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Larval development and allometric growth of the black-faced blenny *Tripterygion delaisi*

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Larval development and allometric growth patterns of the black-faced blenny *Tripterygion delaisi* are described from a larval series (body length, $L_B = 3{\cdot}30–12{\cdot}10$ mm) caught by light traps at the Arrábida Marine Park, Portugal. Larvae of *T. delaisi* possess distinctive morphometric and meristic characteristics which can be used to identify this species from related taxa. Pigmentation is sparse but characteristic, consisting of pigmented eyes, gas bladder pigmentation in the dorsal region, anal pigmentation and a row of regularly spaced postanal ventral melanophores. This pattern is present from as early as the yolk-sac stage and persists throughout all stages with just the addition of head and caudal pigmentation during the flexion and postflexion stages, respectively. The majority of fin development (with the exception of the caudal fin), occurs in the later stages of development. Myomere counts range between 37 and 45 for all stages. Growth is allometric during larval development. When inflexion points of growth were detected, growth was found to be biphasic with the inflexion points occurring within a very narrow range of L_B (8·70–8·90 mm) close to the mean ± s.d. (9·44 ± 1·48 mm L_B) of postflexion larvae. Considering allometric growth patterns and ontogenetic descriptions together, the first developmental phase includes the preflexion and flexion stage larvae, while the second phase characterises the postflexion larvae prior to the transition from larvae to juvenile.

Key words: fish larvae; ontogenetic development; Portugal; Tripterygiidae.

INTRODUCTION

The pelagic larval phase of most marine fishes is a vital stage of their life-cycle with important consequences for recruitment, population dynamics, dispersal and connectivity (Bergenius *et al.*, 2002; Cowen & Sponaugle, 2009). Despite its importance, the accurate identification of fish larvae, which are morphologically very different from the adults, is a major obstacle in many studies aiming at understanding their early life history and ecology. Difficulties in larval sampling, coupled with small larval size and significant changes in body proportions and pigmentation patterns during development, make larval taxonomy an extremely challenging field. Larval descriptions, which continue to play an important role in identification despite the advent of genetic approaches

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(Leis, 2014), are frequently absent or incomplete making identification to the species level tentative at best and many times impossible. These limitations are magnified in temperate nearshore reef environments that are difficult to sample. Consequently, they have received much less attention from marine taxonomists and are thus often less studied than offshore temperate systems and tropical coral reef environments.

One group of fishes that is abundant in the temperate coastal waters of the north-east Atlantic Ocean and Mediterranean Sea is the triplefin blennies belonging to the genus *Tripterygion* Risso 1827 (Family: Tripterygiidae) (Sabatés *et al.*, 2003; Bertoncini *et al.*, 2010). This genus comprises four species including the black-faced blenny *Tripterygion delaisi* (Cadenat & Blache, 1970) which occurs in both the Mediterranean Sea and Atlantic Ocean (Wirtz, 1980; Zander, 1986) and three other species that are endemic to the Mediterranean Sea. Two subspecies of *T. delaisi*, *T. delaisi xanthosoma* and *T. delaisi delaisi*, have been described by Zander (1986), being mainly distinct through male courtship behaviour and colour. Recently, two genetically distinct clades of *T. delaisi* were validated (Carreras-Carbonell *et al.*, 2005; Domingues *et al.*, 2007), with one clade occurring in the Atlantic islands and the other in the Atlantic coast of Europe and the Mediterranean. It remains unknown, however, if these two clades match both subspecies described by Zander (1986) and hence this study was kept focused at the species level.

Previous studies on the composition of larval fish assemblages of the Arrábida Marine Park (AMP), Portugal, indicate that larvae of *T. delaisi* are abundant in this area and that their distribution appears to be restricted to the nearshore environment where they may complete their life-cycle locally, as evident by the co-occurrence of multiple developmental stages (Beldade *et al.*, 2006; Borges *et al.*, 2007, 2009). Due to early life history characteristics (the production of benthic eggs, an inshore larval distribution, limited adult mobility and short pelagic larval duration) which may favour low larval dispersal, this species has been the focus of several studies aiming at investigating population connectivity and the occurrence of self-recruitment and local retention (Carreras-Carbonell *et al.*, 2006, 2007; Galarza *et al.*, 2009; Schunter *et al.*, 2014). The relatively high abundance of *T. delaisi* in the subtidal environment also makes it fairly accessible for both capture and *in situ* observations and hence a good species to investigate various aspects of territoriality (Gonçalves & Almada, 1998), breeding behaviour (De Jonge & Videler, 1989) and habitat selection in rocky reef fish communities (La Mesa *et al.*, 2004, 2006; La Mesa & Vacchi, 2005).

Morphological descriptions of adult and juvenile *T. delaisi* exist (Wirtz, 1980; Zander, 1986; Orlando-Bonaca & Lipej, 2010), but there are no detailed descriptions of the larval stages for this species. At the AMP, *T. delaisi* is the only triplefin blenny present. Relatively calm conditions at this site allow sampling of the very nearshore marine environment, where larvae in different developmental stages can be collected.

Also of relevance during early larval development, is the phenomenon of allometric growth whereby different body parts develop at different rates, leading to complex shape changes during the transition from juvenile to adult (Fuiman, 1983; Osse & van den Boogaart, 1995; van Snik *et al.*, 1997). The objective of this study is therefore to describe the ontogenetic development and allometric growth patterns in the larvae of *T. delaisi* caught by light traps near the reefs inhabited by adults.

MATERIALS AND METHODS

Larvae ($n = 235$) were sampled at the AMP (38° 28′ N; 8° 59′ W), Portugal, using AIMS design light traps (Meekan *et al.*, 2001) deployed for 1 h periods in the extreme nearshore environment, both 1 m below the surface and 1 m above the bottom substratum, at maximum depths of 8 m on the reef, during the period April to September 2007 and 2008. Samples were stored in 4% saline formalin buffered with sodium borate for at least 1 month. To complete the collection, two yolk sac larvae caught in 2013, also by light traps from the AMP, were used only for morphological descriptions of this stage. These specimens, which were preserved in 80% ethanol, were not included in the allometric analyses.

After sorting the plankton samples, larvae of *T. delaisi* were identified, measured (to the nearest 0·01 mm) and assigned to one of four developmental stages (yolk-sac, preflexion, flexion and postflexion) as described by Kendall *et al.* (1984) and by Leis & Carson-Ewart (2000). Each individual was examined in further detail in order to document relevant aspects of ontogenetic development and measure morphological variables. Although all attempts were made to provide as much detail as possible, fin damage during sampling and handling made the accurate counting of fin rays difficult in some instances. Sorting, examination and measurement of all the larvae were conducted using an Olympus SZ-PT stereo microscope equipped with an ocular micrometre and coupled with an Olympus SC35 camera (www.olympus.com). Early recruits ($n = 5$), ranging between 12·98 and 16·61 mm body length (L_B, *i.e.* excluding caudal fin), collected near the reefs using hand nets during scuba diving, in August and September 2014, were included to illustrate the change in melanistic pigmentation patterns during the transformation from postlarva to juvenile. They were fixed and stored in 80% ethanol.

ONTOGENETIC DEVELOPMENT

The description of each individual assigned to a developmental stage was detailed with the main ontogenetic events noted: (1) notochord flexion; (2) fin development; (3) vertebral ossification; (4) changes in pigmentation patterns.

Fuiman's ontogenetic index

Fuiman's ontogenetic index (I_O; Fuiman, 1994) was calculated to express the state of a larva at any point in its ontogeny, as a percentage of a logarithmic developmental period, where $I_O = \log_{10} L_B \, (\log_{10} L_{BJUV})^{-1} 100$ and $L_{BJUV} = L_B$ at the beginning of the juvenile period.

This index represents the percentage of development that has taken place before a given size. Using L_{BJUV} to calculate I_O corrects for interspecific size differences allowing comparisons within and between taxa, while the logarithmic transformation reflects the multiplicative nature of ontogeny (Fuiman & Higgs, 1997).

ALLOMETRIC GROWTH PATTERNS

Allometric growth patterns were examined using bivariate morphological relationships. Morphometric measurements to the nearest 0·01 mm included: L_B from the tip of the snout to the end of the notochord in the preflexion and flexion larvae and to the end of the urostyle or to the mid lateral posterior edge of the hypural plate (standard length) in the postflexion stages; total length (L_T) from the tip of the snout to the end of the caudal fin in postflexion larvae; pre-anal length (L_{PA}) from the snout to the anus; head length (L_H) from the tip of the snout to the end of the operculum; head depth (D_H) from the bottom of the mouth cavity to the top of the head; eye diameter (D_E) in an anterior-posterior plane; body depth at the anus (D_{BA}). Postanal length (L_{POA}) was calculated by subtracting L_{PA} from L_B. Allometric growth was then modelled by a power function of x (where $x = L_B$ or $x = L_H$) using non-transformed data as $y = ax^b$, where y is the measured character or dependent variable and b is the growth coefficient (Fuiman, 1983). Equations were then $\log_{10}$ transformed and the null hypothesis of isometric growth (H_0: $b = 1$) was tested using the t-test for $\alpha = 0{\cdot}05$. Growth is considered to be isometric, positive allometric or negative allometric when $b = 1$, $b > 1$ or $b < 1$, respectively. Additionally, linear regressions were performed on $\log_{10}$-transformed data (L_B or L_H as the independent variable) and the inflection

TABLE I. Larvae of *Tripterygion delaisi* examined according to developmental stage, mean ± S.D. body length (L_B) and range

Stage	*n*	L_B (mm) Mean ± S.D.	L_B (mm) Range
Yolk-sac	2	3·80 ± 0·28	3·60–4·00
Preflexion	98	5·08 ± 0·82	3·30–7·06
Flexion	81	6·98 ± 0·86	5·13–9·19
Postflexion	54*	9·44 ± 1·48	6·10–12·10

n, number of individuals.

*Only 52 were measured for allometric analyses.

points, when existent, represented by the x values where the slope of growth changes, were calculated according to van Snik *et al.* (1997). The x–y data set for each morphological character measured was sorted in ascending order of x. Regression lines were then generated for x_{min} to $x_{intermediate}$ and for $x_{intermediate}$ to x_{max}, where $x_{intermediate}$ varied iteratively from $x_{min} + 2$ to $x_{max} - 2$. T-tests ($\alpha = 0{\cdot}05$) were subsequently performed to determine whether the growth coefficient of each pair of regression lines generated differed significantly. The $x_{intermediate}$ value that iteratively showed the largest t-value was defined as the inflexion point.

RESULTS

ONTOGENETIC DEVELOPMENT

Ontogenetic development is described for the four developmental stages. In total, 235 larvae of *T. delaisi*, ranging between 3·30 and 12·10 mm L_B, were examined (Table I). Yolk-sac larvae [Fig. 1(a)] measured mean ± S.D. 3·80 ± 0·28 mm L_B (range 3·60–4·00 mm, $n = 2$). These larvae were translucent and had a clearly discernible circular yolk sac (*c.* 0·60 and 1·00 mm in diameter, respectively), pectoral fin buds and an open mouth. The body was nearly completely surrounded by a fin fold and the anus, which was also open, was anteriorly located *c.* 33% L_B. Although myomere counts were difficult, at least 12 pre-anal and 33 postanal myomeres were enumerated. Pigmentation at this stage was sparse and limited to pigmented eyes, pigment in the dorsal region of the gas bladder and on the hindgut and a row of regularly spaced postanal ventral punctuate melanophores (visible from the fourth or fifth postanal ventral myomere) [Figs 1(a) and 2]. The otoliths were already clearly visible.

Preflexion specimens [Fig. 1(b)] ranged between 3·30 and 7·06 mm L_B with a mean ± S.D. of 5·08 ± 0·82 mm L_B ($n = 98$). The yolk sac was completely reabsorbed, the entire body was still nearly completely surrounded by its fin fold and, although the caudal fin anlage appeared in 38% of the individuals (mean ± S.D. $L_B = 5{\cdot}83 \pm 0{\cdot}39$ mm, range = 5·00–6·58 mm, $n = 37$) indicating the beginning of caudal fin development (Fig. 2), the notochord had not yet started to flex. The start of vertebral ossification, which began at the urostyle and continued in a posterior–anterior direction, was observed in 56 individuals (57% of preflexion larvae) ranging from 5·03 to 7·06 mm L_B (mean ± S.D. $L_B = 5{\cdot}66 \pm 0{\cdot}48$ mm). The presence of both premaxillary and dentary teeth was observed in 13 individuals (13% of preflexion larvae) ranging from 6·00 to 7·06 mm L_B (mean ± S.D. $L_B = 6{\cdot}34 \pm 0{\cdot}31$ mm). Pigmentation at this stage was

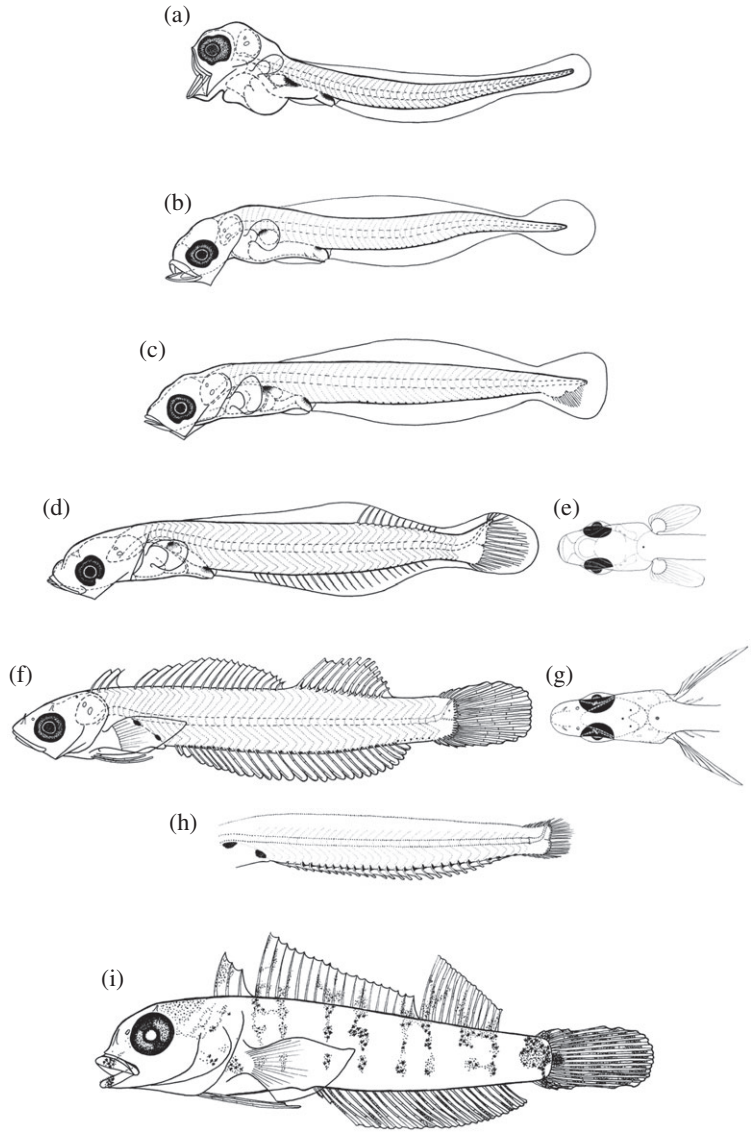

FIG. 1. Larvae of *Tripterygion delaisi* at different developmental stages. (a) Yolk sac larva (body length, *i.e.* excluding caudal fin, L_B 4·00 mm) with a round yolk sac and two conspicuous pigments which are present throughout larval development; (b) preflexion larva (L_B 4·25 mm) with a similar pigmentation to the yolk sac stage; (c) flexion larva (L_B 6·29 mm) showing the beginning of the notochord tip flexion, leading to caudal fin development; (d) late flexion larva (L_B 7·16 mm) with beginning of the third dorsal and anal fin development and with the caudal fin rays nearing the adult longitudinal position; (e) head dorsal view of the larva represented in (d); (f) postflexion larva (L_B 11·61 mm) showing the first, second and third dorsal and anal fins already differentiated and pigmentation on the base of caudal fin; (g) head dorsal view of the larva represented in (f) revealing increased pigmentation; (h) trunk section of a postflexion larva (L_B 11·52 mm), revealing the conspicuous postanal ventral melanophores; (i) early recruit (L_B 13·84 mm) with complete fin development and dermal pigmentation, with the five vertical bars characteristic of the adults.

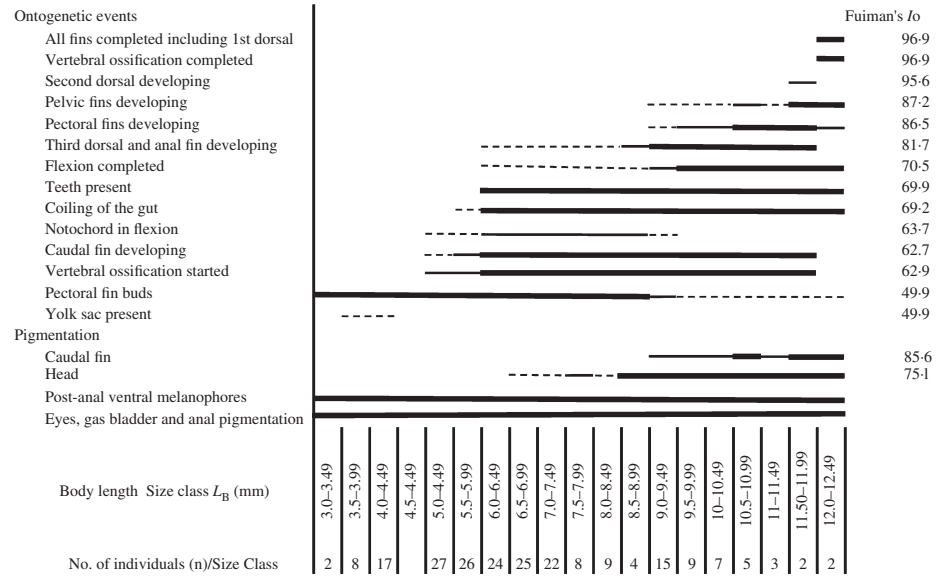

FIG. 2. The sequence of main ontogenetic events relative to body length (*i.e.* excluding caudal fin, L_B), including the development of pigmentation patterns during larval development of *Tripterygion delaisi*. Event–pigmentation: – – –, in <50% of individuals; ——, in 50–99% of individuals; ▬, in 100% of individuals (bar); I_O, Fuiman's ontogenetic index; *n*, sample size.

similar to the yolk-sac stage. Postanal melanophores, which varied between 14 and 27, formed a consistent pattern of about one melanophore per myomere, resulting in a well-defined regular series. Variability in the number of melanophores was due to variability in the starting and ending positions of the series.

The flexion stage specimens ranged between 5·13 and 9·19 mm L_B (mean ± S.D. $L_B = 6{\cdot}98 \pm 0{\cdot}86$ mm, $n = 81$) and were characterized by the upward inclination (flexion) of the posterior part of the urostyle, the coiling of the gut, the continuation of caudal fin development [Fig. 1(c), (d)] and the appearance of dorsal pigmentation in the head region [Figs 1(e) and 2]. Head pigmentation (mean ± S.D. $L_B = 7{\cdot}97 \pm 0{\cdot}67$ mm; range = 6·87–9·19 mm; $n = 15$) comprised either a single internal midline melanophore on the nape or this single melanophore together with one or two additional external melanophores over the hindbrain. In some specimens (mean ± S.D. $L_B = 8{\cdot}54 \pm 0{\cdot}39$ mm, range = 8·13–9·19 mm, $n = 6$) the soft rays of the third dorsal fin (rays added from an anterior to posterior direction) and the anal fin had commenced development [mid-posterior to anterior direction; Fig. 1(d)]. These individuals were in the late stage of flexion with the notochord tip having almost reached its final angle of *c.* 45° and the principal caudal fin rays and supporting skeletal elements nearing the adult longitudinal position.

The postflexion stage [Fig. 1(f); mean ± S.D. $L_B = 9{\cdot}44 \pm 1{\cdot}48$ mm, range = 6·10–12·10 mm, $n = 54$] was mainly characterized by fin development. Both the third dorsal and anal fins had begun development in all postflexion specimens examined (Fig. 2). Throughout development these two fins developed in close synchrony and were always present together. Based on a comparison of the extent of ray development, it is most likely that the third dorsal started to develop just prior to the anal fin. The pectoral fin