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Trophic morphology, feeding performance and prey use in the polymorphic fish *Herichthys minckleyi*

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ABSTRACT

Question: How does pharyngeal jaw morphology influence feeding performance and prey use in the trophically polymorphic cichlid fish *Herichthys minckleyi*?

Organism: *Herichthys minckleyi* exhibits two discrete pharyngeal jaw morphologies. Molariforms possess flattened teeth and enlarged pharyngeal muscles, whereas papilliforms exhibit more gracile jaws, pointed teeth and smaller muscles.

Data: We combined anatomy, experiments, diet analyses and a review of molluscivory to examine the relationships between morphology, feeding performance and prey use.

Conclusions: Handling time differed only slightly between morphotypes. Papilliforms shredded plants more finely than molariforms, and only molariforms readily crushed snails. Molariforms employed their maximum force-producing capabilities to crush snails in the wild. Comparisons with other molluscivorous fish suggested that the amount of hard-shelled prey molariform *H. minckleyi* ingest is not unusual, but its pharyngeal muscle mass and the force used to crush snails are extreme.

Keywords: body size, Cichlidae, co-evolution, Cuatro Ciénegas, Hydrobiidae, maximum performance.

INTRODUCTION

Trophic polymorphisms, in which discrete phenotypes co-exist in a single species, offer ideal systems to examine how changes in trophic morphology influence both feeding performance and diet (Smith, 1982; Sutherland, 1987; Smith, 1990). Although it is clear that differences in jaw morphology can influence feeding performance (Wainwright, 1996) and feeding performance can determine prey use (Rosenzweig and Sterner, 1970; Wainwright 1996), unambiguously assigning variation

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in diet to changes in jaw structure is difficult. However, in polymorphic species like the cichlid fish *Herichthys minckleyi*, the alternativee jaw phenotypes are sufficiently distinct to rigorously test intraspecific differences in feeding performance. Additionally, these alternative phenotypes occur in the same populations (Minckley, 1969), thereby excluding the possibility that differences in diet associated with phenotypic variation are due to changes in prey availability or abundance (Mittelbach, *et al.*, 1999). Furthermore, testing feeding performance on the prey consumed in the native habitat of a polymorphic species guarantees trade-offs in performance between alternative morphotypes that are ecologically relevant to both phenotypes (Wainwright, 1996). Finally, comparisons of feeding performance in a species such as *H. minckleyi* should also elucidate the potentially unique evolutionary and ecological factors maintaining its alternative jaw morphologies (Smith and Skúlason, 1996). To identify how feeding performance links jaw morphology to diet in this discrete polymorphism, we examined trophic morphology, feeding abilities and utilization of potentially co-evolved prey in *H. minckleyi*.

Herichthys minckleyi, like other teleost fish, has both oral jaws used to capture prey as well as pharyngeal jaws – modified gill arches in the throat – used to process prey. However, unlike most fish, *H. minckleyi* has two distinct pharyngeal morphologies (Kornfield and Taylor, 1983). This single species includes 'molariforms', which possess a robust pharyngeal jaw structure with flattened teeth and enlarged muscles, as well as 'papilliforms', which have more gracile pharyngeal morphology exhibiting pencil-like teeth and small muscles (Fig. 1) (Liem and Kaufman, 1984). *Herichthys minckleyi* occurs only within a single isolated valley called Cuatro Ciénegas that contains approximately 200 pools in the centre of the Mexican Chihuahuan desert (Minckley, 1969). These small spring-fed pools, where both morphotypes are always present, range in size from a maximum of 500 m to only 10 m in diameter and their invertebrate fauna is highly endemic (Hershler, 1985). The degree of endemism in Cuatro Ciénegas, coupled with the morphological bimodality of the pharyngeal jaws in *H. minckleyi* likely contributed to early notions that this fish was two distinct species (Minckley, 1969). However, allozyme studies (Kornfield and Koehn, 1975; Sage and Selander, 1975) and observations of breeding in the wild between pharyngeal morphotypes (Kornfield *et al.*, 1982) indicate that the two morphotypes represent alternative phenotypes within a single species.

The jaw polymorphism in *H. minckleyi* has been present for at least 40 years (Minckley, 1969). Also, in over 40 pools that have been examined, both morphotypes are found (Kloeppel, 2002). This temporal and spatial consistency suggests that the functional abilities the pharyngeal

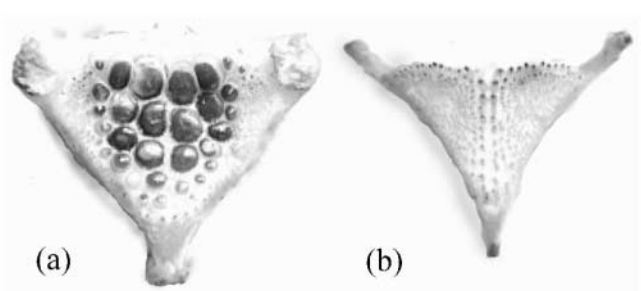

Fig. 1. The lower pharyngeal jaw morphotypes of *H. minckleyi*: (a) molariform jaw morphology and (b) papilliform jaw morphology. The presence or absence of molariform teeth is the character used to diagnose the two pharyngeal morphotypes.

jaws confer should translate into ecological differences that help to maintain the polymorphism. Indeed, it has been hypothesized that the papilliform jaw type is morphologically specialized to feed on arthropods and leafy detritus (Sage and Selander, 1975). Nonetheless, molariform individuals ingest substantial amounts of both prey types (Smith, 1982; C.D. Hulsey *et al.*, unpublished). Similarly, molariform individuals are thought to be adapted to feed on snails. But, papilliform individuals also ingest snails (Sage and Selander, 1975; Smith, 1982). One explanation for the apparent overlap in feeding niche is that the discrete pharyngeal morphologies may differ only slightly in the feeding abilities they confer. It is feasible that the molariforms are simply a little better at quickly crushing snails, while papilliforms are faster at ingesting plant material (Meyer, 1989; Huckins, 1997). At the other extreme, because the pharyngeal jaws are used to crush or macerate prey, it is possible that both of the morphotypes can process prey that the other cannot.

Because the pharyngeal jaw differences decrease intraspecific competition within *H. minckleyi* (Swanson *et al.*, 2003), it is likely that trade-offs in feeding performance do provide the mechanisms for the alternative trophic phenotypes to co-exist (Schoener, 1976, 1986; Chase and Belovsky, 1994). But, because morphological specialization does not often translate linearly into differences in performance or ecology (Arnold, 1983; Robinson and Wilson, 1998; Wainwright *et al.*, 2001; Hulsey and Wainwright, 2002; Bolnick *et al.*, 2003), it is important to test these performance abilities explicitly to mechanistically link jaw morphology to feeding ecology. With this link we can better evaluate what evolutionary and ecological factors promote and currently maintain the polymorphism.

To use the pharyngeal morphology as an indicator of feeding ecology, it is important to quantify what aspects of *H. minckleyi*'s trophic morphology most likely confer differences in feeding ability. There is extensive variation in the entire pharyngeal apparatus of *H. minckleyi* (Liem and Kaufman, 1984), but most quantitative analyses of the morphology of *H. minckleyi* have concentrated on hard structures such as dentition or the morphometrics of skeletal elements (Sage and Selander, 1975; Stephens and Hendrickson, 2001). These hard structures may modify or resist forces *H. minckleyi* uses to process prey such as the hard-shelled snails of Cuatro Ciénegas. However, it is the jaw muscles that generate the forces used to crush or macerate prey. If molariform individuals are specialized to crush snails, we might expect their jaw muscles to be considerably more massive than the muscles of the papilliforms. However, there are no quantitative estimates of pharyngeal musculature in *H. minckleyi*, or estimates of how these muscles change through ontogeny. Furthermore, the forces the pharyngeal muscles are capable of generating may provide a mechanistic explanation for divergence in the feeding ecology of the molariforms and papilliforms.

Understanding the ecological and evolutionary advantages of different jaw structures is difficult without assessing how those structures influence the functional challenges *H. minckleyi* encounters when consuming prey native to Cuatro Ciénegas. In the Cuatro Ciénegas valley, most of the aquatic plant and snail species are endemic (Minckley, 1969), and these potential prey are likely to be heavily defended from *H. minckleyi* predation. Interestingly, the three most abundant snail species in these freshwater desert pools may be involved in a co-evolutionary arms race with *H. minckleyi*. This claim of co-evolution was made because of the snails' very high densities (Hershler, 1985) and because their thick shells, ribbing and sculpturing is more characteristic of marine snails (Vermeij and Covich, 1978; Vermeij, 1993). If maximum feeding performance is routinely used to exploit the most durable snails available in the pools of Cuatro Ciénegas, this might lend support to the hypothesis that these snails and *H. minckleyi* are involved in a co-evolutionary arms race. Additionally, comparing

laboratory-measured maximal crushing performance on these unique snails to prey use in the wild should provide insights into whether crushing performance is critical to the ecological divergence in *H. minckleyi* (West *et al.*, 1991).

Although within species trophic divergence of the magnitude found in *H. minckleyi* is considered relatively uncommon, teleosts with pharyngeal jaws modified for crushing snails have evolved a large number of times (Palmer, 1979; Grubich, 2003). The repeated independent evolution of this feeding apparatus facilitates comparisons of the extent to which *H. minckleyi* is morphologically, functionally and ecologically specialized to eat snails. The extent of specialization on molluscs in *H. minckleyi* is interesting, as the abundance of the snails in the Cuatro Ciénegas pools, up to several hundred individuals per square metre (Hershler, 1985), provides the possibility that *H. minckleyi* crushes hard-shelled prey more often than most mollusc-feeding fish. Alternatively, molariform *H. minckleyi* may not be able to feed effectively on these snails, and therefore utilize snail prey only rarely. Additionally, it is possible that the force used to crush the robust snails of Cuatro Ciénegas and the morphology responsible for this ability is exceptional. If so, this would support the hypothesis that the interactions between *H. minckleyi* and its snail prey have strongly influenced the evolution of each other's unusual phenotypes.

We used a combination of anatomy, laboratory experiments, diet analyses and a comparative review of pharyngeal molluscivory to examine the relationships between morphology, feeding performance and prey use in *H. minckleyi*. First, we measured the time it took the two *H. minckleyi* morphotypes to process different prey types. We next assessed if each morphotype was able to shred plant material and crush small snails. Then we determined the relationship between shell length and force needed to crush the three snail species *H. minckleyi* most commonly consumes. Using this information, we also experimentally tested maximum crushing ability of molariform *H. minckleyi*. We then used snail opercula found in gut contents to assess the extent to which the maximum crushing abilities of the two morphotypes translates into prey use in the wild. Finally, to contextualize this unique system, we compared the proportion of hard-shelled molluscs in the diet, force production and pharyngeal muscle mass in *H. minckleyi* to values reported for other molluscivorous and non-molluscivorous species pairs.

MATERIALS AND METHODS

Ontogeny of muscle mass

To assess muscle masses, we collected and preserved wild-caught *H. minckleyi* in 10% formalin in January 2003. We measured the standard length of fish to the nearest 0.1 mm. The presence or absence of large flattened molariform teeth that are present both on the lower and upper pharyngeal jaws of molariform individuals was identified during dissection and clearly separated the two pharyngeal morphotypes into discrete categories. Two sets of muscles were dissected from an ontogenetic series (papilliform range: 65.4–137.6 mm; molariform range: 69.9–146.0 mm) of both pharyngeal morphotypes (Fig. 2). To contrast the differences we expected in the pharyngeal musculature, we dissected the adductor mandibulae complex from the oral jaws (molariform, $n = 14$; papilliform, $n = 13$). Because the adductor mandibulae is the primary closing muscle of the oral jaws, we expected it to be independent of variation in the pharyngeal jaws. We then removed bones covering the gill arches and their associated muscles. The exposed levator posterior and levator externus

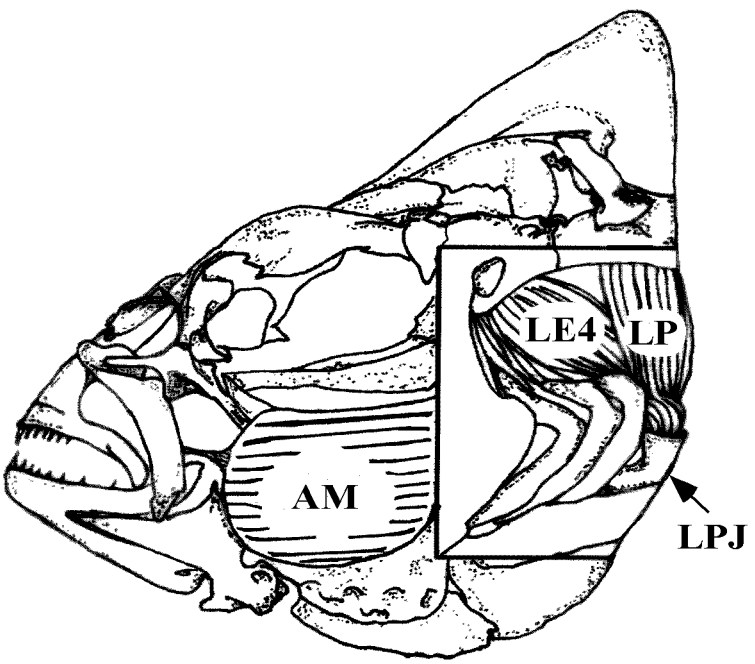

Fig. 2. Pharyngeal and oral jaw musculature of *H. minckleyi*. The adductor mandibulae (AM) complex is nested within the oral jaws and serves as their primary closing muscles. The opercular series is diagramatically cut away to expose the pharyngeal musculature and morphology. The levator posterior (LP) and levator externus 4 (LE4) are depicted and run from the neurocranium down to their attachments on the dorsal horns of the lower pharyngeal jaw (LPJ). The alternative dentition of *H. minckley*is pharyngeal jaws is not visible.

4 muscles, which are strongly associated with one another and which are thought to provide the primary crushing forces in molluscivorous cichlids (Liem 1973; Liem and Kaufman, 1984), were dissected whole from the pharyngeal apparatus (molariform, $n = 14$; papilliform, $n = 15$). The dissected muscles were then placed in 70% ethanol. Subsequently, the muscles were patted dry twice on a paper towel and weighed to the nearest 1 mg. The mass of the muscles and the standard length of the fish were both log-transformed. An analysis of covariance using standard length as a covariate was used to test if there were significant differences in the muscles of the two pharyngeal morphotypes throughout ontogeny. *Post-hoc* analyses of the muscle mass differences between morphotypes were performed on non-significant relationships to assess the power we had to detect differences. These and all subsequent statistical analyses were performed using JMP (SAS Institute Inc., Cary, NC, USA).

Pharyngeal handling time

All experiments were performed on wild-caught individuals collected from the Cuatro Ciénegas valley over 2 months (January to March 2002). Once collected, fish were individually housed in 50-litre aquaria and acclimated over a period of several days to a temperature of 27°C. We identified the morphotype of fish using an otoscope placed into

the buccal cavity. We then scored for the presence or absence of the conspicuous molariform teeth on the lower pharyngeal jaw to identify the fish morphotype. Fish were held without food for 96 h before the experiments and care was taken to avoid performing feeding trials on satiated fish. For the experiments examining handling time, plant shredding and ability to crush small snails, we used individuals drawn from a limited size range of *H. minckelyi* to minimize performance variation due to body size. The average standard length (SL) of the papilliforms ($n = 8$) used was 97.2 mm (range: 78.7–114.8 mm) and that of the molariforms ($n = 8$) used was 100.5 mm (range: 77.7–116.1 mm). Because only a limited number of *H. minckleyi* could be collected, these same eight individuals of each morphotype were used in all experiments. The size of fishes used ensured the pharyngeal bones (Stephens and Hendrickson, 2001) and pharyngeal muscles (see below) were fully developed.

Handling time trials on isopods and plants and the ability to crush snails for an individual were performed randomly with respect to one another. When all other abilities had been measured, the single measure of an individual's plant shredding performance was assessed. For handling times, the amount of time it took individuals to capture and subsequently swallow prey was timed to the nearest second. We measured pharyngeal jaw handling time from the moment the fish captured the prey with its oral jaws until the point when the pharyngeal jaws ceased movement and the prey item was assumed ingested. Many different groups of arthropods are occasionally eaten by both morphotypes (Sage and Selander, 1975). We therefore tested the handling times on isopods (nr *Cylisticus*) because we believed they represented a reasonable approximation of the feeding challenge posed by generalized arthropods. Both morphotypes were offered five pieces of isopod that had been split in half along their mid-section. This provided a standardized amount of the dorsoventrally flattened isopods roughly 0.5 cm^2 in surface area. To maximize our statistical power, we assessed differences in isopod handling time using an analysis of variance (ANOVA) in which the five trials of an individual were nested within morphotype (main effect). The isopods also provided a control for handling time effects described below when feeding abilities of fish were tested on both plants and snails.

The time it took the subject fish to handle and shred plant material was also recorded. For the experiments, leaves of the water lily, *Nymphae* sp., were cut into 1.0 cm^2 sections. As fish appear only to eat decaying plant material in the wild (personal observation), these plant pieces were placed in purified water for 2–3 weeks and allowed to deteriorate and accumulate a bacterial film. The period provided for these leaves to break down was intended to generate leaves that simulated the natural state of detrital *Nymphae* leaves in the pools where *H. minckleyi* is native. However, even with the leaves in this decomposing state, it was difficult to get either morphotype to readily take the plant material alone into their buccal cavity. Therefore, the small section of leaf was tightly rolled and one end was placed in half of an isopod. This ensured the fish would swallow the plant material. The amount of time it took the fish to ingest the *Nymphae* was quantified in the same manner as the half of isopod measured above for three separate feeding events. Only trials in which the fish swallowed the entire section of leaf were recorded. We assessed differences in plant handling time using an ANOVA framework in which the three trials of an individual were nested within morphotype (main effect). For all non-significant results, *post-hoc* analyses of power (Cohen, 1988) were used to assess Type II error or the probability of incorrectly accepting the null hypothesis of no differences in handling time between the two morphotypes.

Plant shredding performance

The ability of the two morphotypes to shred *Nymphae* was also assessed (molariform, $n = 8$; papilliform, $n = 8$). After the first two feedings on plant material, we waited at least 6 h for the foreguts of the fish to be cleared. Directly following the third successful feeding event on *Nymphae* timed above, the fish was removed from its aquarium and given an overdose of anaesthetic (MS222, Finquel, Argent, Washington, USA). After 5 min, the fish was frozen in a −20°C freezer to halt digestion. After 30 min, the fish's entire digestive tract was removed. The third test portion of *Nymphae* was carefully extracted from the gut and the number of pieces this 1.0 cm^2 piece of *Nymphae* leaf was shred into was counted. The pieces were reassembled into the 1.0×1.0 cm square to ensure all pieces were recovered. If the 1.0 cm^2 piece of *Nymphae* was recovered whole, it was counted as one piece for the *t*-test examining differences between the morphotypes.

Snail crushing performance

We also tested the ability of both morphotypes to crush snails using the eight individuals of each morphotype used in the handling time trials and using one additional similarly sized individual of each morphotype. Although clearly motivated to feed, fish of either morphotype would not readily swallow snails in a consistent manner in the laboratory. Therefore, the snails were measured to the nearest 0.1 mm and placed inside half of an isopod. Individuals were each offered three *Mexithauma quadripaludium* snails between 2.0 and 3.0 mm in length, a size both morphologies were assumed to be able to crush. When the fish captured the isopod containing a snail, the fish was monitored. When fish crushed even the smallest of snails, the collapse of the snail shell was clearly audible as a loud crack. If a positive crushing event was heard and the fish swallowed the snail, the feeding event was recorded as a positive crushing event. Snail parts that were spit out were examined to determine if the fish ingested the snail flesh. If the fish spit the snail out without crushing it, this was recorded as an unsuccessful crushing event. In a few cases, the papilliform individuals swallowed the snails without crushing them and these trials were excluded. The three snail trials for each fish were pooled, and if a fish successfully crushed any of the three snails, this was recorded as an ability to crush snails. We tested the probability that individuals with either of the two pharyngeal jaw types (molariform, $n = 9$; papilliform, $n = 9$) were equally likely to crush these small snails with a chi-square test.

Assessing whether use of snail prey reflects maximal performance can be difficult partly because the feeding abilities of predators and the defences of prey often scale strongly with body size (Vermeij, 1987; West *et al.*, 1991; Osenberg *et al.*, 1992; Koehl, 1996; Wainwright, 1996). Snails frequently become more resistant to crushing as they increase in length (Vermeij, 1993). Therefore, we measured the crushing resistance of a wide size range of *Mexithauma quadripaludium* ($n = 67$), *Mexipyrgus churinceanus* ($n = 60$) and *Nymphophilus minckleyi* ($n = 65$) collected from the Mojarral system of the Cuatro Ciénegas basin. The length from the shell apex to the bottom of the aperture of each snail was measured to the nearest 0.1 mm with calipers. The snails were then crushed between two force plates of an Accuforce Cadet force guage (0–500 N; Ametek, Inc., Pennsylvania, USA) and the force in newtons (N) needed to crush the snails was recorded. After each snail was crushed, the operculum of the snails was isolated and its length measured to the nearest 0.1 mm using dial calipers. The length of the shell, the length of the operculum and the force required to crush the snail were all

log-transformed. Using standard regressions, we determined how well both the length of the shell and operculum estimated snail resistance to being crushed and used this information in the analyses of *H. minckleyi* crushing ability.

Because only molariform individuals were readily able to crush small snails, the maximum force production abilities of only this morphotype when feeding on *M. quadripaludium* were determined experimentally. Since the crushing muscles of molluscivorous predators that generate crushing forces often scale with body size (Wainwright, 1987; Wainwright *et al.*, 1991), nine molariform individuals (range: 77.7–137.0 mm) were used in our crushing performance trials to estimate ontogenetic changes in force production. To avoid satiation effects, fish were offered a maximum of 15 snails a day. Snails were offered to fish in no particular order with respect to the length of shells to randomize any bias with respect to motivation or learning. However, the distribution of snail lengths proffered to fish was chosen purposefully to bracket the crushing abilities of individuals. Forty feeding trials that could be scored as either successful or unsuccessful crushing events were performed on each individual fish over a period of 5 days.

A logistic regression was fitted to the relationship between crushing success and snail length for all 40 feeding trials of each experimental fish tested above (Fig. 3). Maximum force generation for each molariform fish was estimated from the compressive force at which 50% of snails of a given length would be crushed according to the logistic equation. This reduced all 40 crushing trials for a given individual into a single value of snail size (p50). The shell size at which 50% of snails are crushed is considered an appropriate performance metric for maximum abilities (Wainwright, 1987), because at any shell size about half of all snails of a given length will not fail under the expected crushing load. For this reason, an individual *H. minckleyi* will only have the ability to crush half of the shells of the length corresponding to its expected maximum force production capabilities. The standard length

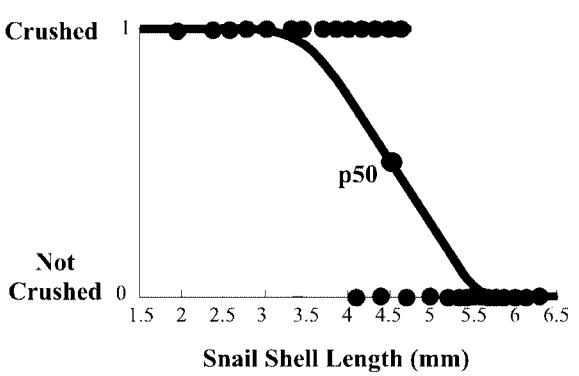

Fig. 3. Logistic regression of snail crushing. We fit a logistic regression to an experimental examination of snail crushing in an ontogenetic series of molariform *H. minckleyi* ($n = 9$; range 77.7–137.0). Snails were measured to the nearest 0.1 mm and introduced into aquaria containing individual fish. Feeding events were scored as either crush (1) or no crush (0). The logistic regression reduced all 40 crushing trials for a given individual into a single value of snail size (p50). The shell size at which 50% of snails are crushed is considered an appropriate performance metric for maximum abilities (Wainwright, 1987), because at any shell size about half of all snails of a given length will not fail under the expected crushing load. For this reason, an individual *H. minckleyi* will only have the ability to crush half of the shells of the length corresponding to its expected maximum force production capabilities.

of all molariform *H. minckleyi* used in the trials was measured when the trials were completed. We then regressed log-transformed maximum crushing ability, p50, on the log-transformed standard length of fish ($n = 9$). Error from the p50 logistic regressions and regression of shell length versus force needed to crush a snail were not included in the error estimates of the p50 versus fish standard length regression.

To determine the force used to crush snails in the wild, we examined the gut contents of a series of 73 molariform (range: 55–156 mm SL) and 85 papilliform (range: 55–169 mm SL) wild-caught *H. minckleyi* for the presence and size of snail opercula. A number of researchers collected the fish over several years (1998 to 2001) from Cuatro Ciénegas and housed them in the Texas Memorial Museum at the University of Texas at Austin. The percent contribution of snails to the gut contents of both molariforms and papilliforms was also quantified. Snail opercula were isolated because they are tightly attached to the snail flesh and should provide a proxy for the number of snails ingested. In our analyses, we used only the opercula clearly identifiable as belonging to the three most abundant snail species in the gut contents: *Mexithauma quadripaludium*, *Mexipyrgus churinceanus* and *Nymphophilus minckleyi*. The length of the snail opercula taken from the guts was then measured using an ocular micrometer under a dissecting microscope. The length was transformed using the regression equation of opercula length versus force for each snail species into an estimate of force production. We estimated the force required to crush 488 snails recovered from gut contents. Then for each individual fish containing opercula, we calculated the maximum force this fish used to crush snails. The single maximum estimate of force production, from the approximately 15 snails an average snail-eating molariform crushed, and the standard length for each fish were log-transformed. The regression of maximum snail hardness on standard length was used to estimate the ontogenetic changes in *H. minckleyi* maximum force production employed in the wild. Finally, an analysis of covariance (ANCOVA) using standard length as a covariate was used to test if there was a significant difference between estimates of molariform maximum force production generated experimentally and molariform crushing estimates from the wild. Acceptance of the null hypothesis of no difference between our estimates of maximum performance was potentially the most interesting result we could recover. Therefore, for the average molariform standard length examined, we assessed the amount of Newtons of crushing force the ANCOVA could detect with a power (β) of 0.95. If the regressions of maximum crushing abilities versus standard length were not statistically distinguishable at $P < 0.05$, this value would determine the minimum amount the two measures of crushing ability could differ and we would not commit a Type II error in 95% of analyses performed.

Comparisons to other molluscivores

We also reviewed all other published studies of fish that crush snails with their pharyngeal jaws. We report results from species in which we could extract the proportion of their diet composed of hard-shelled molluscs, their maximum force production capabilities, and the mass of their levator posterior/levator externus 4 (LP/LE4) pharyngeal muscles. We included reported values for closely related species that are non-molluscivores. Because all families examined are putatively monophyletic, the conclusions we draw from these comparisons should be broadly phylogenetically independent. For the pharyngeal muscle mass of 104 Great Barrier Reef labrids (Wainwright *et al.*, 2005), we regressed the mean standard length versus the mean LP/LE4 muscles to generate an unpublished regression

equation and estimated the average LP/LE4 muscle mass for this group. To make all comparisons size-independent, we scaled the published measures of maximum force production and the mass of the pharyngeal crushing muscles to 100 mm standard length for each species. For studies that only reported force production and mass of pharyngeal muscles versus mass for a given fish species, we used 32 g as the estimate of the mass of 100 mm standard length fish. Finally, we compared the values obtained with our results for *H. minckleyi*.

RESULTS

Ontogeny of muscle mass

The LP/LE4 was strongly correlated with standard length in both the molariforms (slope = 3.29; $n = 14$; $r^2 = 0.83$; $P < 0.001$) and papilliforms (slope 3.54; $n = 15$; $r^2 = 0.95$; $P < 0.001$) (Fig. 4). There was no interaction between morphotype and standard length ($P = 0.59$), suggesting that the slopes of the relationship between LP/LE4 mass and fish size are the same throughout the ontogenetic range examined here for both morphotypes. However, the ANCOVA testing for the differences in LP/LE4 mass between the morphotypes was highly significant ($P < 0.001$), suggesting that the size of the muscle differs significantly between similarly sized molariforms and papilliforms. The ANCOVA of adductor mandibulae mass between the morphotypes with standard length as a covariate was not significant ($P = 0.076$). However, the adductor mandibulae of both morphotypes together ($n = 27$) changed significantly with standard length (slope 3.13; $r^2 = 0.91$; $P < 0.001$). The minimum detectable difference between the adductor mandibulae mass of the two morphotypes was 17.6 mg, but our ability to reject the hypothesis of no difference was low ($\beta = 0.40$). The

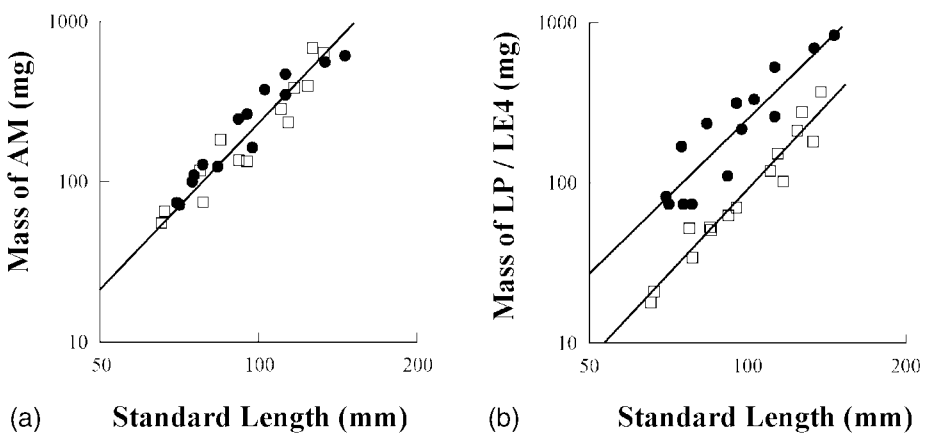

Fig. 4. Morphotype jaw muscle masses through ontogeny. (a) The log mass of the adductor mandibulae (AM) versus standard length is not significantly different between the molariforms (●) and papilliforms (□) ($P = 0.076$). However, the AM mass is clearly correlated with standard length of the fish ($P < 0.001$). (b) The log LP/LE4 mass is also correlated with standard length ($P < 0.001$). However, as one might expect for individuals that differ in their abilities to crush hard-shelled snails, an ANCOVA indicates that the mass of the LP/LE4 is significantly larger in molariforms than papilliforms ($P < 0.001$).

negligible differences in the adductor mandibulae contrasted sharply with the LP/LE4 in the molariforms, which was approximately three times the mass of the same muscles in the papilliforms throughout ontogeny.

Pharyngeal handling time

Papilliform fish had lower handling time than molariforms (Table 1) when feeding on isopods alone (mean ± standard error: papilliform, 21 ± 3 s; molariform, 26 ± 3 s; $P = 0.03$), but the two did not differ in handling times of plants placed inside isopods (molariform, 51 ± 16 s; papilliform, 45 ± 19 s; $P = 0.57$). Although differences as small as 5.1 s could have been found to be significant, the variation in handling times coupled with the small number of individuals examined provided little power to reject the idea that handling time on plants was different between the morphotypes ($\beta = 0.45$). The handling time of both morphotypes when feeding on snails is not reported because of the general inability of the papilliforms to crush snails.

Plant shredding performance

The two morphotypes differed in their capacity to shred plant material (Table 2). The difference in the number of pieces the morphotypes shredded the *Nymphae* into was highly significant ($t_7 = 4.0$; $P = 0.003$). The papilliforms were always able to shred the plant material and on average shredded it into more pieces (5.3 ± 2.2) than the molariforms (1.8 ± 0.8). The molariforms frequently ingested the plant material without shredding it at all. Additionally, the *Nymphae* was generally ragged and clearly more extensively processed when recovered from the experimental papilliform guts.

Table 1. Handling time trials (seconds) between molariform and papilliform *H. minckleyi* (mean ± standard error)

Prey item		Molariform ($n = 8$)	Papilliform ($n = 8$)	d.f.	F	P
Isopod	Individual			14	0.846	0.619
	Morphotype	21 ± 3	26 ± 3	1	7.973	0.0063
Nymphae	Individual			14	2.605	0.012
	Morphotype	51 ± 16	45 ± 19	1	1.569	0.219

Note: The time it took an individual to process and swallow items was timed to the nearest second. Five trials per individual were recorded on isopods and three trials per individual were recorded on *Nymphae*. A nested ANOVA was applied to the data with the multiple feeding trials per individual nested within morphotype (main effect).

Table 2. The number of pieces into which each pharyngeal morphotype shredded a 1.0 × 1.0 cm leaf section of *Nymphae*, water lily during prey processing

	Molariforms ($n = 8$)	Papilliforms ($n = 8$)	t_7	P
Number of pieces	1.8 ± 0.8	5.3 ± 2.2	4.0	0.003

Note: If the fish did not shred the *Nymphae*, this was recorded as one piece. A *t*-test was used to examine differences between the morphotypes in the number of *Nymphae* pieces that resulted from pharyngeal processing.

Snail crushing performance

The molariforms were clearly able to crush snails more frequently than the papilliform individuals ($\chi^2 = 6.4$; d.f. = 1; $P = 0.011$). Every molariform individual ($n = 9$) crushed at least one of the small snails offered to it in the three trials; however, out of all 27 papilliform feeding trials ($n = 9$), only a single event was scored as a possible crushing event.

The three snails in Cuatro Ciénegas examined here required extensive force to crush (Fig. 5). The relationship between compressive force resistance and shell length for *Mexithauma quadripaludium* (slope = 1.40; $n = 66$; $r^2 = 0.68$; $P = 0.001$), *Mexipyrgus carranzae* (slope = 1.11; $n = 60$; $r^2 = 0.46$; $P = 0.001$) and *Nymphophilus minckleyi* (slope = 2.20; $n = 65$; $r^2 = 0.87$; $P = 0.001$) was highly significant. The shell length of *Mexithauma quadripaludium* should be strongly indicative of the force used to crush snails. The relationship between snail opercula length and the force needed to crush the snails, using the same sample sizes as above, was also significant for all three species: *Mexithauma quadripaludium*

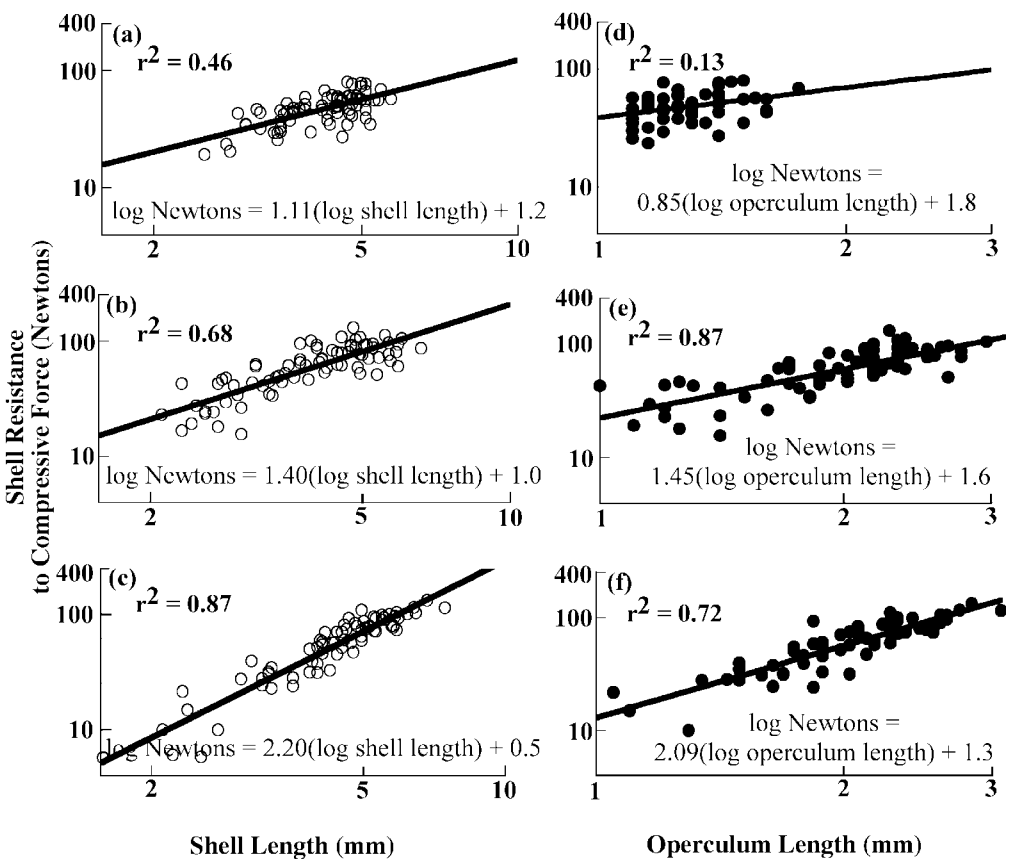

Fig. 5. Shell resistance to crushing versus shell length (a, b, c) and operculum length (d, e, f). The force needed to crush (a) *Mexipyrgus carranzae* ($P < 0.001$), (b) *Mexithauma quadripaludium* ($P < 0.001$) and (c) *Nymphophilus minckleyi* ($P < 0.001$) increased significantly for all three species of snails as their shell length increased. We used *Mexithauma quadripaludium* in experimentally estimating crushing performance.

(slope = 1.45; $r^2 = 0.87$; $P = 0.001$), *Mexipyrgus carranzae* (slope = 0.85; $r^2 = 0.13$; $P = 0.013$) and *Nymphophilus minckleyi* (slope = 2.09; $r^2 = 0.72$; $P = 0.001$). Thus, opercula size should provide a useful proxy for the force used to crush snails in the wild.

The logistic regressions examining maximum crushing ability in molariforms were significant for all nine individuals ($n = 40$ for each of the nine individuals; $r^2 = 0.32$ to 0.69; $P = 0.014$ to 0.001). The smallest molariform (77.7 mm SL) examined could on average crush snails only up to 2.6 mm in length, and even the largest molariform tested (137.0 mm SL) could rarely crush snails over 4.7 mm in length. The relationship between force production measured in the laboratory and the standard length of the molariforms was significant ($n = 9$; slope = 1.56; $r^2 = 0.89$; $P < 0.001$), suggesting molariform maximum force production increases greatly with standard length (Fig. 6). Our laboratory estimates suggest the largest individual (137.0 mm) could produce a maximum of 113.0 N of crushing force, and the smallest individual (77.7 mm) could produce a maximum of only 49.0 N of crushing force, with its pharyngeal jaws.

The gut contents of 44% of the 73 wild caught molariforms contained snail opercula. The average number of opercula found in the guts of snail-eating molariform fish was 14.9 ± 11.2, while only 3 of the 85 papilliforms contained even a single snail operculum. The relationship between estimated force production from opercula and standard length for molariforms was highly significant ($n = 33$; slope = 1.34; $r^2 = 0.67$; $P < 0.001$), while the three maximum crushing estimates for the papilliform morphotype seemed haphazardly

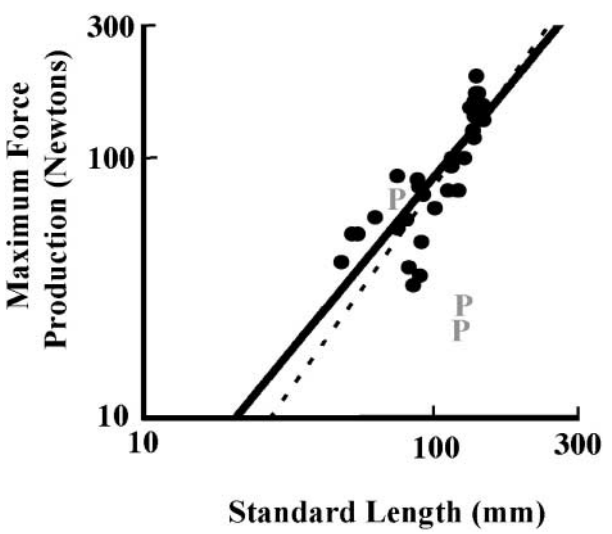

Fig. 6. Maximum crushing performance. Snail opercula were isolated from the gut contents of *H. minckleyi* and identified to species. The length of the operculum was measured to the nearest 0.1 mm and then transformed into force estimates. The maximum estimate of force production (●) from an individual and its standard length were log-transformed and used to estimate changes in maximum force production in the wild as the standard length of *H. minckleyi* increased, depicted by the solid line (——). The crushing estimates for the three papilliforms found to contain opercula is also shown (P). The regression for the experimentally determined relationship between molariform standard length and maximum crushing force measured experimentally is depicted by the dotted line (- - -).

distributed (Fig. 6). The single-factor ANCOVA found no significant interaction between molariform maximum force estimates used in the wild to subdue snails and the experimentally obtained estimates of maximum force production ($P = 0.31$). Our ANCOVA analysis had power of 0.95 to predict a difference of 14.1 N between the experimental and field measurements of maximum force production for the average length molariform *H. minckleyi* (102.6 mm SL) examined. A molariform of this standard length on average should be able to produce 72.7 N. Therefore, we would commit a Type II error in only 5% of cases if molariforms were using at least 80% of their maximum abilities in the wild over the potentially short time period the prey in the gut contents were ingested. The combined maximum force production estimates from both experiments and gut contents of molariforms were significantly related to standard length ($n = 42$; slope = 1.35; $r^2 = 0.67$; $P < 0.001$).

Comparisons with other molluscivores

Many molluscivorous fish have a diet composed of 60–90% hard-shelled molluscs (Table 3). Snails contributed 28% and 0.5% to the gut contents of *H. minckleyi* molariforms and papilliforms, respectively. The masses of the LP/LE4 pharyngeal crushing muscles are generally larger in more molluscivorous fish than in the closely related taxa. The regression for the relationship between standard length and mass of the pharyngeal crushing muscles in the 104 Great Barrier Reef labrids is log(mass) = 4.55(log 100 mm) – 7.84. The mass of molariform *H. minckleyi* pharyngeal crushing muscles is larger than the LP/LE4 mass of any of the other fish examined. Surprisingly, the size-specific mass of the pharyngeal muscles of papilliform *H. minckleyi* is not wildly different from that found in several species considered to be specialized molluscivores. Interestingly, the difference between the mass of molariform and papilliform *H. minckleyi* pharyngeal muscles is much greater than the variation found in the phenotypically plastic *Lepomis gibbosus*. However, several putatively closely related molluscivores and non-molluscivores in various fish families are clearly differentiated in the mass of their LP/LE4 to the degree found in *H. minckleyi*. The ability of the molariform *H. minckleyi* to generate 72.4 N of crushing force is the highest size-specific estimate of force production known for a fish that uses its pharyngeal jaws to crush molluscs.

DISCUSSION

Pharyngeal muscle mass

The *H. minckleyi* morphotypes differ substantially in their pharyngeal musculature. The mass of the LP/LE4 in molariforms is consistently about three times greater than the same muscles in similarly sized papilliforms (Fig. 4). However, there is no significant difference in the adductor muscles of the two morphotypes. Our power to reject a difference between the morphotypes in their adductor mandibulae mass is low, but the lack of substantial difference between these oral jaw muscles and the clear difference between the pharyngeal muscles is stark. Because the force a muscle generates scales strongly with its mass (Calow and Alexander, 1973; Wainwright, 1987), the difference in the mass of the LP/LE4 alone argues molariforms are capable of generating much more force with their pharyngeal jaws than can the papilliforms. However, the mass of the papilliform's pharyngeal muscles at approximately

Table 3. Comparison of *H. minckleyi* mollusc consumption, LP/LE4 muscle mass, and force production to other groups containing molluscivorous fish

Species	Volumetric contribution of molluscs to the diet (%)	Mass of LP/LE4 for 100 mm fish (mg)	Maximum force for 100 mm fish (N)	Study
Cichlidae				
H. minckleyi molariform	28	259.4	72.4	Present study
H. minckleyi papilliform	0.5	89.1	?/<46.7	Present study
Centrarchidae				
Lepomis microlophus (132 mm)	87	73.2	22.7	Huckins (1997), Lauder (1983)
Lepomis gibbosus	70	74.9	11.9	Osenberg *et al.* (1992) Wainwright *et al.* (1991)
Lepomis gibbosus (high density)	<10	39.6	?	Wainwright *et al.* (1991)
Lepomis macrochirus (132 mm)	0	19.7	?	Lauder (1983)
Lepomis cyanellus (127 mm)	0	35.1	?	Lauder (1983)
Labridae				
Halichoeres pictus	0	3.9	1.3	Wainwright, 1988
H. maculipinna	5.5	13.4	2.0	Wainwright, 1988
H. garnoti	27.4	47.8	8.9	Wainwright, 1988
H. poeyi	23.6	72.4	21.0	Wainwright, 1988
H. bivittatus	29.8	7.6	4.7	Wainwright, 1988
H. radiatus	65.8	72.4	11.7	Wainwright, 1988
Lachnolaimus maximus	81.0	129.3	54.4	
Average Labridae on the Great Barrier Reef	variable	82.4	?	Wainwright *et al.* (2005)
Sciaenidae				
Pogonias cromis	85	31.0	?	Randall (1967), Grubich (2003)
Sciaenops ocellatus	0	7.0	?	Randall (1967), Grubich (2003)
Carangidae				
Trachinotus carolinus	90	30.5	?	Randall (1967), Grubich (2003)
Caranx hippos	0	7.5	?	Randall (1967), Grubich (2003)
Haemulidae				
Anisotremus surinamensis	17.3	8.2	?	Randall (1967), Grubich (2003)
Anisotremus virginicus	9.3	7.3	?	Randall (1967), Grubich (2003)

100 mm standard length is the same mass as a molariform's at 60 mm standard length. Molariforms as small as 60 mm are crushing snails. Therefore, muscle mass alone cannot explain why more papilliforms are not crushing snails at larger sizes.

Although other non-morphological factors may play a role, at least two non-mutually exclusive hypotheses based on the functional consequences of their pharyngeal morphology could explain the lack of papilliform snail crushing. First, the small teeth and gracile jaws of the papilliforms may not be able to withstand the forces necessary to crush snails in Cuatro Ciénegas. Additionally, the pinnation angle and fibre length of a muscle both contribute to a muscle's force production capabilities (Calow and Alexander, 1973). These two architectural facets of the pharyngeal muscles may also differ between the morphotypes and influence how much force the molariforms and papilliforms exert on their prey. Although additional characterization of the pharyngeal morphology in *H. minckleyi* could further elucidate the anatomical basis of functional differentiation between the morphotypes, it is clear their structural differences influence feeding performance.

Handling time

The time it takes to process and swallow prey has been suggested to be important to the maintenance and evolution of fish pharyngeal jaw morphology. In both closely related species (Huckins, 1997) and morphotypes within a species (Meyer, 1989), the time spent ingesting prey is thought to be costly because it reduces the time that could be spent pursuing other activities critical to fitness (Schoener, 1976). As previous studies would lead one to predict, the time the papilliforms spent ingesting the experimentally offered isopods and *Nymphae* was less than that of the molariforms (Table 1). However, only the time spent processing isopods was statistically different, although we had very little power to distinguish handling time differences between the two morphotypes. Yet, regardless of the power we had to detect difference in handling by the two pharyngeal types, the difference in handling times on both prey types is minimal.

Furthermore, it is unclear if the shorter handling time the papilliforms need to process prey contributes significantly to trophic differentiation. The more rapid handling time of the comparatively gracile papilliform jaws would likely confer its greatest advantages when arthropods were abundant. However, arthropods of all types are relatively uncommon in the pools of Cuatro Ciénegas (Dinger, 2001). Therefore, caution about the contribution of handling time to niche partitioning between the two morphotypes may be necessary. Furthermore, the speed with which *H. minckleyi* processes prey may be less critical than whether the morphotypes are able to process particular prey types at all.

Plant shredding

While molariforms were virtually unable to shred the experimentally offered plant material (Fig. 6), papilliforms were able to do so extensively. This difference in shredding ability was striking, especially considering that there was no substantial difference in the time the morphotypes spent ingesting *Nymphae* (Table 2). It is important to note that the ability to shred *Nymphae* may only coarsely estimate how effectively the morphotypes process detrital plant material. Nevertheless, plant material in the gut contents of papilliforms appears to be shredded to a much greater extent than plant material found in the gut contents of molariforms (personal observation).

The greater ability of the papilliforms to shred plants should not be too surprising, as it is easy to imagine that the bulky jaws, flat teeth and robust musculature of molariforms makes macerating plant material difficult. Whereas papilliforms' pointed teeth likely can easily pierce and shred plant material, the flattened molariform teeth are likely poorly modified to shred prey. Furthermore, the smaller jaws and muscles of the papilliforms may greatly enhance their mobility and thus efficiency in processing most prey. Shredding plant material, which is generally difficult to digest and nutrient poor (Sturm and Horn, 1998), increases its surface area and, therefore, the ability to extract from it what few nutrients are available. It would be ideal if we could quantitatively assess if the experimentally measured shredding ability of papilliforms reflects greater or more efficient prey use in the wild because it would bolster the hypothesis that shredding ability is critical to *H. minckleyi* diet differentiation. Although we did not match plant feeding performance to prey use in this way, we did do so for snail crushing ability.

Snail crushing

Papilliforms were incapable of crushing snails that molariforms of an equivalent size consistently crush. Only one papilliform tested experimentally may have crushed a single small (<3.0 mm) snail. Furthermore, we found that very few papilliforms ingested snails in the wild (Table 3). Also, in two of the three instances in which snail opercula were recovered from wild caught papilliforms, we inferred the snails to be relatively weak: under 30 N (Fig. 6). The unexpected instances of papilliforms occasionally swallowing a snail whole during our experiments also suggest papilliforms may occasionally ingest small snails in the pools without crushing them. This could explain the occasional inclusion of snails in the diet of papilliforms. However, swallowing snails whole is unlikely to be an effective means of exploiting snails as prey. Because opercula cover and protect a snail's easily digestible body from the outside environment (Vermeij, 1993), it is possible that these snails would survive passage through the digestive tract of papilliforms (Norton, 1988). Both our experiments and estimates of snail crushing in the wild suggest only molariforms are able to effectively crush and feed upon the numerous and robust snails in their native habitat.

Like several other molluscivorous fish (Wainwright, 1987, 1988; Osenberg and Mittelbach, 1989), the body size of molariforms predicted which snails they were able to crush (Fig. 6). Our estimates of the hardness of snails included in the molariform diet suggest molariforms of all sizes are constrained in their ability to eat snails beyond a particular hardness. Yet, it is conceivable that abilities other than maximum force generation could determine which snails *H. minckleyi* uses in the wild. For example, it is commonly assumed in many predator–prey interactions that gape determines the size of prey available to a predator (Wainwright, 1987). Furthermore, many animals are able to modulate their behaviour to overcome constraints morphology imposes (Jayne and Irschick, 2000). For instance, some cichlid fishes, which are probably incapable of crushing snails, have been documented to pull the soft body parts of snails from their shells (Vermeij, 1993). Although none of these other predatory abilities were tested here, *H. minckleyi* in the wild utilizes basically only those snails whose hardness does not exceed our experimentally measured maximum crushing abilities.

However, some caution about the relationship between crushing abilities and fish size are warranted. Learning during the experiments, differences in motivation of individual fish, the inaccuracy of maximum force used in the wild due to the number of snails

crushed, error from the regressions of snail hardness on size, as well as variation around the p50 of maximum crushing ability were not included in our ontogentic estimates of how crushing performance scales with fish size. Importantly, our analysis accepts the null hypothesis that there is no difference in experimentally measured maximal performance and maximal crushing abilities used in the wild. A difference may therefore exist that we failed to detect between maximal abilities measured in the wild and performance measured in the laboratory. However, our analyses do have the power ($\beta = 0.95$) to suggest that the average sized molariform examined with an average maximum crushing ability of 72.7 N is likely to be able to crush snails within 14.1 N of this maximum. The power analysis suggest that only 5% of the time should Type II error, wrongly accepting the null hypothesis when it is in fact false, be a problem if molariforms often use at least 80% of their maximum abilities to crush snails in the wild. Therefore, we believe our results (Fig. 6) are consistent with the hypothesis that the size-specific crushing strength of molariforms determines which snails they are capable of exploiting as prey. Furthermore, the fact that molariforms are routinely using crushing abilities very close to their maximum in the wild suggests that the ecological factors favouring the molariform morphology may be currently influencing the dynamics of the polymorphism.

Co-evolutionary implications

At all sizes, the three most abundant snails in Cuatro Ciénegas require an exceptional amount of force to be crushed (Fig. 5). The distinctiveness of the force needed to crush these freshwater snails is made clear when they are compared with common snails in temperate lakes in North America. The shells of most temperate lake snails are easily crushed well before they are subjected to 50 N of compressive force (Osenberg and Mittelbach, 1989; Osenberg *et al.*, 1992). Our force estimates, combined with the maximum recorded shell lengths of *Mexipyrgus carranzae* (7.3 mm), *Mexithauma quadripaludium* (8.0 mm) and *Nymphophilus minckleyi* (8.3 mm) collected to date (Hershler, 1985), indicate these snails may reach shell strengths of about 140, 180 and 300 N, respectively.

The robustness of these snails' shells may be due to several factors. The high amount of carbonates in the pools of Cuatro Ciénegas (Minckley, 1969; Dinger, 2001) likely makes producing thick shells metabolically inexpensive compared with many other freshwater habitats (Vermeij, 1993). Additionally, many of the pools are thermal (Minckley, 1969), and warmer water favours the production of more extensive calcium carbonate snail shells because unlike most substances this mineral is less soluble at warmer temperatures (Vermeij, 1993). Historical interactions between the snails and *H. minckleyi*, their only known predator (Minckley, 1969), may also have strongly influenced the evolution of the robust shells that result in the snails' resistance to being crushed (Vermeij and Covich, 1978). The morphology of the Cuatro Ciénegas snails and their resistance to crushing are clearly unusual, and further study of their role in influencing the phenotypic diversity of *H. minckleyi* is clearly warranted.

The abundance of snails in the pools of Cuatro Ciénegas and the match between *H. minckleyi* size and the ability to crush snails, begs the question of whether limits on molariform force production may provide larger snails with a refuge from predation. If the three species of snails were less resistant, they would undoubtedly be more susceptible to *H. minckleyi* predation. Importantly, the largest *H. minckleyi* found in Cuatro Ciénegas are approximately 200 mm in length (Artigas-Azaz, 1992). Based on our experiments, these fish

could produce a maximum crushing force of about 200 N (Fig. 6). Additionally, because *H. minckleyi* over 150 mm standard length make up less than 2% of the population (D.A. Hendrickson, unpublished), most molariforms in a pool are unable to crush the larger and thus more durable members of each snail species. This suggests that snail prey would always be available to molariform individuals if they were to evolve increased crushing abilities. Additionally, although other predator defences may be critical to the snails' survival, their 'force refuge' from *H. minckleyi* predation likely contributes to their continued existence in the confined desert pools of Cuatro Ciénegas.

The concordance between the maximum feeding abilities estimated experimentally and in the wild indicate that the current use of snails favours the maintenance of the robust crushing abilities of molariform *H. minckleyi*. However, neither *H. minckleyi* morphotype consumes an extraordinary number of snails for a molluscivorous fish (Table 3). In fact, the diet of molariform *H. minckleyi* includes a smaller proportion of hard-shelled molluscs than many other species of molluscivores. This highlights what appears to be extraordinary about *H. minckleyi*: the size-specific mass of its pharyngeal crushing muscles. For their size, the molariform *H. minckleyi* have larger pharyngeal crushing muscles than any other fish for which comparative data are available. Also, although there are only data from a limited number of taxa, the force-producing abilities of the molariform *H. minckleyi* may be truly exceptional. The polymorphism in *H. minckleyi* itself is unusual, but what may not have been appreciated is the comparative uniqueness of the pharyngeal crushing musculature and feeding abilities of molariform *H. minckleyi*.

CONCLUSIONS

Feeding performance trade-offs likely contribute to the maintenance of the molariform and papilliform pharyngeal morphologies within *H. minckleyi*. Papilliforms are much better at shredding plant material than molariforms, and molariforms are able to crush snails the papilliforms cannot. Furthermore, molariforms consistently use their maximum crushing abilities in the wild, which suggests current prey use favours the maintenance of the extremely robust molariform morphotype. The mechanistic understanding of feeding performance gained here also lends support to the hypothesis that predator–prey interactions between the extremely durable snails of Cuatro Ciénegas and *H. minckleyi* are responsible for each of their unusual phenotypes. Examining the feeding performance of alternative phenotypes within the same species on functionally challenging prey and determining if these feeding abilities are used in the wild provides a powerful means to examine the mechanisms that maintain phenotypic diversity.

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