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Oral shelling within an adaptive radiation of pupfishes: Testing the adaptive function of a novel nasal protrusion and behavioural preference

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Abstract

Dietary specialization on hard prey items, such as mollusks and crustaceans, is commonly observed in a diverse array of fish species. Many fish consume these types of prey by crushing the shell to consume the soft tissue within, but a few fishes extricate the soft tissue without breaking the shell using a method known as oral shelling. Oral shelling involves pulling a mollusc from its shell and it may be a way to subvert an otherwise insurmountable shell defence. However, the biomechanical requirements and potential adaptations for oral shelling are unknown. Here, we test the hypothesis that a novel nasal protrusion is an adaptation for oral shelling in the durophagous pupfish (*Cyprinodon brontotheroides*). We first demonstrate oral shelling in this species and then predict that a larger nasal protrusion would allow pupfish to consume larger snails. Durophagous pupfish are found within an endemic radiation of pupfish on San Salvador Island, Bahamas. We took advantage of closely related sympatric species and outgroups to test: (a) whether durophagous pupfish shell and consume more snails than other species, (b) if F1 and F2 durophagous hybrids consume similar amounts of snails as purebred durophagous pupfish, and (c) if nasal protrusion size in parental and hybrid populations increases the maximum size of consumed snails. We found that durophagous pupfish and their hybrids consumed the most snails, but did not find a strong association between nasal protrusion size and maximum snail size consumed within the parental or F2 hybrid population, suggesting that the size of their novel nasal protrusion does not provide a major benefit in oral shelling. Instead, we suggest that the nasal protrusion may increase feeding efficiency, act as a sensory organ, or is a sexually selected trait, and that a strong feeding preference may be most important for oral shelling.

KEYWORDS
adaptive radiation, craniofacial, durophagy, foraging, novelty, performance, speciation

1 | INTRODUCTION

Dietary specialization is thought to be one way to reduce competition for a food source or to forage more optimally (Futuyman & Moreno, 1988; Pyke, 1984; Robinson & Wilson, 1998). One form of dietary specialization, especially among fishes, is the increased consumption of hard-shelled prey items, such as mollusks and crustaceans (hereafter referred to as durophagy), and both freshwater and marine fishes include durophagous specialists. There are two main ways that fish consume hard-shelled prey items. First, fish may crush or break the outer shell to consume the soft tissue within. Some fishes, such as black carp (*Mylopharyngodon picesus*), pumpkinseed sunfish (*Lepomis gibbosus*), redear sunfish (*Lepomis microlophus*), black drum (*Pogonias cromis*), Florida pompano (*Trachinotus carolinus*) and the black margate (*Anisotremus surinamensis*), use their pharyngeal jaws to crush the shells of snails and other mollusks in order to consume them (Gidmark *et al*., 2015; Grubich, 2003; Lauder, 1983). Others, such as the striped burrfish (*Chilomycterus schoepfi*), use their fused oral teeth to manipulate and crush shells (Ralston & Wainwright, 1997; Winterbottom, 1974). The biomechanical constraints of crushing hard shells is well documented in fishes. For example, body mass (g), bite force (N) and pharyngeal jaw gape size are understood to limit the upper size of prey in the Caribbean hogfish (*Lachnolaimus maximus*), where larger fish generally produce both larger gapes and increased crushing force, allowing them to crush larger or thicker shells (Wainwright, 1987, 1991). Similarly, the upper prey size consumed by black carp is limited by: (a) the amount of force produced by its pharyngeal jaw closing muscle (*medial levator arcus branchialis V*) (Gidmark *et al*., 2013) and (b) the size of the pharyngeal jaw gape (Gidmark *et al*., 2015).

An alternative and much rarer method of consuming hard-shelled prey, primarily documented in cichlids endemic to Lake Malawi (*Metriaclima lanisticola*), Lake Victoria (*Haplochromis xenognathus*, *H. sauvagei*, and *Macropleurodus bicolor*), and Lake Edward (*H. concilians*, *H. erutus*, and *H. planus*), is to extract the soft tissue of the gastropod from its shell *via* wrenching or shaking, known as "oral shelling" (Lundeba *et al*., 2011; Madsen *et al*., 2010; Slootweg, 1987; Vranken *et al*., 2019). It is typically thought that oral shelling is a way to circumvent the force and pharyngeal gape size requirements for consuming large mollusks because oral shelling does not require a fish to break a mollusc's shell; however, very few studies have investigated oral shelling in general (but see: Slootweg, 1987; De Visser & Barel, 1996) nor have they investigated adaptations for oral shelling.

One possibility may be that fish use morphological adaptations to create a mechanical advantage during oral shelling. For example, one hypothesis is that the fleshy snout of *Labeotropheus* cichlids is used as a fulcrum, allowing fish to more easily crop algae from rocks *versus* the bite-and-twist method observed in other cichlid species (Conith *et al*., 2018; Konings, 2007), and specifically that increased snout depth may help create this mechanical advantage (Conith *et al*., 2019). A similar method may be used during oral shelling to amplify force while removing snails from their shells. Thus, we predicted that larger nasal fulcrums should provide greater mechanical advantage for successfully oral shelling larger prey.

The durophagous pupfish (*Cyprinodon brontotheroides*) is an excellent species for testing whether a novel morphological trait provides a mechanical advantage for oral shelling. Durophagous pupfish are found within an adaptive radiation of pupfish endemic to the hypersaline lakes of San Salvador Island, Bahamas, which also includes a generalist pupfish (*C. variegatus*) and a scale-eating pupfish (*C. desquamator*; Martin & Wainwright, 2011, 2013a). Geological evidence suggests that the hypersaline lakes of San Salvador Island, and thus the radiation itself, are less than 10,000 years old (Hagey & Mylroie, 1995; Martin & Wainwright, 2013a, 2013b). Phylogenetic evidence also indicates that: (a) generalist pupfish found outside San Salvador Island are outgroups to the entire San Salvador clade and (b) durophagous pupfish cluster near generalists from the same lake populations, indicating that there is extensive admixture between these young species (Lencer *et al*., 2017; Martin, 2016; Martin & Feinstein, 2014; Richards & Martin, 2017). Gut content analyses indicated that durophagous pupfish consume approximately 5 times the number of mollusks and crustaceans (specifically ostracods) as generalists and fewer shells, suggesting that durophagous pupfish may be orally shelling their prey (Martin & Wainwright, 2013b). In addition to their dietary specialization, durophagous pupfish also possess a novel nasal protrusion not observed in other pupfish species (Martin & Wainwright, 2013a). This nasal protrusion is a skeletal expansion of the maxilla and extends rostrally over the upper jaws (Hernandez *et al*., 2018). It is plausible that this nasal protrusion is an adaptation for oral shelling used by the durophage as a fulcrum.

We investigated oral shelling behaviour in the laboratory and tested if the nasal protrusion of durophagous pupfish is an adaptation for oral shelling. We measured snail consumption across six groups in the laboratory: outgroup generalists, generalists from San Salvador Island, scale-eaters, durophages, and F1 and F2 durophage hybrids (produced by crossing purebred durophages and generalists in the laboratory). If the novel nasal protrusion is adapted for oral shelling, we expected that durophages would consume significantly more snails than generalists and scale-eaters. We explicitly took advantage of the ease of hybridization in this system to test predictions about the underlying genetics of the nasal protrusion and snail-eating behaviour using F1 and F2 hybrids. If the nasal protrusion or snail-eating behaviour is an additive trait, then we expected that F1 hybrids would show intermediate snail consumption and intermediate nasal protrusion size between the parental species, and that F2 hybrids would show greater variation in snail consumption and nasal protrusion size compared to parental species. Finally, we also investigated the relationship between nasal protrusion size and snail-shelling performance by asking if individuals with larger nasal protrusions could consume larger snails in laboratory-reared populations of both durophages and F2 hybrids. Again, we took advantage of F2 hybrids because we could test a wider variety of nasal protrusion sizes and because recombination may have broken up the association between nasal protrusion size and snail-eating behaviour in the F2 generation.

Ultimately, we found that, contrary to our predictions, purebred durophages and F1 and F2 hybrids all shelled significantly more snails than other pupfish species and we did not find evidence that larger nasal protrusion enabled durophages to consume larger snails. Instead, we discuss alternative explanations for the novel nasal protrusion such as a putative function in foraging efficiency, sexual selection, olfaction, or increased area for superficial neuromasts.

2 | MATERIALS AND METHODS

2.1 | Collection and care

During the summer of 2017, we used seine nets to collect generalist, durophage, and scale-eater pupfishes from Crescent Pond (24.113102,

−74.458204), Little Lake (24.101137, −74.482333), Osprey Lake (24.111895, −74.465260), and Oyster Pond (24.108591, −74.462730), all on San Salvador Island, Bahamas. We also collected generalist pupfish from Lake Cunningham (25.060154, −77.405679; Nassau, Bahamas) to use in outgroup comparisons. We transported fish back to the University of North Carolina, Chapel Hill, where they were maintained in mixed-sex stock tanks (37–75 l) in approximately 26°C water at approximately 5–10 parts per thousand salinity (Instant Ocean salt mix). In the laboratory, we produced F1 and F2 hybrid offspring using snail-eater and generalist parents. Wild-caught individuals were also allowed to breed and produced F1–F3 purebred offspring. Hybrid and purebred offspring were used in our feeding assays. We fed all fish a diet of commercial pellet foods, frozen bloodworms and mysis shrimp daily.

We also maintained a colony of freshwater sinistral snails (*Physella* sp.). We kept snails in a 7 l stock tank containing the same water used in pupfish tanks. All snails were acclimated to 5–10 parts per thousand salinity for at least 48 h before being used in a feeding trial. We fed snails a diet of bloodworms every 48 h. We ran multiple control trials without fish alongside feeding trials to track natural snail mortality rates.

2.2 | Morphological measurements

We measured the standard length of each fish by measuring the distance from the tip of the upper jaw to the posterior end of the hypural plate. We also measured nasal protrusion size for a subset of fish (9 generalists, 50 durophages, 17 F1 hybrids and 62 F2 hybrids) using image processing software (Schindelin *et al*., 2012). Scale-eating pupfish do not exhibit even marginal nasal protrusion, and therefore we did not include them in this analysis. We measured fish nasal protrusion size by drawing a tangent line aligning the most anterior dorsal point of the premaxilla with the neurocranium and measuring a perpendicular line at the deepest part of the nasal region (Figure 1c).

2.3 | Feeding assay

We quantified the number of snails consumed by all three species of pupfish and hybrids using feeding assays. Prior to a feeding assay, fish were removed from stock tanks and isolated in 2 l trial tanks which contained one synthetic yarn mop to provide cover for the fish. We allowed fish to acclimate in trial tanks for at least 12 h before the start of a feeding assay. After the acclimation time, we haphazardly chose five snails from our snail stock tank and added them to each feeding assay tank. We added one bloodworm to each tank to ensure that even fish which did not consume any snails had an adequate diet. Fish were allowed to feed freely on snails for 48 h with no additional food source. At the end of the 48 h assay period fish were removed from trial tanks, photographed and placed back into mixed-sex stock tanks. We then recorded the number of snails that were consumed (empty shells remaining) and unconsumed. Finally, we measured the size of each snail shell from the anterior tip of the shell's aperture to farthest tip of the spire (mm) using digital calipers and image processing software. In total, we measured feeding success for 13 outgroup generalists, 20 generalists, 55 durophages, 20 scale-eaters, 25 F1 hybrids, and 63 F2 hybrids. We sampled purebred durophages and F2 hybrids more densely (*i.e*., testing all available individuals from our laboratory colony) because we anticipated needing increased power to detect how continuous variation in nasal protrusion size affected snail consumption compared to the power required to detect differences among species. Out of the 196 trials, only 11 finished the trial period with four snail shells instead of the given five, suggesting that at most 3.5% of snail consumption involved also eating the shell.

2.4 | Data analyses

2.4.1 | No differences between fully consumed and partially consumed snails

We noticed that a portion of the snails were only partially consumed (*i.e*., part of the snail tissue remained in the shell *versus* a completely empty shell after 48 h) and therefore used a generalized linear mixed model (GLMM) with a binomial response distribution to determine if partially consumed snails should be analysed separately from fully consumed snails. We included: (a) whether snails were fully or partially consumed as the response variable (binomial data), (b) species designation as a fixed effect, (c) population and fish ID as random effects, and (d) log standard length as a covariate. We found that the pattern of partially and fully consumed snails did not vary across species ($\chi^2 = 2.73$, d.f. = 5, $P = 0.74$), and therefore included all partially consumed snails in the general "consumed" category for the remainder of our analyses.

2.4.2 | Statistical analysis

We used a linear mixed model to investigate the relationship between nasal protrusion distance and species. For this analysis we used a subset of our data which includes 9 generalists, 50 durophages, 17 F1 hybrids, and 62 F2 hybrids. Our model included: (a) log nasal protrusion size as the response variable, (b) species designation, log standard length and their interaction as fixed effects, and (c) population as a random effect. We also used Tukey's HSD to make *post hoc* comparisons across species.

We used a GLMM with a negative binomial distribution to explore whether the number of snails consumed varied among species. We included: (a) whether snails were consumed or unconsumed as the response variable (binomial data), (b) species designation as a fixed effect, (c) population and fish ID as random effects, and (d) log standard length as a covariate. We made additional *post hoc* comparisons between groups using Tukey's HSD.

We used a linear mixed model to determine if the size of snails varied by whether they were consumed or unconsumed and whether that varied among species. We included: (a) snail size (mm) as the response variable, (b) whether snails were consumed or unconsumed, species designation and their interaction as fixed effects,

(c) population and fish ID as random effects, and (d) log standard length as a covariate. We made additional *post hoc* comparisons between groups using contrasts and a false discovery rate correction.

Finally, we investigated if nasal protrusion distance affected the maximum size snail an individual could consume as an estimate of snail-shelling performance. For this analysis we only considered purebred durophages and F2 hybrids (separately) as they had the largest observed variance in nasal protrusion size and only included individuals that consumed at least one snail during the feeding trial. For each group, we used a linear model with: (a) the size of the largest consumed snail for each individual as the response variable, (b) log nasal protrusion size, log standard size, and their interaction as fixed effects, and (c) the residuals from a linear model investigating the relationship between snail size and nasal protrusion size as a covariate. We included this additional covariate because we found a strong positive relationship between mean snail size provided during trials and nasal protrusion in both purebred durophages (Linear Model: $P = 1.72 \times 10^{-9}$, adjusted $R^2 = 0.14$) and F2 hybrids (LM: $P = 5.58 \times 10^{-10}$, adjusted $R^2 = 0.12$), and wanted to account for this variation in the model (Supporting Information Figure S2). This variation reflected our attempt to provide some larger snails in trials with larger fish to better assess performance. We additionally included the random effect of population in our durophage model.

2.4.3 | Ethical statement

This study was conducted with the approval of the Animal Care and Use Committee of the University of North Carolina, Chapel Hill, NC, USA (protocol# 15–179.0). All wild fish were collected with a research and export permit from the Bahamas BEST commission, renewed annually since 2011.

3 | RESULTS

3.1 | Nasal protrusion size does not vary between purebred durophages and hybrids

Our linear mixed model indicated that nasal protrusion size is significantly associated with log standard length ($\chi^2 = 27.63$, d.f. = 1, $P = 1.47 \times 10^{-7}$; Supporting Information Figure S1), but that this relationship does not vary between purebred and hybrid durophages ($\chi^2 = 3.22$, d.f. = 3, $P = 0.36$). *Post hoc* analysis indicated that generalists had smaller nasal protrusions than durophages ($P < 0.0001$) and F1 hybrids ($P = 0.016$; Figure 1a).

3.2 | Purebred durophages and their hybrids consume the most snails

We found that species designation was a significant predictor for the number of snails an individual consumed (GLMM; $\chi^2 = 35.61$, d.f. = 5, $P = 1.129 \times 10^{-6}$). Specifically, we found that durophages, F1 hybrids, and F2 hybrids consumed more snails than the generalist outgroup population (Lake Cunningham, New Providence Island, Bahamas) and scale-eating pupfish (Figure 1b). Durophages, F1 hybrids, and F2 hybrids also consumed twice as many snails as generalists, but this difference was not significant.

3.3 | Consumed snails were larger than unconsumed snails

In general, we found that the size of snails varied (a) by whether they were consumed ($\chi^2 = 4.002$, d.f. = 1, $P = 0.045$) and (b) across species ($\chi^2 = 24.79$, d.f. = 5, $P = 0.00015$). Specifically, we found that consumed snails were on average 0.12 mm larger than unconsumed snails ($P = 0.046$). Generalists and scale-eaters received snails that were approximately 17% larger than other groups (generalists $P = 0.016$, scale-eaters $P = 0.02$; Figure 1d). Although this was unintentional due to the available size distributions of snails in our colony over the 10 month course of the feeding trials, we believe that it did not introduce a significant bias because (a) larger snails were more likely to be consumed (in fact there was only an 8% difference between the mean size of snail given to generalists and scale-eaters *versus* the mean size of consumed snails) and (b) generalists and scale-eaters were excluded from analyses which examined how nasal protrusion affected a fish's ability to consume snails.

3.4 | Nasal protrusion size did not significantly increase the maximum snail size consumed

We found no effect of log nasal protrusion size, log standard length, or their interaction on the size of the largest consumed snail for either durophages ($P_{\log(\text{nasalprotrusionsize})} = 0.49$, $P_{\log(\text{standardlength})} = 0.61$, $P_{\text{interaction}} = 0.56$; Figure 2a) or F2 hybrids ($P_{\log(\text{nasalprotrusionsize})} = 0.83$, $P_{\log(\text{standardlength})} = 0.66$, $P_{\text{interaction}} = 0.91$; Figure 2b).

4 | DISCUSSION

We present the first strong evidence in any cyprinodontiform fish that the durophagous pupfish is an oral sheller, shaking snails free from their shells rather than crushing or ingesting the whole shell. This is consistent with their notably non-molariform pharyngeal jaws relative to generalists and snail-crushing species (Figure 3). We then tested the hypothesis that the durophagous pupfish's novel nasal protrusion is an adaptation for removing snails from their shells, potentially functioning as a fulcrum. We predicted that durophagous pupfish would (a) consume more snails than other groups and (b) consume larger snails than other groups. We found that both durophages and their F1 and F2 hybrid offspring consumed the most snails compared to other groups (Figure 1b), indicating that any substantial amount of durophagous genetic ancestry increases the number of snails consumed over a 48 h feeding trial. However, contrary to our

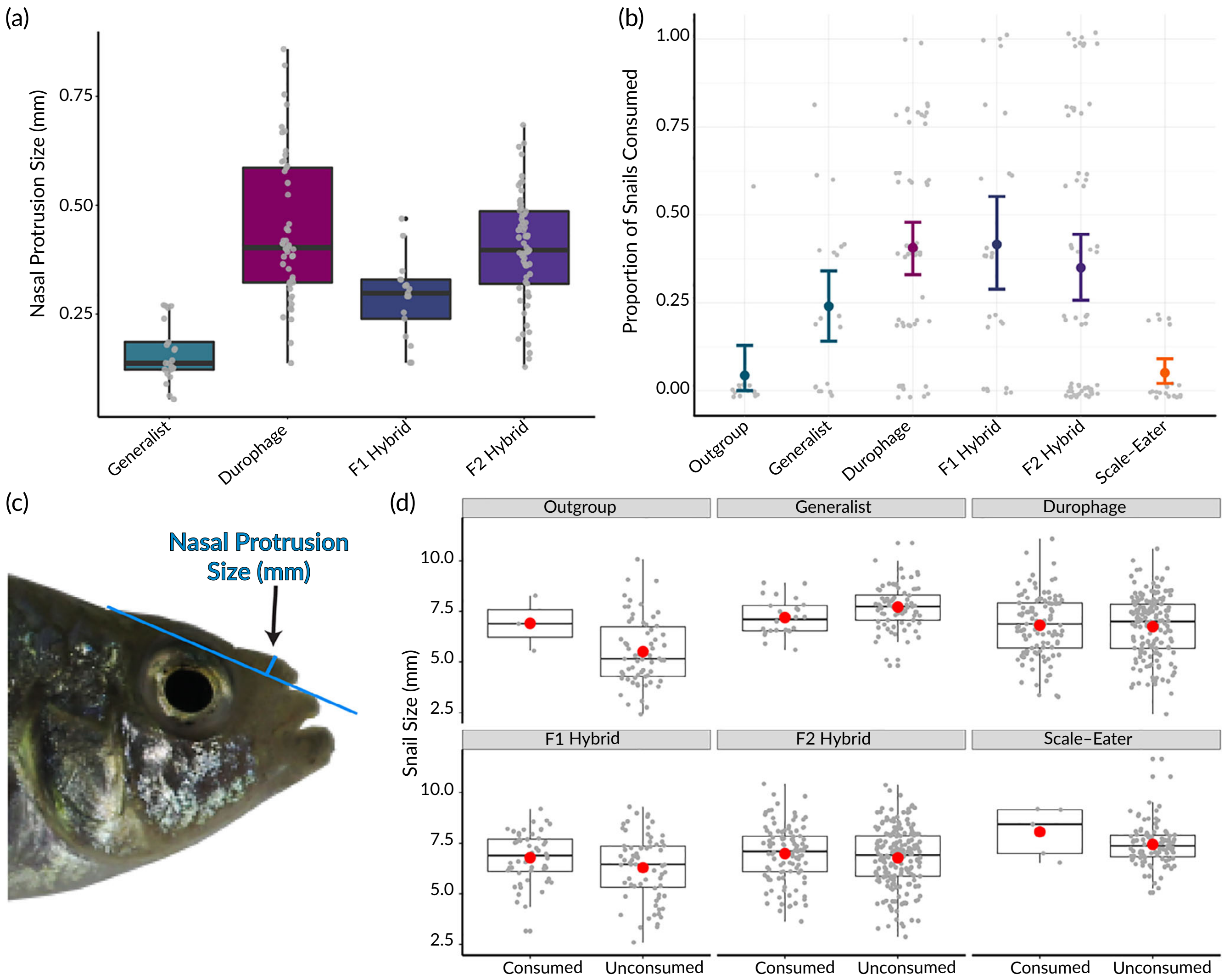

FIGURE 1 Snail consumption, nasal protrusion size and snail size by species. (a) Variation in nasal protrusion size across pupfish groups. Grey dots represent individual fish. (b) Proportion of snails consumed across six groups of pupfish. Coloured dots represent mean proportion and error bars represent 95% confidence intervals (bootstrapping: 1000 iterations). (c) Visualization of how nasal protrusion size was measured (pictured: durophagous pupfish). (d) Visualization of the size of consumed and unconsumed snails for each species. Grey dots represent individual snails and red dots represent the mean snail size

expectations, we found no significant evidence that larger nasal protrusions within hybrid or parental durophagous pupfish populations enabled the fish to consume larger snails (Figure 2).

4.1 | Durophages have a stronger behavioural preference for snails compared to other species

One explanation for the observed pattern is that durophagous pupfish have a stronger preference for snails which is independent from their novel nasal protrusion. We see some support for this within our data. Generalist pupfish from San Salvador Island consumed significantly more snails than a generalist population found outside of the radiation on New Providence Island, and even consumed statistically similar amounts of snails as purebred durophages despite having much smaller nasal protrusions (Figure 1a,b). It could be that extensive geneflow between generalists and durophages on San Salvador Island spread alleles for snail-eating preference throughout both pupfish species (Martin & Feinstein, 2014). Alternatively, the common ancestor of durophages and generalists may have had a strong preference for snails (Martin & Feinstein, 2014; Richards & Martin, 2017). The increased aggression of both male and female durophages toward conspecifics by potentially alternative genetic pathways to scale-eaters, as shown in a recent study (St. John *et al*., 2019), could also be associated with their stronger preference for aggressively attacking snails to flip them over before gripping the body of the snail in their oral jaws and shaking them free from their shells (Supporting Information Video SS1).

Liem's hypothesis and subsequent work has long supported the idea that morphological specialization need not coincide with trophic

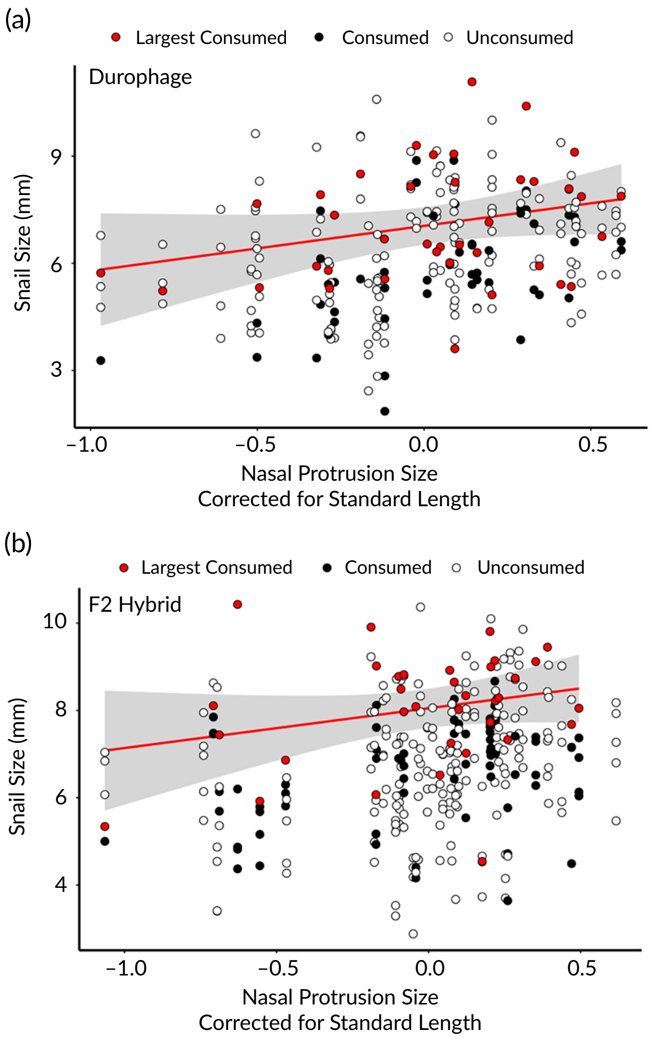

FIGURE 2 The maximum prey size a pupfish can consume was not affected by nasal protrusion size. The *x* axis shows nasal protrusion size corrected for standard length, while the *y* axis shows snail size (mm). Red dots show the size of largest consumed snail from each trial, the red line represents the linear model describing the relationship between nasal protrusion size and the largest consumed snails, and the grey area represents the 95% confidence interval. Closed circles show the size of other snails that were consumed during trials; open circles show the size of unconsumed snails

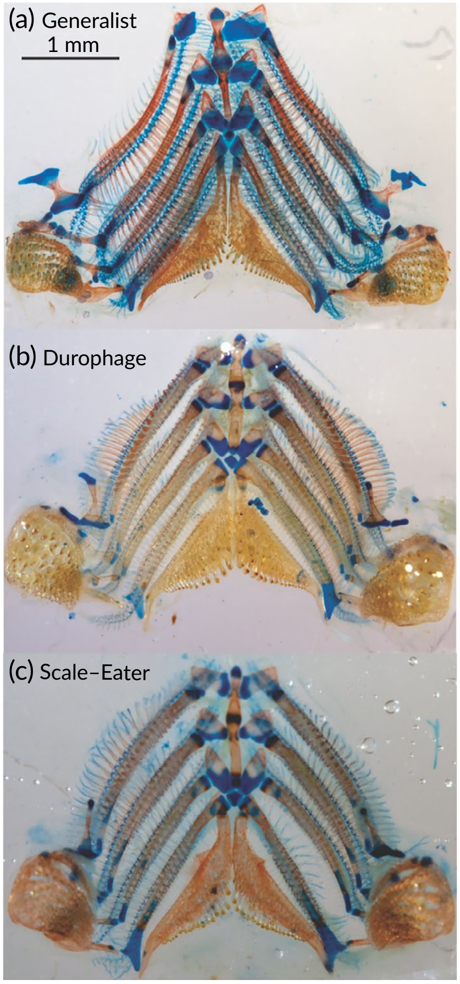

FIGURE 3 Branchial skeleton and pharyngeal teeth of all three San Salvador Island species. Image of the dissected branchial skeleton and pharyngeal jaws of (a) generalist, (b) durophage and (c) scale-eating pupfish. The scale (1 mm) is shown in (a) and is consistent across all three photos. From these three individuals, the representative durophage has pharyngeal teeth that are 50% longer and 75% wider than the generalist or scale-eating individuals

specialization, or *vice versa*. For example, *Tropheops tropheops* and *Metriaclima zebra*, two cichlids from Lake Malawi that are morphologically specialized for scraping algae, often fill a generalist ecological niche, consuming zooplankton, benthic invertebrates, and phytoplankton (Liem, 1978, 1980; McKaye & Marsh, 1983), particularly during periods of resource abundance (Martin & Genner, 2009). An analogous argument can be made for individual dietary specialization within a population (Bolnick *et al*., 2003). For example, Werner and Sherry (1987) found that individual Cocos Island finches specialize on a wide variety of taxa including crustacea, nectar, fruit, seeds, mollusks and lizards, and that individual dietary specialization is most likely driven by behavioural differences. Similarly, increased levels of individual specialization in sticklebacks are driven by shifts in forager density or intraspecific competition (Araújo *et al*., 2008; Svanbäck & Bolnick, 2005, 2007). Thus, individual specialization is often driven entirely by differences in behaviour, feeding preference, or other external factors and can be divorced from adaptive differences in morphology (Werner & Sherry, 1987).

4.2 | Alternative functions of the novel nasal protrusion

We investigated whether an increase in nasal protrusion size affected the maximum size snail an individual could consume (Figure 2). However, it could be that the novel nasal protrusion is related to feeding efficiency, for example in handling time per snail, or is a sensory organ used for locating snails more efficiently with potentially increased numbers of superficial neuromasts (Shibuya *et al*., 2020). There are several examples of nasal protrusions that are used for this purpose. The unique rostrums of paddlefish (Polydontidae), sturgeon (Acipenseridae), and sawfish (Pristidae) are all used as sensory organs, containing electroreceptors, lateral line canals, and even barbels for detecting prey items (Miller, 2006; Wueringer *et al*., 2012). The novel nasal protrusion of the durophagous pupfish may also be a sensory organ, but whether the nasal protrusion has an increased number of superficial neuromasts is still unknown.

Alternatively, the novel nasal protrusion may allow durophagous pupfish to orally shell snails more quickly, increasing their feeding efficiency. For example, Schluter (1993) documented that benthic sticklebacks with deep bodies, large mouths, and few short gill rakers were more efficient at consuming benthic prey items, while limnetic species of stickleback, with slender bodies, small mouths, and many long gill rakers, were more efficient at consuming limnetic prey items. Interestingly, Schluter (1993, 1995) also found that F1 hybrids had decreased efficiency feeding on both limnetic and benthic prey items which was primarily due to their intermediate phenotypes and suggested that reduced fitness in hybrids helps maintain species boundaries between benthic and limnetic species. It could be that the durophage F1 and F2 hybrids have similar preferences for gastropods, but cannot consume snails as efficiently due to their intermediate phenotype. However, we found no strong evidence suggesting that the nasal protrusion is adapted for oral shelling (Figure 2). Future work should investigate other traits that may be adaptive for oral shelling, such as the strength of the dorsal head of the maxilla which comprises the skeletal basis of the novel nasal protrusion, structural differences in the mandibular symphysis, coronoid process or the articular bones, which may all provide additional strength or stabilization during biting, or tooth variation in the durophage pharyngeal jaws (Figure 3). Indeed, there is subtle variation apparent in the pharyngeal teeth and jaws of durophages compared to other pupfish species (Figure 3) which has not been previously reported, suggesting that pharyngeal jaws may be adapted for processing hard-shelled prey.

4.3 | The novel nasal protrusion may be a sexually selected trait

Finally, the novel nasal protrusion may be unrelated to oral shelling and instead may be used in species recognition or mate preference functions. Exaggerated traits, like the novel nasal protrusion in durophage pupfish, commonly arise *via* sexual selection. For example, forceps size in earwigs (Simmons & Tomkins, 1996), major claw size in fiddler crabs (Rosenberg, 2002) and the size of the sword tail ornament present in swordtail fish (Rosenthal & Evans, 1998) are all thought to be sexually selected traits. Two commonly invoked hallmarks of a sexually selected trait are (a) allometric scaling compared to body size and (b) that the trait is sexually dimorphic (Kodric-Brown *et al*., 2006; Kodric-Brown & Brown, 1984; Shingleton & Frankino, 2013). In pupfish, there is a weak positive relationship between standard length and nasal protrusion size observed for generalists (Supporting Information Figure S1A, $\text{generalist}_{\text{slope}} = 0.35$). Generalist pupfish mostly likely resemble the most recent common ancestor for the radiation, making the observed slope a good null expectation for how nasal protrusion size should scale with body size in pupfish. In durophages, we observe much stronger positive allometry of the nasal protrusion (Supporting Information Figure S1B, $\text{durophage}_{\text{slope}} = 0.93$), in which large durophage individuals have nasal protrusion sizes more than twice as large as those in large generalists. However, we found no significant difference in nasal protrusion size between male and female durophages when accounting for these size differences (linear model, $P = 0.96$).

5 | CONCLUSION

In conclusion, we did not find evidence to support that the novel nasal protrusion observed in durophagous pupfish is adapted for consuming large snails. Instead, we found that purebred durophages and their F1 and F2 hybrids have stronger preferences for consuming snails than other species. We suggest that the novel nasal protrusion may be adapted for other aspects of oral shelling, such as feeding efficiency, or that variation in other traits, such as the pharyngeal jaws (Figure 3), may play a larger role in oral shelling. Alternatively, this may be an example of trophic specialization due to behavioural specialization (*i. e*., feeding preference).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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