

Larval rearing of an African catfish, *Heterobranchus longifilis*, (Teleostei, Clariidae): a comparison between natural and artificial diet

Nanthawat Kerdchuen and Marc Legendre (*)

ORSTOM/GAMET, Groupe Aquaculture Continentale Méditerranéenne et Tropicale, BP 5095, 34033 Montpellier Cedex 1, France.

(*) Author to whom all correspondence should be addressed.

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Abstract

Two feeding experiments were conducted to evaluate growth and survival rates of African catfish (*Heterobranchus longifilis* Valenciennes, 1840) larvae fed with different diets: live *Artemia* nauplii, frozen *Artemia* nauplii, live zooplankton (*Moina micrura*), frozen zooplankton (*Moina micrura*), a dry diet based on yeast powder and beef liver, and a commercial trout starter diet. The larvae were fed in excess, six times per day every 4 hours, from the onset of exogenous feeding up to an age of 14 days. In both experiments the final mean weight and specific growth rate of fish fed live *Artemia* nauplii were significantly higher than those of fish fed other diets ($p < 0.05$). Survival rates of fish fed *Artemia* (live or frozen), *Moina micrura* (live or frozen) and dry diet did not significantly differ ($p > 0.05$) and ranged from 79 to 92% and 61 to 73% for experiment 1 and experiment 2, respectively. The commercial trout diet led to significantly lower growth and survival rates in comparison to all other diets tested. Although it led to a lower growth rate than that obtained with *Artemia*, *Moina micrura* proved suitable for first feeding of *H. longifilis* larvae. Locally available in West African waters, this cladocera could constitute a valuable alternative for larval rearing when a shortage of *Artemia* is experienced. The results also showed that the artificial feed based on yeast powder and beef liver leads to survival rates as high as those obtained with living diets and thus represents a promising way for feeding *H. longifilis* fry. However, further studies on the nutritional requirements of the larvae remain necessary in order to improve the efficiency of dry diet in terms of growth performance.

Keywords: *Heterobranchus longifilis*, *Artemia*, *Moina*, compound diet, feeding, larval rearing.

Élevage larvaire d'un silure africain, Heterobranchus longifilis (Teleostei, Clariidae): une comparaison entre aliments naturels et artificiels.

Résumé

Deux expériences ont été réalisées pour évaluer la croissance et la survie des larves de silure africain (*Heterobranchus longifilis* Valenciennes, 1840) nourries avec les aliments suivants: nauplii d'*Artemia* vivantes, nauplii d'*Artemia* congelées, zooplancton vivant (*Moina micrura*), zooplancton congelé (*Moina micrura*), un aliment sec à base de levures et de foie de bœuf et un aliment commercial pour truitelle. Les larves ont été nourries en excès, six fois par jour toutes les 4 heures, depuis leur entrée en phase trophique jusqu'à l'âge de 14 jours. À l'issue des deux expériences, le poids moyen et le taux de croissance spécifique des larves nourries avec les nauplii d'*Artemia* vivantes sont plus élevés que ceux des larves nourries avec les autres aliments ($p < 0,05$). En revanche, les taux de survie ne montrent pas de différence significative pour les larves nourries avec les *Artemia* (vivantes ou congelées), les *Moina* (vivantes ou congelées) et l'aliment sec ($p > 0,05$). L'aliment pour truitelle amène à une croissance et une survie significativement plus faible que celles obtenues avec les autres aliments testés. Bien que conduisant à une croissance plus faible que l'*Artemia*, *Moina micrura* s'est avérée un bon aliment de départ pour les larves de *H. longifilis*. Localement disponible dans les eaux ouest-africaines, ce cladocère pourrait constituer une alternative intéressante pour l'alimentation larvaire lorsque l'approvisionnement en *Artemia* est difficile. Les résultats montrent aussi que l'aliment sec à base de levures et de foie de bœuf permet d'atteindre des taux de survie aussi élevés que ceux obtenus avec les proies vivantes et représente de ce fait une voie prometteuse pour l'alimentation des larves de *H. longifilis*. Des études complémentaires des besoins nutritionnels des larves demeurent cependant nécessaires en vue d'une optimisation des performances de croissance.

Mots-clés : *Heterobranchus longifilis*, *Artemia*, *Moina*, aliment composé, alimentation, élevage larvaire.

INTRODUCTION

The economic importance of Clariid catfishes as food fish has been demonstrated in most Asian and African countries (Hogendoorn, 1983; Areerat, 1987; Legendre, 1992). In Asia, Clariid catfishes of the genus *Clarias*, especially *C. batrachus* (Linnaeus, 1758) and *C. macrocephalus* Günther, 1864 have been used, while in Africa *C. gariepinus* (Burchell, 1822) and more recently *Heterobranchus longifilis* Valenciennes, 1840, have generally been propagated.

The great potential of *H. longifilis* for fish culture was demonstrated a few years ago in Côte-d'Ivoire (Ivory Coast). It is a hardy omnivorous fish that can be cultured either in freshwater or in brackish water up to 10 g.l⁻¹ salinity (Legendre, 1983; 1992). A method of artificial reproduction has also been established and large quantities of eggs and larvae can be obtained throughout the year (Legendre, 1986; 1992). In addition, a comparative study on the growth rates of *H. longifilis* and *C. gariepinus* has shown a faster growth for the former species (Legendre *et al.*, 1992).

Until recently, larval rearing of this species under hatchery conditions has been performed by using *Artemia* nauplii which was found to be an excellent first feed (Legendre *et al.*, 1991). However, the use of *Artemia* may not really be suitable in many African countries since *Artemia* is expensive, has to be imported and requires specific facilities and skill for nauplii production. To avoid this constraint, other diets should be developed as substitutes for *Artemia*.

Successful rearing of fish larvae using live or frozen zooplankton has been reported for several species (Watanabe *et al.*, 1983; Dabrowski, 1984). Among the various species of zooplankton, cladocerans of the genus *Moina* are known to be suitable as feed for successful larval rearing of *Chanos chanos* (Villegas, 1990) and *Clarias macrocephalus* (Fermin and Bolivar, 1991). An investigation on gut contents of fish reared in ponds in the Côte d'Ivoire also indicated that *H. longifilis* larvae feed essentially on zooplankton during their first ten days of life and that, during this period, the cladoceran *Moina micrura* constitutes a prey preferentially selected (Legendre, 1992).

It has also been reported that some freshwater fish larvae can be reared exclusively on artificial diets from first feeding, as is the case of *Clarias gariepinus* (Hecht, 1981; Uys and Hecht, 1985; Appelbaum and Van Damme, 1988), *Coregonus* sp. (Bergot *et al.*, 1986; Dabrowski *et al.*, 1986) or *Cyprinus carpio* (Charlon and Bergot, 1984; Bergot, 1986; Charlon *et al.*, 1986). The purpose of the present study was to evaluate the growth and survival rates of *H. longifilis* larvae fed zooplankton (*Moina micrura*) or dry diets under hatchery conditions. The success of larval rearing was compared to that obtained with *Artemia* which served as a reference.

MATERIALS AND METHODS

The larvae used in this study were obtained from the Layo Aquaculture Research Station (Côte-d'Ivoire) using the procedure of artificial reproduction established by Legendre (1986). On the day after hatching the larvae were counted and transferred to the rearing facilities. Feeding started on the evening of the second day, when the yolk sac was nearly completely absorbed. At this stage, the larvae were approximately 7 mm in total length and 2 mg mean body weight.

Experimental diets

The following diets were tested:

1. Live *Artemia* nauplii, obtained from cysts (Bio-marine, California) incubated in hatching jars containing tapwater with marine salt added to give a salinity of 30 g.l⁻¹. After 28-32 hours of incubation at 30°C, the nauplii were collected, separated from the cyst shells and fed to the fish. The size of nauplii ranged between 390 and 630 µm.

2. Frozen *Artemia* nauplii, prepared by placing the newly hatched nauplii in ice boxes and frozen at -18°C until used. The maximum conservation time was about two weeks.

3. Live *Moina micrura*, produced in concrete tanks (2 m³) filled with filtered water from a pond. The water, stagnant in the tanks, was aerated and fertilized with chicken manure. Each tank was sown with a monospecific population of *Moina*, previously obtained from the ponds of the Layo station and grown in aquaria. After population development in the tanks (2,000-4,000 individuals per litre), *Moina* were collected every day and distributed to the fish. The size of the harvested *Moina* ranged between 490 and 750 µm.

4. Frozen *Moina micrura*, prepared by harvesting the mass-produced *Moina* in tanks with a fine mesh net, then transferred to ice boxes and frozen at -18°C until used.

5. A compound dry diet (48.3% crude protein) provided by INRA, France. The diet composition was as follows: "Protibel" yeast powder (50%), beef liver (35%), cod liver oil (5%), vitamin mixture (5%) and mineral mixture (5%) (Bergot *et al.*, 1986). Two series of microparticles were used: 100-200 µm and 200-400 µm.

6. A commercial trout feed (Trouvit 000, TROUW France; 55% crude protein) which was first ground and sieved to obtain two particle size fractions of 100-200 µm and 200-400 µm.

Experimental procedures

Two experiments with different rearing conditions were carried out at the Centre de Recherches Océanologiques d'Abidjan (Côte d'Ivoire) in order to compare growth and survival rates of *H. longifilis* fry fed with the different experimental diets. Each experiment lasted 12 days.

Experiment 1: The six diets were tested. Eighteen groups of 60 larvae (three replications per treatment) were maintained in 1-litre plastic buckets filled with stagnant tapwater. Water temperature ranged between 26 and 27°C and dissolved oxygen varied from 2 to 5 mg.l⁻¹. Feeding larvae on non-living diets in this stagnant water system tended to induce deterioration of water quality. In order to limit this phenomenon and to prevent the development of flagellates, ciliates and other organisms which may constitute extra food source for the larvae, all the rearing buckets were cleaned carefully and the water totally changed 2-4 times daily.

Experiment 2: The aim of the second experiment was to evaluate the growth and survival of fry in conditions of constant and good water quality. All the diets were tested, except the commercial trout diet (Trouvit 000). Ten groups of 300 larvae (two replications per treatment) were stocked in the 40-l tanks of a tapwater recirculating system. During the experiment, water temperature was maintained between 26 and 28°C and dissolved oxygen ranged from 6 to 8 mg.l⁻¹. Each tank was cleaned twice daily by siphoning off feces and uneaten food.

In both experiments, the tapwater was stocked in an intermediate open tank for at least 48 h in order to remove chlorine before using it in the rearing facilities. The larvae, strongly photophobic (Legendre, 1992), were held in darkness. They were fed to excess six times per day every 4 hours. With the dry diets, fish were fed sieved food particle size of 100-200 µm diameter from 2 to 7 days of age and 200-400 µm diameter from 8 to 14 days of age.

Data collection and analysis

Every three days, starting with 5-day-old larvae, ten fish from each tank were randomly sampled. They were caught one by one with a piece of fine mesh net, gently placed on paper towels in order to absorb most of the adhering water through the net and individually weighed to the nearest 0.1 mg in a receptacle containing water. This method of manipulation and measurement was safe for the fish and they could be returned to their respective tanks without any loss. On the last day of the rearing period, twenty (exp. 1) to thirty fish (exp. 2) from each tank were sampled and individually weighed. Survival rates were determined by counting the remaining fish of each tank. On each day of weight measurement, five fish per treatment were also preserved in 4% formalin for gut content observations.

Final mean weights, specific growth rates [SGR = 100(LnW₂ - LnW₁)/t; with W: mean body weight and t: time in days] and survival rates were compared by one way ANOVA, followed by Duncan's Multiple Range Test to determine significant differences among means ($p < 0.05$). When necessary, analyses were performed after logarithmic transformation for weights or angular transformation for survival rates in order to stabilize residual variance.

RESULTS

All groups of fish fed aggressively on the experimental diets throughout the rearing period, except those fed with trout diet which was poorly ingested during the first week of rearing. With the dry diet based on yeast powder and beef liver, all larvae ingested feed particles until the gut was almost completely filled. Most of the larvae fed on the bottom or along the walls of the tank and, to a lesser degree, in the mid-water.

Gut content observations under binocular microscope confirmed that *H. longifilis* larvae aged 3 days are able to ingest prey of more than 500 µm in length, like *Artemia* nauplii or *Moina*, as the first feed. For larvae of 11 and 14 days of age, it was also observed that stomach and intestine of fish receiving live *Moina* contained numerous undigested ephippial eggs, as well as some copepods (*Mesocyclops*) that began to develop in the plankton culture at that time. By contrast, both *Moina* ephippial eggs and copepods were absent from the digestive tract of fish fed frozen *Moina*.

At the end of the 12-day feeding period, the average fish weights were significantly different ($p < 0.05$) between all the test diets in the two experiments.

In experiment 1 (table 1, fig. 1), fish fed live *Artemia* nauplii gave the highest SGR and reached 84 mg average body weight, whereas fish fed frozen *Artemia* nauplii, frozen *Moina* and live *Moina* reached 51, 39 and 33 mg, respectively. With the dry diets, fish displayed poorer growth rates in comparison to those fed *Artemia* or *Moina*. They reached 29 mg and 13 mg average body weight when fed with dry diet based on yeast powder and beef liver, and commercial trout diet, respectively.

Due to better water quality and more favorable rearing conditions, the growth rates of fry obtained in experiment 2 were higher than those obtained in experiment 1. However, the ranking of growth data obtained for the different experimental diets remained the same. The fry fed live *Artemia* nauplii had a significantly superior SGR than those fed other test diets ($p < 0.05$, table 2). After the 12-days rearing period, fish receiving live *Artemia* nauplii were 236 mg mean weight, while they weighed only 142 mg, 78 mg, 57 mg and 30 mg when frozen *Artemia* nauplii, frozen *Moina*, live *Moina* and dry diet based on yeast powder and beef liver were distributed, respectively (fig. 2).

Table 1. – Mean body weight, specific growth rate (SGR) and survival rate for *H. longifilis* after 12 days of feeding with the various experimental diets.

Feed	Experiment 1				Experiment 2			
	Body weight (mg)		SGR	Survival rate	Body weight (mg)		SGR	Survival rate
	mean	CV	(%.d ⁻¹)	(%)	mean	CV	(%.d ⁻¹)	(%)
Live <i>Artemia</i> nauplii	84 ^a	18	31 ^a	79 ^a	236 ^a	26	40 ^a	65 ^a
Frozen <i>Artemia</i> nauplii	51 ^b	18	27 ^b	92 ^a	143 ^b	25	36 ^b	73 ^a
Live <i>Moina</i>	33 ^c	28	23 ^{cd}	82 ^a	57 ^c	29	28 ^c	71 ^a
Frozen <i>Moina</i>	39 ^d	18	25 ^c	86 ^a	78 ^d	36	31 ^c	69 ^a
Yeast + beef liver diet	29 ^e	33	22 ^d	83 ^a	30 ^e	33	23 ^d	61 ^a
Commercial trout diet	13 ^f	50	16 ^e	32 ^b	–	–	–	–

Figures with same superscripts in the same column are not significantly different ($p < 0.05$).

CV: coefficient of variation for individual weight.

In both trials, although fish fed with live *Artemia* nauplii had better growth rate than fish fed frozen *Artemia* nauplii, it was noted that the growth performance of fish fed live *Moina* was inferior to that of group fed frozen *Moina* (fig. 1 and 2). This difference in mean body weight was noticeable after day 8 and became statistically significant at the end of the trial. Fish fed frozen *Moina* were about 1.3 times bigger than fish fed live *Moina*. In both experiments,

the heterogeneity in individual weight of fry in the different fish groups tended to increase with feed leading to the slowest growth rates (table 1).

At the end of the first feeding trial, the survival rates were not significantly different between treatments, except in the case of fish fed commercial trout diet which led to a significantly lower survival ($p < 0.05$). The survival rates ranged between 79 and 92% for the first five diets and were only 32% for commercial trout diet. In experiment 2, the survival rates ranged

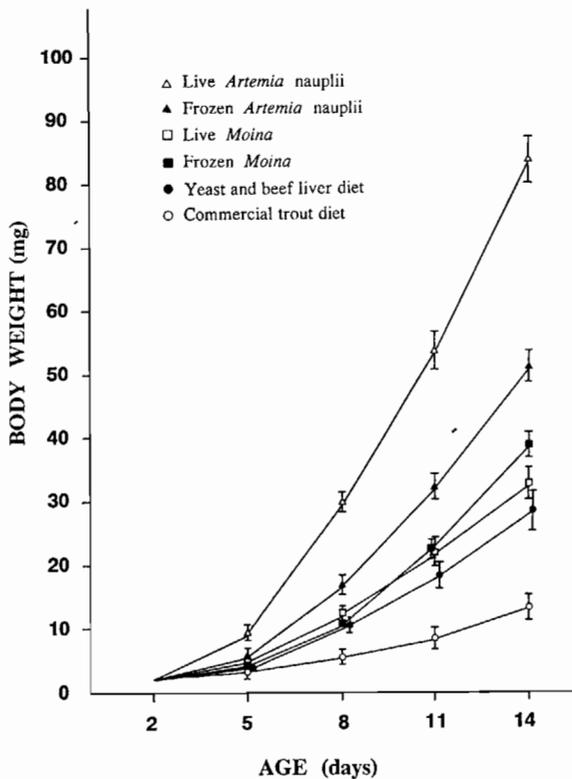


Figure 1. – Growth of *H. longifilis* from first feeding up to 14 days of age as a function of different experimental diets in experiment n° 1. Vertical bars indicate 95% confidence limits for individual mean weight.

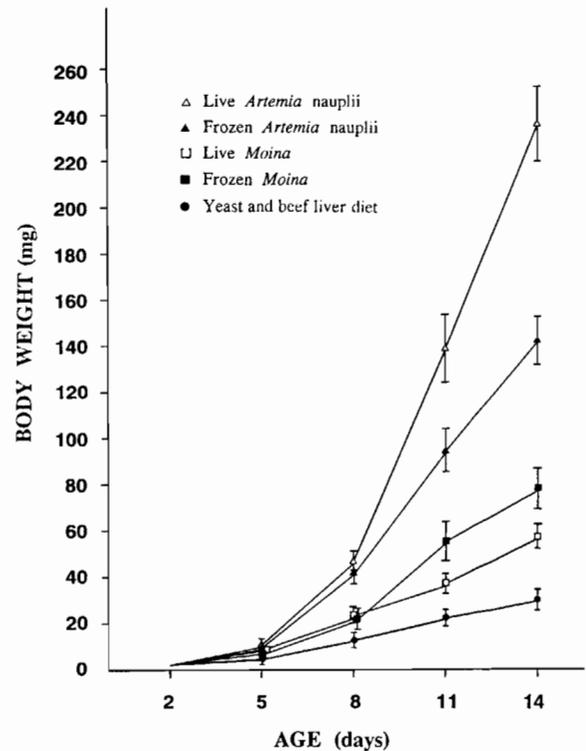


Figure 2. – Growth of *H. longifilis* from first feeding up to 14 days of age as a function of different experimental diets in experiment n° 2. Vertical bars indicate 95% confidence limits for individual mean weight.

between 61 and 73% for all test diets and were not significantly different ($p > 0.05$).

DISCUSSION

In the present study, despite differences in the rearing conditions and overall fish growth, the results obtained in the two experiments were consistent and showed the same ranking for the different experimental diets. Live *Artemia* nauplii have once more proved to be an excellent feed for successful rearing of *H. longifilis*. However, the survival rates of fish groups fed either zooplankton (*Moina micrura*) or the yeast and beef liver dry diet were as high as those fed *Artemia* nauplii although the growth performance obtained with the two former feeds were lower than that obtained with the latter. In practice, as far as this initial lesser growth has no impact on later fish performance, this indicates the possibility of using *Moina* or dry diet for feeding *H. longifilis* larvae when a shortage of *Artemia* is experienced.

Successful rearing of fish larvae using *Artemia* nauplii has also been reported for many fish species (Watanabe *et al.*, 1983; Léger *et al.*, 1986). Hogendoorn (1980) reported better results in rearing *Clarias gariepinus* larvae using live or frozen *Artemia* nauplii alone or in combination with a trout starter than using other diets without *Artemia*. Similarly, *Clarias batrachus* larvae fed *Artemia* nauplii during the first 7 days of rearing displayed the best growth response and survival rates in comparison to other food sources such as rotifers, cladocerans or ground fish meal (Knud-Hansen *et al.*, 1990).

In *H. longifilis*, fish fed either live or frozen *Artemia* nauplii had higher growth rate than fish fed live or frozen *Moina*. A similar result was noted with another clariid catfish, *Clarias macrocephalus*, in which the growth performance of fish fed *Moina macrocopa* was lower than that obtained with fish fed a mixed diet consisting of *Artemia* and dry feed (Fermin and Bolivar, 1991). By contrast, Adeyemo *et al.* (1994) reported faster growth rates and higher survival rates for *Heterobranchus bidorsalis* and *C. gariepinus* fry fed *Moina dubia* than when fed *Artemia* nauplii after a 7-day nursing period. However, the highest average specific growth rates obtained by those authors remained very low and did not exceed 6.1 and 5.1%.d⁻¹ in *H. bidorsalis* and *C. gariepinus*, respectively. In the present study, the average specific growth rates calculated over the 12-day feeding period reached a maximum of 40%.d⁻¹ for *H. longifilis* fry fed live *Artemia* nauplii in the second experiment (table 1). This result is very close to the maximum average SGR values (40.7-41.9%.d⁻¹) calculated in *C. gariepinus* after a 10-day feeding period with decapsulated cysts of *Artemia* (Verreth and Den Bieman, 1987) and suggests that *H. longifilis* and *C. gariepinus* fry have a similar growth potential. Legendre *et al.* (1992) found no difference in the growth rates of these two species

after 15 days of larval rearing in the same conditions, despite the evidence of a faster growth rate in *H. longifilis* than in *C. gariepinus* at more advanced stages of development.

Our results also indicated that fish fed frozen *Artemia* had lower growth performance than those fed live *Artemia*. A decrease in nutritive value due to oxidation of fatty acids during storage at -18°C or to leaching during the process of thawing may be a cause of reduced performance. Grabner *et al.* (1981) reported that upon feeding when frozen zooplankton was introduced into water, rapid leaching of proteins and amino acids occurred. Webster and Lovell (1990) also found that growth and survival of *Morone saxatilis* fed freeze-dried brine shrimp and shock-frozen brine shrimp were lower than when they were fed live brine shrimp. By contrast, the growth rate of *H. longifilis* fry fed frozen *Moina* was higher than those fed live *Moina* during the second week of the rearing period. This was probably due to the quality of harvested *Moina*. For frozen *Moina*, harvest and freezing was performed massively during the first 7 days of the cladoceran culture period in concrete tanks, while live *Moina* were harvested day by day until the end of the larval rearing period. At that time, the cladoceran culture was three to four weeks old. It was observed that, after 7 to 10 days of culture in the concrete tanks, the *M. micrura* production tends to decrease and that this cladoceran begins to produce resting or ephippial eggs (Saint-Jean, comm. pers.). The presence of numerous undigested ephippial eggs found in the digestive tract of 11- and 14-day-old *H. longifilis* fry fed with live *Moina* may be an explanation for the slower growth rate observed in comparison to larvae fed frozen *Moina*. In the latter case, the *M. micrura* were collected during their first week of culture and still had a parthenogenetic mode of reproduction. Ephippial eggs of cladoceran have been found resistant to the digestive process in many fish species (Mellors, 1975).

The commercial trout diet tested in the first experiment led to both low survival and growth rates and proved to be poorly utilized by *H. longifilis* larvae. This is in agreement with previous results of Hogendoorn (1980), and Verreth and Van Tongeren (1989) who also found that trout starter diet was not suitable for early feeding of *Clarias gariepinus*. Verreth *et al.* (1993) stated that the requirement for a specific starter diet in *C. gariepinus* is related to a lack of functional stomach, and particularly the absence of pepsin digestion during the first days of exogenous feeding. In *H. longifilis*, trout diet has been shown to be efficiently utilized by the fry when distributed after an initial 6-8-day feeding period with *Artemia* (Legendre *et al.*, 1991).

In the present study, the compound dry diet based on yeast and beef liver was well accepted by *H. longifilis* larvae from first exogenous feeding. The survival rate was as high as that obtained with groups fed *Artemia* or *Moina*, but the growth rate was lower than that

observed with the latter. High survival rate but poor growth rate for larvae fed only dry diets containing yeast in their composition has also been reported in *Clarias gariepinus* (Hecht, 1981; Uys and Hecht, 1985). On the other hand, the same compound dry diet as in the present study is reported by successful, in terms of both growth and survival rates, for rearing larval stage of other fish species like *Coregonus* sp. (Bergot *et al.*, 1986) and *Cyprinus carpio* (Charlon *et al.*, 1986). Bergot *et al.* (1986) reported that larvae of *Coregonus schinzi palea* accepted this dry diet based on yeast powder and beef liver as the first food and that it led to good results of survival (88-92%) and growth (260-290 mg) after 54 days of rearing. This discrepancy in the efficiency of this dry diet for rearing *H. longifilis* and *Coregonus* sp. larvae may be related to differences in the nutrient requirements of these two species or to differences in the rearing method.

In conclusion, although the use of *Artemia* nauplii for feeding *H. longifilis* larvae led to the highest

growth rate, *Artemia* may not be the most appropriate feed for larval rearing in many African countries for practical and economical reasons. As an alternative, *Moina micrura* also proved to be successful for first feeding of larvae and has the advantage of being locally available in West African waters. However, for a large-scale production of fry, the use of *Moina micrura* may raise the problem of being either not fully reliable with possible loss of nutritional quality (production of ephippial eggs) or linked to the development of an improved technology for prey culture in controlled conditions. In this context, the use of dry diet appears to be a promising way, since survival rates obtained with larvae fed yeast and beef liver dry diet were as high as those obtained with *Artemia*. Further investigations are in progress to identify the specific nutritional requirements of *H. longifilis* larvae and improve the efficiency of dry diet in terms of growth performance.

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