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RESEARCH ARTICLE

Brains over brawn: experience overcomes a size disadvantage in fish social hierarchies

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ABSTRACT

Life experiences can alter cognitive abilities and subsequent behavior. Here we asked whether differences in experience could affect social status. In hierarchical animal societies, high-ranking males that typically win aggressive encounters gain territories and hence access to mates. To understand the relative contributions of social experience and physical environment on status, we used a highly territorial African cichlid fish species, *Astatotilapia burtoni*, that lives in a dynamic lek-like social hierarchy. *Astatotilapia burtoni* males are either dominant or submissive and can switch status rapidly depending on the local environment. Although dominant males are innately aggressive, we wondered whether they modulated their aggression based on experience. We hypothesized that as males mature they might hone their fighting tactics based on observation of other males fighting. We compared males of different ages and sizes in distinctly different physical environments and subsequently tested their fighting skills. We found that a size difference previously thought negligible (<10% body length) gave a significant advantage to the larger opponent. In contrast, we found no evidence that increasing environmental complexity affected status outcomes. Surprisingly, we found that males only a few days older than their opponents had a significant advantage during territorial disputes so that being older compensated for the disadvantage of being smaller. Moreover, the slightly older winners exploited a consistent fighting strategy, starting with lower levels of aggression on the first day that significantly increased on the second day, a pattern absent in younger winners. These data suggest that experience is an advantage during fights for status, and that social learning provides more relevant experience than the physical complexity of the territory.

KEY WORDS: Cichlid, Fish, Hierarchy, Social status

INTRODUCTION

How do animals in a hierarchically organized society gain higher status? Specifically in social systems where males fight for social status, what is the contribution of physical size as opposed to fighting strategy? When males are closely matched for size, does experience or exposure to environmental complexity contribute more to winning outcomes? Over 65 years ago, Hebb (Hebb, 1947) reported that rats allowed to roam free in his house were better at problem solving than rats reared in cages in his laboratory. This anecdotal result suggested a role for experience in shaping behaviors. Subsequent systematic studies by Krech et al. (Krech et al., 1960) demonstrated that rearing rats in barren or rich environments produced measurable differences in brain structures,

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behavior and learning abilities, suggesting that the rearing environment could play a role in development of cognitive abilities. Thus, as social animals grow and develop, behavior and brain structures are shaped by both social and environmental experiences. But, it is not clear what contributes more to social success, the experience of social interactions associated with an enriched environment or the enriched environment itself.

Most vertebrate species that live in social groups, from fish to mammals, have evolved dominance hierarchies where dominant males have increased access to food and mates, making it crucial to achieve dominance. Fish comprise ~50% of all vertebrate species and are increasingly appreciated as models for understanding the complexities of social behavior (reviewed in Brown et al., 2011). Many studies have shown that fish species, particularly those with social structures, have the capacity for environmental and social cognition. Examples of environmental influences include development of foraging skills (Brännäs and Eriksson, 1999), tool use (Timms and Keenleyside, 1975; Paśko, 2010), and spatial memory and manipulation of the environment (Hughes and Blight, 1999). Examples of social intelligence in fish have been measured by how they interact in group-living (Balshine-Earn et al., 1998), enhance offspring survival with bi-parental care (Alonzo et al., 2001; Van den Berghe and McKaye, 2001; Gross and Sargent, 1985; Hourigan, 1989), co-operate in hunting (Vail et al., 2013; Diamant and Shpigel, 1985) and share information about predator inspection (Pitcher et al., 1986). Female fish make mate choices based on social information (Doutrelant and McGregor, 2000; Clement et al., 2005) and male fish use transitive inference to assess fighting abilities of competitors (Grosenick et al., 2007). Fish can intervene between members of their group when new members of the opposite sex are being added (Schradin and Lamprecht, 2000). Interestingly, naïve fish group members can learn schooling locations, mating sites and foraging routes through observation (Warner, 1988; Warner, 1990; Laland and Reader, 1999).

Because most fish have indeterminate growth (grow continuously) and growth rates change based on population density, size rather than chronological age has been typically used by fishery biologists and behaviorists as a way to pair fish. However, age has been reported as an important component in learning. Bisazza et al. (Bisazza et al., 2010) demonstrated that guppies have an innate ability from birth to discriminate between small numbers, but only after sexual maturation and social experience can they discriminate between larger numbers indicating age-related changes in performance. In addition, Kotrschal and Taborsky (Kotrschal and Taborsky, 2010) showed that environmental changes during rearing can affect later outcomes in cognitive abilities. Strand et al. (Strand et al., 2010) also suggested that enrichment of the environment could increase learning in fish. We wondered what effects social experience gained by age (chronological time spent in social interactions) and rearing conditions (complexity of rearing environment) would have on social dominance.

To assess the role of experience in fighting ability in *A. burtoni* males, we measured the effects of chronological age, size and environmental experience on achieving dominance. *Astatotilapia burtoni* offers key advantages for assessing the value of experience in establishing social rank because it lives in a highly organized social system where behavioral interactions directly regulate reproductive success. Although there is clear evidence that their highly aggressive behavior is innate (Fernald, 1980), it was not known whether and how their fighting strategies might develop with age.

Previously we showed that *A. burtoni* males use transitive inference, a skill once thought exclusive to mammals and birds, to construct a virtual social hierarchy from incomplete information gathered as bystanders in territorial disputes (Grosenick et al., 2007). Moreover, males of this species observe local social interactions and respond by switching between dominant and subordinate behavioral phenotypes in seconds (Burmeister et al., 2005; Maruska et al., 2013). These data suggest that *A. burtoni* can learn social skills through observation and also use social information gained through observation to modify their behavior.

Physiologically and behaviorally, dominant and subordinate males are quite distinct. Dominant males comprise ~10–30% of the population at one time, are brightly colored, defend territories, and actively court and spawn with females. In contrast, subordinate males are drably colored, similar to females, do not hold territories, and are reproductively suppressed (reviewed in Fernald, 2012). In their natural habitat in East Africa, rapid changes in bottom cover and predation create a high turnover of territory ownership through fighting (Fernald and Hirata, 1977a). Thus, there is a premium on the ability to adapt to a novel environment, detect and defeat a weaker opponent and, consequently, attract females to spawn. These attributes of the environment and *A. burtoni* social life predict that increased social experience would confer an advantage during subsequent aggressive encounters.

Here we asked whether and what differences in early rearing conditions might influence individual success in subsequent battles over territory and the resulting rise in status. *Astatotilapia burtoni* live in shallow shore pools amongst their food source, decaying vegetation. Dominant males dig spawning shelters in this detritus but otherwise there is little else in their habitat (Fernald and Hirata, 1977b). Would access to defined shelters or more time watching conspecifics engaging in social displays and fights provide more valuable experience? We assessed the effects of the complexity of the physical environment on dominance by rearing animals in conditions that differed only by the presence or absence of shelters. Age was our proxy for experience differences, as older animals had more time for additional interactions. We measured population density effects in our laboratory conditions and we kept equal densities in competing groups to prevent any bias in opportunities for social interactions, specifically in claiming and defending physical shelters during rearing, as a confounding factor.

RESULTS

Physical complexity of rearing environment does not affect aggression or exploration

We hypothesized that having access to shelters would allow males to experience territory acquisition and defense that might lead to differences in overall aggressive behavior. We quantified aggressive behaviors of individual dominant fish in both non-sheltered (NS) and sheltered (S) tanks for 9 weeks from the onset of sexual maturity (weeks 10–19 after release of brood from the mother's mouth) and found no significant differences between groups in any of the aggressive behaviors measured ($P>0.05$, bootstrap t-test).

We hypothesized that males reared in an environment with shelters would be more active explorers when presented with a novel environment. Comparing frequency of shelter entries and movements across compartments in a novel tank, we found no differences between NS and S groups during the 15 min period ($P>0.05$, bootstrap t-test). Comparing behavioral data in 5 min intervals, there were still no significant differences between groups ($P>0.05$, bootstrap t-test within each time bin; $P>0.05$ for environment, time bin, environment–time interaction; two-way repeated-measures ANOVA).

Age difference predicts dominance but rearing environment complexity does not

We staged territorial fights in dyads to test the hypothesis that prior experience with shelters would result in more wins by S than NS males. Contrary to our prediction, in 15 out of 21 dyads (~71%) the NS fish won ($P=0.013$, Fisher's exact test). We tested whether there was a difference in gonadosomatic index (GSI) between winners and losers because high GSI is correlated with dominance (Davis and Fernald, 1990). As expected, GSIs of both S and NS winners ($n=18$) were significantly greater than that of the losers ($n=11$; $P=0.00097$, bootstrap t-test); however, we found no difference in GSI between the S and NS winners, indicating that the rearing environment did not affect this predictor of dominance. Next, we tested whether relative age had an effect on fighting success. Across all 21 dyads, being older significantly increased NS fishes' chances of winning ($P=0.012$, Fisher's exact test). To control for age differences, we compared outcomes of territorial fights where S and NS fish were age-matched ($n=11$), and found no effect on dominance; six S fish and five NS fish won their respective fights ($P=1$, Fisher's exact test). In the remaining dyads ($n=10$), NS winners were older than their S opponents. Taken together, these findings showed that although there was no effect of rearing environment on winning, relative age was a strong predictor of dominance.

Small differences in body length predict social dominance

Relative body length is a reliable predictor of dominance in many species of fish (e.g. Bisazza et al., 1996). That is, when two males with a large size difference engage in agonistic interactions, the larger male will almost always win, in some cases without even a fight. In a previous study with *A. burtoni*, pairs were considered size-matched if their body sizes were within ±10% of each other (Desjardins et al., 2010). Here, although pairs of males tested were well within that range (±3 mm; <8% size difference), we analyzed whether small differences in body length could be an advantage for the larger male and found a significant effect of relative body length on winning. Males larger than their opponent won in 14 out of 21 fights ($P=0.003$, Fisher's exact test). Of the seven other winners, four were smaller than their opponent (one S, three NS) and three were of equal length (one S, two NS). This predictive value of size in territorial disputes is consistent with previous studies (Bisazza et al., 1996; Candolin and Voigt, 2001). However, the small size difference sufficient to predict a victory was unexpected.

Relationship of population density and age with *A. burtoni* size

Like most fish, *A. burtoni* has indeterminate growth, that is, fish grow continuously over their life and their rate of growth can vary with environmental conditions. For this reason, the relationship between size, age and time of sexual maturity is plastic and dependent on small changes in animal density in the aquarium. We measured, under laboratory conditions, the quantitative relationship
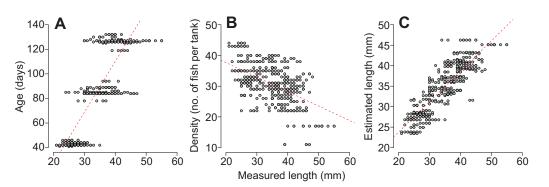
Fig. 1. Age and population density predict body length in the African cichlid *Astatotilapia burtoni*. Scatterplots comparing (A) age in days, (B) rearing tank density and (C) body length (estimated by a linear model based on age and rearing tank density; *y*-axis) with actual measured length (*x*-axis). Each filled circle represents a fish (*n*=425; 226 males + 54 females + 145 juveniles). Red dashed lines represent the linear regression of age, density or estimated length on measured length. Pearson correlation (with *P*-values based on Fisher's *z* transformation) showed that age alone is a strong positive predictor of body length (A; *r*=0.8, *P*=7×10^{-96}), i.e. older fish tend to be larger, and combining age with information on rearing tank density, which correlates negatively with body length (B; *r*=–0.53, *P*=3.7×10^{-32}), increases predictive power (C; *r*=0.86, *P*=1.2×10^{-125}). A linear model of body length was constructed using the lm() function in R, with age and rearing tank density as independent factors. Coefficients (age=0.1367, density=–0.3736) and intercept (32.1303) values from the model were combined with age and density measurements to estimate body lengths, which are shown in C to correspond with the actual lengths better than age or density alone.

between size, density and chronological age (from hatching). Over the course of 25 months, we collected data to determine the effects of population density on growth. At approximately 6-week intervals, we measured population density, body mass and body length of 34 populations. Starting densities of all populations were 22–44 fish per tank. We found no effect of density on sex ratio (*r*=0.19, *P*=0.49) or any correlation between density and onset of sexual maturation (*r*=0.19, *P*=0.7), and 19 of 26 populations had at least one male at week 6. In Fig. 1 we show an empirically derived relationship between age, size and density for *A. burtoni*. Fig. 1C shows that our model can predict fish size when specifying population density and age.

Being older than an opponent is a better predictor of social dominance than size

What is the relationship between size and age of winners? Because size and age both had positive effects on victory, we tested how age

differences related to differences in body length. Across all dyads, we found a significant negative correlation between the relative ages and body lengths of winners (Pearson correlation=–0.53, *P*=0.013), such that winning fish with large age advantages tended to have less of a length advantage, or none at all, and winning fish with the largest length advantages tended to be from an age-matched dyad. This effect was even stronger within the 15 NS–winner dyads (Pearson correlation=–0.66, *P*=0.0074), where five out of 15 winners were actually smaller or of equal length compared with their opponent. Across all 21 dyads (Table 1), the length advantage of winners decreased as a function of increasing age advantage; age-matched winners had the greatest average size advantage, winners that were 6 days older than their opponent had a more modest size advantage, and winners with the greatest age advantage (19 days) were mostly smaller than their opponent (*P*=0.048, Kruskal–Wallis; Fig. 2). These results show that dominant males with an age advantage can overcome a size disadvantage and win territorial

Table 1. Summary of territorial fight winners

	Age difference (days)	Age (days)	Rearing condition	Length difference (mm)	Winner length (mm)
Age-matched	0	115	NS	0.1	4.7
			S	0.2	4.9
			S	0.1	4.7
			S	0	4.6
		143	S	0.2	5.35
			S	0.2	5.35
			NS	0.2	4.1
			S	–0.2	4.1
			NS	0.2	4.2
			NS	0.2	4.2
			NS	0.1	4.1
Older than opponent	6	183	NS	0.05	4.3
			NS	0.05	4.3
			NS	–0.1	4.15
			NS	0.15	4.35
			NS	0.25	4.2
	19	155	NS	–0.2	3.8
			NS	–0.3	4
			NS	0.1	4
			NS	0	4
			NS	0	3.75

Twenty-one territorial fights were staged between animals that were age-matched or older than their opponent. We show the absolute age of the winner and the age difference between the winner and the loser. Length difference is winner length minus loser length. NS, non-sheltered; S, sheltered.
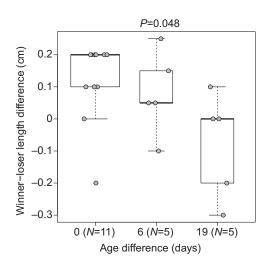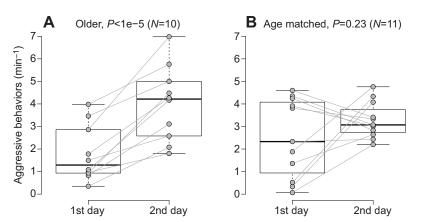
Fig. 2. Social experience offsets size in territorial fights in *A. burtoni*. There was a significant effect of age difference on size advantage across winners of territorial disputes in all dyads ($P=0.048$, Kruskal–Wallis one-way ANOVA). The *y*-axis is the length of the winning fish minus the length of the losing fish (cm), thus negative values indicate that the winner was smaller. The *x*-axis is the relative ages of winners across all dyads. No fish that were younger than their opponent won any territorial disputes. Gray circles represent winner–loser size difference in each fight; top and bottom of boxes represent the first and third quartiles, respectively; whiskers extend to the most extreme data points no more than 1.5 times the interquartile range from the box; and horizontal lines within the boxes represent group medians. All but one of the age-matched winners was larger than their opponent, while animals with a greater age difference (6 or 19 days) tended to be smaller.

disputes, suggesting that age is at least as important if not more so than size. Thus, the additional experience gained with age may confer a greater advantage than a larger size.

Experience affects fighting strategy in dominance fights

What advantage did age confer on winners that were smaller than their opponents? We hypothesized the older winners were more aggressive in fighting over territory, even though we did not see differences in aggression during rearing in either environment. There was no significant relationship between absolute age and the number of aggressive behaviors per minute in each of the first 3 days of the dyad encounters. However, among all winners there was a significant increase in the number of aggressive acts per minute from the first to second day in the dyad ($n=21$, $P=0.0015$, bootstrap paired *t*-test), an effect that was even stronger in dyads where the winner was older than his opponent ($n=10$, $P<1\times10^{-5}$; Fig. 3A), but was not found in age-matched winners ($n=11$, $P=0.23$; Fig. 3B),

suggesting a role for experience in deciding when to attack aggressively.

DISCUSSION

We hypothesized that *A. burtoni* males growing up in a physically enriched environment would have an advantage in territorial disputes, but to our surprise, we found that small differences in age and size had greater effects than the physical environment. Animals reared with shelters did not have any significant advantage over those reared in tanks with only gravel. Animals that were older than their opponent won territory in every case, even some that were smaller than their opponent – an unexpected result. We posit that this age advantage reflects the experience gained in social interactions, giving fish more time to practice and observe territorial disputes. Despite the relatively small differences in age, the high rates of behavioral interactions in male *A. burtoni* (~40–60 aggressive interactions per hour) means that even slightly older males have experienced hundreds more social interactions than younger counterparts. Our males were young adults, an age that may be a critical learning period. Previous work where age was not monitored and size was larger suggested that size difference was a certain predictor of victory. In an extreme case where one male was four times larger and there was no physical interaction, the smaller opponent surrendered without a fight (Chen and Fernald, 2011). We have found a critical time period where social interaction differences can result in differential outcomes in social rank. Interestingly, we found that at ages between 150 and 183 days, a very small size difference (<8%), previously considered adequate to 'size-match' *A. burtoni* males, was sufficient to give an advantage to the slightly larger male, with a predictive value of ~66%. It will be interesting to see whether at older ages these small differences become moot and the effect of experiences is not related to age but to interactions among dominant males. The ability of males to detect size differences of less than 10% suggests that relative size difference affects competition to a degree that was previously unknown. Size differences previously believed to match opponents fairly may result in a confounding effect, suggesting that experimental matches be based on both age and size. We showed how population density affects the size distribution in *A. burtoni* between birth and week 24 and we disentangled the relationship of size, onset of sexual maturity and age in *A. burtoni* under our laboratory conditions. At the population densities used for our dominance assay, we saw no effect of sexual maturity or sex ratio in populations, showing that our fish were well paired without confounding effects of time from sexual maturity. It would be interesting to test larger age differences between competitors to see if there is an upper limit to learning how to fight more successfully.

Fig. 3. Older fish show an increased rate of aggressive behaviors from day 1 to day 2 while age-matched animals do not. (A) Among winners that were older than their opponent, there was a significant increase in rate of aggressive behaviors (*y*-axis) on day 2 of the encounters compared with day 1 ($P<1\times10^{-5}$, bootstrap paired *t*-test). This was not seen in age-matched winners (B; no winners were younger than their opponent), suggesting time in social interactions provides experience to develop a strategy. (B) Among age-matched winners, there was no difference in the rate of aggressive behaviors from day 1 to day 2 (*y*-axis). Each gray circle is the day 1–day 2 difference for a single winner fish. Top and bottom of boxes represent the first and third quartiles, respectively; whiskers extend to the most extreme data points no more than 1.5 times the interquartile range from the box; and horizontal lines within the boxes represent group medians.

How can smaller *A. burtoni* males beat larger but younger opponents? Previous work in salmon (*Salmo salar*) based on variability in the relation between dominance and size suggested that early social interactions may depend on behavioral experience rather than size, and that the larger size of dominant fish was a consequence and perhaps not a cause of higher status (Huntingford et al., 1990). A parametric exploration assessing asymmetries in prior experience and size in green swordtail fish (*Xiphphorus helleri*) found that among fish within 10% in size, animals with prior experience winning defeated animals with prior experience losing; however, when matched with much larger animals (10–20%), larger size determined the outcome (Beaugrand et al., 1996). In cichlids, there have been several studies exploring parameters affecting fight outcomes. In analyzing in a South American cichlid (*Aequidens rivulatus*), Maan et al. (Maan et al., 2001) found that prospective winners postpone escalation because of the high cost of fighting and proposed that fights in which prospective fighters could see one another allowed animals to use prior experience to judge their strategy. Similarly, Neat et al. (Neat et al., 1998a) measured the proximate costs of fighting in *Tilapia zillii* and recorded decisions about whether to escalate or give up. In the same species, Neat et al. (Neat et al., 1998b) found that in territorial fighting, relative gonadal weight was a stronger predictor of fight outcome than body size. They hypothesized that males with larger gonads fight harder to defend their territory. In *A. burtoni*, there is a significant difference between the gonadal size of dominant and non-dominant fish, but in our case, the gonad differential we measured was a consequence of the loser remaining in a subordinate position for an extended period of time (3–4 weeks).

We have previously shown that fighting males assess the relative strength of animals they observe fighting and can use this information to predict winners (Grosenick et al., 2007). We suggest that the ability to evaluate opponents in addition to physical strength makes the difference for establishing dominance. Younger winners did not have a consistent strategy, some having higher aggression on day one than day two and vice versa. Older fish, however, consistently began with low levels of aggression followed by significantly increasing the number of attacks 24 h into the challenge. This consistency may reflect accumulated experience, a skill that would benefit males in the wild where the physical environment is dynamic and there is a regular turnover of status among males.

In this study, we have not disambiguated whether age alone, perhaps as a late developmental stage as seen in mammals, may lead to the delayed attack response. However, in birds, the ability to store and forage food was not based on age, but on the experience of storing and retrieving food, suggesting that this was a learned effect and not an innate quality (Clayton and Krebs, 1994). We speculate that younger, less experienced males are testing strategies with high levels of aggression attempting to intimidate less experienced fish, a strategy that may not work on older opponents.

Our data suggest that in a sophisticated fish social system, males learn and practice skills that are crucial to their success in becoming dominant as they become older. After sexual maturity, learning from social interactions may play a greater role than environmental enrichment in determining fight outcomes. It is conceivable that this socially relevant learning is developmentally regulated and may be occurring during a specific critical period. As much as male *A. burtoni* show an innate drive to fight for mating territory, male songbirds will sing to attract mates even if they are raised in isolation. However, 'isolate song' is not effective for courting females, and young male birds typically need to learn well-formed song from an adult tutor in order to successfully attract mates (Williams et al., 1993). Song learning requires the acquisition and integration of socially relevant sensory information during crucial developmental phases that can be shifted in time by manipulating incoming sensory information (Morrison and Nottebohm, 1993; Funabiki and Konishi, 2003). We suggest a parallel in *A. burtoni*, where the onset of sexual maturation increases the innate drive to obtain a territory, but the efficacy of fighting is dependent on experience. Designing experiments that disambiguate these effects, e.g. comparing the fighting strategies of age-matched males raised in isolation versus community tanks, will be necessary to identify the relative contributions of the social experience and absolute age in shaping behavior.

The division of labor is linked to age in the social insect systems of both the honeybee (Lindauer, 1953) and ants (Mersch et al., 2013). Older individuals perform more complex tasks than younger ones, suggesting a chronology that could be a developmentally controlled in the brain and dependent on experience and juvenile hormones (Withers et al., 1995). However, more recently, research in honeybees shows that more mature foragers can revert into nursing, a task performed by more immature bees, if the hive is depleted of nurses. Remarkably, this plastic behavioral response is linked to changes in DNA methylation in the mushroom bodies (Lockett et al., 2012). This suggests that chronological age is required to mature and perform certain tasks, but that the molecular mechanism is at least in part reversible and mediated by social stimuli. In *A. burtoni*, where dominance is in flux and there is a benefit to reverting to more subordinate (immature) behaviors, it will be very interesting in future studies to identify how age and ethologically relevant experience may shape the brain in a vertebrate social system.

MATERIALS AND METHODS
Animals
Astatotilapia burtoni (Günther 1894), derived from wild-caught stock [Lake Tanganyika, East Africa (Fernald and Hirata, 1977a)], were maintained in aquaria under conditions mimicking their natural habitat (28°C, pH 8, 12 h:12 h light:dark cycle, constant aeration and water chemistry similar to that in Lake Tanganyika). Fish were fed cichlid flakes (AquaDine, Healdsburg, CA, USA) and brine shrimp once a day.

Determining density effects on growth rate and sex ratios in laboratory conditions
Over the course of 25 months, we analyzed the growth rate of 34 populations in tanks with a starting density of 20–44 fish per tank. Broods were released from their mothers' mouths into rearing tanks (121 l) after ~14 days of brooding. For each population, every 6 weeks, we collected five animals at random and determined body mass (g) and length (mm). We re-counted fish to determine changes in the population density over time (deaths) and checked fish for secondary sexual characteristics, such as egg spots and body coloration, as indicators of gender (Table 2).

Sex characteristics arise earlier in some fish than others (color and egg spots in males), but sex ratios continue to change as males mature. We counted the number of males appearing at week 6 as an indicator of onset of sexual maturation in a population and we determined gender ratios at week 18. Prior to week 18, we designated non-males as juveniles (these could be females or immature males).

We measured and recounted one population at week 6, four populations at week 12 and three populations at week 18. From one population each we collected measurements and population densities at week 6 and 12, week 6 and 18 and week 12 and 18. Twenty-one populations were measured and recounted at week 6, 12 and 18 and two populations at week 6, 12, 18 and 24.

Table 2. Timepoints sampled for population density effects on growth rate

Populations scored	Week 6	Week 12	Week 18	Week 24
1	X			
4		X	X	
1	X	X		
3			X	
1	X		X	
1		X	X	
21	X	X	X	
2	X	X	X	X

Rearing conditions

Animals were raised in one of two conditions: with or without shelters. As noted, in their natural habitat, dominant males occupy and defend makeshift shelters dug into the substrate of decaying vegetation. These constructed shelters served as spawning sites for dominant males, while non-dominant males and females school together above the territories. Otherwise there is little else in these pools (Fernald and Hirata, 1977b). *Astatotilapia burtoni* is a mouth brooder, meaning that eggs are kept in the mother's mouth until they are released ~2 weeks after fertilization. Approximately 70 days after the brood is released from the mother's mouth, males can be distinguished from females by body coloration and behavior (Fraley and Fernald, 1982). At this time, successful males will actively defend a territory (i.e. shelter) and display a typical repertoire of dominant behaviors [e.g. chasing conspecifics, fighting with other males and courting females (Fernald, 1977)].

Fish reared with shelters (S)

Fish (~35 per tank) were reared in four 114 l aquaria (30.5×55.9×30.5 cm, width × depth × height) with four to eight terra cotta pots cut in half lengthwise (11×11×5.5 cm, length × width × height) simulating the natural shelters and placed on the gravel substrate. Dominant males typically dug into the gravel beneath the shelter.

Fish reared without shelters (non-sheltered; NS)

Fish (~35 per tank) were reared under identical conditions except that terra cotta pots were absent. Dominant males typically dug pits in the corners of the aquaria.

Behavioral measurements

To test whether there were any behavioral differences between NS and S dominant males, we scored the number of aggressive behaviors displayed by dominant males (n=4–6 males) starting at week 10 after brood release, the first week fish show signs of aggressive behavior. Additional observation weeks were 11, 12, 15 and 19. We chose to analyze the following aggressive behaviors defined in the *A. burtoni* ethogram (Fernald, 1977): threat displays, border fights, chases, bites and nips. Male fish were classified as dominant if they were brightly colored, displayed an eye-bar and defended a territory (with or without shelters).

Exploration assay

We developed an assay to test whether rearing conditions had measurable effects on exploratory behavior. To eliminate differences in the type and/or frequency of recent social interactions as confounding factors, S and NS fish were housed in isolation for 24 h prior to the exploratory assay. Thus, regardless of rearing condition, all fish experienced the same environment on the day prior to test day. Isolation tanks were 28 l (18×57×30.5 cm, width × depth × height), with gravel substrate but no shelters. After 24 h, each fish was transferred to a 76 l (61×55.9×30.5 cm, width × depth × height) test tank with shelters and placed inside an opaque bottomless plastic cylinder (3 min) to allow the fish to acclimate after handling and transfer. The test period (15 min) began once the opaque cylinder was lifted and the fish was free to explore. To assess activity, we placed marks that delineated three compartments of identical size on the front side of the aquarium, and the number of times the fish traversed between compartments, as well as the frequency of shelter entries, was scored at 5 min intervals.

Dominance assay

Establishment of dominance requires winning a fight against another male. We used pairwise fights (dyads) to assess which animals from each rearing environment could become dominant. Twenty-four hours after the exploration assay, one NS and one S dominant male were size matched (within 3 mm, mean fish size=4.31 cm) and transferred to a single 30 l tank (30.5×55.9×30.5 cm, width × depth × height) that contained three females and one shelter. Under these conditions, size-matched animals will fight to establish a territory that includes the shelter, and the winner becomes the dominant male. We recorded three 15 min periods: immediately after transfer to the test tank, and 24 and 48 h post-transfer. We clipped the top dorsal fin uniquely to identify each fish.

Age differences

To assess dominance, we paired S and NS fish in dyads where the fish were either age-matched (N=11) or the NS fish was older (N=10). Fish in age-matched dyads were either 115 or 143 days old. In the NS–older dyads, 155-day-old NS fish were paired with 136-day-old S fish, and 183-day-old NS fish were paired with 177-day-old S fish. Thus, NS animals were either 0, 6 or 19 days older than their S dyad partners. Using age as a proxy for experience, we calculated how much more experience in defending territory older males might have: as males begin active dominance fights at ~70 days, the analysis groups had 0% (0 days), 8% (6 days) or 27% (19 days) more experience than their relatively younger opponents. To put this in perspective, dominant males perform approximately three aggressive acts per minute, averaging ~2000 aggressive acts per day.

Statistics

All statistical analysis was performed using the R language and environment for statistical computing (www.r-project.org). The likelihoods that categorical variables (rearing environment: S versus NS; relative age: 0 versus 6 versus 19 days difference; relative length: bigger versus smaller versus equal) had some effect on establishing dominance were computed using Fisher's exact test, implemented with the R function 'fisher.test'. Pearson correlations, e.g. between age and length differences, were computed using the R function 'cor.test'. A Kruskal–Wallis one-way ANOVA, implemented with the R function 'kruskal.test', was used to assess the effect of categorical age differences (0 versus 6 versus 19 days) on length differences. For the exploration assay, a two-way repeated-measures ANOVA, implemented with the R function 'aov', was used to test for effects of rearing environment and time bin on shelter entries or compartment crossings, and whether there was a significant interaction between rearing environment and time bin.

Tests for differences between two sets of independent measurements, e.g. GSI of dominant versus non-dominant fish, were conducted via a permutation-based, or bootstrap, procedure that is comparable to an unpaired t-test. Similarly, tests for within-subjects differences at two time points, e.g. the frequency of aggressive behaviors for the same fish on different days, were conducted via another bootstrap procedure comparable to a paired t-test. We did not perform standard t-tests because assumptions about data normality and variance could not be verified with enough confidence to assure validity of the test results. Bootstrap tests do not require any assumptions about the form of the data and are thus valid in all cases, including those that violate assumptions of standard statistical tests (Efron and Tibshirani, 1991). Bootstrap tests were implemented with custom R functions, available on the Fernald laboratory website (www.stanford.edu/group/fernaldlab).

Briefly, to compare independent datasets, we first computed the mean value of each dataset, and the difference between group means was taken as the test statistic. To assess the likelihood of observing this value of the test statistic under the null hypothesis, i.e. that both groups were actually subsets of the same underlying population, the datasets were combined and resampled with replacement to yield pseudo-groups that were the same sizes as the real groups. Then, the difference between pseudo-group means was recomputed. This process was repeated 100,000 times, generating 100,000 pseudo test statistics that together made up the null distribution. After resampling, the actual test statistic was projected onto the null distribution, its reflection across the mean of the distribution was computed, and the

number of pseudo test statistics more extreme than the actual test statistic or its reflection, divided by 100,000, was taken as the *P*-value.

To compare dependent datasets, we first computed, for each subject, the difference between its measurements at time 1 versus time 2, and the mean of these within-subject differences was taken as the test statistic. To assess the likelihood of observing this value of the test statistic under the null hypothesis, i.e. that the signs (increase versus decrease from time 1 to time 2) of within-subject differences were random, a resampling procedure was repeated 100,000 times to build a null distribution of the test statistic; in each iteration, the actual within-subject differences were multiplied by −1 or 1 (chosen randomly), effectively randomizing the sign of the change for each subject, and the mean within-subject difference was recomputed. The 100,000 pseudo-within-subject differences generated during this process made up the null distribution. The actual test statistic was projected onto the null distribution, its reflection across the mean of the distribution was computed, and the number of values more extreme than the actual test statistic or its reflection, divided by 100,000, was taken as the *P*-value.

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