

32 **Significance Statement**

33 Specialization on hard-shell prey items (i.e. durophagy) is a common dietary niche among fishes.

34 Oral shelling is a rare technique used by some durophagous fish to consume prey items like

35 snails; however, adaptations for oral shelling are still unknown. Here, we document the first

36 evidence of oral shelling in a cyprinodontiform fish, the durophagous pupfish (*Cyprinodon*

37 *brontotheroides*), and experimentally test whether its novel nasal protrusion is an adaptation for

38 oral shelling using hybrid feeding trials.

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55 **Abstract**

56 Dietary specialization on hard prey items, such as mollusks and crustaceans, is commonly
57 observed in a diverse array of fish species. Many fish consume these types of prey by crushing
58 the shell to consume the soft tissue within, but a few fishes extricate the soft tissue without
59 breaking the shell using a method known as oral shelling. Oral shelling involves pulling a
60 mollusk from its shell and may be a way to subvert an otherwise insurmountable shell defense.
61 However, the biomechanical requirements and potential adaptations for oral shelling are
62 unknown. Here, we test the hypothesis that a novel nasal protrusion is an adaptation for oral
63 shelling in a durophagous pupfish (*Cyprinodon brontotheroides*). We first demonstrate oral
64 shelling in this species and then predicted that a larger nasal protrusion would allow pupfish to
65 consume larger snails. Durophagous pupfish are found within an endemic radiation of pupfish on
66 San Salvador Island, Bahamas. We took advantage of closely related sympatric species and
67 outgroups to test: 1) whether durophagous pupfish shell and consume more snails than other
68 species, 2) if F1 and F2 durophagous hybrids consume similar amounts of snails as purebred
69 durophagous pupfish, and 3) to determine if nasal protrusion size in parental and hybrid
70 populations increases the maximum diameter snail consumed. We found that durophagous
71 pupfish and their hybrids consumed the most snails, but did not find a strong association between
72 nasal protrusion size and maximum snail size consumed within the parental or F2 hybrid
73 population, suggesting that the size of their novel nasal protrusion does not provide a major
74 benefit in oral shelling. Instead, we suggest that nasal protrusion may increase feeding efficiency,
75 act as a sensory organ, or is a sexually selected trait, and that a strong feeding preference may be
76 most important for oral shelling.

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78 **Introduction**

79 Dietary specialization is thought to be one way to reduce competition for a food source or to
80 forage more optimally (Pyke 1984; Futuyman and Moreno 1988; Robinson and Wilson 1998).
81 One form of dietary specialization, especially among fishes, is the increased consumption of
82 hard-shelled prey items, such as mollusks and crustaceans (hereafter referred to as durophagy),
83 and both freshwater and marine fishes include durophagous specialists. There are two main ways
84 that fish consume hard-shelled prey items: First, fish may crush or break the outer shell to
85 consume the soft tissue within. Some fishes, such as black carp (*Mylopharyngodon picesus*),
86 pumpkinseed sunfish (*Lepomis gibbosus*), redear sunfish (*Lepomis microlophus*), black drum
87 (*Pogonias cromis*), Florida pompano (*trachinotus carolinus*), and the black margate
88 (*Anisotremus surinamensis*), use their pharyngeal jaws to crush the shells of snails and other
89 mollusks in order to consume them (Lauder 1983; Grubich 2003; Gidmark et al. 2015). Others,
90 such as the striped burrfish (*Chilomycterus schoepfi*), use their fused oral teeth to manipulate and
91 crush shells (Winterbottom 1974; Ralston and Wainwright 1997). The biomechanical constraints
92 of crushing hard shells is well documented in fish. For example, body mass (g), bite force (N),
93 and pharyngeal jaw gape size are understood to limit the upper size of prey in the Caribbean
94 hogfish (*Lachnolaimus maximus*), where larger fish generally produce both larger gapes and
95 increased crushing force, allowing them to crush larger or thicker shells (Wainwright 1987,
96 1991). Similarly, the upper prey size consumed by black carp is limited by 1) the amount of
97 force produced by its pharyngeal jaw closing muscle (*medial levator arcus branchialis V*)
98 (Gidmark et al. 2013) and 2) the size of the pharyngeal jaw gape (Gidmark et al. 2015).

99 An alternative and much rarer method of consuming hard-shelled prey, primarily
100 documented in cichlids endemic to Lake Malawi (*Metriaclima lanisticola*), Lake Victoria

101 (*Hapochromis xenognathus*, *H. sauvagei* and *Macropleuroodus bicolor*), and Lake Edward (*H.*
102 *concilians* sp. nov., *H. erutus* sp. nov. and *H. planus* sp. nov), is to extract the soft tissue of the
103 gastropod from its shell via wrenching or shaking, known as ‘oral shelling’ (Slootweg 1987;
104 Madsen et al. 2010; Lundebea et al. 2011; Vranken et al. 2019). It is typically thought that oral
105 shelling is a way to circumvent the force and pharyngeal gape size requirements for consuming
106 large mollusks because oral shelling does not require a fish to break a mollusk’s shell; however,
107 very few studies have investigated oral shelling in general (but see: Slootweg 1987; De Visser
108 and Barel 1996) nor have they investigated adaptations for oral shelling.

109 One possibility may be that fish use morphological adaptations to create a mechanical
110 advantage during oral shelling. For example, one hypothesis is that the fleshy snout of
111 *Labeotropheus* cichlids is used as a fulcrum, allowing fish to more easily crop algae from rocks
112 versus the bite-and-twist method observed in other cichlid species (Konings 2007; Conith et al.
113 2018), and specifically that increased snout depth may help create this mechanical advantage
114 (Conith et al. 2019). A similar method may be used during oral shelling to amplify force while
115 removing snails from their shells. Thus, we predicted that larger nasal fulcrums should provide
116 greater mechanical advantage for successfully oral shelling larger prey.

117 The durophagous pupfish (*Cyprinodon brontotheroides*) is an excellent species for testing
118 whether a novel morphological trait provides a mechanical advantage for oral shelling.
119 Durophagous pupfish are found within an adaptive radiation of pupfish endemic to the
120 hypersaline lakes of San Salvador Island, Bahamas, which also includes a generalist pupfish (*C.*
121 *variegatus*) and a scale-eating pupfish (*C. desquamator*; Martin and Wainwright 2011, 2013a).
122 Geological evidence suggests that the hypersaline lakes of San Salvador Island, and thus the
123 radiation itself, are less than 10,000 years old (Hagey and Mylroie 1995; Martin and Wainwright

124 2013*b*, 2013*a*). Phylogenetic evidence also indicates that: 1) generalist pupfish found outside San
125 Salvador Island are outgroups to the entire San Salvador clade, and 2) that durophagous pupfish
126 cluster near generalists from the same lake populations, indicating that there is extensive
127 admixture between these young species (Martin and Feinstein 2014; Martin 2016; Lencer et al.
128 2017; Richards and Martin 2017). Gut content analyses indicated that durophagous pupfish
129 consume approximately 5.5 times the number of mollusks and crustaceans (specifically
130 ostracods) as generalists and fewer shells, suggesting that durophagous pupfish may be orally
131 shelling their prey (Martin and Wainwright 2013*b*). In addition to their dietary specialization,
132 durophagous pupfish also possess a novel nasal protrusion not observed in other pupfish species
133 (Martin and Wainwright 2013*a*). This nasal protrusion is an expansion of the maxilla, and
134 extends rostrally over the upper jaws (Hernandez et al. 2018). It is plausible that this nasal
135 protrusion is an adaptation for oral shelling used by the durophage as a fulcrum.

136 We investigated oral-shelling behavior in the laboratory and tested if the nasal protrusion
137 of durophagous pupfish is an adaptation for oral shelling. We measured snail consumption across
138 6 groups in the laboratory: outgroup generalists, generalists from San Salvador Island, scale-
139 eaters, durophages, and F1 and F2 durophage hybrids (produced by crossing purebred
140 durophages and generalists in the lab). If the novel nasal protrusion is adapted for oral shelling,
141 we expected that durophages would consume significantly more snails than generalists and scale-
142 eaters. We also expected that F1 hybrids would show intermediate snail consumption between
143 the parental species and that F2 hybrids would show greater variation in snail consumption
144 compared to parental species. To directly tie nasal protrusion size to snail-shelling performance,
145 we also investigated whether individuals with larger noses could consume larger snails in lab-
146 reared populations of both durophages and F2 hybrids. Ultimately, we found that, contrary to our

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

152

153 **Methods**

154 *Collection and Care*

155 During the summer of 2017, we used seine nets to collect generalist, durophage, and scale-eater
156 pupfishes from Crescent Pond, Little Lake, Osprey Lake, and Oyster Pond (San Salvador Island,
157 Bahamas). We transported fish back to the University of North Carolina, Chapel Hill, where they
158 were maintained in mixed-sex stock tanks (37-75 l) in approximately 26° C water at
159 approximately 5-10 ppt salinity (Instant Ocean salt mix). In the lab, we produced F1 and F2
160 hybrid offspring using snail-eater and generalist parents. Wild caught individuals were also
161 allowed to breed and produced F1-F3 purebred offspring. Hybrid and purebred offspring were
162 used in our feeding assays. We fed all fish a diet of commercial pellet foods, frozen bloodworms,
163 and mysis shrimp daily.

164 We also maintained a colony of freshwater sinistral snails (*Physella sp.*). We kept snails
165 in a 7 liter stock tank containing the same water used in pupfish tanks. All snails were acclimated
166 to 5-10 ppt salinity for at least 48 hours before being used in a feeding trial. We fed snails a diet
167 of bloodworms every 48 hours. We ran multiple control trials without fish alongside feeding
168 trials to track natural snail mortality rates.

169

170 *Morphological Measurements*

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

179
180 *Feeding Assay*

181 We quantified the number of snails consumed by all three species of pupfish and hybrids using
182 feeding assays. Prior to a feeding assay, fish were removed from stock tanks and isolated in 2L
183 trial tanks which contained one synthetic yarn mop to provide cover for the fish. We allowed fish
184 to acclimate in trial tanks for at least 12 hours before the start of a feeding assay. After the
185 acclimation time, we haphazardly chose 5 snails from our snail stock tank and added them to
186 each feeding assay tank. We added one bloodworm to each tank to ensure that even fish which
187 did not consume any snails had an adequate diet. Fish were allowed to feed freely on snails for
188 48 hours with no additional food source. At the end of the 48-hour assay period fish were
189 removed from trial tanks, photographed, and placed back into mixed-sex stock tanks. We then
190 recorded the number of snails that were consumed (empty shells remaining) and unconsumed.
191 Finally, we measured the size of each snail shell from the anterior tip of the shell's aperture to
192 farthest tip of the spire (mm) using digital calipers and image processing software . In total, we
193 measured feeding success for 13 outgroup generalists, 20 generalists, 55 durophages, 20 scale-

194 eaters, 25 F1 hybrids, and 63 F2 hybrids. Out of the 196 trials, only 11 finished the trial period
195 with four snail shells instead of the given five, suggesting that at most 3.5% of snail consumption
196 involved also eating the shell.

197

198 *Data Processing*

199 *No differences between fully consumed and partially consumed snails*

200 We noticed that a portion of the snails were only partially consumed (i.e. part of the snail tissue
201 remained in the shell versus a completely empty shell after 48 hours) and therefore used a
202 generalized linear mixed model (GLMM) with a binomial response distribution to determine if
203 partially consumed snails should be analyzed separately from fully consumed snails. We
204 included 1) whether snails were fully or partially consumed as the response variable (binomial
205 data), 2) species designation as a fixed effect, 3) population and fish ID as random effects, and 4)
206 log standard length as a covariate. We found that the pattern of partially and fully consumed
207 snails did not vary across species ($\chi^2= 2.73$, $df=5$, $P=0.74$), and therefore included all partially
208 consumed snails in the general “consumed” category for the remainder of our analyses.

209

210 *Statistical Analysis*

211 We used a linear mixed model to investigate the relationship between nasal protrusion distance
212 and species. For this analysis we used a subset of our data which includes: 9 generalists, 50
213 durophages, 17 F1 hybrids, and 62 F2 hybrids. Our model included 1) log nasal protrusion size
214 as the response variable, 2) species designation, log standard length, and their interaction as fixed
215 effects, and 3) population as a random effect. We also used Tukey’s HSD to make *post hoc*
216 comparisons across species.

217 We used a GLMM with a negative binomial distribution to explore whether the number
218 of snails consumed varied between species. We included 1) whether snails were consumed or
219 unconsumed as the response variable (binomial data), 2) species designation as a fixed effect, 3)
220 population and fish ID as random effects, and 4) log standard length as a covariate. We made
221 additional *post hoc* comparisons between groups using Tukey's HSD.

222 We used a linear mixed model to determine if the size of snails varied by whether they
223 were consumed or unconsumed and whether that varied between species. We included 1) snail
224 size (mm) as the response variable, 2) whether snails were consumed or unconsumed, species
225 designation, and their interaction as fixed effects, 3) population and fish ID as random effects,
226 and log standard length as a covariate. We made additional *post hoc* comparisons between
227 groups using contrasts and an FDR correction.

228 Finally, we investigated if nasal protrusion distance affected the maximum size snail an
229 individual could consume as an estimate of snail-shelling performance. For this analysis we only
230 considered purebred durophages and F2 hybrids (separately) as they had the largest observed
231 variance in nasal protrusion size and only included individuals that consumed at least one snail
232 during the feeding trial. For each group, we used a linear model with 1) the size of the largest
233 consumed snail for each individual as the response variable, 2) log nasal protrusion size, log
234 standard size, and their interaction as fixed effects, and 3) the residuals from a linear model
235 investigating the relationship between snail size and nasal protrusion size as a covariate. We
236 included this additional covariate because we found a strong positive relationship between mean
237 snail size provided during trials and nasal protrusion in both purebred durophages (LM: $P=1.72 \times$
238 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
239 account for this variation in the model (Figure S2). This variation reflected our attempt to

240 provide some larger snails in trials with larger fish to better assess performance. We additionally
241 included the random effect of population in our durophage model.

242

243 *Ethical Statement*

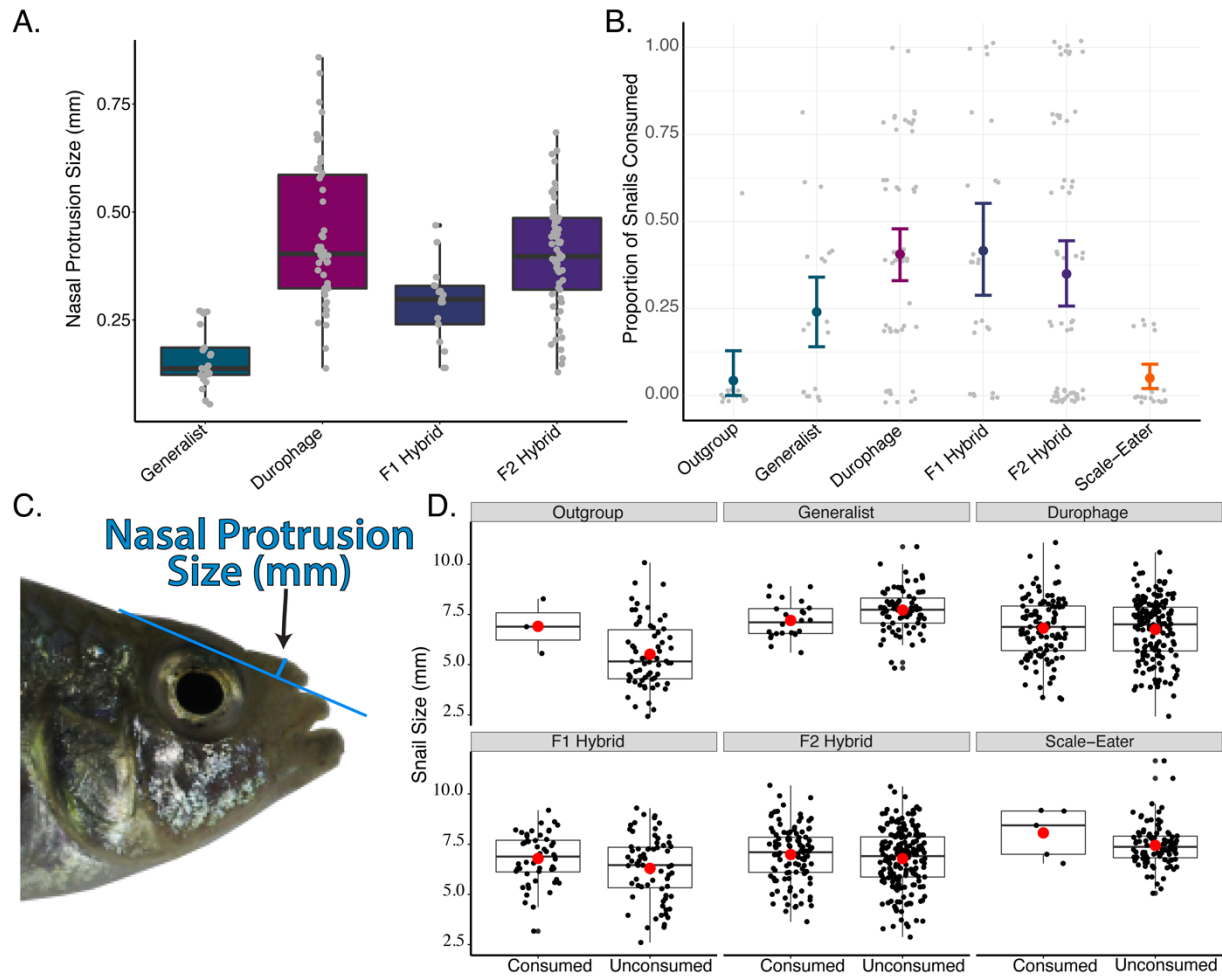
244 This study was conducted with the approval of the Animal Care and Use Committee of the
245 University of North Carolina, Chapel Hill, NC (protocol# 15–179.0). All wild fish were collected
246 with a research and export permit from the Bahamas BEST commission, renewed annually since
247 2011.

248

249 Results

250 *Nasal protrusion size does not vary between purebred durophages and hybrids*

251 Our linear mixed model indicated that nasal protrusion size is significantly associated with log
252 standard length ($\chi^2= 27.63$, $df=1$, $P=1.47 \times 10^{-7}$), but that this relationship does not vary between
253 purebred and hybrid durophages ($\chi^2= 3.22$, $df=3$, $P = 0.36$; Figure 1A & S1). *Post hoc* analysis
254 indicated that generalists had smaller noses than durophages ($P < 0.0001$) and F1 hybrids ($P =$
255 0.016).



256

257 **Figure 1. Snail consumption, nasal protrusion size, and snail size by species.** A) Variation in

258 nasal protrusion size across pupfish groups. Grey dots represent individual fish. B) Proportion of

259 snails consumed across six groups of pupfish. Colored dots represent mean proportion, and error

260 bars represent 95% confidence intervals (bootstrapping: 1,000 iterations). C) Visualization of

261 how nasal protrusion size was measured (pictured: durophagous pupfish). D) Visualization of the

262 size of consumed and unconsumed snails for each species. Black dots represent individual snails

263 and red dots represent the mean snail size.

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266

267 *Purebred durophages and their hybrids consume the most snails*

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

274

275 *Consumed snails were larger than unconsumed snails*

276 In general, we found that the size of snails varied 1) by whether they were consumed ($\chi^2= 4.002$,
277 $df=1$, $P=0.045$), and 2) across species ($\chi^2= 24.79$, $df=5$, $P=0.00015$; Figure S1). Specifically, we
278 found that consumed snails were on average 0.12 mm larger in diameter than unconsumed snails
279 ($P=0.046$). Generalists and scale-eaters received snails that were approximately 17% larger than
280 other groups (generalists: $P=0.016$; scale-eaters: $P=0.02$). Although this was unintentional due to
281 the available size distributions of snails in our colony over the ten month course of the feeding
282 trails, we believe that it did not introduce a significant bias because 1) larger snails were more
283 likely to be consumed (in fact there was only an 8% difference between the mean size of snail
284 given to generalists and scale-eaters vs the mean size of consumed snails) and 2) generalists and
285 scale-eaters were excluded from analyses which examined how nasal protrusion affected a fish's
286 ability to consume snails.

287

288 *Nasal protrusion size did not significantly increase the maximum snail size consumed*

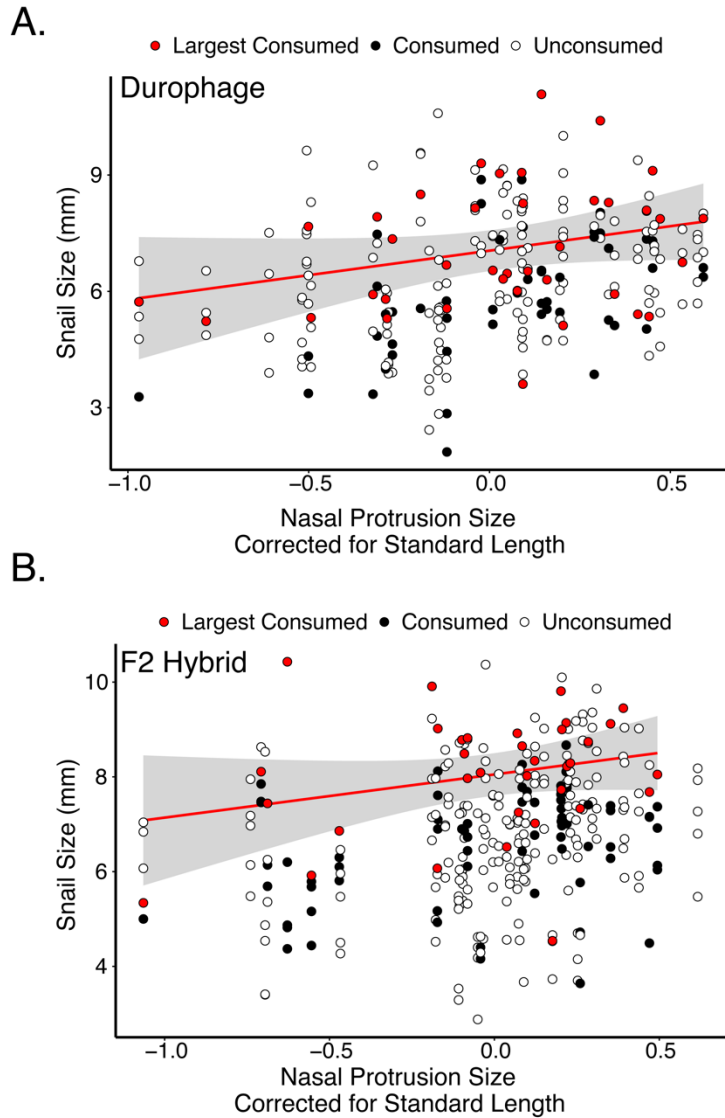
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290 We found no effect of log nasal protrusion size, log standard length, or their interaction on the
291 size of the largest consumed snail for either durophages ($P_{\log(\text{nasalprotrusionsize})}=0.49$,

292 $P_{\log(\text{standardlength})}=0.61, P_{\text{interaction}}=0.56$) or F2 hybrids ($P_{\log(\text{nasalprotrusionsize})}=0.83,$

293 $P_{\log(\text{standardlength})}=0.66, P_{\text{interaction}}=0.91$; Figure 2).

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296

297 **Figure 2. The maximum prey size a pupfish can consume was not affected by nasal**
298 **protrusion size.** The X-axis shows nasal protrusion size corrected for standard length while the
299 Y-axis shows snail size (mm). Red dots show the size of largest consumed snail from each trial,
300 the red line represents the linear model describing the relationship between nasal protrusion size
301 and the largest consumed snails, and the grey area represents 95% CI. Closed circles show the
302 size of other snails that were consumed during trials; open circles show the size of unconsumed
303 snails.

304 **Discussion**

305 We present the first strong evidence in any cyprinodontiform fish that the durophagous pupfish is
306 an oral sheller, shaking snails free from their shells rather than crushing or ingesting the whole
307 shell. This is consistent with their notably non-molariform pharyngeal jaws relative to generalists
308 and snail-crushing species (Figure 3). We then tested the hypothesis that the durophagous
309 pupfish's novel nasal protrusion is an adaptation for removing snails from their shells, potentially
310 functioning as a fulcrum. We predicted that durophagous pupfish would 1) consume more snails
311 than other groups, and 2) consume larger snails than other groups. We found that both
312 durophages and their F1 and F2 hybrid offspring consumed the most snails compared to other
313 groups (Figure 1B), indicating that any substantial amount of durophagous genetic ancestry
314 increases the number of snails consumed over a 48-hour feeding trial. However, contrary to our
315 expectations, we found no significant evidence that larger nasal protrusions within hybrid or
316 parental durophagous pupfish populations enabled the fish to consume larger snails (Figure 2).

317

318 *Durophages have a stronger behavioral preference for snails compared to other species*

319 One explanation for the observed pattern is that durophagous pupfish have a stronger preference
320 for snails which is independent from their novel nasal protrusion. We see some support for this
321 within our data. Generalist pupfish from San Salvador Island consumed significantly more snails
322 than generalists found outside of the radiation on New Providence Island, and even consumed
323 statistically similar amounts of snails as purebred durophages despite having much smaller nasal
324 protrusions (Figure 1A&B). It could be that extensive gene flow between generalists and
325 durophages on San Salvador Island spread alleles for snail-eating preference throughout both
326 pupfish species (Martin and Feinstein 2014). Alternatively, the common ancestor of durophages

327 and generalists may have had a strong preference for snails (Martin and Feinstein 2014; Richards
328 and Martin 2017). The increased aggression of both male and female durophages toward
329 conspecifics by potentially alternate genetic pathways to scale-eaters, as shown in a recent study
330 (St. John et al. 2019), could also be associated with their stronger preference for aggressively
331 attacking snails to flip them over before gripping the body of the snail in their oral jaws and
332 shaking them free from their shells (Supplemental Video 1).

333 Liem's hypothesis and subsequent work has long supported the idea that morphological
334 specialization need not coincide with trophic specialization, or *vice versa*. For example,
335 *Tropheops tropheops* and *Metriaclima zebra*, two cichlids from Lake Malawi that are
336 morphologically specialized for scraping algae often fill a generalist ecological niche, consuming
337 zooplankton, benthic invertebrates, and phytoplankton (Liem 1978, 1980; McKaye and Marsh
338 1983), particularly during periods of resource abundance (Martin and Genner 2009). An
339 analogous argument can be made for individual dietary specialization within a population
340 (Bolnick et al. 2003). For example, Werner and Sherry (1987) found that individual Cocos
341 Island finches specialize on a wide variety of taxa including crustacea, nectar, fruit, seeds,
342 mollusks, and lizards, and that individual dietary specialization was most likely driven by
343 behavioral differences. Similarly, increased levels of individual specialization in sticklebacks are
344 driven by shifts in forager density or intraspecific competition (Svanbäck and Bolnick 2005,
345 2007; Araújo et al. 2008). Thus, individual specialization is often driven entirely by differences
346 in behavior, feeding preference, or other external factors and can be divorced from adaptive
347 differences in morphology (Werner and Sherry 1987).

348

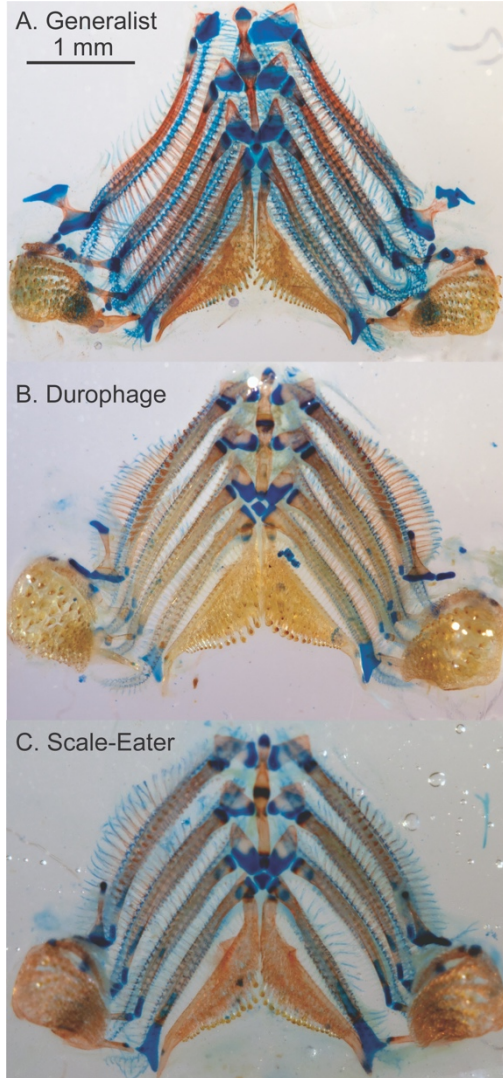
349 *Alternative functions of the novel nasal protrusion*

350 We investigated whether an increase in nasal protrusion size affected the maximum size snail an
351 individual could consume (Figure 2). However, it could be that the novel nasal protrusion is
352 related to feeding efficiency, e.g. in handling time per snail, or is a sensory organ used for
353 locating snails more efficiently with potentially increased numbers of superficial neuromasts
354 (Shibuya et al. 2019). There are several examples of nasal protrusions that are used for this
355 purpose. The unique rostrums of paddlefish (Polydontidae), sturgeon (Acipenseridae), and
356 sawfish (Pristidae) are all used as sensory organs, containing electroreceptors, lateral line canals,
357 and even barbels for detecting prey items (Miller 2006; Wueringer et al. 2012). The novel nasal
358 protrusion of the durophagous pupfish may also be a sensory organ, however, whether the nasal
359 protrusion has an increased number of superficial neuromasts is still unknown.

360 Alternatively, the novel nasal protrusion may allow durophagous pupfish to orally shell
361 snails more quickly, increasing their feeding efficiency. For example, Schluter (1993)
362 documented that benthic sticklebacks with deep bodies, large mouths, and few, short gill rakers
363 were more efficient at consuming benthic prey items, while limnetic species of stickleback, with
364 slender bodies, small mouths, and many, long gill rakers, were more efficient at consuming
365 limnetic prey items. Interestingly, Schluter (1993, 1995) also found that F1 hybrids had
366 decreased efficiency feeding on both limnetic and benthic prey items which was primarily due to
367 their intermediate phenotypes and suggested that reduced fitness in hybrids helps maintain
368 species boundaries between benthic and limnetic species. It could be that the durophage F1 and
369 F2 hybrids have similar preferences for gastropods, but cannot consume snails as efficiently due
370 to their intermediate phenotype. However, we found no strong evidence suggesting that the nasal
371 protrusion is adapted for oral shelling (Figure 2). Future work should investigate other traits that
372 may be adaptive for oral shelling such as the strength of the dorsal head of the maxilla which

373 comprises the skeletal basis of the novel nasal protrusion, structural differences in the
374 mandibular symphysis, coronoid process, or the articular bones which may all provide additional
375 strength or stabilization during biting, or tooth variation in the durophage pharyngeal jaws (Fig.
376 3). Indeed, there is subtle variation apparent in the pharyngeal teeth and jaws of durophages
377 compared to other pupfish species (Figure 3) which has not been previously reported, suggesting
378 that pharyngeal jaws may be adapted for processing hard-shelled prey.
379

380



381

382 **Figure 3. Branchial skeleton and pharyngeal teeth of all three San Salvador Island species.**

383 Image of the dissected branchial skeleton and pharyngeal jaws of A) generalist, B) durophage,

384 and C) scale-eater pupfish. Scale (1mm) is shown in Figure A and is consistent across all three

385 photos. From these three individuals, the representative snail-eater has lower pharyngeal teeth

386 that are 50% longer and 75% wider than the generalist or scale-eating individuals.

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390 *The novel nasal protrusion may be a sexually selected trait*

391 Finally, the novel nasal protrusion may be unrelated to oral shelling and instead may be used in
392 species recognition or mate preference functions. Exaggerated traits, like the novel nasal
393 protrusion in durophage pupfish, commonly arise via sexual selection. For example, forceps size
394 in earwigs (Simmons and Tomkins 1996), major claw size in fiddler crabs (Rosenberg 2002),
395 and the size of the sword tail ornament present in swordtail fish (Rosenthal and Evans 1998) are
396 all thought to be sexually selected traits. Two commonly invoked hallmarks of a sexually
397 selected trait are 1) allometric scaling compared to body size and 2) that the trait is sexually
398 dimorphic (Kodric-Brown and Brown 1984; Kodric-Brown et al. 2006; Shingleton and Frankino
399 2013). In pupfish, there is a weak positive relationship between standard length and nasal
400 protrusion size observed for generalists (Figure S1A, $\text{generalist}_{\text{slope}} = 0.35$). Generalist pupfish
401 mostly likely resemble the most recent common ancestor for the radiation, making the observed
402 slope a good null expectation for how nasal protrusion size should scale with body size in
403 pupfish. In durophages, we observe much stronger positive allometry of the nasal protrusion
404 (Figure S1B, $\text{durophage}_{\text{slope}} = 0.93$), in which large durophage individuals have nasal protrusion
405 sizes more than twice as large as those in large generalists. However, we found no significant
406 difference in nasal protrusion size between male and female durophages when accounting for
407 these size differences (LM, $P=0.96$).

408

409 **Conclusion**

410 In conclusion, we did not find evidence to support that the novel nasal protrusion observed in
411 durophagous pupfish is adapted for consuming large snails. Instead, we found that purebred
412 durophages and their F1 and F2 hybrids have stronger preferences for consuming snails than

413 other species. We suggest that the novel nasal protrusion may be adapted for other aspects of oral
414 shelling such as feeding efficiency, or that variation in other traits, such as the pharyngeal jaws
415 (Figure 3), may play a larger role in oral shelling. Alternatively, this may be an example of
416 trophic specialization due to behavioral specialization (i.e. feeding preference).
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418 **References**

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