam. Spawning was induced by treatment with human chorionic gonadotropin hormone (hCG). When hatched larvae were 24 hours old, 500 of them were placed into each of twelve 50 L aquarium that were aerated and had a continuous flow of well water at a rate of 0.4-0.5 $L.mn^{-1}$. Feeding started from 48 hours post hatching when the yolk sac was not completely absorbed. Fish weight and total length at that time ranged from 3.7 to 4.0 mg and from 8.7 to 9.0 mm, respectively.

Water current through aquarium was maintained constant at 0.4-0.5 $L.mn^{-1}$ and an aeration of water was done during the experiment. Dissolved oxygen and pH were measured twice a week with DO meter (YSI model 518) and pH meter (Hana HI 8424). Ammonia and nitrite, were measured by colorimetry method (Aquaquant 14423, 14424). Temperature, monitored twice a day at 8h.00 and 15h.00, ranged from 28 to 30°C. Dissolved oxygen concentration was always higher than 5 $mg.l^{-1}$. pH values varied in a range of 7.0-7.5. Ammonia and nitrite were from 0.1 to 0.3 $mg.l^{-1}$ and from 0.01 to 0.04 $mg.l^{-1}$, respectively.

Three experiments were carried out in the present study to evaluate different feed on growth performances, survival rates in *P. bocourti* larval rearing and the weaning time with a trout feed starter.

1. The first experiment tried four diets, including *Artemia* nauplii, tubifex worms, cladocerean and trout feed starter.
2. The second experiment tested three diets, including *Artemia* nauplii, decapsulated *Artemia* cysts and dry diet based on beef liver and yeast.
3. The third experiment determined the suitable weaning time in *P. bocourti*. In the experiment, fish were fed either *Artemia* nauplii or a trout starter diet. There were eight treatments which were assigned to 0, 1, 2, 3, 4, 5, 6 and Art. For instance, treatment 2 means that larvae were fed during two days with *Artemia* nauplii before being shifted directly to dry diet. Treatment "O" or "Art" means that larvae were fed exclusively with dry diet or *Artemia* nauplii respectively.

   Each treatment had three replications.

Artemia nauplii obtained from cysts (San Francisco Bay strain), were inoculated and cultured in Vietnam since 1982. Cysts were incubated in $10g.L^{-1}$ saline water for 24 hours at a temperature of 30°C. Newly hatched *Artemia* were kept in aerated saline water. To ensure the provision of the nauplii stage, *Artemia* were used within a period of 12 hours. These *Artemia* nauplii have a size of 146-250 µm in width and 411-450 µm in total length.

Cladocereans were cultured in earthen ponds fertilised with pig manure. They were daily collected and treated with formalin for 1-2 minutes to eliminate disease germs. They were still alive and moved actively after treatment. A small part of other zooplankton organisms were recorded in the *Moina* collection, including *Eucyclops* and some

ephippial eggs. Cladocereans were mostly *Moina sp.* with a size of 288-300 µm in width and 850-900 µm in total length.

Tubifex worms (*Tubifex tubifex*) were collected on river bank. They were treated with formalin for 1-2 minutes and then chopped into small pieces of 800-900 µm in width and 900-1000 µm in length.

Dry diet used in the first experiment, was a trout starter diet (Aqualim, France) composed of fish meal, cereal, terrestrial animal product, fat, oil, vitamin and mineral premix. The proximate composition of the dried feed was 55% protein, 18% lipid, 8% mineral. The size of feed particles was 0.2-0.4 mm.

Another dry feed (dry diet 2), based on beef liver and yeast with the following formula (INRA) and the proximate composition, was used in the second experiment. The formula was:

- «Protibel» yeast powder	50%
- Beef liver	35%
- Soybean oil	5%
- Vitamin premix	5%
- Mineral premix	5%

Proximate composition (dry basis):

- Moisture	7.83%
- Crude protein	35.69%
- Crude lipid	10.97%
- Ash	11.73%

Artemia cysts were decapsulated in 25% hypochloride solution for 5-10 minutes until the orange colour appearance and were then washed in freshwater until disappearance of the chloride odour. Decapsulated cysts were then kept in refrigerator (10°C) for daily feeding. Fish were fed six times a day at 8h:00, 12h:00, 16h:00, 20h:00, 24h:00 and 4h:00. Live *Artemia* nauplii, *Moina* and tubifex were fed at 160% of fish biomass (wet feed basis), based on the last fish sampling and increased arbitrarily by 50% at each following day. The adjustment was made on the basis of fish weights registered every three days. The dry diet was distributed at 20% of fish biomass and increased by 50% at each following day. Every three days, 30 larvae were randomly sampled. They were placed on paper towels, in order to absorb water and weighed in batch of 30 fishes at an accuracy of 0.1 mg, according to the procedure of Kerdchuen and Legendre (1994). Weighed larvae were not further used in the feeding experiment. At the end of the experiment, 50 fishes were sampled per aquarium. On each sampling day, five supplementary larvae were also caught 30 minutes after feeding and fixed in formalin 10% for further gut content analysis and mouth size measurement.

Mouth height was the distance from lower jaw to upper jaw, for larvae with the mouth open at 45 or 90°. Mouth width was the width of the lower jaw. Measurement were performed under a binocular lens with an accuracy of 0.01 mm. Survival rates were calculated by taking into account the remaining and discarded larvae.

Histological study was carried out on larvae fed exclusively on *Artemia* nauplii in a separated aquarium with temperature range of 28-30°C. Sampling of 10 larvae occurred at 0, 24, 36, 48 hours post hatching and later on 3, 4, 5, 7 and 10 days old. Larvae were fixed in either buffered formalin (10%) or Hollande Bouin and then dehydrated and embedded in paraffin and cut at sections of 5-10 µm. The section was coloured with Hematoxycline-Eosin or PAS. The pH gut measurement was carried out by injecting a methyl red or Congo red fluid into the gut of larvae.

Dead larvae were recorded and siphoned out two times a day at 8h:00 and 20h:00. The observed mortality was defined as the observed dead larvae ratio to the larvae amount. The cumulative mortality rate was denoted as the cumulate of daily observed mortality. The cannibalism rate was also defined in the present study as the missing larvae during the experiment, cannibalism rate % = 100 – (survival rate % + observed mortality %).

Mean weight, specific growth rate and survival rates were subjected to one way ANOVA, followed by Duncan's Multiple Range test to determine the significant difference among treatments with the help of the software Statgraphics version 5.0.

RESULTS

Comparison between Artemia *nauplii, tubifex worms, Cladocerean and trout starter feed*

The larvae fed voraciously all experimental diets during the rearing period. They swam actively at the moment of feeding, searching feed on the bottom. Gut content analysis showed that *P. bocourti* larvae started to feed 48 hours after hatching at the temperature of 28-30°C, while yolk

sac has not yet completely absorbed. At first feeding, the mouth height opening at 45° and 90° was 0.55 mm and 0.95 mm respectively and the width of lower jaw was 1.00 mm (Table 1). It is clear that *Pangasius bocourti* have a mouth size large enough to ingest different types of natural diets including *Moina* sp and tubifex worms. Cladocereans were always alive and available in the water column while dry feed and tubifex worms settled down quickly. *Artemia* nauplii remained alive for 5-6 hours in freshwater but they all concentrated at the bottom of aquarium.

Mean weight, specific growth rate and survival rate at the end of the experiment showed that *Artemia* nauplii and tubifex worms were excellent diets for *P. bocourti* larval rearing (Table 2). During the three initial days of feeding, *Artemia* nauplii led to the highest growth performances compared to tubifex worms. Nevertheless, larvae fed tubifex worms grew fast in further days and caught up the growth of larvae fed *Artemia* nauplii (Fig. 1). Starting eight days after hatching (6 days from exogenous feeding) onwards, larvae fed tubifex did not show any significant difference in mean weight when compared to larvae fed *Artemia*. Cladocereans led to a lower growth rate than those fed *Artemia* nauplii or tubifex worms. Artificial diet led to lower growth performances than all live feeds. This low growth was already apparent after the first 3 days of feeding (D5).

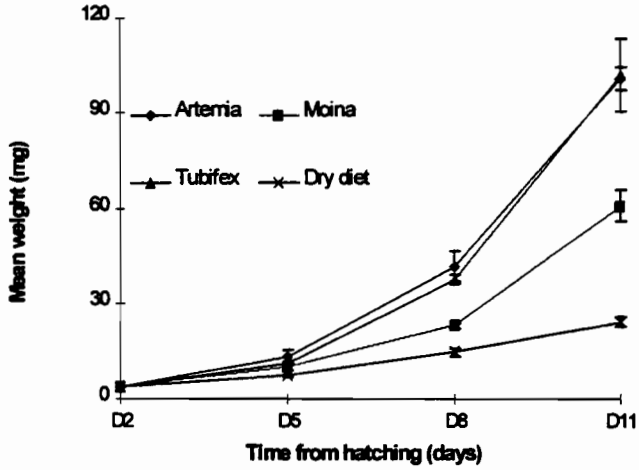


Figure 1: Growth curves of *P. bocourti* larvae fed live brine nauplii (*Artemia sp.*), *Moina sp.*, tubifex worms and a trout starter diet in the first experiment.

The survival and cannibalism rate of larvae fed live feed including *Artemia* nauplii, tubifex worms and cladocereans were not significantly different, while trout starter led to the lowest survival rate (67.5%) and the highest cannibalism (10.4%). Cumulative mortality (Fig. 2) indicated that larvae fed live feed had high mortality during the three initial days of feeding (D2 to D5), compared to further days (D5 to D11). By contrast, artificial feed caused an increased mortality until the end of the experiment. However, the mortality of larvae fed artificial diet tended to diminish after the 5th day, in comparison to the period from D2 to D5.

Age from hatching	Total length (mm)	Mouth size (mm)		
		opening at 45°	opening at 90°	width of lower jaw
48 h (D2)	8.7	0.5	0.9	1.0
72 h (D3)	9.6	0.7	1.2	1.3
96 h (D4)	10.6	0.8	1.3	1.3

Table 1: Mouth size of *Pangasius bocourti* larvae at first feeding. (48 hours after hatching). Distance from lower to upper jaw was measured for larvae with mouth opened at 45° or 90°.

Feeding treatments	*Artemia* nauplii	*Moina* sp	Tubifex worms	Trout starter
Initial weight at D2	3.7	3.7	3.7	3.7
Weight at D5	13.3 ± 1.7 ^{a}	9.9± 1.2 ^{b}	11.0 ± 0.4 ^{b}	7.2 ± 0.4 ^{c}
Weight at D8	41.5 ± 4.6 ^{a}	22.9 ± 0.7 ^{b}	37.6 ± 1.3 ^{a}	14.6 ± 1.3 ^{c}
Final weight at D11	100.7 ± 0.7 ^{a}	60.8 ± 5.1 ^{b}	101.5 ± 11.7 ^{a}	24.3 ± 1.7 ^{c}
SGR (%. d^{-1})	36.0 ± 0.4 ^{a}	31.0 ± 0.9 ^{b}	36.7 ±1.3 ^{a}	20.8 ± 0.8 ^{c}
Survival rate (%)	91.7 ± 5.2	93.7 ± 2.4	92.7 ± 2.1	67.5 ± 13.6
Cannibalism rate (%)	3.9 ^{a}	1.6 ^{a}	2.6 ^{a}	10.4 ^{b}

Figures in the same line having same superscripts are not significantly different ($p<0.05$). Mean ± SD.
SGR = 100x(Ln(W2)-Ln(W1))/(T2-T1)

Table 2: Mean weight (mg), specific growth rate (SGR, %.day^{-1}) and survival rate (%) of *Pangasius bocourti* larvae fed artificial diet or live feed (*Artemia* nauplii, *Moina* sp., tubifex worms) after nine days of experiment from first feeding.

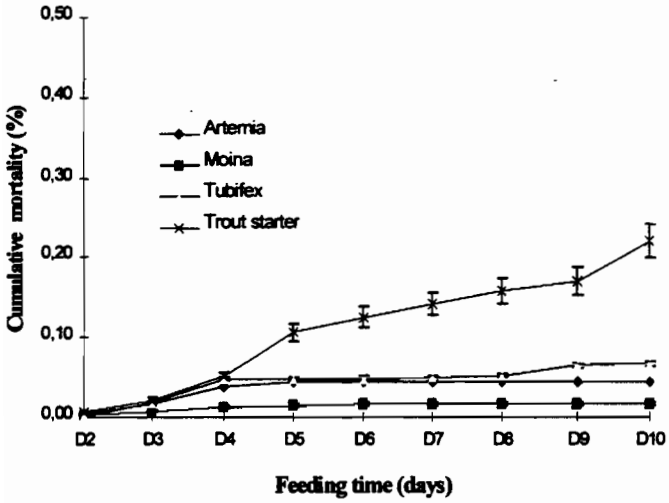


Figure 2: Cumulative mortality of *P. bocourti* larvae in response to live feed or trout feed starter. Feeding started 48 hours after hatching (D2).

Comparison between* Artemia *nauplii, decapsulated* Artemia *cysts and dry diet based on yeast and beef liver.

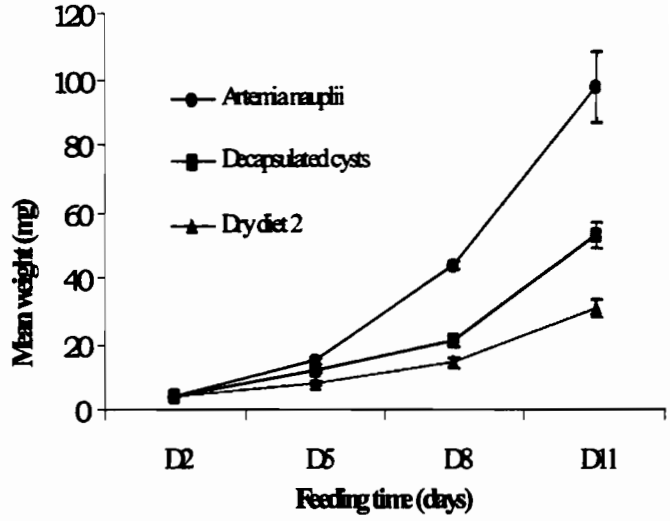
In the second experiment, The SGR of larvae fed on *Artemia* nauplii was 35.4%.d^{-1}, higher than those obtained with decapsulated cysts (28.7%.d^{-1}) or dry diet based on yeast (22.7%.d^{-1}). Thus, the growth performance of fish fed decapsulated cysts or dry diet based on yeast, was inferior to that of fish fed Artemia nauplii (Table 3; Fig. 3). However, survival rates in fish fed decapsulated cysts or dry diet based on yeast, were 90.4% and 86.6% respectively, not significantly different to that of fish fed Artemia nauplii (90.5%). Cannibalism rates were not significantly different among the three treatments.

When growth performance and survival rates in fish fed trout starter or dry diet based on yeast and beef liver were compared, it was clear that the latter ameliorates the survival rate while the growth was similar with the two dry diets.

Weaning time and digestive tract development

Weaning time

After 9 days of feeding, the highest growth performance was found in larvae fed exclusively on *Artemia* nauplii (101.9 mg and 36.7%.day^{-1}). However, the final mean weight of larvae weaned to artificial feed after 4 days and 6 days of *Artemia* feeding were 101.3 mg and 99.7 mg respectively, not significantly different from those fed *Artemia* nauplii. Larvae weaned after 3 days had a mean weight of 94.8 mg, relatively lower than those fed exclusively on *Artemia* but not significantly different from those weaned after 5 days.



Vertical bars refer to standard error.

Figure 3: Growth of *P. bocourti* larvae fed *Artemia* nauplii, decapsulated *Artemia* cysts or dry diet based on yeast and beef liver (dry diet 2) in the second experiment.

It was not surprising to notice that larvae fed exclusively on artificial diet or weaned as early as 1 day of *Artemia* feeding resulted in the lowest growth performances with final mean weights of 49.3 mg and 48.0 mg and specific growth rates of 28.6%.day^{-1} and 28.3%.day^{-1}, respectively. Larvae weaned after 2 days presented an intermediate growth rate between the group fed on artificial diet and the group fed on *Artemia*. In general, larval mean weights at the end of the experiment illustrated a trend curve that reached approximately an horizontal line starting with larvae weaned after 3 days of *Artemia* feeding (Fig. 4).

Larvae fed exclusively on a commercial trout feed, had the lowest survival rate (67.2%). Nevertheless, weaning after 1 day or 2 days resulted in improving the survival (78.0% and 76.0% respectively). With the exception of larvae that fed exclusively on artificial diet or weaned after 1 or 2 days of *Artemia* feeding, survival rates were consistently high and varied between 88.1% and 96.2%. Figure 4 also pictured the trend of survival rates of different treatments that reached approximately an horizontal line starting with larvae weaned after 3 days of *Artemia* feeding.

Feeding treatments	*Artemia* nauplii	Decapsulated *Artemia* cysts	Yeast and beef liver
Initial weight (mg)	3,7	3,7	3,7
Final weight (mg)	$97,6 \pm 10,7^{a}$	$53,0 \pm 3,8^{b}$	$30,9 \pm 2,6^{c}$
SGR (%. d^{-1})	35.3 ± 2.2^{a}	28.7 ± 0.8^{b}	22.7 ± 0.2^{c}
Survival rate (%)	$90,5 \pm 4,8^{a}$	90.4 ± 7.7^{a}	$86,6 \pm 1.5^{a}$
Cannibalism rate (%)	3.2 ± 4.2^{a}	5.5 ± 4.8^{a}	$7.0 + 1.4^{a}$

Figures in the same line having same superscripts are not significantly different. ($p<0.05$). Mean ± SD.
SGR = 100x(Ln(W2)-Ln(W1))/(T2-T1)

Table 3: Mean weight (mg), specific growth rate (%.d^{-1}) and survival rate (%) of *P. bocourti* larvae fed *Artemia* nauplii, decapsulated *Artemia* cysts or dry diet based on yeast and beef liver.

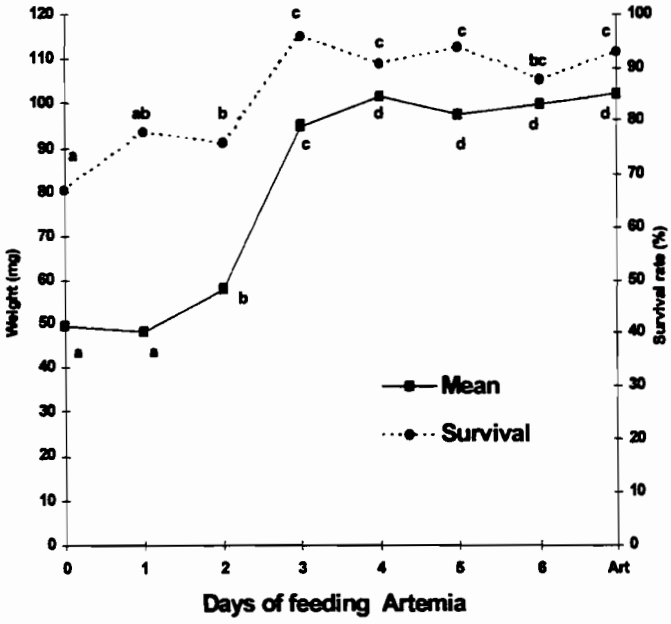


Figure 4: Mean weight (mg) and survival rate in *P. bocourti* larvae weaned at different days after hatching. Fish were fed either *Artemia* nauplii and then shifted directly to a commercial trout starter at different age.

Digestive tract development

At hatching, the digestive tube is only a rudimentary undifferentiated segment lying on yolk sac. Twenty-four hours later (D1), pancreas and liver are present as a clump of cells in posterior part of the digestive tract. At the exogenous feeding (D2), the stomach is an enlarged part with isometric epithelium cells, which are different from elongated cells in intestinal part. The stomach is also well distinguished with intestine due to the presence of a thick circular muscular layer in stomach and high folds of epithelium in intestine. Also at that time, zymogens were detected in pancreas in form of purple coloured granules in Hematoxycline-Eosin section. Gastric glands were not yet found in the stomach at that moment. It is only 24 hours later that these glands started to develop and were well distributed beneath the gastric epithelium on the day 4 post hatching (D4). Like other carnivorous species, surface epithelial cells in the stomach of *P. bocourti* contain muco-polysaccharides. Therefore, these cells may be responsible for the PAS positive coloration, which is visible on the stomach section on the day 5 (D5). At that time, the gastric secretion pH falls to 3.3 (changed colour of Congo red indicator) and the pyloric sphincter was found.

These observations indicated that the stomach then attained its morphological and probably functional completeness. In respect to absorption, droplets of lipids were detected twenty-four hours after feeding in the posterior part of intestine and liver cells had basal nucleus, showing that liver started to accumulate stored materials.

DISCUSSION

Growth performances and survival rates in response to different type of feed

In the first and second experiment, *Artemia* nauplii proved to be an excellent feed in terms of growth and survival rates for larval rearing of *P. bocourti*. Nevertheless, the survival rate of larvae fed *Artemia* was not significantly different from those fed other live food such as Cladocerean or tubifex. That implies that the use of other live feed than *Artemia* is possible. Using *Artemia* for successfully larval rearing was reported for several species. Hogendoorn (1980) reported superior results for larval rearing of *Clarias gariepinus* when using live *Artemia*, or a combination of *Artemia* and dry diet, as first feeds. Kerdchuen and Legendre (1994) showed the best growth

performance in *Heterobranchus longifilis* larvae fed live or frozen *Artemia* nauplii when compared to other diets. For these species, the specific growth rate (SGR) after a 14-day period was 40%.d^{-1}, which is close to the one obtained in *P. bocourti,* 36%.d^{-1} for an 11-day duration. Fermin and Bolivar (1991) who carried out experiment on *Clarias macrocephalus* also demonstrated that *Artemia* plus dry diet led to the best growth performance, even if the SGR was only 12.4%.d^{-1}. Knud-Hensen *et al.* (1990) concluded that *Clarias batrachus* larvae fed *Artemia* during 7 days displayed the best growth performance and survival in comparison to other live and dry feed. Thus, like most other tropical catfish, *P. bocourti* larvae had a maximal growth and an optimal survival rate when fed live *Artemia* nauplii.

Larvae fed *Moina* had a lower growth performance than those fed *Artemia* nauplii and tubifex worms. The SGR was only 22.9%.d^{-1}. The same conclusion was reported in *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994). When fed exclusively on *Moina macrocopa,* Fermin and Bolivar (1991) showed that *Clarias macrocephalus* also had a lower growth than fish fed a mixture of *Artemia* and dry feed. However, Adeyemo *et al.* (1994) had an opposite conclusion in two other catfishes, *Heterobranchus bidorsalis* and *Clarias gariepinus*. They reported that fish fed *Moina dubia* had a higher growth and survival than those fed *Artemia* nauplii. However, in their experiments, the SGRs of fish fed *Artemia* were surprisingly low, 5.1%.d^{-1} and 6.1%.d^{-1} for a 7-day duration in *Heterobranchus bidorsalis* and *Clarias gariepinus* respectively. Kerdchuen and Legendre (1994) observed the presence of numerous undigested ephippial eggs in the digestive tract of *Heterobranchus longifilis* fed *Moina,* which could lead to the low growth. *Moina* used in the present experiment, were collected in earthen ponds. We observed some *Eucyclops* and ephippial eggs in larval stomach content analysis. Then, their presence may explain, at least for a part, the lower growth observed in *P. bocourti* larvae as ephippial eggs are resistant to digestion in many fish species (Mellors, 1975).

Tubifex worms have been used as a live feed for nursing European catfish (*Silurus glanis*) in Hungary. Horvath *et al.* (1981) proposed the application of tubifex worms in large scale rearing which proved to be the most economical diet. However, many fish larvae cannot ingest such a large prey. Based on the measurement of mouth opening at 45° and 90°, food size suitable for first feeding of *P. bocourti* larvae was 0.6-1.0 mm. Therefore, mouth opening in *P. bocourti* permits to ingest tubifex worms if they are chopped. Starting from 8 days of age, larvae fed tubifex did not present any growth differences with the ones fed *Artemia.* Nevertheless, their mean weight at 5 days of age was still lower than those of fish fed *Artemia* (Table 1). Hence, the study confirmed the feasibility of completely replacing *Artemia* by tubifex worms for larval rearing of *P. bocourti.*

The poor growth and survival of *P. bocourti* larvae fed a commercial trout starter diet are in agreement with findings in other catfish such as *Clarias gariepinus* (Hogendoorn, 1980; Verreth & Van Tongeren, 1989) and *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994). This may be related to the feed quality and the digestibility of the dry diet or to the primary development of digestive systems at the first feeding. When fed trout starter feed, larvae of *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994) and *Clarias gariepinus* (Msiska, 1981) showed low survival rates of 32% and 12% respectively. In the present study, *P. bocourti* apparently showed a better survival rate of 67.5%. This indicates that this species has a high potential for using artificial diet.

In the present study, the yeast and beef liver based dry diet was well accepted by *P. bocourti* larvae and led to survival rates as high as that obtained with *Artemia* nauplii or other live feed. Dry diet based on yeast proved comparative to *Artemia* nauplii in term of survival rate in several fish species such as *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994), *Clarias gariepinus* (Applebaum & Van Dame, 1988). The improved survival of yeast based dry feed could be attributed only to better digestibility of protein in the diet. Secondly, the inactive yeast are roasted during the manufacturing process, thereby, denaturing the protein to a significant degree, which in turn facilitates their digestion (Hecht, 1996). This would explain the unsatisfactory result obtained when using other protein sources as dry feed ingredients for the *P. bocourti* larval rearing.

The use of decapsulated cysts as a direct food source for an initial stage of larvae has been

proposed as a reference diet in larval nutritional studies (Verreth *et al.*, 1987). However, in the present study, live *Artemia* nauplii resulted in higher growth than decapsulated *Artemia* cysts. Highly nutritional value of decapsulated cysts was confirmed by several workers. They contain 30-50% energy higher than freshly hatched nauplii (Vanhaecke *et al.*, 1983).

It is clear that the nutritive value of feeds did not account for the difference observed in body gain of larvae fed live *Artemia* nauplii and decapsulated cysts. In the present study, *P. bocourti* larvae showed a higher feed ingestion of live nauplii than decapsulated cysts (Fig. 5). This may be linked to movements of live *Artemia* nauplii and quick sedimentation of decapsulated cysts that become less available for the larvae. Therefore, the stomach analysis can elucidate the reduced growth in larvae fed decapsulated cysts in comparison to live *Artemia*. The same phenomenon was also observed in *Heterobranchus longifilis* (unpublished data). It reflects a difference in feed ingestion and preference more than in nutritional quality of the feed.

Trout starter feed in the first experiment resulted in a higher cannibalism rate (10.4%) when compared to live food (1.6-4.7%). Fermin and Bolivar (1991) also observed a high cannibalism rate in a dry diet treatment (21.7%) in comparison to a combination of *Artemia* with dry diet (9.7%).

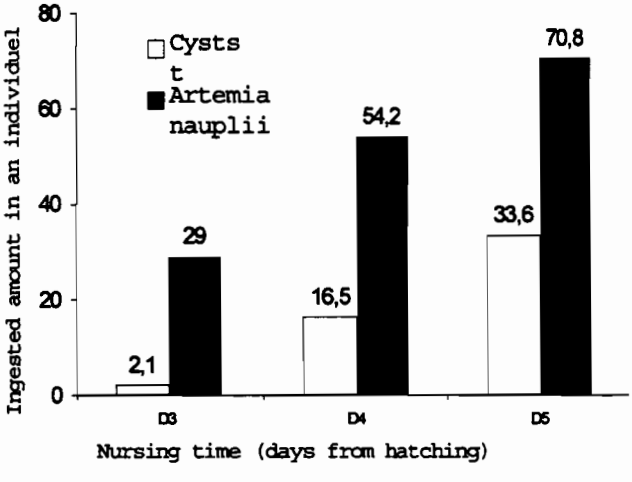


Figure 5: Amount of *Artemia* nauplii or decapsulated cysts found in stomach of 3 to 5 days old *P. bocourti* larvae 30 minutes after feeding.

Cannibalism was reported in most larval rearing. Hecht and Appelbaum (1987) demonstrated that cannibalism in *Clarias gariepinus* contributed greater to larval mortality than natural mortality.

Weaning time and digestive tract development

From results of the third experiment, it seems that *P. bocourti* larvae require 3 days of *Artemia* feeding before being shifted directly to trout starter feed without effects on growth performance. In respect to survival rate, it requires only one day. When compared to other freshwater species, *P. bocourti* larvae tend to have an earlier weaning time since at the temperature of 30°C, Verreth and Tongeren (1989) indicated that *Clarias gariepinus* could be weaned at 4.1 days and 1.8 days in respect to growth performance and survival respectively. Fermin *et al.* (1995) reported the weaning time of the Asian catfish (*Clarias macrocephalus*) to be 4 days after live zooplankton feeding. Bryant and Matty (1981) demonstrated that common carp larvae may be reared on a commercial trout starter from initial weight of approximately 15 mg, which is what they called "adaptation weight". The weaning size of different species were reported as 5-6 mg in Chinese carps, 18 mg in African catfish and 11-14 mg in Asian catfish (Dabrowski, 1984; Verreth & Tongeren, 1989; Fermin *et al.* 1995). In *P. bocourti* larvae, the earliest weaning size was 10-13 mg. Cyprinids larvae have a lowest weight at weaning (5-6 mg); however, when taking weaning time into account, the adaptation weight correspond to 4 days in common carps and 8 days in grass carp (*C. idella*) and silver carp (*H. molitrix*).

A question arises concerning the use of dry feed versus live feed for larval rearing; why fish larvae require live feed during the initial feeding? There has been a debate on the matter since the publication of Appelbaum's papers for larval rearing of carps (Hecht, 1996). According to Dabrowski and Culver (1991), larvae of some catfish have no functional stomach or gastric gland at initial feeding but whole digestive system differentiates during a complex metamorphosis. Therefore, the fish would depend on exogenous enzymes in live food to compensate for the impaired activity of proteolysis enzymes during the initial feeding (Dabrowskii & Glogowski, 1977; Lauff & Hofer, 1984). Moreover, Dabrowski (1992) indicated that the weaning period often corresponds to the moment at which the stomach becomes functional, with a switch from a digestion

exclusively intestinal to a mainly digestive active stomach. However, *Cyprinus carpio* does not have a functional stomach during its larval stage nor throughout its life. Theoretically, therefore, the larvae of carps should not be able to digest dry diet. However, the work undertaken by Appelbaum (1976a, b), Appelbaum and Dor (1978) and Dabrowskii *et al.* (1978) conclusively showed that carp larvae can indeed be reared as successfully on dry feed as on live feed.

In the present study, at the exogenous feeding fish larvae had a functional pancreas. Yet, larval gastric glands in stomach wall do not develop until 2 days after exogenous feeding. In addition, the stomach attains the functional and physiological perfection together with the appearance of a pyloric sphincter and a fall in pH value to 3.3 occurring 3 days after first feeding. When compared to the development of digestive tract in *Clarias gariepinus* larvae, *P. bocourti* larvae have a functional stomach earlier, 3 days after feeding instead of 5 days in *C. gariepinus*. It is obvious that digestive tract development of *P. bocourti* larvae is still going on during the initial feeding.

However, more fundamental studies are needed to elucidate whether the reduced growth of *P. bocourti* larvae fed on dry diet is due to a deficiency in gastric proteolysis enzyme during its initial feeding or to other reasons. That needs the mentioned debate. Yet, few work have paid attention to the fact that larval preference of live feed rather than dry diet may be due to the movement of live prey. As a result, feed intake of dry diet feeding is constantly lower than that obtained in live feeding, especially during the initial feeding when larval fins are not yet well developed. This is possibly an important reason for the unsuitable dry diet for larval rearing in many fish species.

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EFFECTS OF TYPE OF PREY, FEEDING LEVEL, PREY ACCESSIBILITY AND WATER AERATION ON GROWTH AND SURVIVAL OF *PANGASIUS HYPOPHTHALMUS* LARVAE (SILUROIDEI, PANGASIIDAE)

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Abstract

Two feeding experiments were carried out on *Pangasius hypophthalmus* larvae reared in a recirculating water system during 8 days. The first experiment was done at the Can Tho University (Vietnam) and the second at the RIFF Sukamandi station (Indonesia).

The main of the first experiment was to compare survival and growth performance of *P. hypophthalmus* larvae fed with either *Moina* sp. or *Artemia* nauplii and with either strong or moderate aeration. Strong aeration was tested in order to homogenise prey repartition in the water column, since *Artemia* and *Moina* sp. tended normally to be concentrated in corners or at the bottom of the tanks. Two feeding rates (150 and 4000% of fish biomass) were tested.

The second experiment aimed at an evaluation of optimal feeding level with *Artemia* nauplii, by considering growth, number of ingested preys and survival rates of larvae. Each of three feeding levels (R1, R2 and R3) were tested at three different fish stocking densities (10, 30 and 90 larvae.L^{-1}). The feeding level R1 was adjusted daily according to a pre-established model of the number of *Artemia* ingested as a function of age in *P. hypophthalmus* larvae. The rations R2 and R3 were respectively 3 and 9 times greater than R1. The prey accessibility (number of prey per litre), which varied as a function of the different combinations of feeding level x fish stocking density, was also considered as a parameter that could influence larval growth and survival.

The results of the first experiment indicated that both *Artemia* and *Moina* sp. could be used as a first feed for *P. hypophthalmus* larvae. However larvae fed *Artemia* nauplii presented a faster growth rate than those fed *Moina*. Perturbation of the rearing media by strong water aeration resulted systematically in reduced larval survival rates, whatever the type of prey and feeding rate used. The results of the second experiment showed that: 1) at a same feeding level, the stocking density did not affect the growth or survival of larvae; 2) at a same stocking density, larval growth and survival increased significantly with an increase of the feeding level, except for the highest feeding level at the highest stocking density (90 larvae.L^{-1}) which resulted in excessive feed quantity in the tanks; 3) at a same feed accessibility, growth and survival were decreased with a decrease in feeding level associated to an increase in stocking density. Therefore, in the range tested, the feeding level had a predominant effect on larval growth and survival in comparison to stocking density or prey accessibility. In the most favourable conditions, the larvae showed a very high growth rate, reaching up to 50 mg mean body weight at 8-days of age. The optimal feeding levels of *P. hypophthalmus* are discussed in regards to the observed number of *Artemia* nauplii ingested as a function of age of larvae in the different situations tested.

INTRODUCTION

Originating from the Mekong River, *Pangasius hypophthalmus* is the most widely cultured fish species in ponds in the Mekong Delta. Its production reaches several ten thousands tons per year in Vietnam. The species was introduced from Thailand to Indonesia in 1972 (Hardjamulia *et al.*,

1981) where it has been well adapted to local conditions and is appreciated by consumers.

Induced spawning of *P. hypophthalmus* was initially reported in Thailand in 1976 (Charoen Panil, 1977), in Indonesia in 1981 (Hardjamulia *et al.*, 1981) and in Vietnam in 1981 (My Anh *et al.*, 1981). Although *P. hypophthalmus* has been cultured for about 30 years, larval rearing of this species remains problematic (Subagja *et al.*, 1999). It is generally recognised that the first 8 days of life represents the most critical period, during which a marked cannibalistic behaviour was considered as the main cause of mortality. However, Subagja *et al.* (1999) showed that bacterial disease and female parents had more influence on survival rates of *P. hypophthalmus* larvae than direct effect of cannibalism.

Investigations carried out in Indonesia (Yuniardi, 1987) showed that *P. hypophthalmus* larvae fed on *Artemia* nauplii gave higher survival and growth rates than those fed on *Daphnia carinata*. However, *Artemia* is still the most expensive live food in South East Asia and some others natural feed should be found as substitutes.

Feeding level and prey accessibility play also an important role in growth and survival during the larval rearing.

In the present study, two different experiments were conducted:

- The first one with *Artemia* and *Moina* sp. as first feed distributed at two different rations. As these preys tend to be concentrated in corners or at the bottom of the tanks after being distributed to the fish, strong water aeration was tested as a possible mean of increasing prey accessibility by homogenising their distribution in the water column. It has been observed that young *P. hypophthalmus* larvae have a predominantly pelagic behaviour.
- The second one to determine the optimal feeding rates with *Artemia* nauplii, and the effects of prey accessibility for *P. hypophthalmus* larvae by considering growth, ingested number of prey and survival rates at different fish stocking densities.

MATERIAL AND METHODS

Experiment 1

The first experiment was carried out at the Can Tho university (Vietnam) and was designed to compare the effects of a low (150% of fish biomass) and high (4000% of fish biomass) feeding ration on growth and survival of *P. hypophthalmus* larvae fed either with *Artemia* nauplii or *Moina sp.* It aimed also to determine the effects of a strong water aeration on the prey accessibility by homogenising live food in water column. Therefore, the larvae were placed in the following rearing conditions:

- larvae fed with *Artemia* at 150% of fish biomass with strong aeration
- larvae fed with *Artemia* at 150% of fish biomass with slight aeration
- larvae fed with *Artemia* at 4000% of fish biomass with strong aeration
- larvae fed with *Artemia* at 4000% of fish biomass with slight aeration
- larvae fed with *Moina* at 150% of fish biomass with strong aeration
- larvae fed with *Moina* at 150% of fish biomass with slight aeration
- larvae fed with *Moina* at 4000% of fish biomass with strong aeration
- larvae fed with *Moina* at 4000% of fish biomass with slight aeration.

The larvae were obtained from broodfish held in earthen ponds at the Can Tho University (Vietnam). Oocyte maturation and ovulation were induced after hormonal treatment with hCG (human chorionic gonadotropin; Campet, 1997). Twenty four hours after hatching, larvae were individually counted and transferred to the larval rearing structures.

All treatments were tested with three replications. The larvae were reared in 50 litre aquarium at a stocking density of 10 larvae.L^{-1} with a water flow of 0.4-0.5 L.min^{-1}. The experiment was monitored for 8 days and feeding started at 48 hours post-hatching when yolk sac was not completely absorbed. The feeding frequency was 6 meals per day at 08:00, 12:00, 16:00, 20:00, 24:00 and 4:00. Each three day, in each treatment the larvae were weighed in batch in order to readjust the quantity of *Artemia* and *Moina* distributed according to feeding rates.

During the experiment, the water temperature was measured daily with a minimal-maximal thermometer and varied between 28 and 30°C. Water quality was monitored twice a week during the period of larval rearing, pH varied between 7.0 to 7.5 and dissolved oxygen was in all cases higher than 5.0 mg.L^{-1}. Ammonia and nitrite concentrations were determined at the same time

using Aquaquant© kits (Merck 14423, 14424) and ranged between 0.007 and 0.02 mg.L^{-1}, and between 0.01 and 0.04 mg.L^{-1}, respectively. Each aquarium was cleaned every day by siphoning off faeces and uneaten food.

On the last day of rearing period (day 8), thirty larvae from each tank were sampled and weighed in batch at an accuracy of 0.1 mg, the fishes being previously placed on paper towels in order to absorb adhering water. Survival rates were determined by counting all the remaining larvae in each aquarium.

Experiment 2

The second feeding experiment was carried out at the Sukamandi station of the Research Institute for Freshwater Fisheries, (West Java, Indonesia). It was designed to evaluate the optimal level of feeding with *Artemia* nauplii for larval rearing of *P. hypophthalmus*. Each of three feeding rations (R1, R2 and R3) were tested at three stocking densities (10, 30 and 90 larvae.L^{-1}).

Feeding levels and fish densities were chosen to ensure that several combinations of these two factors resulted in a same prey accessibility (the level of prey accessibility, which is the product of the feeding level by the fish stocking density, represents the density of *Artemia* available per litre of water, see Table 2). This was done in order to be able to separate, in the effects of feeding level on larval growth and survival, those related directly to the amount of feed distributed (number of *Artemia* per larvae) from those that may result from changes in feed accessibility (number of *Artemia* per litre).

A supplementary treatment (R'3) consisted in larvae stocked at the lowest density (10 larvae.L^{-1}) and fed with the highest quantity of *Artemia* nauplii used in this experiment (ration R3 given to larvae stocked at 90 fish.L^{-1}). These two treatments resulted in the same high prey accessibility (A1 x 81, see Table 2).

The feeding level was basically defined from observations of the number of *Artemia* nauplii ingested as a function of age in *P. hypophthalmus* larvae fed in excess. These observations, were done at the AGIFISFH hatchery, Vietnam (Slembrouck, 1997). The relationship between age and observed-number of *Artemia* nauplii ingested was modelised (Fig. 1) and served as a reference to defined the lowest feeding level used in this experiment. Medium ration (R2) was three times greater than this low ration (R1) and high ration (R3) was three times greater than the medium ration (R2). Feeding level was adjusted daily as a function of this model (Fig. 1) and feeding rate (% of fish biomass) was calculated from an estimated *Artemia* mean body weight of 15 µg (Sorgeloos *et al.*, 1986).

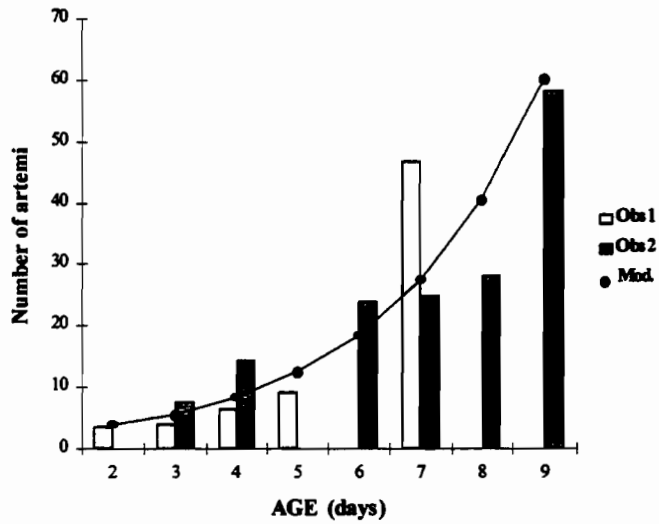


Figure 1: Number of *Artemia* ingested by *Pangasius hypophthalmus* as a function of age of larvae observed in AGIFISH hatchery in 1996 (Slembrouck, 1997). The modelised relationship between number of *Artemia* ingested and age of larvae is of the form: Y=e^(a+bX), with a=0,563541 and b=0,392637(r2 = 0,873).

The larvae used in this experiment were obtained from 3-5 years old *P. hypophthalmus* brooders held in ponds at the Sukamandi station. Induced breeding, artificial fertilisation and incubation of eggs were made following the procedure described by Legendre *et al.* (1999). Twelve hours after hatching, the larvae were individually counted and transferred to the experimental facilities.

All treatments were tested in duplicate and larvae were placed in 30 L tanks of a recirculating water system with mechanical and biological filters. Water flow through the tanks were of 0.25 L.min^{-1} up to 3-day then 0.5 L.mn^{-1}. Oxytetracycline at dose of 10 mg.L^{-1} was applied as a permanent bath to the larvae from the first day up to 8-days of age (Subagja *et al.*, 1999).

Larvae were fed with *Artemia* nauplii starting from 40 hours after hatching. Up to 8-days of age, the feeding frequency was of 8 meals per day at 09:00, 12:00, 15:00, 18:00, 21:00, 24:00, 03:00 and 06:00. At each feeding time, the water flow was stopped during 30 minutes in order to maintain the living preys in the tanks.

During the experiment, the water temperature was measured continuously and varied between 28.4 and 30.9°C. Water quality was monitored daily, dissolved oxygen and pH varied in the range of 4.4-7.8 $mg.L^{-1}$ and 8.4–8.5, respectively. Ammonia and nitrite concentrations were determined at the same time using Aquaquant© kits (Merck 14423, 14424) and ranged between 0.00 and 0.22 $mg.L^{-1}$, and between 0.005 and 0.012 $mg.L^{-1}$, respectively. Each aquarium was cleaned daily by siphoning off dead larvae and uneaten *Artemia* nauplii.

Every two days from the 2nd day until the 8th day of age, ten larvae from each tank were randomly sampled 30 minutes after the 6:00 PM meal and weighed in batch at an accuracy of 0.1mg according to the procedure of the first experiment. Then, the larvae were fixed in 5% formalin, individually dissected, and the total number of *Artemia* nauplii present in the first part of their digestive tract was counted. On the last day, survival rates were determined by counting all the remaining larvae in each tank.

Statistical analysis

Final mean body weights and survival rates of larvae were subjected to three way ANOVA (kind of prey x ration x water aeration) in the first experiment and two way ANOVA (ration x density) in the second one. Data from the supplementary treatment (10 $larvae.L^{-1}$; R'3) in the second experiment were compared to those from the treatment with R3 at stocking density of 90 $larvae.L^{-1}$ by one way ANOVA. When necessary, angular transformation of data expressed as percentage was carried out in order to stabilised the residual variance.

RESULTS

Experiment 1

Mean body weight and survival rates of larvae obtained after 8 days of age as a function of feed type (*Artemia* or *Moina)*, feeding level and conditions of water aeration are given in Table 1.

Survival rate

Results of analysis of variance indicated that aeration ($p<0.0001$) and ration ($p<0.001$) had a great influence on survival rates of *P. hypophthalmus* larvae. However, no effect of the type of prey (*Artemia* or *Moina*) were found ($p>0.05$).

When larvae were fed with the low ration (150%), the survival rates obtained with a strong water aeration (21% and 18%) were lower than those obtained with a slight aeration (40 and 27%). The survival rates were higher when larvae were fed with *Artemia* nauplii rather than *Moina* at low ration with strong or slight water aeration and at high ration with strong aeration. However, the highest survival rate (62%) was obtained when larvae were fed with *Moina* at a high ration (4000%) with slight aeration.

Growth

Analysis of variance showed no significant effect of the aeration conditions on the larval growth ($p>0.05$). Mean body weights were significantly higher ($p<0.0001$) for larvae fed *Artemia* nauplii than for those fed *Moina*. The growth rate of larvae was significantly increased with higher ration ($p<0.001$), except for larvae fed *Moina* in the treatment with strong aeration.

Experiment 2

Survival rate

The survival rates ranged between 20% and 60.5% (Table 2). The highest values were observed in treatments where larvae were fed with a high ration (R3) at density of 10 $larvae.L^{-1}$ (60.5%) or 30 $larvae.L^{-1}$ (52.0%). The analysis of variance indicated that the survival rates of *P. hypophthalmus* larvae increased significantly according to the ration ($p<0.001$). However, the treatment at ration R3 and high density (90 $larvae.L^{-1}$) showed a lower survival rate (33.0%), as was also observed in the supplementary treatment (10 $larvae.L^{-1}$; R'3). This indicated a negative effect of too high concentration of *Artemia* nauplii on survival rates.

For a same feeding rate (except for R3 with high stocking density, see upper), there was no effect of the larval stocking density on the survival rates, despite prey accessibility increased with increasing fish stocking densities. For a same larval stocking density, survival rates increased according to the ration, therefore according to the prey accessibility. However, considering a same prey accessibility we observed that the survival rates had a tendency to decrease with higher stocking density, i.e. with a lower feeding rate.

The result obtained (37.5%) with the supplementary treatment (10 $larvae.L^{-1}$; R'3) was close to the one at density of 90 $larvae.L^{-1}$ and

Feeding	Water aeration	Feeding rate (% biomass)	Final body weight (mg)	Survival rate (%)
Artemia	**Strong**	150	13.9	20.7
Artemia	Slight	150	11.2	40.4
Artemia	**Strong**	4000	19.9	28.1
Artemia	Slight	4000	20.6	48.9
Moina	**Strong**	150	5.0	18.4
Moina	Slight	150	7.3	27.3
Moina	**Strong**	4000	6.1	22.3
Moina	Slight	4000	10.9	62.3

Table 1: Body weight and survival rate of *P. hypophthalmus* larvae reared at a stocking density of 10 larvae.L^{-1} at the age of 8 days as a function of prey (*Artemia*, *Moina*), feeding level and water aeration (mean for three replications per treatment).

Fish stocking density (larvae.L^{-1})		Corresponding feeding rate on the first day of feeding (% biomass .day^{-1}) (*)	Feeding level at the first feeding (Nb *Artemia* .larvae^{-1} .feeding^{-1})	Accessibility at the first feeding (Nb *Artemia* .litre^{-1} .feeding^{-1})	Level of prey accessibility	Final body weight (mg)	SGR (%.d^{-1})	Survival rate (%)
10	R1	40	3	30	A1	20.3	44.5	23.5
10	R2	120	9	90	A1 x 3	29.1	50.1	38.0
10	R3	360	27	270	A1 x 9	43.9	56.1	60.5
30	R1	40	3	90	A1 x 3	23.8	47.1	20.0
30	R2	120	9	270	A1 x 9	33.3	52.0	31.5
30	R3	360	27	810	A1 x 27	49.8	57.9	52.0
90	R1	40	3	270	A1 x 9	22.5	46.3	21.5
90	R2	120	9	810	A1 x 27	33.0	51.9	39.5
90	R3	360	27	2430	A1 x 81	46.9	57.1	33.0
10	R'3	3240	81	2430	A1 x 81	51.3	58.9	37.5

(*) Calculated considering an individual *Artemia* weight of 15µg (Sorgeloos *et al.*, 1986).

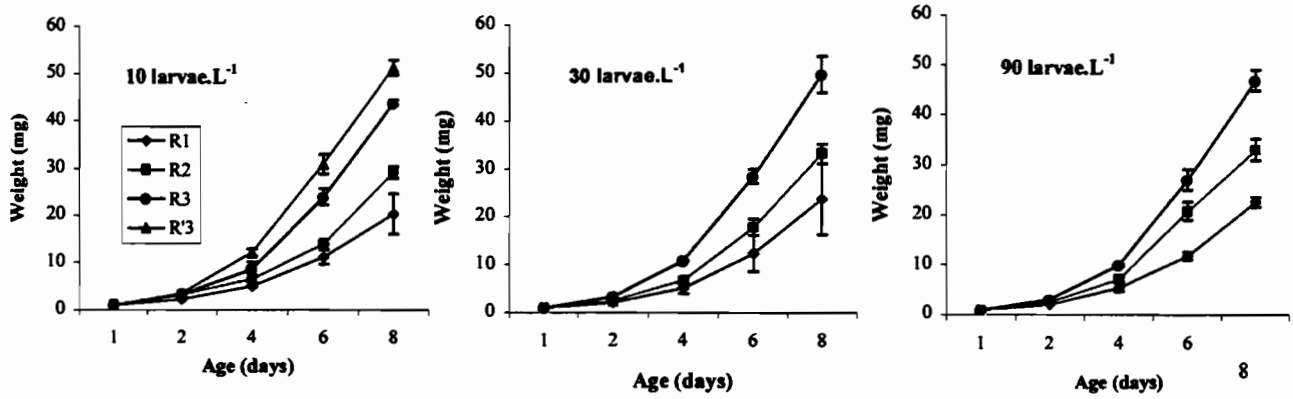
Table 2: Ration, accessibility, mean body weight, specific growth rate (SGR) and survival rate of *P. hypophthalmus* larvae fed with *Artemia nauplii* after 8 days of rearing at different stocking densities (mean for two replications).

ration R3 (33.0%). Then, larvae fed with the highest *Artemia* accessibility (A1 x 81) presented equivalent and rather low survival rates whatever the stocking density (10 or 90 larvae.L^{-1}).

Growth

Growth performances were positively correlated with feeding ration ($p<0.0001$; Table 2 and Fig. 2). By contrast, there was no effect of the stocking density on the growth performances. Considering the same accessibility of prey (A1 x 9 and A1 x 27), the final body weight tended to decrease according to stocking density and to increase according the ration (Table 2).

Growth data were examined per period of two days (Fig. 3), in terms of specific growth rate [SGR=100(LnW2-LnW1)/t]. During a first period from 60 hours to 5 days after hatching, the Figure 3 indicated that the highest SGR were obtained with the highest rations (R3 and R'3). Afterwards, SGR for the high rations generally decreased faster than for the low rations until the end of the experiment. From the 4th day of larval rearing the difference of SGR between high and low rations were reduced and SGR values tended to be very close. It appeared (Fig. 3) that the growth differential observed between larvae fed with ration R1, R2, R3 and R'3 (Fig.2) concerned only the first four days of larval rearing.



Vertical bars indicate range between replicates.

Figure 2: Growth of *Pangasius hypophthalmus* larvae at the age of 8 days from the first feeding as a function of feeding level with *Artemia* nauplii and fish stocking densities.

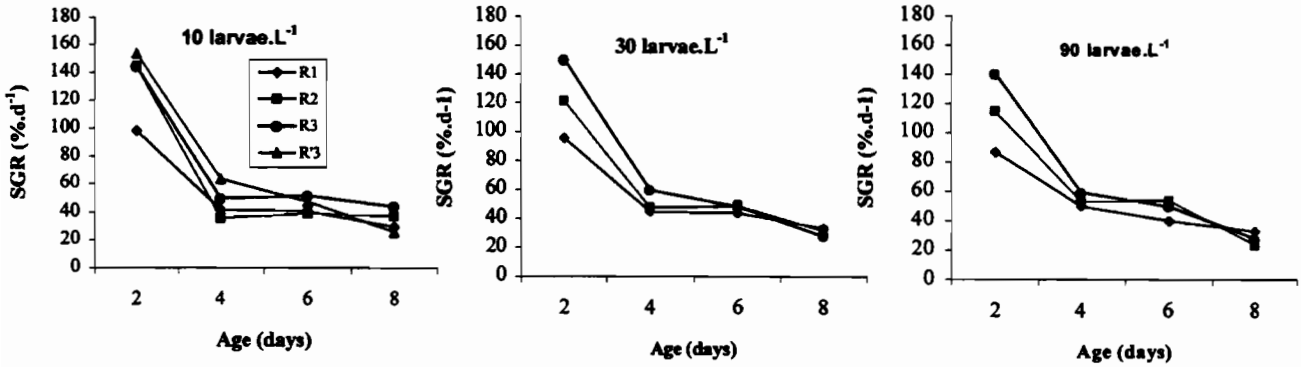


Figure 3: Specific growth rate (SGR) of *P. hypophthalmus* as a function of age of larvae at each two days sampling periods for the different feeding level and densities.

Number of ingested preys

The evolution of the number *Artemia* nauplii ingested by *P. hypophthalmus* larvae as a function of their age, at each two days of sampling, is presented in Figure 4. The number of ingested nauplii increased with the ration given. However, the number of ingested preys decreased at the 8th day of rearing whatever the treatment.

For the stocking density of 10 larvae.L^{-1}, the numbers of ingested preys at the 2nd, 4th and 6th day of larval rearing at the high rations were 13.7, 27.0 and 64.4 *Artemia* nauplii (R3) and 14.5, 29.1 and 70.3 *Artemia* nauplii, (R'3). This showed that increasing the feeding level by a 9 factor (from R3 to R'3) did not led to a strong increase in the number of ingested preys. Therefore, the ration R3 could be considered as a maximal level of rationing.

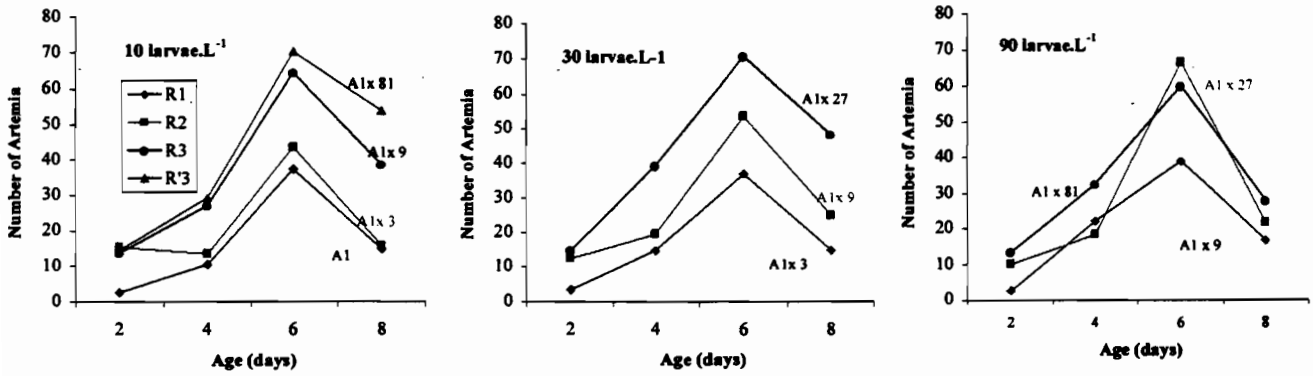
Independently from the stocking density, the mean number of *Artemia* nauplii ingested by larvae fed with the ration of reference (R1) at the 2nd and 4th days were respectively 2.8 and 16 and corresponded approximately to the model curve (Fig. 1). However, after the 4th day and whatever the stocking density, the observed numbers of *Artemia* nauplii ingested were higher (35 to 70) than predicted by the reference curve (23).

When the ration was multiplied by 9 at a same stocking density (from R1 to R3 at 10 or 30 larvae.L^{-1}), the number of ingested *Artemia* was multiplied by 5 at day 2, by 3 at day 4 and by 2 at day 6. For larvae reared at a stocking density of 90 per litre, increasing the ration from R1 to R3 resulted in a lower increase of the number of ingested preys, which was multiplied by 1.5 only from the 4th day of rearing (Fig. 4).

The number of *Artemia* nauplii ingested by larvae fed with equal conditions of prey accessibility decreased when feeding rate decreased, which was also accompanied by an increase in stocking density.

DISCUSSION

The first experiment showed that *P. hypophthalmus* larvae were fragile and sensitive to mechanical chocks and perturbations of the media. In addition, the homogenisation of the preys by strong aeration did not seemed to improve



(A1, A1 x 3, ... : corresponding levels of prey accessibility)

Figure 4: Number of *Artemia* ingested by *P. hypophthalmus larvae* as a function of age at each two days sampling periods for the different feeding level and densities.

the ingestion, as no difference in the final mean body weight of larvae were found between the two levels of water aeration tested. In this experiment the low survival rates were probably not related to feeding (ration and type of food) or distribution of the preys in the water column. Some other factors such as feeding frequency, rearing conditions, water quality or diseases may have resulted in low growth or survival rates. As a matter of fact, *Aeromonas hydrophila* was identified as a pathogenic agent for *P. hypophthalmus* larvae (Hambali *et al.*, 1999). Occurrence of bacterial infection and its negative effect on the survival rate of *P. hypophthalmus* larvae was already demonstrated by Subagja *et al.* (1999). However, these authors did not find any effects of bacterial infection on the growth rate of larvae in their study, as final body weights were equivalent when larvae were reared with or without antibacterial treatment.

In this first experiment, no difference of survival rates was found between larvae fed *Artemia* and *Moina* sp. However, mean body weight were higher for larvae fed *Artemia* nauplii, indicating that this prey was more adapted for first feeding of *P. hypophthalmus* larvae. Yunardi (1987) also demonstrated a better growth and survival for *P. hypophthalmus* larvae fed *Artemia* (21mg) than for those fed *Daphnia carinata* (11mg) at a feeding rate of 200-250% of fish biomass. *Artemia* nauplii was also considered as the best live feed for larval rearing of *Heterobranchus longifilis* (Legendre *et al.*, 1991), *Clarias macrocephalus* (Fermin & Bolivar, 1991) and *Pangasius bocourti* (Hung *et al.*, 1999).

When larvae were reared at a stocking density of 10 larvae.L^{-1} and fed with *Artemia* nauplii in the first experiment, the results did not show difference of survival rates between low (150%) and high feeding rates (4000%). By contrast, the second experiment indicated a decreasing of survival rates of *P. hypophthalmus* larvae when they were fed *Artemia* at a high feeding level (10 fish.L^{-1}; R'3, A1x 81; corresponding to 3240% of initial fish biomass). Similar observations were also made for the treatment with a stocking density of 90 larvae.L^{-1} and ration R3, which resulted in the same high quantity of *Artemia* nauplii (A1 x 81) in the tanks. These results indicated a negative effect of too high quantities of *Artemia* nauplii on larval survival rate. By comparison, the high survival rate of larvae (62%) fed *Moina* distributed at 4000 % of fish biomass in the first experiment could result from the capacity of *Moina* to survive in fresh water, while *Artemia* nauplii die after 3-4 hours in this media and decayed. Thus it can be assumed that larvae fed very high rations of *Artemia* were reared in inadequate hygiene as the tanks were cleaned only once a day.

The highest mean final body weight of larvae obtained in the first experiment was 21 mg only. Similar final weights (20-24 mg) were also observed in the second experiment with the lowest feeding rate. These values are in accordance with those previously reported (20-25 mg) for *P. hypophthalmus* larvae at the age of 10 days (Prihastowo, 1987; Yuniardi, 1987) or 8 days (Subagja *et al.*, 1999). However, observations carried out previously at the Sukamandi station (unpubl. Data) and in Vietnam (see Fig. 1) for larvae fed *Artemia* nauplii in excess, but without antibacterial treatment, showed lower levels of prey ingestion (7-14 *Artemia* nauplii at day 4-5) than for larvae fed high rations in the second experiment reported here (32 *Artemia* nauplii at day 4). Therefore, the lower final weights of larvae (20-25 mg at day 8-10) in these different trials could have been related to a low rate of prey

ingestion whatever the feeding level used. The present results showed that, with appropriate rearing conditions, a mean body weight as high as 50 mg can be expected at the age of 8 days.

The results of the second experiment showed that, in the range tested, fish stocking density did not influence growth and survival of larvae. By contrast, both were positively related to the feeding level up to a limit corresponding to an excessive quantity of feed in the tanks.

Until the age of 4 days *P. hypophthalmus* larvae swim with the mouth open and close their jaws only when meeting a prey. Therefore the search for food seems to be passive phenomenon during this early period, and it is only afterwards that larvae search actively for their preys in all the water column (Hardjamulia *et al.*, 1981; Legendre, unpubl. data). These observations suggested that the prey concentration (or accessibility) could be an important parameter to consider for larval rearing, particularly for young stages. However, at a same feeding ration, the larval growth and survival remained roughly the same despite prey accessibility was increased by 3 or 9 times when fish stocking density was increased in the same ratio. As an example, the mean body weight, survival and numbers of ingested preys remained unchanged in fish fed ration R1 and reared at stocking density of 10, 30 or 90 per litre, corresponding to prey accessibility of 30, 90 and 270 *Artemia* per litre (see Table 2 and Fig. 4). Conversely, for the same prey accessibility, the growth and survival of larvae, and the number of ingested *Artemia* nauplii, increased as a function of the feeding ration given. Therefore, the lowest level of prey accessibility (A1 = 30 *Artemia* nauplii.L^{-1}.feeding^{-1}) tested in this experiment was probably above the threshold at which this parameter could have been a limiting one for the larvae.

Hung *et al.* (unpublished data) showed a progressive and important increase of ingested preys as a function of age in *Heterobranchus longifilis* when larvae were fed *Artemia* nauplii (a mean of 221 preys per fish was observed at 9 days of age). By contrast, the present investigation showed a diminution of the number of preys ingested by *P. hypophthalmus* larvae between day 6 and day 8 (Fig. 4). It was noticed however that at 8 days of age, the larvae regurgitated a part of the ingested *Artemia* nauplii when they were fixed in 5% formalin. This reaction, which biased the counting of ingested preys at this moment, was not observed during the samplings of the 2nd, 4th and 6th days. This phenomenon was concomitant to morphological changes in the digestive tract, corresponding to the development of an individualised stomach. However, a detailed investigation remains necessary to precise the ontogeny of the digestive tract in *P. hypophthalmus*. In an other pangasiid species, *Pangasius bocourti*, the stomach attains its functional and physiological achievement 3 days after the first feeding (Hung *et al.*, 1999).

The specific growth rate of larvae calculated over the whole experimental period was increased at higher feeding rate. However, examining the evolution of SGR by period of two days showed that the growth differential observed between larvae fed with ration R1, R2 and R3 concerned mostly the first four days of larval rearing (see Fig. 3). The same trend was also observed when considering the number of *Artemia* ingested: the differential in number of prey ingested between larvae fed the different rations was reduced in older fish in comparison to younger ones. These results suggest that feed rationing would be more efficient (i.e. leading to high growth rates for a lower total quantity of *Artemia* distributed) if an initial high ration (R3) is reduced to R2 between 4 and 6 days of age, than reduced to R1 between 6 and 8 days of age.

CONCLUSION

The present study showed that both *Artemia* nauplii and *Moina* sp., which led to similar survival rates, could be used as a first feed for larval rearing of *P. hypophthalmus*. However, larvae fed *Artemia* nauplii systematically displayed a faster growth rate than those fed *Moina*. *Pangasius hypophthalmus* larvae were rather fragile and sensitive to strong water aeration used as a mean to homogenise the distribution of preys in the water column.

In the range tested, the feeding level using *Artemia* had a predominant effect on larval growth and survival in comparison to fish stocking density or prey accessibility. This positive relationship between feeding level and larval growth and survival was associated to an increase in the mean number of *Artemia* nauplii ingested by the larvae. In the most favourable conditions, the larvae showed a very high growth rate, reaching up to 50 mg mean body weight at 8-days of age.

Although, growth performance and survival rates increased according to the feeding level, the ration R3 (corresponding 27 *Artemia* per larvae per feeding, or 360% of fish biomass, on the first day of exogenous feeding) appeared as the maximal efficient ration for *P. hypophthalmus* larvae. Further investigations are presently carried out to precise the optimal feeding level according to the age and body development of the larvae.

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ANALYSIS OF PRECOCIOUS MORTALITY OF *PANGASIUS HYPOPHTHALMUS* LARVAE (SILURIFORMES, PANGASIIDAE) DURING THE LARVAL REARING AND PROPOSITION OF APPROPRIATE TREATMENTS[1]

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Abstract

Both in Indonesia and Vietnam, larval rearing of *Pangasius hypophthalmus* remains problematic due to variable and generally low survival rates obtained. The first week represents the most critical period for these larvae and up to now the cannibalistic behaviour was considered as the main cause of mortality.

In the present study, two experiments were carried out in order to better understand the evolution and causes of mortality of *P. hypophthalmus* larvae from hatching up to 8 days of age and find out measures to improve survival rates.

The first experiment was designed to evaluate the importance of cannibalism and differences in mortality when larvae from two different females were reared either in groups of 30 individuals or in isolated condition (30 larvae reared separately). In both cases, the culture was carried out either with or without antibiotic. The aim of the second experiment was to test Oxytetracycline and different disinfectants (Chloramine-T, formalin and "formalin + Malachite Green Oxalate") at different dosages to prevent bacterial outbreaks in the culture.

The results indicated that the survival rates of *P. hypophthalmus* larvae was dependant on the initial quality of larvae or eggs and that larval mortality was more a consequence of pathogenic infection than a direct effect of cannibalism. The present study demonstrated that the survival rates of larvae were systematically improved when rearing was carried out in water containing antibiotic (Oxytetracycline at a dose of 5 to 20 mg.L^{-1}). Survival rates and final mean body weights of larvae as high as those obtained using antibiotic were also reached with applications of disinfectants such as Chloramine-T and formalin. The use of these disinfectants is recommended for an application in commercial *P. hypophthalmus* hatcheries.

INTRODUCTION

Fish breeding in Indonesia has rapidly developed and is characterised by the emergence of numerous small-scaled hatcheries. Among cultured fish species, *Pangasius hypophthalmus* (Sauvage, 1878), synonimised with *Pangasius sutchi* since a recent systematic revision of Pangasiidae (Roberts & Widthayanon, 1991) and originating from the Mekong River, was introduced from Thailand to Indonesia in 1972 (Hardjamulia *et al.*, 1981). The species has been well adapted to local conditions and is appreciated by consumers.

Induced spawning of *P. hypophthalmus* was initially reported in Thailand in 1976 (Charoen Panil, 1977) and in Indonesia in 1981 (Hardjamulia *et al.*, 1981). However, larval rearing

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of this species remains problematic because variable and low survival rates of larvae are generally experienced. In West Java, the survival rate of larvae after 2 weeks from hatching generally falls in the range of 10-15%. During the first week of larval rearing, Slembrouck (1996) observed high variations of survival rates (0-79%) at the Chau Doc hatchery, Vietnam. Similar variations of survival rate were also observed at the Can Tho University, Vietnam (unpublished data) and at the RIFF Sukamandi station in West Java, Indonesia (0-83%). A marked cannibalistic behaviour, sometimes leading to the disappearance of more than 90% of the population (Campet, 1997), was considered as the main cause of mortality of *P. hypophthalmus* larvae. Observations carried out during the larval rearing showed that cannibalism started as early as 40 hours post hatching, before complete absorption of yolk sack, and stopped 3 to 4 days after hatching (Slembrouck, 1996).

Although *P. hypophthalmus* has been cultivated for about 30 years, published information on the larval rearing of this species remains very scarce. It is known however that the first 8 days of life represent the most critical period, afterwards the mortality rate decreases (Yuniardi, 1987). Observations carried out at the Sukamandi station indicated that two peaks of mortality generally occur during this period. The first mortality peak was observed at 2-3 days of age during the period of cannibalism and represented about 30-50% of initial fish number, while the second peak, occurring at 5-7 days of age and representing 50-60% mortality, seemed to be due to other causes than cannibalistic behaviour. Previous works indicated that the cannibalistic behaviour and feed intake during the first 3-4 days of age were passive phenomena (Hardjamulia *et al.*, 1981) and that the survival rate could be increased when the fry were fed *Artemia* nauplii at a daily rate of 250% of fish biomass, with a minimum of 5 distributions per day during the first 5 days of rearing (Prihastowo, 1987; Yuniardi, 1987). However, after the first week of larval rearing, the survival rate still showed very high variability in both private and research stations, even when fry were abundantly and frequently fed with *Artemia* nauplii.

Therefore, the objectives of the present study were to better understand the evolution and causes of precocious mortality of *P. hypophthalmus* larvae from hatching up to 8 days of age and find out feasible measures to improve survival rates.

MATERIAL AND METHODS

The larvae were obtained from 3-5-years old *P. hypophthalmus* brooders held in ponds at the RIFF Sukamandi station (West Java, Indonesia). Ovulation was induced using two injections of salmon gonadotropin-releasing hormone analogue and domperidone (Ovaprim©, Syndel Laboratories, Canada) with a total dose of 0.9 $ml.kg^{-1}$ body weight. Males received a single Ovaprim injection (0.4 $ml.kg^{-1}$) in order to increase the volume of milt collected. After stripping of gametes, artificial fertilisation was performed and eggs were incubated in happas placed in 5 m^3 concrete tanks. Twelve hours after hatching, the larvae were individually counted and transferred to their respective rearing containers. Two larval rearing experiments were carried out.

Experiment 1

The first experiment was designed to evaluate the importance of cannibalism and differences in mortality when larvae were reared either in groups of 30 individuals or in isolated condition (30 larvae reared separately). In both cases, the culture was carried out either without antibiotic or in water containing permanently Oxytetracycline at a dose of $5mg.L^{-1}$. Therefore, the fish were placed in the following situations :

- isolated larvae with antibiotic,
- isolated larvae without antibiotic,
- group of larvae with antibiotic,
- group of larvae without antibiotic.

A supplementary treatment consisting in groups of larvae without feeding and without antibiotic was done for comparison of the evolution of daily mortality with other treatments tested.

Each treatment was tested on larvae obtained from the eggs of two different females fertilised with sperm pooled from two males. The groups of 30 larvae were reared in 300 ml plastic containers with 3 replications for each treatment x female combination. Each isolated larvae was placed in a 150 ml plastic container, 30 larvae being individually followed for each treatment x female combination.

Experiment 2

The second experiment was designed to test the efficiency of Oxytetracycline and different disinfectants at different dosages to prevent bacterial outbreaks in the culture. The following treatments and doses were compared:

- Oxytetracycline at doses of 5, 10, 15 and 20 $mg.L^{-1}$,
- Chloramine-T at doses of 1.5, 2.0 and 2.5 $mg.L^{-1}$,
- formalin at doses of 1.5, 2.0 and 2.5 $mg.L^{-1}$,
- solution of 4 g of Malachite Green Oxalate (MGO) in 1 litre of formalin at final doses of 1.5, 2.0 and 2.5 $mg.L^{-1}$,
- untreated water (control).

All treatments were tested with 3 replications on groups of 30 larvae reared in 300 ml plastic containers and obtained from one female and one male. Oxytetracycline was applied as a permanent bath to the larvae from the first day up to 8-days of age, while all treatments using disinfectants were applied every two days during a period of 24 h.

In both experiments, the larvae were reared in stagnant spring water, and fed in excess with *Artemia* nauplii starting from 36 hours after hatching up to 8 days of age. The feeding frequency was of 8 meals per day at 09:00, 12:00, 15:00, 18:00, 21:00, 24:00, 03:00 and 06:00.

Water of each plastic container was changed twice a day at 10:00 and 22:00. During the experiments, water temperature was measured continuously (Optic StowAway©) and fluctuated between 27.5 and 29.8°C. Dissolved oxygen and pH were measured at day 3, 5 and 7 before water changing and varied in the range of 4.6-6.3 $mg.L^{-1}$ and 7.0-7.4, respectively. Ammonia and nitrite concentrations were determined at the same time using Aquaquant© kits (Merck 14423, 14424) and ranged between 0.002 and 0.027 $mg.L^{-1}$, and between 0.011 and 0.016 $mg.L^{-1}$, respectively.

Died larvae were removed and counted twice a day, simultaneously to water changes, to estimate the percentage of observed mortality per period of 24 h. On the last day of experiment (day 8), all the remaining larvae were individually counted for calculation of actual survival rate. The percentage of missing larvae was calculated as: 100-survival (%)-total observed mortality (%).

At the end of the experiment, the larvae were weighed at an accuracy of 0.1mg, the fishes being previously placed on paper towels in order to absorb adhering water.

Final mean body weights and survival rates of larvae were subjected to three way ANOVA (female x type of fish stocking x antibiotic) in the first experiment and to one way ANOVA followed by Duncan's multiple range test to determine significant differences among treatments in the second experiment. When necessary, angular transformation of data expressed as percentage was carried out in order to stabilise the residual variance.

RESULTS

Experiment 1

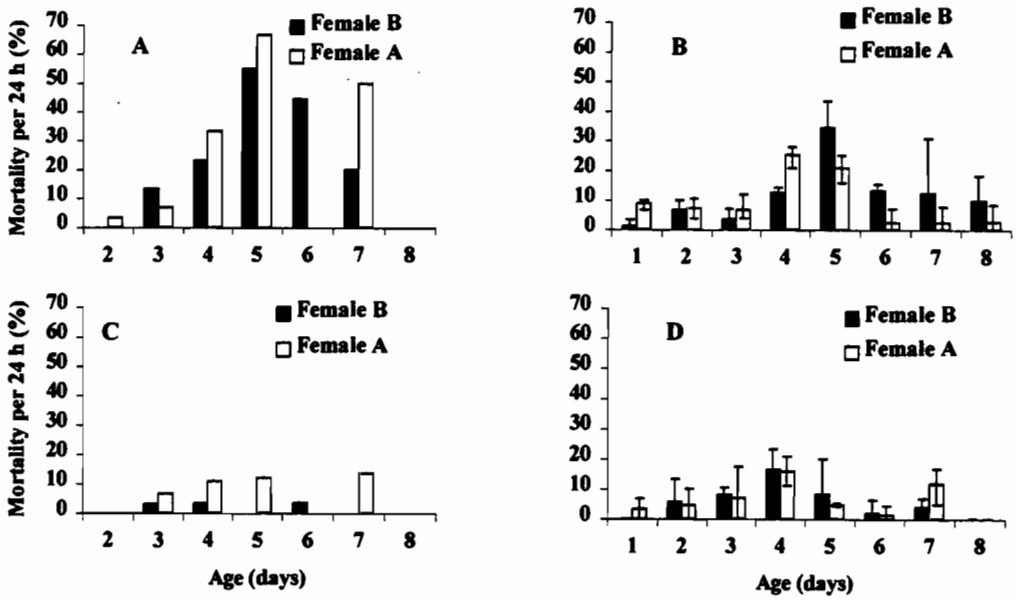
Evolution of daily mortality.

The evolution of mortality per period of 24 h for larvae obtained from the two different females, reared in group or isolated, with antibiotic or not, is given in Figure 1. The mortality of the isolated larvae was recorded from the second day only (Fig. 1A, 1C), while some dead larvae were already counted in the groups of fish (Fig. 1B, 1D). This is because the quasi totality of isolated larvae surprisingly died during the first night after stocking in their containers; these larvae were immediately replaced for a new starting at day 1.

From Figure 1, the mortality of isolated larvae from both females seemed to be higher than that observed in the group of larvae during the experimental period. However, this was not confirmed when the percentages of missing larvae were taken into account (see *infra* and Table I).

In the treatments without antibiotic, the percentage of dead fish per 24 h increased progressively from day 1 to day 5 and slightly decreased afterwards. The highest daily mortality was observed at 5 days of age for both isolated larvae (60-70%) and groups (20-35%). It should be noted that in this experiment, the first peak of mortality, which is generally observed at day 2 during larval rearing of *P. hypophthalmus*, did not occurred (Fig. 1B).

The treatment with Oxytetracycline allowed a clear lowering of larval mortality (Fig. 1A, 1C). For the isolated larvae, the percentage of dead fish per period of 24 h remained very low during the whole experimental period, not exceeding 5% and 15% for larvae from female B and A, respectively.



Vertical bars indicate range between replicates.

Figure 1: Evolution of mortality per period of 24 h up to the age of 8 days in *P. hypophthalmus* larvae obtained from two different females and reared isolated (A) or in group (B) without antibiotic, and isolated (C) or in group (D) with antibiotic.

Broodfish	Fish stocking	Feeding	Antibiotic OTC (5 mg.L^{-1})	Final body weight (mg)	Survival rate (%)	Missing larvae (%)
Female A	Isolated	*Artemia*	No	18.0	6.7	0.0
Female B	Isolated	*Artemia*	No	15.3	13.3	0.0
Female A	Isolated	*Artemia*	Yes	21.9	51.9	0.0
Female B	Isolated	*Artemia*	Yes	21.6	83.3	0.0
Female A	Group	*Artemia*	No	-	0.0	43.3
Female B	Group	*Artemia*	No	17.0	10.0	25.6
Female A	Group	*Artemia*	Yes	18.4	30.0	30.0
Female B	Group	*Artemia*	Yes	18.9	53.3	10.0
Female A	Group	No feeding	No	-	0.0	51.0
Female B	Group	No feeding	No	-	0.0	31.0

Table I : Mean body weight, survival rate and percentage of missing fish after 8 days of rearing for *P. hypophtalmus* larvae obtained from two different females, reared in group or isolated with antibiotic or not in the first experiment.

Similarly, for larval groups, the daily mortality was generally lower than 10%, except on day 4 at which values of 16% were observed for both females.

Survival rate

The survival rates obtained at the end of the experiment ranged between 0% and 83% depending on rearing conditions and female parent (Table I). The results of analysis of variance indicated significant differences in larval survival depending on the female parent ($p<0.01$), stocking conditions (isolated or grouped larvae; $p<0.05$) and antibiotic treatment ($p<0.001$). No interaction was found between these three factors.

The highest survival rate (83%) corresponded to the isolated larvae from female B reared using antibiotic. In all rearing situations, except in absence of feeding, the survival of larvae from female B was systematically higher (about 10 to 30%) than that of larvae from female A. Group rearing condition resulted in lower survival than

isolated rearing in water containing antibiotic or not. The use of Oxytetracycline led to a clear improvement of the survival of *P. hypophthalmus* larvae after 8 days of culture. The increases in survival rates when using antibiotic were of 45% and 30% for larvae from female A and of 70% and 43% for larvae from female B, in isolated conditions and in groups respectively.

No survival was observed for larvae from female A fed with *Artemia* and reared in groups without antibiotic, as well as for groups of larvae from both female A and B without feeding.

Missing larvae

When larvae were reared in isolated conditions, no missing fish were registered. In all group-rearing treatments, the percentage of missing fish was always about 20% higher for larvae from female A compared to larvae from female B (Table I). The percentage of fish that disappeared during the experiment were similar for both females in the groups without antibiotic fed with *Artemia* nauplii and in groups without feeding. Missing fish tended to be less numerous in the groups reared with antibiotic in comparison to groups without antibiotic (Table I).

Growth

At the end of the 8-days rearing period, the mean body weight of larvae was comprised between 15.3 and 21.6 mg and did not differ significantly between treatments (Table I).

Experiment 2

Evolution of daily mortality

The evolution of daily mortality for all treatments during the experiment is given in Figure 2.

The control showed 2 peaks of mortality at day 2 (up to 41%) and at day 7 (up to 31%). These peaks were not observed when Oxytetracycline (maximum 14,2%) or Chloramine-T (maximum 13,2%) were used, whatever the dose applied.

At day 2, the highest mortality (up to 83%) was observed with the treatment "formalin + MGO" at 2 $mg.L^{-1}$ (Fig. 2). The lowest mortality obtained during the first 3 days using this medicine corresponded to the lowest dosage (1,5 $mg.L^{-1}$).

The treatment with formalin at doses of 1.5 and 2 $mg.L^{-1}$ resulted in two peaks of mortality at day 2 and day 6 respectively. With a higher dose of formalin (2.5 $mg.L^{-1}$), the daily mortality remained low (less than 10%) during the whole experiment (Fig. 2).

The mortality stopped completely at day 3 with Oxytetracycline at a dose of 20 $mg.L^{-1}$, day 4 with Chloramine-T at 2.5 $mg.L^{-1}$, day 7 with formalin at 2.5 $mg.L^{-1}$ and day 5 with "formalin + MGO" at a dose of 2,5 $mg.L^{-1}$.

Survival rate

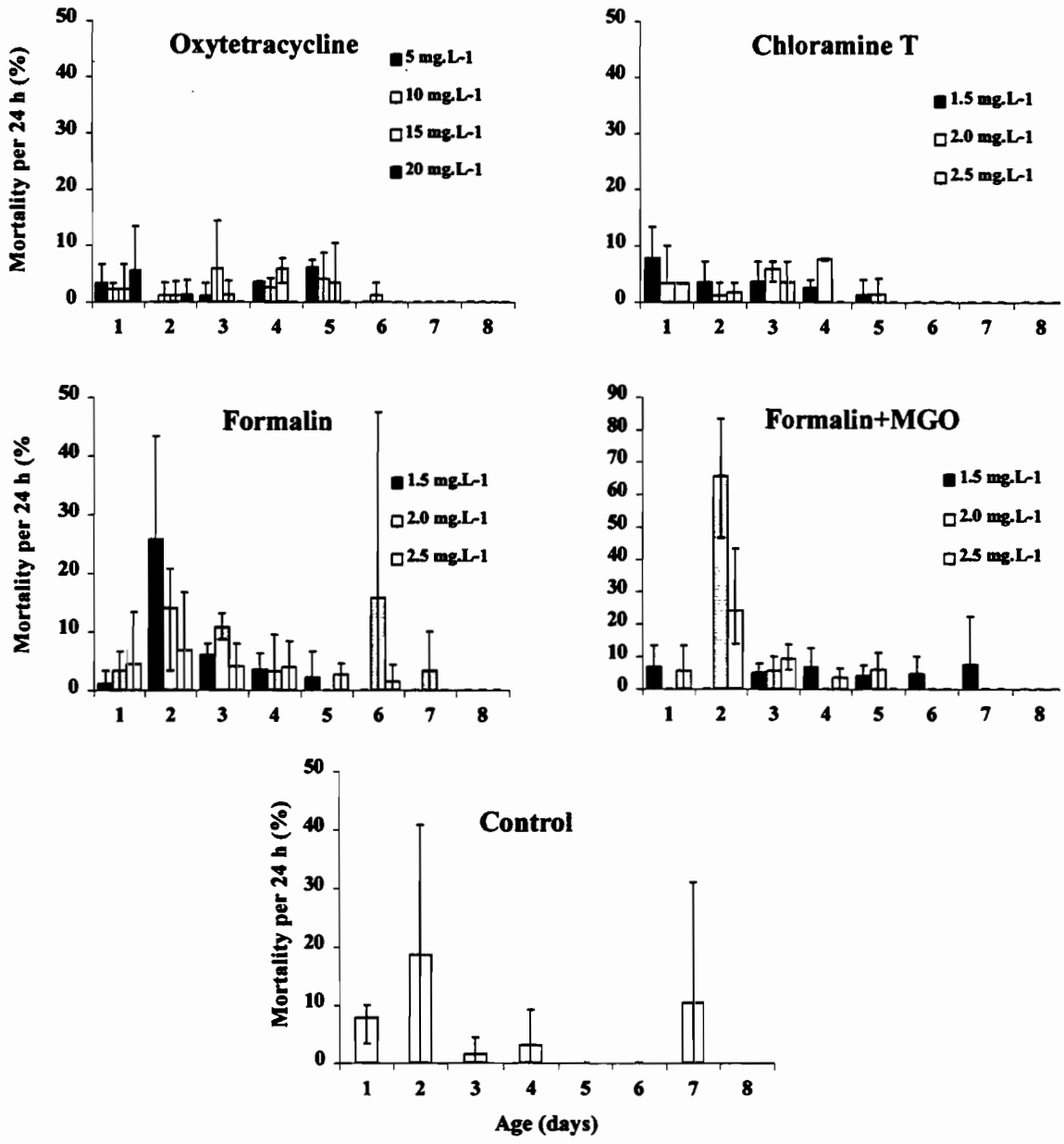
The survival rate of *P. hypophthalmus* larvae was not significantly different when using Oxytetracycline and Chloramine-T at any of the doses tested, and formalin at 1.5 or 2.5 $mg.L^{-1}$ (Table 2). The lower mean survival observed using formalin at 2.0 $mg.L^{-1}$ was due to a low value in one of the replicates. The best results were obtained with Oxytetracycline at all doses (81-87%), Chloramine-T at doses of 1.5 and 2.5 $mg.L^{-1}$ (81 and 87%) and formalin at 2,5 $mg.L^{-1}$ (76%). Survival rates obtained using Oxytetracycline at 5, 15 or 20 $mg.L^{-1}$ and Chloramine-T at 2.5 $mg.L^{-1}$ were significantly higher than that of the control groups (52%). Formalin at doses of 1.5, 2.0 and 2.5 $mg.L^{-1}$ did not led to significant difference from the control, even though higher dose gave a downward trend of mortality. Application of "formalin + MGO" at a dose of 2 $mg.L^{-1}$ led to the lowest survival rate (29%).

Missing larvae

The percentages of missing larvae were low for all treatments, ranging from 1 to 10% (Table 2). Although missing larvae tended to be more numerous in the control (10%), no significant difference was found between all treatments.

Growth

At the end of the 8-day experiment, the mean body weight of larvae reared with any doses of Oxytetracycline (21.5-22.2 mg), Chloramine-T (21.7-23.7 mg) and formalin (21.8-22.3 mg) were not significantly different from each other and from the control (21.3 mg). However, the mean larval body weight obtained with all doses of "formalin + MGO" (12.5-18.4 mg) were significantly lower than that of larvae reared with all other medicines tested (Table 2).



Vertical bars indicate range between replicates.

Figure 2: Evolution of mortality per period of 24 h up to the age of 8 days in *P. hypophthalmus* larvae reared with Oxytetracycline, Chloramine-T, formalin, formalin + MGO and without medicine.

DISCUSSION

In this study, the results obtained in the control groups of the two experiments carried out (no prophylactic treatment, feeding with *Artemia* nauplii) illustrated once again the problems generally encountered during the larval rearing of *P. hypophthalmus* : low survival rates of larvae after one week with two peaks of mortality occurring generally at 2 and then 5-7 days of age. The present study demonstrated, however, that the survival rate of *P. hypophthalmus* larvae were systematically improved when rearing was carried out in water containing antibiotic. The use of Oxytetracycline proved to be highly efficient in preventing the two peaks of mortality observed at 2 and 5-7 days of age in the control situation without prophylactic treatment. This result suggests that mortality of larvae at these stages was mostly a consequence of pathogenic infection and not of cannibalistic behaviour.

Several supplementary arguments indicated that the influence of cannibalistic behaviour was limited in the two experiments: 1) direct observations of cannibalism were only occasional, 2) the evolution of daily mortality in isolated and

Treatment	Dose ($mg.L^{-1}$)	Final body weight (mg)	Survival rate (%)	Missing larvae (%)
Oxytetracycline	5	22.0 ^{d}	83.5 ^{cd}	3.3 ^{a}
Oxytetracycline	10	22.2 ^{d}	81.1 ^{bcd}	3.3 ^{a}
Oxytetracycline	15	21.5 ^{d}	83.5 ^{cd}	2.2 ^{a}
Oxytetracycline	20	21.6 ^{d}	86.7 ^{d}	6.7 ^{a}
Formalin	1,5	22.1 ^{d}	60.0 ^{bcd}	5.6 ^{a}
Formalin	2,0	21.8 ^{d}	55.6 ^{abc}	5.6 ^{a}
Formalin	2,5	22.3 ^{d}	75.5 ^{bcd}	3.3 ^{a}
Chloramine-T	1,5	23.7 ^{d}	81.1 ^{bcd}	1.1 ^{a}
Chloramine-T	2,0	23.2 ^{d}	71.1 ^{bcd}	8.9 ^{a}
Chloramine-T	2,5	21.7 ^{d}	86.7 ^{d}	5.0 ^{a}
Formaldehyde + MGO	1,5	18.4 ^{bc}	71.5 ^{bcd}	1.1 ^{a}
Formaldehyde + MGO	2,0	12.5 ^{a}	28.9 ^{a}	1.1 ^{a}
Formaldehyde + MGO	2,5	17.0 ^{b}	58.1 ^{abc}	4.4 ^{a}
Control	-	21.3 ^{cd}	52.2 ^{ab}	10.0 ^{a}

Figures with the same superscripts in the same column are not significantly different ($p<0.05$).

Table 2: Survival rate, mean body weight and percentage of missing larvae as a function of prophylactic treatment for *P. hypophthalmus* after 8 days of larval rearing in the second experiment.

grouped larvae followed similar kinetics and 3) the fact that percentages of missing larvae were equivalent in fish fed *Artemia* or unfed (first experiment) tended to confirm that the missing larvae were not ingested by congeners. A reduction of cannibalism was reported in both *P. hypophthalmus* (Hardjamulia *et al.*, 1981) and *Clarias gariepinus* (Hecht & Appelbaum, 1988) when the larvae received adequate and abundant feeding.

In this investigation, Oxytetracycline was used at doses ranging between 5 $mg.L^{-1}$ and 20 $mg.L^{-1}$ during 8 days. No significant difference in survival rates and mean body weight of the larvae was found as a function of the dose used. However, the follow up of daily mortality (Figure 2) showed that the mortality stopped sooner (day 3) with a dose of 20 $mg.L^{-1}$ than with the other doses. The dosage recommended by De Kinkelin *et al.* (1985) for treatment of fish was also 20 $mg.L^{-1}$ during 6-8 days.

The pathogenic agent responsible of the infection of *P. hypophthalmus* larvae was identified as *Aeromonas hydrophila*, which could be isolated from the larvae as early as 2 days of age (Hambali *et al.*, 1999). *Aeromonas hydrophila* is widespread in the environment and can even been found in the intestinal flora of fish without pathogenic consequences. Generally, pathogenic effects become manifest when the fish are adversely effected by some other factor. The primary prophylactic measure against *A. hydrophila* is stress avoidance. Stress can result from protozoan infection, inadequate hygiene, abundance of particulate matter in the water, handling and crowding, low oxygen content and chronic exposure to various pollutants (Kabata, 1985).

In the present experiments, the larvae were reared in limited living space in regularly changed stagnant water. However, it is assumed that these rearing conditions were not deleterious to the larvae because similar survival rates were observed in many occasions when larvae from the same spawn were reared in 30 L tanks supplied with water of high quality in a re-circulating system (unpubl. data).

It has been observed that at the age of 2-4 days the larvae search for food in a passive manner; they swim actively with the mouth open and close their jaws when meeting a prey or a congener (Hardjamulia *et al.*, 1981; unpublished observations). This behaviour may result in cannibalism, but very often the bitten congener escapes and continues to swim. It was hypothesised that wounded body of larvae resulting from this behaviour may facilitate the entrance of pathogens and subsequent mortality of larvae. In that case bacterial infection could be, at least for a part, a secondary consequence of fish behaviour. The fact that survival of larvae was significantly higher for isolated larvae than for

larvae reared in groups (exp. 1) tended to support this hypothesis. Nevertheless, further investigations on interaction between behaviour and survival of larvae remain necessary to fully clarify this question.

In any cases, treatments using antibiotic have to be administrated at the effective dosage and during enough time to ensure elimination of bacteria (De Kinkelin *et al.*, 1985). As a consequence of inappropriate use of an antibiotic, bacteria, such as *Aeromonas hydrophila* (Aoki *et al.*, 1971) and *Aeromonas salmonica* (Popoff & Davaine, 1971), can develop resistance to this antibiotic which is transmitted to the next generations. Therefore the systematic use of antibiotics did not appear as a sustainable way for larval rearing of *P. hypophthalmus* at the production scale and alternative solutions had to be found.

The present study demonstrated that survival rates and final mean body weight as high as those obtained using antibiotics could be reached with applications of disinfectants such as Chloramine-T and formalin.

With Chloramine-T results obtained at doses of 1.5 and 2.0 $mg.L^{-1}$ did not significantly differed from the control. Therefore, the higher dose of 2.5 $mg.L^{-1}$ is recommended for a routine application of this disinfectant.

Although no significant differences in survival and body weight of larvae were found when using formalin at doses of 1.5 and 2.5 $mg.L^{-1}$, the results tended to be improved at the highest dose. It should be noted that the peak of mortality observed at day 2 was clearly reduced with 2.5 $mg.L^{-1}$ formalin, while it remained quite high with the lower doses (Fig. 2). De Kinkelin *et al.* (1985) recommended treatments at a dose of 25-40 $mg.L^{-1}$ of formalin during 24-48 hours in closed water, although the corresponding species and age of fish were not given. It is thus possible that higher dose of formalin than those tested in the present study could lead to further improvement of the results. Complementary investigations remain to be performed in order to identify the optimal dose of formalin for larval rearing of *P. hypophthalmus*. This latter disinfectant presents a particular interest because it is inexpensive and very easy to obtain.

By contrast, the larvae of *P. hypophthalmus* appeared sensitive to "formalin + MGO", this disinfectant leading to similar or even lower survival than in the control and to the lowest final body weights. In *Silurus glanis*, it was assumed that treatments containing malachite green were toxic for the larvae and could be administered at dosage not exceeding 0.1 $mg.L^{-1}$ for a maximum period of 1 hour (Schlumberger, 1993). De Kinkelin *et al.* (1985) recommended a dosage of 0.1 $mg.L^{-1}$ of MGO for a continuous treatment in closed water. Bastiawan, (1988) showed that *Saprolegnia* sp. were sensitive to MGO at doses of 1-5 $mg.L^{-1}$. However, dose of 1 $mg.L^{-1}$ represented a threat for newly hatched carp larvae. In the present investigation, MGO was used in association with formalin at doses of 0.006, 0.008 and 0.01 $mg.L^{-1}$, respectively. Therefore the dosages used were not high but the duration of treatment (24 h) appeared to be too long for the *P. hypophthalmus* larvae. For these reasons, "formalin + MGO" can hardly be recommended for an application during larval rearing of *P. hypophthalmus*, particularly when compared to other disinfectants such as Chloramine-T and formalin.

In this study, the survival rates at day 8 were respectively 52, 10 and 0% for larvae reared without antibiotic, and then 84, 53 and 30% when the same larvae were reared in water containing permanently 5 $mg.L^{-1}$ of Oxytetracycline. As the rearing conditions were strictly equivalent in the different experiments performed, these results indicate that the survival rates of *P. hypophthalmus* larvae was strongly dependent on the initial quality of the larvae or eggs. This may explain, at least for a part, the survival variability observed in the preliminary investigations and in productions on farms. The causes of these discrepancies are unknown and further investigations would be necessary to identify criteria that may allow assessment of the initial quality of the larvae.

CONCLUSIONS

The present study indicated that bacterial disease and female parents had more influence on survival rates of *P. hypophthalmus* larvae than a direct effect of cannibalistic behaviour.

Survival rates and mean body weights of the larvae were considerably improved in water treated with either Oxytetracycline (5-20 $mg.L^{-1}$) and Chloramine-T (2,5 $mg.L^{-1}$) in comparison to

control situation without medicine. A clear tendency of improved survival rates was also observed with the use of formalin (2.5 $mg.L^{-1}$). As it is known that the use of antibiotics may induce bacteria resistance when applied in an inappropriate manner, the use of these disinfectants is recommended for safer treatment or prevention against bacterial diseases in commercial *P. hypophthalmus* hatcheries.

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EFFECTS OF FREQUENCY AND PERIOD OF FEEDING ON GROWTH AND FEED UTILISATION IN TWO ASIAN CATFISHES, *PANGASIUS BOCOURTI* (SAUVAGE, 1880) AND *PANGASIUS HYPOPHTHALMUS* (SAUVAGE, 1878)

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Abstract

Feeding to satiation in *P. bocourti*, resulted in higher growth with increased meal frequency. However, growth performance of two-meal or three-meal feeding was not significantly different at all. Moreover, feeding three meals a day led fish to consume a relatively higher amount of feed than fish administered two meals a day. When compared feed intake of the feeding at 12:00 to those of the feeding at 17:00, *P. bocourti* tends to take more food at 17:00 feeding, even though both feedings had the same interval between meals. It is obvious that besides feeding frequency, feeding time is an important factor affecting increased feed intake in *P. bocourti* when they were subjected to feed to satiation.

When fed an equal fixed ration, *P. bocourti* and *P. hypophthalmus* had growth performance and feed efficiency which did not alter so much with increased meal frequency. However, one-meal feeding in the morning resulted in a lower growth and reduced feed utilisation than multiple feeding treatments. The reduced growth and lower feed utilisation here were apparently caused by feeding period rather than by frequency effect, since one-meal feeding during the night gave the same growth and feed efficiency as other feeding regimes in the night-time. Therefore, feeding in early morning resulted in lower feed intake and reduced assimilation efficiency as well. Moreover, feeding period during the night exhibits a higher growth and better feed efficiency than diurnal feeding in both species. Increased meal frequency and nocturnal feeding of an equal fixed ration usually resulted in a relatively higher fat deposition in both species with a higher adipose-somatic and hepato-somatic index.

When combined data of feeding during the day and during the night, it showed that continuous feeding in *P. bocourti* and *P. hypophthalmus* led to a significantly lower growth performance and reduced feed efficiency than fractionated feeding. In addition, when fed a continuous regime, *P. bocourti* had a significantly higher coefficient of variation of final weight than other fractionated feedings.

INTRODUCTION

Pangasius bocourti and *Pangasius hypophthalmus* are two indigenous fish species living in the Mekong River (Roberts & Vidthayanon, 1991). The culture of *P. bocourti* in floating cages has been a production with an annual figure of 13 000 tons (Cacot, 1994). The culture of latter species in earthen ponds has been a long traditional activity with an estimated figure of several tens thousand tons.

When supplied sufficiently feed in quality as well as in quantity, fish has growth rate depending upon two main factors: voluntary feed intake and feed assimilation efficiency (Brett, 1979; Diana *et al.*, 1988). Feeding frequency is one of important factor affecting both voluntary feed intake and assimilation efficiency (Brett, 1971). There are many previous studies on frequency in which fish was fed to satiation; thus, difference in fish growth and feed efficiency reflects an influence of frequency on feed intake rather than on feed assimilation efficiency.

Besides frequency, feeding period also has a great influence on growth and feed utilisation in fishes (Brett, 1979), especially in catfish which is usually said to have a mainly nocturnally trophic activity. Many studies confirmed a better growth

performance in catfish fed during the night: *Heterobranchus longifilis* (Kerdchuen & Legendre, 1991), *Silurus glanis* (Authouard *et al.*, 1987), *Clarias gariepinus* (Hogendoorn, 1981), *Ictalurus punctatus* and *Ictalurus melas* (Boet, 1981). Nevertheless, it appears that the influence of light/dark alternation on trophic activity of some catfish may be masked under restricted conditions of temperature or dissolved oxygen (Boujard & Luquet, 1996)

Therefore, the objective of the study is to evaluate effects of frequency and period of feeding on growth and feed utilisation when *Pangasius bocourti* and *Pangasius hypophthalmus* are allowed to feed to satiation or an equal fixed ration.

MATERIALS AND METHOD

Experiment fish and culture facilities

Experimental fish were obtained from broodfish cultured in earthen ponds. They were induced spawning with a human gonadotropin hormone injection. Fingerlings originated from one female, were acclimatised in one week for *P. bocourti* and two weeks for *P. hypophthalmus* since this species is more sensitive to confined condition. *P. bocourti* fingerlings of 7.3-7.5 g (first experiment) and of 4.8-5.0 g (second experiment) were cultured at a stocking density of 25 fish per 50 L aquarium in a recycling water system. *P. hypophthalmus* fingerlings of 4.8-5.0 g were reared in outdoor concrete tanks (1 x 1 x 0.6 m) at a stocking density of 25 fish per tank. Water in tank was pumped from a storage earthen pond (600 m^2). The pond contains microphyte, tilapia and silver carp. The lightning condition is natural with a 13D/11N photoperiod.

Water in aquarium were aerated and exchanged at a flow rate of 4-5 $L.min^{-1}$. Dissolved oxygen and pH were monitored twice a week, ranged from 4.3 to 5.5 $mg.L^{-1}$ and from 7 to 7.5, respectively. Ammonia and nitrite, monitored once a week, varied from 0.3 to 0.8 $mg.L^{-1}$ and from 0.01-0.04 $mg.L^{-1}$, respectively. Water temperature in aquarium ranged from 28°C to 30°C.

Water in concrete tanks were exchanged at a flow rate of 10–12 $L.min^{-1}$. Dissolved oxygen in tanks, measured early in the morning, ranged from 3.0-3.5 $mg.l^{-1}$, pH from 7.0 to 7.3. Ammonia and nitrite varied from 0.2 to 0.4 $mg.L^{-1}$ and from 0.02 to 0.04 $mg.L^{-1}$, respectively. Water temperature in tanks ranged from 28°C to 33°C.

Feed and feeding

Fish were fed with pelleted feed which were produced with the following formula:

Fish meal	70%
Soybean meal	20%
Rice starch	8%
Soybean oil	1%
Vitamin and mineral premix	1%

The feed has a proximate composition as follows:

Moisture	3.10%
Protein	37.17%
Lipid	5.41%
Mineral	23.50%
Cellulose	8.96%

There are three experiments in the present study. The first experiment was a satiation feeding trial for *P. bocourti*. Feed was distributed by hand one, two or three meals a day piece by piece until fish stopped feeding. Consumed feed was daily registered. There are three replicates of each treatment for the experiment.

Feeding procedure:

One meal feeding	7:00		
Two meal feeding	7:00	17:00	
Three meal feeding	7:00	12:00	17:00

In the second experiment (for *P. bocourti*) and third experiment (for *P. hypophthalmus*), fish were fed an daily equal fixed ration of 5-6% biomass (5% for the first week and 6% for the following). Feeding adjustment took place every two weeks. The feeding level was sufficient that fish fed one meal a day and consume completely distributed feed. Both second and third experiment had 8 treatments, including 1, 2, 3 meals or continuous feeding which were distributed during the day or during the night. There are two replicates of tanks or aquarium for each treatment.

Sampling and data analysis

Each experiment lasted six weeks. Fish were weighed in batch every two weeks to adjust feeding for the second and third experiment. Initial and final weight were taken individually at a precision of 0.01g. At the end of the experiment, ten fish in each tank or aquarium were sacrificed to measure liver and abdominal fat in order to determine hepato-somatic index: HSI = (liver weight/total weight) x 100 and adipo-somatic index: ASI = (abdominal fat/total weight) x 100.

Treatment	Meal number	Feeding period	Feeding time		
1D	1 meal	Daytime	7:00		
2D	2 meals		7:00		17:00
3D	3 meals		7:00	12:00	17:00
CTD	Continuous		7:00	to	17:00
1N	1 meal	Night time	19:00		
2N	2 meals		19:00		05:00
3N	3 meals		19:00	24:00	05:00
CTN	Continuous		19:00	to	05:00

Feeding procedure for the second experiment (*P. bocourti*) and the third experiment (*P. hypophthalmus*). Continuous feeding were done with an automatic feeder.

Ten other fish were subjected to chemical carcass analysis.

Data were analysed using the SPSS software for statistical analysis. Final mean weights, specific growth rates (SGRs), coefficient of variance of final mean weigh (CV%); HSI and ASI were subjected to GLM analysis (General Linear Model) and Duncan's multiple range test to determine the significant difference at 0.05 level.

RESULT

Frequency and period of feeding in Pangasius bocourti

Growth performance and feed efficiency

The first experiment, in which *P. bocourti* is allowed to consume feed to satiation, demonstrated that fish fed three meals a day, had a highest specific growth rate of 3.63%.d^{-1} when compared to one meal feeding (2.61%.d^{-1}) or two-meal feeding (3.39%.d^{1}). However, the SGR in three meal frequency was not significantly different from those in fish fed two meals a day (Table 1). Therefore, feeding to satiation resulted in higher growth with increased meal frequency. Concerning to a total daily feed intake, Figure 1 demonstrated that feeding one meal a day resulted in lower feed intake than fish administrated two or three meals a day.

Moreover, feeding three meals a day led fish to consume a relatively higher amount of feed than fish administered two meals a day. When compared feed intake of the feeding at 12:00 to those of the feeding at 17:00, *P. bocourti* tends to take more food at 17:00 feeding, even though both feedings had the same interval between meals. It is obvious that besides feeding frequency, feeding time is an important factor affecting increased feed intake in *P. bocourti* when they were subjected to feed to satiation. Increased frequency of feeding to satiation also raised food conversion ratio (FCR) but the FCRs in two or three meal feeding was not significantly different. They were 2.12 and 2.19, respectively. The coefficient of variance of final weight (CV%) was not significantly different among 1, 2 or 3 meal frequencies.

The second experiment in which *P. bocourti* received an equal fixed ration, demonstrated that fish growth and its feed efficiency did not alter so much with increased meal frequency. One meal

Growth and feed efficiency	One Feeding	Two feedings	Three Feedings
Initial weight (g)	7,52 ± 0.15 ^{a}	7,50 ± 0.23 ^{a}	7,35 ± 0.06 ^{a}
Final weight (g)	22.57 ± 1.84 ^{a}	31.19 ± 3.29 ^{b}	34.60 ± 0.77 ^{b}
CV (%)	16.59 ± 2.26 ^{a}	15.50 ± 0.15 ^{a}	13.43 ± 1.65 ^{a}
SGR (%.day^{-1})	2.61 ± 0.18 ^{a}	3.39 ± 0.21 ^{b}	3.63 ± 0.12 ^{b}
Hepato-somatic index (%)	2.42 ± 0.34 ^{a}	3.09 ± 0.44 ^{ab}	3.66 ± 0.36 ^{b}
Adipo-somatic index (%)	2.01 ± 0.22 ^{a}	2.46 ± 0.44 ^{ab}	3.61 ± 0.46 ^{b}
Feed intake per fish (g)	25.58 ± 0.25 ^{a}	49.58 ± 2.28 ^{b}	57.90 ± 2.00 ^{c}
FCR: food conversion ratio	1.71 ± 0.18 ^{a}	2.12 ± 0.35 ^{b}	2.19 ± 0.11 ^{b}

Mean ± SD; CV(%): coefficient of variance of final weight; SGR: specific growth rate. Figures in the same row followed by similar superscripts letter, are not significantly different (p>0.05).

Table 1: Growth performance and feed efficiency in *P. bocourti* fed to satiation in function to increased meal frequency. Fish were fed 1, 2 or 3 meals a day at 7:00, 12:00 or 17:00. Fish intake was calculated per individual in a period of 42 days.

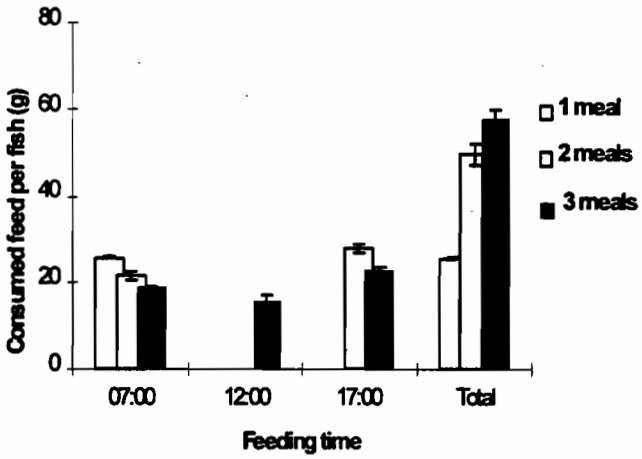

Figure 1: Amount of ingested feed by each fish (*P. bocourti*) in a period of 42 days in response to feeding time. Fish were fed to satiation with 1 meal, 2 meals or 3 meals a day either at 7:00, 12:00 or 17:00.

feeding in the day resulted in a lower growth and reduced feed utilisation than multiple feeding treatments. The SGR was only 3.39%.d^{-1} , lowest when compared to other treatments (3.6-4.0%. day^{-1}) and the FCR was 1.56, highest when compared to others. The reduced growth and lower feed utilisation here were apparently caused by feeding period rather than by frequency effect. Since one meal feeding during the night gave the same growth and feed efficiency as other feeding regimes. The SGR of 4.00%.d^{-1} in one feeding at 19:00 was similar to figures of nocturnal feedings, ranging from 3.66 to 4.02%.d^{-1} (Table 2).

In contrary to fractionated feeding, continuous feeding during the day or the night in the second experiment usually gave a tendency of lower growth and reduced feed efficiency in comparison to fractionated meal feeding. When combined data of feeding during the day and during the night, it showed that continuous feeding led to a significantly lower growth performance and reduced feed efficiency than fractionated feeding (Table 2). In addition, when fed a continuous regime, *P. bocourti* had a significantly higher coefficient of variation of final weight (CV%) than other fractionated feedings. The CV% varied from 32.07% in the day to 22.52% in the night, compared to CV% of other feeding treatments ranging in 11.19% to 15.05%. Concerning to feeding period, the second experiment demonstrated that nocturnal feeding in *P. bocourti* gave evidently higher growth rates. SGRs were 3.68-4.02%.d^{-1} in night time feeding, when compared to 3.39-3.62%.d^{-1} in daytime feeding. Food conversion ratio were 1.21-1.37 in night-time, when compared to 1.39–1.56 in daytime regardless of either continuous or fractionated feeding.

Fat deposition in abdominal cavity

When allowed to feed to satiation in *P. bocourti*, increased feeding frequency obviously led to an increase in adipose-somatic index (ASI) and hepato-somatic index (HSI). The differences were statistically significant in one-meal feeding compared to two or three meal feeding and there were not significantly different between two and three meal feeding (Table 1). Meanwhile an equal fixed ration in *P. bocourti* particularly resulted in a

	Daytime				Night time			
	1 meal	2 meals	3 meals	Continuous	1 meal	2 meals	3 meals	Continuous
Initial weight (g)	5.76 ^{a}	5.76 ^{a}	5.71 ^{a}	5.79 ^{a}	5.72 ^{a}	5.53 ^{a}	5.65 ^{a}	5.71 ^{a}
Final weight (g) *, &	23.93 ^{a}	26.24 ^{bc}	26.11 ^{bc}	24.43 ^{ab}	30.60 ^{d}	29.89 ^{d}	29.90 ^{d}	26.74 ^{c}
CV (%) &, μ	14.93 ^{ab}	15.05 ^{ab}	12.66 ^{a}	32.07 ^{c}	14.22 ^{ab}	14.53 ^{ab}	11.97 ^{a}	22.52 ^{b}
SGR (%.day^{-1}) *, &	3.39 ^{a}	3.61 ^{a}	3.62 ^{a}	3.43 ^{a}	4.00 ^{c}	4.02 ^{c}	3.97 ^{bc}	3.68 ^{ab}
FCR *, μ	1.56 ^{c}	1.39 ^{abc}	1.39 ^{abc}	1.45 ^{bc}	1.22 ^{a}	1.21 ^{a}	1.25 ^{ab}	1.37 ^{bc}
HIS (%) *, &	3.25 ^{a}	3.42 ^{a}	3.44 ^{a}	3.54 ^{a}	4.28 ^{c}	3.83 ^{abc}	3.54 ^{abc}	4.19 ^{bc}
ASI (%) &, μ	2.18 ^{a}	2.14 ^{a}	2.22 ^{a}	2.55 ^{ab}	2.38 ^{a}	2.25 ^{a}	2.26 ^{a}	2.80 ^{b}

CV: coefficient of variance of final weight. SGR: specific growth rate. FCR: food conversion ratio. HSI: hepato-somatic index. ASI: adipo-somatic index. Figures followed by same superscript letters, are not significantly different at 5% statistical level (P>0.05).

* : a significant difference between night time to daytime feeding.

& : a significant difference among feeding frequency.

μ : a significant interaction between frequency and period of feeding.

Table 2: Growth performance, feed efficiency and fat accumulation in *P. bocourti* in response to frequency and period of feeding. Fish were fed an equal fixed ration of 1, 2, 3 meals a day or a continuous feeding. Feeding time were 7:00, 12:00, 17:00 for daytime feeding or 19:00, 24:00, 05:00 for night time feeding.

relatively higher fat deposition with increased meal frequency than a single feeding, but the difference in ASI and HSI among treatments was not statistically significant (P>0.05). However, continuous feeding gave a significantly higher ASI and a trend of higher in HSI than those in fractionated feeding (Table 2). In addition, nocturnal feeding of an equal fixed ration in *P. bocourti*, resulted in a significantly higher HSI and a tendency of higher ASI than those fed in the daytime (Table 2).

Frequency and period of feeding in Pangasius hypophthalmus

Growth performances and feed efficiency

The third experiment in which *P. hypophthalmus* fed an equal fixed ration led to the similar result as in *P. bocourti*. Increased feeding frequency did not improve fish growth and feed efficiency. However, one meal feeding in daytime resulted in a lowest fish growth and a reduced feed assimilation. The SGR were only 2.65%.d^{-1} compared to SGRs of other treatments (3.12-3.52%.d^{-1}) and the FCR was 1.82 higher than others treatments (1.34-1.50). The reduced growth and lower feed utilisation here were apparently caused by feeding period rather than by frequency effect. Nocturnal feeding in *P. hypophthalmus* resulted in a higher fish growth and a better feed efficiency than diurnal feeding. The SGRs during the night were in a range of 3.27-3.52%.d^{-1}, compared to 2.65-3.37%.day^{-1}.in the daytime. The FCRs in the night were in a range of 1.34-1.41, lower than figures of 1.38-1.82 in daytime (Table 3). Continuous feeding in *P. hypophthalmus* even had a tendency of inferior growth and a reduced feed efficiency when compared to fractionated feeding. However, The CV% in the continuous feeding was not significantly different from those of fractionated feeding. That was different from the case in *P. bocourti* (Figure 2). Moreover, one meal feeding in *P. hypophthalmus* led to a relatively higher CV% than other treatments. The CV% in one meal feeding during the day or the night were 19.57% and 23.24% respectively, relatively higher than others (11.74%-17.96%).

In summary, when an equal feed ration occurred in *P. bocourti* and *P. hypophthalmus* feeding frequency does not have important influence on fish growth and feed efficiency, except for one feeding in the morning. Conversely, feeding period during the night exhibits a higher growth and better feed efficiency than diurnal feeding in both species. Feeding in early morning resulted in lower feed intake and reduced assimilation efficiency as well.

Fat deposition in abdominal cavity

Increased feeding frequency in *P. hypophthalmus* did not alter so much the HSI and ASI. These figures were not significantly different at 0.05 level (Table 3). However, continuous feeding resulted in a tendency of higher fat deposition in abdominal cavity (Figure 3). On

Treatments	Daytime feeding				Night time feeding			
	1 meal	2 meals	3 meals	Continuous	1meal	2 meals	3 meals	Continuous
Initial weight	4,81 ^{a}	4,83 ^{a}	4,78 ^{a}	4,84 ^{a}	4,91 ^{a}	4,81 ^{a}	4,79 ^{a}	4,82 ^{a}
Final weight *, &	14,56 ^{a}	19,44 ^{c}	19,73 ^{cd}	17,92 ^{b}	20,26 ^{cd}	20,99 ^{d}	19,52 ^{cd}	18,96 ^{bc}
SGR *, &	2,65 ^{a}	3,34 ^{cd}	3,37 ^{cd}	3,12 ^{b}	3,37 ^{cd}	3,52 ^{d}	3,44 ^{cd}	3,27 ^{bc}
CV (%) &, μ	19,57 ^{ab}	14,45 ^{ab}	11,73 ^{a}	12,93 ^{a}	23,04 ^{b}	15,82 ^{ab}	13,56 ^{a}	17,96 ^{b}
FCR *,&	1,82 ^{c}	1.40 ^{ab}	1,38 ^{ab}	1,50 ^{b}	1,40 ^{ab}	1,34 ^{a}	1,37 ^{a}	1,41 ^{b}
HSI &	2,75 ^{b}	2,69 ^{b}	2,48 ^{a}	2,87 ^{bc}	3,04 ^{c}	2,71 ^{b}	2,72 ^{b}	2,86 ^{bc}
ASI *, &, μ	0,42 ^{ab}	0,44 ^{abc}	0,41 ^{a}	0,55 ^{bcd}	0,61 ^{d}	0,56 ^{cd}	0,4 6 ^{cd}	0,59 ^{d}

CV: coefficient of variance of final weight. SGR: specific growth rate. FCR: food conversion ratio. HSI: hepato-somatic index. ASI: adipo-somatic index. Figures, followed by same superscript letters, were not significantly different at 5% statistical level (P>0.05).

*: a significant difference between night time to daytime feeding.

&: a significant difference among feeding frequency.

μ: a significant interaction between frequency and period of feeding.

Table 3: Growth performance, feed efficiency and fat accumulation in *P. hypophthalmus* in response to frequency and period of feeding. Fish were fed an equal fixed ration of 1, 2, 3 meals a day or a continuous feeding. Feeding time were 7:00, 12:00, 17:00 for daytime feeding or 19:00, 24:00, 05:00 for night-time feeding.

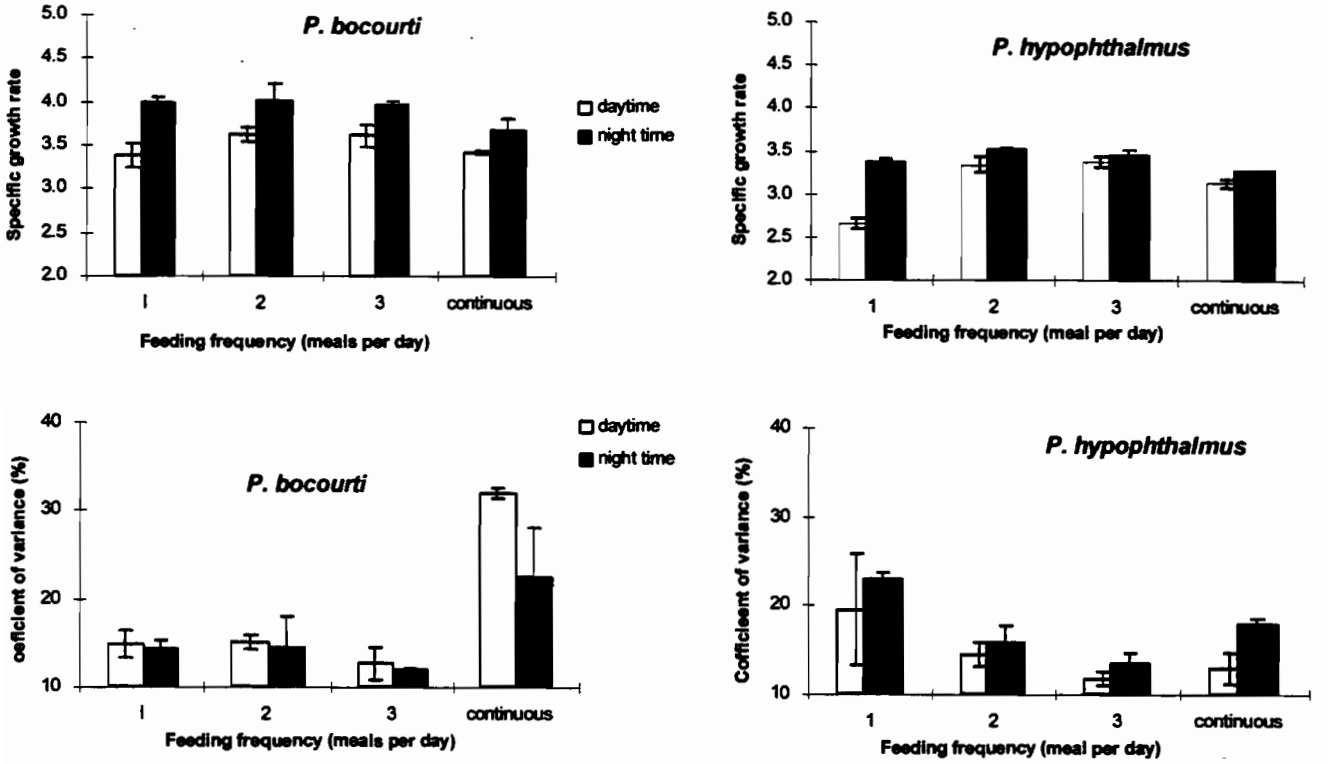

Figure 2: Effects of feeding frequency and period of feeding on the growth (SGR) and the coefficient of variance (CV%) of final weight in *P. bocourti* and *P. hypophthalmus*. Fish are fed an fixed equal ration at the feeding frequency 1, 2, 3 meals or a continued feeding in daytime or in the night-time.

the other hand, period of feeding had more clear effect on fat deposition in *P. hypophthalmus*. The HSI and ASI in the nocturnal feeding were relatively higher than those in diurnal feeding (Table 3).

DISCUSSION

When fed to satiation, *P. bocourti* consumed an amount of food that was raised by increasing feeding frequency. Increased feed intake with higher feeding frequency was reported in fishes (Adrews & Page, 1975; Grayton & Beamish, 1977; Lovell, 1979; Hogendoorn, 1981; Marian *et al.*, 1981; Sampath, 1982). However, the amount of feed consumed by increasing frequency reaches to a maximal feed intake due to the stomach content limitation. *P. bocourti* fed to satiation of two meal frequency, nearly reached to a maximal feed intake. Even thought three meal feeding had a relatively higher feed intake but the growth was not significantly different from those of two feedings. Increasing feed intake in three feeding was to one part possibly due a higher feed loss. Fish are fed by hand distribution to satiation, so feed distribution only stopped when observed a little bit of uneaten feed. Another part, the feed efficiency in high meal frequency tends to be reduced. It was reported that increased feeding frequency results in either a reduction of individual meal size or defecation of material before being fully digested, thus a lower feed assimilation occurred due to higher deamination cost and apparent specific dynamic action (Buurma & Dina, 1994).

Fish growth and feed utilisation in *P. bocourti* and *P. hypophthalmus* fed an equal fixed ration, do not rise with increased meal frequency. That is in accordance with studies in *Heterobranchus longifilis* (Kerdchuen & Legendre, 1991), *Salmo gairdneri* (Grayton & Beamish, 1977). However, some other studies have reverse results like in *Clarias gariepinus* (Hogendoorn, 1981), *Clarias fuscus* (Buurma & Diana, 1994), *Heteropneustes fossilis* (Marian *et al.*, 1981) and *Cyprinus carpio* (Huisman, 1974). Buurma and Diana (1994) demonstrated that *Clarias fuscus* fed three meals a day resulted in significantly higher growth than fish fed single feeding but fish fed two or three meals were not significantly different. It seems that lower growth in one meal feeding of their study was caused by feeding period rather than by changes in feeding frequency. The same

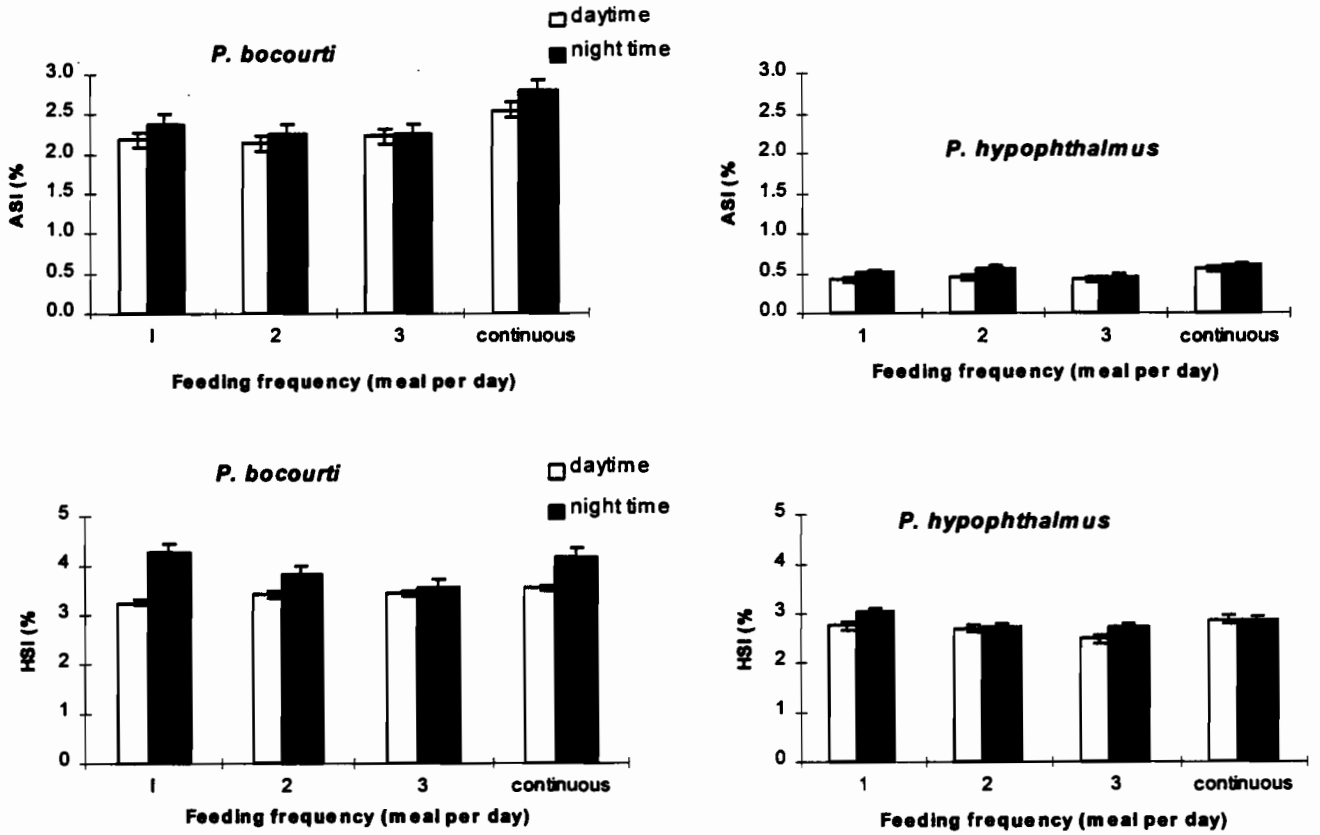

Figure 3: Effects of feeding frequency and period of feeding on adipose-somatic index (ASI) and hepato-somatic index (HSI) in *P. bocourti* and *P. hypophthalmus*.

phenomenon was observed in *P. bocourti* and especially in *P. hypophthalmus* in the present study. One meal feeding during the day (7:00) gave a lower growth but one meal during the night did not significantly differ from two or three meal feeding. It is usually impossible to determine whether the better growth observed in catfish with increased meal frequency is due to an augmentation in voluntary feed intake or to changes in nutrient retention efficiency (Boujard & Luquet, 1995). Mortality did not occur in our experiment and distributed feed was completely consumed as well, so fish were supposed to have an equal feed intake. When supplied an equal feed intake, *P. bocourti* and *P. hypophthalmus* did not alter fish growth and feed efficiency with increased meal frequency. Thus, it is possible to say that increased meal frequency of an equal ration does not have any effect on assimilation efficiency in both species. On the contrary, when fed to satiation fish tends to have faster growth with increased meal frequency. The higher growth in increased meal frequency is clearly due to increased feed consumption.

When fed a continuous feeding with an equal feed intake, *P. bocourti* and *P. hypophthalmus* do not ameliorate their growth performances and feed efficiency. The same result was observed in many fish species in which fish growth increased parallel to higher meal frequency and it rapidly get stable at a level of several limited meals per day. That corresponds to one daily meal in *Channa striatus* (Sampath, 1984), two meals a day in trout (Luquet *et al.*, 1981) and *Ictalurus punctatus* (Andrews & Page, 1975), one to two meals in *Heteropneustes fossilis* (Marian *et al.*, 1981). On the other hand, Kerdchuen and Legendre (1991) found that *Heterobranchus longifilis* had higher growth with continuous feeding and a same result was likewise found in *Clarias gariepinus* (Hogendoorn, 1981). Fractionating a daily distributed feed into many tiny meals in continuous feeding, therefore has divergent results on fish growth and feed efficiency of different fish species. The difference is possibly related to its feeding behaviour, especially to the presence of a distinct stomach of digestion physiology. When used a demand-feeder, Boujard and Leatherland (1992) found the aggregation of food demand in *Oncorhychus mykiss*. They explained that it is probably related to the presence of a district stomach, playing a predominantly storage role with little or no mixing

or digestive function. For fish having periodic access to food sources, the storage capacity is essential. Conversely, in browsing fish such as *Oreochromis aureus* (Hargreaves *et al.*, 1988) and *Carassius auratus* (Rozin & Mayer, 1961) the absence of a marked gut storage site is associated with a continuous feeding. It is strange to know that *Heterobranchus longifilis* and *Clarias gariepinus* have a marked stomach but these fish expose a higher fish growth in continuous feeding. More studies thus should be needed to explain the phenomenon. In our results, *P. bocourti* fed a continuous feeding exhibited a higher heterogeneity of fish size at the end of the experiment in comparison to fractionated feeding. The phenomenon was not observed in *P. hypophthalmus*. There is a hypothesis for such an observation. *P. bocourti* is a highly greedy species. The fish rapidly rushes to get feed in short period and therefore stronger and faster fish receive more feed distributed bit by bit in a continuous feeding. Conversely, *P. hypophthalmus* ingests slowly feed, more fish thus receives a small amount of feed distributed little by little in continuous feeding.

Increased meal frequency is usually applied in aquaculture to obtain highest growth with an acceptably lower feed efficiency. *P. bocourti* and *P. hypophthalmus* do not improve their feed assimilation efficiency with increased feeding frequency. However, one meal feeding is not enough to have an optimal growth since its stomach fullness is limited. *P. bocourti* fed to satiation, consumed only 5-6% body weight with one meal feeding but two or three feedings induced fish to ingest 12-15% of their biomass. In practice, aquaculturist thus can do the feeding at a two meal frequency to get much benefit from higher feed intake.

Results of the present study likewise demonstrated that nocturnal feeding resulted in higher growth and better feed efficiency than diurnal feeding in *P. bocourti* and *P. hypophthalmus*. Our result is in concordance with other species of Siluriformes (Kerdchuen & Legendre, 1991; Authouard *et al.*, 1987; Hogendoorn, 1981; Boet, 1981). Boujard and Luquet (1996) likewise marked several restricted condition which may effect feeding behaviour in fishes that possibly changes nocturnal feeding activity. Randolph and Clemens (1976) reported that *Ictalurus punctatus* in limited condition of temperature or dissolved oxygen depends much on two limited factors rather than on night/day alternation. In the present study, *P. bocourti* were cultured in a recycling water system with a dissolved oxygen of higher than 4 $mg.l^{-1}$ and *P. hypophthalmus* is an air breathing species (Browman & Kramer, 1985). Water temperature ranged in 28–30°C (*P. bocourti*) and in 28–33°C (*P. hypophthalmus*). Therefore, there was not any restricted condition in feeding trial for both species. The higher feed efficiency when fed in night-time may be in relation to a circadian variation metabolism and its hormonal control (Spieler, 1979; Noeske & Spieler, 1983). Leatherland *et al.* (1974) demonstrated that growth hormone and plasma prolactin in *Oncorhynchus nerka* were higher during the night. A same observation was reported in *Ictalurus punctatus* with a higher plasma corticoid concentration in night-time (Goudie *et al.*, 1983; Davis *et al.*, 1984). Sheridan, *et al.* (1986) found that the corticoid takes place in lipogenesis in fishes and the hormone likely takes part in protein and lipid metabolism (Donaldson *et al.*, 1979).

In the present study, one meal feeding in the morning in *P. bocourti* and *P. hypophthalmus* seemed not to be suitable. These fish had inferior fish growth performances and reduced assimilation efficiency in comparison to others of one meal feedings either at 17:00, 19:00 or 5:00. In *P. bocourti*, voluntary intake in the morning was lower than other feedings as well. Kerdchuen and Legendre (1991) also observed the same result in *Heterobranchus longifilis* when fed at 8:00. It is obvious to conclude that besides feeding period, feeding time likely plays an important role in stimulating voluntary feed intake as well as assimilation efficiency in both species.

Frequent feeding generally improved fish growth due to increased feed intake. That leads to elevate lipid deposition and change body composition. The event was observed in channel catfish (Noeske-Hallin *et al.*, 1985), rainbow trout (Grayton & Beamish, 1977) and hybrid Tilapia (Tung & Shiau, 1991). Satiation feeding in *P. bocourti* apparently induced higher fat deposition through a higher hepato-somatic and adipo-somatic index. However, when fed an equal feed ration, there are likewise a relative tendency of increased fat deposition with increased frequency in both *P. bocourti* and *P. hypophthalmus* . As a result, when fed highly increased meal frequency in continuous feeding these fish resulted in more clearly higher fat

accumulation than single feeding. Tung and Shiau (1991) observed hybrid Tilapia, *Oreochromis niloticus x O. aureus*, fed six times a day had higher fat body content compared to fish fed twice a day. They also observed the higher 6-phosphogluconate dehydronase in fish fed six times a day. Conversely, there are some other studies in which higher feeding frequency suppressed lipid deposition in *Epinephelus akaara* (Kayano *et al.*, 1993), *Plecoglossus altivelis* (Yao *et al.*, 1994) and *Heterobranchus longifilis* (Kerdchuen & Legendre, 1991).

Kerdchuen and Legendre (1991) even showed that *Heterobranchus longifilis* fed during the night exhibited a lower lipid deposition. However, both *P. bocourti* and *P. hypophthalmus* in the study showed a tendency of higher lipid deposition in abdominal cavity during nocturnal feeding.

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PROTEIN AND ENERGY UTILISATION IN TWO MEKONG CATFISHES, *PANGASIUS BOCOURTI* AND *PANGASIUS HYPOPHTHALMUS*

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Abstract

Fish unlike terrestrial animal, use a large amount of protein as an energy source for their energy metabolism. When fed on a high protein ration, the fish itself may choose which amount of protein in food will be converted into body growth and which will be burned through the catabolism. Therefore, in the study one diet was designed with a high protein content and fed to fish with different levels: 0, 5, 15, 25, 35 and 45g crude protein $kg^{-1}.d^{-1}$.

P. bocourti has a higher growth rate than *P. hypophthalmus* at the same food intake. However, the higher growth observed in *P. bocourti* is obviously associated to higher fat accumulation in the body. The protein efficiency, NPU and PER, tends to be reduced at higher protein intake in both species.

Energy and protein requirements for maintenance were calculated to be 128 and 92 $kJ.kg^{-1}.day^{-1}$, and 5.16 and 3.24 $g.kg^{-1}.day^{-1}$, in *P. bocourti* and *P. hypophthalmus*, respectively. *P. bocourti* has nearly a double protein and energy requirement for maintenance. The event may be linked to the fact that *P. bocourti* has higher growth rate and higher ability to synthesise a large amount of body lipid than *P. hypophthalmus*.

An estimate of protein requirement was in range of 12-13 and 11-12 $g.kg^{-1}.day^{-1}$ in *P. bocourti* and *P. hypophthalmus*, respectively. The DP/DE ratio was estimated to be 18 and 17 $mg.kJ^{-1}$ in *P. bocourti* and *P. hypophthalmus*, respectively. These low DP/DE ratios may be related to the fact that the protein requirement was relatively low in the two species.

INTRODUCTION

Pangasius bocourti Sauvage, 1880 and *Pangasius hypophthalmus* Sauvage, 1887 are two indigenous fish species living in the Mekong River (Roberts & Vidthayanon, 1991). The culture of *P. bocourti* in floating cages represents a production with an annual figure of 13 000 tons (Cacot, 1994). The culture of *P. hypophthalmus*, done mainly in earthen ponds, has been a long traditional activity with an estimated production of several ten thousand tons a year.

Optimal protein requirement for growth was estimated for some catfishes reared in Asia (Madu & Tsumba, 1989; Degani *et al.*, 1989). Optimal dietary levels of crude protein ranged from 25% in *Pangasius sutchi* (Chuapoehuk & Pothissong, 1985) to 50% in *Clarias gariepinus* (Henken *et al.*, 1986). Even in the same species, there are also differences from one author to another. One of reason for such discrepancies may lie in the level of feed intake, amount of non-protein energy in the feed and also the quality of the dietary protein (Wilson & Moreau, 1996).

When feed quality and quantity are not limiting factors, three main factors have an influence on growth rate: feeding rate, protein level and energy content in the diet. They are all related and influenced by each other. Therefore, optimal level of protein may be defined for one feeding rate with a fixed energy level. Thus, requirement must be define using consecutive experiments where one of the factor is fixed in each case. To reduce the influence of feeding rate, absolute ration which is the combination of feeding rate with nutrient content, may be used (Moreau *et al*, 1995).

In attempt to install an experiment with the aim to determine protein-energy requirement, the best way is to avoid any interference between protein and energy content in the feed. Fish unlike terrestrial animal, use a large amount of protein as an energy source for their energy metabolism (Velas, 1981). Therefore, fish may be fed on a high protein ration as it is commonly done when trash fish is provided. In this case, the fish may choose itself which amount of protein in food will be converted into body increase and which will be burned through the catabolism. Fish will be fed on a diet where the content could be used either for protein supply or energy supply. Thus, the diet was designed with a very high protein content and gave varying amount to the fish. Then, fish itself could select and determine which ratio of energy to protein to be fixed or burned. The sum of a nutrient amount fixed for optimal growth and a nutrient amount required for maintenance (i.e. situation with no increase in body weight) has been defined as the protein and energy requirement for optimal growth.

Therefore, the objective of the present study is to estimate the protein and energy requirement for optimal growth in *P. bocourti* and *P. hypophthalmus* using a single diet formulation with only one experiment for each species, and to compare their growth performance as well.

MATERIAL AND METHODS

Fish and facilities

Experimental fish were obtained from sexually mature broodfish cultured in earthen ponds. The fish were induced to spawn using human gonadotropin hormone injection. The fish selection for a homogenous population was done and then fish were acclimatised to experimental facilities during one week for *P. bocourti* and two weeks for *P. hypophthalmus* since the later species is more sensitive to confined condition. *Pangasius bocourti* and *P. hypophthalmus* were on average 6.68 g and 7.69 g at initial day of the experiment, respectively.

Pangasius bocourti fingerlings were cultured in 50-liter aquarium in a recirculating water system at a stocking density of 20 fish per aquarium. *Pangasius hypophthalmus* fingerlings were cultured in concrete tank (1 x 1 x 0.6 m) at a density of 20 fish per tank. Water in tanks was supplied from a deep well. Water in aquarium was aerated and exchanged at a flow rate of 2-3 L. min^{-1}. Dissolved oxygen and pH, monitored twice a week, ranged between 3.5-5.5 $mg.L^{-1}$ and 7.0-7.5, respectively. Ammonia and nitrite in aquarium were measured once a week, varied from 0.1 to 0.8 $mg.L^{-1}$ and 0.01 to 0.03 $mg.L^{-1}$, respectively. Aquarium temperature ranged from 28 to 30°C. Water in tanks was exchanged at a flow rate of 10-15 $L.min^{-1}$. Dissolved oxygen was monitored twice a week and ranged from 3.0 to 3.5 $mg.L^{-1}$, pH ranged 7.0-7.2. Ammonia and nitrite ranged 0.1-0.4 $mg.L^{-1}$ and from 0.01 to 0.03 mg. L^{-1}, respectively. Temperature in tanks varied between 28 and 32°C.

Feed and feeding

A high protein diet was designed in which vitamins, minerals as well as essential fatty acids were added to avoid any nutrient deficiency. Therefore, fish were fed a pelleted feed containing high amount of fishmeal plus vitamin and mineral premix as well as soybean oil. Ingredients and composition are given in Table 1.

Ingredients	% dry matter
Fish meal (70% crude protein)	91
Vitamin premix	1
Mineral premix	4
Soybean oil	3
Sodium alginate (as binder)	1

Feed composition for each species (% dry matter)		
	P. hypophthalmus	*P. bocourti*
Crude protein	65.7	63.0
Crude fat	10.9	10.4
Ash	17.2	16.3

Table 1: Formulation of the diet and composition obtained for each species.

In the present study, six rations (0, 5, 15, 25, 35 and 45 g protein per kg of fish per day) were chosen in order to provide different amounts of protein and then energy intake. The protein amount is given with the aim to provide protein and energy as well. Therefore, presenting result only in term of amount of protein intake, may lead to confusion even if it is done this way. So, treatments will be referred later as R0, R5, R15, R25, R35 and R45 for convenience. Each treatment had three replications. Fish were fed

twice a day at 8:00 and at 18:00. The experiment lasted for 4 weeks.

Sampling and data analysis

Fish were weighed individually every week to adjust the feeding at a precision of 0.1g. Initially 10 fishes were kept in freezer and at the end of the experiment 10 fishes in each tank or aquarium were also kept in freezer for carcass analysis.

Carcass and feed composition were determined for crude protein (Kjeldahl, nitrogen x 6.25), crude lipid (Soxhlet, chloroform extract), ash (residue after burning 4-5 hours at 550°C) and moisture (weight loss after drying at 105°C for 4-5 hours).

Growth performances and feed efficiency were expressed using usual parameters as following:

- Specific growth rate: SGR= (Ln(final weight) - Ln(initial weight)) / number of days.
- Food conversion ratio: FCR = dry food intake / increase in body weight.
- Protein efficiency ratio: PER = increase in body weight / protein intake.
- Net protein utilisation: NPU = increase in body protein / protein intake.
- For the purpose of this experiment and to match NPU, a new index regarding fat utilisation was introduced as followed: net fat utilisation: NFU = increase in body fat / fat intake.

Data analysis was done using SAS GLM and MIXED procedures.

RESULTS

***Growth performance and feed utilisation of* Pangasius bocourti *and* Pangasius hypophthalmus**

Higher level of diet intake induces higher increase in body weight for both species (Figure 1). As intake is equal to R25 and above, the growth reaches a plateau for *P. hypophthalmus*. Meanwhile the fish growth in *P. bocourti* is still increasing until the ration R35. Comparing growth rate between the two species shows that *P. bocourti* has higher growth than *P. hypophthalmus* for the same intake. It can be seen also from Figure 1 that variation in body weight, obtained with treatment R5, is not or slightly different from zero, for both species.

Variation in the amount of food intake induces changes in body composition. Considering an

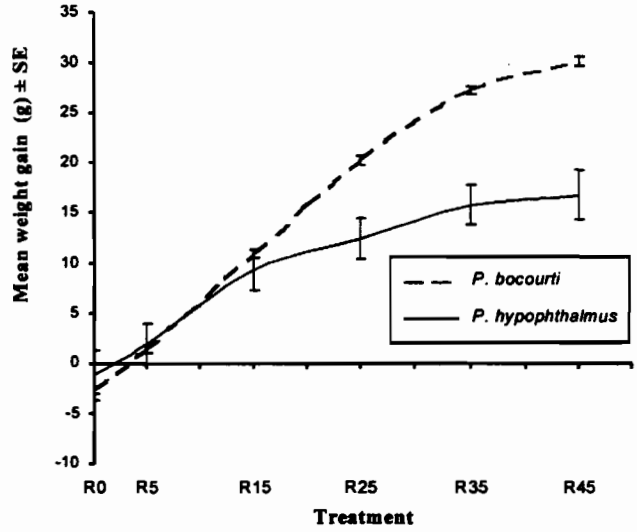
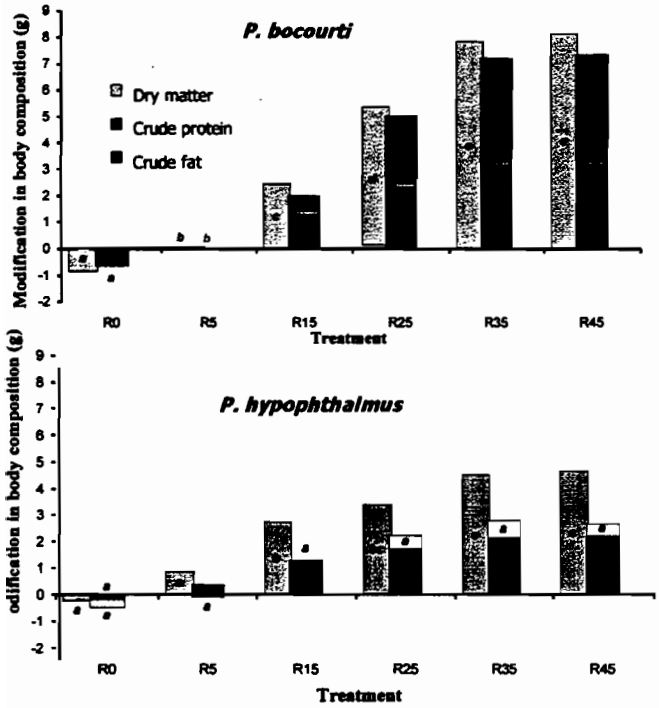


Figure 1: Mean weight gain of *P. bocourti* and *P. hypophthalmus* in response to treatments.

average fish for each treatment, changes in body composition such as dry matter, protein and fat may be calculated from difference between initial and final body composition (Figure 2). Similarly to growth performance, body dry matter and body protein increased with higher diet intake. However, body dry matter and body protein did not increase more when feed intake is over treatment R35 and R25, for *P. bocourti* and *P. hypophthalmus* respectively. For *P. bocourti*, higher feed intake induces high lipid retention that represents more than fifty percent of dry body mass increase. For fish receiving the treatment R5, body composition did not change so much for both species.

Values for common indices of growth performance and food utilisation are summarised in Table 2. Food conversion ratio (FCR) increased with higher food intake when ration was above R15 for both species. Yet, FCR is at its highest level with treatment R5, 1.14 for *P. bocourti* and 0.96 for *P. hypophthalmus*. In both species, efficiency indicators of protein intake like protein efficiency ratio (PER) or net protein utilisation (NPU) are at their highest level for treatment R15 to R25. Whereas, increase of SGR are still observed with higher diet intake, treatment R45 and R35, for *P. bocourti* and *P. hypophthalmus*, respectively. It suggests that fish accumulate a high proportion of other nutrient instead of body protein for treatments over R15 and R25. It is quite noticeable to find the high lipid retention, above 100%, with *P. bocourti* (Table 2). That refers the fish can deposit a large amount of fat. On the other hand, lower lipid deposition is



For each component, same letter indicates value not significantly different (α=0.05).

Figure 2: Modification of body composition in each species for an average fish according to the amount of diet supplied.

observed in *P. hypophthalmus*. The highest value was about 40% for treatment R25 in comparison to 150% for the same treatment with *P. bocourti*.

Based on each index, optimal condition may be determined for each species. Considering growth performance, optimal condition was encountered for group of treatments having high growth performance with the lower food intake. This is obtained with treatment R45 for *P. bocourti* and R35 for *P. hypophthalmus*. Similarly, treatment R25 and R35 are optimal, for *P. hypophthalmus* and *P. bocourti* respectively, when one considered protein increase and NPU which are higher among the group of treatment with the highest SGR. Optimal condition was then consequently determined for each index such as SGR, Protein increase, FCR, PER, NPU and NFU (Table 3). When all indices were considered, treatment R35 was found generally optimal for *P. bocourti* and R25 for *P. hypophthalmus*.

The choice between R25 and R35 will refer not only to general growth performance as well as food utilisation. Some others considerations such as acceptance of high fat content in fish by the consumer or the commercialisation way of fish (live, fresh or frozen fillet), may influence the fish farmers and lead them to produce fat or lean fish. Therefore both treatments, R25 and R35, will be further considered for the two species.

Nutrient and energy utilisation in P. bocourti *and* P. hypophthalmus

Nutrient utilisation (Table 4) indicated that *P. bocourti* can synthesise 6.8 to 7.74g protein. $kg^{-1}.day^{-1}$ in ration corresponding to 25 to 35g protein.$kg^{-1}.day^{-1}$. While *P. hypophthalmus* can fix only 5.78 to 6.52g protein.$kg^{-1}.day^{-1}$. Thus, at intake levels ranging from R25 to R35, *P. bocourti* can synthesise some more protein than *P. hypophthalmus*. However, *P. hypophthalmus* uses dietary protein more efficiently since the fish fix 23% to 31% dietary protein when compared to

Indices	Treatment					
	R0	R5	R15	R25	R35	R45
P. bocourti						
SGR	-2.68 ^{a}	0.67 ^{b}	3.41 ^{c}	4.89 ^{d}	5.64 ^{e}	6.01 ^{f}
FCR		1.14 ^{a}	0.59 ^{c}	0.67 ^{bc}	0.80 ^{abc}	1.03 ^{a}
PER		1.42 ^{a}	2.59 ^{c}	2.26 ^{bc}	1.84 ^{ab}	1.48 ^{a}
NPU		0.01 ^{a}	0.28 ^{b}	0.26 ^{b}	0.21 ^{b}	0.16 ^{b}
NFU		0.05 ^{a}	0.55 ^{a}	1.50 ^{b}	1.65 ^{b}	1.24 ^{b}
P. hypophthalmus						
SGR	-0.62 ^{a}	0.80 ^{b}	2.88 ^{c}	3.45 ^{cd}	4.03 ^{de}	4.13 ^{e}
FCR		0.96 ^{a}	0.71 ^{a}	0.99 ^{a}	1.16 ^{ab}	1.53 ^{b}
PER		1.79 ^{ab}	2.27 ^{a}	1.65 ^{ab}	1.39 ^{b}	1.05 ^{b}
NPU		0.34 ^{a}	0.31 ^{a}	0.23 ^{a}	0.19 ^{a}	0.14 ^{a}
NFU		-0.53 ^{a}	0.08 ^{a}	0.40 ^{a}	0.35 ^{a}	0.18 ^{a}

Table 2: Growth performance and feed utilisation indices obtained for *P. bocourti* and *P. hypophthalmus*. Values with different letters in the same line are significantly different.

21%-26% in *P. bocourti* for the same fed intake.

With regard to lipid utilisation, both species cannot synthesise any body lipid at R5 intake level. To maintain a minimal requirement, the fish have to mobilise all dietary lipid. Yet, *P. hypophthalmus* has still synthesised body protein but neither *P. bocourti* for the same ration. It implies that *P. bocourti* has still mobilised dietary protein for maintenance at dietary protein intake as low as R5. At higher intake, R25-R35, *P. bocourti* has fixed 6.48 to 10.02g lipids per kg of fish per day. Meanwhile, *P. hypophthalmus* has fixed only1.65 to 2.03g lipid.kg^{-1}.day^{-1}. It showed that *P. bocourti* has a high ability to accumulate lipid, 4-5 times greater than that of *P. hypophthalmus*. The deposit lipid in *P. bocourti* was higher than the daily lipid intake (Table 2). It means that the fish has to transfer a large amount of dietary protein to deposit lipids. With regard to energy utilisation, the protein and lipid loss in fasted fish indicated that the energy requirement for maintenance in *P. bocourti* and *P. hypophthalmus* were 128 and 92 kJ.kg^{-1}.day^{-1}, respectively (Table 5). Energy utilisation for growth in term of synthesised protein and lipid of the fish was calculated for R25 and R35 feed intake. *P. bocourti* can synthesise 151 and 172 kJ.kg^{-1}.day^{-1} as fixed protein and *P. hypophthalmus* can synthesise a little lower, 128 and 145 kJ. kg^{-1}.day^{-1} as fixed protein at the same feeding levels. Similarly, *P. bocourti* can synthesise 252 and 390 kJ.kg^{-1}.day^{-1} body lipid and the latter species can fix only 64 and 79 kJ. kg^{-1}.day^{-1}.

An estimate of protein requirement was denoted as the addition of protein requirement for maintenance, R5 treatment, and fixed protein, R25 or R35 treatment. That was 248 and 269 kJ. kg^{-1}.day^{-1} in *P. bocourti* and 189 and 206 kJ. kg^{-1}.day^{-1} in *P. hypophthalmus*, when one convert protein to energy value on digestible basis regarding maintenance and on gross basis regarding fixed protein. Finally an estimated protein requirement was 12-13 g.kg^{-1}.day^{-1} for *P. bocourti* and 11-12 g.kg^{-1}.day^{-1} for *P. hypophthalmus*.

DISCUSSION

The present study demonstrated that *P. bocourti* has a higher growth rate than

Species	Indices					
	SGR	Protein increase	FCR	PER	NPU	NFU
P. bocourti	R45	R35	R35	R25	R35	R15
P. hypophthalmus	R35	R25	R35	R25	R25	R35

Table 3: Treatment gave optimal results for each index.

Species		*P. bocourti*			*P. hypophthalmus*		
Treatment		R5	R25	R35	R5	R25	R35
Received feed	g.kg^{-1}.day^{-1}	7.97	39.68	55.56	7.94	39.68	55.56
Protein	g.kg^{-1}.day^{-1}						
- received	g.kg^{-1}.day^{-1}	5.21	26.07	36.50	4.94	24.68	34.56
- fixed	g.kg^{-1}.day^{-1}	0.06	6.80	7.74	1.70	5.78	6.52
	%	1%	26%	21%	34%	31%	23%
- lost	g.kg^{-1}.day^{-1}	5.16	19.27	28.76	3.24	18.91	28.03
	%	99%	74%	79%	66%	69%	77%
Lipids							
- received	g.kg^{-1}.day^{-1}	0.87	4.33	6.06	0.83	4.13	5.78
- fixed	g.kg^{-1}.day^{-1}	0.04	6.48	10.02	0.00	1.65	2.03
from feed	g.kg^{-1}.day^{-1}	0.04	4.33	6.06	0.00	1.65	2.03
from body	g.kg^{-1}.day^{-1}	0.00	2.16	3.96	0.00	0.00	0.00
- lost	g.kg^{-1}.day^{-1}	0.82	0.00	0.00	0.83	2.48	3.74
from feed	g.kg^{-1}.day^{-1}	0.82	0.00	0.00	0.83	2.48	3.74
from body	g.kg^{-1}.day^{-1}	0.00	0.00	0.00	0.00	0.00	0.00

Table 4: Nutrient utilisation according to diet intake in *P. bocourti* and *P. hypophthalmus*.

Species		*P. bocourti*		*P. hypophthalmus*	
		Energy utilisation at maintenance level			
Treatment		**R5**		**R5**	
Burned as protein	$kJ.kg^{-1}.day^{-1}$	97		61	
Burned as lipids	$kJ.kg^{-1}.day^{-1}$	31		31	
Total	$kJ.kg^{-1}.day^{-1}$	128		92	
		Energy utilisation at optimal growth level			
Treatment		**R25**	**R35**	**R25**	**R35**
Received	$kJ.kg^{-1}.day^{-1}$	653	914	620	867
Fixed as protein	$kJ.kg^{-1}.day^{-1}$	151	172	128	145
	%	23	19	21	17
Fixed as lipids	$kJ.kg^{-1}.day^{-1}$	252	390	64	79
	%	39	43	10	9
Lost	$kJ.kg^{-1}.day^{-1}$	250	353	427	644
	%	38	39	69	74
		Estimation of requirements			
Treatment basis		**R25**	**R35**	**R25**	**R35**
Protein	$kJ.kg^{-1}.day^{-1}$	248	269	189	206
	$g.kg^{-1}.day^{-1}$	12	13	11	12
Protein/energy ratio (DP/DE)	$mg.kJ^{-1}$	18	14	17	13

Table 5: Energy utilisation and estimation of protein requirement for *P. bocourti* and *P. hypophthalmus*. Energy equivalents are calculated according to Luquet & Moreau (1989): 18.8 $kJ.mg^{-1}$ digestible protein (DP); 37.7 $kJ.mg^{-1}$ digestible lipids, 22.2 $kJ.mg^{-1}$ crude protein (CP) and 38.9 $kJ.mg^{-1}$ crude lipids.

P. hypophthalmus. However, the higher growth observed for *P. bocourti* is obviously associated to higher fat accumulation in the body. The protein efficiency, the NPU and the PER, tends to be reduced at higher protein intake in both species. This trend was similar to those found in *Cyprinus carpio* (Ogino & Saito, 1970), *Ctenopharyngodon idella* (Dabrowski, 1977), *Leptobarbus hovenii* (Pathmasothy & Omar, 1982). The highest protein retention was found in the present study to be 28% and 31% in *P. bocourti* and *P. hypophthalmus*, respectively. The figure was lower than those in other fish species as protein retention of 60% were obtained with *Clarias gariepinus* (Machiels, 1987) and 54% with Nile tilapia, *Oreochromis niloticus* (Kaushik *et al.*, 1995). Yet, the protein retention observed in the present study may not be related to values obtained in other studies as protein are supplied to cover all energy metabolism needs and other energy sources were not provided. Regarding estimate, fixed protein represents 60, 70% of intake for *P. bocourti* and *P. hypophthalmus*, respectively. Data in the nutrient utilisation showed that *P. bocourti* can synthesise body lipid, as they store more lipid than what they fed in diet. In that case, some of dietary protein was, therefore, transferred to body lipid. Comparing results obtained with R15 treatment and over, a higher protein loss was observed in *P. bocourti*. That must be associated to the fact that the fish tends to deposit a large amount of body lipid. It is interesting to realise that *P. bocourti* fingerlings can synthesise a large amount of body lipid at young stage.

Energy and protein requirement for maintenance was calculated to be 128 and 92 $kJ.kg^{-1}.day^{-1}$, and 5.16 and 3.24 $g.kg^{-1}.day^{-1}$ in *P. bocourti* and *P. hypophthalmus*, respectively. Many authors have determined the protein and energy maintenance needs in other fish species. That was found to be in the range of 1-1.32 g. $kg^{-1}.day^{-1}$ in channel catfish (Gatlin *et al.*, 1986), 1-2 $g.kg^{-1}.day^{-1}$ in common carp (Kaushik, 1995) and about 2 $g.kg^{-1}.day^{-1}$ in Nile tilapia when cultured at 28°C. The energy and protein maintenance requirement in *P. hypophthalmus* was nearly comparable to channel catfish and tilapia. It is noticed that the culture temperature in the study

was 28-32°C, higher than the cultured condition of channel catfish and tilapia. That may be the reason for such a higher energy and protein requirement in *P. hypophthalmus*, since Schwarz and Kirchgessner (1984) found that the energy need for maintenance in common carp was reduced at lower temperature. Moreover, when comparing the two species of Mekong catfish, *P. bocourti* and *P. hypophthalmus*, it is obvious that *P. bocourti* has nearly a double protein and energy requirement for maintenance. The event may be linked to the fact that *P. bocourti* has higher growth rate and the fish has a high ability to synthesise a large amount of body lipid. Anyway, treatment R5 was retained for the maintenance estimate even if a slight growth was observed. This will be necessary to have an estimate of maintenance requirement by having a slight growth since one could calculate the maintenance requirement by interpolation between R0 and R5 treatment. Such estimates are safer regarding the purpose of the present study.

An estimate of protein requirement was in range of 12-13 and 11-12 $g.kg^{-1}.day^{-1}$ in *P. bocourti* and *P. hypophthalmus*, respectively. Daily protein requirement for most tropical catfish species ranges from 15-25 $g.kg^{-1}.day^{-1}$ (Wilson & Moreau, 1996), but it can be as low as 12 $g.kg^{-1}.day^{-1}$ in *Clarias batrachus* (Mollah & Hussain, 1990) or 10 $g.kg^{-1}.day^{-1}$ in *Clarias gariepinus* (Henken *et al.*, 1986). The value of 8.75 $g.kg^{-1}.day^{-1}$ was also reported to obtain maximum growth of channel catfish (Gatlin *et al.*, 1986). Therefore, the daily protein requirement in *P. bocourti* and *P. hypophthalmus* was in the lower range when compared to most catfishes.

The optimal protein to energy ratios for catfishes ranged from 20 to 30 $mg.kJ^{-1}$ (DP/DE) (Wilson & Moreau, 1996). The DP/DE ratio was estimated to be 18 and 17 $mg.kJ^{-1}$ in *P. bocourti* and *P. hypophthalmus*, respectively. These values are closer to the value of Nile tilapia (Kaushik *et al.*, 1995). In the present study, the low DP/DE ratio may be related to the fact that the protein requirement was relatively low in the two species. Even if such study appears to be a good way to compare performance between species, more studies remain necessary to investigate the energy utilisation in both species, regarding mainly the starch utilisation as an energy substitute in diet and the regulation of lipid metabolism.

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EFFECTS OF FEEDING LEVELS ON THE GROWTH AND FEED CONVERSION EFFICIENCY OF *PANGASIUS BOCOURTI* FINGERLINGS

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Abstract

An experiment was conducted to investigate the effects of different feeding levels on the growth, survival and feed conversion efficiency of *Pangasius bocourti* fingerlings (initial weight of 13.3-14.0 g). The experiment was conducted in a water flow-through and "self cleaning system" consisting of 15 forty litre plastic tanks. Each container was stocked with 20 fingerlings. Fish were fed daily with a pelleted diet (32% protein) at five levels of 1, 3, 6, 9 and 12% body weight, with three replications per treatment. Growth was monitored every 7 days for 28 days.

Final mean weight of fish was significantly lower for the 1% treatment (17.3 g) than for all other treatments (27.3 g to 41.2 g), while no significant difference was found between treatments of 6% to 12% feeding rate. The mean daily weight gain of fish was significantly lower in the 1% feeding level treatment (0.12 $g.day^{-1}$) than in all other feeding treatments (0.48-0.99 $g.day^{-1}$). The feed per gain ratio was improved at lower feeding levels; it was significantly lower with the 1% (1.12) and 3% (1.01) feeding levels than with other treatments (1.33-2.62). Altogether, the results indicate that the maximum growth corresponded to a feeding level of 9.36%, optimum growth to 3.17%, and forecasted maintenance (growth=0) to 0.50%.

INTRODUCTION

Pangasius bocourti is an important species for intensive culture in cage in the Great Lake of Cambodia and in the Vietnamese part of the Mekong Delta (Pantulu, 1979; Rainboth *et al.*, 1976; Mekong River Commission, 1992). The culture of *P. bocourti* in floating cages in the Mekong Delta has been rapidly expanding since the last few years, and the total production reached 27 000 tons in 1995 (Phuong, 1996). In practice, fish are mostly fed moist or home-made diets with irregular feeding levels depending on the availability of foodstuff supply (Hieu & Cuong, 1996), then fish overfeeding or underfeeding are situations commonly observed. How this could affect the growth and feed conversion efficiency of *Pangasius* catfish has not been assessed yet. Suitable feeding strategy is important not only in terms of improvement of fish growth but also in terms of reduction of feed cost and environmental problems. In cage culture of fish, feeding level becomes more important since any overfeeding will certainly cause wastage of feed and negative impact on the water environment. For such reasons, a number of studies have been conducted on feeding level and feeding frequency of different species of fish.

Zamal and Ollevier (1995) studied the effects of feeding and lack of food on the growth and biochemical and fatty acid composition of juvenile catfish, *Clarias gariepinus*. Santiago *et al.* (1987) reported that the growth of Nile tilapia fry (*Oreochromis niloticus*) was improved at high feeding level of 30-40% body weight. Henken *et al.* (1985) studied the effects of feeding levels on apparent digestibility of dietary dry matter, crude protein and gross energy in African catfish (*Clarias gariepinus*) and found that at high feeding level, the feed was less digested and/or absorbed by the fish. Meer *et al.* (1997) studied the effects of three feeding levels on feed losses and feed utilisation of soya and fish meal diets in *Colossoma macropomum* and concluded that there was no significant difference in terms of feed passage rate in the digestive tract of the fish at different feeding levels but found a relation between feed ration and feed utilisation.

The objective of the present study was to investigate the effects of different feeding levels on the growth and feed utilisation parameters of *Pangasius bocourti* fingerlings in controlled

environment in order to propose suitable feeding levels for maximum or optimal growth of the fish and define low cost feeding strategies.

MATERIALS AND METHODS

Tested animals: good quality *Pangasius bocourti* fingerlings (13.3-14.0 g) produced from hatchery were used for the study. The fish were nursed to fingerling size in concrete tanks by using a pelleted feed. They were then acclimatised in the experimental systems and fed a commercial pellet containing 32% protein for one week prior to start the experiment.

Experimental system: the experiment was conducted in a water flow-through and self-cleaning system consisting of 15 forty litre plastic tanks. The system was supplied with filtered and aerated deep-well water at a flow rate of 2.8 L. mn^{-1}.

Water management: plastic tanks were completely cleaned weekly at the moment of fish sampling by removing water and scrubbing the tank wall. Temperature and pH were measured daily while dissolved oxygen was checked weekly. The measurement was taken place at 7:30 am. During the experiment, the water temperature varied from 28.6-30.1°C, dissolved oxygen from 4.3-5.7 $mg.L^{-1}$ and pH from 7.5-8.0.

Experimental design: the experiment was conducted using completely randomised design procedure. Five graded feeding levels (1, 3, 6, 9, and 12%) with triplicate containers for each level were designed. Twenty fish were randomly released into each plastic container one week prior to start the experiment. During the experiment, the fish of all treatments were fed a same diet containing 32% protein (formulated from fish meal, soybean meal, wheat floor, Vitamin-mix, vegetable oil and Carboxyl methyl cellulose-CMC). Weight gain of fish was measured at the beginning of the experiment and subsequently every 7 days for 28 days. Fish were fed four times daily (7:00, 10:30; 13:30 and 17:30 h). The fish even at high feeding levels ingested all the feed distributed. Two morning feedings were cancelled at the sampling dates.

Statistical analysis: data on daily weight gain (DWG) and feed per gain ratio (FGR) were subjected to one-way analysis of variance and differences in treatment means were compared by Duncan's new multiple range test using Statgraphics computer program ($p<0.05$). Maximum and optimum feeding levels were estimated using method described by Brett *et al.* (1969).

RESULTS

Effects of feeding level on survival rate and growth of fish

The survival rate of fish was 100% in all treatment. Final weight, specific growth rate (SGR) and daily weight gain (DWG) increased up to a plateau as the feeding level increased. SGR and DWG of fish fed at 6, 9 and 12% feeding levels were significantly higher than that of fish of the 1 and 3% treatments, but no significant difference was found among fish of the 6 to 12% treatments (Table 1, Fig. 1).

The maximum, optimum and forecasted maintenance feeding levels, estimated from Figure 2, were of 9.36% and 3.17% and 0.50%, respectively.

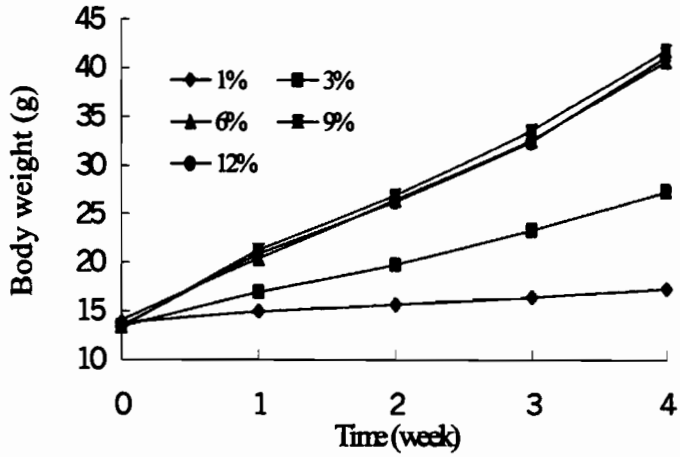

Figure 1: Weight gain of *Pangasius bocourti* fingerlings as a function of varying feeding level.

Feed conversion efficiency (Table 2, Fig. 3): the feed per gain ratio (FGR) obtained at a feeding level of 3% was the lowest (1.01), although it was not significantly different from FGR obtained at 1% feeding rate (Table 2). The highest FGR corresponded to the 12% treatment (2.62) and was significantly different in comparison with all other treatments. The FGRs corresponding to the feeding levels for maximum and optimum growth were 1.94 and 1.09, respectively (Fig. 3).

Feeding levels (%)	Initial body weight (g) (1)	Final body weight (g)	Survival rate (%) (2)	SGR (%.day^{-1}) (3)	DWG (g.day^{-1}) (4)
1	13.8 ± 0.53	17.3 ± 0.84^{a}	100	0.77 ± 0.04^{a}	0.12 ± 0.01^{a}
3	13.5 ± 1.16	27.3 ± 1.82^{b}	100	2.44 ± 0.17^{b}	0.48 ± 0.04^{b}
6	14.0 ± 2.10	40.7 ± 3.46^{c}	100	3.69± 0.22^{c}	0.92 ± 0.05^{c}
9	13.3 ± 0.59	41.8 ± 1.57^{c}	100	3.94 ± 0.27^{c}	0.99 ± 0.60^{c}
12	13.5 ± 0.74	41.2 ± 3.38^{c}	100	3.78 ± 0.25^{c}	0.93 ± 0.50^{c}

(1) Mean ± sd
(2) Survival rate = 100 * (No. of fish harvested / No. of fish stocked)
(3) Specific growth rate (SGR) = 100*(ln Wf -ln Wi)/t
(4) Daily weight gain (DWG) = (Wf-Wi)/t; with Wf: final weight, Wi: initial weight, and t: time (day)
Values in each column sharing same superscript letter are not significantly different (p<0.05)

Table 1: Mean final body weight, survival rate, specific growth rate (SGR) and daily weight gain (DWG) of *Pangasius bocourti* fingerlings fed varying levels after 28 days of experiment.

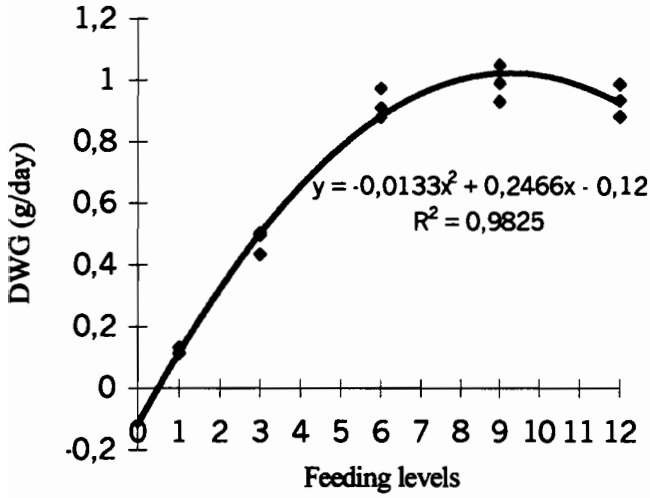

Figure 2: Evolution of daily weight gain (DWG) of *P. bocourti* fingerlings as a function of feeding level after 28 days of experiment.

Feeding level (%)	FGR (1)
1	1.12 ± 0.06^{ab}
3	1.01 ± 0.05^{a}
6	1.33 ± 0.09^{b}
9	1.92 ± 0.15^{c}
12	2.62 ± 0.24^{d}

(1) Feed per gain ratio (FGR) = feed fed/weight gain, values with the same superscript are not significantly different (p<0.05).

Table 2: Feed per gain ration (FGR) of *Pangasius bocourti* fingerlings fed varying levels after 28 days of experiment (mean ± std).

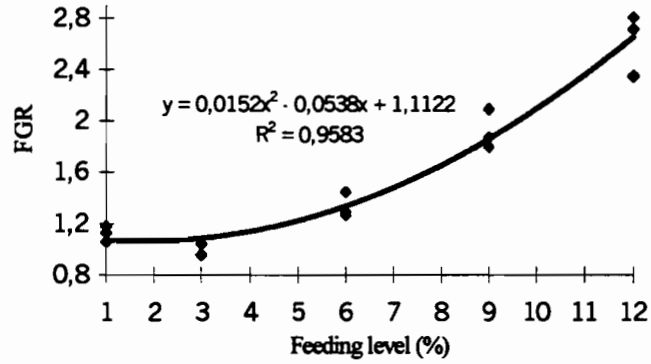

Figure 3: Evolution of feed per gain ration (FGR) of *Pangasius bocourti* fingerlings as a function of feeding level after 28 days of experiment.

DISCUSSION

The growth rate of *Pangasius bocourti* fingerlings was not significantly improved at feeding levels above 6% body weight, and even started to reduce at the feeding level of 12%. This is in agreement with results reported on Florida red tilapia (*Ochreochromis urolepis* x *O. mossambicus*) indicating that the growth increased as feeding level increased to a maximum level above which growth was unchanged and feed per gain ratios increased (Campbell, 1985). Chakraborty *et al.* (1995) stated that the growth of common carp (*Cyprinus carpio*) increased with ration level and with dietary protein level, and there was an approximately linear increase of growth with ration level for any given diet. In respect to energy, the same study also showed that increasing the feeding level resulted in a larger amount of ingested energy available for growth.

The feed per gain ratios obtained in the present study were higher at higher feeding levels. This is in accordance with the results of Chakraborty *et al.* (1995). Increase of feed per gain ratios at high feeding rates may result from the incomplete digestion of feed (Balarin & Haller, 1982). Henken *et al.* (1985) discussed that at higher feeding levels, the passage rate of the dietary materials through the digestive tract is thought to be higher, causing less materials to be digested and/or absorbed, and the correlation between digestibility and feeding

level was significant indicating that digestibility was depressed at higher feeding levels.

Pangasius bocourti can take a large size meal, up to 5-8% body weight per each meal (Hieu & Cuong, 1986). In comparison with the results of this study, the 5-8% is properly over suitable level, particularly for one meal, then the low digestion and absorption of nutrients can result in low growth of fish and high feed per gain ratio. However, feeding levels have to be adapted depending on diets quality and feeding frequency.

CONCLUSIONS

This present study demonstrates that the growth and feed conversion efficiency of *Pangasius bocourti* was affected by feeding levels. A feeding rate of 6% could be suitable for growth of fingerlings of this species with diets containing 32% protein. This can be used to modify the recent practical feeding scheme of *P. bocourti* cultured in cages, the 5-8% feeding levels of moist feed can be used but splitting into several meals may help to improve the digestion, absorption and the growth of fish. Further study on feeding frequency and feeding level with different dietary protein would be important and useful for developing suitable feeding management in *P. bocourti* culture.

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THE USE OF PLANT PROTEIN (SOYBEAN MEAL) AS A REPLACEMENT OF ANIMAL PROTEIN (FISH MEAL AND BLOOD MEAL) IN PRACTICAL DIETS FOR FINGERLING OF *PANGASIUS BOCOURTI*

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Abstract

A series of feeding trials was conducted to determine the feasibility of using plant protein (soybean meal) to partially replace animal protein (fish meal and blood meal) in practical diets for *Pangasius bocourti* fingerlings. Test diets were formulated with soybean meal to replace 25, 43, 56, or 67% of the fish meal. All diets were iso-caloric (4.3 $Kcal.g^{-1}$) and iso-nitrogenous (34% protein). Fish were fed 4-5% body weight, feeding ration being adjusted weekly after fish sampling. Growth and feed conversion efficiency were compared to a fish meal based control diet.

At the end of 5-weeks trial, the growth of fish was found to decreased as the percentage of replacement of fish meal by soybean meal in the diets increased. The SGRs obtained with diets in which 43, 56 and 67% of fish meal was replaced by soybean meal were 1.75, 1.66 and 1.51%.day^{-1}, respectively. These growth rates were significantly lower compared to the fish meal based diet (or control) (2.13%.day^{-1}) ($p<0.05$). However, there was no significant difference in fish growth between 25% soybean meal and control diets, and between 25% and 43% soybean meal treatments. The feed per gain ratio (FGR) and protein efficiency ratio (PER) were better in the diets in which less fish meal was replaced by soybean meal. The FGRs obtained with diets containing soybean meal (2.00 to 2.42) were significantly higher compared to control diet (1.54), but no significant difference were found between diets in which 25% and 43%, and 56 and 67% of fish meal was replaced by soybean meal. The PER was the highest with the fish meal based diet, but no significant difference was found between 25% and 43% soybean meal diets and 43 to 67% soybean meal diets. No significant differences in percentage of body protein and dry matter were found among treatments. However body lipid percentages of fish receiving 56 and 67% soybean diets were significantly higher than those of fish fed fish meal based diet. The present study demonstrates that the replacement of not more than 25% fish meal by untreated soybean meal in practical diet for *Pangasius bocourti* is applicable.

INTRODUCTION

Intensive aquaculture is becoming more and more an industrial practice incorporating novel technologies at all different operational stages. From the economical point of view, feed cost still appears to be one of the major constraints against the greater expansion of aquaculture (Kaushik, 1990). For instance, the feed cost in *Pangasius* intensive cage culture shared about 40-50% of total costs (unpubl. data). From the nutritional point of view, it has been established since a long time in all fish diets, protein is quantitatively the most important dietary nutrient and that fish meal remains the major source of dietary protein. However, fish meal is decreasing in supply (Tacon, 1993) and increasing in costs (Gallagher, 1994). Given this context, serious efforts are made continuously to reduce fish meal cost in practical diets through identification of alternative sources of protein. Tacon (1994) gave a substantial review of data concerning the partial or total replacement of fish meal with alternative protein sources.

In Vietnam, the use of fish meal for fish and shrimp feeds culture is rapidly increasing while the local supply of fish meal is unable to meet the requirement. Therefore, a large amount of fish meal has to be imported from other countries (*e.g.*, from Peru). The use of fish meal in the diet for *Pangasius* intensively cultured in cage is increasing to compensate the existing reduction of trash fish source. To sustain the protein level

supplied in fish diets, plant protein has been considered as a valuable substitute to animal protein in practical diets for fish culture, with a special regard to the intensive culture of carnivorous fish reared in cage. However, some of them have received more attention than the others. Among the main alternative protein sources considered, soybean meal (either in full fat or defatted) has become promising due to its relatively low cost and sustainable supply. Moreover, among over plants used as feed ingredients, soybean meal is considered as the most nutritious and used as a major protein source in many fish diets (Lovell, 1988). However, the proper use of this ingredient has to be determined for each fish species, *e.g.* the appropriate ratio in the diet or treatment to avoid anti-nutritional factor (trypsine).

A number of research has been carried out to replace fish meal protein by less expensive protein sources, especially plant protein sources (Hoops *et al.*, 1981; Akiyama, 1988 and 1991; Appler & Jauncey, 1983). Some plant ingredients have limited value as protein sources in fish feed due to their improper amino acid balance, the presence of enzyme inhibitors and the lack of energy availability (Gallagher, 1994). The total or partial replacement of fish meal protein by soybean meal protein has been studied with varying success (Webster *et al.*, 1992; Gallagher, 1994; Hughes, 1991). The growth of blue catfish was greater when fed diet containing 13% fish meal and 48% soybean meal than when fed diets containing higher percentages of soybean meal and less fish meal (Webster *et al.*, 1992). Balogun and Ologhobo (1989) conducted an experiment on fingerlings of African catfish (*Clarias gariepinus*) of 65 g initial body weight by replacing 25%, 50%, 75% and 100% of fish meal of the control diet with cooked and raw soybean meal. They concluded that the best nutrient utilisation and growth of fish was obtained with the control diet, but there was no significant difference between control and cooked soybean meal replaced diets. However, the cooked soybean dietary inclusion was superior to all diets containing raw soybean meal in all respects.

There have been also a number of studies focussing on the use of soybean meal in diet of fish species other than catfishes. Shiau *et al.* (1987) reported that the replacement of 67% of fish meal protein by soybean meal protein in tilapia feed had no adverse effects on growth. Kaushik *et al.* (1995) reported that replacement of 33 to 100% of fish meal by protein soybean concentrate (obtained after hot water-ethanol extraction) did not affect the growth performance or nutrient utilisation of rainbow trout (*Oncorhynchus mykiss*) of 83 g initial body weight. Shiau *et al.* (1987) found that in hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) fish meal can be replaced partially by commercial hexane-extracted soybean meal when the dietary protein level is sub-optimal for tilapia growth, but at the optimal dietary protein level (32%), partial replacement of fish meal protein with protein from soybean meal depresses both growth and feed conversion.

Studies in the United States (Smith, 1977) demonstrated the potential use of full-fat soybean (steam cooked or toasted) as a total alternative protein source to fish meal in rainbow trout diets. Tacon *et al.* (1983) found some differences between soybean meals depending on the extraction solvent used and other processing techniques. They found that puffed full-fat soybean faired much better than other soybean meals. Kaushik (1990) found that the extrusion treatments can improve significantly the apparent digestibility coefficient values of soybean meal proteins.

This study seeks to evaluate the possibility of partial or total replacement of fish meal and blood meal by full-fat soybean in practical diet for *Pangasius* catfishes in order to reduce feed costs.

MATERIALS AND METHODS

Experimental system: the experiment was conducted in a water flow-through and self-cleaning system consisting of 15 four hundred litre concrete tanks. The system was supplied with aerated deep-well water at a flow rate of 2.8 L. mn^{-1}.

Tested animal: good quality *Pangasius bocourti* fingerlings (weighing 58.7-61.2 g/fish) produced from hatchery were used for the study. The fish were nursed to fingerling size in concrete tanks using pelleted diet. The fish were also acclimated in the experimental systems and fed a pelleted diet containing 34% protein for one week prior to the experiment.

Test diets: five 34% protein diets (Phuong & Moreau, unpublished data), which were iso-caloric and iso-nitrogenous, containing various replacement levels of fish meal by soybean meal

(0-67%) were computer-formulated. The diets were prepared orderly by thoroughly mixing weighted dry ingredients in a mixer, adding oil and distilled water until a moisture mixture resulted. Then, they were passed through a mincer with a 3 mm die and dried in 50°C oven. The dried diets were broken up into suitable size and stored in freezer at about -18°C before use. The composition and proximate analysis of the test diets are presented in Table 1.

Chemical composition of fish carcass and test diets were analysed for their crude protein (Kjeldahl, nitrogen x 6.25); crude lipid (Soxhlet chloroform extraction); ash (residue after burning 5 minutes and heating 4-5 hrs. at 550°C); moisture (loss on drying at 105°C for 4hrs.) and NFE (Nitrogen free extract, subtraction).

Water management: tanks were completely cleaned at the sampling of fish by removing water and scrubbing the tank wall. Temperature and pH were measured daily while dissolved oxygen was checked weekly. The measurement was taken place at 7:30 am. During the experiment, the water temperature varied from 26.7-28.3°C, dissolved oxygen 4.5-5.9 $mg.L^{-1}$ and pH 7.5-7.8.

Experimental design: the experiment was conducted by using completely randomised design procedure. Five treatments with various replacement levels of fish meal by soybean meal were tested with three replications per treatment. Twenty fish were randomly released into each tank one-week prior to start the experiment. Before beginning of experiment, 30 fish (2 per replicate) were removed and frozen for carcass composition analysis. Weight gain of fish was measured at the beginning of the experiment and subsequently every 7 days for a period of 35 days. At the end of the experiment six fish were taken from each treatment and subjected to proximate analysis. Fish were fed four times daily (7:00, 10:30; 13:30 and 17:30 h) with a daily feeding level of 4-5%. Two morning feedings were cancelled at the sampling dates.

Statistical analysis: data on growth, feed per gain ratio (FGR), protein efficiency ratio (PER) and body composition were subjected to one-way analysis of variance using Statgraphics computer program. Differences in treatment means were compared by Duncan's new multiple range test ($p<0.05$).

Ingredients (%)	Experimental diets (% protein)				
	Diet 1 0% soy.	Diet 2 25% soy.	Diet 3 43% soy.	Diet 4 56% soy.	Diet 5 67% soy.
Fish meal [1]	46	41	36	31	26
Soybean meal	0	13.6	26.7	39.8	52.7
Wheat floor	43.2	35	28.6	22	16
Vegetable oil	3.39	2.57	1.44	0.31	0
Vitamin [2]	2	2	2	2	2
CMC	5.4	5.8	5.28	4.39	3.33
Gross energy ($Kcal.g^{-1}$) [3]	4.32	4.26	4.24	4.2	4.26
Proximate analysis					
Crude protein	33.8	34.2	33.5	33.7	34.1
Crude lipid	8.34	8.04	8.5	8.35	9.31
NFE	38.7	36.8	36	35.7	34.2
Ash	13.4	13.5	13.2	13.1	13.2
Crude fibre	5.76	7.46	8.55	9.25	9.19

(1) Comprising two fish meals and one blood meal.

(2) Vitamin mixture produced by Phone-Poulenc, namely VEMEVIT. No. 9. In 1 kg of mixture, there are Vit. A: 2 000 000 IU; Vit. D3: 400 000 IU; Vit. E: 12 000 mg; Vit. K: 480 mg; Vit. B1: 800 mg; Vit. B2: 800 mg; Vit B6: 500 mg; Nicotinic acid: 5 000 mg; Calcium D pantothenate: 2 000 mg; Vit. B12: 2 000 mg; Folic acid: 160 mg; Microvit H 2000: 1 000 mg; Vit. C: 100 000 mg; Fe^{++}: 1 000 ppm; Zn^{++}: 3 000 ppm; Mn^{++}: 2 000 ppm; Cu^{++}: 100 ppm; Iodine: 20 ppm; Co^{++}: 10 ppm; Methionine: 30 000 mg; Lysine 25 000 mg; Sulfathiazole: 10 000 mg and Antioxidan (BHT): 2,000 mg.

(3) Gross energy was calculated based on: protein = 5.65, lipid = 9.45, and NFE = 4.20

Table 1: Composition and proximate analysis of experimental diets.

RESULTS

Effects of various replacement levels of fish meal by soybean meal on the survival rate and growth of fish (Table 2, Fig. 1 and 2)

The survival rates of fish varied from 83.3 to 96.7% but there was no significant difference among treatments. Final body weight and specific growth rate (SGR) tended to decrease as the soybean level in the diet increased. However both final mean weight and SGR of fish fed 25% soybean diet (2.00%.day^{-1}) was not significantly different compared to those of fish fed fish meal based diet (2.13%.day^{-1}) and 43% soybean diet (1.75%.day^{-1}). There was also no significant difference of SGR between fish receiving 56% and 67% soybean meal diets.

Feed conversion efficiency (Table 3)

There was a significantly lower feed efficiency when fish meal was replaced by soybean meal in the diet. Food conversion ratio (FGR) increased as the soybean levels in the diet increased, but there were no significant differences between 25% and 43%, and between 56% and 67% soybean treatments. The protein efficiency ratio (PER) was significantly higher in fish meal based diet (2.15) compared to other diets containing soybean meal (1.35 to 1.74), but no significant differences were found between 25% and 43%, and between 43% to 67% soybean treatments. The best FGR and PER were obtained with the diet containing only fish meal and no soybean meal.

Body composition (Table 4)

The body composition of fish fed various replacement levels of fish meal with soybean meal did not show any difference in percentages of crude protein and dry matter. The percentages of

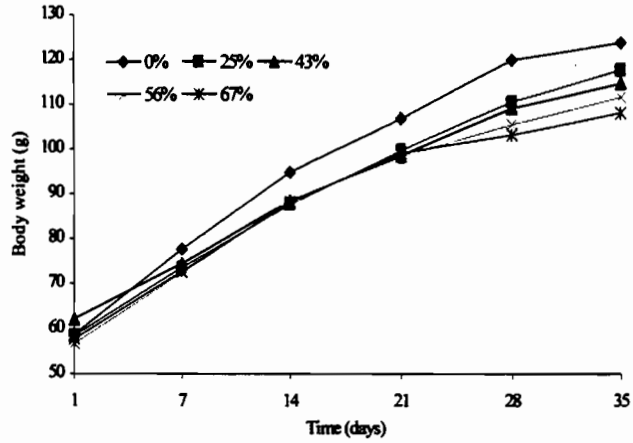

Figure 1: Growth of *Pangasius bocourti* fingerlings fed diets with varying replacement levels of fish meal and blood meal by soybean meal.

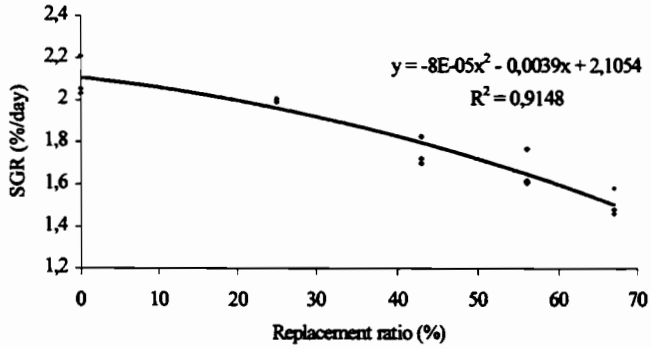

Figure 2: Evolution of specific growth rate (SGR) of *Pangasius bocourti* fingerlings as a function of varying replacement levels of fish meal and blood meal by soybean meal in the diet.

lipid in fish body tended to increase as the soybean meal replacement levels in the diets increased but there were no significant differences among fish fed diets with different levels of soybean meal. The body lipid content significantly increased in fish fed diets containing 56 and 67% soybean meal compared to those receiving the fish meal based diet. No difference was found among

Diets	Initial body Weight (g) (1)	Final body Weight (g)	Survival rate (%) (2)	SGR (%.day^{-1}) (3)
Diet 1 (0% soy.)	58.6 ± 2.56	123 ± 5.51^{a}	83.3 ± 10.4	2.13 ± 0.50^{a}
Diet 2 (25% soy.)	58.3 ± 1.94	117 ± 3.93^{ab}	86.7 ± 7.64	2.00 ± 0.01^{ab}
Diet 3 (43% soy.)	61.2 ± 1.92	114 ± 4.27^{b}	95.0 ± 5.00	1.75 ± 0.07^{b}
Diet 4 (56% soy.)	56.6 ± 2.02	101 ± 0.46^{c}	90.0 ± 0.00	1.66 ± 0.09^{c}
Diet 5 (67% soy.)	57.7 ± 0.84	97.8 ± 3.53^{c}	96.7 ± 5.77	1.51 ± 0.06^{c}

(1) Mean ± sd
(2) Survival rate = 100 * (No. of fish harvested / No. of fish stocked)
(3) Specific growth rate (SGR) = 100*(ln Wf -ln Wi)/time (day); Wf: final weight; Wi: initial weight
Values in each column sharing same superscript are not significantly different ($p<0.05$)

Table 2: Mean body weight, survival rate and specific growth rate (SGR) of *Pangasius bocourti* fingerling fed diets with fish meal replaced by soybean meal after 35 days of experiment.

Dietary protein (%)	FGR [1]	PER [2]
Diet 1 (0% soy.)	1.54 ± 0.17^{a}	2.15 ± 0.26^{c}
Diet 2 (25% soy.)	2.00 ± 0.30^{b}	1.74 ± 0.28^{b}
Diet 3 (43% soy.)	2.05 ± 0.08^{b}	1.60 ± 0.06^{b}
Diet 4 (56% soy.)	2.35 ± 0.08^{c}	1.39 ± 0.05^{a}
Diet 5 (67% soy.)	2.42 ± 0.03^{c}	1.35 ± 0.02^{a}

(1) Feed per gain ratio (FGR) = Feed fed/weight gain
(2) PER = (Wf-Wi)/protein fed
Values in each column with the same superscript are not significantly different ($p<0.05$).

Table 3: Feed per gain ratio (FGR) and protein efficiency ration (PER) of *Pangasius bocourti* fingerlings fed diets with fish meal partially replaced by soybean meal during 35 days of experiment.

Dietary protein (%)	Body composition (% on wet weight basis)		
	Dry matter	Crude protein	Crude lipid
Diet 1 (0% soy.)	32.2 ± 0.98	36.4 ± 1.22	27.6 ± 3.65^{a}
Diet 2 (25% soy.)	32.6 ± 0.80	35.2 ± 1.64	30.1 ± 5.08^{ab}
Diet 3 (43% soy.)	31.2 ± 0.83	37.2 ± 1.84	35.0 ± 2.93^{ab}
Diet 4 (56% soy.)	30.6 ± 1.29	35.5 ± 3.38	37.2 ± 1.89^{b}
Diet 5 (67% soy.)	32.1 ± 2.27	36.0 ± 1.23	37.9 ± 4.27^{b}

Values with the same superscript are not significantly different ($p<0.05$)

Table 4: Body composition of *Pangasius bocourti* fingerlings fed varying levels of soybean diets for 35 days of experiment (means for three replicates).

treatments of 25, 43% soybean and fish meal based diets.

DISCUSSION

Up to now, soybean meal has not been widely used as an ingredient in the practical diets for *Pangasius* catfish cultured in cage in the Mekong Delta, Vietnam. The present results showed no significant difference in growth rate and lipid deposition between fish fed fish meal based diet and those receiving a diet in which 25% of the fish meal was replaced by soybean meal. This suggests some possibilities of replacing fish meal by soybean meal in the compound diet for *Pangasius bocourti*. However the feed efficiency, estimated through both FGR and PER, was significantly lower in fish fed the 25% soybean diet than in fish receiving the fish meal based diet. When more than 25% of fish meal was replaced by soybean meal in protein diet did not differ after 6 week from that of fish fed diet without soybean, and there was also no significant difference among diets in which fish meal was replaced by 25-75% soybean meal.

According to Webster *et al.* (1992), the growth has been often reduced in direct proportion to the percentage of soybean in the diet. Two hypotheses have attempted to explain these results: (i) sub-optional amino acid balance and inadequate the diet, the growth of *P. bocourti* fingerlings was significantly reduced.

Webster *et al.* (1992) replaced fish meal with an increasing level of soybean meal in diets for blue catfish (from 7.9g to 37.5g). No significant difference in survival rate was found, even when soybean replaced 100% of fish meal. However, the growth of fish fed diet containing 13% fish meal and 48% soybean meal was significantly higher than in fish fed higher soybean levels. Mohsen and Lovell (1990) also found that fish meal improved the growth of channel catfish when it was substituted in the soybean-corn basal diet. Reinitz (1980) reported a growth reduction in rainbow trout fed a diet containing 65% soybean meal and 0% fish meal (cited by Webster *et al.*, 1992). Similar results were reported by Nandheesha *et al.* (1989) on Common carp (*Cyprinus carpio*). Gallagher (1994) reported that the growth of hybrid striped bass (*Morone saxatilis* x *M. chrysops*) (starting from 5g) fed 25% soybean available phosphorus in soybean meal, and (ii) presence of anti-nutritional factors (including trypsin inhibitors) in soybean meal. Soybean meal is deficient in available phosphorus, but the diets of the present study contained supplemental phosphorus from fish meal. Dabrowski *et al.* (1989) stated that amino acid availability, especially methionine, was reduced if soybean meal protein was used in excess of 50% of the diet.

Balogun and Ologhobo (1989) reported that the cooked soybean was superior to all diets containing raw soybean meal in all respects.

The FGR increased and PER decreased as soybean meal in the diets increased. In the 25% soybean meal diet, the FGR increased by 30% and the PER decreased by 20% in comparison to the fish meal based diet. The replacement of 25 to 43% or 56% to 67% fish meal by soybean meal in the diets gave similar results in terms of FGR and PER (Table 3). Webster *et al.* (1992) reported that FGR and PER of channel catfish fed diets with varying replacements of fish meal by soybean meal were not significantly different, but there was a trend in increasing FGR and decreasing PER in diet containing higher soybean levels. Gallagher (1994) stated that FGR, PER and body composition did not differ in different size groups of hybrid striped bass (5 g, 100 g and over 150 g) either the fish were fed diets with or without soybean.

The percentages of dry matter and protein contents in fish body did not seem to be related to different levels of soybean meal in the diets for fish. The lipid content tended to increase according to the increase of soybean meal level in the diets, although all test diets contained similar protein and energy.

CONCLUSIONS

The present study demonstrates that the replacement of not more than 25% fish meal by untreated soybean meal in practical diet for *Pangasius bocourti* is applicable. The body lipid increases slightly accordingly with the increase of the soybean replacement levels. Further investigations on the replacement of fish meal by treated soybean meal and amino acid supplementation will be carried out in a near future in order to precise the present results.

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REARING OF *PANGASIUS* CATFISH FRY (*PANGASIUS BOCOURTI*) FED DIFFERENT DIETS IN CONCRETE TANKS

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Abstract

An experiment was conducted in concrete tank to determine technology and possibility for mass rearing of *Pangasius bocourti* fry using locally available feeds in large scale condition. The experiment was carried out for 4 weeks in running water concrete tanks. The fry of *Pangasius bocourti* were fed Tubifex worm, commercial feed and home made feed. The survival rate of fish fed home made feed was very low (4.63%) and significantly lower than that obtained in the other two treatments. There were no significant difference in final mean body weight of fish fed tubifex and fish fed the commercial feed. There was indication that nursing *Pangasius bocourti* fry in concrete tank and using artificial feed can be applied in mass production of fingerling for culture.

INTRODUCTION

Pangasius catfish (*Pangasius bocourti*) is a common fish species cultured in cages in the Mekong River Delta, Vietnam. Its annual yield is about 13,400 tons (Cacot, 1994). Fingerling supply of this species for culture depends mainly on wild sources. Artificial propagation and larval rearing is, therefore, necessary to the sustainable development of the cage culture industry. Induced spawning of *P. bocourti* was obtained successfully for the first time at Cantho University in 1995 (see Cacot, 1999). In 1996, 350,000 fingerlings of two *Pangasius* species *P. bocourti* and *P. hypophthalmus* were produced in hatcheries at Cantho University and at AGIFISH company (Thuy, 1998). Nursing technology of *P. bocourti* was studied mainly in small scale, aquarium system. Hung *et al.* (1999) reported that *Artemia* nauplii and *Artemia* decapsulated cysts were adequate food for larval rearing of *P. bocourti*, but its price was quite high. Hung and Tam (1997) also showed the possibility of complete replacement of *Artemia* nauplii by tubifex worms or *Moina*, these preys leading to equivalent survival rate of larvae in comparison to those obtained with *Artemia*. For fry rearing, an artificial diet in which proteins were provided by fish meal and a limited proportion of soybean meal could be used efficiently for feeding *Pangasius* catfish fingerlings (Phuong *et al.*, 1999).

However, studies on the methods of fry and fingerling rearing of *P. bocourti* in systems applicable for production, using large size concrete tanks and earthen ponds, have not been conducted yet. The present study was conducted in order to find out technology and possibility for mass rearing of *Pangasius bocourti* fry and fingerlings using locally available feeds in large scale conditions.

MATERIALS AND METHODS

Source of fry: The experiment was carried out at the Cantho University using artificially propagated larvae. Larvae were fed *Artemia* nauplii for the first 3 days, then *Moina* until they reached the age of 7 days. Five days after hatching, the larvae were counted and released into concrete tanks of 4 m^2 with a stocking density of 3,500 larvae per tank. Average initial weight of fish at the beginning of the experiment was 6.24 mg.

Experimental design: The experiment was conducted with three feed treatments and three replications per treatment applying completely randomised design procedure. The feed tested were the following: (i) tubifex worms, collected from sewage system in Cantho city. The worm was chopped into small pieces as smaller as one mm and treated by 4% formalin in 1-2 minute; (ii) a dry

home made feed formulated with 50% fish meal, 30% soybean meal, 10% rice bran, 7% wheat flour and 3% soya oil; and (iii) a commercial feed, available in the market. The chemical compositions of the home made and commercial feeds are given in Table 1.

Rearing system: The experiment was conducted in running water concrete tanks of 4 m^2 each. The water flow provided to the tanks was of 6-8 $L.mn^{-1}$. During the experiment, water temperature was maintained within the range of 28-30°C, dissolved oxygen concentration ranged between 6.2 and 7.6 $mg.L^{-1}$ and pH was 7-7.5.

Feeding program: between 7 and 14 days of age, the fish were fed 4 times per day. Feeding rates were 150% of fish biomass for tubifex, and 100% for home made and commercial feeds. From the day 15th until 28th, the feeding rates were reduced to 60% of fish biomass for tubifex and 30% for home made and artificial feeds. The quantity of feed distributed was increased daily by 25% of the previous day until the next sampling. The weight of fish was measured at the beginning of the experiment and every 7 days. Thirty fish were caught randomly at each sampling for measuring weight. Data on growth and survival rates were subjected to one way ANOVA, followed by Duncan's multiple range test to determine difference in treatment means using Statgraphics computer program.

RESULTS AND DISCUSSION

The survival rate of fish fed the dry home made feed was very low (4.63%) and significantly lower than that obtained in the other two treatments (Table 2). The survival rate of fish fed tubifex was the best (93.1%) compared to 80.1% obtained with the commercial feed. The survival rate of fish fed tubifex was as high as that obtained when feeding the fish larvae with *Artemia* (Hung & Tam, 1997). There were no significant difference in final mean body weight of fish fed tubifex (577 mg) and fish fed the commercial feed (486 mg). But they were significantly higher than that of fish fed the home made diet (240 mg) ($p<0.05$). The very poor survival and growth rates of the fish fed the dry home made diet from day 7 to 21 could be due to an inappropriate preparation and size of feed particles that may have resulted in a lower feed intake and feed efficiency.

Hung and Liem (1997) nursed *P. bocourti* larvae in aquarium using different feed (*Artemia*, artificial feed and a combination of *Artemia* and artificial feed), the results showed that the growth performance of fish fed *Artemia* was higher compared to artificial feed. The mean weight of *P. bocourti* at 11 days of age was about 98 mg and 12 mg for *Artemia* and artificial feed, respectively. When compared with the above cited data, the final mean weights of fish at 14 days of age in the present study were lower in all three treatments than that of fish fed *Artemia* but they were much higher than that of fish fed artificial diet.

Figure 1 shows that, between 21 and 28 days of age, growth was equivalent for fish fed either tubifex or commercial diet. Thus, this commercial feed can be used for growing fish of more than 21 days of age. The growth rate of the fish in the experiment using dry home made or commercial feed increased. This can be understood that before

Feed	Dry matter	Crude protein	Crude fat	Ash
Dry home made feed	92.0	44.1	13.6	18.1
Commercial feed	89.7	45.3	7.45	13.5

Table 1: The proximate composition of the artificial feed (in %).

No		Dry home made feed		Commercial feed		Tubifex	
	Mean weight (mg)						
1	Initial (7 days old)	6.24		6.24		6.24	
2	day 14th	23.90	(7.06) [(1)]	51.90	(15.7)	62.60	(12.1)
3	day 21st	50.30	(17.4)	142.70	(59.7)	230.10	(51.0)
4	day 28th	239.70	(88.4)	486.22	(168.3)	576.70	(185.6)
5	**Survival (%)**	4.63	(0.67)	80.10	(6.42)	93.10	(3.48)

(1): Mean (sd)

Table 2: Growth performance and survival rate of *P. bocourti* fry fed tubifex worms and two types of artificial feed.

21 days old the fry of *P. bocourti* might not digest protein from soybean meal. In other hand, the trypsin inhibitors, present in soybean meal have been show to be partially responsible for decreased digestive enzyme activity and reduced growth rates of channel catfish (Robert & William, 1985). It can be suggested that the *P. bocourti* fish can be fed commercial feed or natural feed (as Tubifex) during the small stage, then they will grow well with other kinds of artificial feeds.

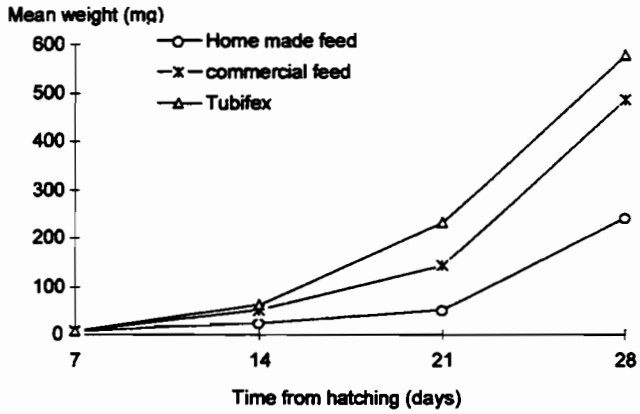

Figure 1: Growth of *P. bocourti* fry as a function of feed used between 7 and 28 days of age.

CONCLUSION

Nursing *P. bocourti* fry in concrete tanks with flow-through water can be applied for the mass production of fingerlings of this species. Artificial feed can be used for rearing *P. bocourti* fry when they are older than 21 days of age, while the combination of natural and artificial feeds could be useful for earlier age.

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SOME BIOLOGICAL CHARACTERISTICS OF *CLARIAS BATRACHUS* AND PRELIMINARY RESULTS ON THE HYBRIDISATION BETWEEN *CLARIAS BATRACHUS* AND *CLARIAS GARIEPINUS*

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Abstract

Clarias batrachus is mature all year round when reared in ponds located in the Mekong Delta (Vietnam). However, the proportion of mature fish (stage IV of gonad development) appears higher from April to July, which seems to be the main reproduction season. The hybrid crosses between *C. batrachus* and *C. gariepinus* failed in both reciprocal directions. Hybrid embryos stopped their development a few hours after fertilisation, while the eggs were fertilised and hatched successfully in both intra-specific parental crosses.

INTRODUCTION

The walking catfish, *Clarias batrachus* (Clariidae, Siluriformes), is naturally distributed not only in Mekong delta (Vietnam) but also in several other countries in Southeast Asia. Farming of this species takes an important part of aquaculture activity in Vietnam as well as in Southeast Asia in general (Chinabut *et al.*, 1991; Csavas, 1994).

The artificial propagation of other Asian catfish and the crossbreeding between *Clarias macrocephalus* and the African species, *Clarias gariepinus*, have been done successfully in Thailand and neighbouring countries. The hybrid between *Clarias macrocephalus* and *Clarias gariepinus* grows well in different farming systems and also with different diets. However, the information on crossbreeds between other Asian species and the African *Clarias* remains quite limited.

In Vietnam, studies related to *C. batrachus* has not been carried out so far. The present study provides some preliminary results on the seasonal variations of sexual maturation and artificial propagation of this species and examines the possibility of its hybridisation with *C. gariepinus*. A comparison of growth rates of *C. batrachus* and *C. gariepinus* fry is also presented.

MATERIAL AND METHODS

Fish origin and maintenance

Broodfish were collected from fish farms and stocked at 34-35 kg.m^{-3} in floating cages hanged in ponds at the Can Tho University (Mekong delta, Vietnam). The initial average fish body weight was of 250-300 g and 350-400 g for *C. batrachus* and *C. gariepinus*, respectively. The fishes were fed a home-made diet containing 35% crude proteins distributed twice a day, at a feeding rate of 4-5% of fish biomass, during 2 months before induced spawning trials. The sex-ratio was 3 males for 1 female.

Seasonal variations of sexual activity

During the study which lasted 10 months (from January to October 1997), the evolution of the ovary development was assessed monthly by calculation of the gonado-somatic index [GSI (%) = (gonad weight x 100) / fish body weight] and measurements of egg diameter.

The fish condition was estimated by calculating the two following indexes: Fulton index = P x 100/L^3 and Clark index = P_O x 100/L^3, with L= fish standard length, P= total fish body weight, P_O= weight of eviscerated fish.

Induced spawning and hybridisation trials

Female broodfish were induced to ovulate by two successive injections of a solution containing a mix of carp pituitary and LH-RHa. Eight to ten hours after the last injection, ova were stripped and fertilised using the dry fertilisation method. The fertilised eggs were then spread on the surface of mosquito screen at a density of 1.0-1.5 egg.mm^{-2}.

Thereafter the screen were suspended in

running water until hatching.

The two following hybrid crosses were tested:

- *C. batrachus* (female) x *C. gariepinus* (male)
- *C. gariepinus* (female) x *C. batrachus* (male).

Fish nursing

Fish larvae were nursed in concrete tanks (2x2x 0.5 m, each) at a stocking density of 1000 fish.m^{-2}.

During the first week, they were fed with blood worms (Tubifex) distributed daily at 25-30% of total biomass and then shifted to an artificial diet (CP Group, 30% crude proteins), distributed twice a day with a daily feeding rate of 5-7% of fish biomass.

Fish Body weight and length of fish were measured every 15 days for 45 days. The growth rate was estimated by the daily weight gain [DWG = $(W_2-W_1)/(t_2-t_1)$].

RESULTS AND DISCUSSION

***Evolution of sexual maturity of* Clarias batrachus** (Table 1)

Clarias batrachus was mature all year round. However, seasonal variations in the reproductive state of the fish were observed. Egg diameter increased from January and reached a maximal size of about 1.0-1.1mm in the period from March to July. Similarly, the proportion of gonad at the stage IV of development was the highest from April to July, which corresponds to the rainy season and seems to be the main season of reproduction. After that period, egg diameter and ovary development tended to be reduced.

A relationship between fish size and sexual maturity was observed. The GSI was lower in fish of about 250 g (1.3%) than in bigger specimens of about 750 g (5.3%). The fecundity also tented to increase with the size the fish, from 1650 eggs.g^{-1} at 270 g to 1720 eggs.g^{-1} at 750 g.

Relationship between sexual maturation and fish condition index (Table 2)

There was a negative relationship between sexual maturation of the fish and its condition index. This result is in agreement with the findings of Tri *et al.* (1981) on *Clarias macrocephalus* in Vietnam and with those of Trong (1997) on Cambodian catfish.

The mean GSI increased from Jan-97 (1.2%) and reached its maximal value in July-97 (11.6%), thereafter it decreased progressively until November. Both condition indexes showed an inverse evolution.

Crossbreeding trials (Table 3)

The hybrid cross between *C. batrachus* and *C. gariepinus* failed in the two reciprocal directions. The hybrid embryos stopped their development a few hours after fertilisation, while eggs were fertilised and hatched successfully in both intra-specific parental crosses. However, Tarnchalanukit (1986) reported that high fertility and hatching rates (66-99%) could be obtained when crossing *C. batrachus* with *C. macrocephalus*.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
Egg diameter	0.65 ±0.1	0.85 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	1.1 ± 0.2	1.1 ±0 .15	1.1 ± 0.15	0.95 ± 0.15	0.90 ± .12	0.85 ± 0.2
Gonad stage	II-III	II-III	III-IV	III-IV	IV	IV	IV	IV-III	IV-III	III-IV
(%)	65.3	68.4	75.5	94.2	95.4	95.3	93.2	75.3	70.1	65.5

Table 1: Seasonal variation of sexual maturation in *C. batrachus*.

Indexes	Month									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Nov
GSI (%)	1.2 ±0.2	1.2 ±0.1	3.5 ±0.2	7.5 ±0.4	8.5 ±0.3	11.2 ±0.2	11.6 ±0.3	8.4 ±0.4	7.5 ±0.2	6.5 ±0.3
Fulton (%)	2.1 ±0.1	2.2 ±0.1	2.1 ±0.2	1.9 ±0.2	1.8 ±0.1	1.8 ±0.1	1.7 ±0.2	1.7 ±0.1	1.9 ±0.2	1.9 ±0.1
Clark (%)	1.7 ±0.1	1.8 ±0.2	1.7 ±0.1	1.6 ±0.2	1.5 ±0.1	14 ±0.1	1.4 ±0.2	1.4 ±0.2	1.6 ±0.2	1.6 ±0.2

Table 2: Seasonal variations of Gonado-somatic index (GSI) and condition indexes of Fulton and Clark in *C. batrachus*.

No	Factors Crossing forms	Fertilisation rate (%)	Hatching rate (%)	Duration of incubation (h)
1	*C. batrachus*(F) x *C. batrachus* (M)	75.3 ± 5.4	80.3 ± 5.6	24.5
2	*C. batrachus* (F) x *C. gariepinus* (M)	-	-	-
3	*C. gariepinus* (F) x *C. gariepinus*(M)	80.2± 5.1	85.2 ± 4.5	24.5
4	*C. gariepinus* (F) x *C. batrachus* (M)	-	-	-

Table 3: Results of intra- and inter-specific crosses.

Nursing Period	*C. batrachus*		*C. gariepinus*	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Initial	0.72 ± 0.02	0.0045 ± 0.001	0.73 ± 0.03	0.0048 ± 0.002
After 15 days	2.32 ± 0.5	0.89 ± 0.16	2.35 ± 0.2	0.86 ± 0.17
DWG (from day 1-15)	0.16	0.059	0.16	0.058
After 30 days	3.86 ± 0.5	1.45 ± 0.16	3.82 ± 0.28	1.45 ± 0.21
DWG (from day 15-30)	0.1	0.037	0.1	0.034
After 45 days	5.52 ± 0.21	1.82 ± 0.18	5.65 ± 0.24	2.15 ± 0.046
DWG(from day 30-45)	0.11	0.024	0.012	0.046

DWG = Daily weight gain

Table 4: Growth in length and weight of *C. batrachus* and *C. gariepinus* fry.

Growth and survival of fry (Table 4)

The weight and length of the fish were measured every 15 days during the 45 days of nursing. During the first 30 days, the growth (weight and length) of *C. batrachus* and *C. gariepinus* was not significantly different ($p>0.05$). However, between day 30 and day 45, the growth in weight of *C. gariepinus* was faster than that of *C. batrachus* ($p<0.05$).

After 45 days of rearing, the survival rate of fry was of 72.5% and 65.3% in *C. gariepinus* and *C. batrachus* respectively. Although the survival rate of *C. batrachus* was already remarkably high, further investigations should be done to improve it.

CONCLUSION

Although *C. batrachus* could be reproduced all year round, a major reproductive season does exist from April to July. During this period, the GSI (%) increases while the condition of the fish tend to decrease.

The two reciprocal hybrid crosses between *C. batrachus* and *C. gariepinus* were found to be unsuccessful. In both cases, no hybrid larvae could be obtained.

The growth rate of *C. batrachus* and *C. gariepinus* larvae did not differ significantly during the first 30 days of rearing. However, a faster growth in weight of *C. gariepinus* fry was observed between day 30 and day 45.

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EVALUATION OF HYBRIDISATION IN FIVE *CLARIAS* SPECIES (SILURIFORMES, CLARIIDAE) OF AFRICAN (*C. GARIEPINUS*) AND ASIAN ORIGIN (*C. BATRACHUS*, *C. MELADERMA*, *C. NIEUHOFII* AND *C. TEIJSMANNI*)

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Abstract

Ten crosses between five *Clarias* species of African (*C. gariepinus*) and Asian origin (*C. batrachus*, *C. meladerma*, *C. nieuhofii* and *C. teijsmanni*) were evaluated on the basis of the fertilisation and hatching rates obtained. Zootechnical performances (growth, survival) of *C. batrachus* and *C. gariepinus* larvae were compared. Viable hybrids growth and survival were followed up until the age of 11 weeks. The genotype of the reciprocal crosses between *C. gariepinus* and *C. meladerma* was studied and the gonad development of two-years-old *C. meladerma* x *C. gariepinus* hybrids was examined (*inter-specific crosses are always given with the female parent in the first position*).

No hybrid between *C. gariepinus* and *C. batrachus* could be obtained. *Clarias gariepinus* x *C. nieuhofii* and *C. gariepinus* x *C. teijsmanni* hybrids did not survive more than a few hours after hatching and larvae obtained from *C. gariepinus* x *C. meladerma* cross were non viable beyond a few days after hatching. In the latter case, protein electrophoresis of progeny carried out at two loci diagnostic for *C. gariepinus* and *C. meladerma* (PGI and PGM) indicated that the larvae resulted from an haploid gynogenetic development. The crosses between female *C. meladerma* and male *C. gariepinus* or *C. nieuhofii* or *C. teijsmanni* produced viable hybrids. *Clarias gariepinus* and *C. batrachus* zootechnical performances comparison during larval rearing indicated that the growth potential of these two species is equivalent until the age of 12 days, though *C. gariepinus* is favoured by its larvae bigger initial size. The comparison of *C. gariepinus* and *C. meladerma* x *C. gariepinus* growth and survival until the age of 11 weeks showed good performances in the hybrid compared to the Asian species but slightly lower than the ones observed in *C. gariepinus*. The gonads observation made in *C. meladerma* x *C. gariepinus* pointed out the complete sterility of the hybrids that were sampled. On account of the low growth rates observed in *C. meladerma* and *C. meladerma* x *C. nieuhoffi* or *C. teijsmanni*, those fish seem to present only a limited interest for aquaculture.

INTRODUCTION

Species belonging to the *Clarias* genus are freshwater catfishes characterised by their ability to utilise atmospheric air and walk on land for several hundred meters using their pectoral spines (Teugels, 1996). *Clarias* species are present from Africa to south-east Asia, where they are frequently exploited by fishermen and produced in farms. Essential source of proteins from animal origin, they have gained a major economic importance (Legendre, 1992).

In Indonesia, five species from the *Clarias* genus are generally recognised (Kottelat *et al.*, 1993). The yellow and flabby flesh of *C. batrachus* is highly appreciated by consumers and this species was the first to be used for aquaculture on the archipelago. Its farming, based mainly on natural reproduction in captivity, is most often carried out in ponds or small tanks, as a side-line. At the beginning of the 90's, Indonesian researchers succeeded in reproducing a second local species, *C. meladerma*, in captivity (Sumastri *et al.*, 1994). That latter species potential for aquaculture has not been evaluated yet. However, generally speaking, Asian *Clarias* species seems to present a lower resistance to pathogens and a lower growth rate than the African *Clarias*,

C. gariepinus, whose potential for aquaculture is clearly established. The robust, omnivorous and fast growing African *Clarias* was introduced into Indonesia in 1985, via Taiwan (Sudarto & Sumastri, 1994). Its performances in aquaculture led to a rapid development of its farming but this species, whose flesh is tougher and whiter than *C. batrachus* one, remains less appreciated by the local population and is sold half-price on markets. Looking for a fish which could satisfy producers and consumers at the same time leads to think about the opportunities given by inter-specific hybridisation between the different *Clarias* species consumed in Indonesia.

Hybridisation can be attractive for aquaculture in many ways (Chevassus, 1983). First, it may produce sterile animals, avoiding growth loss or fragility related to sexual maturation. Hybrids sterility reduces potential interactions between domestic and wild fishes. Secondly, hybridisation may lead to one-sex population production which might be an advantage in case of a differential growth between males and females or in species whose proliferation has to be avoided (e.g. in tilapias). Finally, even if hybrids growth and survival are rarely higher than the ones of both of their parental species, they might present the growth rate of the faster growing parental species and one (or several) characteristic(s) sought-after in the other parent (robustness, salinity tolerance, morphology, flesh quality...).

It is in Thailand, at the end of the 70's that a first cross between *C. gariepinus* and an Asian *Clarias* species, *C. macrocephalus*, enabled farmers to associate the zootechnical performances of the African *Clarias* to the flesh quality of the species the most appreciated by the local population, *C. macrocephalus* (Csavas, 1994; Lazard, 1994). The hybrid *C. macrocephalus* x *C. gariepinus*[1], whose performances in culture and flesh quality are halfway between its parental species ones may have largely contributed to catfishes production expansion in that country, gone from 5,000 t in 1976 to 39,500 t in 1992, 26,500 t of which from *Clarias* spp. (Csavas, 1994). At the same period, *C. macrocephalus* was crossed with *C. batrachus* (Boonbrahm *et al.*, 1977) and in the 80's, the production of the hybrid *C. fuscus* x *C. gariepinus* developed in Northern Vietnam (Csavas, 1994). Three crosses between genera, involving *C. gariepinus* or *C. batrachus* have also been achieved: *C. gariepinus* x *Heterobranchus longifilis* (Hetch & Lublinkhof, 1985; Legendre *et al.*, 1992), *C. gariepinus* x *H. bidorsalis* (Salami *et al.*, 1993) and *C. batrachus* x *Heteropneustes fossilis* (Mukhopadathy & Dehadrai, 1987). Finally, Tarnchalanukit *et al.* (1986) crossed *C. batrachus* and *C. macrocephalus* with *Pangasius hypophthalmus*.

In such a context, a viable hybridisation between *C. batrachus* and *C. gariepinus* could have been expected and might have been fruitful for catfishes production in Indonesia. However, while crossing these two species was reported to be successful in Bangladesh (Ahmed & Sarder, 1994; Rahman *et al.*, 1995), hybrids between the African *Clarias* and an Indonesian stock of *C. batrachus* could not be obtained (Richter *et al.*, 1995). At the same period, Indonesian researchers studied hybridisation opportunities between *C. meladerma* and *C. gariepinus*. The first hybrids that were obtained between female *C. meladerma* and male *C. gariepinus* seem to present a growth potential higher than that of the local *Clarias* species. However, this latter hybridisation remains to be described accurately in terms of hatching results, zootechnical performances, biological characteristics and consumers appreciation of its products.

This study focused on an African *Clarias* species, *C. gariepinus*, and four Indonesian *Clarias* species, *C. batrachus*, *C. meladerma*, *C. nieuhofii* and *C. teijsmanni*. The feasibility of different crosses between these species was tested. Zootechnical performances (growth, survival) of *C. batrachus* and *C. gariepinus* larvae were compared. The growth and survival of the hybrids which could be obtained were followed up until the age of 11 weeks. The genotype of the reciprocal crosses between *C. gariepinus* and *C. meladerma* was studied and the gonad development of two-years-old *C. meladerma* x *C. gariepinus* hybrids was examined.

MATERIAL AND METHODS

Four hybridisation experiments were carried out between March and July 1997 at the Sukamandi RIFF research station, West Java, Indonesia. In that region at low altitude, climate is characterised by high temperatures, high atmospheric humidity and two main seasons: a dry season, from May to

[1] Inter-specific crosses are always given with the female parent in the first position.

September, and a wet season, from October to April.

Broodstock management

Clarias gariepinus broodstock used in this study had been bought from Sukamandi region farms. *Clarias batrachus* one also came from reproduction in captivity and descended from a wild Western Java stock. Part of the *C. meladerma* broodstock had been produced at Depok station of the Research Institute For Fisheries, from individuals initially caught in the wild near Jambi (Sumatra Island) on the Batang Hari River system. The rest of *C. meladerma* broodstock and *C. nieuhofii* and *C. teijsmanni* broodstocks were wild fish caught in the same area. Every stocks specimens were identified following Teugels (1986) and Kottelat *et al.* (1993). Finally, observations were made on hybrids between female *C. meladerma* and male *C. gariepinus* that had been produced at the Depok station in 1995 and whose parents came respectively from the Batang Hari River system and a RIFF stock. All specimens of brooders used in this study were deposited at the Musée Royal de L'Afrique Centrale (MRAC), Tervuren.

Every species or hybrids were stocked (males and females together), at a maximum density of 10 fish.m^{-3}, in 2 m^3 net cages set up in a 200 m^2 pond. During the experiments, the pond water temperature fluctuated between 26.5 and 32.0°C and pH between 7.5 and 8.5. Fish were fed a 35% crude protein pelleted feed, distributed twice per day and six days a week at a daily rate of 2% of fish biomass.

Artificial reproduction

Four hybridisation experiments were carried out (Table 1). For each experiment, spawners were selected on the day of reproduction. Genital papilla dimorphism between males and females, allowed to chose rapidly two to five males (depending on the species and the quantity of sperm required). For *C. gariepinus* and *C. batrachus*, females were selected according to the diameters measured on about thirty oocytes sampled by intra-ovarian biopsy. For a female to be chosen, observed diameters had to be homogeneous, with a mode higher than 1.5 mm and 1.2 mm for *C. gariepinus* and *C. batrachus*, respectively. Those diameters correspond to oocytes ending vitellogenesis and apt to respond to hormonal treatment for inducing maturation and ovulation. For *C. meladerma*, in which most of the broodstock came from natural environment, some atresia was observed, probably related to the stress induced by capture and captivity. The lack of individuals showing an advanced maturity stage led to the selection of small females, on which biopsies could not be made. Those females were chosen according to outward signs of maturity (rounded and supple abdomen, turgescent genital papilla).

However, oocytes could be sampled by intra-ovarian biopsy on the three biggest females, coming from Depok research station. Measured oocytes were grouped around a mode of 2 mm, close to the maximal diameter observed on prespawning females caught in natural environment (Catfish Asia Project, unpublished data).

Selected females were placed in individual aquariums or plastic tanks (from 40 to 300 L, depending on the fish size). Temperature was continuously taken by a data logger thermometer. Oocytes maturation and ovulation were induced by a single hCG (human chorionic gonadotropin) injection of 4.0 UI.g^{-1} body weight (Eding *et al.*, 1983; Zonneveld *et al.*, 1989). These authors' recommendations for *C. gariepinus* and *C. batrachus* being almost equivalent, a same latency time for all the species (depending on water temperature) was applied between injection

Experiment	Hybridisation attempts
1	*C. batrachus* x *C. gariepinus* and *C. gariepinus* x *C. batrachus* *C. gariepinus* x *C. meladerma* and *C. meladerma* x *C. gariepinus* *C. batrachus* x *C. meladerma* and *C. meladerma* x *C. batrachus*
2	*C. gariepinus* x *C. meladerma* and *C. meladerma* x *C. gariepinus* *C. gariepinus* x *C. nieuhofii*, *C. gariepinus* x *C. teijsmanni* *C. meladerma* x *C. nieuhofii*, *C. meladerma* x *C. teijsmanni*
3	*C. batrachus* x *C. gariepinus* and *C. gariepinus* x *C. batrachus*
4	*C. gariepinus* x *C. meladerma*

Table 1: Hybridisation attempts in the four experiments carried out between March and June 1997 (crosses are given with the female parent in the first position).

and eggs collection by abdominal stripping. Latency time ranged from 10 to 12 h for mean temperatures between 26 and 29°C.

Clariidae male genital apparatus is characterised by seminal vesicles, composed of many lobes and well individualised from the testis (Legendre & Jalabert, 1986). This can explain the troubles met when trying to collect sperm by abdominal stripping (Legendre, 1986). Thus, males had to be killed for testis dissection and sperm collecting. In *C. gariepinus*, testis multiple incisions let the sperm leak and the amounts collected from all the males (0.6 to 2 ml per male) were diluted 10 times in a NaCl 0.9% solution for a better preservation. In *C. batrachus*, *C. meladerma*, *C. nieuhofii*, *C. teijsmanni*, intra-testicular sperm quantity was too low to use this method. Thus, wide incisions were made on testis and the latest were rinsed successively in a known quantity of NaCl 0.9%. Sperm preparations were kept at 5°C during the few hours required for fertilisation trials, knowing that, in *C. gariepinus* and *Heterobranchus longifilis*, sperm can be kept this way at least 24 h without loss of its fertilising ability (Hogendoorn & Vismans, 1980; Legendre and Otémé, 1995). Spermatozoa concentrations in the sperm preparations were evaluated using a Thomas's hematimeter after a second dilution (dilution rate from 2.10^{-2} to 2.10^{-3}) in a NaCl 0.9% solution. Active spermatozoa proportion and motility duration were evaluated after mixing 2 µL of sperm with 50 µL of spring water on a microscope slide (x 200, black background).

In each experiment, hybrid and intra-specific crosses were made at the same time with gametes coming from the same parents. Batches of 100 to 300 eggs (two replications per cross), weighed beforehand (± 0,1 mg) were mixed with 200 µl of diluted sperm in a dry small plastic receptacle. Spermatozoa were then activated by adding 6 ml of spring water. After one minute of moderated shaking, eggs were rinsed to withdraw sperm excess and spread in another receptacle holding 300 ml of spring water for incubation.

The eggs of all the females (two to three per species) that were used were first tested individually in intra-specific fertilisation. In the first two experiments, the eggs of the different females coming from the same species were then mixed with a feather before intra and inter-specific fertilisations. However, a negative effect of this practice was observed on hatching rates. During the last two experiments, eggs coming from two females were fertilised individually in all the crosses that were made.

Incubation was carried out in stagnant water. The receptacles containing the oocytes were put to buoy on large aquariums in order to reduce temperature variations. Water temperature was recorded by a data logger thermometer during embryonic development.

During the third experiment, the embryonic development of *C. batrachus*, *C. gariepinus* and their crossed-fertilisation products was followed up on batches of about thirty eggs spread in Pétri dishes that held 100 ml of spring water at the laboratory temperature (29 to 29.5°C). Two batches per female and per cross were observed simultaneously by the authors, under two binocular microscopes, at 1h and 3h hours after fertilisation and then every 5 hours until hatching. Such a chronology, based on Legendre et Teugels (1991) and Legendre *et al.* (1992) observations on *Heterobranchus longifilis* and *C. gariepinus*, had been chosen in order to take into account all the main embryonic development stages. For estimating embryonic mortality, development was considered as aborted only when eggs were beginning to turn white. At the same time, hatching kinetics of the different products obtained in incubation containers (28.2°C to 29.5°C) was followed up on one batch of about 200 eggs per female and per cross, twice an hour from the beginning to the end of the hatching period. During the third and the fourth experiments, fertilisation rates of the different batches under observation were estimated by embryos percentages one hour after fertilisation.

After hatching, the proportions of normal and deformed larvae were determined for each batch of eggs (two replications per cross) by observations and counting over an illuminated table.

Growth and survival

During the first experiment, only larvae obtained from *C. batrachus* and *C. gariepinus* intra-specific crosses were numerous enough to make growth and survival comparisons. The day after hatching (D1), normal larvae were sorted out and shared out in batches of 400 individuals (two replications per species) in 30 L tanks of a recirculating water system. They were observed during 12 days. From D2 (i.e. the beginning of exogenous feeding) larvae were fed 6 times a day with *Artemia* nauplii, on the basis of a daily food ration equal to 200% of estimated biomass. From

D9 to D13, *Artemia* was progressively replaced by a 35% crude protein pelleted feed (President n°1). During the whole of that period, 20 individuals per batch were weighed every 3 days to the nearest 0.1 mg. On the last day of larval rearing, all the fish remaining in each tanks were counted to determine survival rates.

After the second experiment, two batches of 50 *C. gariepinus* larvae and two batches of 50 hybrid larvae from *C. meladerma* x *C. gariepinus* cross were placed in the 30 L tanks of a recirculating water system. Those batches were fed 6 times a day with *Artemia* nauplii until D9, and then with President n°1 pellets, progressively introduced in food rations. Twenty individuals per replicates were individually weighed on D3, D5, D8, D9 (to the nearest 0.1 mg). After 15 days (D16), each batch was counted again and all larvae individually weighed. Respecting the mean weights obtained for each kind of larvae, fish were shared out again in two batches of 30 individuals per cross type and placed in 30 L aquariums, filled with stagnant oxygenated water renewed every two days. They were fed in excess, 4 times a day with 35% crude protein pellets (President n°1 and n°2,) until the age of 78 days. During that period, all the larvae in every batches were counted and individually weighed on D29, D48 and D78.

During the same experiment, the low quantities of normal larvae obtained after hatching in the other crosses, whether intra-specific (*C. meladerma*) or inter-specific (*C. meladerma* x *C. nieuhofii* and *C. meladerma* x *C. teijsmanni* hybrids) did not allow making two batches of 50 individuals, as in the case of *C. gariepinus* and *C. meladerma x C. gariepinus*. Nevertheless, those fish growth and survival were followed up to the same age, in the same conditions except for stocking densities and absence of replicate.

The main physicochemical characteristics of the water during larval rearing in the first two experiments were recorded every day and proved to be particularly stable (Table 2).

	Minimum	Maximum
Temperature (°C)	26	28
O_2 (mg.L^{-1})	7.5	7.7
pH	8.6	8.7
NH_4^+ (mg.L^{-1})	0.2	0.2
NO_2 (mg.L^{-1})	0.012	0.012
Water flow	8 L.h^{-1} (D2) to 40 L.h^{-1} (D15)	

Table 2: Main water characteristics during larval rearing in the first two experiments.

Genetic characterisation of* C. gariepinus, C. meladerma *and their reciprocal hybrid progenies

The genotypes of the progenies obtained from the reciprocal crosses between *C. gariepinus* and *C. meladerma* were compared between them and with their parents ones, studying phospho-gluco-isomerase (PGI) and phospho-gluco-mutase (PGM) expression polymorphism on starch gel. Both protein systems (PGI and PGM) are diagnostic for the two studied species, which means that each species is characterised by an original allelic form for each enzymatic system studied. This is revealed by a different electrophoretic migration of each allelic form. If allelic segregation is respected during gamete association, an heterozygous state of hybrid progenies must be expected.

One batch of larvae coming from the cross *C. gariepinus* x *C. meladerma* was ground in distilled water and centrifuged (6,000 *g*, 30 minutes, 4°C) in order to extract proteins. Eye samples coming from *C. gariepinus*, *C. meladerma* and hybrids of *C. meladerma* x *C. gariepinus* underwent the same treatment. Electrophoresis of the proteins extracts was made on starch gel (15%) using a Ridgway type migration buffer. The PGI and PGM systems revelation was carried out using the method of Pasteur *et al.* (1987).

Gonad development in* C. meladerma *x* C. gariepinus *hybrid

Eleven hybrids coming from a cross between female *C. meladerma* and male *C. gariepinus* (made by the RIFF in 1995) were killed in order to examine their gonads. Those were observed with the naked eye and under a binocular microscope. Besides, intra-testicular sperm samples were subject to a meticulous exam, using a microscope, in the same conditions as previously described. Ovaries and testis of the seven females and four males (531 to 900 g individual body weight) were weighed to the nearest 0.1 g, in order to calculate gonado-somatic index (GSI = 100 x ovaries weight or testis weight / body weight).

Statistical analysis

Experimental data were compared using Student t test or one way ANOVA. When analysis of variance concluded to a significant difference, homogeneous experimental groups were sought using Duncan multiple-range test ($p<0.05$). When necessary, analyses were performed after angular

Species	Oocyte diameter (mm)	Ova diameter (mm)	Weight of ova (mg)
C. batrachus	1.23	1.28	0.91
C. gariepinus	1.61	1.72	1.75
C. meladerma	2.00	2.08	3.11

Table 3: Mean size of oocytes (before induced ovulation) and mean size and weight of ova in three *Clarias* species used in the hybridisation study (2 to 10 females per observations).

(arc-sine) transformation of data in order to stabilise residual variance.

RESULTS

Gametes used in the different crosses

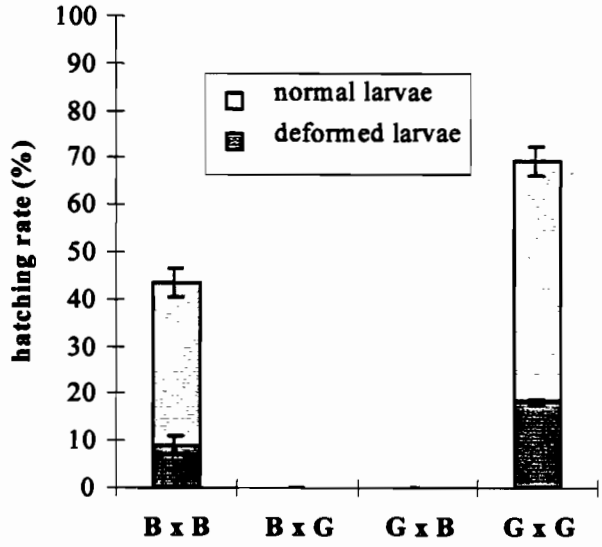
The three species that were used as female parent clearly differed by their egg size. Weight of *C. meladerma* ova was three times greater than that of *C. batrachus*, whereas *C. gariepinus* ova presented intermediate values (Table 3).

Among the five species used as male parent, *C. gariepinus* appeared clearly less oligospermic than the Indonesian species. As a matter of fact, the sperm quantities collected after rinsing testis of the Indonesian species were always very low in comparison with the sperm quantities obtained by simples incisions on the African *Clarias* testis. This has resulted in different numbers of spermatozoa per ovum during the fertilisation trials, from 1 to 5×10^6 spz per ovum with *C. gariepinus* sperm, 3×10^4 to 1×10^5 with *C. batrachus*, 1 to 2×10^4 with *C. nieuhofii* and 3 to 7×10^5 with *C. meladerma*. Spermatozoa concentration was not quantified when using *C. teijsmanni* sperm but it was also low. Moreover, the percentage of motile spermatozoa after dilution in water was usually lower than 50% in the local species, while it was generally greater than 90% in *C. gariepinus*. For those reasons, number of active spermatozoa per ovum in the artificial fertilisations carried out with sperm of the Asian species was always lower than in the ones carried out with sperm of *C. gariepinus*. However, spermatozoa motility duration (usually between 40 and 60 sec.) did not noticeably differ between the five species.

Cross-fertilisation success and embryonic development

Reciprocal crosses between C. batrachus *and* C. gariepinus

At 27°C (first experiment), hatching time ranged between 20h and 24h30 after fertilisation in *C. gariepinus* and between 25h15 and 29h30 after fertilisation in *C. batrachus*. In the third experiment, where incubation temperature was noticeably higher (28.2-29.5°C), hatching started earlier in the two species but did not end before 30h after fertilisation in *C. batrachus*. During those two experiments, no hybrid between *C. batrachus* and *C. gariepinus* was obtained (Fig. 1 and 2). With hatching rates rising up to 83.7% (of which 8.5% deformed larvae) in *C. batrachus* and to 73.6% (of which 10.3% deformed larvae) in *C. gariepinus*, gametes quality could not be responsible for the non-viability of the inter-specific crosses carried out.

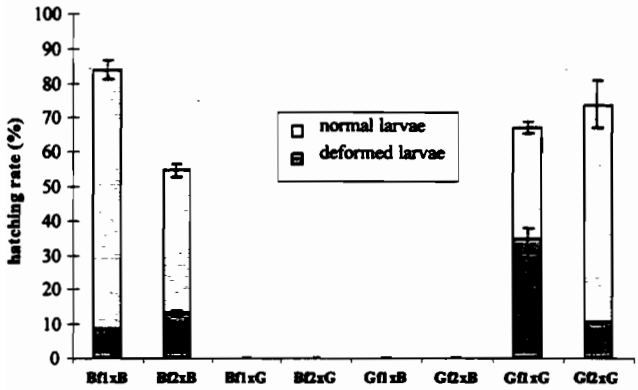


Vertical bars indicate range between replicates.

Figure 1: Mean hatching rates in *C. batrachus* (BxB), *C. gariepinus* (GxG) and their hybrid crosses (BxG and GxB) in the first experiment (pool of ova from 3 *C. batrachus* and 2 *C. gariepinus* females).

A more precise examination of embryonic development in the third experiment showed that the cross between female *C. batrachus* and male *C. gariepinus* was characterised by a very low fertilisation rate (9.3%), about 10 times lower than that obtained in the two parental species (Fig. 3.).

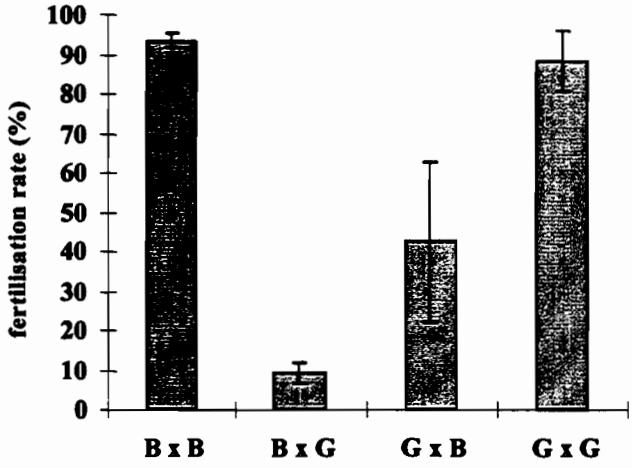
In the reciprocal cross, *C. gariepinus* x *C. batrachus*, fertilisation rate was noticeably higher (42.4%) but remained lower than the ones obtained in intra-specific crosses. In the two intra-specific crosses, most of the embryonic mortality occurred between beginning of gastrulation and the first somites apparition (Fig. 4). In *C. gariepinus* x



Vertical bars indicate ranges between replicates.

Figure 2: Mean hatching rates in *C. batrachus* (BxB), *C. gariepinus* (GxG) and their hybrid crosses (GxB and BxG) in the third experiment (crosses using individual females).

C. batrachus cross, the development of eggs containing embryos went on until gastrulation. However, all the observed gastrulations presented major anomalies (disorganised cells structures) and no embryo could develop beyond closure of the blastopore. In the reciprocal cross, *C. batrachus* x *C. gariepinus*, anomalies were observed as early as morula stage (cells dispersal in the perivitelline space) and only 9.1% of fertilised eggs entered gastrulation stage. In the two cases, all the embryos were dead (white eggs) 22h after fertilisation.



Vertical bars indicate range between replicates.

Figure 3: Mean fertilisation rates in *C. batrachus* (BxB), *C. gariepinus* (GxG) and their hybrid crosses (GxB and BxG) in the third experiment (two females per species, two replicates per female).

Reciprocal crosses between C. gariepinus *and* C. meladerma

In the first experiment, at an incubation temperature of 27°C, embryos of *C. gariepinus* were the first to hatch (20 to 24h30 after fertilisation). In *C. meladerma*, hatching occurred between 29h30 to 36h after fertilisation. In the hybrids, incubation times were in-between the parental species ones: 22h to 26h30 in *C. gariepinus* x *C meladerma* and 26 to 31h in *C. meladerma* x *C. gariepinus*. During that experiment, only one out of the three *C. meladerma* females presented an egg quality good enough to get hatching (21.7% hatching, of which 6.7% deformed larvae). Afterwards, eggs pools led to very low hatching rates, around 1% in intra-specific crosses and 5.4% after fertilisation with *C. gariepinus* sperm. In *C. gariepinus*, intra-specific control batches proved to be a lot better (69.2% hatching of which 26.5 deformed larvae; Fig. 1). After fertilisation with *C. meladerma* sperm, the same eggs pool gave only 28.0% hatching, with a particularly high proportion of deformed larvae (69.4%).

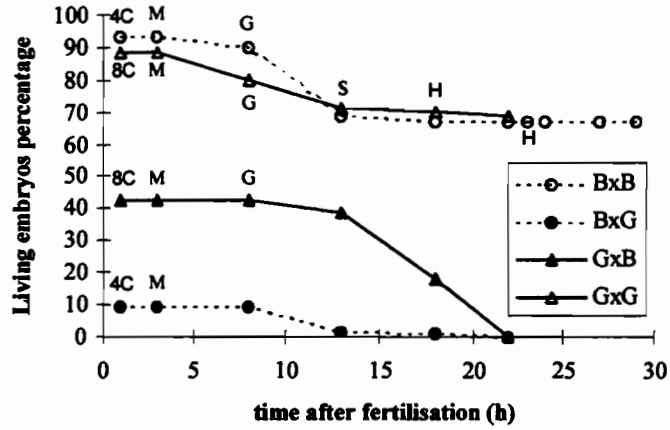
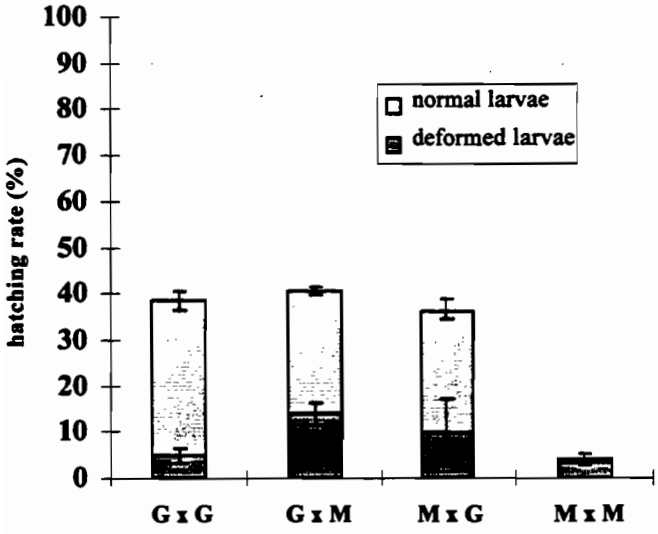


Figure 4: Kinetic of embryonic development and embryonic mortality at 29-29.5°C in *C. batrachus* (BxB), *C. gariepinus* (GxG) and their reciprocal hybrid crosses in the third experiment (4 or 8C: 4 or 8 cells stage; M: morula stage; G: gastrulation; S: somites stage; H: beginning of hatching).

During the second experiment, hatching rates obtained in *C. meladerma* intra-specific crosses were only slightly higher than those observed in the first experiment (Fig. 5). However, hatching rates obtained in inter-specific crosses with *C. gariepinus* rose up to 36.1% (of which 24.7% abnormal larvae). *Clarias meladerma* and *C. gariepinus* x *C. meladerma* hatching rates did not significantly differ but all the hybrid batches between male *C. gariepinus* and female *C. meladerma* presented more than one third strongly deformed larvae.

During the fourth experiment, without any ovulation in *C. meladerma*, the hybrid cross *C. gariepinus* x *C. meladerma* only could be achieved. The ova coming from the two females of

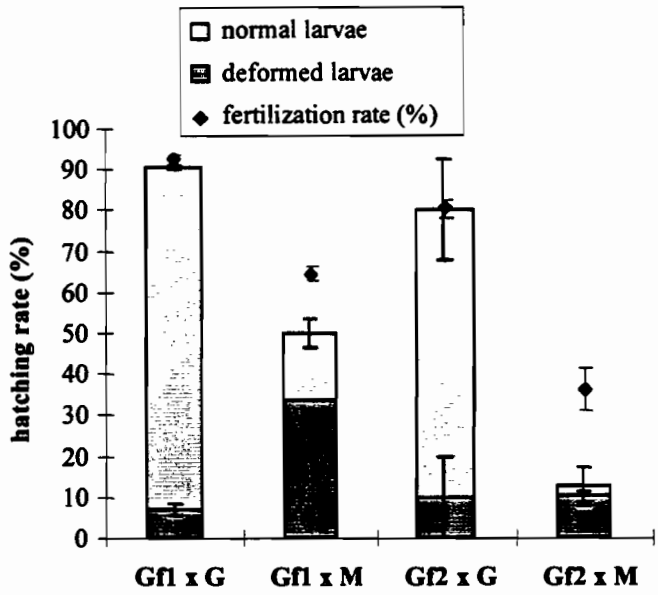


Vertical bars indicate range between replicates.

Figure 5: Mean hatching rates in *C. gariepinus* (GxG), *C. meladerma* (MxM) and their hybrid crosses (GxM and MxG) in the second experiment (pool of ova from 2 *C. gariepinus* and 3 *C. meladerma* females).

C. gariepinus were not mixed and the hatching rates obtained after intra-specific fertilisation underlined the quality of the gametes used (Fig. 6). The more meticulous examination of embryonic development showed that the hybrid cross was characterised by fertilisation rates significantly lower than those obtained in the female parent. Many hybrid embryos were abnormal during gastrulation and more than 40% died before hatching. After hatching, 67.8% of the hybrids coming from the first female and 85.7% of the hybrids coming from the second female presented major deformations.

In the three experiments, non deformed larvae obtained in crossing female *C. gariepinus* and male *C. meladerma* were characterised by a spherical yolk sac and a much more frail appearance than *C. gariepinus* larvae. As soon as D1, a very low percentage of that larvae were still active. Their eyes were relatively smaller and their barbels noticeably less developed than those of *C. gariepinus* larvae. On D2, many motionless larvae presented an abnormal, curvilinear vertebral column sketch. More than 60% of the hatched larvae did not survive after 72h. On D3, alive larvae which were still moving did not seem to have started yolk sac absorption and the scarce larvae still moving on D4 had not started eaten yet whereas, in *C. gariepinus*, the beginning of exogenous feeding had been observed on D2. On D5, no larvae were still alive.



Vertical bars indicate range between replicates.

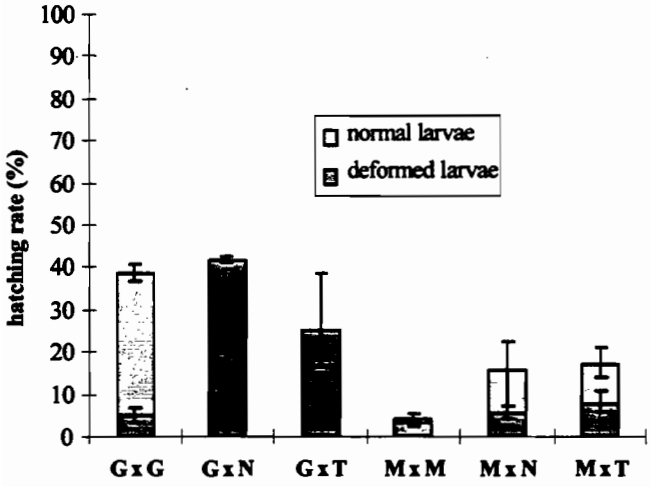
Figure 6: Mean fertilisation rates in eggs from two *C. gariepinus* females (Gf1 and Gf2) fertilised with *C. gariepinus* (G) or *C. meladerma* (M) sperm (fourth experiment).

Crosses between female C. gariepinus *or* C. meladerma *and male* C. nieuhofii *or* C. teijsmanni

Due to oocytes atresia observed on *C. nieuhofii* and *C. teijsmanni* wild stocks, the hybridisation experiments were restricted to the crosses between female *C. gariepinus* or *C. meladerma* and male *C. nieuhofii* or *C. teijsmanni* (experiment n°2). Larvae were obtained in the four crosses (Fig. 7). Hatching rates obtained in *C. gariepinus* x *C. gariepinus*, *C. gariepinus* x *C. nieuhofii*, and *C. gariepinus* x *C. teijsmanni* did not significantly differ. However, in the two inter-specific crosses, 100% of hatched larvae presented deformations and mortality began soon after hatching. In the best case, larvae did not survived more than a few hours. In that experiment, 3.9% of *C. meladerma* eggs only went to hatching in intra-specific cross. In inter-specific crosses with male of *C. nieuhofii* or *C. teijsmanni*, hatching rates passed over 15%, with a fraction of deformed larvae around 44% in the two crosses. These larvae proved to be viable.

Reciprocal crosses between C. batrachus *and* C. meladerma

In the first experiment, no hybrid between female *C. meladerma* and male *C. batrachus* was obtained. In the reciprocal cross, hatching rates were lower than 1% in the two replicates and the larvae did not survive. However, considering the



Vertical bars indicate range between replicates.

Figure 7: Mean hatching rates in *C. gariepinus* (GxG), *C. meladerma* (MxM) and their hybrid crosses with *C. nieuhofii* (N) or *C. teijsmanni* (T) males (pool of ova from 2 *C. gariepinus* and 3 *C. meladerma* females, second experiment).

poor results obtained in *C. meladerma* intra-specific cross during the same experiment, supplementary observations remain necessary before expressing any conclusion.

Growth and survival

C. batrachus *and* C. gariepinus *larval growth and survival*

Growth and survival of two batches of 400 *C. gariepinus* larvae and two batches of 400 *C. batrachus* larvae were compared from hatching up to 13 days of age. During that period, the mean body weight difference between the two species (already in favour of *C. gariepinus* at hatching) increased progressively. Mean weights of twelve-days-old fry were 81 ± 7 mg in *C. gariepinus* and 33 ± 4 mg in *C. batrachus*. However, specific growth rates (SGR) and relative weight gain (RWG) between D0 and D12, or between the beginning of exogenous feeding and D12, were very close in the two species and did not differ significantly (Table 4). Thus, final weight differences seemed to result essentially from the initial specific difference between larval weight of the two species. After 13 days of larval rearing, survival rate exceeded 93% in *C. gariepinus* while it was less than 27% in *C. batrachus*.

Growth and survival until 78 days in C. gariepinus *and hybrids between female* C. meladerma *and male of* C. gariepinus, C. nieuhofii, *or* C. teijsmanni

Growth and survival of two batches of 50 *C. gariepinus* larvae and two batches of 50 *C. meladerma* x *C. gariepinus* larvae were compared from hatching up to 16 days of age. Just after hatching, on account of the difference of egg size between the two species, *C. gariepinus* larvae mean weight was much lower than that of hybrid larvae (Table 5). However, this handicap was compensated as soon as D5, age at which *C. gariepinus* had reached a significantly higher weight than the hybrid. Mean weights of 16-days-old larvae were 494 ± 43 mg in *C. gariepinus* and 214 ± 17 mg in the hybrid. *Clarias gariepinus* growth was significantly higher than that of the hybrid during the whole period (Table 5). After 16 days of larval rearing, *C. gariepinus* and the hybrid survival rates were higher than 60% and did not differ significantly.

During the same experiment, growth and survival in small batches (without replicates) of *C. meladerma* x *C. meladerma*, *C. meladerma* x *C. nieuhofii* and C. *meladerma* x *C. teijsmanni* (respectively 13, 31 and 27 fish) was followed up in the same conditions except for stocking densities. The low quantities of fry available for these groups were due to the very low hatching rates obtained in those crosses. After 16 days, the mean body weight obtained for each kind of cross was below 90 mg (Table 5). Their growth rates were thus lower than that of the hybrid *C. meladerma* x *C. gariepinus*. On the other hand,

Species	Initial fish number	Initial body weight (mg)	Final body weight (D12) (mg)	SGR (%day^{-1})	RWG (%day^{-1})	Survival rate at 13 days (%)
C. batrachus	(2x) 400	D0 : 0.7 D2*: 1.6	33.0 ± 3.9	from D0 : 32.1 from D2 : 30.3	from D0 : 461.4 from D2 : 196.3	94.3
C. gariepinus	(2x) 400	D0 : 1.8 D2*: 3.4	81.1 ± 7.1	from D0 : 31.7 from D2 : 31.7	from D0 : 440.3 from D2 : 228.4	26.0

(*): beginning of exogenous feeding

Table 4: Mean growth from 0 to 12 days and mean survival rates after 13 days for *C. batrachus* and *C. gariepinus* larvae in the first experiment (SGR = specific growth rate, RWG = relative weight gain).

Species or hybrids	Initial fish number	Initial body weight (D0) (mg)	Final body weight (D16) (mg)	SGR (%day^{-1})	RWG (%day^{-1})	Survival rate at 16 days (%)
C. gariepinus	(2x) 50	1.8	497 ± 43	35.1	1708	69.0
C. meladerma x *C. gariepinus*	(2x) 50	3.3	214 ± 17	26.1	399	61.0
C. meladerma	13	3.3	65 ± 17	18.6	116	61.5
C. meladerma x *C. nieuhofii*	31	3.3	89 ± 7	20.6	162	48.1
C. meladerma x *C. teijsmanni*	27	3.3	85 ± 13	20.3	155	61.3

Table 5: Mean growth and survival rates from 0 to 16 days in *C. gariepinus*, *C. meladerma* and *C. meladerma* x *C. gariepinus* or x *C. nieuhofii* or x *C. teijsmanni* in the second experiment (SGR = specific growth rate, RWG = relative weight gain).

the survival rates of the hybrids between female *C. meladerma* and male of *C. gariepinus*, *C. nieuhofii* or *C. teijsmanni* did not differ significantly from that observed in *C. meladerma*.

After the 16 days of larval rearing, two batches of 30 *C. gariepinus* and two batches of 30 *C. meladerma* x *C. gariepinus* were reared in aquarium during two months. At the end of this period, the mean body weight of *C. gariepinus* fingerlings (8.4 ± 1.9 g) was still higher than that of hybrid fingerlings (6.2 ± 0.8 g). However, during that period, a bacterial infection developed in some aquariums. Mortality began on D25 in *C. gariepinus* and spread (at a lower level) to the hybrids batches a few days latter. This could explain a growth decrease observed between D29 and D48, particularly obvious in *C. gariepinus*. Although SGR on the whole period was higher in the hybrid than in *C. gariepinus* (Table 6), the last weighing period revealed a higher growth in *C. gariepinus* from D48 to D78. Survival rates, accounting for the mortality observed during the bacterial infection, were higher in the hybrid (93.3%) than in C. *gariepinus* (63.3%).

From 16 to 78 days, *C. meladerma* fingerlings and hybrids between female *C. meladerma* and male of *C. nieuhofii* or *C. teijsmanni* were also transferred to aquariums and presented SGR similar to that of *C. meladerma* x *C. gariepinus* (Table 6). However, on account of their growth lateness on D16, their mean body weights remained very low in comparison with *C. meladerma* x *C. gariepinus* one until D78. Within those three crosses, *C. meladerma* x *C. teijsmanni* only presented a high mortality during bacterial infection.

Genetic characterisation of* C. gariepinus, C. meladerma *and their reciprocal hybrid progenies

The PGI and PGM locus revelations led to the same results (see Fig. 8 for PGI locus). As expected hybrids genotypes resulting from the cross *C. meladerma* x *C. gariepinus* were heterozygous. They displayed allelic forms from both parental species. However, in the reciprocal cross, *C. gariepinus* x *C. meladerma*, progeny genotype was identical to the one observed on the female parent (*C. gariepinus*) and revealed no trace of *C. meladerma* genes.

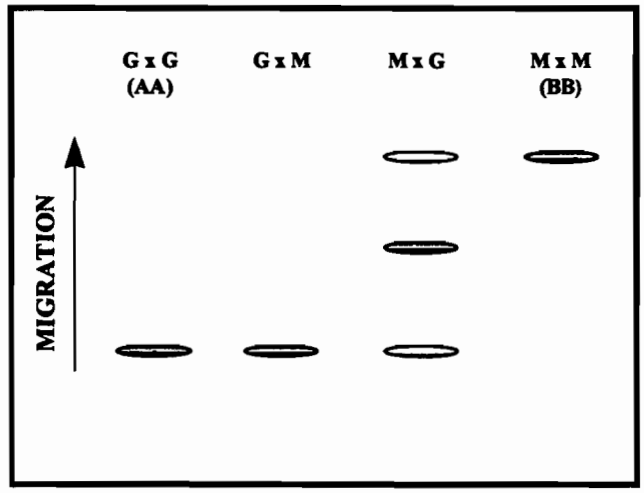


Figure 8: Electrophoretic profile (starch gel) at the PGI diagnostic locus in *C. gariepinus* (G x G), *C. meladerma* (M x M), *C. gariepinus* x *C. meladerma* (G x M) and *C. meladerma* x *C. gariepinus* (M x G).

***Gonad development in the hybrids between female* C. meladerma *and male* C. gariepinus**

The *C. meladerma* x *C. gariepinus* hybrids that were observed for their gonad development were about two-years-old. Females weighed between 647g and 900g and males between 531 and 879g. The sexual dimorphism of the genital papilla was much less obvious in these individuals than in their parental species at the same age. The maximum GSI observed on females was 0.68%. This value is much lower than GSI reported in *C. gariepinus* at the same age (generally around 15%) and much lower than GSI observed on wild *C. meladerma* females as well (4-11%). Gonads of four out of the

Species or hybrids	Initial fish number	Initial body weight (D16) (g)	Final body weight (D78) (g)	SGR (%day^{-1})	RWG (%day^{-1})	Survival rate From D16 (%)
C. gariepinus	(2x) 30	0.493	8.4 ± 1.9	4.6	25.9	63.3
C. meladerma x *C. gariepinus*	(2x) 30	0.214	6.2 ± 0.8	5.4	45.1	93.3
C. meladerma	8	0.064	1.6	5.2	38.7	100
C. meladerma x *C. nieuhofii*	13	0.089	1.9	4.9	32.8	100
C. meladerma x *C. teijsmanni*	19	0.085	2.3	5.3	42.0	36.8

Table 6: Mean growth and survival rates from 16 to 78 days in *C. gariepinus*, *C. meladerma* and *C. meladerma* x *C. gariepinus* or x *C. nieuhofii* or x *C. teijsmanni* in the second experiment (SGR = specific growth rate, RWG = relative weight gain).

seven female hybrids examined were reduced to two white long filaments. In the three other females, gonads looked like small pinkish sacs surrounded with blood vessels. Within the most developed gonads, some translucent and vacuolated areas of the ovarian tissue presented an organisation similar to that of perivisceral adipose tissue. In any case, no developing oocytes could be observed, even in the biggest ovaries,. The GSI of the 4 male hybrids were low (0.02-0.05%) in comparison to those of the *C. gariepinus* (0.32-0.89%) and *C. meladerma* (0.06-0.16%) males used during this study. By contrast, three hybrids presented particularly well developed seminal vesicles. The pinkish and almost translucent testis observed in the hybrids contained no spermatozoa and backcross fertilisation attempts using *C. gariepinus* ova did not lead to any fertilisation. At the same age, *C. meladerma* broodstock coming from the Depok station had reached full sexual maturity. In farm reared *C. gariepinus*, the first sexual maturation occurs when fish are 5 to 7 months old (Legendre *et al.*, 1992).

DISCUSSION - CONCLUSION

In this study, ten crosses between four Asian *Clarias* species and one African *Clarias*, *C. gariepinus*, were evaluated according to the fertilisation and hatching rates that were obtained. Zootechnical performances (growth, survival) of *C. batrachus* and *C. gariepinus* were compared in larval rearing. Viable hybrids were followed up until 11 months old. The genotypes of the reciprocal crosses between *C. gariepinus* and *C. meladerma* were studied and sexual maturation state of two years-old hybrids between female *C. meladerma* and male *C. gariepinus* was examined.

On a methodological point of view, analysis of fertilisation trials underlined a decrease of egg quality after mixing the eggs coming from several females in *C. batrachus* and *C. gariepinus*. Hatching rates of mixed eggs were 15 to 40% lower than the mean hatching rates observed in individual intra-specific fertilisations. This phenomenon may be explained by the low fluidity of the ova masses that were collected by abdominal stripping (almost complete absence of ovarian fluid), in favour of mechanical shocks during gametes homogenisation. The low quantities of sperm that could be obtained in the oligospermic Asian species raises the issue of the minimum number of spermatozoa required to achieve fertilisation in optimal conditions in the different female parental species that were used. The few data available on Clariidae point out that, in *C. macrocephalus*, required number of spermatozoa per ovum is about 40,000 to 80,000 (Tambasen-Cheong *et al.*, 1995). In *Heterobranchus longifilis*, a decrease in hatching rate is observed under 50,000 spermatozoa per ovum (Legendre, 1992). In some of the fertilisations made with *C. batrachus* or *C. meladerma* sperm, mean number of spermatozoa per ovum were close to those critical values, or even lower. Moreover, in some fish families (such as Salmonidae), large egg size, lengthening spermatozoa trajectory to the micropyle, may result in an increase of the required number of spermatozoa per ovum (Billard, 1990). The low spermatozoa concentration observed when fertilising with *C. meladerma* sperm, associated with the relatively large egg size of this clariid, may partly explain the low hatching rates obtained in this species after intra-specific fertilisation in comparison to those observed after fertilisation with *C. gariepinus* sperm.

Table 7 summarises the results obtained in terms of hatching achievement and offspring viability for the different crosses tested. The two hybridisation experiments between *C. batrachus*

Cross tested (female x male)	Hatching	Offspring viability
C. gariepinus x *C. batrachus*	NO	/
C. gariepinus x *C. meladerma*	YES	NO
C. gariepinus x *C. nieuhofii*	YES	NO
C. gariepinus x *C. teijsmanni*	YES	NO
C. batrachus x *C. gariepinus*	NO	/
C. batrachus x *C. meladerma*	NO	/
C. meladerma x *C. gariepinus*	YES	YES
C. meladerma x *C. batrachus*	less than 1%	NO
C. meladerma x *C. nieuhofii*	YES	YES
C. meladerma x *C. teijsmanni*	YES	YES

Table 7: Hatching achievement and offspring viability in the different crosses tested.

and *C. gariepinus* did not lead to any hatching. In *C. batrachus* x *C. gariepinus*, this result was expressed by a significant decrease of fertilisation rates compared to the intra-specific control cross and by the mortality of the scarce embryos that were obtained. In the reciprocal cross, fertilisation rate decrease was not significant but none of the embryos was viable. Theses observations match with the ones made by Richter *et al.* (1995) on an Eastern Java *C. batrachus* stock. These authors assigned the fertilisation rate decrease in the cross between female *C. batrachus* and male *C. gariepinus* to the relatively small size of the first species micropyle compared to the latter species spermatozoa size. The success reported in the hybridisation of these two species in Bangladesh (Ahmed & Sarder, 1994; Rahman *et al.*, 1995) could then only be explained by a genetic differentiation between Indian and Indonesian populations of *C. batrachus* from Indian Peninsula and Indonesia, or by the fact that fish called "*C. batrachus*" in these two areas may not correspond to the same species. The latter hypothesis is actually supported by recent caryotype and morphometric comparison analyses, suggesting that "*C. batrachus*" from the Indian Peninsula may in fact be misidentified and could correspond to *C. fuscus* (Garcia-Franco, 1993).

Larvae obtained in the crosses between female *C. gariepinus* and male of *C. nieuhofii* or *C. teijsmanni*, all strongly deformed, did not survive more than a few hours. Mortality that was observed in *C. gariepinus* x *C. meladerma* within the first five days of life seems to come from a different cause. Those larvae, of which many were not deformed, presented no trace of *C. meladerma* genes at the PGI and PGM loci, whereas in the reciprocal cross *C. meladerma* x *C. gariepinus*, the expected hybrid genotype was actually observed. This kind of situation (no paternal genome contribution and early mortality) recalls the one observed in artificial gynogenesis by sperm irradiation without diploidism restoration (Chourrout, 1980). In Salmonidae, haploid larvae that are obtained are characterised by a short body, few blood vessels around vitellus and small eyes. Those individuals die before yolk sac resorption. Larvae obtained after crossing female *C. gariepinus* and male *C. meladerma* presented similar characteristics and could therefore have resulted from haploid gynogenetic development of *C. gariepinus* ova. The fact that no embryo could be obtained from non fertilised *C. gariepinus* ova suggests that the developments obtained in that cross were activated by *C. meladerma* spermatozoa. Establishing those larvae caryotype would allow to fully confirm the haploid state of their genome.

By contrast, *Clarias meladerma* ova were successfully fertilised by spermatozoa coming from three different species: *C. gariepinus*, *C. nieuhofii* and *C. teijsmanni*. In all cases, obtained larvae proved to be viable.

Clarias batrachus and *C. gariepinus* larval rearing showed that those two species possessed an equivalent growth potential until the age of 12 days. Thus, mean body weight differences observed at the end of the experiment should have resulted from the initial differences in eggs and larvae size (bigger in *C. gariepinus*). A similar conclusion is given by Verreth and Eding (1993), who specify that *C. batrachus* growth potential could be under-exploited in Indonesian farms. Growth and survival of these two species should therefore be studied, under optimal farming conditions and until getting the size required on local market, in order to specify their relative interest for fish culture in that area.

Growth and survival comparisons between the hybrid *C meladerma* x *C. gariepinus* and its male

parental species until the age of 11 weeks showed a higher global growth potential in *C. gariepinus*. However, the hybrid reached a noticeably higher weight than its female parental species and than the two hybrids between Asian *Clarias* species that were obtained (*C. meladerma* x *C. nieuhofii* and *C. meladerma* x *C. teijsmanni*). Gonads examination of two years-old *C. meladerma* x *C. gariepinus* hybrids showed an abnormal development of the ovaries and testis that were observed: very low GSI, presence of adipose-like tissue in ovaries and total absence of spermatozoa in testis. These hybrids seem therefore completely sterile but this result has to be specified by (ongoing) histological analysis of samples taken on the gonads that were observed. It would be also interesting to extend the growth comparison that was made between this hybrid and the African *Clarias* until the latter species reaches sexual maturity, in order to test for an eventual effect of gonads development in *C. gariepinus* on the relative growth of these two fishes.

However, it should be noted that three Indonesian populations of *C. meladerma* were found to present a sufficient genetic distance to be considered as three different species inside the Clariidae family (Catfish Asia Project, 1997). The present study used one of these stocks, originating from Jambi (Sumatra Island). New hybridisation experiments with the two other "*C. meladerma*" groups that were identified could lead to different results.

Generally speaking, *C. meladerma* and its hybrids with *C. nieuhofii* and *C. teijsmanni* do not seem to present a high fish farming potential on account of the very low growth rates that were observed. On the opposite, relative zootechnical performances of *C. batrachus*, *C gariepinus* and *C. meladerma* x *C. gariepinus* are worth being specified. They have to be analysed taking into account the flesh and organoleptic characteristics of these fishes, compared to local market requirements. The sterility of the hybrid *C. meladerma* x *C. gariepinus* would be an important advantage as it could be used in aquaculture without possible impacts on gene pools of wild species.

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PRELIMINARY RESULTS ON GROWTH AND BODY COMPOSITION IN *CLARIAS MACROCEPHALUS*, *CLARIAS GARIEPINUS* AND THEIR HYBRID (*C. MACROCEPHALUS* FEMALE X *C. GARIEPINUS* MALE)

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Abstract

The local catfish *Clarias macrocephalus*, the African one *Clarias gariepinus* and their hybrid were compared for their growth rate and body composition during a 4 months rearing period. The African catfish and the hybrid proved to be the best in term of growth rate both for length and weight gain in comparison with *C. macrocephalus*. At the end of the experiment, starting from 8-10 g fingerlings, the mean length and weight of the African catfish, the local catfish and their hybrid were 27.8 cm, 20.4 cm, 26.6 cm and 183 g, 94,1 g and 176 g, respectively. This corresponded to a daily weight gain of 0.7 $g.d^{-1}$ in *C. macrocephalus* and 1.4 $g.d^{-1}$ in both *C. gariepinus* and the hybrid.

The muscle and carcass composition of the fish were studied for different size groups. The protein content of the muscle was higher than in carcass, while the inverse situation was observed for lipids. The fish composition varied as a function of fish size. The water content was the highest (79.3-83.3%) in the smaller fish (8-10g). In this fish group, the protein content of the muscle was of 82.7, 70.6 and 81.4% of dry matter (DM) for *C. macrocephalus*, *C. gariepinus* and their hybrid, respectively. In comparison with *C. gariepinus* and the hybrid, the muscle of *C. macrocephalus* presented the highest mean protein content (83.0% DM) and the lowest mean lipid content (3.7% DM). The hybrid had the highest lipid level in comparison to the other fish studied, and its highest lipid value (17.3% DM) was found for fish of 200-250 g body weight.

INTRODUCTION

Clarias catfish is one of the commercially important fish groups reared in Vietnam, where it is represented by 4 species, the Clarias catfish (*Clarias macrocephalus*), the African catfish (*Clarias gariepinus*), the Black catfish (*Clarias fuscus*) and the Walking catfish (*Clarias batrachus*), and by one hybrid *Clarias macrocephalus* (female) x *Clarias gariepinus* (male). These fishes have a short culture period, high meat quality, high prices and are highly tolerant to a large range of environmental conditions. Moreover, they can be cultured in small-scale fish farming. The Clarias catfish (*Clarias macrocephalus*) has the highest meat quality but the slowest growth in comparison with the others. On the other hand, the African catfish reaches large size and presents a high growth rate, but its flesh is poorly appreciated by consumers. The hybrid, *C. macrocephalus* x *C. gariepinus*, has combined good characteristics from its parents as fast growth, high tolerance to bad environmental conditions and esteemed meat quality. For these reasons, its culture has known an important development in Vietnam.

In order to evaluate their respective performances and flesh quality, the aim of the present study was to compare the growth and composition of muscle and carcass of *C. macrocephalus*, *C. gariepinus* and their hybrid.

MATERIALS AND METHODS

Experimental conditions

Groups of *C. macrocephalus*, *C. gariepinus* and their hybrids were reared for 4 months at a stocking density of 30 $fish.m^{-2}$ in hapas implemented in a pond. The fish were fed twice a day at a daily feeding rate of 5% of their body weight. The composition of the feed was 63% rice bran, 35% fishmeal and 2% mineral premix. For each fish category, thirty individuals were sampled every month for length and weight determination.

The temperature and pH of the pond water were

measured every day while DO, COD and $N\text{-}NH_4^+$ were measured once a week.

Test animals

Fish of 8-10g initial weight were used in this study. For the measurement of carcass composition, fish were sampled at three size groups: 100-150 g, 200-250 g and 300 g. A supplementary group (8-10 g) referred to the initial situation of the fish. In the case of the fourth group of *Clarias macrocephalus*, specimens were not caught in the hapa but directly in the pond, because no specimens of 300 g could be obtained in the experimental fish at the end of this study.

Analysis of muscle and carcass composition

Chemical composition of fish (muscle and whole carcass) and diets were determined for crude proteins (Kjeldahl, nitrogen x 6.25); crude lipids (Soxhlet chloroform extraction); ash (residue after burning 5 minutes and heating 4-5 hrs at 550 °C); moisture (loss on drying at 105 °C for 4 hrs) and NFE (Nitrogen free extract, subtraction).

Statistical analysis

Data on daily weight gain (DWG) and feed per gain ratio (FGR) were subjected to one-way analysis of variance and differences in treatment means were compared by Duncan's new multiple range test ($p<0.05$) using the Statgraphics software.

RESULTS AND DISCUSSION

Water management

The evolution of water quality parameters in the pond during the experiment is presented in Table 1.

Growth of fish (Table 2)

During the first month of the experiment, the specific growth rate (SGR) of *C. gariepinus* and the hybrid were superior (4.5 and 4.6 $\%.day^{-1}$, respectively) to that of *C. macrocephalus* (2.9 $\%.day^{-1}$). However, during the last two months (months 3 and 4), the SGR of *C. macrocephalus* and hybrid were higher than that of *C. gariepinus*.

The daily weight gain observed for *C. gariepinus*, *C. macrocephalus* and their hybrid ranged from 0.9 to 2.1 g, from 0.4 to 1.2 g and from 0.7 to 2.2 g, respectively. When considering the whole experimental period, *C. macrocephalus* displayed the lowest daily weight gain (0.7 $g.d^{-1}$). This DWG value was two times lower than those observed for both *Clarias gariepinus* and the hybrid (1.4 $g.d^{-1}$). For the hybrid, Long (1995) reported values of SGR and DWG of 1.25-1.40%.day^{-1} and 0.53-0.56 $g.d^{-1}$, respectively.

Muscle and carcass composition

The carcass composition is of major importance to evaluate the nutritional value of the products. The results of the analysis of muscle and carcass composition of *C. gariepinus*, *C. macrocephalus* and their hybrid, carried out during the four months of grow-out, are given in Table 3 and 4.

The results indicates that, after four months, water and protein levels in carcass were lower than in the muscle, while lipid and mineral levels were higher. Can *et al.* (1987) reported that the lipid level varies usually in opposition to water level in the fish body. The lipid level in fish body is higher than in the muscle because we may find high levels of lipids in the skin, intestine and liver (Tuan, 1982).

During the experiment, the protein levels in the muscles of *Clarias macrocephalus*, *Clarias gariepinus* and their hybrid were of 79.0-85.8% DM (or 14.3-17.7% in wet weight), 62.5-82.3% DM (or 13.9-16.5% in wet weight) and 72.0-81.4% DM (or 13.9-14.6% in wet weight), respectively. According to Can *et al.* (1987) protein level of catfish is about 70-80% DM. Phuong (1997) reported protein contents of 12.9% (in wet weight) for *Pangasius bocourti* and Tram (1996) values of 14-28% (in wet weight) in various sea fish.

The mean lipid content appeared much lower in the muscle of *C. macrocephalus* (3.7% DM) than in the muscles of *C. gariepinus* (6.8% DM) or of the hybrid (11.1% DM). These values were equivalent to about 0.5 to 3% of lipid content in wet weight. Can *et al.* (1987) reported lipid levels of less than 1% (in wet weight) for *C. macrocephalus* and *C. gariepinus*. In contrast, a particularly high value of 17.1% (in wet weight) was reported for the Mekong catfish *Pangasius bocourti* (Phuong, 1997).

The chemical composition of the studied catfish changed with the size groups (Table 3 and 4, Fig. 1 and 2). For the first size group (8-10g), the protein level in the muscle of the three fish was high (70.6-82.7%), while the lipid level was low (4.1-6.6%). The highest protein level in the muscle was found for *C. macrocephalus* in the second group (100-150 g) and for *C. gariepinus* in the third group

Parameters	Month 1	Month 2	Month 3	Month 4
Temperature (°C)	28.0 ± 0.8	30.0 ± 1.3	29,5 ± 1.3	29.0 ± 1.5
pH	6,5 ± 0.4	7.0 ± 0.4	7.0 ± 0.1	7.0 ± 0.4
O_2 ($mg.l^{-1}$)	2.4 ± 0.5	3.2 ± 0.4	3.6 ± 0.4	3.0 ± 0.3
COD (mgO2/l)	9.6 ± 0.8	13.6 ± 1.4	12.0 ± 0.9	8.0 ± 1.3
$N\text{-}NH_4^+$($mg.l^{-1}$)	0.7 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	0.8 ± 0.1

Table 1: Water quality of experimental pond.

Time (month)	*C. macrocephalus*	*C. gariepinus*	Hybrid
Body weight (g)			
Initial weight	9.2 ± 0.7 a	9.0 ± 2.2 a	8.7 ± 0.5 a
1 month	22.9 ± 1.8 a	35.6 ± 7.8 b	35.6 ± 3.2 b
2 month	35.0 ± 7.8 a	75.2 ± 12.9 b	56.9 ± 8.1 b
3 month	58.9 ± 16.0 a	118.6 ± 5.9 b	108.8 ± 3.5 b
4 month	94.1 ± 13.0 a	183.2 ± 22.1 b	176.1 ± 11.1 b
SGR ($\%.day^{-1}$) (1)			
1 month	2.9 ± 0.5	4.5 ± 1.5	4.6 ± 0.5
2 month	1.3 ± 0.5	2.4 ± 1.2	1.4 ± 0.7
3 month	1.7 ± 1.4	1.6 ± 0.7	2.2 ± 0.6
4 month	1.6 ± 1.2	1.4 ± 0.3	1.6 ± 0.1
DWG ($g.day^{-1}$) (2)			
1 month	0.4 ± 0.1 a	0.9 ± 0.3 b	1.0 ± 0.1 b
2 month	0.4 ± 0.2 a	1.3 ± 0.7 b	0.7 ± 0.3 ab
3 month	0.8 ± 0.7 a	1.7 ± 0.6 a	1.7 ± 0.4 a
4 month	1.2 ± 0.9 a	2.1 ± 0.6 a	2.2 ± 0.2 a
Whole period	0.7 ± 0.1 a	1.4 ± 0.2 b	1.4 ± 0.1 b

(1) Specific growth rate (SGR) = 100*(Ln Wf -Ln Wi)/time (day);
(2) Daily weight gain (DWG) = (Wf-Wi)/time (day); Wf: final weight; Wi: initial weight
Values in each line having same letter (a, b, c, d) are not significantly different ($P<0.05$)

Table 2: Comparison of different growth parameters of *C. macrocephalus*, *C. gariepinus* and their hybrid.

Species	Size groups	Water	Minerals (% DM)	Proteins (% DM)	Lipids (% DM)
C. macrocephalus	8-10 g	83.3	11.5	82.7	4.1
	100-150 g	79.4	6.5	85.8	6.0
	200-250 g	81.9	9.3	79.0	3.2
	300 g	84.7	9.9	84.1	1.4
	Average	82.3	9.3	82.9	3.7
C. gariepinus	8-10 g	80.3	18.0	70.6	6.2
	100-150 g	80.8	10.7	78.8	10.5
	200-250 g	80.0	8.4	82.3	3.1
	300 g	77.8	9.8	62.5	7.3
	Average	79.7	11.7	73.6	6.8
Hybrid	8-10 g	81.8	10.7	81.4	6.6
	100-150 g	80.6	11.7	72.0	17.3
	200-250 g	77.7	8.3	75.1	11.8
	300 g	78.2	9.4	75.2	8.8
	Average	79.6	10.0	75.9	11.1

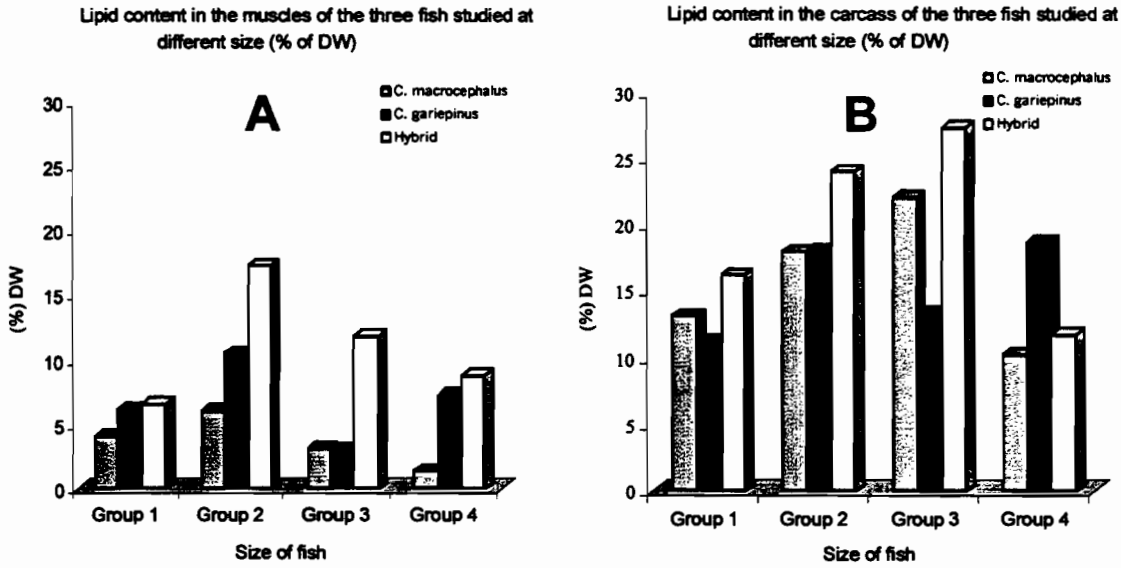
(data expressed in % of dry matter)

Table 3: Chemical composition of the muscles in the three studied fish at different sizes.

Species	Size groups	Water	Minerals (% DM)	Proteins (% DM)	Lipids (% DM)
C. macrocephalus	8-10 g	83.3	18.0	67.9	13.2
	100-150 g	74.3	13.5	67.8	18.0
	200-250 g	77.5	17.5	60.3	22.1
	300 g	79.1	16.8	72.7	10.3
	Average	78.5	16.4	67.2	15.9
C. gariepinus	8-10 g	79.3	20. 6	62.0	11.2
	100-150 g	76.0	21.3	60.2	18.1
	200-250 g	74.8	27.7	48.7	13.4
	300 g	73.6	20.1	60.4	18.8
	Average	76.0	22.4	57.8	15.4
Hybrid	8-10 g	79.2	19.3	64.2	16.3
	100-150 g	79.1	21.4	54.4	24.1
	200-250 g	75.9	12.2	60.2	27.4
	300 g	75.1	18.2	69.5	11.8
	Average	76.3	17.8	62.1	19.9

(data expressed in % of dry matter)

Table4: Chemical composition of the carcass in the three studied fish at different sizes.



(group 1 = 8-10 g; group 2 = 100-150 g; group 3 = 200-250 g; group 4 = 300 g)

Figure 1: Lipid content in the muscles (A) and the carcass (B) of the three fish studied as a function of different size groups.

(200-250 g).

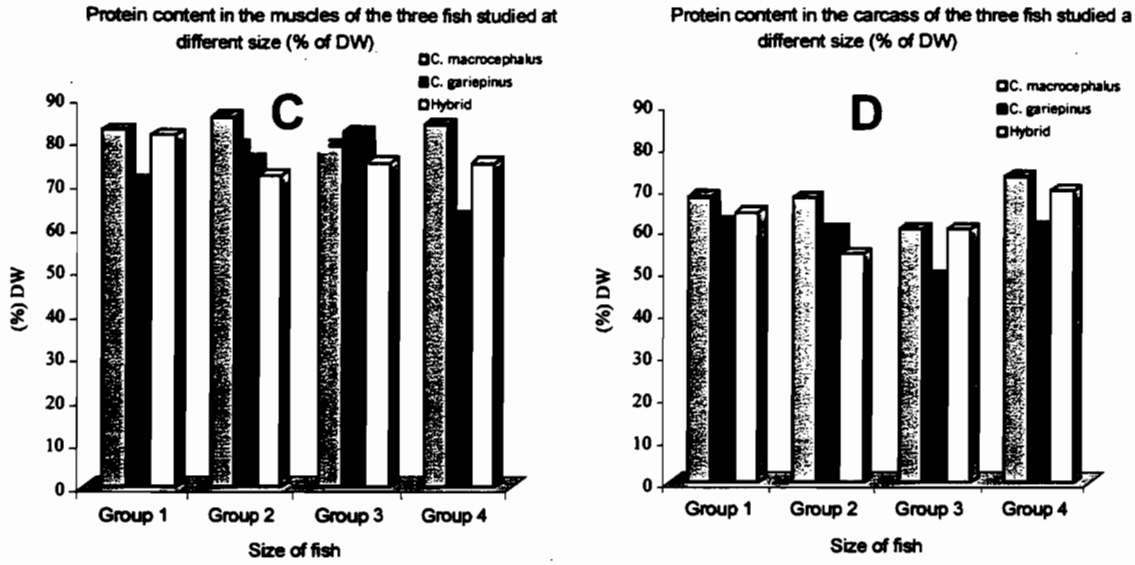
By contrast, in the final group (300g) the protein level of *C. gariepinus* is the lowest (62.48%).

CONCLUSIONS

The African catfish and the hybrid proved to be the best in term of growth rate both for length and weight gain in comparison to *C. macrocephalus*. The daily weight gain observed for *C. macrocephalus* (0.7 $g.d^{-1}$) during the 4 months experiment was two times lower than that of *C. gariepinus* and the hybrids (1.4 $g.d^{-1}$ in both cases).

Concerning fish composition, the water content was the highest (79.3-83.3%) in the smaller fish (8-10g). In this fish group, the protein content of the muscle was of 82.7, 70.6 and 81.4% DM for *C. macrocephalus*, *C. gariepinus* and their hybrid, respectively.

In comparison with *C. gariepinus* and the hybrid, the muscle of *C. macrocephalus* presented the highest mean protein content (83.0% DM) and the lowest mean lipid content (3.7% DM). The hybrid had the highest lipid level in comparison to the other fish studied.



(group 1 = 8-10 g; group 2 = 100-150 g; group 3 = 200-250 g; group 4 = 300 g)

Figure 2: Protein content in the muscles (C) and the carcass (D) of the three fish studied as a function of different size groups.

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PRELIMINARY OBSERVATIONS ON THE INFECTION OF THE GILL OF CULTIVATED *PANGASIUS HYPOPHTHALMUS* BY MONOGENEA

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Abstract

Preliminary study on the determination of Monogenea species present on *Pangasius hypophthalmus* gills in culture conditions (Sukamandi station, Indonesia) was carried out in ten fish of about 1.0 kg body weight and one year old. One species was found and was identified as *Thaparocleidus caecus* (syn. *Silurodiscoides caecus*).

INTRODUCTION

Pangasius hypophthalmus was introduced from Thailand to Bogor (Indonesia) in 1972. The first reproduction by induced spawning using common carp pituitary gland was done in 1978 and led to the production of about 5.000 larvae (Hardjamulia, pers. comm.). Nowadays *P. hypophthalmus* is one of the most popular species to be cultured in Indonesia, particularly in Sumatra.

It is well known that fish culture is important for a populous country like Indonesia because fish become the main source of protein for the people. Aquaculture is also important because it can be a source of income for people who involve in this activity directly or indirectly.

One of the major limiting factor that has to be considered in fish culture is disease. Disease caused by parasites can result in death of fish or can reduce fish growth (Bauer, 1970; Needham & Wootten, 1978).

Gill is an essential organ; it has a multifunction which involves ion and water transfers as well as oxygen, carbon dioxide and ammonia exchange (Hoar & Randal, 1984). Irritation of the gills by parasites or pollution will disrupt its function and affect the condition of fish (Ferguson, 1989).

Monogenea are among the parasites most commonly found on fish gills. Lim (1990) reported that there are two species of Monogenea infecting the gills of *Pangasius hypophthalmus* in Malaysia. Pariselle and Komarudin (1999) also found two species of Monogenea on the gills of specimens of this species from the Mekong Delta (Vietnam).

The objective of this study is a preliminary determination of the species of monogenetic Trematodes infecting *Pangasius hypophthalmus* in culture condition in Java, Indonesia.

MATERIALS AND METHODS

Ten *P. hypophthalmus* specimens of about 1.0 kg body weight and one year old were obtained from the experimental ponds of the Research Institute for Freshwater Fisheries (Sukamandi, West Java, Indonesia). The right gills of each fish were dissected and placed in a deep freezer before determination of the parasites.

The parasites were identified by examining fresh died and semi-permanent mounted specimens. The parasites were teased out from the gill filaments using fine needle and then placed on a glass-slide added with Malmberg (1957) solution, covered with coverslip and mounted by Entelan for microscopic examination and measurements.

RESULTS AND DISCUSSION

One species of *Thaparocleidus* Jain, 1952 (syn. *Silurodiscoides* Gussev, 1961) was found which was identified as *T. caecus* (Mizelle & Kritsky, 1969). *T. caecus* was identified from the following measurements (in µm) and observations: size 737 (500-920) x 106 (60-140), marginal hook 14.3

(12.5-15.0), dorsal anchor 55.8 (55.0-62.5), ventral anchor 23.8 (17.5-25.0), dorsal bar 40.8 (32.5-50.0), ventral bar 25.8 (17.5-30), four granulated eyespot.

Similar finding of *T. caecus* has also been reported from Malaysia and Vietnam on the same fish (Lim ,1990; Pariselle & Komarudin, 1999). However, these authors also reported the presence of another species of Monogenea (*T. siamensis*) on the gill of *P. hypophthalmus*. The absence of this latter species from *P. hypophthalmus* sampled in the Sukamandi station may be a result of:

⇒ the difference in age of fish examined in Malaysia and Vietnam compared to Indonesia,

⇒ the low number of fish individuals examined at this stage,

⇒ the peculiar water conditions in Sukamandi (only place sampled).

An other hypothesis is the loss of one species of parasite during the first transfer between Thailand and Indonesia. This is possible due to the fact that parasitic species are not randomly distributed within hosts and one species could have been absent from the living fishes introduced to Indonesia.

To be able to culture catfishes successfully attention has to be given to the presence of Monogenea because they were considered as dangerous for the fish. Lewis and Lewis (1970) reported that *Gyrodactylus* (Monogenea) can cause mortality independent of secondary infection. The effect of Monogenea on the fish is due to its attachment (Lester, 1972; Cusack & Cone, 1986) and feeding activity (Cone & Odense, 1984).

Further study has to be carried out to precise the species of Monogenean infecting *P. hypophthalmus* in other locations in Indonesia and determine the effects of these worms on the fish and their prevention and control methods.

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PRELIMINARY STUDY OF THE SOURCE OF *AEROMONAS HYDROPHILA* INFECTION ON *PANGASIUS HYPOPHTHALMUS* LARVAE

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Abstract

One of the main problems that has to be faced by *Pangasius hypophthalmus* breeders is the high mortality of the larvae, which can be a consequence of both cannibalism and bacterial diseases. The aim of this study was to explore the possible sources of *Aeromonas hydrophila* that may result in an infection of *Pangasius hypophthalmus* larvae. Isolation of bacteria were made from water source, *Artemia* culture medium, *Artemia* nauplii used as feed, ova (unfertilised eggs), sperm, and fed as well as unfed larvae that were collected every day from hatching, up to the age of 7 days. Characterisation of bacteria were done on the basis of morphological, physical and biochemical characteristics. The serological method of identification using polyclonal *Aeromonas hydrophila* antiserum was used. The results indicated an absence of bacteria from sperm. By contrast, bacteria *Alcaligenes* sp. were isolated from source of water, unfertilised eggs and from 1-day-old larvae. *Proteus* sp. were isolated from the 1st day of age in fed larvae and from the 7th day in unfed larvae. *Aeromonas hydrophila* were isolated from larvae of 2 days of age, and *Aeromonas punctata* from 2-days-old unfed larvae, meanwhile *Plesiomonas shigelloides* were identified from fed larvae of 2 and 4 days of age, and from 2-days-old unfed larvae.

INTRODUCTION

During the last few years, fish health problem became a major concern to aquaculturist in all over the world. In south-east Asian countries, fish production was badly affected by the outbreak of fish disease, such as Epizootic Ulcerative Syndrome (EUS) in 1980. Important bacterial fish pathogens, including *Aeromonas* spp., *Pseudomonas* spp. and *Flexibacter columnaris*, are regularly isolated from fish and become primary pathogenic agents frequently reducing the production of cultured freshwater fish. In late 1980, a total of 125 tons of carp were lost in Java (Indonesia) due to bacterial disease infection (Djajadiredja *et al*., 1983). The disease was caused by bacterium *Aeromonas hydrophila*. This bacterium was not only causing mortality on common carp but also on catfishes and snakehead fish. In Indonesia, the outbreak of this disease was firstly reported from West Java but since then it has spread out to all over Java, Kalimantan and Sumatra.

Catfishes are important commonly cultured freshwater fish species besides common carp and tilapias. Intensive culture of walking catfish (*Clarias batrachus*) in Indonesia is already practised. Fish farmers are encouraged to culture catfish because it is highly profitable. African catfish (*Clarias gariepinus*) was introduced into Indonesia and is now being cultured extensively. *Clarias gariepinus* grow faster than the local species and is therefore preferred by farmers for culture. However, the development of catfish culture in recent years has been hampered by the frequent occurrence of bacterial disease.

Another species of catfish that was introduced to Indonesia 26 years ago is the Thai catfish (*Pangasius hypophthalmus*). This species of catfish resemble to local pangasiid species. Because of breeding technique of local *Pangasius* has not been developed, the Thai catfish has been cultivated as a substitute to local species. One of the main problems that has been faced by the breeder is the important mortality of the larvae, which is suspected to be a consequence of both cannibalism and bacterial disease (Subagja *et al*, 1998).

Fish diseases are often associated with intensive fish culture. Interaction leading to bacterial disease in fish depends on the presence of the pathogen, the quality of environment and the general status of the fish. Balance of these conditions can ensure

fish health without the use of chemotherapeutic agent. Health is promoted by ensuring good water quality, appropriate stocking densities and providing balanced feed.

Bacterial haemorrhagic septicaemia due to strain of *Aeromonas hydrophila* may be transmitted through the water, diseased and healthy fish, other affected vertebrates, and favoured by external as well as internal parasites. A reservoir of potential pathogens probably exists in all natural and artificial water bodies (Newman, 1982).

The present paper aims at a preliminary exploration of the possible sources of *Aeromonas hydrophila* that may result in infection of *Pangasius hypophthalmus* larvae.

MATERIALS AND METHODS

Pangasius hypophthalmus used for the experiment were bred at the Research Institute for Freshwater Fisheries (RIFF) in Sukamandi, Subang West Java.

The larvae were obtained from 3-5 years old *P. hypophthalmus* brooders held in earthen ponds and supplied with water from irrigation. Fish were artificially induced to ovulated using Ovaprim and eggs were fertilised using the procedure described by Legendre *et al.* (1999). Twelve hours after hatching, the larvae were individually counted and transferred to the experimental facility. they were reared in stagnant spring water and maintained in fibreglass container and aquarium.

Isolation of bacteria

The isolation of bacteria was made from different sources:

- water (ground water which is normally used for larval rearing),
- the medium of *Artemia* culture,
- *Artemia* nauplii themselves,
- unfertilised eggs collected just after stripping of females,
- from sperm collected just after stripping of males,
- from fertilised eggs in incubation, collected at 2 h after fertilisation.

Larvae were collected every day, from hatching up to the age of 7 days. Two groups of larvae were followed, one group was totally starved during the whole duration of experiment and the other one was fed with *Artemia* nauplii. This was done in order to test for a possible direct or indirect disease transmission through feeding. Fed larvae received *Artemia* nauplii in excess, starting from 36 hours after hatching up to 8 days of age. The feeding frequency was of 8 meals per day at 09:00, 12:00, 15:00, 18:00, 21:00, 24:00, 03:00 and 06:00.

Isolations of bacteria from water, and *Artemia* medium were carried out by serial dilution-agar plating procedure. A total of 25 µl of water or *Artemia* medium was inoculated onto melted Tryptic Soy Agar (TSA) (Dipco) and then plated onto sterile Petri dish.

Isolation of bacteria from sperm was done by inoculation of an aliquot onto the TSA plate.

The surface of unfertilised eggs, fertilised eggs, larvae at different ages either fed or unfed larvae and *Artemia* nauplii, were separately sterilised by washing with ethanol 70% and then re-washed with sterile water. Each specimen was then crushed by using a sterile tissue grinder and then inoculated onto TSA plate.

All isolations were made in three replicates and incubated at 28°C.

Identification of bacteria

Identification of *Aeromonas hydrophila* was done by serological method using polyclonal *Aeromonas hydrophila* antiserum. Antiserum used was prepared from *Aeromonas hydrophila* strain No. 26 of RIFF collections, produced by a standard procedure (Burrell, 1979).

Identification of other bacteria was carried out by morphological, physical and biochemical characterisation as describes by Cappucino and Sherman (1987) and Cowan (1985). Gram stain was done to determine the shape of bacteria and gram reaction. Motility of bacteria was determined by the "hanging drop" method.

RESULTS AND DISCUSSION

A number of colonies were observed after the isolation and 12 h of incubation. The colour of the colonies varies from buff to yellowish. The results after further identification indicated that various bacteria were able to be identified. All isolated bacteria belonged to the genera *Alcaligenes, Proteus, Plesiomonas* and *Aeromonas*. Isolated and identified bacteria are listed in Table 1.

Alcaligenes sp. identified from unfertilised eggs was considered as coming from the water source, in which it was also found. So, in that case, the surface sterilising process was not properly done.

Source of sample	Isolated bacteria
Water	*Alcaligenes* sp
Sperm	None
Ova	*Alcaligenes* sp
Fertilised eggs	None
Artemia culture medium	None
Artemia nauplii	None
Unfed larvae	
Age 2	*Plesiomonas shigelloides, Aeromonas punctata*
Age 7	*Proteus* sp.
Fed larvae	
Age 1	*Proteus* sp., *Alcaligenes* sp.
Age 2	*Aeromonas hydrophila, Plesiomonas shigelloides*
Age 4	*Plesiomonas shigelloides*

Table I: Isolated and identified bacteria from water, *Artemia* culture medium, *Artemia* nauplii and *Pangasius hypophthalmus* larvae.

Bacterium *Alcaligenes* sp. was also isolated from the samples. The bacterium was isolated from water, then found in fertilised egg and finally isolated in 1-day-old larvae. According to Kersters and De Ley (1963), this bacterium occurs in water and soil. Recently Austin *et al.* (1981) described a new group (subspecies) of bacteria isolated from moribund lobster and named them as *Alcaligenes faecalis* subsp. *homari*

Some of isolated bacteria were gram positive, and cocci shape. Those bacteria were considered to be non-pathogenic bacteria. As revealed by Richard and Roberts (1978), the majority of fish pathogens are Gram-negative rod but there are some Gram-positive pathogens, including a few which are acid fast.

There was no *A. hydrophila* isolated from water, sperm, ova, fertilised eggs, nor from 1-day-old fed and unfed larvae. The absence of bacterium *A. hydrophila* in the water reservoir, sperm, ova, fertilised eggs and *Artemia*, revealed that those sources were not at the origin of infection of larvae. Also *A. hydrophila* were absent in 1-day-old larvae. However, *A. hydrophila* were discovered to infect the larvae aged of 2 days. This could be coming from other sources such as planktonic organisms, fish parasites or other organisms, as Newman (1982) stated that a reservoir of potential pathogens probably exists in all natural and artificial water bodies. Besides the role of external and internal parasites in the transmission of the disease is probably much greater than it is generally assumed. Dombrowski (1953) isolated *A. liquifaciens* from copepods (*Argulus foliaceus*) and from leeches (*Piscicola geometra*). The state of contamination of equipment and operators may also play an important role in the transmission of disease agents.

Other species of bacteria predominantly isolated from the samples was *Plesiomonas shigelloides*. According to Habs and Schubert (1962), this bacterium was formerly classified in the genus *Pseudomonas* and then transferred to the genus *Aeromonas*. This was followed by its transfer to the newly created genus *Plesiomonas*. The bacterium occurs in fish and other aquatic animal.

CONCLUSION

The transmission of disease agent seems to be not through the water source, ova, sperm, *Artemia* culture medium or *Artemia* nauplii but could be due to contamination of equipment and operators.

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PRELIMINARY RESULTS OF THE STUDY OF PARASITIC AND RED SPOT DISEASES ON HIGH ECONOMICAL VALUABLE CATFISH SPECIES IN THE MEKONG DELTA

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Abstract

The parasitic diseases and bacteria causing red spot disease in the Mekong Delta were studied in three catfishes used in aquaculture: *Pangasius bocourti*, *P. hypophthalmus* and *Clarias* hybrid (*C. macrocephalus* x *C. gariepinus*). *Ichthyophthirius multifiliis*, *Trichodina* sp., *Dactylogyrus* sp., *Gyrodactylus* sp. and *Oodinium* sp. are the parasites most often found in skin, fins, and gills of diseased fish, particularly in fry nursed in cement tanks and earthen ponds. The "white spots" disease caused by *Ichthyophthirius multifiliis* occurred in both *Pangasius bocourti* and *P. hypophthalmus*. Red spot disease has also been recorded as the most common disease on catfish at the grow-out stage in cage culture. Most species of bacteria were found to be Gram negative and motile Aeromonads such as: *Aeromonas hydrophila*, *A. caviae*, *Pseudomonas fluorescens*, *Edwadsiella tarda* and *Vibrio* sp.

INTRODUCTION

As wild fish stocks tend to decline continuously, there is an increasing need to expand aquaculture in order to satisfy the demand for high quality protein to feed the world's growing population. To obtain high productivity, the diseases of cultured species need to be understood and controlled. In this prospect, some fast growing culture fish need to be considered and studied such as *Pangasius* and *Clarias* genera that were cultured commonly in Mekong Delta. Their main favourable characteristics for aquaculture are the ability to tolerate high stocking densities, fast growth and high yield as well as their good palatability and high market value. As the farming intensity increases, diseases, which are normally present in wild populations, become much more evident in the confined condition. Fish diseases caused by pathogenic organisms such as protozoa, fungus, bacteria and virus often occur during the culture period. However, a particular attention should be given to parasitic and bacterial diseases, as they have been considered as a serious problem on catfish.

In some cases, outbreaks of bacterial diseases have been related to stress factors. Circumstantial evidence suggests that *Aeromonas hydrophila* may be a secondary invader of parasite induced injuries (Kumar, 1986; cited by Pai *et al.*, 1995). In addition, many bacterial and parasitic pathogens have been reported in the Walking catfishes *Clarias batrachus*, *C. macrocephalus*, and the hybrid *C. macrocephalus* x *C. gariepinus* (Tonguthai *et al.*, 1993; Angka, *et al.*, 1994). The symptoms of the disease are similar in appearance to those found in other bacterial haemorrhagic septicaemia and differentiated into three main categories: acute with few gross symptoms, acute form with dropsy, chronic ulcerous form. It is believed that *A. hydrophila* is an opportunistic organism that contributes to secondary infection of the lesions; this bacterium has been also isolated from Epizootic Ulcerative Syndrome (EUS) (Anonymous, 1986; Llobrera & Gacutan, 1987). However, Supriyadi *et al.* (1995) indicated that Walking catfish (*C. batrachus*) can be protected from *A. hydrophila* by vaccination.

Much of the previous effort in studying and controlling diseases on catfishes such as *Pangasius bocourti, P. hypophthalmus* and the hybrid *Clarias macrocephalus* x *C. gariepinus* in Vietnam have been dissipated through a lack of complete scientific data. Therefore, the study of parasitic diseases and bacteria causing red spot disease (bacterial haemorrhagic septicaemia) on these catfish of economical importance have been considered as an important goal in the project. This paper presents the preliminary results obtained in this field.

MATERIALS AND METHODS

Studied sites: the survey was conducted in Chau Doc town (An Giang province), Vinh Long and Can Tho provinces. Samples of *Pangasius* at the grow-out stage were collected from cage culture in Chau Doc town with three different period during both the dry and the rainy seasons. At the same time, fish farmers were interviewed using adapted questionnaire forms. Information and data related to disease, culture techniques and health management, were recorded on the farms and at the fisheries processing plant (AGIFISH CO.).

Two hatcheries, located at Can Tho University and Chau Doc town (My Chau hatchery), were involved in the propagation and nursing of *P. bocourti* and *P. hypophthalmus* in the Mekong Delta. Fry and fingerling samples with parasitic diseases were collected from indoor cement tanks and examined every month.

Study of pathogens: method of study of fish parasites of Dogiel (1933) was applied in this study. The following methods were used for the collection of fish samples: (1) live diseased fish were kept in nylon bags and stored in ice container, samples being analysed as soon as possible (within 24 hours); (2) fish samples were transported to the laboratory in nylon bags supplied with oxygen; (3) diseased fish were also processed on the field, Tryptone Soy Agar plates (TSA) was then used for initial inoculation. Rimler-shortts Agar (RS), Decarboxylase (Arginie Lysine and Ornithine), Oxidation fermentation medium (O/F), Triple Sugar Iron Agar were used for biochemical tests to identify bacteria. The work was carried out at the Fish disease laboratory of the College of Agriculture, Can Tho University. Fish bacteria were studied according to the methods of Plumb (1983) and Frerich (1984).

RESULT AND DISCUSSIONS

Parasitological study: the commonest ecto-parasites were recorded on the cultured catfish studied (Table 1). Some protozoan (*Trichodina* sp, *Oodinium* sp), were observed on *Clarias* hybrid fry in earthen ponds and on *P. bocourti* fingerling in nursing cement tanks. White spot disease caused by *Ichthyophthirius multifiliis* occurred in both *P. hypophthalmus* and *P. bocourti*. In addition, Monogenetic trematodes consisting of *Dactylogyrus* and *Gyrodactylus* sp, attacked skin and gills causing high mortality on small fish, during the rainy season. Flashing movements, pin-point haemorrhages, excessive mucus production, obstacle to oxygen uptake, impaired feeding and lethargy have been commonest clinical signs observed. Specific signs as "white spots" were caused by *Ichthyophthirius multifiliis*.

The mortality rate caused by parasitic diseases

Infected catfish	Location	Stage	Parasites	No. of fishes tested	Degree of infestation
P. bocourti	Gills, fins and skin	Fingerlings	*Ichthyophthyrius*	40	++++
	Gills, fins and skin		*Trichodina*	30	++
	Skin and gills		*Dactylogyrus*	15	+++
	Gills and skin		*Gyrodactylus*	10	+++
	Gills and skin	Grow-out stage	*Dactylogyrus*	25	++
	Skin and gills		*Gyrodactylus*	10	++
	Tract intestine		*Balantidium*	7	++
	Tract intestine		*Trematoda*	11	+
	Tract intestine		*Nematoda*	6	+
P. hypophthalmus	Gills, fins and skin	Fingerlings	*Trichodina*	20	+++
			Ichthyophthyrius	14	++++
			Dactylogyrus	5	++
			Gyrodactylus	5	++
Clarias hybrid	Skin, fins, gills and muscle	Fingerlings	*Trichodina*	50	+++
			Ichthyophthyrius	20	+++
			Oodinium	10	++
		Juveniles	*Metacercaria*	5	+

+ = light, ++ = medium, +++ = heavy, ++++ = very heavy

Table 1: Parasites found on cultured catfish in the Mekong delta.

reached 80-90% at hatchery of Can Tho University and 50% at My Chau hatchery in Chau Doc town. *Ichthyophthirius multifiliis* was responsible for most of the mortality observed in *P. hypophthalmus* and *P. bocourti*. In general, external parasites attack skin, fins, and the gills causing acute mortality due to their direct life cycle or rapid multiplication. Unbalanced diets also appeared as one of the reasons for diseases and mortality of catfish.

In addition, *Balantidium* and Nematodes were observed in the intestinal tracts of *P. hypophthalmus* and *P. bocourti* and *Clarias* hybrid. These parasites did not cause high mortality on catfish. Immature Digenea (metacercaria) were also found under skin or muscle.

Bacteria isolated from diseased fish: seven strains of *Aeromonas hydrophyla*, three strain of *A. caviae* and *Pseudomonas sp.* were identified. These bacteria caused red spot disease on *P. bocourti*, *P. hypophthalmus* and *Clarias* hybrid. All strains of bacteria isolated from these catfishes are given in Table 2.

Besides pathogens, several other factors were responsible for outbreaks of disease in catfish farms. They include particularly overcrowding, poor environment quality, unbalanced diets and poor sanitary measures. Though the development of aquaculture and the desire to increase farm production, farmers often forget the importance of maintaining the delicate balance between the host and the environment. In hatcheries, changes in physico-chemical and microbiological quality of water also generate stress in fish making them more susceptible to invasion by pathogens. Subasinghe (1995) shown that the best quality environment with respect to an aquatic organism refers to water body close to its natural environment. This includes provision of good physical, chemical and microbial quality of water, adequate swimming space and feeding with a nutritionally balanced diet.

CONCLUSIONS

White spot disease (Ich) is still a serious problem in small catfish farming, including fingerling and even juvenile stages. Therefore, study of chemotherapeutants application methods, as well as environment management for prevention and treatment should be carried out. Prevention and control of fish disease through environmental manipulation is far more economical and effective than other methods of control. Therefore, attention should be paid for water treatment in nursing tank systems in hatcheries, in order to reduce mortality caused by ectoparasites. Unbalanced diets may be related to outbreak of some diseases, especially lack of vitamin and specific minerals in diets. An experiment on supplying Vitamin C for prevention of white spot disease should be carried out in near future. Hemorrhage disease (red spot disease) have caused a reduced production of catfish at the grow-out stage. Study on cause, treatment and precautionary measures have to be considered.

Bacteria isolated	Site of isolation	Infected species	Clinical signs	No. of fishes examined
A. hydrophila		*P. bocourti*,	Haemorrhages on the muscle	10
A. caviae		*P. hypophthalmus*,	and internal organs or at the	
		Clarias hybrid	base of fins; red or yellow	
			fluid in abdominal cavity and	
	Kidney		fin rot	
Pseudomonas	Liver	*P.bocourti*,		2
fluorescens	Spleen	*Clarias* hybrid		
	Lesion			
Vibrio sp.				2
Streptococcus.sp		*P. bocourti*	White nodules in the internal	3
			organs and protruded anus	
Edwadsiella				4
tarda				

Table 2: Bacteria isolated from diseased catfishes cultured in the Mekong delta.

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