

# Use of otolith microincrements for estimating the age and growth of young armoured catfish *Hoplosternum littorale*

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At  $27.0-28.0^{\circ}$  C, the lapilli of *Hoplosternum littorale* developed rapidly in the embryo between 35 and 21 h before hatching. At hatching, lapilli averaged 78 µm on their longest axis and 69 µm on their shortest axis, and had up to three faint narrow microstructures. Primordia were fused and the large core was surrounded by a conspicuous discontinuous zone, formed at hatching, and visible in both sagittal and transverse preparations. The deposition rate of microincrements, counted in transverse thin sections of lapilli, was daily at least for the first 50 days and the innermost microincrements were deposited from hatching on. The growth rates of *H. littorale* differed significantly between two different rice field habitats in Suriname.

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Key words: otolith microstructures; daily deposition; juvenile; Siluriformes; Suriname; South America.

## INTRODUCTION

Hoplosternum littorale (Hancock, 1828) is a medium-sized armoured catfish (family Callichthyidae), of maximal  $L_{\rm S}$  20 cm (Singh, 1978), endemic to neotropical fresh waters (Mol, 1994; Reis, 1997). It is fished extensively in northern South America (Singh, 1978; Novoa, 1982; Ouboter & Mol, 1994) and has great promise for aquaculture (Boujard *et al.*, 1988; Luquet *et al.*, 1989; Ramnarine, 1994*a,b*). Callichthyid catfish are among the best known neotropical fishes due to studies of their systematics (Gosline, 1940; Reis, 1997), distribution and salinity tolerance (Mol, 1994), food (Winemiller, 1987; Mol, 1995), reproduction (Mol, 1993, 1996*a*; Hostache & Mol, 1998), mineral metabolism (Mol *et al.*, 1999), and predation (Mol, 1996*b*). The annual growth of *H. littorale* in natural habitats has been studied using transverse sections of the pectoral spine (Singh, 1978; Boujard & Meunier, 1991).

In their natural habitats, predation pressure from invertebrate and vertebrate -predators on young armoured catfish decreases from hatching to the end of their first 2 months of life (Mol, 1996b). So the faster they grow the better should be their survival. Accurate age estimation is mandatory for testing such a hypothesis, but the well-established techniques of examination of daily micro-increments in fish otoliths (Stevenson & Campana, 1992) have never been applied to Siluriformes.

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FIG. 1. Dorsal, external, ventral and internal views of left otoliths extracted from a 92 mm  $L_s$ Hoplosternum littorale caught in Suriname. Lapilli are easily recognized by their characteristic curved sulcus (S) and their rostrum (R) pointing laterally. The black dot corresponds to the approximate position of the core inside the lapillus. The dotted lines indicate where the otolith was cut for transverse preparation.

The present study aimed to verify that increments are deposited daily in the otoliths of young *H. littorale* and that these increments are deposited from hatching on, and to apply this technique to estimate the growth performance of young *H. littorale* in some of their natural habitats in Suriname.

## MATERIALS AND METHODS

Only the lapilli of *H. littorale* were used because they are larger than the thin V-shaped sagittae and the discoid and curved asterisci (Fig. 1).

# OTOLITH DEVELOPMENT AND VALIDATION EXPERIMENT

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Eggs of *H. littorale* were obtained from nests built naturally by adults reared in vegetated ponds of the company SA Gabriel Guyane at Roura, French Guiana (Fig. 2). The development of the otoliths in the embryos was studied from eggs that were transferred carefully to a  $30 \times 30 \times 20$  cm cage (mesh size  $500 \,\mu\text{m}$ ) suspended in a 2001 plastic tank. Eggs were aerated with air bubbles coming from an air stone and a constant water flow was maintained in the tank. Eggs were sampled randomly and fixed in 90%



FIG. 2. Sampling stations in Suriname: 1, Lelydorp Swamp; 2, large Boeroe Swamp; 3, Coronie district with the swamp and the two nearby rice fields. V, Location of the company SA Gabriel Guyane at Roura, French Guiana, where the validation experiment took place.

ethanol 34, 21 and 10 h before hatching and just at hatching. During these 2 days, water temperature in the tank varied between 27.0 and 28.0° C, pH between 6.7 and 7.2, conductivity between 54 and 89  $\mu$ S cm<sup>-1</sup>, and oxygen between 6.0 and 6.7 mg l<sup>-1</sup>.

A second nest was placed in a 1 m<sup>3</sup> cage (mesh size 500  $\mu$ m) installed in the vegetated pond where the adults were living. Randomly chosen larvae were sampled and fixed in 90% ethanol at hatching and then 3, 7, 10 and 14 days after hatching. After 2 weeks, the young fish were transferred gently in two 1 m<sup>3</sup> cages covered with a 2 mm mesh size net placed near the first cage. Every week, individuals of both cages were caught randomly and fixed in ethanol until 77 days after hatching. During the 11 week experiment, the water temperature in the pond near the cages varied between 27.8 and 29.6° C, pH between 5.5 and 7.4, conductivity between 42 and 77  $\mu$ S cm<sup>-1</sup>, oxygen between 4.3 and 6.3 mg 1<sup>-1</sup>, and turbidity between 89 and 124 NTU.

### COLLECTION LOCALITIES IN SURINAME

Young *H. littorale* were collected in natural habitats in Suriname with a small seine  $(2.5 \times 1 \text{ m}, 2 \text{ mm} \text{ unstretched mesh size})$  while large juveniles were collected with a cast net. The fish were fixed immediately in 90% ethanol. Young *H. littorale* can be distinguished easily from other catfish in the swamps by their pigmentation pattern and forked caudal fin (Mol, 1996b).

In Suriname, H. littorale is restricted to herbaceous swamps of the coastal plain (Mol, 1994). Young H. littorale were collected in the Lelydorp Swamp, the Boeroe Swamp and in the Coronie district (Fig. 2). The Lelydorp Swamp in the Wanica district, c. 20 km south of Paramaribo, is a narrow east-west running swamp dominated by dense stands of *Eleocharis interstincta*. The large Boeroe Swamp in the Saramacca district is situated at c. 5 km from the Atlantic coast and between the estuaries of the Suriname River and the Coppename River. The Boeroe Swamp is <1 m deep and dominated by Typha angustifolia, Cyperus giganteus, Heliconia psittacorum and Montrichardia arborescens. The Boeroe Swamp was sampled at Weg Naar Zee and at the Sara-Maria plant of State Oil. The third locality was the huge Coronie Swamp in the Coronie district. Young H. littorale were collected near Ingikondre, both in the swamp itself and in two rice fields at the edge of the swamp. The swamp site was dominated by *Eleocharis*. With the exception of a few patches of Nymphaea the two rice fields were cleared of vegetation. Although pre-germinated rice seeds were broadcast seeded only a few days before collecting took place, the fields were inundated several weeks earlier for tillage and weed control. The three collection sites in Coronie were within 5 km of each other.

# LAPILLI PREPARATION AND EXAMINATION

The lapilli of embryos and larvae of  $L_{\rm S} < 10$  mm were extracted with needles under a binocular microscope equipped with polarized light. Lapilli were identified by their size and by their lateral position with respect to the sagittae. For each individual both lapilli were extracted, cleaned, dried and, given their small size, immersed immediately sagitally into a drop of polyester resin (Sody33 from Escil, Chassieu, France) placed on a cover glass. After polymerization of the resin (24 h), the lapilli were polished finely dorsally with aluminium oxide slurry (0.3  $\mu$ m placed on a polishing cloth).

For individuals  $L_{s} \ge 10$  mm, the lapilli were extracted following the right-between-theeyes method (Secor et al., 1992). First the fish head was severed behind the operculum and then cut sagitally. The brain was removed from each half of the head, the labyrinth was located, and the utricular vestibule containing the lapillus was extracted. Each lapillus was cleaned in ethanol and either embedded immediately in polyester resin or stored dry in a vial and processed later. In the resin, the longitudinal axis of the lapillus was oriented parallel to the long axis of the mould. After polymerization, each otolith was prepared to obtain a thin transverse section following Secor et al. (1992). Briefly, each block was cut transversally with an Isomet<sup>®</sup> (Buehler, U.S.A.) low speed saw equipped with a 300-µm diamond blade (ref D100 from Escil, Chassieu, France) to leave 0.8-1.0 mm of material on each side of the core (Fig. 1). The embedded section of otolith was glued with thermoplastic glue (CrystalBond<sup>®</sup> 509, Buehler, Lakebluff U.S.A.) to a  $1 \times 1$  cm piece of glass fixed on a microscope slide (Secor *et al.*, 1992). The side of the block containing the sectioned lapillus was ground rapidly with wet sandpaper of 800 and 1200 grit sizes and then polished finely up to the otolith core using successively aluminium oxide slurry of 3, 1 and  $0.3 \,\mu\text{m}$  mixed with water on polishing cloths. When the core was reached the preparation was cleaned, dried, turned and prepared the same way on the other side. Finished preparations were  $15-35 \,\mu m$  thick.

Lapilli preparations were observed at  $\times 400$ ,  $\times 600$  or  $\times 1000$  magnification with an Olympus BX40 light microscope equipped with a Sony 3CCD DXC-930C colour video camera. Measurements were performed with the TNPC (Traitement Numérique des Pièces Calcifiées) module of the Visilog<sup>®</sup> image analysis software (Noesis SA, Courtaboeuf, France) using calibrated images. Increments were counted manually on a Sony PVM-2950QM Triniton video monitor. For counting the number of increments, two counting areas appeared most appropriate with respect to increment clarity: one corresponding to the exterior of the fish, and one corresponding to the interior of the animal (Fig. 3). Rarely were increments counted along a straight line but the count paths always remained within the defined area and maintained increment continuity. A minimum of two complete counts were performed for each area: one count starting at the first increment after the hatching check and towards the otolith edge, the second starting from the otolith edge and towards the core.



FIG. 3. Transverse preparation of the left lapillus of a 35 mm  $L_{\rm S}$  Hoplosternum littorale individual caught in rice field no. 1 in the Coronie district, Suriname. The dotted lines delimit the sectors where microincrements were counted on the exterior (A) and interior (B) side of the otolith. The horizontal bar represents 0.1 mm. D, Dorsal and V, ventral side of the otolith; E, exterior and I, interior of the fish.

#### RESULTS

# VALIDATION OF DAILY INCREMENT FORMATION

Embryonic development of *H. littorale* from fertilization to hatching is dependent on water temperature (Hostache *et al.*, 1992) and lasted *c*. 48 h in the present experiment. In total 30 embryos were examined, fixed between 43 h before hatching and hatching. The lapilli developed rapidly between 35 h before hatching, when they were not detected under polarized light, and 21 h before hatching when they measured  $61 \pm 1 \mu m$  on their longitudinal axis and  $47 \pm 4 \mu m$ on their transverse axis (n=5). Newly hatched larvae had lapilli that averaged  $78 \pm 6 \mu m$  on their longest axis and  $69 \pm 7 \mu m$  on their shortest axis (n=7) and presented up to three very faint and narrow microstructures [Fig. 4(a)].

In *H. littorale* lapilli, primordia are fused and the large core is surrounded by a conspicuous discontinuous zone. The discontinuous zone was observed in both sagittal and transverse preparations of lapilli of fish reared in the pond [Fig. 4(b)]. The length of the long axis of the core prepared transversally fell between the lengths of the short and long axis observed sagittally (Fig. 5). Thus, the discontinuous zone observed in sagittal and transverse preparations correspond to the same structure formed at hatching.

In the cage placed in the fish pond, the daily growth of young *H. littorale* averaged  $0.20 \text{ mm day}^{-1}$  from hatching to day 7 and thereafter increased to  $0.41 \text{ mm day}^{-1}$  until day 49. Older individuals had a lower average daily growth of only  $0.33 \text{ mm day}^{-1}$  [Fig. 6(a)]. The lapilli of 43 individuals between 0 and 77 days old were examined. These lapilli showed broad increments from the date of hatching but were counted more easily in the transverse sections. In transverse preparations, the number of microincrements in each area did not differ

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FIG. 4. Core of the lapillus of *Hoplosternum littorale* with its multiple primordia (P) and the discontinuous zone formed at hatching (H) that defines the limit of the core and corresponds to the onset of incremental growth. (a) Lapilli observed sagittally at hatching; (b) lapilli prepared transversally from a >14-day-old individual. The white bar represents 0.01 mm. LL', the long axis (sagittal and transverse view); SS', short axis (sagittal view only).

significantly with the direction of counting, core to edge or edge to core (paired *t*-test, P=0.998 for the exterior area and P=0.289 for the interior area), and so the two counts were averaged. The two counts in each area did not differ between areas (paired *t*-test, P=0.582) and so their grand mean was used as the observed number of microincrements.

For fish 14–49 days old, the number of microincrements (n), as a function of the true age (A) of the fish, was fitted well by a linear model: n=0.985 \* A - 1.653



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FIG. 5. Size of the core of lapilli of *Hoplosternum littorale* delimited by the conspicuous discontinuous zone. The length of the long axis of the core of lapilli prepared transversally (individuals older than 7 days, n=19) is comprised between the lengths of the short and long axis observed sagittally (individuals between 0 and 7 days old, n=23). Upper and lower limits of the boxes correspond to the first and third quartiles respectively, horizontal bars indicate the median, whiskers the 10th and 90th percentile, and dots the outliers. See Fig. 4 for the location of the short and long axis as measured on the core.

(n=14, F=442.6, P<0.001). For older individuals, the number of microincrements underestimated their true age [Fig. 6(b) and (c)]. The slope of the model did not differ significantly from 1 (*t*-test, P>0.5) and the intercept did not differ significantly from 0 (*t*-test, P>0.1). Adding individuals aged 0, 3 and 7 days did not improve the fit of the model.

#### GROWTH IN NATURAL HABITATS IN SURINAME

Only 51 wild-caught individuals (all  $L_{\rm S} \leq 100$  mm) could be used for estimating the number of lapillar microincrements (Fig. 7). Fish caught in the Lelydorp and Boeroe swamps had >50 microincrements, i.e. were outside of the range of validity of the linear model derived earlier, but they aligned to a general pattern of size-at-age for Surinamese young *H. littorale*. Assuming size at hatching was 5 mm  $L_{\rm S}$  (Machada-Allison, 1986), the size of young *H. littorale* caught in the two Coronie rice fields was related to their estimated age by exponential models (Fig. 8). The slopes of the models differed significantly between these two rice fields (ANCOVA with log-transformed  $L_{\rm S}$ , P=0.022). The estimated average growth from hatching to day 25 was 1.04 mm day<sup>-1</sup> in rice field no. 1 and 0.36 mm day<sup>-1</sup> in rice field no. 2.

# DISCUSSION

The daily increment technique has been used widely since the early 1980s to estimate the age of different fish species from high latitudes to the tropics and from fresh- to oceanic waters, but this is the first time that the microincrements of otoliths have been used to estimate the age in days of a neotropical freshwater



FIG. 6. (a) Standard length; (b) number of microincrements counted on lapilli; (c) difference between observed and predicted number of microincrements on lapilli as a function of the true age in days of young *Hoplosternum littorale* reared in cages. The dotted line indicates the 1:1 relationship between number of increments (n) and true age (A) and the solid line represents the model n=0.985 \* A - 1.653 established for individuals between 14 and 49 days old ( $\Box$ ). Data outside the range of the model ( $\bigcirc$ ) are presented for comparison.

fish or of young Siluriformes. Fagade (1980) described the microincrements in the otoliths of *Chrysichthys nigrodigitatus* (Lacépède) but did not use them for age estimation. With respect to South American freshwater fish, some studies addressed the annual growth of catfishes such as the loricariid *Rineloricaria latirostris* Boulenger (Barbieri, 1995), the ariid *Netuma barba* Bleeker (Reis, 1982, 1986) and even *H. littorale* (Singh, 1978; Boujard & Meunier, 1991) but never the daily growth. Age estimation of neotropical fishes is developing only slowly and has been limited so far to adults. For example, studies have been conducted using scales or otoliths of Characiformes (Meunier, 1994; Loubens & Panfili, 1995, 1997) or spines and opercula of Siluriformes (Meunier *et al.*, 1994). Microincrements should be particularly useful for studying neotropical fish



FIG. 7. Standard length v. number of microincrements on the lapilli of young *Hoplosternum littorale* caught in Suriname in Lelydorp (∇) and Boeroe (■) swamps in 1998 and in the Coronie district in the swamp (♥) and in two nearby rice fields (●, no. 1; ○, no. 2) in 1999.



FIG. 8. Size v. number of microincrements used as an estimator of age for young Hoplosternum littorale caught in two different rice fields in Coronie district, Suriname. The growth of the young H. littorale in the two rice fields were adjusted to exponential models where size at hatching is assumed to be 5 mm  $L_{\rm S}$  [rice field no. 1 (•): n=12,  $F=1373\cdot2$ ,  $P<0\cdot001$ ; rice field no. 2 (O): n=15,  $F=1041\cdot5$ ,  $P<0\cdot001$ ]. —,  $L_{\rm S}=5e^{0\cdot073}$  estimated age; ---,  $L_{\rm S}=5e^{0\cdot041}$  estimated age.

because in many species (including *H. littorale*, Singh, 1978) most growth occurs in the first year, and many species are small and have a short life span (Weitzman & Vari, 1988).

Before estimating the age of young fish, the rate of formation of otolith microincrements must be determined (Geffen, 1992). One of the most rigorous and reliable methods for validating this rate is the examination of otoliths from individuals of known age, subjected to environmental conditions close to natural ones. Following Geffen (1992), the outdoor enclosures were considered optimal

environments for the validation experiment during the first 50 days. From this experiment it was concluded that the deposition rate of microincrements in transverse thin sections of lapilli of young *H. littorale* was daily and the innermost microstructures were deposited from hatching on.

In wild fish  $L_s>100$  mm, microstructures near the edge of the lapilli were difficult to discriminate (Fig. 3) and the counts never exceeded 90 microstructures whatever the size of the fish. Scanning electronic microscopy (model Philips XL30) of these edges did not reveal any microincrements not observed with light microscopy (D. Ponton & J. H. Mol, unpubl. data). This narrowing of the increments suggests that the growth rate of *H. littorale* in natural habitats can decrease drastically after age 3 months.

Although limited to the first 1.5 months, precise ageing of young *H. littorale* could be important for ecological studies and for the management of their natural habitats. For example, during every dry season, some areas of herbaceous coastal swamps are burned intentionally in Suriname (Teunissen, 1993) and in French Guiana. Age, and thus growth, estimations of young *H. littorale* caught in different areas of these swamps during the next rainy season will be a valuable tool for studying the consequences of this practice.

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