ONTOGENY OF THE BALASHARK *Balantiocheilos melanopterus* BLEEKER, 1851 (CYPRINIDAE)

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ABSTRACT

The Balashark is a highly praised ornamental species that has been overfished to the point that it has become endangered in most of its distribution area, especially in Indonesian waters. Captive propagation has reduced the threats upon wild populations of balashark. Nevertheless, knowledge on the biology of this species is still scarce, in particular as regards to its ontogeny. This information is crucial for the identification of larvae and small juveniles in the wild, as well as for improving its culture. Balashark eggs average 1.2-1.3 mm in diameter and hatch after 13 hours (26-28°C), giving birth to 4.5-mm embryos, with a yolk sac of *circa* 1.1 mm^3. At the end of the endogenous feeding period (day 4 after hatching [AH]), larvae attain 6.3 mm TL. The mouth opens on day 2 AH (5.8 mm total length, TL). At the time of first feeding (day 4 or 5 AH, 6.3 mm TL), their mouth and gape already average 0.5 mm and 0.27 mm, respectively, and they grow up to 10.2 and 6.3% TL, respectively, on day 8 AH. The swim bladder starts forming as early as day 2 AH and is filled by day 3 AH (5.9 mm TL), but the separation between the anterior and posterior chambers does not take place before 9.5 mm TL (day 9 or 10 AH). The fin development sequence is typical of cyprinids, and follows a caudal-to-cranial pattern (i.e.; caudal, dorsal and anal, pelvic then pectorals), except for the anlagen of pectoral fins, which are present in 1-day old fish (5.3 mm TL). Based on the vanishing of the abdominal and caudal fin fold, and on the lengths of the fins relative to fish size, the transition between the larval and juvenile stage occurs at *circa* 17 mm TL (corresponding standard length: 13.7 mm). At this stage, the scale cover is not developed yet, but juveniles already exhibit the typical pigmentation pattern of adults on their dorsal, anal, pelvic and caudal fins, while pigmentation on the pelvic fins is still in its very early stages.

KEYWORDS: ***Balantiocheilos melanopterus*, cyprinid, ontogeny, morphology, larva**

INTRODUCTION

In the vast majority of fish species, the bulk of mortality occurs during the early life stages, both in the wild and in captivity. A series of species have evolved fast growing strategies while others have seemingly privileged life styles that rely on energy conservation, to the detriment of growth but giving the embryo or larva the opportunity to survive more or less prolonged periods of food shortage. In order to understand to what extent ontogenetic patterns match environmental constraints, and thus the threats upon species resilience in man-modified environments, one should be capable of identifying a particular species at all stages of its ontogeny. Genetic tools nowadays enable the rapid and accurate determination of fish species, but their application to broad numbers of fish is tedious and excessively expensive when applied to (very) large samples, as those needed in ecological studies, thereby emphasizing the need for thorough morphological descriptions.

These principles have fostered research on the ontogeny of fish, which was originally driven by evolutionary purposes, and resulted

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in the production of taxonomic guides, which permit the univocal determination of who is who for all or most life stages. Generally, these guides are produced on a river basin (e.g. Hogue et al., 1976; Margulies, 1983) or country basis (e.g. Pinder, 2001). The degree of taxonomic resolution may not be as accurate as for adults or juveniles, essentially because discriminating criteria are generally less obvious in embryos and larvae than in juveniles or adults. Nonetheless, ontogeny is a dynamic process, the description of which may enable an equally accurate taxonomic resolution if a sufficient number of traits are under scrutiny.

Currently, such taxonomic guides are not available for tropical freshwater fish communities, largely because accurate descriptions of fish ontogeny are scarce in these regions. The comparison between the sizes of the databases in FishBase (Froese & Pauly, 2006) and LarvalBase emphasises this paucity of information. The development of aquaculture production and research, in which an increasing number of species are bred and raised in captivity for bioconservation, ornamental or food purposes, provides a heuristic context for bridging this gap and providing information that might contribute to identify the causes of a species' decline and the solutions to be implemented. With aquaculture-bases studies, fish ontogeny can be traced instead of being inferred from scant captures in the wild. , Experimental facilities give the opportunity of studying live specimens, and thus their colour patterns, which may vanish totally or partly in preserved specimens that originate from the wild. Furthermore, aquaculture-based studies permit studying species that are currently endangered in their natural distribution area without jeopardizing their populations

Here, we provide the first description of the ontogeny of the silver shark *Balantiocheilos melanopterus* Bleeker 1851 (Cyprinidae), also known as tricolour shark minnow or balashark in Indonesia. The balashark is a highly praised ornamental species that has been overfished to the point that it has become endangered in most of its distribution area, especially in Indonesian waters (Ng & Tan, 1997).

MATERIAL AND METHODS

The fish that were analysed in this study originate from broodfish from a Thai population of balashark that was introduced in Indonesia. Eggs were obtained following a hormonal treatment of females (HCG 300 IU.kg^{-1}, then Ovaprim 0.5 mL.kg^{-1} of female 5 hours later), followed by artificial fertilization. Embryos and larvae were raised in 80-L (100 x 40 x 20 cm) tanks and fed with brine shrimp nauplii. Water temperature during the study averaged 26°C at night and 28°C during the day (12L:12D). Fish were sampled at daily intervals, transferred to the LR-BIHAT Depok station, and photographed under the stereomicroscope (magnification: 8-100 x) and the light microscope for closer shots, using top, bottom and side views. Dimensions were measured to the nearest pixel from digital photographs, by reference to a graduated scale that was photographed at the same magnification(s). The morphomeristic variables under scrutiny were those that are critical to feeding and locomotion: i.e. fish size (total, fork and standard lengths), body depth, fin length and growth of finrays, head width and length, upper and lower jaw lengths; gape height was estimated from jaw length, on the assumption of a 90° opening capacity, which is a generally used as a standard for describing the ingestion capacity of fish larvae and small juveniles (Shirota, 1970).

Additional information was collected on the rate of yolk absorption and on the ontogeny of the swimbladder, in particular as regards the specialisation of the swimbladder into two distinct chambers, and the variation of their respective proportions during the ontogeny. For both structures, the contour was traced on digital side views of the fish, thereby enabling the direct calculation of the surface (pixels), which is later expressed by reference to the actual size of the fish, so the surface can be converted into mm^2. Thereafter, the volume is reconstructed from an ellipsoidal approximation.

RESULTS

Growth. Balashark eggs averaged 1.2-1.3 mm in diameter. They hatched 13 hours after fertilization (26-28°C), giving birth to embryos of *circa* 4.5 mm in total length (TL), with a yolk sac of *circa* 1.1 mm^3. During the first two days after hatching (hereafter AH), embryos absorbed their yolk at a rapid pace (0.36 mm^3 day^{-1}) and exhibited fast growth (0.7 mm TL day^{-1}). During the next two days, the rate of yolk absorption decreased by *circa* 50% (0.2 mm^3 day^{-1}) and growth slowed down as well (0.25 mm TL day^{-1}).

Exogenous feeding commenced on day 4 or 5 AH, when larvae averaged 6.3 mm TL. The growth on the first feeding day was slow (0.3 mm day^{-1}) then it increased rapidly and averaged 0.7-0.8 mm day^{-1} during the next 9 days, i.e. almost the same pace as during the fast growing embryonic period. Thereafter, a parasitic outbreak took place and compromised growth considerably before it was mitigated with a 20-ppm formaldehyde treatment (day 17 AH). Following the treatment, growth resumed at the same pace as before the parasitic outbreak and fish attained 17.2 mm TL when aged 23 days AH.

Because the growth of balashark was slowed down or halted during several days, no reference to age was attempted for fish older than two weeks. However, this shortcoming is not of utmost importance since in most fish species; ontogeny refers most closely to size than to age, except for the very early life stages. This was empirically verified in balashark by comparing the developmental stages of fish of different ages but of similar body lengths.

Fins. At hatching, only a continuous, non-structured finfold was present. It extended over 58.5 and 51.5% of the embryo's length on the dorsal and ventral side, respectively, and its surface amounted to 0.47 mm^2. The next day (5.2 mm TL), the surface finfold had almost doubled (0.87 mm^2), essentially because of the development of the anlage of the caudal fin. Thereafter, the finfold exhibited no further specialisation until 6 days AH (6.6 mm TL), when the first finrays of the caudal fin started growing ventrally. Caudal finrays started differentiating on day 5 AH, before the flexure of the notochord, which was tenuous (5°) at 7 days AH (6.9 mm TL) but attained its definitive bend (32-33°) at 8 days AH (8.1 mm TL). The caudal fin became forked at 10 days AH. Throughout this period, the caudal fin length exhibited a rapid positive allometric growth up to *circa* 20% TL at 15 mm TL, and a slower growth thereafter, with a maximum of 24% TL in a 75-mm juvenile.

The finfold of the ventral and dorsal regions exhibited no specialisation before 7 days AH, when the anlage of the dorsal fin started differentiating. The anlage of the anal fin was not distinctly shaped before day 9 AH. From these moments onwards, these two fins exhibited a rapid allometric growth (Figure 1), which was supported by the concomitant growth of their finrays (starting on day 10 AH; Table 1). The growth of the anal fin became isometric in fish greater than 17.2 mm TL (*circa* 8.2% TL) while the dorsal fin continued its allometric growth

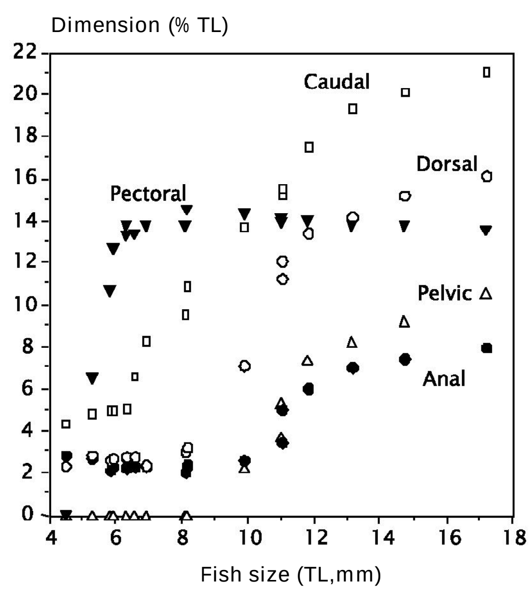

Figure 1. Fin development sequence and growth allometries of fins in *Balantiocheilos melanopterus*. In fish less than 8 mm TL, the dimensions given for the dorsal and anal fins are the heights of the dorsal and ventral finfold, respectively

Table 1. Variations in the number of finrays during the ontogeny of balashark *Balantiocheilos melanopterus*. All finrays were fully elongated on day 13 after hatching (AH). The first ray of the dorsal and anal fins is a supporting ray, which is at least twice shorter than the second ray, and is noted +1

Age (days AH)	Size (TL, mm)	Caudal	Dorsal	Anal	Pelvic	Pectoral
4	6.3	0	0	0	0	0
5	6.3	4	0	0	0	0
6	6.6	9	0	0	0	0
7	6.9	14	0	0	0	0
8	8.1	19	0	0	0	0
9	8.2	19	0	0	0	0
10	9.9	19	7	2	0	0
11	11	19	8	4	8	9
12	11	19	9+1	6+1	8	9
13	11.8	19	9+1	6+1	8	9

and attained 20.2% in the 75-mm juvenile that was used as a standard in this study.

In regards to the paired fins, the pectoral fins were absent at hatching but on the next day, their anlagen had differentiated and already attained 6.5 % TL. Their growth was faster than that of other fins, as they attained their maximum dimension (13.5-14.0 % TL) as early as 5 days AH (6.3 mm TL). By contrast, the pectoral finrays did not develop before the age of 11 or 12 days AH (11.0 mm TL). The anlagen of the pelvic fins were the last to grow, at 11 days AH, but their finrays elongated rapidly from the next day. The pelvic fins had attained their maximal dimension (10.5% TL) at 17.2 mm TL, a size at which the remnants of the caudal and abdominal finfold had vanished.

Cephalic region. The length and width of the head in hatchlings averaged 15.1 and 9.7% TL, respectively. Both variables showed a rapid allometric growth during the embryonic period and attained their largest dimension relative to body length at the start of exogenous feeding; thereafter, they exhibited an isometric growth during the rest of the larval and early juvenile stages.

The gill arches appeared on day 2 AH, while the opercula did not form before day 4 AH. The eyes were present at birth, but almost non-pigmented, except for the lowest margin. Two days later, the eyes were fully pigmented. The eye diameter at hatching was 4.1 % TL. Thereafter, the eyes exhibited a positive allometric growth throughout the juvenile and larval stages and averaged 6.3 % TL at 17.2 mm TL. A similar ratio was observed in a 75-mm TL juvenile from an older progeny. The otic capsule was also present at birth and two otoliths were clearly visible. One day later, the semicircular canals had already developed. In hatchlings, the otic capsule was caudal to the head (16.3% TL from the snout). On the next day, it had migrated forward (11.1% TL from the snout), above the location of the forthcoming first gill arches. The diameter of the otic capsule was 2.7% TL at hatching, and increased rapidly during the next few days, up to a maximum of 5.6% TL at 5 days AH. Because of the development of pigmentation, the ontogeny of the otic capsule could not be traced later on.

The mouth of balashark was still closed on day 1 AH, but opened on day 2 AH (5.9 mm TL). As in most fish species, the lower jaw (LJ) was originally shorter than the upper jaw (UJ). The LJ:UJ ratio increased progressively from 72 to 87% at 15 mm TL and then exhibited almost no or little variation thereafter. Mouth width and gape (90° opening) at 2 days AH were 6.7 and 3.9 % TL, respectively. Both dimensions exhibited a positive allometric growth during the rest of the embryonic period (Figure 2), resulting in absolute dimensions of 0.50 (width) and 0.27 mm (gape) at the start of exogenous feeding. Both dimensions make it possible for balashark

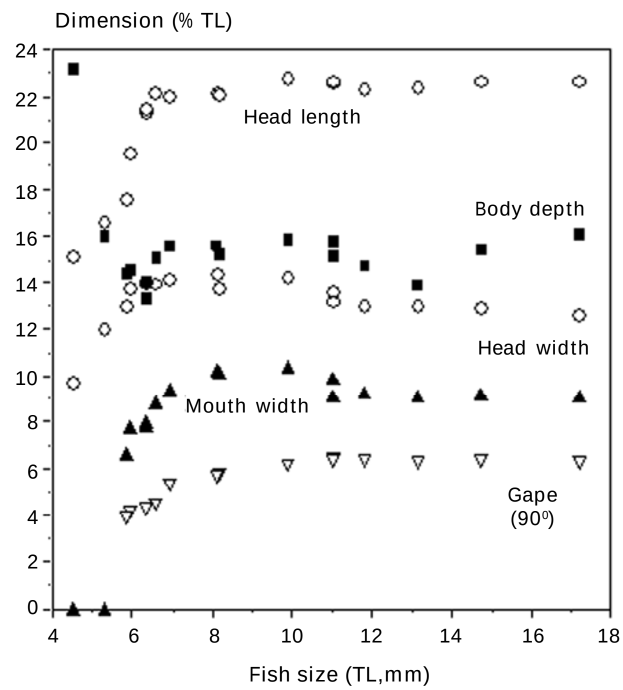

Figure 2. Growth allometries during the ontogeny of *Balantiocheilos melanopterus*, focusing on the cephalic region

to ingest large prey, such as brine shrimp nauplii (0.6 mm x 0.15 mm in diameter), which were readily ingested by 5-day old larvae and permitted to fuel a relatively fast growth. The allometric growth of mouth width and gape continued during the early larval period, until larvae attained 9 mm TL, when the two dimensions averaged 10.2 and 6.3% TL, respectively. Thereafter, gape remained unchanged while mouth width exhibited a slightly negative allometric growth that paralleled that of head width.

Thoracic and abdominal region. As other cyprinids, balashark possess an ill-defined stomach, so the ontogeny of their gut could not be traced from morphological criteria, contrary to the situation in many other fish taxa. The swimbladder started forming as early as day 2 AH and but was not filled before the next day (5.9 mm TL). The volume of the swimbladder expanded rapidly from 0.017 to 0.050 mm^3 during the next two days. Thereafter, its growth was isometric (by reference to the cube of the fish body length) until 17.2 mm TL (the contour of the swimbladder could no longer be traced in larger fish because of the development of the pigmentation pattern and scale cover). Until day 9 AH, the swimbladder displayed no specialisation. From day 10 AH onwards (9.5-9.9 mm TL), the anterior chamber started differentiating and its volume (relative to that of the entire swimbladder) increased rapidly up to 70% at 13 mm TL. Thereafter, this proportion remained almost stable until 17.2 mm TL (71%; Figure 3).

Body dimensions and pigmentation pattern. From the size and age when the caudal fin became forked, the relationships between the total TL, standard (SL) and fork lengths (FL) in balashark were described by simple linear relationships:

FL (mm) = 1.026 [0.077] + 0.755 [0.003] TL (mm) (R^2=0.999, df=16)

SL (mm) = 1.301 [0.068] + 0.818 [0.002] TL (mm) (R^2=0.999, df=16)

SL (mm) = -0.235 [0.045] + 0.924 [0.002] FL (mm) (R^2=0.999, df=16)

Fish body depth at birth was 23.2% TL, but diminished rapidly as the yolk was absorbed, and attained 13.2% at the start of exogenous feeding. Thereafter, it increased in a curvilinear way, up to 16.1% TL at 17.2 mm TL. The positive allometric growth of body depth pur-

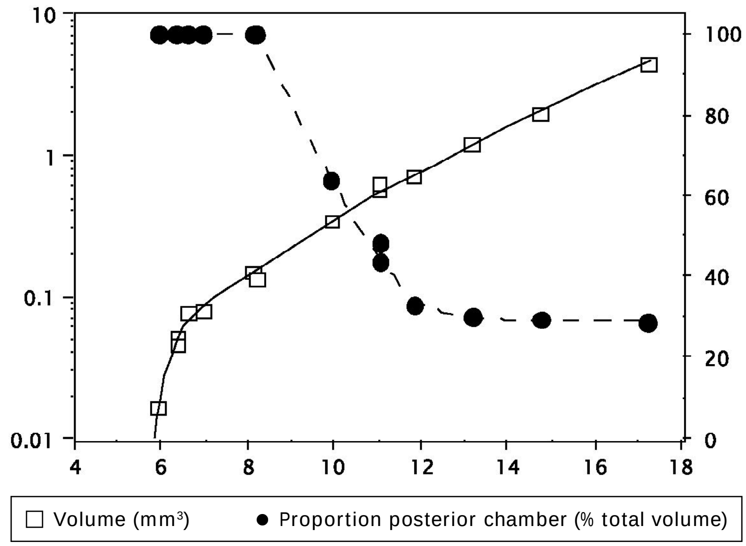

Figure 3. Variation of the volume of the swimbladder and of the proportion of its posterior (hydrostatic only) chamber during the ontogeny of *Balantiocheilos melanopterus*

sued later on, since it attained *circa* 22% TL at 75 mm TL. No model is proposed for this variable, because the pathological outbreak that took place during this study produced a loss of condition in balashark and caused body depth to shrink slightly during this period (Figure 1).

The variation of the pigmentation pattern during the ontogeny of balashark is illustrated on plate I. The chromatophores first appeared above the swimbladder on day 2 AH, then as a ventral row, below the epaxial and caudal musculature on day 3 AH. Thereafter, chromatophores developed in the cephalic region, then in the fins, either before finrays (caudal), slightly after finrays (dorsal, anal) or much later than finrays (pelvic).

DISCUSSION AND CONCLUSION

Based on the criterion of finfold vanishing, balashark becomes juveniles at a size of *circa* 17 mm TL or slightly before this. This cut-off size is also consistent with the growth patterns of fins, which are almost isometric by then. However, many characters remain to appear or develop in fish greater than 17 mm TL. At first, the scale cover has not developed yet, balashark are still slender and the pigmentation of the fins is yet to come (pelvic fins) or expand (caudal, anal, and dorsal). Another notable feature is the fact that the first full-length finray of the dorsal fin is not serrated yet.

The ontogenetic sequence in balashark is shared by many other fish species, probably because many species face similar constraints (i.e. escaping predation and feeding). At first, developing a more efficient propeller and structures that provide balance (namely the pectorals), which both permit improving locomotion and escape from predation. Next the swimbladder, which provides buoyancy, the gills, and the mouth and gut, which are proportionally less crucial than other structures while the fish can still rely on its yolk. Except for the anlagen of the pectoral fins, which grow from a very young age, the fin development sequence in balashark follows a caudal-to-cranial pattern: i.e. caudal, dorsal and anal, pelvic then pectoral fins. To the best of our knowledge, all Cypriniformes and almost all Otophysi exhibit this pattern, with slight variations (i.e. the anal fin and finrays may grow at the same time as or slightly after the dorsal fin and finrays). However, this pattern has also been reported in a broad series of taxa, and comprises over 50% of the species in which this sequence has been described (*circa* 800 species, E. Baras, unpublished data).

Currently, we have not identified any criteria that makes the embryo and larvae of balashark so typical that could make their identification straightforward and univocal, as for example the striped pigmentation pattern of the clown loach *Chromobotia macracanthus* (a cobitid that also originates from rivers and streams in Sumatra), which appears at a very early age and is not shared by many species (if any) in its range (Legendre *et al.*, 2005). Nonetheless, this study provided a complete description of the variations of meristic characters and relative proportions of a series of morphometric variables, including some that are generally neglected, such as the respective proportions of the two chambers of the swimbladder. Hopefully, the combination of these traits will enable the definition of discriminating criteria with other Indonesian fish species, for which the ontogeny is still to be scrutinised.

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Plate I. Illustration of the ontogeny of *Balantiocheilos melanopterus*