

## Early developmental and allometric patterns in the electric yellow cichlid *Labidochromis caeruleus*

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The electric yellow cichlid *Labidochromis caeruleus* is a mouth-brooding haplochromine cichlid from Lake Malawi and one of the most popular cichlids in the ornamental fish industry. To investigate the early development of *L. caeruleus* from hatching until the juvenile stage, we studied its morphological development and allometric growth patterns. In newly-hatched larvae, most organs and body parts were not yet differentiated and continued to develop until 15 days post hatching (dph). The yolk sac was depleted at 13 dph. There was allometric growth, primarily in the anterior and posterior regions of the body, and inflection points when trajectories of allometric growth changed. Head and tail growth was prioritized, suggesting that body parts linked to feeding and swimming behaviour mature earlier than the rest of the body. Additionally, growth patterns revealed that development of organs related to vital functions such as branchial respiration, sensation, exogenous feeding and swimming was prioritized. Comparisons with other African and Neotropical cichlids revealed differences in ontogenetic processes and allometric growth along the anterior–posterior axis as well as variation in developmental timing. These results indicate how early morphological development and ontogenetic processes might respond to the distinctive parental care observed in mouth-brooding cichlids.

Key words: Cichlidae; growth coefficient; mouth-brooding; ontogeny; organogenesis.

### INTRODUCTION

Knowledge of the early development and growth patterns of fishes is important for the assessment and optimization of hatchery production (Koumoundouros *et al.*, 1999; van Maaren & Daniels, 2000) and for a better understanding of the molecular and developmental mechanisms driving phenotypic diversification (Meyer, 1986, 1987, 1988; Salzburger & Meyer, 2004; Henning & Meyer, 2014; Kratochwil & Meyer, 2015; Kratochwil *et al.*, 2015). Early larval development depends on morphometric changes that are regulated by genetic and environmental factors (Gilbert & Bolker,

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2003). Ontogenetic allometric shifts (*i.e.* differences in relative growth rates between body parts) contribute to both fixed and phenotypically plastic morphological differences (Simonovic *et al.*, 1999; Shingleton *et al.*, 2007; Muschick *et al.*, 2011; Schneider & Meyer, 2017). These shifts are linked to environmental conditions and ecology (Dettlaff *et al.*, 1993; Osse *et al.*, 1997). Allometric and isometric growth patterns can be revealed by plotting the size of individual body parts against body size (Simonovic *et al.*, 1999; Shingleton *et al.*, 2007). This is of interest because adult morphological differences are commonly generated through variation in allometric and isometric juvenile growth patterns (Osse & van den Boogaart, 1995; van Snik *et al.*, 1997). Furthermore, developmental staging and growth patterns allow comparisons of developmental processes and growth patterns between model and non-model species, thus revealing underlying mechanisms of evolutionary change (Meijide & Guerrero, 2000; Pena & Dumas, 2009). However, despite the availability of developmental data on hundreds of fish species, relatively few studies have focused on the diverse and species-rich family Cichlidae and studies of haplochromine cichlids are particularly sparse (Balon, 1977; Richards & Leis, 1984; Holden & Bruton, 1994; Britz, 1997; Stiassny & Meyer, 1999; Lopes *et al.*, 2015).

With 2500 to 3000 species, cichlids are one of the most species-rich families of teleosts (Fryer & Iles, 1972; Meyer *et al.*, 1991; Sturmbauer & Meyer, 1992; Meyer, 1993; Farias *et al.*, 2000; Snoeks, 2000; Turner *et al.*, 2001) and include many important ornamental fishes. Cichlids demonstrate exceptional variation in body shapes and colour patterns (Meyer, 1993; Kornfield & Smith, 2000). In particular, body shape variation can be ultimately linked to variation in allometric growth during development. Cichlids can be categorized into two alternative reproductive strategies that probably influence patterns of development: substratum-brooders, which display parental care and guarding of fertilized eggs and developing young, and mouth-brooders, which carry fertilized eggs and developing young within the buccal cavity (Barlow, 1991; Keenleyside, 1991). Generally, mouth-brooders have been described as precocial, *i.e.* larger completely developed young receiving more parental care (in need of parental), whereas substratum-brooders have been described as altricial (Noakes, 1991), *i.e.* smaller incompletely developed young receiving little parental care (selfsupporting).

The target species for this study, the electric yellow cichlid *Labidochromis caeruleus* Fryer 1956, is a popular freshwater aquarium fish native to the coastal area of Nkhata Bay in Lake Malawi, Malawi (Konings, 2001). Lake Malawi is estimated to contain 800–1000 endemic cichlid species (Turner *et al.*, 2001), most of which are believed to have evolved from a single ancestral species (Meyer *et al.*, 1990; Verheyen *et al.*, 2003; Turner, 2007) that underwent an adaptive radiation into many striking forms (Lee *et al.*, 2014). This diversity makes Lake Malawi cichlids an ideal model for understanding the role of developmental differences, molecular mechanisms and ecological factors in phenotypic diversification (Salzburger & Meyer, 2004; Seehausen, 2006).

The object of this paper is to present a comprehensive description of the early morphological development of *L. caeruleus*. Laboratory-reared specimens were studied from hatching until 51 days post hatching (dph) to study allometric growth patterns. The study tested the hypotheses that growth is prioritized in the anteriormost and posteriormost parts of body (*i.e.* the head and tail) and that the mouth-brooding *L. caeruleus* exhibits differences in morphological development compared with substratum-spawning cichlids (Meyer, 1986, 1987, 1988). Lastly, the study relates early developmental morphology with specific behaviours such as swimming,

feeding and prey detection and constitutes a baseline for future studies on the early morphological development, systematics and phylogenetics of cichlids.

## MATERIALS AND METHODS

Three breeding pairs of *L. caeruleus* were obtained from a local ornamental fish farm and transferred to glass aquaria for spawning. Water variables of the spawning aquaria were: temperature  $28 \pm 1^\circ \text{C}$ , pH  $7.5 \pm 0.4$ , hardness  $170 \pm 10 \text{ mg l}^{-1}$  and dissolved oxygen  $5.9 \pm 0.3 \text{ mg l}^{-1}$ . Hatching occurred 3 days post fertilization and larvae were transferred to new aquaria for rearing. Water variables of the rearing aquaria were the same as the spawning aquaria. Newly hatched larvae began actively swimming 3–5 days post hatching (dph) and were fed with *Artemia* nauplii and micro-worms from 1 to 5 dph, then with a mixture of nauplii and commercial food pellets (Biomar A/S; www.biomar.com: 58% protein, 15% lipid) twice a day from one to 51 dph.

Development was characterized at different stages starting at 1 dph (12 h after hatching) until 51 dph following the terminology, descriptions and developmental criteria used by Balon (1977, 1986, 1999) and Mejjide & Guerrero (2000). Five randomly-selected larvae were collected once a day for the first 5 dph, then every other day until 51 dph. Specimens were sacrificed with an overdose of clove-powder solution, photographed, then fixed in 4% buffered formalin solution. Morphological characteristics of specimens, including the presence of myotomes, yolk sac absorption, eye and body pigmentation, mouth and anus opening, notochord flexion, fin development, appearance of scales and lateral-line development were examined under a Leica MC5 Stereozoom microscope (www.leica-microsystems.com).

Allometric growth patterns were characterized for the following morphological characteristics: total length ( $L_T$ ), head length ( $L_H$ ), trunk length ( $L_{Tr}$ ), tail length ( $L_{Ta}$ ), maximum body depth ( $D_B$ ), eye diameter ( $D_E$ ) and snout length (measured from tip of premaxilla to anterior edge of the eye;  $L_{Sn}$ ). All measurements were made to the nearest 0.01 mm from pictures taken of the left side of fixed specimens using ImageJ 1.240 (www.imagej.nih.gov) following the protocol of Leis & Trnski (1989) (Fig. 1).

Inflection points on growth curves were calculated according to van Snik *et al.* (1997) and Gisbert (1999) in which the inflection point is the  $x$  value where the slope changes. To calculate the inflexion point, the  $x$ - $y$  data set was sorted according to increasing  $x$ , where  $x$  is  $L_T$ . Then,  $L_T$  was divided into two different regression groups: a group bounded by  $L_{Tmin}$  and  $L_{Tintermediate}$  and the second group was bounded by  $L_{Tintermediate}$  and  $L_{Tmax}$ . (1) A  $t$ -test was used to determine whether the two slopes (2) for these small and large groups were significantly different at the level of  $P = 0.05$ .  $L_{Tintermediate}$  was defined as a value that ranged from  $L_{Tmin} + 2$  to  $L_{Tmax} - 2$ . Note that  $L_{Tintermediate}$  could not equal  $L_{Tmin}$ ,  $L_{Tmin} + 1$  and  $L_{Tmax}$  because a regression of less than three points has no statistical meaning (Müller & Videler, 1996; van Snik *et al.*, 1997).

Allometric growth was calculated using simple linear regressions according to the equation  $\log y = \log a + b \log L_T$ , where  $y$  is  $L_H$ ,  $L_{Tr}$ ,  $L_{Ta}$ ,  $D_B$ ,  $D_E$ ,  $L_{Sn}$ ,  $a$  is the intercept and  $b$  is the growth coefficient. We considered growth as isometric when  $b = 1$ , positively allometric when  $b > 1$  and negatively allometric when  $b < 1$  (Huxley, 1950; Nogueira *et al.*, 2014).

## RESULTS

### MORPHOLOGICAL DEVELOPMENT

The increase in  $L_T$  of *L. caeruleus* from hatching to 51 dph was linear ( $r^2 = 0.92$ ) (Fig. 2).

#### *Post-hatching stage*

At the point of hatching (0 dph), newly hatched individuals had a closed mouth and anus, 23 myomeres were visible and the eye was fully pigmented. Up to 4 dph the

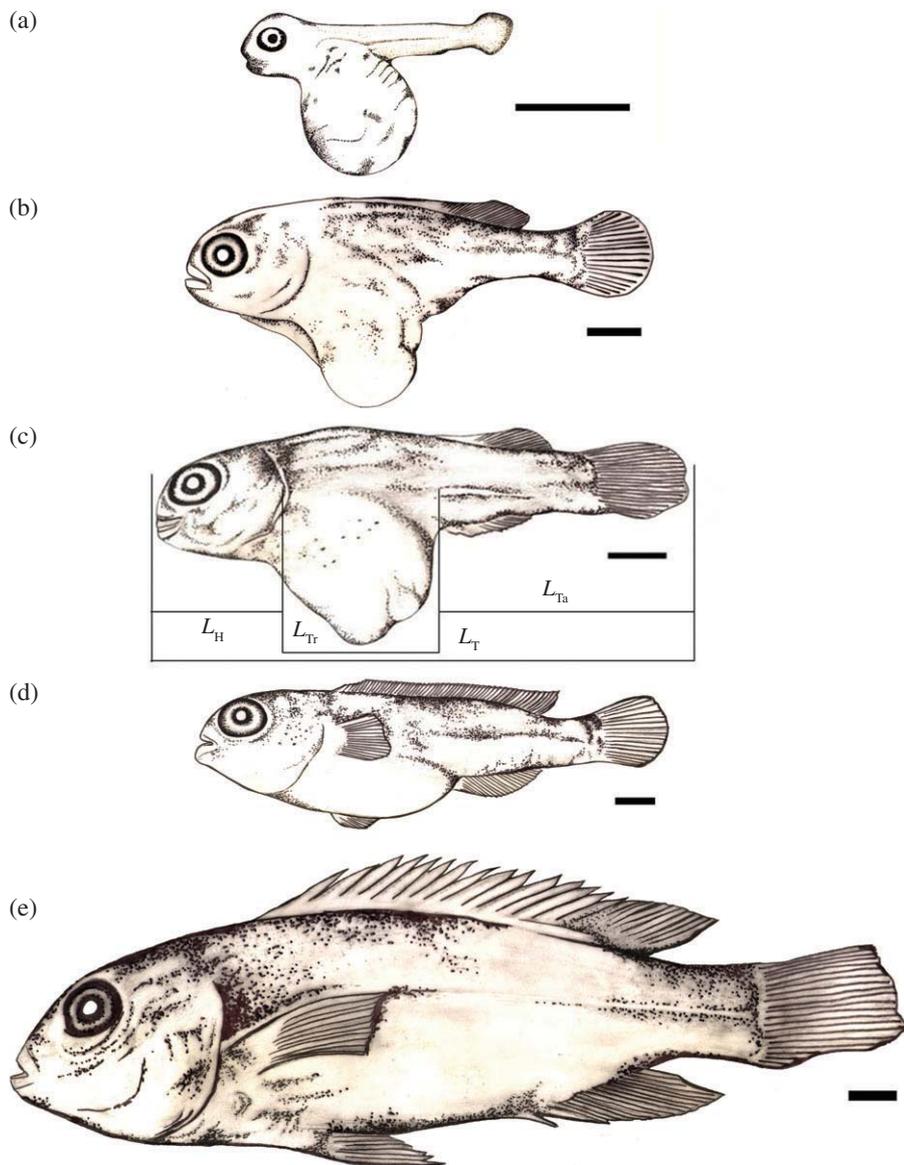


FIG. 1. Early ontogenetic development of *Labidochromis caeruleus*: (a) 1 day post hatching (dph), (b) 5 dph, (c) 7 dph, (d) 9 dph and (e) 19 dph when fin rays and juvenile morphology were complete. (c) Morphometric characteristics measured:  $L_T$ , total length, ( $L_H$ , head length, ( $L_{Tr}$ , trunk length, ( $L_{TA}$ , postanal tail length. Scale bar = 1 mm.

newly hatched larvae [3.4 mm  $L_T$ ; Figs 1(a) and 3(a)–(d)] were almost non-pigmented with scarce melanophores on the snout and on the surface of the yolk sac. The first melanophores (excluding eye melanophores) appeared on the surface of the yolk-sac. Stellate melanophores covered the head, anterior part of the trunk and dorsal parts of the body. At 5 dph a caudal expansion could be seen [6.5 mm  $L_T$ ; Figs 1(b) and 4(e)].

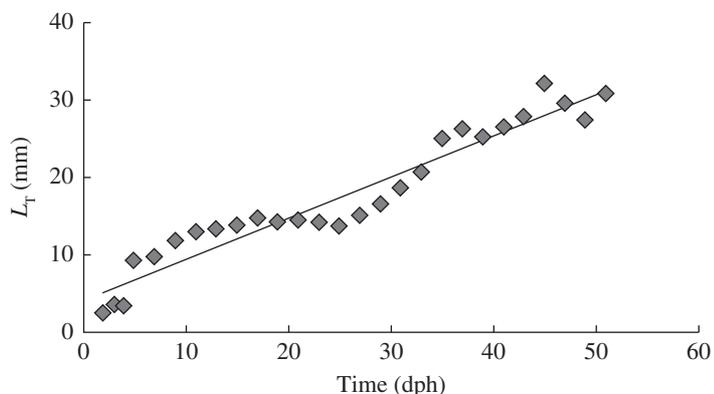


FIG. 2. Relationship between total length ( $L_T$ ) and days post hatching (dph) in *Labidochromis caeruleus* ( $y = 0.5336x + 4.0927$ ,  $r^2 = 0.92$ ,  $P < 0.05$ ).

Some larvae had curved bodies during the first few days, but after the 4th day all became straight [Figs 1(b) and 3(e)]. Patchy pigmentation covered the upper part of the body.

#### *Opened-mouth stage*

At 5 dph primordial dorsal and anal fins appeared and were pigmented, caudal and anal ray rudiments appeared and the mouth and anus opened. The operculum also began developing [Fig. 1(b)] and a pair of small, disc-shaped pectoral-fin buds appeared. Caudal-fin rays appeared at 7 dph [Figs 1(c) and 3(f)].

#### *Yolk sac stage*

By 9 dph about half of the yolk sac was absorbed and the pelvic and pectoral fins were developed [Fig. 3(h)], notochord flexion was complete with an oblique angle (c.  $30^\circ$ ) [Fig. 3(g)] and caudal, dorsal and anal-fin rays became distinguishable [Figs 1(d) and 3(h)].

#### *Free swimming stage*

At 11 dph, a large proportion of the yolk sac was depleted and pectoral fins were functionally developed [Fig. 3(i)].

#### *Unpaired fin stage*

By 13 dph dorsal and anal-fin rays were differentiated and developed. Pelvic-fin ray count was five or six, the yolk sac was barely visible and the first scales were observed.

#### *Juvenile stage*

Body shape and pigmentation started to resemble the adult phenotype. Scales fully covered the body and the lateral line was visible. Specimens already showed sexual dimorphisms in pigmentation patterns by 15 dph [Fig. 3(j), (k)]: Male pigmentation pattern [Fig. 3(l), (m)] with vertical bars on the upper part of the male trunk and female uniformly coloured pale yellow. Fin ray counts were: caudal fin 19–27, dorsal fin

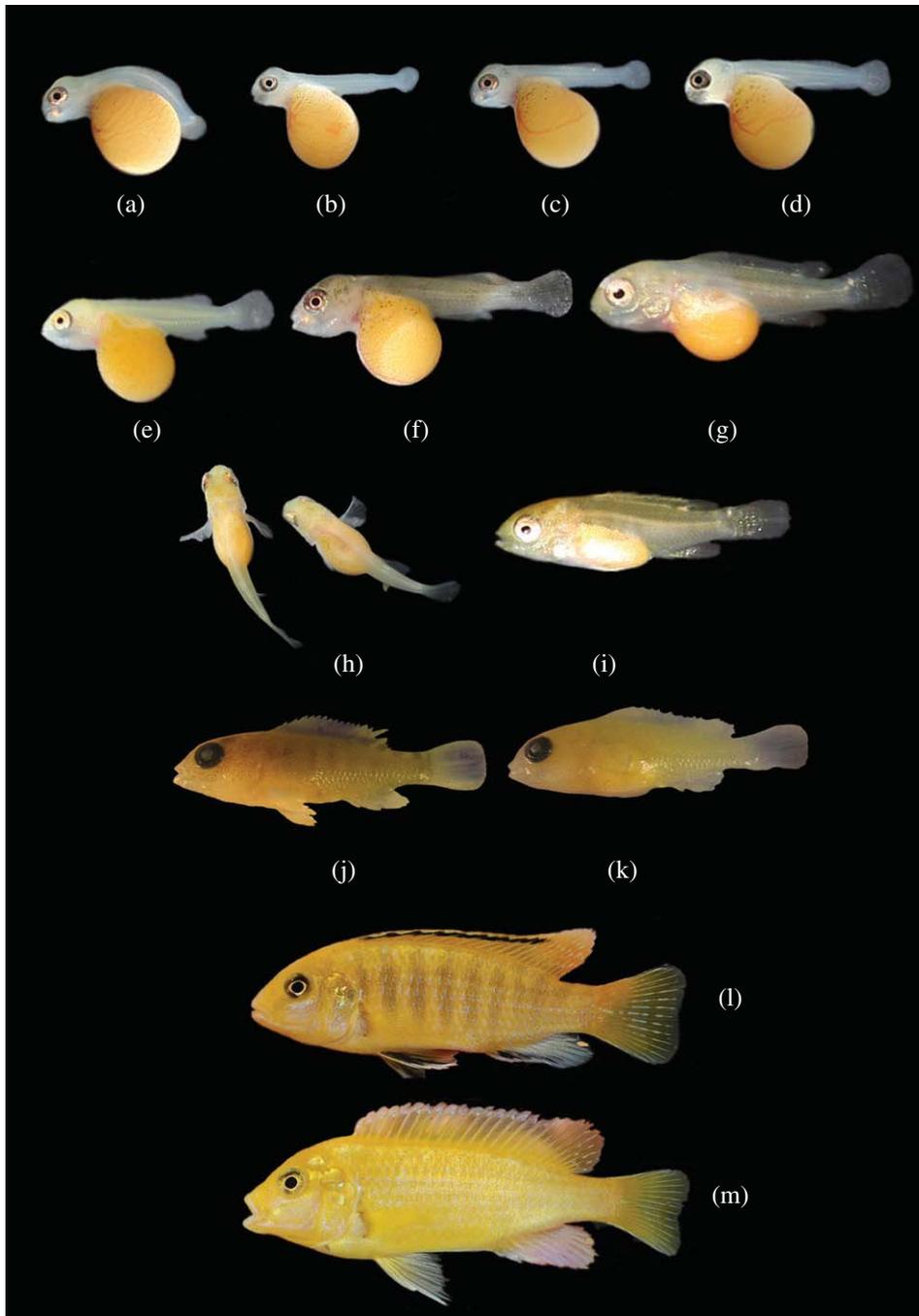


FIG. 3. Developmental stages of *Labidochromis caeruleus*: (a) newly hatched larva (2 mm total length,  $L_T$ ), (b) 1 day post hatching (dph) (2.1 mm  $L_T$ ), (c) 2 dph (2.5 mm  $L_T$ ), ((d) 3 dph (3.5 mm  $L_T$ ) (e) 5 dph (6.5 mm  $L_T$ ), (f) 7 dph (9.8 mm  $L_T$ ), (g) 9 dph (11.1 mm  $L_T$ ), (h) dorsal view 9 dph, (i) 11 dph, ((12.1 mm  $L_T$ ), (j & k) distinctive coloration pattern in male and female by 15 dph, ((13.5 mm  $L_T$ ), (l & m) distinctive coloration pattern in mature male and female (8.5 cm  $L_T$ ).

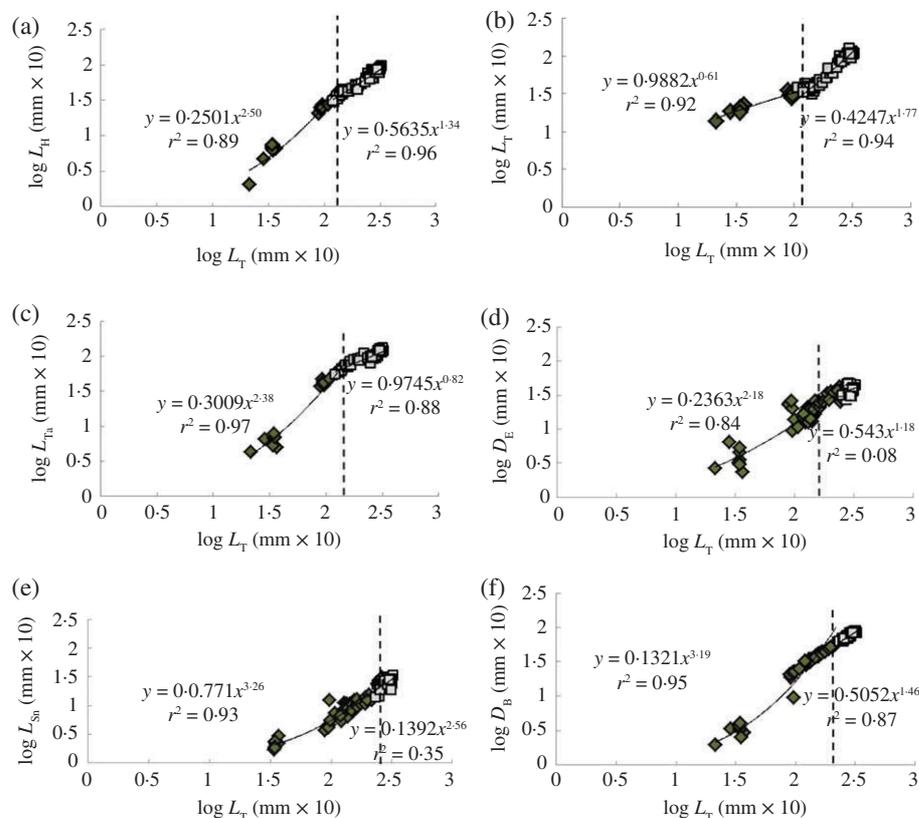


FIG. 4. Pre (◆) and post-inflexion (■) growth relationships between total length ( $L_T$ ) and mean (a) head length ( $L_H$ ), (b) trunk length ( $L_{Tr}$ ), (c) tail length ( $L_{Ta}$ ), (d) eye diameter ( $D_E$ ), (e) snout length ( $L_{Sn}$ ) and (f) body depth ( $D_B$ ) of young *Labidochromis caeruleus* during early development between hatching and 51 days post hatch. |, Growth inflexion point.

24–30, anal fin 10–12, pelvic fin 5–6 and pectoral fin 8–12. Thereafter, unbranched rays were completely differentiated in dorsal, anal and pelvic fins. Unbranched fin-ray counts were: dorsal fin 16–19, anal fin four to six and pelvic fin two or three. The lateral line became visible at 19 dph [Fig. 1(d)], after which most structures became fully developed and only body size increased [Fig. 1(d)].

#### ALLOMETRIC GROWTH PATTERN

Head length ( $L_H$ ) showed positive allometric growth ( $b = 2.50$ ) prior to its inflection point at 13 dph, then continued with positive though reduced allometry ( $b = 1.34$ ) up to 51 dph [Fig. 4(a)]. Growth of the trunk ( $L_{Tr}$ ) was negatively allometric ( $b = 0.61$ ) prior to its inflection point at 7 dph [Fig. 4(b)], then positively allometric afterwards ( $b = 1.77$ ). In the first 7 days, the proportion of  $L_{Tr}$  relative to  $L_T$  decreased from 64 to 31%, then dropped further to between 24 and 34%  $L_T$  during the post-flexion period (Table I).

TABLE I. Slopes of developmental rates and ranges of various morphometric subunits of larval *Labidochromis caeruleus* before and after inflexion (all regressions significant at  $P < 0.05$ )

	Pre-inflexion slope	Pre-inflexion range (% $L_T$ )	Post-inflexion slope	Post-inflexion range (% $L_T$ )
Head length ( $L_H$ )	2.5	13.5–28.3	1.34	27.2–23.8
Trunk length ( $L_{Tr}$ )	0.6	64–31%	1.77	24.0–34.0
Tail length ( $L_{Ta}$ )	2.3	23–48%	0.82	46.0–38.0
Eye diameter ( $D_E$ )	2.1	9.7–15.2	1.18	15.7–11.6
Snout length ( $L_{Sn}$ )	3.2	1.2–8%	2.56	9.0–11.0
Body depth ( $D_B$ )	3.2	10.5–25.5%	1.46	25.6–27.05

Tail length ( $L_{Ta}$ ) showed positive allometric growth ( $b = 2.38$ ) prior to its inflection point at 19 dph, increasing from 23 to 48%  $L_T$ . Post inflection,  $L_{Ta}$  showed negative allometric growth ( $b = 0.82$ ) and the proportion of  $L_{Ta}$  to  $L_T$  decreased from 46 to 38% [Table I and Fig. 4(c)]. Eye diameter ( $D_E$ ) had positive allometric growth ( $b = 2.18$ ) prior to its inflection point at 41 dph, then subsequently grew isometrically ( $b = 1.18$ ). Moreover, the proportion of  $D_E$  to  $L_T$  increased from 9.7 to 15.2% before inflection at 41 dph, then decreased from 15.7 to 11.6% from inflection to 51 dph [Table I and Fig. 4(d)].

Snout length ( $L_{Sn}$ ) showed positive allometric growth prior to ( $b = 3.26$ ) and after ( $b = 2.56$ ) its inflection at 37 dph. Snout length was 1.2%  $L_T$  at 1 dph and reached 8% of  $L_T$  by 18 dph. After inflection, snout growth increased, reaching nine to 11% of  $L_T$  [Fig. 4(e)]. Body depth ( $D_B$ ) showed positive allometric growth ( $b = 3.19$ ) prior to its inflection at 33 dph, with  $D_B$  being 10.5% of  $L_T$  at 1 dph and expanding to 25.5%  $L_T$  by 33 dph. By 35 dph,  $D_B$  showed positive but reduced allometric growth ( $b = 1.46$ ) [Table I, Fig. 4(f) and Supporting Information in Table S1].

## DISCUSSION

It is hypothesized that mouth-brooding cichlid species, such as *L. caeruleus*, differ from substratum spawning cichlids in their life history and morphological development including colour pattern formation, craniofacial development and development of adult body shape (Meyer, 1986, 1987, 1988). For example, *L. caeruleus* colour patterns develop later than in model teleost fishes as zebrafish *Danio rerio* (Hamilton 1822) or medaka *Oryzias latipes* (Temminck & Schlegel 1846) (Kimmel *et al.*, 1995; Iwamatsu, 2004). Even in embryos, coloration patterns may be important for providing camouflage to decrease predation risk (Sanchez *et al.*, 1999; Hilsdorf *et al.*, 2002). Our results suggest that changes in the onset of color pattern development may be linked to decreased predation risk in well-protected mouth-brooding cichlid larvae such as those of *L. caeruleus*.

A close relationship between the timing of mouth opening and yolk-sac absorption has been previously shown (Lasker *et al.*, 1970). However, there is a transitional phase from endogenous to exogenous feeding (Sato *et al.*, 2003), at the end of which, around 13 dph in the present study, larvae of *L. caeruleus* attained a suitable size and degree of organ development to support their survival in unpredictable environmental conditions

(Kamler & Keckeis, 2000). As a freshwater mouth-brooding cichlid, *L. caeruleus* took longer to absorb its yolk sac than substratum-brooding cichlids (Meyer, 1986, 1987, 1988; Meijide & Guerrero, 2000; Kratochwil *et al.*, 2015) and non-haplochromine mouth-brooders such as the Nile tilapia *Oreochromis niloticus* (L. 1758), which is characterized by high fecundity and small eggs numbering in the hundreds per clutch, compared with typically 30–40 in *L. caeruleus* (Fujimura & Okada, 2007). Furthermore, the remnant of the yolk sac can still be observed at juvenile stages.

Juvenile *L. caeruleus* already resemble adults in morphology and feeding behavior hinting at a longer transitional phase from endogenous to exogenous feeding in mouth-brooding cichlids in comparison with substratum-spawning cichlids. It is known that Lake Malawi cichlids produce fewer, larger eggs than substratum-brooders (Morrison *et al.*, 2001), possibly explaining the longer period for yolk exhaustion in *L. caeruleus*. Larvae with higher maternal investment and longer periods of maternal care are able to begin feeding, as well as avoid being fed upon, at later stages of ontogeny. It has been suggested that the duration of the larval stage strongly depends on available food (Strathmann, 1986), but if larval fishes have access to nutrients and food resources, they should grow faster (Swearer *et al.*, 1999). Alternatively, other authors have suggested that a long larval phase might be essential to complete particular developmental processes (Radtke *et al.*, 2001). As a mouth-brooder with a relatively long larval period ( $\pm 2$  week), *L. caeruleus* showed parental care that is dependent on food availability, as it is known that females experimentally raised in poor conditions produce larger eggs and young (Taborsky, 2006a, 2006b). In turn, egg size may increase in response to an increase in care because larger eggs have longer developmental times during which they are protected (Shine, 1978; Sargent, 1997).

In the present study, paired and unpaired fins developed at different larval stages. The primordial fin fold is likely to improve locomotion and respiration by increasing surface area (van Snik *et al.*, 1997; Geerinckx *et al.*, 2008). Development of fin rays may be related to a transition in swimming style from an anguilliform larval type to a sub-carangiform juvenile type (van Snik *et al.*, 1997; Gisbert, 1999; Gisbert *et al.*, 2002; Pena & Dumas, 2009), which occurs prior to the start of exogenous feeding in *L. caeruleus* (Meijide & Guerrero, 2000; Korzelecka-Orkisz *et al.*, 2012). Starvation and predation are known as the main factors for high mortality rate in early developmental stages (Chambers & Trippel, 1997), so the timing and coordination of transitions in locomotion and feeding are probably important (Domenici & Kapoor, 2010).

Positive allometric growth of the head from hatching to inflection can be related to development of the brain, sensory systems (*e.g.* vision and olfaction), respiratory system and food processing organs needed to find and obtain food (van Snik *et al.*, 1997; Gisbert *et al.*, 2002). Although positive allometric growth of the head in *L. caeruleus* decreased after inflection, coefficient values were still higher compared to isometric growth trends in other teleosts (Osse & van den Boogaart, 2004). The large head size of mouth-brooding cichlids such as *L. caeruleus* may be explained by positive allometric head growth for a longer developmental period.

Positive tail allometry is mostly related to development of the caudal fin and ossification of fin rays (Betti *et al.*, 2009; Maciel *et al.*, 2010). Trunk length ( $L_T$ ) showed a negative allometric growth pattern for up to 7 dph, which is similar to other teleosts (Osse & van den Boogaart, 1999; Osse & van den Boogaart, 2004; Nogueira *et al.*, 2014). Osse & van den Boogaart (1995) suggested that rapid growth of the head and

tail could be associated with the reduction of drag on the body and improvement of swimming ability. Afterward, positive trunk growth may be linked to the completed development and functionality of the digestive system (Asgari *et al.*, 2014). In addition, positively allometric growth of body depth correlates with the development of the digestive tract and its functional glands (Pena *et al.*, 2003). These changes also coincided with the shift to exogenous feeding and improved swimming capacity (Gisbert *et al.*, 1999) allowing for food capture and predator avoidance (Osse & van den Boogaart, 1995, 1999, 2004). Thus, selective organogenesis may act as a survival strategy during early life (Osse *et al.*, 1997; van Snik *et al.*, 1997; Gisbert, 1999).

We hypothesized that *L. caeruleus* would exhibit several developmental changes in organogenesis that are adaptations to the life history of mouth-brooding cichlids. For example, allometric growth of the head, trunk and tail of *L. caeruleus* showed the same trends prior to and after inflection points relative to Neotropical substratum-brooding cichlids such as *Cichlasoma dimerus* (Heckel 1840), *Pterophyllum scalare* (Schultze 1823) and *Nannacara anomala* Regan 1905, but with variation in the magnitude and time of inflection (Meijide & Guerrero, 2000; Kupren *et al.*, 2014; Eagderi *et al.*, 2017). Head length allometry and inflection of *L. caeruleus* (2.50) were weaker and later (day 11) than *P. scalare* ( $b = 3.19$ ; 6 dph). Tail growth inflection of *L. caeruleus* (19 dph) was also later than *N. anomala* (4 dph), *C. dimerus* (Meijide & Guerrero, 2000) and *P. scalare* (Korzelecka-Orkisz *et al.*, 2012) (6 dph). Variation among substratum-brooding cichlids could be partly explained by maximum adult size (adult *Cichlasoma* Swainson 1939 and *Pterophyllum* Heckel 1840 are at least twice as large as *Nannacara* Regan 1905) and rearing conditions (*i.e.* temperature and feeding schedule) (Kupren *et al.*, 2014).

In conclusion, differences in the timing and magnitude of ontogenic processes during the early development of *L. caeruleus* allow for a novel perspective on phenotypic divergence and adaptation. Our study reveals longer yolk-sac depletion periods and the prioritization of several organogenetic processes as characteristics of *L. caeruleus* specifically and mouth-brooding haplochromine cichlids more generally.

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## References

- Asgari, R., Rafiee, G., Eagdei, S., Shahrooz, R., Pourbagher, H., Agh, N. & Gisbert, E. (2014). Ontogeny of the digestive system in hatchery produced beluga (*Huso huso* Linnaeus, 1758); a comparative study between Beluga and genus Acipenser. *Aquaculture Nutrition* **20**, 595–608. <https://doi.org/10.1111/anu.12113>
- Balon, E. K. (1977). Early ontogeny of *Labeotropheus* Ahl 1927 (Mbuna, Cichlidae, Lake Malawi), with a discussion of advanced protective styles in fish reproduction and development. *Environmental Biology of Fishes* **2**, 147–176. <https://doi.org/10.1007/BF00005370>

- Balon, E. K. (1986). Types of feeding in the ontogeny of fishes and the life-history model. *Environmental Biology of Fishes* **16**, 11–24. <https://doi.org/10.1007/BF00005156>
- Balon, E. K. (1999). Alternative ways to become a juvenile or a definitive phenotype (and on some persisting linguistic offenses). *Environmental Biology of Fishes* **56**, 17–38. <https://doi.org/10.1023/A:1007502209082>
- Barlow, G. W. (1991). Mating systems among cichlid fishes. In *Cichlid Fishes: Behavior, Ecology and Evolution* (Keenleyside, M. H. A., ed), pp. 173–190. London: Chapman & Hall.
- Betti, P., Machinandiarena, L. & Ehrlich, M. D. (2009). Larval development of Argentine hake *Merluccius hubbsi*. *Journal of Fish Biology* **74**, 235–249. <https://doi.org/10.1111/j.1095-8649.2008.02136.x>
- Britz, R. (1997). Egg surface structure and larval cement glands in nandid and badid fishes with remarks on phylogeny and biogeography. *American Museum Novitates* **3195**, 1–7.
- Chambers, R. C. & Trippel, E. A. (1997). *Early Life History and Recruitment in Fish Populations*, 1st edn. London: Chapman & Hall.
- Dettlaff, T. A., Ginsburg, A. S. & Schmalhausen, O. I. (1993). *Sturgeon Fishes: Developmental Biology and Aquaculture*. Berlin: Springer-Verlag. <https://doi.org/10.1007/978-3-642-77057-9>
- Domenici, P. & Kapoor, B. G. (2010). *Fish Locomotion: An Eco-Ethological Perspective*. Boca Raton, FL: Science Publishers.
- Eagderi, S., Moshayedi, F. & Mousavi-Sabet, H. (2017). Allometric growth pattern and morphological changes of *Pterophyllum scalare* (Schultze, 1823) during the early development. *Iranian Journal of Science and Technology* **41**, 965–970. <https://doi.org/10.1007/s40995-017-0318-6>
- Farias, I. P., Meyer, A. & Ortí, G. (2000). Total evidence: molecules, morphology and the phylogenetics of cichlids fishes. *Journal of Experimental Zoology* **288**, 76–92.
- Fryer, G. & Iles, T. D. (1972). *The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution*. Edinburgh: Oliver & Boyd.
- Fujimura, K. & Okada, N. (2007). Development of the embryo, larva and early juvenile of Nile tilapia *Oreochromis niloticus* (Pisces: Cichlidae). Developmental staging system. *Development, Growth & Differentiation* **49**, 301–324. <https://doi.org/10.1111/j.1440-169X.2007.00926.x>
- Geerinckx, T., Verhaegen, Y. & Adriaens, D. (2008). Ontogenetic allometries and shape changes in the suckermouth armoured catfish *Ancistruscf. triradiatus* Eigenmann (Loricariidae, Siluriformes), related to suckermouth attachment and yolk-sac size. *Journal of Fish Biology* **72**, 803–814. <https://doi.org/10.1111/j.1095-8649.2007.01755.x>
- Gilbert, S. F. & Bolker, J. A. (2003). Ecological developmental biology: preface to the symposium. *Evolution & Development* **5**, 3–8. <https://doi.org/10.1046/j.1525-142X.2003.03002.x>
- Gisbert, E. (1999). Early development and allometric growth patterns in Siberian sturgeon and their ecological significance. *Journal of Fish Biology* **54**, 852–862. <https://doi.org/10.1111/j.1095-8649.1999.tb02037.x>
- Gisbert, E., Williot, P. & Castello-Orvay, F. (1999). Behavioural modifications of Siberian sturgeon (*Acipenser baerii* Brandt) during early life stages of development: their significance and use. *Journal of Applied Ichthyology* **15**, 237–242. <https://doi.org/10.1111/j.1439-0426.1999.tb00242.x>
- Gisbert, E., Merino, G., Muguét, J. B., Bush, D., Piedrahita, R. H. & Conklin, D. E. (2002). Morphological development and allometric growth patterns in hatcheryreared California halibut larvae. *Journal of Fish Biology* **61**, 1217–1229. <https://doi.org/10.1111/j.1095-8649.2002.tb02466.x>
- Henning, F. & Meyer, A. (2014). The evolutionary genomics of cichlid fishes: explosive speciation and adaptation in the postgenomic era. *Annual Review of Genomics and Human Genetics* **15**, 1–516. <https://doi.org/10.1146/annurev-genom-090413-025412>
- Hilsdorf, A. W. S., Penman, D. J., Farias, E. C. & McAndrew, B. (2002). Melanophore appearance in wild tilapia embryos. *Pigment Cell & Melanoma Research* **15**, 57–61. <https://doi.org/10.1034/j.1600-0749.2002.00058.x>
- Holden, K. K. & Bruton, M. N. (1994). The early ontogeny of the southern mouth-brooder, *Pseudocrenilabrus philander* (Pisces, Cichlidae). *Environmental Biology of Fishes* **41**, 311–329. <https://doi.org/10.1007/BF02197852>

- Huxley, J. S. (1950). Relative growth and form transformation. *Proceedings of the Royal Society B* **137**, 465–469.
- Iwamatsu, T. (2004). Stages of normal development in the medaka *Oryzias latipes*. *Mechanisms of Development* **121**, 605–618. <https://doi.org/10.1016/j.mod.2004.03.012>
- Kamler, E. & Keckeis, H. (2000). Reproduction and early life history of *Chondrostoma nasus*: implications for recruitment. *Polski Archive Hydrobiologie* **47**, 73–85 (a review).
- Keenleyside, M. H. A. (1991). Parental care. In *Cichlid Fishes: Behavior, Ecology and Evolution* (Keenleyside, M. H. A., ed), pp. 191–208. London: Chapman & Hall.
- Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics* **203**, 253–310. <https://doi.org/10.1002/aja.1002030302>
- Konings, A. (2001). *Malawi Cichlids in their Natural Habitat*, 3rd edn. El Paso, TX: Cichlid Press.
- Kornfield, I. & Smith, P. F. (2000). African cichlid fishes: model systems for evolutionary biology. *Annual Review of Ecology and Systematics* **31**, 163–196. <https://doi.org/10.1146/annurev.ecolsys.31.1.163>
- Korzelecka-Orkisz, A., Szalast, Z., Pawlos, D., Smaruj, I., Tański, A., Szulc, J. & Formicki, K. (2012). Early ontogenesis of the angelfish, *Pterophyllum scalare* Schultze 1823 (Cichlidae). *Neotropical Ichthyology* **10**, 567–576.
- Koumoundouros, G., Divanach, P. & Kentouri, M. (1999). Ontogeny and allometric plasticity of *Dentex dentex* (Osteichthyes: sparidae) in rearing conditions. *Marine Biology* **135**, 561–572. <https://doi.org/10.1007/s002270050657>
- Kratochwil, C. F. & Meyer, A. (2015). Closing the genotype – phenotype gap: emerging technologies for evolutionary genetics in ecological model vertebrate systems. *Bioessays* **37**, 213–226. <https://doi.org/10.1002/bies.201400142>
- Kratochwil, C. F., Sefton, M. M. & Meyer, A. (2015). Embryonic and larval development in the Midas cichlid fish species flock (*Amphilophus* spp.): a new evo-devo model for the investigation of adaptive novelties and species differences. *BMC Developmental Biology* **15**, 12. <https://doi.org/10.1186/s12861-015-015-0>
- Kupren, K., Ska, M. P., Źarski, D., Krejszeff, S. & Kucharczyk, D. (2014). Early development and allometric growth in *Nannacara anomala* Regan 1905 (Perciformes: Cichlidae) under laboratory conditions. *Neotropical Ichthyology* **12**, 659–665. <https://doi.org/10.1590/1982-0224-20130104>
- Lasker, R., Feder, H. M., Theilacher, G. H. & May, R. C. (1970). Feeding, growth and survival of *Engraulis mordax* larvae reared in the laboratory. *Marine Biology* **5**, 345–353. <https://doi.org/10.1007/BF00346901>
- Lee, M. N., Ye, C., Villanim, A. C., Raj, T., Li, W., Eisenhaure, T. M., Imboywa, S. H., Chipendo, P. I., Ran, F. A., Slowikowski, K., Ward, L. D., Raddassi, K., McCabe, C., Lee, M. H., Frohlich, I. Y., Hafler, D. A., Kellis, M., Raychaudhuri, S., Zhang, F., Stranger, B. E., Benoist, C. O., De Jager, P. L., Regev, A. & Hacohen, N. (2014). Common genetic variants modulate pathogen-sensing responses in human dendritic cells. *Science* **343**, 1246980. <https://doi.org/10.1126/science.1246980>
- Leis, J. M. & Trnski, T. (1989). *The Larvae of Indo-Pacific Shorefishes*. Sydney: New South Wales University Press.
- Lopes, T. M., Oliveira, F. G., Bialetzki, A. A. & Agostinho, A. (2015). Early development in the mouth-brooding cichlid fish *Satanoperca pappaterra* (Perciformes: Cichlidae). *Revista de Biologia Tropical* **63**, 139–153.
- van Maaren, C. C. & Daniels, H. V. (2000). A practical guide to the morphological development of southern flounder, *Paralichthys lethostigma*, from hatch through metamorphosis. *Journal of Applied Aquaculture* **10**, 1–9. [https://doi.org/10.1300/J028v10n02\\_01](https://doi.org/10.1300/J028v10n02_01)
- Maciel, C. M. R. R., Lanna, E. A. T. L., Maciel, A. Jr., Donzele, J. L., Neves, C. A. & Menin, E. (2010). Morphological and behavioral development of the piracanjuba larvae. *Revista Brasileira de Zootecnia* **39**, 961–970. <https://doi.org/10.1590/S1516-35982010000500004>
- Meijide, F. J. & Guerrero, G. A. (2000). Embryonic and larval development of a substrate-brooding cichlid *Cichlasoma dimerus* (Heckel 1840) under laboratory conditions. *Journal of Zoology* **252**, 481–493. <https://doi.org/10.1111/j.1469-7998.2000.tb01231.x>

- Meyer, A. (1986). Changes in behavior with increasing experience with a novel prey in fry of the Central American cichlid, *Cichlasoma managuense* (Teleostei: Cichlidae). *Behaviour* **98**, 145–167.
- Meyer, A. (1987). Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* **41**, 1357–1369.
- Meyer, A. (1988). Plasticity in morphology and performance in the trophically polymorphic cichlid fish *Cichlasoma citrinellum*. PhD Thesis, University of California, Berkeley, CA.
- Meyer, A. (1993). Phylogenetic relationships and evolutionary processes in east-African cichlid fishes. *Trends in Ecology and Evolution* **8**, 279–284. [https://doi.org/10.1016/0169-5347\(93\)90255-N](https://doi.org/10.1016/0169-5347(93)90255-N)
- Meyer, A., Kocher, T. D., Basasibwaki, P. & Wilson, A. C. (1990). Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**, 550–553. <https://doi.org/10.1038/347550a0>
- Meyer, A., Kocher, T. D. & Wilson, A. C. (1991). African fishes – a replay. *Nature* **351**, 467–468.
- Morrison, C. M., Miyake, T. & Wright, J. R. Jr. (2001). Histological study of the development of the embryo and early larva of *Oreochromis niloticus* (Pisces: Cichlidae). *Journal of Morphology* **247**, 172–195. [https://doi.org/10.1002/1097-4687\(200102\)247:2<172::AID-JMOR1011>3.0.CO;2-H](https://doi.org/10.1002/1097-4687(200102)247:2<172::AID-JMOR1011>3.0.CO;2-H)
- Müller, U. K. & Videler, J. J. (1996). Inertia as a ‘safe harbour’: do fish larvae increase length growth to escape viscous drag? *Reviews in Fish Biology and Fisheries* **6**, 353–360.
- Muschick, M., Barluenga, M., Salzburger, W. & Meyer, A. (2011). Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evolutionary Biology* **11**, 116. <https://doi.org/10.1186/1471-2148-11-116>
- Noakes, D. L. G. (1991). Ontogeny of behaviour in cichlids. In *Cichlid Fishes: Behavior, Ecology and Evolution* (Keenleyside, M. H. A., ed), pp. 209–224. London: Chapman & Hall.
- Nogueira, L. B., Godinho, A. L. & Godinho, H. P. (2014). Early development and allometric growth in hatcheryreared characin *Brycon orbignyanus*. *Aquaculture Research* **45**, 1004–1011.
- Osse, J. V. M. & van den Boogaart, J. G. M. (1995). Fish larvae, development, allometric growth and aquatic environment. *ICES Marine Science Symposium* **201**, 21–34.
- Osse, J. W. M. & van den Boogaart, J. G. M. (1999). Dynamic morphology of fish larvae, structural implications of friction forces in swimming, feeding and ventilation. *Journal of Fish Biology* **55**, 156–174. <https://doi.org/10.1111/j.1095-8649.1999.tb01053.x>
- Osse, J. W. M. & van den Boogaart, J. G. M. (2004). Allometric growth in fish larvae: timing and function. In: *The Development of Form and Function in Fishes and the Question of Larval Adaptation* (by J.J. Govoni), pp. 167–194. American Fisheries Society, Symposium 40, Bethesda, MD: American Fisheries Society.
- Osse, J. W. M., van den Boogaart, J. G. M., van Snick, G. M. J. & van der Sluys, L. (1997). Priorities during early growth of fish larvae. *Aquaculture* **155**, 249–258. [https://doi.org/10.1016/S0044-8486\(97\)00126-9](https://doi.org/10.1016/S0044-8486(97)00126-9)
- Pena, R. & Dumas, S. (2009). Development and allometric growth patterns during early larval stages of the spotted sand bass *Paralabrax maculatofasciatus* (Percoidei: Serranidae). *Scientia Marina* **73S1**, 183–189. <https://doi.org/10.3989/scimar.2009.73s1183>
- Pena, R., Dumas, S., Ortíz-Galindo, J. L. & Villalejo-Fuerte, M. (2003). Ontogenetic development of the digestive tract in reared spotted sand bass *Paralabrax maculatofasciatus* larvae. *Aquaculture* **219**, 633–644. [https://doi.org/10.1016/S0044-8486\(02\)00352-6](https://doi.org/10.1016/S0044-8486(02)00352-6)
- Radtke, R. L., Kinzie, R. A. & Shafer, D. J. (2001). Temporal and spatial variation in length of larval life and size at settlement of the Hawaiian amphidromous goby *Lentipes concolor*. *Journal of Fish Biology* **59**, 928–938. <https://doi.org/10.1111/j.1095-8649.2001.tb00162.x>
- Richards, W. J. & Leis, J. M. (1984). Labroidei: development and relationships. In *Ontogeny and Systematics of Fishes* (Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W. & Richardson, S. L., eds), pp. 542–547. Lawrence, KS: Allen Press. American Society of Ichthyologists and Herpetologists Special Publication 1

- Salzburger, W. & Meyer, A. (2004). The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften* **91**, 277–290. <https://doi.org/10.1007/s00114-004-0528-6>
- Sanches, P. V., Nakatani, K. & Bialecki, A. (1999). Morphological description of the developmental stages of *Parauchenipterus galeatus* (Linnaeus, 1766) (Siluriformes, Auchenipteridae) on the floodplain of the upper Parana river. *Revista Brasileira de Biologia* **59**, 429–438. <https://doi.org/10.1590/S0034-71081999000300007>
- Sargent, R. C. (1997). Parental care. In *Behavioural Ecology of Teleost Fishes* (Godin, J.-J. J., ed), pp. 292–315. Oxford: Oxford University Press.
- Sato, Y. N., Fenerich-Verani, A. P. O., Nuner, H. P. & Godinho, J. R. (2003). Padões reprodutivos de peixes da bacia do São Francisco. In *Águas, Peixes e Pescadores do São Francisco das Minas Gerais* (Godinho, H. P. & Godinho, A. L., eds), pp. 224–268. Belo Horizonte: PUC Minas.
- Schneider, R. F. & Meyer, A. (2017). How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. *Molecular Ecology* **26**, 330–350. <https://doi.org/10.1111/mec.13880>
- Seehausen, O. (2006). African cichlid fish: a model system in adaptive radiation research. *Proceedings of the Royal Society B* **273**, 1987–1998. <https://doi.org/10.1098/rspb.2006.3539>
- Shine, R. (1978). Propagule size and parental care: the “safe harbor” hypothesis. *Journal of Theoretical Biology* **75**, 417–424.
- Shingleton, A. W., Frankino, W. A., Flatt, T., Nijhout, H. F. & Emlen, D. J. (2007). Size and shape: the developmental regulation of static allometry in insects. *Bioessays* **29**, 536–548. <https://doi.org/10.1002/bies.20584>
- Simonovic, P. D., Garner, P., Eastwood, E. A., Kovac, V. & Copp, G. H. (1999). Correspondence between ontogenetic shifts in morphology and habitat use in minnow *Phoxinus phoxinus*. *Environmental Biology of Fishes* **56**, 117–128. [https://doi.org/10.1007/978-94-017-3678-7\\_9](https://doi.org/10.1007/978-94-017-3678-7_9)
- van Snik, G. M. J., van den Boogaart, J. G. M. & Osse, J. W. M. (1997). Larval growth patterns in *Cyprinus carpio* and *Clarias gariepinus* with attention to finfold. *Journal of Fish Biology* **50**, 1339–1352. <https://doi.org/10.1111/j.1095-8649.1997.tb01657.x>
- Snoeks, J. (2000). How well known is the ichthyodiversity of the large East African lakes? *Advances in Ecological Research* **31**, 17–38. [https://doi.org/10.1016/S0065-2504\(00\)31005-4](https://doi.org/10.1016/S0065-2504(00)31005-4)
- Stiassny, M. L. J. & Meyer, A. (1999). Cichlids of the rift lakes. *Scientific American* **280**, 64–69.
- Strathmann, R. R. (1986). What controls the type of larval development? *Bulletin of Marine Science* **39**, 616–622.
- Sturmbauer, C. & Meyer, A. (1992). Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature* **359**, 578–581.
- Swearer, S. E., Caselle, J. E., Lea, D. W. & Warner, R. R. (1999). Larval retention and recruitment in an island population of a coral-reef fish. *Nature* **402**, 799–802. <https://doi.org/10.1038/45533>
- Taborsky, B. (2006a). Mothers determine offspring size in response to own juvenile growth conditions. *Biology Letters* **2**, 225–228.
- Taborsky, B. (2006b). The influence of juvenile and adult environments on life-history trajectories. *Proceedings of the Royal Society B: Biological Sciences* **273**, 741–750.
- Turner, G. F. (2007). Adaptive radiation of cichlid fish. *Current Biology* **17**, R827–R831. <https://doi.org/10.1016/j.cub.2007.07.026>
- Turner, G. F., Seehausen, O., Knight, M. E., Allender, C. J. & Robinson, R. L. (2001). How many cichlid fishes are there in African Lakes? *Molecular Ecology* **10**, 793–806. <https://doi.org/10.1046/j.1365-294x.2001.01200.x>
- Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. (2003). Origin of the cichlid fishes from Lake Victoria, East Africa. *Science* **300**, 325–329. <https://doi.org/10.1126/science.1080699>