

Neglected Patterns of Variation in Phenotypic Plasticity: Age- and Sex-Specific Antipredator Plasticity in a Cichlid Fish

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ABSTRACT: The ability of organisms to plastically respond to changing environments is well studied. However, variation in phenotypic plasticity during ontogeny is less well understood despite its relevance of being an important source of phenotypic variation in nature. Here, we comprehensively study ontogenetic variation in morphological antipredator plasticity across multiple traits in *Pelvicachromis taeniatus*, a western African cichlid fish with sexually dimorphic ornamentation. In a split-clutch design, fish were raised under different levels of perceived predation risk (conspecific alarm cues or distilled water). Morphological plasticity varied substantially across ontogeny: it was first observable at an early juvenile stage where alarm cue-exposed fish grew faster. Subsequently, significant plasticity was absent until the onset of sexual maturity. Here, alarm cue-exposed males were larger than control males, which led to deeper bodies, longer dorsal spines, larger caudal peduncles, and increased eye diameters. Sexual ornamentation emerged delayed in alarm cue-exposed males. In later adulthood, the plastic responses receded. Despite small effect sizes, these responses represent putative adaptive plasticity, as they are likely to reduce predation risk. In females, we did not observe any plasticity. In accordance with theory, these results suggest fine-tuned expression of plasticity that potentially increases defenses during vulnerable developmental stages and reproductive output.

Keywords: *Pelvicachromis taeniatus*, *Pelvicachromis kribensis*, alarm cues, predation risk, ontogenetic plasticity, morphology.

Introduction

Adaptive phenotypic plasticity allows genotypes to express different phenotypes dependent on environmental conditions (West-Eberhard 2003). Ontogenetic processes may substantially influence phenotypic plasticity, which represents an important source of phenotypic variation. For example, different selection pressures among ontogenetic stages may favor age-specific phenotypic plasticity (Wright and McCon-

naughay 2002). Moreover, developmental constraints limit the plastic expression of an optimal phenotype at any given time (DeWitt et al. 1998; Auld et al. 2010; Murren et al. 2015). During ontogeny, different plastic traits may either be correlated with each other, as predicted by the pace-of-life syndrome hypothesis (Réale et al. 2010), or develop independently, leading to different relationships between traits among ontogenetic stages (DeWitt et al. 1999; DeWitt and Scheiner 2004; Van Kleunen and Fischer 2005). Hence, even under constant environmental conditions during ontogeny, the degree and direction of phenotypic plasticity are not necessarily expressed evenly throughout individual development. Instead, plastic responses can be present only at certain ontogenetic stages or their strength may differ between ontogenetic stages. Therefore, the common approach to measure plasticity by assessing certain phenotypes in different environments only at a single point in ontogeny may greatly over- or underestimate the degree of plasticity present in nature, which may lead to incorrect assumptions about plasticity and may distort our view of the ability of organisms to cope with environmental change (sensu Wright and McConnaughay 2002). Only a few studies have investigated ontogenetic changes in the plasticity of single traits (Pigliucci 1997; Hjelm et al. 2001; Koumoundouros et al. 2001; Ostrowski et al. 2002; Ruell et al. 2013; Nilsson-Örtman et al. 2015). Comprehensive studies that consider many plastic traits concurrently throughout ontogeny remain scarce.

Age-specific plasticity theory predicts that adaptive plasticity has two peaks (Fischer et al. 2014); due to strong viability selection, one is during early life, where organisms are most susceptible to environmental change (e.g., Sullivan 1989; Blaustein et al. 2005; Dybala et al. 2013). Also, at early life stages, the first information about the environment has been collected by young organisms, which is a basic requirement for adaptive plasticity to occur (Fischer et al. 2014). Afterward, fecundity selection leads to a second peak shortly before reproduction when more information about variation in the environment is accumulated and plastic responses during sexual selection can increase reproductive output. Af-

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ter the reproductive period, plasticity is assumed to decline with increasing age (Dufty et al. 2002; Fischer et al. 2014). This is because developmental constraints canalize phenotypes with increasing age (epiphenotype problem; DeWitt et al. 1998) as a result of weaker selection for plasticity at later developmental stages due to decreased reproductive output (Callahan et al. 2008).

Predation is an important, variable selective force (Lima and Dill 1990; Lima 1998; Sih et al. 2000). Accordingly, predator-induced phenotypic plasticity is widespread in prey animals (Bourdeau and Johansson 2012). Because predator-induced mortality is often most pronounced for certain age and/or size classes (Charlesworth 1980), predation risk varies substantially among ontogenetic stages, which may favor the evolution of age-specific antipredator plasticity. However, comprehensive studies focusing on antipredator plasticity and its consequences throughout ontogeny are scarce (Hoverman et al. 2005; Kishida et al. 2010; Auld et al. 2011). At juvenile life stages, animals are particularly prone to predation, which favors the development of plastic antipredator responses (Crowl and Covich 1990; Reznick et al. 1996; Sommer 2000), whose adaptive benefits are well known (Nilsson et al. 1995; Urban 2007; Weber et al. 2012). In contrast, the extent of morphological antipredator plasticity during adult developmental stages is only poorly understood. For example, the development of conspicuous sexual ornaments may have viability costs because it increases predation risk (Zuk and Kolluru 1998; Godin and McDonough 2003; Stuart-Fox et al. 2003; Husak et al. 2006; Roberts et al. 2007). Accordingly, one study has suggested that under predation, sexual ornament development is plastically delayed (Ruell et al. 2013). Furthermore, sexual ornament development requires a reallocation of limited resources, which is traded off against investment into other traits (Zahavi 1975; Kodric-Brown and Brown 1984; Zeh and Zeh 1988; Winemiller 1992; Baldauf et al. 2014). Similarly, morphological antipredator plasticity in one trait may affect the expression of other traits due to genetic or phenotypic links as well as resource trade-offs (Stoks et al. 2006; Selden et al. 2009; Kishida et al. 2010), which makes trade-offs between sexual ornament development and morphological antipredator traits likely, but this topic has also received little attention.

The predation context also appears well suited for investigating sex-specific plasticity, as in many species predation risk often differs between sexes (Andersson 1994; Pocklington and Dill 1995; Reznick et al. 1996; Sommer 2000; Christe et al. 2006), which is predicted to generate sex-specific morphological antipredator plasticity (Gosline and Rodd 2008; Välimäki and Herczeg 2012) and which in turn may affect the strength as well as the direction of sexual selection (Andersson 1982, 1994). Previous studies on sex-specific antipredator plasticity were conducted in animal species with traditional sex roles (males are more conspicuous than females).

However, in mutual mate choice systems where conspicuous ornamentation can evolve in both sexes (Kokko and Johnstone 2002), it remains unknown to which extent predation risk influences the development of sexual ornaments in both males and females.

In this study, we aim to conduct a comprehensive study on variation in plasticity by studying morphology including sexual ornaments throughout ontogeny in fish raised in constant environments that differ in the level of perceived predation risk. In fishes, morphological changes induced by predation risk are well known (Brönmark and Miner 1992; Bourdeau and Johansson 2012; Ruell et al. 2013). For our study, we use the western African river cichlid *Pelvicachromis taeniatus*, a well-established fish model system with sexual dimorphism (Baldauf et al. 2009, 2011) and mutual mate choice (Thünken et al. 2007). We raised *P. taeniatus* in a split-clutch design in which fish were continuously exposed to chemical stimuli, generating different levels of perceived predation risk. Specifically, we exposed experimental fish to either (a) conspecific alarm cues or (b) control conditions. Alarm cues are known to be a primary factor inducing antipredator phenotypic plasticity among diverse taxa (Stabell and Lwin 1997; Laforsch et al. 2006; Chivers et al. 2008, 2012). Over a period of 2 years, ontogenetic changes were regularly documented by photographs. As predicted by age-dependent plasticity theory, we hypothesized that in *P. taeniatus* morphological antipredator plasticity should be particularly pronounced during the youngest developmental stage and shortly before reproduction takes place. Accordingly, we expected to find common plastic antipredator defenses such as increased growth and increased morphological defenses at these developmental stages. Furthermore, we predicted to find less conspicuous ornamentation (i.e., delayed color development) in adult fish exposed to high perceived predation risk.

Methods

Study Species

Pelvicachromis taeniatus is a stream-dwelling, cave-breeding, and socially monogamous cichlid from western African countries such as Nigeria, Benin, and Cameroon (Lamboj 2004). For populations from Cameroon, *Pelvicachromis kribensis* was recently suggested as a revalidated species name (Lamboj 2014). Juveniles of this species are cryptically colored and form shoals (Meuthen et al. 2016b), whereas adult fish feature a conspicuous sexual dimorphism and dichromatism; males are larger and colored differently than females (Baldauf et al. 2009, 2011). As adults, male *P. taeniatus* are territorial and occupy a breeding cave, while females are free swimming and selecting among males (Lamboj 2004). During their complex mutual mate choice, both body size and color orna-

ment intensity are relevant (Baldauf et al. 2011, 2013; Thünken et al. 2012). *Pelvicachromis taeniatus* is sensitive to conspecific alarm cues, which induce behavioral antipredator responses in both juveniles and adults (Meuthen et al. 2014, 2016a, 2016b).

We collected 60 adult *P. taeniatus* from the Moliwe River, Cameroon (4°4'N, 9°16'E), in June 2007. Pairs were then randomly bred at the Institute for Evolutionary Biology and Ecology, University of Bonn. The F1 generation was raised in sibling groups to maturity and subsequently used as parental stock for the current experiments (mean body size \pm SD of breeding fish; males: 7.45 \pm 0.50 cm; females: 5.23 \pm 0.21 cm). Random pairs from different families (to generate outbred clutches) were formed and bred in 50 \times 30 \times 30-cm (length \times width \times height) tanks containing an artificial breeding cave. Eggs were immediately removed on deposition (see below). We continued sampling for 6 months until 12 pairs reproduced with sufficiently large clutches. In one case, we collected two clutches of the same pair (which are thus full siblings), resulting in a total of 13 clutches that were used for the experiments.

Rearing and Documentation Protocol

Each clutch was split into two equally sized groups (13–32 eggs each), and each group was incubated in a 15.5 \times 9 \times 11-cm tank containing an airstone for oxygen supply. Eighty percent of water was replaced with fresh water daily. From hatching onward, siblings were subjected to different levels of perceived predation risk (see below). This split-clutch design controlled for genetic variation while investigating environmentally induced effects (i.e., morphological antipredator plasticity). After passing through the wriggler stage (the first stage after hatching, which is ubiquitous in cichlids; see Barlow 2000) and entering the free-swimming stage, the amount of fry (seven to 28 fry, dependent on egg number) was matched between treatments with a maximum number of 10 fry per tank. When one of the treatment groups of a single family contained less than 10 fry, we reduced the number of fish in all other treatment groups of the same family to the same amount. In the cases where more than 10 fry were available, we created multiple replicates of the same treatment/family combination in different tanks. Tank size was sequentially increased to conform to the increased space requirements of growing fish (age 22–220 days: 20 cm \times 30 cm \times 20 cm; age 220–514 days: 50 cm \times 30 cm \times 30 cm), and 30% water changes were conducted fortnightly. We matched food amounts to fish number and sequentially increased them during ontogeny so as to conform to increasing nutritional requirements, as morphological antipredator responses may be limited by nutrient availability (Chivers et al. 2008): 8–13 days: 10 μ L/fish; 22–27 days: 20 μ L/fish; 50–55 days: 40 μ L/fish; 78–83 days: 60 μ L/fish; 115–122 days:

80 μ L/fish; 150–157 days: 100 μ L/fish; 185–192 days: 120 μ L/fish; 220–227 days: 140 μ L/fish; 255–262 days: 160 μ L/fish; 297–304 days: 180 μ L/fish; 339–346 days: 200 μ L/fish. At first, food consisted of *Artemia* nauplii exclusively; at the transition to 80 μ L, it was replaced by a mix of frozen adult *Artemia* sp. and *Chironomus*, *Culex*, and *Chaoborus* larvae in a ratio of 2:1:0.25:1. Throughout rearing, fish in different tanks had no visual or olfactory contact, water temperature was 24.5° \pm 1.5°C, and illumination was provided by full-spectrum fluorescent tubes (Lumilux Cool Daylight 36W/865; Osram, Munich, Germany) in a 12L:12D light cycle (from 8 a.m. to 8 p.m.).

Perceived Predation Risk Treatment

The two groups split from each clutch were exposed to two different chemical stimuli 5 days a week: alarm cues derived from conspecifics (CON) or distilled water (DW). Conspecific alarm cues are a well-established signal for predator presence (Chivers and Smith 1998; Chivers et al. 2012), the mechanism by which prey animals learn the risk associated with previously unknown cues (Chivers and Smith 1994a, 1994b) as well as one of the major factors in inducing antipredator phenotypic plasticity (Stabell and Lwin 1997; Laforch et al. 2006). Instead, other predator-related cues such as predator odors often have to be learned beforehand (Berejikian et al. 1999; Wisenden 2000), and unlike alarm cues, fish habituate to them (Imre et al. 2016). Distilled water was applied to control for possible effects of frequent water disturbance (as by the introduction of alarm cues).

We produced conspecific alarm cues from adult F1 and F2 lab-bred donor *P. taeniatus*. Previous studies revealed that in cichlids, alarm cues derived from adults do not elicit qualitatively different behavioral responses than those obtained from juvenile fish (Brown et al. 2004). All fish were previously starved for 2 days to exclude diet effects. Alarm cues always originated from four males and four females, controlling for individual and sex effects. Donor fish were euthanized with a blow to the head followed by cervical dislocation in accordance with §4, §8b, and §9(2) of the German animal welfare act (BGB I. I S. 1207, 1313). Whole fish were ground in a mortar, which ruptured cells and released alarm cues. By using whole fish, we considered that additional cichlid alarm cues may be located outside the skin (e.g., Barreto et al. 2013). Homogenates were diluted with distilled water, passed through filter floss, and frozen in 1-mL aliquots at -20°C until use. Consequently, fish were exposed to 1 mL obtained from 7.2 mg (approximately 0.028 cm² skin; until day 22–27) or 43.2 mg (approximately 0.167 cm² skin; from day 22–27 onward due to the higher volume of subsequent rearing tanks) donor fish wet body mass. Similar conspecific alarm cue concentrations elicit clear behavioral and morphological antipredator responses in *P. taeniatus* (Meu-

then et al. 2014, 2016a, 2016b), in several cichlid species (Roh et al. 2004; Pollock et al. 2005; Abate et al. 2010), and in other fish taxa (Chivers and Smith 1994a). Likewise, we prepared 1-mL aliquots of distilled water. To apply treatments, aliquots were thawed and introduced in the respective tanks.

Photographic Documentation

Fish were photographed at six different time points that constitute regular intervals over ontogeny, including three juvenile and three adult stages (fig. 1). In our fish, sex-specific sexual ornaments were expressed for the first time at the fourth developmental stage (213–220 days), which indicates imminent reproduction in *P. taeniatus*. Hence, we refer to this stage as the onset of sexual maturity.

The setup differed between juvenile and adult fish, as 7-mm fry and 100-mm adults cannot be accurately photographed with the same setup. Juveniles were photographed in water-filled, distortion-free, and orthochromatic quartz glass cuvettes selected according to fish size (100-OS [outer dimensions 1.25 cm × 0.5 cm × 4.4 cm, inner dimensions 0.95 cm × 0.5 cm × 4.4 cm] at age 22–27 and 50–55 days; 6030-OG [outer dimensions 1.25 cm × 1.25 cm × 4.5 cm, inner dimensions 0.95 cm × 0.95 cm × 3.5 cm] and 402.000-OG [outer dimensions 4.0 cm × 2.35 cm × 1.5 cm, inner dimensions 3.8 cm × 1.85 cm × 1.3 cm] at age 108–115 days; Hellma, Müllheim, Germany). Cuvettes with fish were placed inside a photobox with standardized illumination provided by two 16-W LED lamps (LDRC1665WE7EUD, 32°, 6500 K; Toshiba, Tokyo) from 45 cm above (light incidence in a 90° angle with the fish). White and size standards were placed close to the cuvette. Multiple pictures per individual were then taken with a digital camera (Nikon D5000 with AF-S Micro Nikkor 105-mm 1:28G macro objective) in RAW format.

Pictures of adult fish were taken inside of a 20 × 30 × 20-cm tank under standardized illumination provided by the same lamps as mentioned before being positioned in a distance of 20 cm at a height of 24 cm (light incidence in a 45° angle with the fish). Fish were held into place within a 9 × 1 × 9-cm central area using a perforated plastic pane with attached sponge stripes. White and size standards were placed inside the water close to the fish. Pictures were taken as described above.

Fish could not be photographed at exactly the same age due to logistical reasons. Differences in age at photography were limited to a few days. However, siblings subject to different treatments were always photographed at the same age. Due to logistical reasons, we photographed a random 50% subset of fish equally distributed among sexes, families, and treatments for the last two developmental stages. In total, 30 groups of six to 10 fish from 12 different families were raised; 1,238 photographs of repeatedly sampled fish were analyzed (table 1).

Data Analysis

We measured body size (from the snout tip to the base of the tail fin) as well as 12 other morphometric distances (fig. 2) with ImageJ software (Rasband 1997–2014, National Institutes of Health, Bethesda, MD), which automatically converts digital dimensions to metric units according to size standards. We applied traditional morphometrics throughout instead of geometric morphometrics because our aim was to interpret phenotypic changes of specific traits with known fitness consequences rather than describing general body shape differences between treatments (Gianoli and Valladares 2012). Moreover, it was often difficult to reliably measure the morphometric distances across the total body within a single photograph because fish were often not plane aligned due to their sudden movements, which can confound consistent landmark placement for geometric morphometry. Although anesthetizing fish during the photographing procedure may have alleviated this issue, we did not do so, because administration of many fish anesthetics is suggested to be stressful for fish, as exemplified by increased cortisol levels, which may facilitate the development of an antipredator phenotype across treatments (Iwama et al. 1989; Thomas and Robertson 1991; Bressler and Ron 2004).

Coloration was obtained from photographs by importing them into Adobe Photoshop CS 5.1 followed by applying the white standard with the “adjust levels” function. From the many color patterns of adult *P. taeniatus*, its belly coloration (see fig. 2), which is highly variable within and among individual *P. taeniatus*, is particularly relevant during mate choice (Baldauf et al. 2011, 2013). Therefore, we obtained values describing the intensity of belly coloration, applying the uniform CIELab color space (Commission Internationale de l’Eclairage 1976). CIELab values, which separate contrast (L^*) from color (a^* , b^*), are standardized, perceptually and device independent (Chen et al. 2004), and thus widely applied to study fish coloration (Craig and Foote 2001; Svensson et al. 2005; Sköld et al. 2008). CIELab values may reflect the carotenoid concentrations underlying color patterns (Humphries et al. 2004; Shatilova 2008). In our species, the examined CIELab values (see below) match hue values from the corresponding spectral reflectance in *P. taeniatus* (D. Meuthen, unpublished data). Utilizing the color sampler tool, we obtained Lab values describing belly color intensity by averaging 12 different points (5 pixels × 5 pixels each) evenly spread over the belly. Because male bellies are distinctly yellow, we focused on the b components (higher b values represent higher intensity of yellow coloration). For females, whose bellies express a purple coloration (a composite color), we calculated a chromaticity value to include both the a value (higher positive a values represent a higher intensity of red coloration; we ignored negative a values that represent green color and thus constitute a measurement

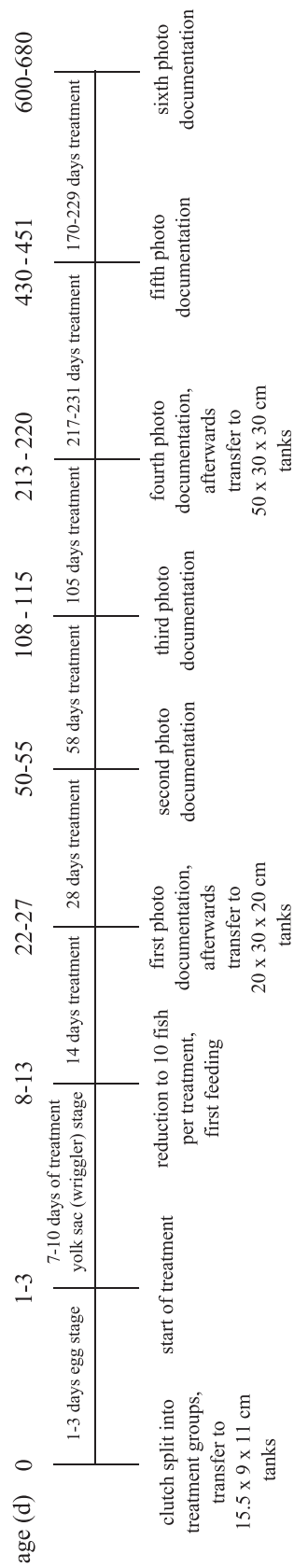


Figure 1: Timeline of the experimental procedure and data collection (inspired by Segers and Taborsky 2012).

Table 1: Sample sizes of fish in the conspecific alarm cue (CON) and the distilled water treatment (DW) at the different ontogenetic stages where they were photographically documented

Age (days)	CON		DW	
	Male	Female	Male	Female
22–27		132 (12)		130 (12)
50–55		125 (12)		123 (12)
108–115	63 (12)	59 (12)	66 (12)	54 (12)
213–220	61 (12)	57 (12)	62 (12)	51 (12)
430–451*	31 (12)	28 (12)	30 (12)	27 (12)
600–680*	36 (11)	40 (11)	31 (12)	32 (12)

Note: Asterisks indicate where sampled fish constituted a subset of available fish. Values in parentheses denote the number of families fish were derived from.

artifact occurring particularly when juvenile fish did not fully express belly coloration) and b value (increasingly negative b values represent a higher intensity of blue coloration; likewise, we ignored positive b values, as they express changes in yellow coloration, which are not present in adult female bellies) by applying the following formula according to Robertson (1977):

$$\text{chromaticity} = \sqrt{a^2 + b^2}.$$

In *P. taeniatus*, using photography has clear advantages over spectrophotometry to assess fish coloration. This is because first *P. taeniatus* rapidly changes its color under stress

(stress-based melanization) and photographs taken quickly underwater minimize stress and thus prevent a loss of color information. Moreover, spectrophotometric data and CIELab data from photographs are highly correlated for this species (see above). All data from this study are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.0p401> (Meuthen et al. 2017).

Statistical Analysis

All analyses were conducted using R 3.2.5 (R Core Team 2016). Throughout analyses, quantile-quantile plots of model

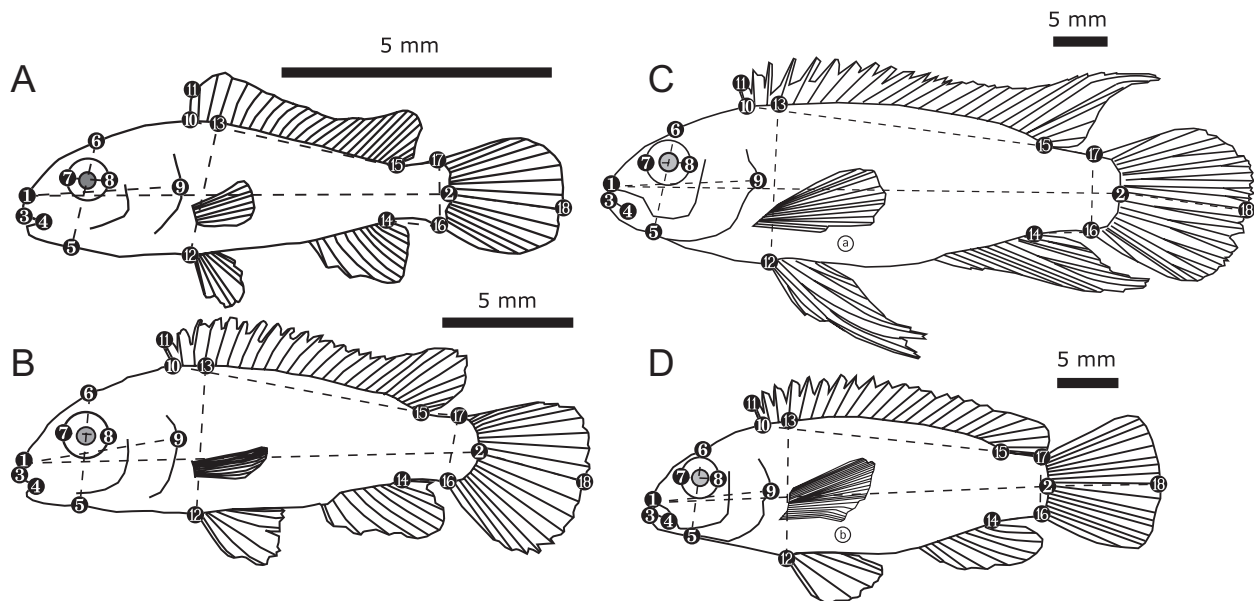


Figure 2: Twelve morphological distances (dashed lines) and two color areas in juvenile (A), subadult (B), adult male (C), and adult female (D) *Pelvicachromis taeniatus*: standard length (1–2), mouth length (3–4), head depth (5–6), eye diameter (7–8), head width (1–9), first dorsal spine length (10–11), body depth (12–13), dorsal fin base length (10–15), ventral caudal peduncle length (14–15), caudal peduncle height (16–17), dorsal caudal peduncle length (15–17), caudal fin length (2–18), male belly coloration (a), and female belly coloration (b). Size standards are displayed in the upper right of each figure.

residuals were inspected visually for deviations from normality. The variables body size and body shape met assumptions of normality throughout and were not transformed. Color variables were subjected to a Box-Cox transformation (Box and Cox 1964) for normalization.

First, fish growth patterns (the development of body size over time) follow a curved rather than a linear relationship (von Bertalanffy 1934). Hence, we fitted a predictive nonlinear mixed model using a logistic function (function *nlme* in the *nlme* package) to statistically compare growth patterns between treatments. We modeled the growth equation developed by Pütter (1920) and von Bertalanffy (1934), which was later modified by Beverton and Holt (1957):

$$y = A(1 - e^{-K(t-t_0)}).$$

This equation contains three relevant parameters for examining growth patterns: (1) the asymptote (A), which equals the body size on day 680; (2) the curvature (K), which equals the growth rate until the asymptote is reached; and (3) the x -intercept (t_0), which equals the theoretical age at body size 0. As the treatment occurred only after hatching, we set $t_0 = -16$ days for all treatments, as this value best fit the predictive curve to our data. To test for effects of perceived predation risk, we included treatment (conspecific alarm cues, distilled water) as a fixed factor on the asymptote and the curvature. Family identity was entered as a random effect (grouping factor) to account for differences in growth patterns between families (see Sofaer et al. 2013 for details). Furthermore, to account for repeated sampling of fish derived from the same families, we included a random effect of family identity on the asymptote and the curvature. Color development over time was instead appropriately described by a linear function rather than a nonlinear one. Hence, we fitted linear mixed-effect models (function *lme* from R library *nlme*) with maximum likelihood algorithms (Pinheiro and Bates 2000) to investigate differences in the rate of color development between treatments.

To account for both the split-clutch design and repeated sampling over time, we included family identity as a random intercept as well as the age of the respective developmental stage (22–680 days) as a random slope. Explanatory variables were body size (standard length), age (22–680 days), and treatment (conspecific alarm cues, distilled water). Here, we focused on analyzing interactive effects between age and treatment (age \times treatment interaction). In addition to quantifying differences caused by treatments in fish growth and coloration over time, we were also interested in whether there were differences in body size, body shape, and coloration intensity at each developmental stage. For this purpose, we ran Bayesian Markov chain Monte Carlo (MCMC) generalized linear mixed-effect models (function *mcmcglmm* from the R package *MCMCglmm*; see Hadfield 2010), which is an ef-

fective statistical approach to analyze animal morphology (Ruell et al. 2013; Seebacher et al. 2016). For all investigated variables, we compared treatments by entering treatment (conspecific alarm cues, distilled water) as a fixed factor. Except for the analysis of body size, body size (standard length) was also included as a covariate in additional analyses to control for effects of body size. Family identity was entered as a random intercept throughout to account for the split-clutch design. Analyses were run with a noninformative inverse-Wishart proper prior (Hadfield 2010) for 500,000 iterations with a burn-in of 150,000 and a thinning interval of 200, which minimized autocorrelation between samples (Hadfield 2010). Means of the fixed effects and the 95% credible intervals (CrI) were estimated using MCMC sampling of their posterior distributions, conditioned on the random effects. Fixed effects were considered significant when the estimated pMCMC value was ≤ 0.05 . The pMCMC values were calculated as two times the smaller of MCMC estimates of, first, the probability that the parameter estimate is greater than 0 and, second, the probability that the parameter estimate is less than 0 (Ruell et al. 2013). A 95% CrI that does not overlap 0 is comparable to a significant frequentist P value (Cumming and Finch 2005).

Unless otherwise stated, all tests of statistical significance were based on likelihood ratio tests (LRTs), which assessed whether the removal of a variable caused a significant decrease in model fit (Zuur et al. 2009). The P values refer to the increase in deviance when the respective variable was removed. Test probabilities are two-tailed throughout.

Results

Overall, *Pelvicachromis taeniatus* displayed age- and sex-specific morphological plasticity during its ontogeny.

Growth

In male *P. taeniatus*, treatment groups differed in the curvature but not in the asymptote of the growth equation ($n = 890$; asymptote: CON: 67.741 ± 0.763 mm, DW: 69.124 ± 0.941 mm, $\chi^2 = 1.856$, $P = .173$; curvature: CON: $2.940 \times 10^{-3} \pm 0.053 \times 10^{-3}$ days $^{-1}$, DW: $2.790 \times 10^{-3} \pm 0.069 \times 10^{-3}$ days $^{-1}$, $\chi^2 = 3.881$, $P = .049$). This was not the case for females ($n = 859$; asymptote: CON: 41.607 ± 0.461 mm, DW: 46.320 ± 0.287 mm, $\chi^2 = 0.976$, $P = .323$; curvature: CON: $5.060 \times 10^{-3} \pm 0.826 \times 10^{-3}$ days $^{-1}$, DW: $5.100 \times 10^{-3} \pm 0.698 \times 10^{-3}$ days $^{-1}$, $\chi^2 = 0.346$, $P = .556$). Fish differed in their body size and shape between treatments primarily during early development (at age 22–27 days; table 2) and in males at the onset of sexual maturity (at age 213–220 days; table 3). At all other developmental stages, morphological differences were miniscule among treatments (tables A1–A4, available online).

Table 2: Summary of the effects of treatment on body size and body shape of juvenile *Pelvicachromis taeniatus* at early development (age 22–27 days) estimated from the posterior distributions of a Bayesian multivariate generalized linear mixed model

Trait	Mean control treatment (95% CrI)	Absolute effect of conspecific alarm cues (95% CrI)	Relative effect of conspecific alarm cues (95% CrI)	Significance level (pMCMC)
Standard length (mm)	7.681 (7.481, 7.911)	+ .102 (.02, .181)	+1.322 (.265, 2.282)%	*
Third dorsal spine length (mm)	.786 (.745, .835)	+ .019 (.003, .036)	+2.444 (.376, 4.373)%	*, ns
Body depth (cm)	2.584 (2.525, 2.656)	+ .001 (−.026, .030)	+ .052 (−1.021, 1.134)%	ns, *
Dorsal caudal peduncle length (mm)	.709 (.677, .741)	+ .005 (−.012, .022)	+ .695 (−1.737, 2.961)%	ns, ns
Caudal peduncle depth (mm)	1.088 (1.061, 1.114)	+ .012 (−.001, .024)	+1.087 (−.103, 2.167)%	ns, ns
Ventral caudal peduncle length (mm)	.806 (.774, .839)	+ .01 (−.015, .034)	+1.21 (−1.968, 4.075)%	ns, ns
Eye diameter (mm)	.827 (.799, .854)	+ .01 (−.002, .019)	+1.173 (−.253, 2.243)%	ns, ns

Note: Family identity was included as a random effect. Ninety-five percent credible intervals (CrIs) are included in parentheses. Estimated posterior means are shown for juveniles continuously exposed to conspecific alarm cues. Estimated levels of significance (pMCMC) are stated for analyses without any covariate (left value) and analyses with standard length as a covariate (right value) by the following values: ns indicates that $P \geq .05$, and an asterisk indicates that $P < .05$.

At the earliest juvenile stage, *P. taeniatus* raised with conspecific alarm cues were larger than controls (fig. 3; table 2). This body size difference also led to longer dorsal spines in fish exposed to conspecific alarm cues (table 2). Moreover, conspecific alarm cues induced a greater body depth relative to body size (table 2).

Likewise, male *P. taeniatus* exposed to alarm cues were larger than controls at the onset of sexual maturity (fig. 4A, 4B; table 3). This body size difference led to longer dorsal spines, a greater body depth, a longer and deeper caudal pe-

duncle, and a greater eye diameter in males exposed to conspecific alarm cues compared to controls (table 3).

Color Development

Male *P. taeniatus* raised under different levels of perceived predation risk differed significantly in color development ($n = 380$, interaction age \times treatment, LRT, difference in slope: $5.730 \times 10^{-5} \pm 2.870 \times 10^{-5}$, $\chi^2 = 4.015$, $P = .045$), whereas females did not ($n = 349$, interaction age \times

Table 3: Summary of the effects of treatment on body size, body shape, and coloration of male *Pelvicachromis taeniatus* at the onset of sexual maturity (age 213–220 days) estimated from the posterior distributions of a Bayesian multivariate generalized linear mixed model

Trait	Mean control treatment (95% CrI)	Absolute effect of conspecific alarm cues (95% CrI)	Relative effect of conspecific alarm cues (95% CrI)	Significance level (pMCMC)
Standard length (mm)	31.837 (31.009, 32.643)	+1.330 (.371, 2.566)	+4.177 (1.196, 7.861)%	*
Third dorsal spine length (mm)	2.714 (2.629, 2.806)	+ .101 (.002, .195)	+3.735 (.070, 6.966)%	*, ns
Body depth (mm)	9.666 (9.421, 9.933)	+ .436 (.071, .802)	+4.511 (.756, 8.076)%	*, ns
Dorsal caudal peduncle length (mm)	3.356 (3.204, 3.513)	+ .164 (.008, .308)	+4.874 (.258, 8.781)%	*, ns
Caudal peduncle depth (mm)	4.556 (4.419, 4.693)	+ .221 (.039, .383)	+4.844 (.873, 8.159)%	** , ns
Ventral caudal peduncle length (mm)	3.729 (3.568, 3.922)	+ .038 (−.116, .195)	+1.017 (−3.254, 4.978)%	ns, ns
Eye diameter (mm)	2.850 (2.778, 2.921)	+ .132 (.040, .223)	+4.614 (1.432, 7.633)%	** , ns
Belly color intensity (CIE Lab <i>b</i> value)	29.661 (24.389, 34.638)	−1.467 (−3.893, 1.045)	−4.946 (−15.962, 3.017)%	ns, *

Note: Family identity was included as a random effect. Ninety-five percent credible intervals (CrIs) are included in parentheses. Estimated posterior means are shown for males continuously exposed to conspecific alarm cues. Estimated levels of significance (pMCMC) are stated for analyses without any covariate (left value) and analyses with standard length as a covariate (right value) by the following values: ns indicates that $P \geq .05$, one asterisk indicates that $P < .05$, and two asterisks indicate that $P < .01$.

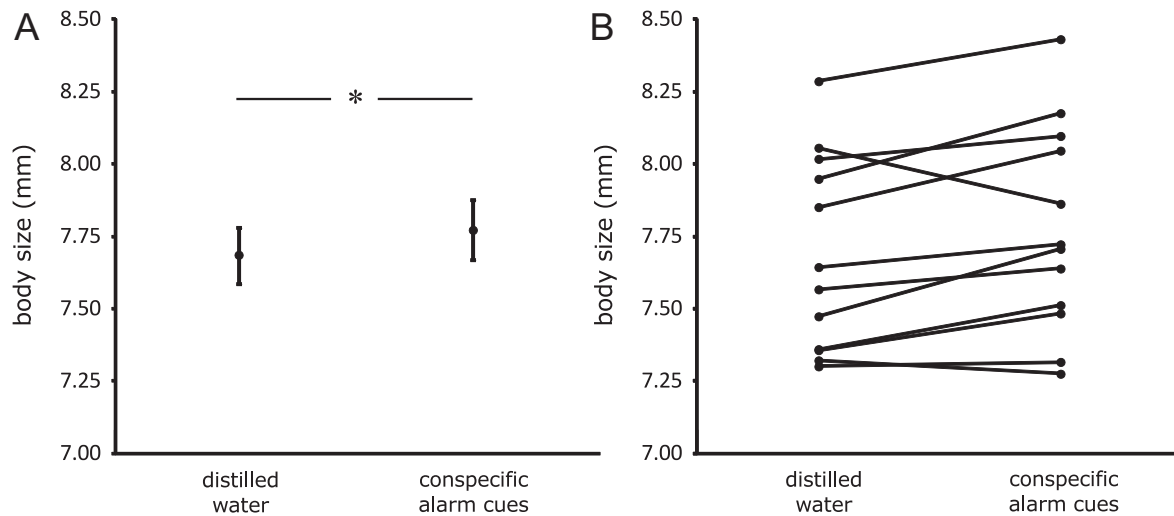


Figure 3: Body size of 22–27-day-old juvenile *Pelvicachromis taeniatus* subject to different levels of perceived predation risk: distilled water ($n = 132$) and conspecific alarm cues ($n = 130$). Shown are mean \pm SE body size of the different treatment groups (A) and reaction norms depicting the average body size of each family (B; $n = 12$) for the distilled water and conspecific alarm cue treatment. An asterisk indicates that $P < .05$.

treatment, LRT, difference in slope: $1.226 \times 10^{-4} \pm 2.737 \times 10^{-4}$, $\chi^2 = 0.200$, $P = .655$). Males from the conspecific alarm cue treatment were less colorful relative to their body size (fig. 4C, 4D; table 3).

Discussion

In our study, morphological antipredator plasticity varied across ontogeny. Despite constant environments, plastic responses were present only at the earliest developmental stage and at the onset of sexual maturity. Here, plasticity was sex-specific; juvenile and male but not female *Pelvicachromis taeniatus* displayed plastic morphological responses to the conspecific alarm cue treatment.

Plasticity was present at the first observed developmental stage shortly after birth, then absent for the two following time points, and reemerged at the onset of sexual maturity. Afterward, the plastic responses again receded. This variation across ontogeny matches the theoretical predictions of age-specific adaptive plasticity. As expected, due to strong viability selection, the first peak occurred during early life when animals first sample their environment (Fischer et al. 2014) and when animals are most susceptible to environmental change (e.g., Sullivan 1989; Blaustein et al. 2005; Dybala et al. 2013). The second peak occurred at the onset of sexual maturity, where animals have accumulated more information about variation in their environment and where they also benefit most from a plastic adjustment due to strong fecundity selection. Afterward, we did not find any significant plastic responses, suggesting that the relative costs of plastic-

ity (DeWitt et al. 1998; Auld et al. 2010; Murren et al. 2015) exceeded its benefits (Fischer et al. 2014).

The fact that the effects of plasticity appeared and disappeared across developmental stages suggests that antipredator plasticity is highly reversible even in constant environments, which is in accordance with expectations stated in other studies (Relyea 2003; Chivers et al. 2008; Kishida et al. 2010). This is because, first, the fitness benefits of antipredator plasticity are likely to vary because viability selection from predation risk is strongest at the ontogenetic stages, where we did observe plastic responses. Small juvenile (Crowl and Covich 1990; Reznick et al. 1996; Arendt 1997; Sommer 2000; Metcalfe and Monaghan 2003; Bell et al. 2011; Beston et al. 2017) and ornamented subadult animals that are not yet fully grown (Zuk and Kolluru 1998; Godin and McDonough 2003; Stuart-Fox et al. 2003; Husak et al. 2006; Roberts et al. 2007) experience the highest selection by predation risk. Thus, age-specific antipredator plasticity is theoretically expected to evolve (Wiedenmayer 2009). Second, plastic responses are usually based on resource reallocation to specific traits (Harvell 1990; Bourdeau and Johansson 2012), which leads to resource trade-offs (Walls et al. 1991; LaFiandra and Babbitt 2004; Teplitsky et al. 2005; Collier et al. 2008; Edgell and Neufeld 2008; Selden et al. 2009). For example, during fast growth, quality is sacrificed for speed, making bodies more prone to developmental errors or weaknesses (Blanckenhorn 2000) as investment into protein maintenance (Morgan et al. 2000) or the repair of molecular damage is neglected (Cichon 1997). Consequently, subsequent to a period of accelerated growth, resources need to be reallocated

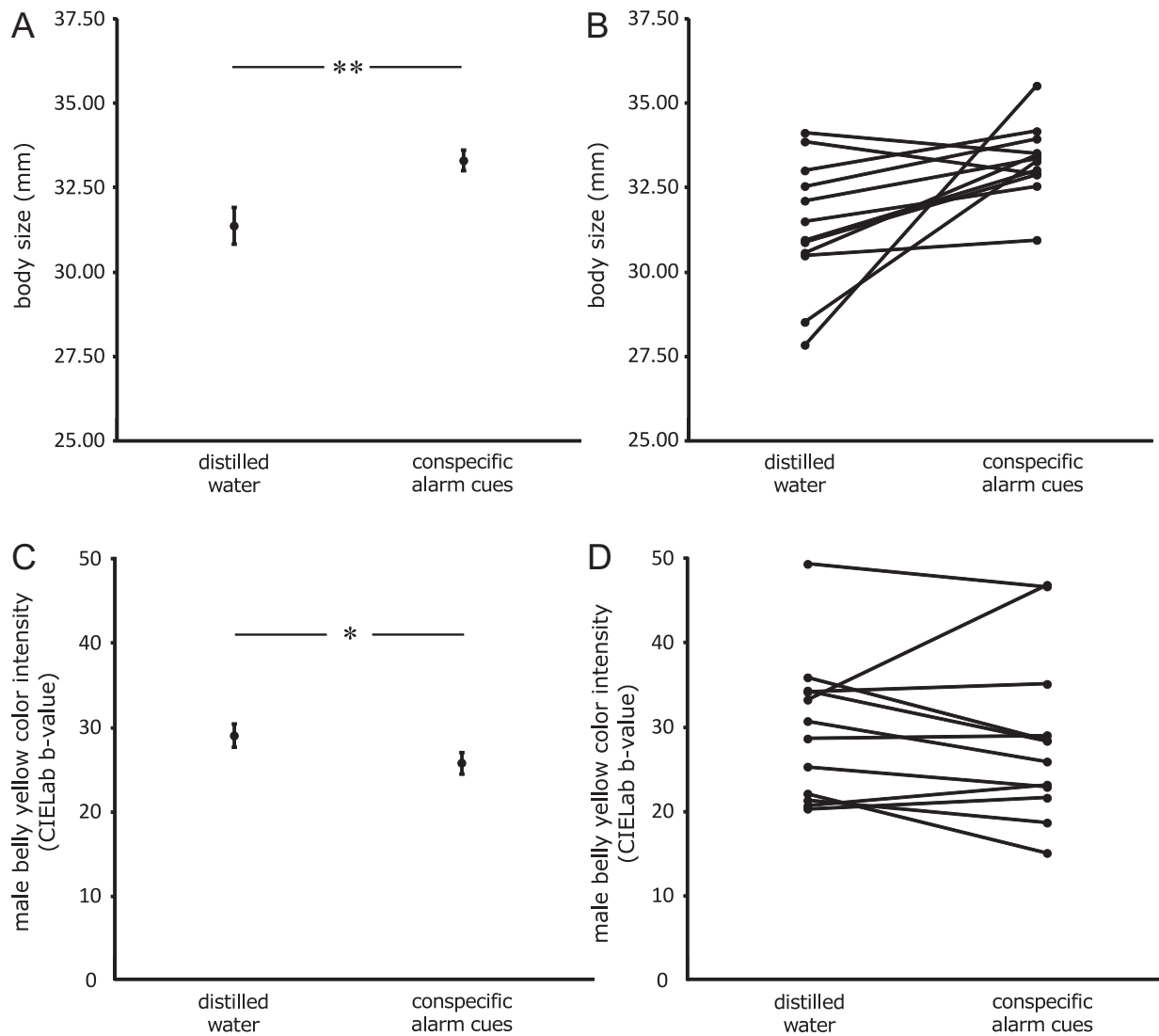


Figure 4: Body size (A, B) and belly color intensity (C, D) of 213–220-day-old adult male *Pelvicachromis taeniatus* subject to different levels of perceived predation risk: distilled water ($n = 62$) and conspecific alarm cues ($n = 61$). Shown are mean \pm SE values for the different treatment groups (A, C) and reaction norms depicting average values of each family (B, D; $n = 12$) for the distilled water and conspecific alarm cue treatment. Two asterisks indicate that $P < .01$, and one asterisk indicates that $P < .05$.

into repairing these developmental errors to allow survival rather than just growing further, which may allow nonplastic individuals periods of catch-up growth, as they do not need to reallocate resources in the same way.

We found accelerated growth in fish exposed to high perceived predation risk at an early juvenile developmental stage and in males at the onset of sexual reproduction. The observed relatively small size differences between treatments (tables 2, 3) are in accordance with other studies that report similar small effect sizes for predator-induced accelerated growth in other fish species (Vøllestad et al. 2004; Stoks et al. 2005; Bell et al. 2011; Frommen et al. 2011). By accel-

erated growth, fish effectively reduce their vulnerable period as they outgrow the limited gape of many piscivore predators (Hambright et al. 1991; Gadomski and Parsley 2005; Urban 2007). This is also successfully achieved by only small differences in body size (Bell et al. 2011). Accelerated growth usually correlates with increased nutritional requirements, which suggests that the observed size differences may be a consequence of a predator-induced increase in foraging frequency. However, in our study fish of both treatments received the same amount of nutrition, and all food provided was consumed throughout. Therefore, in our study different growth rates can clearly be attributed to different resource

allocation rather than increased energy uptake in high-risk fish, which might have occurred if food had been accessible at all times.

Accelerated growth also led to the development of deeper bodies, larger dorsal spines, a longer and deeper caudal peduncle, and increased eye diameters in juvenile and male *P. taeniatus*, suggesting that predator-related benefits of increased growth are not restricted to the benefits of greater body length alone. A deeper body and elongated dorsal spines are common morphological antipredator defenses in fish species (Brönmark and Miner 1992; Eklöv and Jonsson 2007; Januszkiewicz and Robinson 2007; Frommen et al. 2011) that increase prey handling time for predators and thus increase its chance of escape (Hoogland et al. 1956; Brönmark and Miner 1992). Long and deep caudal peduncles effectively enhance escape locomotion, which prevents capture by predators (Langerhans et al. 2004, 2007). Here, individuals may also accrue survival benefits by simply being able to attain a higher burst-swimming speed than nearby conspecifics that are then preyed on rather than being able to outrun predators on their own. A plastically increased eye size may improve visual acuity (sensu Veilleux and Kirk 2014) and thereby enhance predator detection, although it may make individuals more conspicuous to predators (Lönstedt et al. 2013).

Finally, male *P. taeniatus* exposed to high perceived predation risk displayed a delayed ornament development. Thereby, individuals become conspicuous only after they have reached a large body size and have fully expressed morphological defenses protecting them from predation. This is likely an adaptive strategy, as reduced coloration intensity has been shown to enhance survival under predation (Booth 1990; McCollum and van Buskirk 1996; Lindström et al. 2006; Woods et al. 2007; Touchon and Warkentin 2008; Ruell et al. 2013; Ercit and Gwynne 2015). However, this might come at the cost of reduced attractiveness, which might be less relevant in a predatory environment where the selectiveness of females is reduced (Breden and Stoner 1987; Stoner and Breden 1988; Houde and Endler 1990).

The fact that ornament development was delayed at the same developmental stage where we found accelerated growth may suggest a resource trade-off. As both plastically induced morphological defenses (Harvell 1990; Bourdeau and Johansson 2012) and ornament development (Zahavi 1975; Kodric-Brown and Brown 1984; Zeh and Zeh 1988; Winemiller 1992; Hooper et al. 1999) require substantial resource investment, the observed decreased ornament intensity may be a consequence of increased investment into accelerated growth.

While other studies suggest that the scope of plasticity to adapt animals to a changing environment is relatively low and thus leads to only small phenotypic shifts (van Heerwaarden et al. 2016), we are unable to conclude whether the observed relatively small effects across traits that are like-

wise present in other studies of morphological plasticity in fish (Vøllestad et al. 2004; Stoks et al. 2005; Bell et al. 2011; Frommen et al. 2011; Ab Ghani et al. 2016) are actually relevant for individual fitness. This is also because we know little about how changes in different phenotypic traits interact. Different phenotypic effects may interact additively (trait cospecialization), can compensate for each other (trait compensation), can be required to act in an integrated way to maximize effects (trait complementation), or can rely on each other to function (trait codependence; DeWitt et al. 1999). For example, alarm cue-exposed juvenile *P. taeniatus* are more sensitive to novel cues (Meuthen et al. 2016b), and such an increased attention in combination with the observed larger body size is likely to have a greater impact on survival than each of those effects on their own. Similarly, in males at the onset of sexual maturity, reduced conspicuousness caused by slightly decreased coloration intensity in addition to a larger body size may increase survival probability to a greater degree compared to the response in a single phenotypic trait alone.

Theory predicts that phenotypic plasticity can be sex-specific (Teder and Tammaru 2005; Stillwell et al. 2010; Ceballos and Valenzuela 2011). This is because the sex subject to stronger selection by environmental factors should be the more plastic sex (Lande 1980; Stillwell et al. 2010; Connallon and Clark 2014; Connallon 2015; Connallon and Hall 2016). Predators often predate more intensely on the male sex, whose activity patterns and ornamentation regularly make it the more conspicuous sex (Magnhagen 1991; Andersson 1994). This repeatedly leads to female-biased sex ratios in natural populations (Reznick et al. 1996; Sommer 2000; Koga et al. 2001; Christe et al. 2006) and the emergence of male-specific morphological plasticity (Gosline and Rodd 2008; Välimäki and Herczeg 2012). Likewise, we found morphological plasticity in response to conspecific alarm cues in male but not in female *P. taeniatus*. Similar to other species, *P. taeniatus* males actively defend their territory, while females are initially nonterritorial (Lamboj 2004). Therefore, male *P. taeniatus* reach higher activity levels than females (Meuthen et al. 2011), which may increase their conspicuousness toward predators and favor the evolution of male-specific antipredator phenotypic plasticity.

Taken together, our study on phenotypic plasticity across ontogeny and sexes suggests that phenotypic plasticity is not constantly expressed but rather present only at critical developmental stages—during early life and at the onset of sexual maturity in one sex. However, further studies are needed to confirm whether this pattern of variation is comparable across different environmental factors and taxa. Only then can we correctly assess the degree of plasticity present in nature and derive comprehensive conclusions regarding the evolutionary significance of phenotypic plasticity (sensu Wright and McConnaughay 2002). Furthermore, our study also high-

lights the fact that assessing plasticity at early development as well as at the onset of sexual maturity may reveal the maximum expression of phenotypic plasticity and therefore be particularly worthwhile for future studies.

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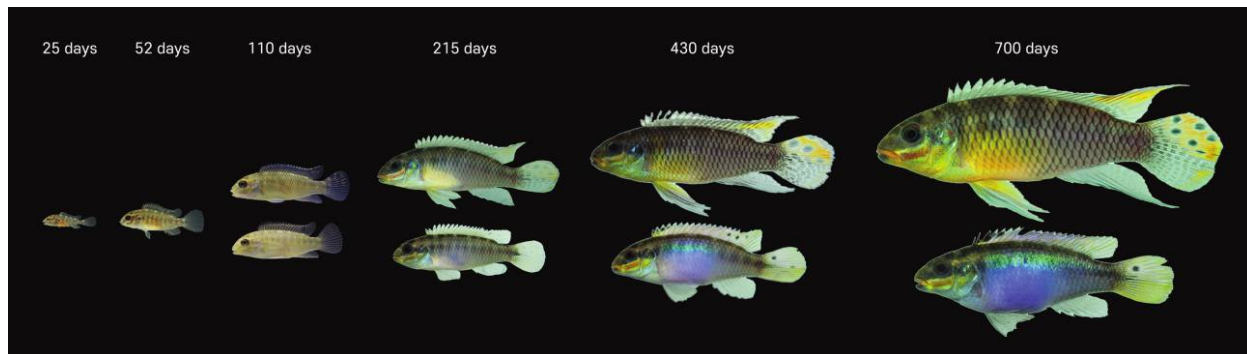
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Male (*top*) and female (*bottom*) *Pelvicachromis taeniatus* at the six developmental stages examined in the present study. Sexes are not distinguishable during the first two developmental stages, thus only one individual is shown, respectively. Photo credit: Denis Meuthen.