Environ Biol Fish (2016) 99:325–333
DOI 10.1007/s10641-016-0476-y

ORIGINAL ARTICLE

# Population-specific co-evolution of offspring anti-predator competence and parental brood defence in Nicaraguan convict cichlids

**Brian D. Wisenden · Anthony D. Stumbo · Daniel C. McEwen · Kurtis McIntire · Justin Scheierl · Jessica Aasand · Heather North · Janna Gilbertson · Diana Grant · Frantz Joseph · Emily Mammenga · Rachel Walsh · Ellen Brisch**

Received: 23 June 2015 / Accepted: 14 February 2016 / Published online: 18 February 2016

**Abstract** Parental care in fishes is a three-way interaction among brood predators, parental brood defence, and the escaping ability of the developing young. Convict cichlids are Neotropical freshwater fish with prolonged biparental brood defence of their eggs and free-swimming larvae. In a previous study, developmental timing of changes in larval swimming performance was correlated with larval skeletal ossification and biparental brood defence in convict cichlids in the Río Cabuyo, a stream in Costa Rica (Wisenden et al. 2015). Here, we repeat this study on a population of convict cichlids from Laguna de Xiloá, a volcanic crater lake in Nicaragua. We found that fish from Laguna de Xiloá also showed correlations among swimming performance and skeletal ossification of the larvae, and brood defence by the parents. However, in Laguna de Xiloá the developmental timing of these events was delayed relative to the Río Cabuyo population. The population difference between Costa Rica and Nicaragua could be an effect of genetic divergence or an artefact of phenotypic plasticity between lab-reared fish (Costa Rica) versus wild-caught fish (Nicaragua) for ossification scores. To resolve this question we repeated study using lab-reared fish of brood stock from Laguna de Xiloá. Comparing among the lab-reared Costa Rican fish, and lab-reared and field-collected Nicaraguan fish, we found that the timing of ossification was significantly delayed for both Nicaraguan samples relative to the Costa Rican samples. These shifts likely reflect population differences in selection on anti-predator competence of the young and, consequently, parental brood defence. These data indicate that larval ontogenetic development, anti-predator performance and parental care co-evolve with site-specific ecological differences.

**Keywords** Parental care · Larval swimming performance · Skeletal ossification · Anti-predator behavior · Population differences

B. D. Wisenden (✉) · A. D. Stumbo · D. C. McEwen · K. McIntire · J. Scheierl · H. North · J. Gilbertson · D. Grant · F. Joseph · E. Mammenga · R. Walsh · E. Brisch
Biosciences Department, Minnesota State University Moorhead, Moorhead, MN 56563, USA
e-mail: wisenden@gmail.com

J. Aasand
Department of Biological Sciences, North Dakota State University, Fargo, ND 58102, USA

## Introduction

Natural selection acts on variation in performance (Arnold 1983; Kingsolver and Huey 2003) with effects manifest at the level of population and community (Post and Palkovacs 2009; Bassar et al. 2012). In two-way interactions such as competition, sender-receiver communication, female-male interactions, predator-prey interactions, parent-offspring interactions, etc., each member of the dyad exerts selection pressure on the performance of its partner as the pair co-evolve within the ecological context of their habitat. Here, we consider the co-evolved interactions between morphology and anti-predator performance in larval convict cichlids, and biparental care behaviors of brood defence, and how this co-evolved relationship differs at two study sites.

## Convict cichlid study system

The convict cichlid (*Amatitlania siquia*) is a small freshwater fish endemic to Central America (Bussing 2002; Schmitter-Soto 2007). They form monogamous pair bonds and deposit their eggs on the roof of a cavity formed under a solid object on the substratum. They have biparental care of their eggs and free-swimming young over a period of four to six weeks (Wisenden 1995). During this time, parents defend their young against a range of brood predators (Wisenden 1994; Wisenden et al. 2015). Both parents contribute to brood defence, although there is some division of labor between the sexes (Itzkowitz et al. 2001). Parents guard a mobile territory around the young as the family roams over the substratum during the day. Young convict cichlids are about 4.5 mm in standard length (SL) when they first emerge from their lair. Parental protection of the young continues until the young reach a SL of 10–12 mm (4–6 weeks post-hatch) in Costa Rican streams (Wisenden 1995), and up to 20 mm in Laguna de Xiloá, Nicaragua (McKaye 1977; Alonzo et al. 2001). In the field, convict cichlid broods form a cohesive two-dimensional disc hovering above the substrate. When brood predators attack, the young at the periphery are at greatest risk and the young quickly retreat to the brood center directly under their parents. Thus, the radius of the brood area is an important variable defining both the cost of parental defence and predation risk to the young. In this system, defence against brood predators is a two-way interaction shared by the adults that repel brood intruders, and the larvae that must evade direct attacks when brood predators inevitably penetrate parental defences (Wisenden et al. 2015). Thus, one may predict that the co-evolved solution between parental care and offspring anti-predator competence may differ in habitats that differ in predator climates.

## Ontogeny of anti-predator competence in larval convict cichlids

Larval convict cichlids are poorly developed and swim weakly at the time of emergence but become better at evading attack as they grow and develop (Wisenden and Keenleyside 1992, 1994; Wisenden et al. 2015). Proximate anatomical structures that contribute to rapid starts in larval fish include neurological, muscular and skeletal changes (Fuiman and Magurran 1994). We quantified the timing of skeletal ossification because the techniques for doing so are well established (Song and Parenti 1995) and because ossification of the axial and appendicular skeleton is necessary to translate the forces of muscular contraction into propulsive forces generated by the fins (Hale 1999). By scoring the timing of the cartilage-bone transition of individual skeletal elements we could then test for a correlation between skeletal ontogeny and changes in swimming performance.

## Parental brood defence in response to larval anti-predator competence

Convict cichlid field ecology has been studied mainly at two sites: the Río Cabuyo and its tributary, Quebrada Amores, in Guanacaste, Costa Rica (Wisenden 1994, 1995) and in the volcanic crater lake Laguna de Xiloá, in Nicaragua (McKaye 1977; Alonzo et al. 2001). The sites differ in many ways but chief among them is greater overall fish density and species diversity at Laguna de Xiloá than is present at Río Cabuyo. Moreover, the specialist brood predator *Gobiomorus dormitor* is present in Laguna de Xiloá but absent from Río Cabuyo. These contrasting predation climates exert different anti-predator selection pressures on developing cichlid larvae that should promote changes in swimming performance, the underlying proximate skeletal support responsible for locomotory behaviors, and the area that two parents can effectively defend.

Wisenden et al. (2015) studied the three-way relationship among offspring development, parental care and brood predators in convict cichlids in the Río Cabuyo. In that system swimming performance, measured as maximum velocity and maximum acceleration, was flat until it rapidly increased when the young were between 6 and 7 mm SL, which coincided with rapid ossification of elements of the axial skeleton associated with the fins and their support structures. The 6–7 mm interval was also when brood diameter ceased expanding, having reached the maximum diameter that parents can efficiently defend. Moreover, 7 mm is the size at which conspecific brood adoption is no longer size selective (Wisenden and Keenleyside 1992) and differential predation on broods of mixed sizes no longer favors the larger size class (Wisenden and Keenleyside 1994).

Here, we report parallel data based on fish from Laguna de Xiloá, Nicaragua. We predicted that the relatively harsh predation climate at Laguna de Xiloá would lead to strong selection for anti-predator competence in the young, which could be achieved through either one of two mechanisms. The first mechanism would be for skeletal ossification and improved swimming performance to occur earlier in Laguna de Xiloá than in Costa Rican streams. However, even fully ossified fish cannot swim very quickly if they ossify while still at a small body size. Salmonids faced with similar selection achieve relatively fast growth (increase in length) by delaying skeletal ossification (Hale 1999). Therefore, an alternative prediction is that high predation risk will lead to selection for delayed ossification so that resources can be allocated into rapid increase in length. In either case, we predict that the timing of skeletal ossification should coincide with timing of improvements in swimming performance. We also predict that parents in Laguna de Xiloá will be forced to defend smaller areas than brood areas observed in the Río Cabuyo.

Population differences may reflect genetic divergence in response to different selection pressures, but they may also reflect phenotypic plasticity in response to any one of a number of environmental differences between Costa Rica headwater streams and an open volcanic crater lake. Previous research has shown that jaw morphology can be influenced by feeding opportunities and rearing environment (Meyer 1987; Gunter et al. 2013). Phenotypic plasticity is therefore a potential source of variation when comparing between fish collected form the wild and those reared in captivity (Solem et al. 2006; Kerschbaumer et al. 2011). Therefore, in this study we collected data from lab-reared and wild-caught offspring to evaluate the potential role, if any, of rearing environment on the timing of skeletal ossification.

## Materials and methods

### Source populations

Fish used in this study were either observed directly in the field or were $F_1$ offspring of wild-caught fish from Laguna de Xiloá, Nicaragua (12° 13′ 16.83″N, 86° 19′ 13.76″ W, elev. 45 m). Laguna de Xiloá is a volcanic crater lake located on a peninsula of Lago de Managua. Laguna de Xiloá is approximately 3.75 km$^2$ in area, and has a mean depth of 60 m and maximum depth of 88.5 m (Stauffer and McKaye 2002). We compared results from Xiloá fish with earlier results obtained from Costa Rican fish (Wisenden et al. 2015).

### Skeletal ossification

We preserved larvae (sizes ranged from 5 to 9 mm SL) from13 broods collected from the littoral zone of Laguna de Xiloá. Fish were preserved in 10 % buffered formalin and brought back to the lab for processing. Offspring were cleared and stained with Alcian Blue to reveal cartilage and Alizarin Red to reveal ossified bone (Song and Parenti 1995). Cleared and stained offspring were stored in glycerol with phenol crystals as a preservative. We were interested in the comparison between fish from Costa Rican streams versus Laguna de Xiloá. The initial comparison revealed a population difference but the comparison was potentially confounded by the effect of rearing environment because Costa Rican data on skeletal ossification was based upon lab-reared stock, while our first sample of Nicaraguan fish was collected from the wild. Therefore, we repeated the skeletal analysis on offspring of lab-reared fish that were $F_1$ or $F_2$ of wild-caught adults from Laguna de Xiloá. If the Costa Rica/Nicaragua difference in skeletal ossification persists for lab-reared fish then the effect is likely due to genetic divergence rather than being an artefact of phenotypic plasticity in response to rearing environment.

High-resolution images of stained offspring were taken with an Olympus Q-color3 digital camera mounted on an Olympus CX31compound microscope as described in Wisenden et al. (2015). We assessed calcification of the following skeletal structures: caudal fin, urostyles (bones that connect the caudal fin to the posterior tip of the vertebral column), dorsal fin rays and its pterygiophores (small bones at base of fin that support and anchor the fin to the underlying musculature), and the anal fin rays and its pterygiophores. We focused on these structures because we hypothesized that calcification of fin support would confer greatest effect on swimming performance. A scale of 0–4 was used to assess the level of ossification for each skeletal element: 0 = all blue, i.e., completely cartilage; 1 = mostly (>67 %) blue; 2 = approximately equal amounts of blue and red (between 33 % and 67 % blue); 3 = mostly (>67 %) red; 4 = all red, i.e., completely ossified.

To compare among the three data sets [Costa Rica lab-reared (CL); Nicaraguan wild-caught (NW); and

Nicaraguan lab-reared (NL)], we used permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to test differences in ossification scores on 37 different bones ($n = 216$ fish) against three levels of population (i.e., CL, NL, and NW) and size as a factor using 6–9 mm fish. We removed fish of the same size and population that had identical scores across bones because they provided no additional variance. This left a total of 216 fish. Our initial model tested the interaction between population and size, but if we found a nonsignificant multiplicative effect, we reran the analysis using the additive model only. While PERMANOVA can give traditional *F*-statistics, it is not useful for post-hoc analyses to determine group differences. In order to do that, we used permutational analysis of multivariate dispersion (PERDISP; Anderson 2006), which is essentially a multivariate extension of Levene's test (1960) that uses multivariate distances rather than raw data as an input to test for main group distances of the main effects, and we used non-metric multidimensional scaling (NMDS) to visualize interactive effect in two dimensions. Individual bones were then correlated using a Spearman rank correlation to the NMDS axes to relate those to the population-size groupings. For both PERDISP and NMDS, we used a modified Gower's similarity coefficient that has been deemed appropriate for ordinal type data to generate the distance matrix (Podani 1999). Because PERDISP essentially tests all pairwise matches, we adjusted scores using a Bonferroni correction. The R 3.2.1 package vegan was used for both PERMANOVA (adonis function) and the PERDISP (betadisp function), each with 9999 iterations; the Gower distance matrix was generated with the package FD (gowdis function), and the package BioDiversityR (NMSrandom function) was used to generate a two-dimensional solution to the ossification scores using 9999 random starts.

### Swimming performance

Methods for assessing the Costa Rican lab-reared fish (CL) fish are given in Wisenden et al. (2015). Wild-caught fish from Laguna de Xiloá were held in the lab for 18 mo before being used in this experiment. Individual $F_1$ convict cichlid larvae (NL) were placed in a circular plastic arena (diam =25.5 cm) containing dechlorinated tap water to a depth of about 2 cm. Startle responses were induced by a gentle prod with a monofilament line and recorded by an overhead digital camcorder at 30 frames per Secon*d. video* play-back of swimming behavior was analyzed for maximum velocity (m/s) using LoggerPro® software. We tested young at 1-mm intervals of standard length (SL) from 5 to 9 mm SL. Maximum velocity and acceleration were analyzed separately using a two-way analysis of variance that included an interaction term with population as the first factor having two levels (i.e., Costa Rica vs. Nicaragua) and standard length as the second factor having five factors (i.e., 5–9 mm). For a significant interaction, we tested population-size differences by creating a composite factor with 10 levels, one for each population-size and tested for differences between levels using Tukey's HSD test. Sample sizes were for Costa Rica 5 mm ($n$ = 23), 6 mm ($n$ = 17), 7 mm ($n$ = 28), 8 mm ($n$ = 13), and 9 mm ($n$ = 12), and for Nicaragua 5 mm ($n = 18$), 6 mm ($n = 20$), 7 mm ($n = 20$), 8 mm ($n$ = 13), and 9 mm ($n$ = 5).

### Brood areas in Laguna de Xiloá

We collected brood diameter data from broods in the littoral zone of Laguna de Xiloá. A total of 37 convict cichlid broods were sampled along approximately 200 m of rocky and sandy shoreline on the northern edge of the lake. All broods sampled were at depths of less than 2 m. We observed each brood for several minutes from a distance so as not to influence brood behaviour, and took note of landmarks on substrate that represented the outer limits of the brood area. We then approached the brood and measured the distance between landmarks using a tape measure. For each brood we measured the standard length (SL) of a subsample of 10 young, and counted the total number of young in the brood. To ensure accurate brood counts, we caught all but several of the young with hand nets and brought them to shore to measure and count. The remaining few young in the water could be counted with certainty with the aid of a mask. Young were returned to their parents immediately after sampling where they reformed as a coherent family shoal (see Wisenden 1995 for description of methods).

## Results

### Skeletal ossification

PERMANOVA showed a significant interaction between population and size on ossification scores ($F_{6,204} = 8.75$, $p < 0.001$). PERMDIST showed for the main effect of sample, the two Nicaraguan samples (NL vs NW) to be not different ($p = 0.123$), but both Nicaraguan samples were different from the Costa Rica population ($p < 0.05$). The main effect for size showed differences in transitioning between 6 and 7 mm ($p < 0.05$), and 7 and 8 mm ($p < 0.05$), but not between 8 and 9 mm ($p = 0.074$). The interaction between sample (CL, NL, NW) and size is shown with the NMDS ordination plots (stress =0.090). Of the explained variation, the majority of the first axis could be explained by fish length (87.7 %) while the second axis represented sample differences (89.8 %). Confidence intervals indicated that the largest change in skeletal ossification occurred between sizes 6 and 7 SL for the Costa Rican population, 7 and 8 mm SL for the Nicaraguan laboratory sample and between 8 and 9 mm SL for the Nicaraguan wild-caught fish (Fig. 1). Ossification scores were statistically indistinguishable from each other for all fish at 9 mm. Dorsal, caudal and anal fin pterygiophores were all identified as skeletal components that ossified earlier in Costa Rican fish than in Nicaraguan fish (Table 1). Of the 22 clusters of bones we analyzed, 13 ossified earlier in Costa Rican fish while only 3 ossified earlier in Nicaraguan fish ($\chi^2 = 6.25, p < 0.05$).

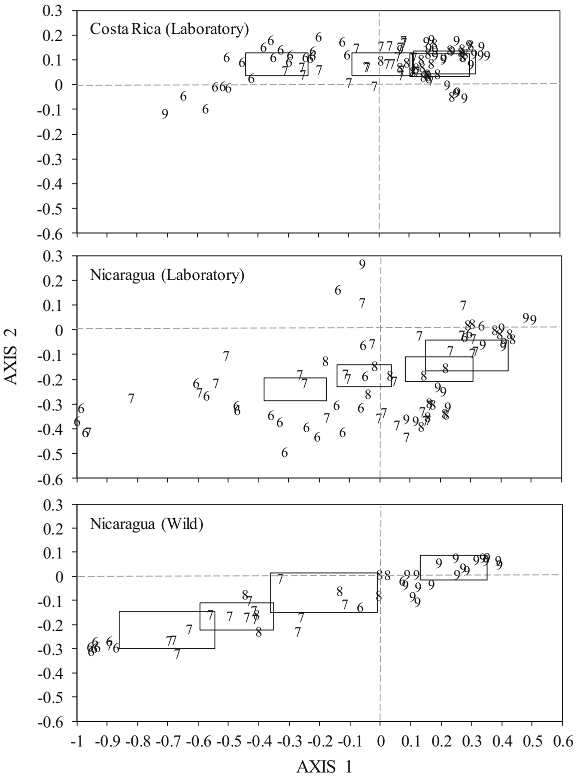


**Fig. 1** Subplots of NMDS (stress =0.907) illustrating the timing of ossification of larval convict cichlids from the three samples, from top to bottom, Costa Rica lab-reared, Nicaragua lab-reared, and Nicaragua wild-caught, and four size categories (6, 7, 8, and 9 mm standard length). *Rectangles* indicate the 95 % confidence intervals and for all subplots the confidence rectangles are ordered from left to right from 6, 7, 8, and 9 mm SL. The centroids of each *rectangle* indicate the average response. Overlapping confidence intervals indicate no significant difference in ossification scores

## Swimming performance

When compared to swimming performance data collected from lab-reared Costa Rican stock, there was a significant interaction between fish length and population for both velocity ($F_{4,159} = 7.516$, $p < 0.001$) and acceleration ($F_{4,159} = 4.191$, $p < 0.01$), indicating that the timing of improvement in swimming performance differed between the populations. Both velocity and acceleration increased rapidly in the transition for the Costa Rican fish between 6 and 7 mm and for the Nicaraguan fish more gradually from 7 to 8 mm and 9–10 mm (Fig. 2).

## Brood areas

The relationship between brood radius and size of young was not linear. A significant change point (Siegel and Castellan 1988) occurred at 7.9 mm SL ($z = 4.26$, $p < 0.001$) in the slope of the trend line defining the effect of young SL on brood radius (Fig. 3). Individual regressions on pre- and post-change point data for each location showed significant slopes for brood radius with respect to size of young only for post-change point broods. Number of young had no significant effect on brood radius ($p > 0.05$).

Anecdotally, we noted that the behavior of young in Laguna de Xiloá differed from that of young in Río Cabuyo. Young in Río Cabuyo emerge from their lair at a size of 4.5 to 5 mm SL whereas in Laguna de Xiloá young generally remained in their lair until they were 6 mm. We found only one brood outside of its lair before the young were 5 mm SL. We dismantled five rock piles in Laguna de Xiloá guarded by parental convict cichlids and discovered shoals of young of mean sizes of 4.5, 5, 5.85, 7.6 and 8.5 mm SL. Thus, differences between the habitats in the care of small young were not due to differences in size at hatch. We also noted that the size at independence from parental care at Laguna de Xiloá

**Table 1** Population comparison of the relative timing of skeletal ossification for bones associated with swimming propulsion in the caudal, dorsal and anal fins using a modification of indicator species analysis (Dufrene and Legendre 1997). '+' indicates earlier ossification. RC, Río Cabuyo; LX, Laguna de Xiloá

| Fin | Skeletal Element | Position | RC > LX | LX > RC |
|---|---|---|---|---|
| Caudal | Urostyles: Upper 1–3 | Proximate | 0 | 0 |
| | | Medial | + | 0 |
| | | Distal | 0 | 0 |
| | Urostyles: Central 1–6 | Proximate | + | 0 |
| | | Medial | + | 0 |
| | | Distal | 0 | 0 |
| | Urostyles: Lower 1–2 | Proximate | + | 0 |
| | | Medial | + | 0 |
| | | Distal | 0 | 0 |
| | Rays | Proximate | + | 0 |
| | | Medial | + | 0 |
| | | Distal | 0 | 0 |
| Dorsal | Spiny rays | | 0 | 0 |
| | Soft rays | | + | 0 |
| | Pterygiophores 1–15 | Proximate | + | 0 |
| | | Medial | 0 | + |
| | | Distal | 0 | 0 |
| Anal | Spiny rays | | + | 0 |
| | Soft rays | | + | 0 |
| | Pterygiophores 1–15 | Proximate | + | + |
| | | Medial | + | + |
| | | Distal | 0 | 0 |
| Total | | | 13 | 3 |

was extended to greater sizes (> 16 ml SL) than at the Río Cabuyo (Fig. 3).

## Discussion

Data from skeletal ossification, swimming performance and parental brood defence behavior are clearly correlated for convict cichlids from Laguna de Xiloá, Nicaragua. Relative to comparable data from the Río Cabuyo (Wisenden et al. 2015), the timing for Laguna de Xiloá fish was delayed for all measures. The switch point in Xiloá fish for swimming performance, skeletal ossification and brood defence was between 8 and 9 mm SL compared to 6–7 mm SL in Cabuyo fish (Wisenden et al. 2015). The overall nonlinear increase of maximum velocity with respect to body length observed in convict cichlids is similar to the pattern seen in zebrafish (Fuiman and Webb 1988). The main determinant of predator evasion by fish larvae is ontogenetic shifts in anti-predator competence because other aspects of predator detection and evasion have been shown to contribute little to escape potential (Fuiman 1994; Fuiman et al. 2006). Muscle mass, fin area and overall body form also change over the larva-juvenile transition, all of which contribute to locomotory performance (Fuiman and Magurran 1994; McHenry and Lauder 2006). However, skeletal ossification is a convenient and easily quantifiable measure of ontogeny and useful to test our question because bone transfers forces of muscular contraction to propulsion more efficiently than cartilage does. We are confident that the population difference between Costa Rican fish and Nicaraguan fish in the timing of skeletal ossification is due mainly to genetic divergence (and not an effect of phenotypic plasticity in response to rearing environment) because wild-caught

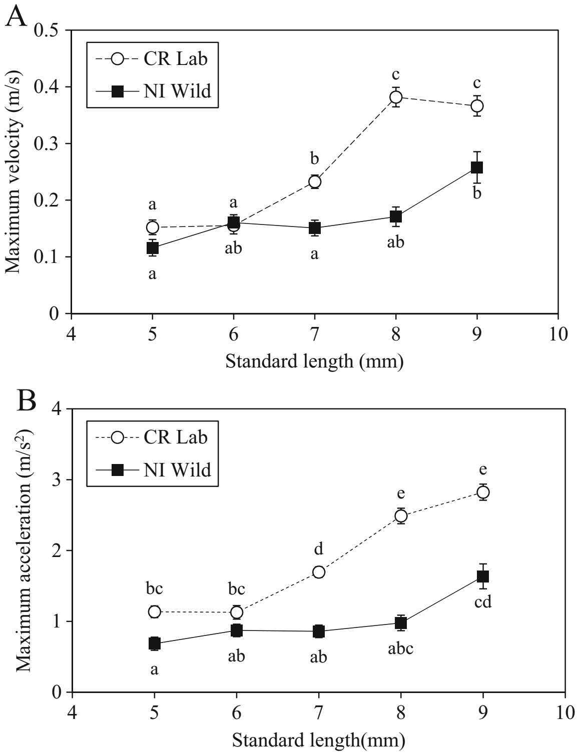


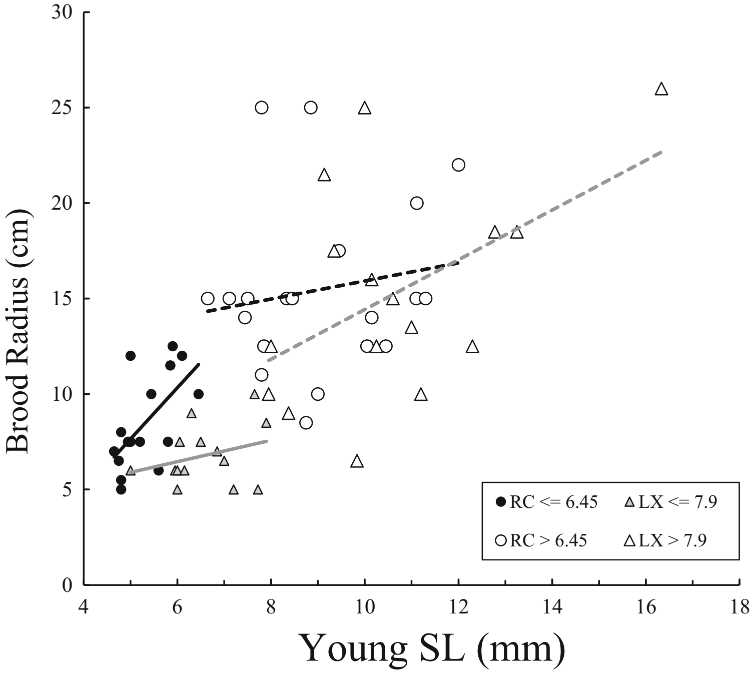
**Fig. 2** (**a**) Maximum velocity and (**b**) maximum acceleration for young convict cichlids from Laguna De Xiloá, Nicaragua (NI) and from Río Cabuyo, Costa Rica (CR) sized 5, 6, 7, 8 and 9 mm in standard length (SL). The *error bars* represent 1 SE. *Shared letters* indicate $p > 0.05$ in post-hoc pair-wise comparisons

and lab-reared offspring of Laguna de Xiloá fish showed similar schedules of ossification.

Our data suggest that predation in Laguna de Xiloá may have selected for fast growth at the expense of skeletal ossification and swimming performance (Hale 1999). Although primary production is greater in Laguna de Xiloá than in Río Cabuyo, this is not likely the root cause of selection for delayed ossification in Laguna de Xiloá because selection for high growth in other contexts (e.g., food limitation imposed by interspecific foraging competition) leads to delayed calcification (Arendt and Wilson 2000) and swimming performance in some species (Hale 1999; Billerbeck et al. 2001). Moreover, swimming performance tests presented in this study for Laguna de Xiloá fish, and those presented by Wisenden et al. (2015) for Cabuyo fish, were all obtained by lab-spawned fish from adults that had been held in captivity for at least 18 mo (i.e., under common environmental conditions) thus, population differences in skeletal ossification and swimming performance reported here likely reflect genetically-based differences.

As with the Río Cabuyo population (Wisenden et al. 2015), there was strong intra-population concordance in Laguna de Xiloá fish between radius of area occupied by the young, the ontogeny of swimming performance by the young and timing of their skeletal ossification.

**Fig. 3** Brood diameter for broods in field sites in the Río Cabuyo, Costa Rica (*circles*) and Laguna de Xiloá (*triangles*) before (*solid symbols*) and after (*open symbols*) switch point in slope as a function of Young SL. Switch point occurs at 6.45 mm SL for Río Cabuyo fish and 7.9 mm SL for Laguna de Xiloá



The significant effect of habitat on these variables strongly suggests different selection pressures on anti-predator competence of the young and the effective defence radius achievable by parental care. *Gobiomorus dormitor*, a specialist brood predator in Laguna de Xiloá, does not occur at the Costa Rican site. Field studies in Laguna de Xiloá show that parental convict cichlids disproportionately attack *G. dormitor* and this species was responsible for 47 % of all brood mortality when parents were experimentally removed (Alonzo et al. 2001). One might anticipate that the presence of *G. dormitor* may alter predation risk to the extent that the optimal strategy for larval convict cichlids at Laguna de Xiloá may be to remain near their parents and close to or inside their lair until they have grown in size to 8 mm SL and ossified their skeleton. The anecdotal observations of medium-sized free-swimming young inside their lair and extension of parental care to late stages of development are both consistent with the hypothesis that the predation climate is more intense in the Nicaraguan lake habitat than it is in the Costa Rican stream habitat.

Experimentally enlarged broods in the Río Cabuyo show suppressed rates of daily growth increments relative to unmanipulated control broods (Wisenden and Keenleyside 1995), indicating that brood radius represents a trade-off between foraging (radius expansion) and the cost of predation risk (radius contraction). In Laguna de Xiloá, the young remained sequestered in a tight shoal under their parents suggesting that predation risk is more intense and that food may be more abundant than in the Río Cabuyo system. Thus, a contributing factor to site differences in the timing of skeletal ossification and swimming performance may be that shade from riparian vegetation limits primary production in Costa Rican streams and forces young to forage over a relatively large area at an earlier stage of development than their counterparts in Laguna de Xiloá.

In the Río Cabuyo, brood diameter increased as free-swimming young grew from 4.5 to approximately 6.45 mm SL. This expansion most likely reflects growing demand for food and not a response to changes in parental defence. After the change point (Fig. 3), brood radius in river habitat was highly variable perhaps depending on whether the young were actively foraging or responding to intruders. In contrast to fish in the Río Cabuyo, fish in Laguna de Xiloá showed no change in brood radius until the young reached 7.9 mm SL, after which brood radius expanded linearly with fish length.

Cichlids are well known for radiation and endemism in the rift valley lakes of East Africa, and, to a much lesser extent, in Central American cichlid fauna (Barluenga et al. 2006; Elmer et al. 2010, 2013; Kautt et al. 2012). Recent systematic revision of Central America cichlids split the species formally known as the “convict cichlid” into four species based upon geographic region (Schmitter-Soto 2007). The sites in Río Cabuyo and Laguna de Xiloá are both within the range of convict cichlids renamed as *Amatitlania siquia*, but clearly even within this subregion there are habitat effects that may well lead to incipient speciation as isolated populations adapt to local conditions.

**Acknowledgments** Field data in Costa Rica were collected under MINAE Pasaporte de Investigaciónes number 00058. Special thanks to Celso Alvarado and Henry Ramirez of the Ministerio del Ambiente y Energía, Sistema Nacional de Áreas de Conservación Arenal Tempisque (ACT MINAE). We are very grateful to Ken R McKaye and Glenn Campbell (The Scuba Shack, San Juan del Sur, Nicaragua) for their generous donation of time and in-kind equipment, lodging and staff, without which our efforts at Laguna de Xiloá would not have been possible. Ossification and startle responses were enabled by research grants from the College of Social and Natural Sciences, MSUM to Brian Wisenden, and the Dille Fund for Excellence (MSUM) to Brian Wisenden and Ellen Brisch. Anusha Mishra, Bree Hamann and Joe Mullins were critical to the early development of the methods used in the final version of the study linking skeletal ossification to escape responses.

**Compliance with ethical standards** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This work was conducted under protocol 07-R/T-BIO-018-N-N-C of the Minnesota State University Moorhead Institutional Animal Care and Use Committee.

**Conflict of interest** The authors declare that they have no conflicts of interest.

## References

Alonzo JJ, McKaye KR, van den Berghe E (2001) Parental defence of young by the convict cichlid, *Archocentrus nigrofasciatus*, in Lake Xiloá, Nicaragua. J Aquaricult Aq Sci 9:208–228

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46

Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62:245–253

Arendt JD, Wilson DS (2000) Population differences in the onset of cranial ossification in pumpkinseed (*Lepomis gibbosus*), a potential cost of rapid growth. Can J Fish Aq Sci 57:351–356

Arnold SJ (1983) Morphology, performance and fitness. Am Zool 23:347–361

Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature 439:719–723

Bassar RD, Ferriere R, López-Sepulcre A, Marshall MC, Travis J, Pringle CM, Reznick DN (2012) Direct and indirect ecosystem effects of evolutionary adaptation in the Trinidadian guppy (*Poecilia reticulata*). Am Nat 180:167–185

Billerbeck JM, Lankford LE, Conover DO (2001) Evolution of intrinsic growth and energy acquisition rates. I. trade-offs with swimming performance in *Menidia menidia*. Evolution 55:1863–1872

Bussing WA (2002) Peces de las aguas continentals de Costa Rica. Editorial de la Universidad de Costa Rica, San Jose

Dufrene M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr 61:53–73

Elmer KR, Fan S, Gunter HM, Jones JC, Boekhoff S, Kuraku S, Meyer A (2010) Rapid evolution and selection inferred from the transcriptomes of crater lake cichlid fishes. Mol Ecol 19(suppl. 1):197–211

Elmer KR, Lehtonen TK, Fan S, Meyer A (2013) Crater lake colonization by neotropical cichlids fishes. Evolution 67: 281–288

Fuiman LA (1994) The interplay of ontogeny and scaling in the interactions of fish larvae and their predators. J Fish Biol 45: 55–79

Fuiman LA, Magurran AE (1994) Development of predator defences in fishes. Rev Fish Biol Fish 4:145–183

Fuiman LA, Webb PW (1988) Ontogeny of routine activity and performance in zebra danios (teleostei: cyprinidae). Anim Behav 36:250–261

Fuiman LA, Rose KA, Cowan JH, Smith EP (2006) Survival skills required for predator evasion by fish larvae and their relation to laboratory measures of performance. Anim Behav 71: 1389–1399

Gunter HM, Fan S, Xiong F, Franchini P, Fruciano C, Meyer A (2013) Shaping development through mechanical strain: the transcriptional basis of diet-induced phenotypic plasticity in a cichlid fish. Mol Ecol 22:4516–4513

Hale ME (1999) Locomotor mechanics during early life history: effects of size and ontogeny on fast-start performance of salmonid fishes. J Exper Biol 202:1465–1479

Itzkowitz M, Santangelo N, Richter M (2001) Parental division of labour and the shift from minimal to maximal role specialization: an experiment using a biparental fish. Anim Behav 61:1237–1245

Kautt AF, Elmer KR, Meyer A (2012) Genomic signatures of divergent selection and speciation patterns in a 'natural experiment', the young parallel radiations of Nicaraguan crater lake cichlid fishes. Mol Ecol 21:4770–4786

Kerschbaumer M, Postl L, Koch M, Wiedl T, Sturmbauer C (2011) Morphological distinctions despite large-scale phenotypic plasticity – analysis of wild and pond-bred juveniles of allopatric populations of *Tropheus moorii*. Naturwissenschaften 98:125–134

Kingsolver JG, Huey RB (2003) Introduction: the evolution of morphology, performance and fitness. Integr Comp Biol 43: 361–366

Levene H (1960) Robust tests for equality of variances. Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling 2:278–292

McHenry MJ, Lauder GV (2006) Ontogeny of form and function: locomotory morphology and drag in zebrafish (*Danio rerio*). J Morphol 267:1099–1109

McKaye KR (1977) Competition for breeding sites between the cichlid fishes of Lake Jiloá, Nicaragua. Ecology 58:291–302

Meyer A (1987) Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. Evolution 41: 1357–1369

Podani J (1999) Extending Gower's general coefficient of similarity for ordinal characters. Taxon 48:331–340

Post DM, Palkovacs EP (2009) Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. Phil Trans R Soc B 364:1629–1640

Schmitter-Soto JJ (2007) A systematic revision of the genus *Archocentrus* (perciformes: cichlidae), with the description of two new genera and six new species. Zootaxa 1603:1–76

Siegel S, Castellan NJ Jr (1988) Nonparametric statistics for the behavioral sciences, 2nd edn. McGraw-Hill, New York

Solem Ø, Berg OK, Kjøsnes AJ (2006) Inter- and intra-population morphological differences between wild and farmed Atlantic salmon juveniles. J Fish Biol 69:1466–1481

Song J, Parenti LR (1995) Clearing and staining whole fish specimens for simultaneous demonstration of bone, cartilage, and nerves. Copeia 1995:114–118

Stauffer JR Jr, McKaye KR (2002) Descriptions of three new species of cichlid fishes (Teleostei: Cichlidae) from Lake Xiloá, Nicaragua. Cuad Investig UCA 12:1–18

Wisenden BD (1994) Factors affecting reproductive success of convict cichlids in costa Rican streams. Can J Zool 72:2177–2185

Wisenden BD (1995) Reproductive behaviour in free-ranging convict cichlids. Environ Biol Fish 43:121–134

Wisenden BD, Keenleyside MHA (1992) Intraspecific brood adoption in convict cichlids: a mutual benefit. Behav Ecol Sociobiol 31:263–269

Wisenden BD, Keenleyside MHA (1994) The dilution effect and differential predation following brood adoption in free-ranging convict cichlids (*Cichlasoma nigrofasciatum*). Ethology 96:203–212

Wisenden BD, Keenleyside MHA (1995) Brood size and the economy of brood defence: testing Lack's hypothesis in a biparental cichlid fish. Environ Biol Fish 43:145–151

Wisenden BD, Stumbo AD, Self PA, Snekser JL, Wisenden PA, Keenleyside MHA, McEwen DC, Itzkowitz M, Brisch E (2015) Co-evolution of offspring anti-predator competence and parental brood defence in convict cichlids. Hydrobiologia 748:259–272