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Sensory cues underlying competitive growth in the clown anemonefish (Amphiprion percula)

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Thesis

SENSORY CUES UNDERLYING COMPETITIVE GROWTH IN THE CLOWN ANEMONEFISH (*Amphiprion percula)*

by

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SENSORY CUES UNDERLYING COMPETITIVE GROWTH IN THE CLOWN ANEMONEFISH (*Amphiprion percula)*

LEAH DESROCHERS

ABSTRACT

In some animal societies, access to breeding depends on the individual's position in a hierarchy, which often depends on an individual's size. In such societies, individuals may engage in competitive growth, trying to outgrow one another to attain a higher rank. This suggests that members of the hierarchy can track changes in the growth and size of potential competitors and respond accordingly. The clown anemonefish, *Amphiprion percula*, is one species known to exhibit competitive growth at the initiation of size hierarchies. Here, we use 5 combinations of sensory cues to determine which cues must be available to initiate competitive growth between size-matched individuals. Our results show that mechanosensory (pressure and/or touch) cues are used to assess size and initiate competitive growth. This study provides an understanding into the relationship between environment and phenotypic response in a social context.

Table of Contents

List of Tables

List of Figures

1. Introduction

Animal societies, in which some individuals breed while others forgo their own reproduction and behave cooperatively are one of the more remarkable products of evolution, and they have provided a context for the evolution of many extraordinary traits (Bourke 1995, Emlen 1997). In many of these societies, access to breeding positions is determined by individuals' social rank (Drummond 2006) which in turn, partially or fully depends on individual's size (Reeve et al. 1998, Clutton-Brock et al. 2006). In such societies, where breeding opportunities depends on individual's social rank and size, selection may favor growth and size modification strategies that maximize an individual's chances of maintaining their current rank or attaining a higher rank (Buston 2003, Wong et al. 2007, Huchard et al. 2016, Reed et al. 2019). For example, in clown anemonefish (*Amphiprion percula*) and emerald coral gobies (*Paragobiodon xanthosomus*) in stable dominance hierarchies, individuals will reduce their growth rate to avoid coming into conflict with their immediate dominants and thereby maximize their chances of inheriting breeding positions (Buston 2003a, Buston 2004a, Wong et al. 2007). In contrast, in Kalahari meerkat (*Suricatta suricatta*) societies, at the formation of dominance hierarchies, size-matched individuals will increase their growth rate in a race to outcompete their rivals and attain the highest rank (Huchard et al. 2016). In these contexts, where growth is a strategic response to social environment, individuals must be able to assess their own size and the size of others within the dominance hierarchy. Thus, the question arises, how are individuals assessing their own size and the size of others in these societies?

Generally, animals can assess their own size and the size of conspecifics via a variety of sensory cues such as mechanosensory (pressure and/or touch), auditory, chemosensory, and visual. Considering mechanosensory cues, for example, male pygmy swordtails (*Xiphophorus nigrensis* and *Xiphophorus multilineatus*) use aggressive encounters to assess size and recognize other males of their species (Morris et al. 1995). Other species, such as male green frogs (*Rana clamitans*), common toads (*Bufo bufo*), or carpenter frogs (*Rana virgatipes*) rely on auditory cues: males use advertisement call frequency to evaluate the size of other males in competition (Davies and Halliday 1978, Given 1987, Bee et al. 1999). Multiple studies of fishes have found that smaller individuals produce higher frequency sounds for a shorter duration than larger individuals, suggesting the potential for fish to use auditory cues to assess size (Myrberg et al. 1985, Lobel and Mann 1995, Connaughton et al. 2000, Colleye et al. 2009). Turning to consider chemosensory cues, male garter snakes use tongue-flicks to detect pheromones of females in a communal den and uses these pheromones to assess the body size of females (Shine et al. 2003). Iberian rock-lizards, (*Lacerta monticola*), use tongue flicks to read chemical cues in order to recognize males and the rate of tongue flick response depends on body size (Aragon et al. 2000). Analogously, pintado catfish (*Pseudoplatystoma coruscans*) adjust barbel movements when placed in water that is conditioned by larger or similarly sized conspecifics indicating that they can discriminate individuals' size by chemical cues (Giaquinto and Volpato 2005). Finally, other animals, such as the big-clawed snapping shrimp (*Alpheus heterochaelis*), rely on visual cues; in this species, males use the size of the open chela of opposing snapping shrimp to assess the body size of the opposition (Hughes 1996). However, despite the large amount of work conducted on the sensory cues underlying size assessment, currently no studies have investigated the sensory cues underlie individuals' size assessment in critical contexts where individuals' growth and size is a strategic response to their social environment.

In the present study, we investigate the sensory cues facilitating competitive growth in the clownfish (*Amphiprion percula*). *Amphiprion percula* is one of 28 species of anemonefish *Amphiprion sp*. that lives in anemones on IndoPacific coral reefs. *Amphiprion percula* live in social groups composed of a breeding pair and zero to four nonbreeders, in which individuals adjust their growth and size in response to other group members (Buston 2003a, Buston and Cant 2006, Reed et al. 2019). Within each group, there is a well-defined size hierarchy: the female is the largest individual, the male is the second largest, and non-breeders get progressively smaller (Buston 2003a). The size hierarchy represents a queue to become a breeding member: if the female of the group dies, then the male changes sex and becomes the new female, the largest non-breeder becomes the new male, and the smallest individual in their hierarchy is the most recent newcomer (Buston 2004a). In stable size hierarchies, clownfish individuals reduce their growth rate to avoid coming into conflict with their immediate dominant and retain their current rank

(Buston 2003a, Buston and Cant 2006, Wong et al. 2016; Branconi et al. in review). At the initiation of size hierarchies, clownfish individuals increase their growth rate to outcompete their rivals and attain the highest dominance rank without conflict (Reed et al. 2019). How clownfish are able to assess their own size, the size of others, and adjust their growth accordingly remains an open question.

Anemonefish are known to use a combination of mechanosensory (pressure and/or touch), auditory, chemosensory, and visual signals for variety of social interactions with conspecifics and heterospecifics (Fricke 1973, Parmentier et al. 2005, Colleye et al. 2009, Colleye and Parmentier 2012, Johnston et al. 2017). Multiple *Amphiprion* species engage in mechanosensory behaviors such as head shaking which may be a behavior of submission or threat. These behaviors have been observed when more than one fish is present in an anemone, especially when multiple juveniles are vying for a spot in an anemone (Allen 1972). Red Sea clownfish (*Amphiprion bicinctus*) use visual cues to recognize individuals and after 10 days in isolation could still distinguish partners from strangers (Fricke 1973). Auditory sensory signals such as dominant frequency and pulse duration convey information regarding fish size and hypothetically could be used to assess size and recognize individuals within a group (Colleye and Parmentier 2012). Here, to assess the type of sensory cues required for clownfish individuals to engage in competitive growth [e.g., mechanosensory (pressure and/or touch), auditory, chemosensory, and/or visual] we use a suite of experiments, in which we size match individuals in an experimental design analogous to that of Reed et al. (2019) but selectively block different types of cues across different treatments. We test four main hypotheses regarding the sensory cues required for size-matched individuals to engage in competitive growth: 1) they must have mechanosensory (pressure and/or touch) cues; 2) they must have auditory cues; 3) they must have chemical cues; 4) they must have visual cues.

2. Materials and Methods

2.1 Adult Broodstock

This experiment was conducted at Boston University (Boston, MA, USA) from July 2019-March 2020. All fish used in this experiment were reared from wild-caught non-breeders (less than 30mm in standard length) in Papua New Guinea and supplied by Quality Marine and Sea Dwelling Creatures. The removal of non-breeders is considered a sustainable practice, because the presence of non-breeders does not influence the birth rate or death rate of breeders (Buston 2004; Barbasch et al. in review). A detailed description of broodstock housing conditions can be found elsewhere (Schmiege et al. 2017, Barbasch and Buston 2018).

2.2 Juvenile Rearing

To investigate competitive growth of size-matched individuals, we raised juvenile clownfish. Egg clutches laid by broodstock pairs were observed each day and after 7-8 days were transferred to a rearing tank. To oxygenate the eggs and stimulate hatching, a stream of air was gently directed over the eggs using an air diffuser positioned underneath the clutch. Upon hatching, and up to 21 days old, larvae were fed *HUFA*-enriched-rotifer *Brachionus rotundiformis* (15 rotifers ml^{-1}), decapsulated *Artemia* nauplii (3 *Artemia* ml^{-1}) and the water was tinted with *Nannochlorpsis* algae paste (Rotigreen Nanno, Reed Mariculture, USA). The water quality of the rearing bins was maintained at a salinity of 33-35ppt, with a temperature of 27-28°C, pH of 8.0-8.3, NH_3 levels of 0-0.25ppt, NO_2 levels of 0ppm, and NO_3 levels of 0ppm. The larvae were reared for approximately two weeks until they settled, then another week until they reached about 7.5mm.

2.3 Juvenile Housing

Experimental juvenile fish were housed in 3L fish tanks which were part of a re-circulating saltwater aquarium system. Flow through each tank was approximately 91 ±0.5 l/h. Abiotic conditions were monitored regularly and maintained as constant as possible: pH = 8.1±0.1, temperature = 26.1±0.6°C and salinity = 34±0.5 ppt were monitored daily; ammonia (0 ppm), nitrite (0 ppm) and nitrate (0 ppm) were monitored weekly (Spectrophotometer and test strips, Sensafe, North America). Approximately 10% of the system water was changed daily. Lighting was provided by ambient fluorescent lighting and the room was maintained on a 12 light : 12 dark light cycle. The life support system consisted of a biomedia bed for biological filtration and a UV sterilizer for disinfection.

2.4 Experimental Design

Our experimental design was based on that of Reed et al. 2019, which provided the first demonstration of competitive growth in clownfish. At three weeks of age, each larval fish was measured for initial standard length (ISL) to within 0.1mm using a dissection microscope, Sedgewick Rafter Cell, and ImageJ. Then, three fish were size matched to within 0.5mm: two of these three fish were housed as a pair, and one fish was housed as a singleton. The fish could not see individuals in other tanks due to the tanks being painted black on one side.

Each pair and their singleton had one of five divider options to assess the sensory cues involved in competitive growth (Table 1). More specifically, to determine the type of sensory cues (mechanosensory via pressure and/or touch, auditory, chemosensory, and visual) required for clownfish individuals to engage in this growth and size modification strategy, we used 5 tests in which we selectively blocked the different types of cues.

First, to test the hypothesis that individuals need mechanosensory (pressure and/or touch) cues to engage in competitive growth, no divider was used (NCB). Given the impossibility of blocking auditory, chemosensory, and visual cues while mechanosensory cues were present, this first experiment allowed all 4 cue types simultaneously. Second, to test the hypothesis that size-matched individuals need only auditory cues to engage in competitive growth, an opaque, solid partition was used (OS). This partition was cemented in place so that no water could pass around the sides, guaranteeing that no chemical cues were transferred and the opacity prevented transmission of visual cues. Third, to test the hypothesis that size-matched individuals need chemosensory cues to engage in competitive growth, an opaque, perforated divider was used (OP). This partition had a mesh insert allowing water to flow from one side of the tank to the other across the divider. The opacity of the divider prevented transmission of visual cues. Fourth, to test the hypothesis that size-matched individuals need visual cues to engage in competitive growth, a clear, solid partition was used (CS). This partition was cemented in place so that no water could pass around the sides, guaranteeing that no chemical cues were transferred, and the clear divider allowed visual sensory cues to remain. Fifth, to test the hypothesis that size-matched individuals need chemosensory and visual cues, a clear, perforated divider was used (CP). This divider allowed chemosensory cues to pass in the water through a mesh insert, and the clear divider allowed visual sensory cues to remain.

All fish were fed the same ration of fish pellets: 0.01g of mixed A and B1 pellets (TDO Chroma Boost, APBreed, Reed Mariculture Inc., CA, USA). To ensure that both members of the pair were fed the same amount, each fish was fed on their side of the partition; to control for the effect of the partition, the singleton was also exposed to a partition. The fish housed in the absence of blocked cues were given a partition during feeding, and the partition was removed after feeding. These steps were repeated daily for four weeks, after four weeks aggression was observed between paired individuals and the trials were stopped (Reed et al 2019).

Table 1. *Hypotheses regarding the sensory cues required for individuals to engage in competitive growth (rows), the tests required to discriminate among those hypotheses (columns), and the predictions of those experiments (competitive growth occurs, Y or N).*

	Tests				
Hypotheses	Test 1. No cues blocked (NCB)	Test 2. Opaque Solid. Visual, chemical, & tactile cues blocked (OS)	Test 3. Opaque Perforated. Visual & tactile cues blocked (OP)	Test 4. Clear Solid. Chemical & tactile cues blocked (CS)	Test 5. Clear Perforated. Tactile cues alone blocked (CP)
H1. Mechanosensory cues required	Y	N	N	N	N
H2. Auditory cues required	Y	Y	Y	Y	Y
H3. Chemical cues required	Y	N	Y	N	Y
H4. Visual cues required	Y	N	N	Y	Y
Replicates	10	9	8	9	9

At the end of the trial period, a final standard length (FSL) was measured to within 0.1mm using a dissection microscope, Sedgewick Rafter Slide, and ImageJ. Within pairs, each individual was assigned a rank (pair-rank-1, P1; pair-rank-2, P2; singleton, S) on the basis of their FSL, with the largest being P1. Number of replicates per test ranged from 8-10.

2.5 Statistical analyses

Separate one-way ANOVAs were performed for each test (Table 1). First, a one-way ANOVA was performed with growth as the dependent variable and type of individual (P1, P2, or S) as the independent variable. Post-hoc Tukey test was used to determine where the differences lay. Second, a one-way ANOVA was performed with growth as the dependent variable and type of individual with pair members combined (P, or S) as the independent variable. In this case, no post-hoc Tukey test was required. Finally, two more one-way ANOVAs were performed with growth of individuals (P or S) as the dependent variable and type of test (NCB, OS, OP, CS, CP) as the independent variable. Again, post-hoc Tukey tests were used to determine where differences lay. It is the evidence from all tests taken together that provides the most compelling case for when competitive growth is and is not occurring.

3. Results

3.1 No Cues Blocked

This experiment blocks no cues. Under these conditions, on average, P1 grew 5.03 ± 0.58mm, P2 grew 3.10 ± 1.00 mm, and S grew 1.61 ± 0.94mm. The type of individual was a significant predictor of growth (Figure 1a; ANOVA: df=2, F=33.92, $p<0.001$): P1 grew faster than P2 and (Tukey's HSD: $p<0.001$) and P2 grew faster than S (Tukey's HSD: $p<0.01$). Furthermore, when combined, paired individuals grew faster than singletons (Figure 1b; ANOVA: df=1, F=29.12, $p<0.001$). Taken together, these results provide compelling evidence of competitive growth when no cues are blocked (see also Reed et al 2019).

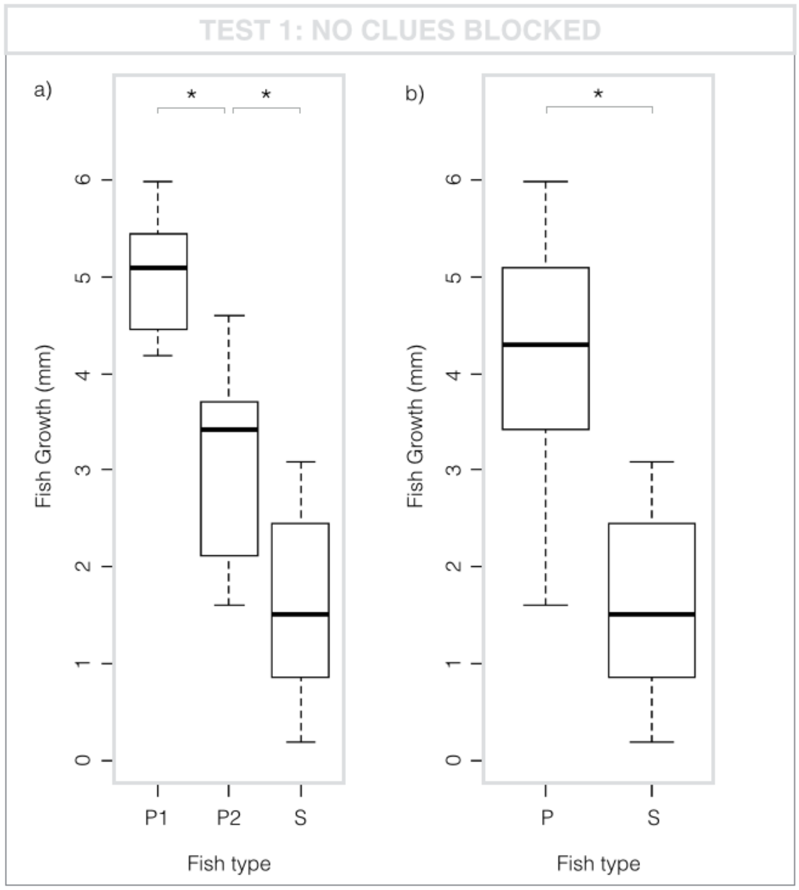

Figure 1. *(a) Growth of clownfish in Test 1 — No Cues Blocked (n= 10 replicates) separated by fish type: largest in pair (P1); smallest in pair (P2); singleton (S); (b) Growth of clownfish in Test 1 — No Cues Blocked (n=10 replicates) separated by fish type: paired individuals (P) and singleton (S). The bold band indicates the median, the box indicates the interquartile range, and the whiskers indicate ± 1.5*IQR.*

3.2 Opaque Solid

This experiment blocks all cues apart from auditory cues. Under these conditions, on average, P1 grew 2.33 ± 1.03mm, P2 grew 0.93 ± 0.60mm, and S grew 1.28 ± 0.66mm. At first it seems that there might be competitive growth because P1 grows more than P2 (Figure 2a: ANOVA df=2, F=7.686, $p<0.01$. Tukey's HSD: $p<0.01$). However, P2 does not grow more than S (Tukey's HSD: $p>0.05$). Furthermore, when combined, paired individuals did not grow faster than singletons (Figure 2b; ANOVA: df=1, F=0.791, $p>0.05$). Taken together, these results suggest that the fish need more than just auditory cues to engage in competitive growth. This leaves us with the question: which cues have to be available to restore competitive growth (Figure 1)?

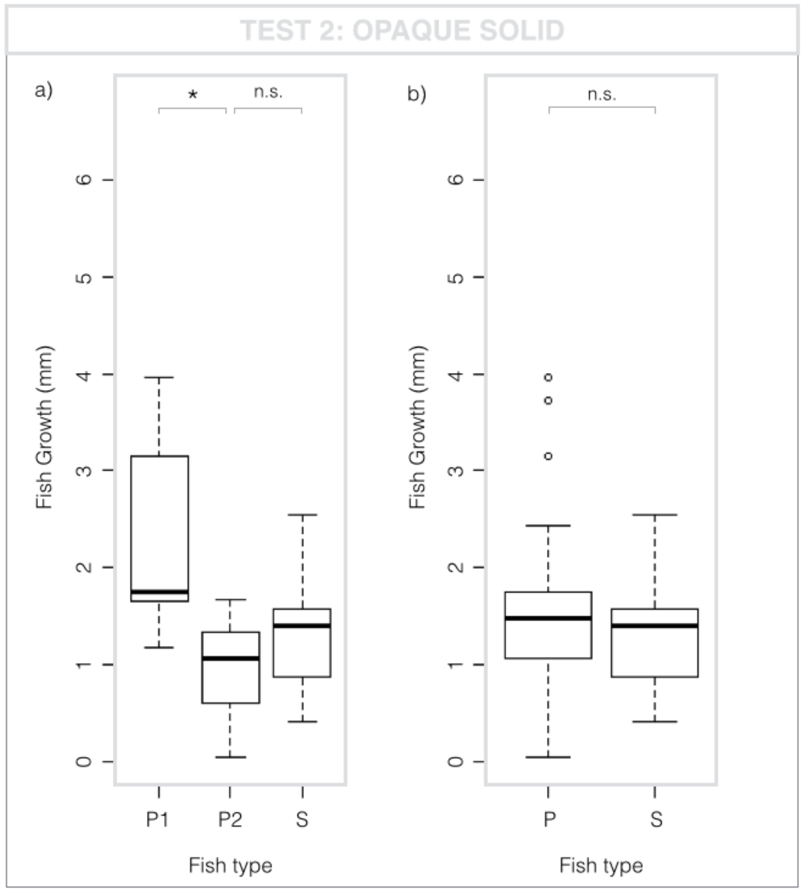

Figure 2. *(a) Growth of clownfish in Test 2 — Opaque Solid (n= 9 replicates) separated by fish type: largest in pair (P1); smallest in pair (P2); singleton (S); (b) Growth of clownfish in Test 2 — Opaque Solid (n=9 replicates) separated by fish type: paired individuals (P) and singleton (S). The bold band indicates the median, the box indicates the interquartile range, and the whiskers indicate ± 1.5*IQR.*

3.3 Opaque Perforated

This experiment makes chemical cues available to the fish. Under these conditions, on average, P1 grew 2.40 ± 1.21mm, P1 grew 1.31 ± 0.91mm, and S grew 2.00 ± 0.80mm. There is no evidence for competitive growth because P1 grows slightly more than P2 (Figure 3a: ANOVA df=2, F=2.494, p>0.05). Furthermore, when combined, paired individuals did not grow faster than singletons (Figure 3b; ANOVA: df=1, F=0.105, p>0.05). Taken together, these results suggest that the fish need more than just auditory and chemical cues to engage in competitive growth.

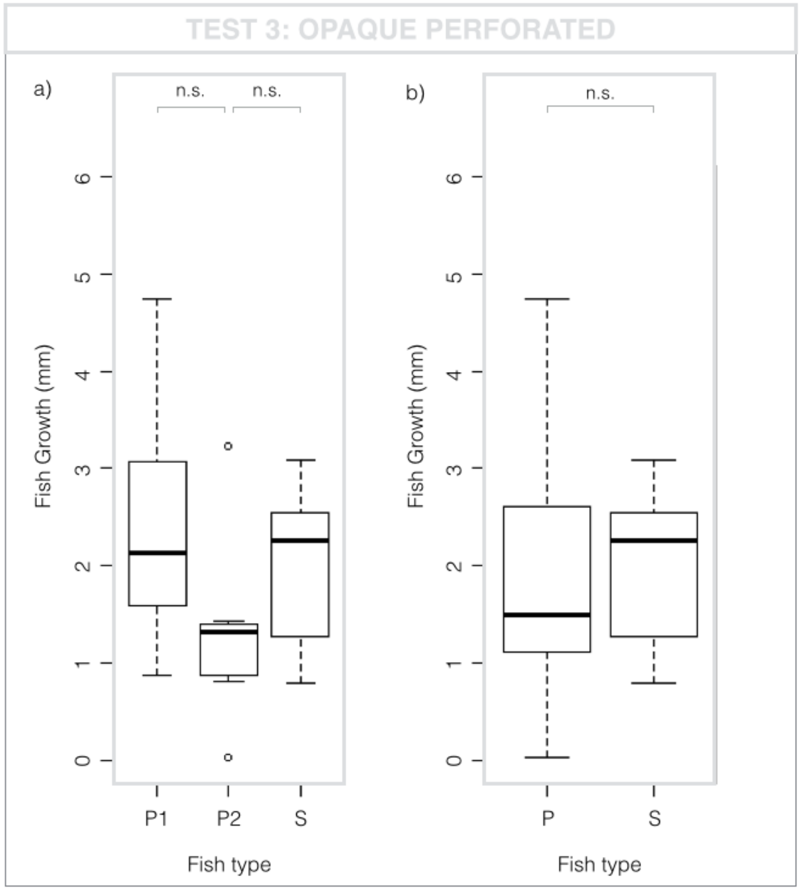

Figure 3*. (a) Growth of clownfish in Test 3 — Opaque Perforated (n= 8 replicates) separated by fish type: largest in pair (P1); smallest in pair (P2); singleton (S); (b) Growth of clownfish in Test 3 — Opaque Perforated (n=8 replicates) separated by fish type: paired individuals (P) and singleton (S). The bold band indicates the median, the box indicates the interquartile range, and the whiskers indicate ± 1.5*IQR.*

3.4 Clear Solid

This experiment also makes visual cues available to the fish. Under these conditions, on average, P1 grew 2.12 ± 0.80mm, P2 grew 1.23 ± 0.66mm, and S grew 1.47 ± 0.49mm. At first it seems that there might be competitive growth, because P1 grows slightly more than P2 (Figure 4a: ANOVA: df=2, F=4.354, $p<0.05$. Tukey's HSD: $p<0.05$). However, P2 does not grow more than S (Tukey's HSD: $p>0.05$). Furthermore, when combined, paired individuals did not grow faster than singletons (Figure 4b; ANOVA: df=1, F=0.47, $p>0.05$). Taken together, these results suggest that the fish need more than just auditory and visual cues to engage in competitive growth.

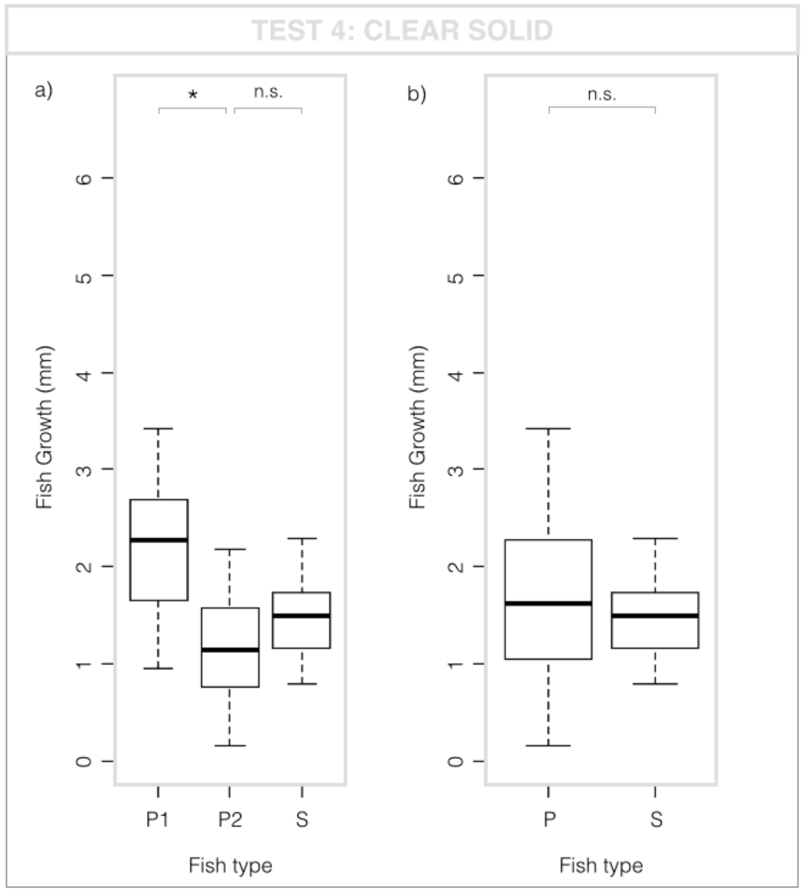

Figure 4. *(a) Growth of clownfish in Test 4 — Clear Solid (n= 9 replicates) separated by fish type: largest in pair (P1); smallest in pair (P2); singleton (S); (b) Growth of clownfish in Test 4 — Clear Solid (n=9 replicates) separated by fish type: paired individuals (P) and singleton (S). The bold band indicates the median, the box indicates the interquartile range, and the whiskers indicate ± 1.5*IQR.*

3.5 Clear Perforated

This experiment makes auditory, chemosensory, and visual cues available to the fish. That is, it allows all cues except mechanosensory (pressure and/or touch). Under these conditions, on average, P1 grew 2.10 ± 0.54mm, P2 grew 1.37 ± 0.44mm, and S grew 1.60 ± 0.69mm. At first it seems that there might be competitive growth, because P1 grows more than P2 (Figure 5a: ANOVA: df=2, F=3.954, $p<0.05$. Tukey's HSD: $p<0.05$). However, once more, P2 does not grow more than S (Tukey's HSD: $p>0.05$). Furthermore, when combined, paired individuals did not grow faster than singletons (Figure 5b; ANOVA: df=1, F=0.292, $p>0.05$). Taken together, these results suggest that the fish need more than auditory, chemosensory, and visual cues to engage in competitive growth.

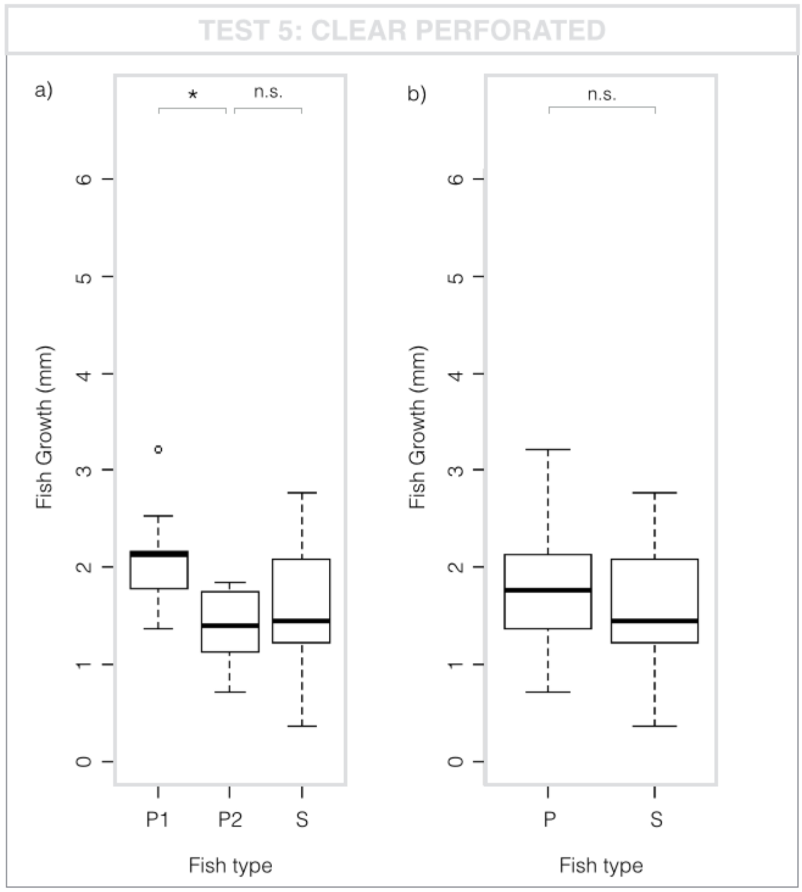

Figure 5. *(a) Growth of clownfish in Test 5 — Clear Perforated (n= 9 replicates) separated by fish type: largest in pair (P1); smallest in pair (P2); singleton (S); (b) Growth of clownfish in Test 5 — Clear Perforated (n=9 replicates) separated by fish type: paired individuals (P) and singleton (S). The bold band indicates the median, the box indicates the interquartile range, and the whiskers indicate ± 1.5*IQR.*

3.6 Synthetic Analysis

Turning to compare growth of P individuals among tests, on average, NCB individuals grew 4.07 ± 1.27mm, OS individuals grew 1.86 ± 1.09mm, OP individuals grew 1.54 ± 1.18mm, CS individuals grew 1.68 ± 0.85mm, and CP individuals grew 1.74 ± 0.61mm. The ANOVA revealed that test type was a significant predictor of growth of P individuals (Figure 6a; ANOVA=4, F=20.23, $p<0.001$). The post hoc tests revealed that NCB individuals grew faster than individuals of all other tests (all Tukey's HSD: $p<0.001$) and individuals in no other tests grew no differently from each other (all Tukey's HSD: $p>0.05$.

Finally, turning to compare growth of S individuals among tests, on average, NCB individuals grew 1.61 ± 0.94mm, OS individuals grew 1.28 ± 0.66mm, OP individuals grew 2.01 ± 0.80mm, CS individuals grew 1.47 ± 0.49mm, and CP individuals grew 1.60 ± 0.70mm. The ANOVA revealed that all singletons grew similar amounts (Figure 6b; ANOVA=4, F=1.11, $p>0.05$). The post hoc tests confirmed that no individuals of any test grew faster than any other test (all Tukey's HSD: $p>0.05$).

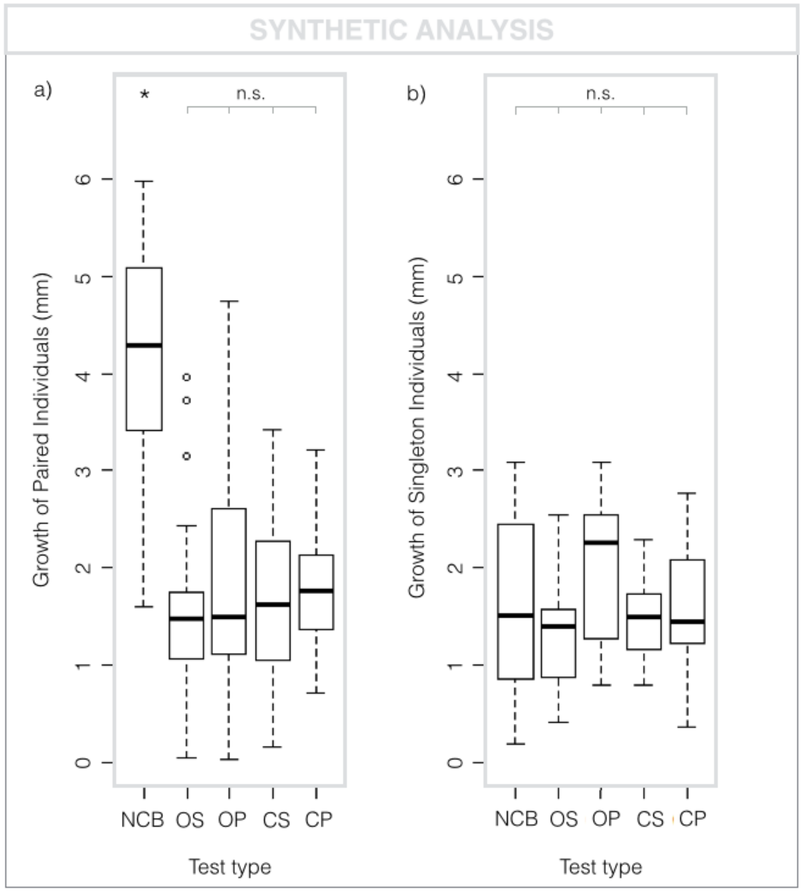

Figure 6. *(a) Growth of paired (P) individuals, separated by test (NCB, OS, OP, CS, CP); (b) Growth of all singleton (S) individuals separated by test (NCB, OS, OP, CS, CP). The bold band indicates the median, the box indicates the interquartile range, and the whiskers indicate ± 1.5*IQR.*

4. Discussion

In animal societies where size determines rank, individuals must be able to assess their own size and the size of other group members so that they can behave appropriately and avoid unnecessary conflict. This is especially true in societies where individuals adjust their growth and size in response to the size of others. Here, we use a series of laboratory experiments to show that juvenile clownfish likely require the use of mechanosensory (pressure and/or touch) cues to assess the size of conspecifics and engage in competitive growth. We found that when auditory, chemosensory, or visual cues were available (tests 2-5), but mechanosensory cues were not available, competitive growth did not occur (Figures 2-5). In contrast, we found that, when mechanosensory (pressure and/or touch) cues were available (test 1), one of the paired clownfish individuals grew faster than the other (Figure 1a), paired individuals grew faster than the singleton (Figure 1b), and the paired individuals grew significantly faster than paired individuals in any other trial (Figure 6), indicating that competitive growth did occur (e.g., see Huchard et al. 2016, Reed et al. 2019).

At first pass, one apparent inconsistency in the results is that, in tests 2-5, we found that, within the paired individuals, P1 often grows faster than P2, which is suggestive of competitive growth. However, when combining both P1 and P2 individuals, they did not grow faster than the solitary individual, S, which indicates that competitive growth did not occur. Therefore, tests 2-5 did not provide evidence of competitive growth. This apparent anomaly can be explained by the fact that we defined P1 as the largest individual in the pair and P2 as the smallest individual in the pair, so the size difference between P1 and P2 on its own is a methodological artefact. It is the combination of i) a difference in size between P1 and P2 in the same trial, ii) a difference in size between P and S in the same trial, and iii) a difference in size between P in one trial and P in another trial (while there is no difference between S in one trial and S in another trial) that provides the most compelling evidence for competitive growth.

Thus, it seems that clownfish need mechanosensory cues (pressure and/or touch), or mechanosensory cues in addition to other cues, to engage in competitive growth. Why? To decide whether to engage in competitive growth, individuals must be able to assess their own size and the size of others. In, *Amphiprion* sp. when individuals of similar size are in close proximity, conflict between individuals can be intense, and combatants sometimes fight until one or both are maimed or killed, (Allen 1972, Wong et al. 2016). In these contexts, when dominants are aggressive toward or chase subordinates (Fricke 1973), it is well-known that subordinates may perform a behavior called “head shaking” toward dominants (Allen 1972). The ethological literature has suggested that *Amphiprion* species may have evolved these threat and submissive behaviors to lessen or circumvent injury during intraspecific conflict (Allen 1972), but does not address why this particular submissive signal is maintained by selection. It seems plausible that this head shaking behavior, displaces water, conveying honest information on size (Johnstone and Grafen 1993) that can be sensed by the opposing clownfish using their lateral line or inner ear, enabling them to make decisions about whether to engage in conflict or competitive growth.

Our experiment suggests that mechanosensory (pressure and/or touch) cues, or mechanosensory cues in addition to other cues, are necessary for competitive growth, however the experiment cannot rule out the possibility that it is some other element of interaction permitted in test 1, e.g., swimming side by side and using visual cues, that might be eliciting competitive growth. To critically test the mechanosensory cue hypothesis, it would be interesting to do a gentamicin knockout (Faucher et al 2008, Van Trump et al 2010, Butler and Maruska 2015). Gentamicin is an antibiotic that can temporarily functionally block the neuromasts of the lateral line and inner ear from transmitting mechanosensory reception. A gentamicin knockout experiment would allow the fish to have proximity and engage in all other aspects of interaction associated with test 1, while negating the ability to detect mechanosensory cues via the lateral line and inner ear. To do such an experiment, the set up could be similar to our test 1, in which no cues are blocked by dividers. Gentamicin could then be added to the water while, allowing the fish to interact, providing a neat experimental test of the hypothesis that the fish need mechanosensory cues in order to engage in competitive growth.

Considering potential next steps to our experiment to understand how, at the physiological level, the fish are able to perform this behavior, we suggest looking into the hormonal mechanisms underlying competitive growth. Endocrine mechanisms might underlie the phenotypic changes in the growth rate of clownfish.

Some potential hormones could be arginine vasotocin, isotocin, sex steroids, and/or growth hormone (Peng and Peter 1997, Norbeck and Sheridan 2011, Kleszczynksa 2012, DeAngelis et al. 2017). The test set up would be similar to our test 1, in which no cues are blocked by dividers, except while feeding. After the month-long trial, clownfish could be euthanized and grouped by growth rate and fish type and combined in order to create a sample large enough to assay. Whole body ELISA assays could be used to analyze the presence of hormones in homogenized samples (Yeh et al. 2013, DeAngelis and Rhodes 2016). Our hypothesis is that the P1 individuals would have significantly different concentrations of the hormone than the P2 or S individuals.

Comparing our results to the known assessments of other organisms, we question if the same mechanism for growth regulation will be used in all taxa. It is known that pygmy swordtail fish use mechanosensory cues to assess size and recognize other males of their species (Morris et al 1995). The number of bites inflicted between males decreased significantly when they interacted with a familiar male. Small males would bite first during aggressive encounters, suggesting that males are learning about the relative size of opponents during aggressive encounters (Morris et al 1995). Prior research has studied auditory, chemical, and visual cues in size assessment (Myrberg et al. 1993, Lobel and Mann 1995, Hughes 1996, Connaughton et al 2000, Shine et al. 2003, Giaquinto and Volpato 2005, Colleye et al. 2009), but less work has been published on the influence of mechanosensory cues in size assessment. This is despite the fact that submissive quivering and head-shaking behaviors are common in fishes (Reddon et al. 2019) and regular physical stimulation of the outer hind femur induces the shift to gregarious behaviors in locusts (Simpson et al. 2001), and perhaps reflects our lack of familiarity with vibrational and mechanosensory signals as humans. We hypothesize that many taxa might be incorporating mechanosensory cues into their assessment of conspecifics.

5. Conclusions

This study is the first of its kind to investigate the sensory cues that vertebrates use when modifying their size and growth in response to social context. Other vertebrate systems in which this kind of behavior (phenotypic plasticity) occurs include meerkats (Russell et al. 2004, Huchard 2016), African cichlid (Hofmann et al. 1999), Lake Tanganyika cichlid (Heg et al 2004), mole-rats (Dengler-Crish and Catania 2007, Young and Bennett 2010), and tomato anemonefish (Hattori 1991). Using an integrative approach to determining the underlying mechanisms will generate new understandings into the phenomena of competitive growth. A mechanistic understanding has the potential to uncover trade-offs undiscoverable from investigations that demonstrate a relationship between environment and phenotypic response alone, leading to more rapid advances in the field.

Ethics

All work was approved by Boston University's Institutional Animal Care and Use Committee (protocol number TR202000002)

Data accessibility

All data have been uploaded with this manuscript and are available as electronic supplementary material.

Authors' contributions

L.D. participated in research design, data collection, data analysis, and writing of the manuscript. R.B. participated in data analysis and writing of the manuscript. E.S. participated in data collection. B.D. participated in data collection. P.B. participated in research design, data collection, data analysis, and writing of the manuscript.

Competing interests

We have no competing interests.

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