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Trophic polymorphism in the pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus): effects of environment on ontogeny

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Abstract. The effects of diet on morphology of the feeding apparatus were explored in the pumpkinseed sunfish, *Lepomis gibbosus* Linnaeus. Adult pumpkinseeds feed primarily on gastropod molluscs that they crush between their pharyngeal jaws. Development of the muscles and bones of the pharyngeal jaws was contrasted in two Michigan (USA) lakes that differed both in snail density and in the numbers of snails in pumpkinseed diets. In least squares regressions of muscle masses, bone masses and bone shape variables on body mass, all structures examined showed isometry or positive allometry, with those structures most directly involved in snail crushing tending to scale with positive allometry. Of nine pharyngeal jaw muscles that were examined, analyses of covariance found six to be significantly larger in fish from snail-rich Three Lakes. The primary crushing muscle, the levator posterior, was more than twice as massive in Three Lakes fish than it was in fish from snail-poor Wintergreen lake. All three of the pharyngeal jaw bones used to exert crushing forces on snails were found to be significantly larger and of different shape in fish from Three Lakes. The masses of one muscle and two bones that do not function in prey crushing showed no differences between lakes. Teeth on the crushing surfaces of the jaw bones were worn down, and thus, shorter

in Three Lakes fish. Ontogenetic variation in growth rates of two muscles, the levator posterior and the retractor dorsalis, occurred in fish from Three Lakes. These muscles exhibited strong positive allometry between 40 and 80 mm standard length (SL), the size range over which fish diets shift from soft-bodied invertebrates to snails, but growth slowed to strong negative allometry in fish larger than 80 mm. These differences between lakes in muscle and bone growth appear to be a direct consequence of the physical regime created by repeatedly crushing snails during ontogeny. This plasticity indicates that transformations in the feeding mechanism may originate as specific responses to the demands of novel diets.

Key-words: Allometry, *Lepomis gibbosus*, ontogeny, phenotypic plasticity

Introduction

It is common for populations of a single species that occur in different environments to diverge phenotypically (e.g. Lindsey, 1981; Lavin & McPhail, 1985; Grant, 1986; Hoogerhoud, 1986; Liveley, 1986; Magnan, 1988). The differences that emerge can be a result of shifts in the genetic make-up of the population (Carl & Healey, 1984; Grant, 1986; Trexler & Travis, 1990), via natural selection, founder effects and other mechanisms, or differences may result from developmental plasticity of the species responding to local conditions (Collins & Cheek, 1983; Liveley, 1986). In the latter case, population-level variation in functionally important characters provides an opportunity to study the role of the environment in determining phenotypic expression. Further, such instances permit a direct assessment of the degree to which particular functional systems may respond independently from other anatomical structures, even those that lie in close physical proximity.

In this study we examine the phenotypic response of the feeding mechanism in the pumpkinseed sunfish, *Lepomis gibbosus* Linnaeus, to interlake variation in their prey resource. Adult

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pumpkinseeds are typically trophic specialists on gastropod molluscs which they crush in their pharyngeal jaws (Lauder, 1983a; Mittelbach, 1984; Osenberg & Mittelbach, 1989). We take advantage of natural variation between lakes in the abundance of snails and document the influence of an environmentally imposed diet shift on the dimensions of the bones and muscles of the pharyngeal jaw apparatus.

Snail crushing in pumpkinseeds offers a particularly promising system to study the effects of diet on the feeding mechanism because of extensive previous research on both the functional morphology of snail crushing (Lauder, 1983a,b; Wainwright & Lauder, 1991) and the general trophic ecology of this species (Keast, 1978; Mittelbach, 1984, 1988; Osenberg & Mittelbach, 1989). Only rarely are studies of polymorphic species made in the light of such detailed knowledge of the functional morphology and ecology of a complex functional system (i.e. Liem & Kaufman, 1984; Meyer, 1989, 1990). These pre-existing data not only strengthen our interpretations of the functional and ecological consequences of morphological differences, but, by understanding the functional morphology of snail crushing, we are able to infer the mechanistic processes that produced the morphological variation.

The system

The pumpkinseed sunfish, *L. gibbosus*, and its sister species *L. microlophus* Gunther are the only molluscivorous members of the endemic North American freshwater fish family Centrarchidae. Mollusc crushing in these species is associated with numerous morphological and neuromuscular specializations (Lauder, 1983a,b, 1986; Wainwright & Lauder, 1990), including hypertrophy of the pharyngeal jaw muscles, bones and teeth between which snails are crushed and a novel pattern of muscle activity that is used during snail crushing. As expected, gastropods commonly make up a large fraction of adult pumpkinseed diets in nature (often > 70% by volume or dry weight; Sadzikowski & Wallace, 1976; Keast, 1978; Mittelbach, 1984; Osenberg & Mittelbach, 1989). Small pumpkinseeds, which are unable to effectively crush snails (Mittelbach, 1984), feed on insect larvae and other soft-bodied invertebrates (Sadzikowski & Wallace, 1976; Keast, 1978).

Although pumpkinseeds show considerable specialization for feeding on snails, they remain flexible in their diet and habitat choice, and may change diets in response to changes in resource

levels and the density of competitors (Werner & Hall, 1979). In this study we take advantage of pronounced differences in pumpkinseed diets between two natural lakes in southern Michigan (USA) to examine the impact of consuming gastropods on the development of the pumpkinseeds' pharyngeal jaws. Wintergreen lake has a very dense population of pumpkinseeds and a depauperate snail fauna (C.W. Osenberg, G.G. Mittelbach & P.C. Wainwright, unpublished observations). In this lake, gastropods make up less than 10% of adult pumpkinseed diets by dry weight. In nearby Three Lakes II (hereafter simply called Three Lakes), snail abundances are more typical of the region (about an order of magnitude higher than in Wintergreen lake) and gastropods are the predominant prey in the diets of large pumpkinseeds (60–100% by dry weight for fish greater than 75 mm SL; Mittelbach, 1984; Osenberg, 1988). We hypothesized that these interlake differences in snail abundance and pumpkinseed diets would affect pharyngeal jaw anatomy. Below we compare the musculature and bone structure of the pharyngeal jaws of pumpkinseeds collected from the two lakes, documenting an increased robustness in these structures with ontogeny in Three Lakes relative to Wintergreen lake. No differences were found between lakes in several structures not involved in snail crushing. Elsewhere we also show that pumpkinseeds from Wintergreen lake are less proficient at crushing snails and have slower adult growth rates than do fish from Three Lakes (C.W. Osenberg, G.G. Mittelbach & P.C. Wainwright, unpublished observations).

Materials and methods

Thirty-three pumpkinseeds, ranging in size from 40 to 132 mm SL, were collected with seines from Three Lakes on 21 and 23 June 1988, and 20 individuals (45–109 mm SL) were collected from nearby Wintergreen lake on 6 July 1988. Eviscerated fish were fixed in 10% buffered formalin for about 4 weeks, transferred to 70% ethanol for storage and weighed to the nearest 0.01 g.

Pharyngeal jaw anatomy was contrasted in the two lake populations through three types of measurements: (1) the mass of pharyngeal jaw muscles; (2) the mass of pharyngeal jaw bones; and (3) aspects of the shape of pharyngeal jaw bones. The entire branchial apparatus was dissected from each preserved fish and the left side members of nine paired muscles and one unpaired muscle were carefully removed and stored separately. A strict protocol was followed during weighing to

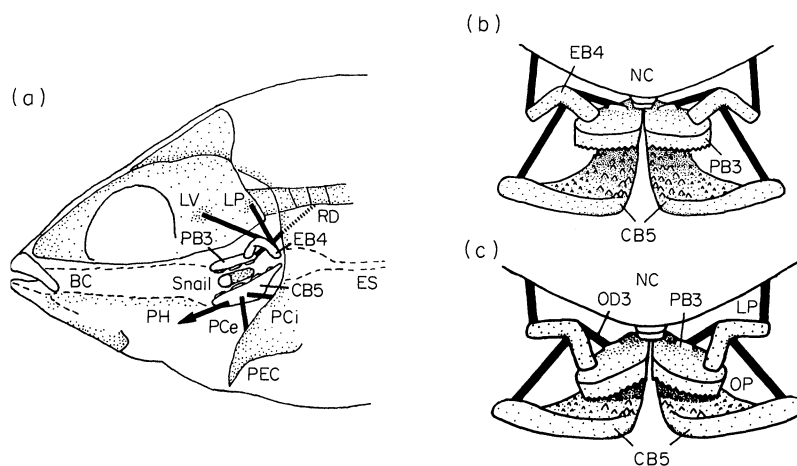


Fig. 1. Illustrations of the snail-crushing mechanism in the pumpkinseed sunfish. (a) Schematic lateral view of the head illustrating the position of the pharyngeal jaw apparatus and the muscles that function during its use. Muscles are shown as thick black lines indicating their attachments. (b) Posterior view diagram of the pharyngeal jaws illustrating the mechanism of upper jaw depression that is crucial to snail crushing. During snail crushing the lower jaw is held relatively stationary and the upper jaw exerts the primary crushing force as it is pressed firmly against the snail shell. Upper jaw depression is caused by rotation of epibranchial 4 about the insertion site of the obliquus posterior muscle. This rotation causes the epibranchial to press against the dorsal surface of the upper jaw, forcing it downward. This crushing action is produced by several muscles, principally the levator posterior, levator externus 3/4, and the obliquus dorsalis. BC, buccal cavity; CB5, fifth ceratobranchial or lower pharyngeal jaw; EB4, fourth epibranchial; ES, oesophagus; LP, levator posterior; LV, fourth levator externus; NC, neurocranium; OD3, third oblique dorsalis; OP, obliquus posterior; PB3, third pharyngobranchial or upper pharyngeal jaw; PCe, pharyngocleithralis externus; PCi, pharyngocleithralis internus; PEC, pectoral girdle; PH, pharyngohyoideus; RD, retractor dorsalis.

minimize measurement errors. Each muscle was weighed twice to the nearest 0.01 mg, after first being patted twice on a dry paper towel to remove excess moisture. The average of these two measurements was used in subsequent analyses on the 10 muscle mass variables (Table 1). The 10 muscles were (see Fig. 1): the levator posterior (LP), a primary upper jaw depressor; the third obliquus dorsalis (OD3), also an upper jaw depressor; the third and fourth levator externus (LE3/4, these muscles are believed to function together as upper jaw depressors [Lauder, 1983a; Wainwright, 1989a] and are difficult to separate physically so they were treated together); the second levator internus (LI2), an upper jaw elevator and protractor; the third levator internus (LI3), also an upper jaw elevator and protractor; the retractor dorsalis (RD), the only muscle that retracts the upper jaws posteriorly; pharyngocleithralis internus (PCi), a lower jaw depressor and retractor; the pharyngocleithralis externus (PCe), also a lower jaw depressor; the pharyngohyoideus (PH), a lower jaw protractor; and the sternohyoideus (SH), the only muscle measured that is unpaired and that is not part of the pharyngeal jaw apparatus. The SH muscle depresses the hyoid bar during suction feeding, and therefore serves a general role during the

capture of all prey types (Lauder, 1985). The SH was selected as a control because it does not function during snail crushing. Thus, it enabled us to determine if any observed differences among lakes in pharyngeal jaw muscle masses were specific to the pharyngeal jaws or simply a correlated response in all cranial muscles.

In addition to the masses of these 10 muscles, an estimate of the physiological cross-sectional area of the levator posterior muscle was obtained so that approximate differences among lakes in the scaling of force-producing capacity of this primary crushing muscle could be made. This is of interest because it is conceivable that muscle masses could differ among lakes while, through organizational changes within the muscle, no differences result in the force-producing capability of the muscle. As force production is presumably the feature of primary importance in limiting snail-crushing ability in these fishes we explored this additional level of potential population divergence. Muscle mass was divided by an estimate of muscle density, 1.05 g cm^{-3} (Lowndes, 1955), to obtain muscle volume and this was then divided by the overall length of the muscle, which was measured to the nearest 0.1 mm, to obtain cross-sectional area. Such a procedure will only produce a reliable estimate of physiological cross-sectional area if the

Table 1. Analyses of covariance results comparing the \log_{10} transformed masses of 10 cranial muscles in pumpkinseed sunfish from two lakes. Body mass is the covariate in all cases. Muscles were divided into three groups depending on their role during snail crushing.

Muscle	Three Lakes			Wintergreen lake			Ratio of adjusted means TL/WG†	ANCOVA tests		
	Slope	SE slope	Intercept	Slope	SE slope	Intercept		(entries are <i>F</i> -ratios)		
								Slope	Intercept	
Active during crushing and generate crushing forces:										
Levator posterior	1.29	0.057	-3.20	1.28	0.036	-3.55	2.33	0.04	109.33*	
Obliquus dorsalis 3	1.09	0.045	-3.19	1.12	0.035	-3.36	1.37	0.25	23.44*	
Levator externus 3/4	1.10	0.044	-3.38	1.06	0.042	-3.40	1.19	0.44	6.65	
Retractor dorsalis	1.08	0.051	-2.96	1.12	0.028	-3.11	1.31	0.28	13.57*	
Pharyngohyoideus	1.05	0.045	-3.29	0.99	0.070	-3.37	1.39	0.63	17.01*	
Active during crushing but do not generate crushing forces:										
Pharyngocleithralis externus	1.18	0.031	-3.22	1.10	0.030	-3.18	1.10	2.63	4.41	
Pharyngocleithralis internus	1.15	0.041	-3.35	1.02	0.032	-3.32	1.29	2.77	16.54*	
Levator internus 2	1.05	0.035	-3.67	1.07	0.048	-3.75	1.14	0.07	5.11	
Levator internus 3	1.15	0.041	-3.59	1.04	0.046	-3.63	1.48	3.25	35.09*	
Not active during crushing:										
Sternohyoideus	1.07	0.039	-1.86	1.12	0.071	-1.95	1.10	0.45	1.53	
LP cross-sectional area	0.92	0.069	-3.62	1.02	0.045	-3.96	2.25	0.18	86.75*	
Standard length	0.32	0.008	1.52	0.33	0.022	1.51	0.98	0.33	0.84	

Sample sizes: Three Lakes, $n = 33$; Wintergreen lake, $n = 20$.

ANCOVA tests degrees of freedom: slope = 1, 49; intercept = 1, 50.

* Significant at $P < 0.005$, the Bonferroni corrected $P = 0.05$.

† Using a pooled slope for the two populations, this column lists the ratio of predicted muscle masses for fish of the same body mass from each population. Ratios are predicted value for Three Lakes fish divided by predicted value for Wintergreen fish.

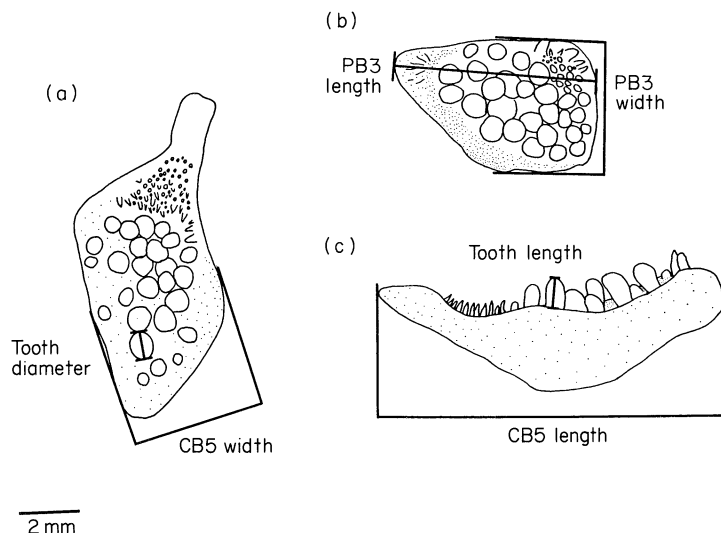


Fig. 2. Diagrams of the toothed pharyngeal jaw bones from a pumpkinseed sunfish illustrating the morphological measurements that were taken to summarize aspects of bone shape. (a) Pharyngobranchial 3, the upper pharyngeal jaw bone and its associated tooth plate, shown in ventral view; (b) ceratobranchial 5, the lower pharyngeal jaw bone and its associated tooth plate, shown in dorsal view; (c) ceratobranchial 5 shown in mesial view.

LP muscle has parallel fibres in the axis of action of the muscle, from origin to insertion. Small deviations from a parallel fibred arrangement will not have a strong effect on estimates of physiological cross-sectional area (Powell *et al.*, 1984). Observations under the dissecting microscope confirmed that the muscle fibres were oriented approximately parallel to the muscle's axis of movement.

Following removal of the muscles, five bones were dissected out of the branchial apparatus from 20 fish from each lake. Each branchial apparatus was first cleared and double stained for bone and cartilage (Dingerkus & Uhler, 1977) to facilitate the recognition and separation of the bones from the pharyngeal jaws. The following bones were removed and weighed: the fifth ceratobranchial (CB5) or lower pharyngeal jaw bone; the third pharyngobranchial (PB3) or upper pharyngeal jaw bone; the fourth epibranchial (EB4); the first epibranchial (EB1); and the first ceratobranchial (CB1). In each case the left side member of the paired bones was taken. Of these bones only the CB5, PB3 and EB4 bones are thought to be directly involved in the snail-crushing movements of the pharyngeal jaws (Lauder, 1983a; Wainwright, 1989a). The EB1 and CB1 bones were selected as controls to ensure that any differences that might be found between lakes in bone weights were not due simply to effects seen in all skeletal elements. All bones were patted twice on a paper towel and weighed to the nearest 0.01 mg. The average of two measurements was used in subsequent analyses.

To gain a more complete understanding of the morphological differences between the two lake populations, nine additional measurements were made on the upper and lower pharyngeal jaw bones (PB3 and CB5) and the bone that pushes the upper jaw ventrally during crushing (EB4). Each bone was viewed under a dissecting microscope that was equipped with a computer interfaced video camera. Desired views were digitized and a computer program aided in making linear or areal measurements. Nine variables were measured that more finely determined the differences between lakes in bone shape (Fig. 2): from PB3 the anterior–posterior length, maximum lateral width and area of the toothed surface were measured; from EB4 the area projected by the surface articulating with PB3; from CB5 the anterior–posterior length, maximum width, area projected in dorsal view, the average diameter of the four widest teeth and the length of the teeth above the deepest portion of the keel of CB5.

Statistical analyses

Because the samples of fish from the two lakes spanned a broad range of body sizes it was necessary to remove the effects of body size from all variables before testing for differences between lakes. All data were \log_{10} transformed to render bivariate relationships approximately linear and to meet assumptions of the parametric statistics used. For each group of variables (muscle mass, bone mass and bone shape) multivariate analyses

of covariance (MANCOVA) were first used to make an overall comparison between the two lakes. Analysis of covariance (ANCOVA) was then employed to compare all morphological variables between the two lake populations. Fish mass (without viscera) was selected as a proxy for body size and used as the covariate in all analyses. Because so many significance tests were conducted on highly correlated variables a Bonferroni correction of the critical probability value of $P < 0.05$ was used in each of the three datasets (Snedecor & Cochran, 1980); muscle masses, bone masses and bone shape. For example, 10 muscle masses were contrasted so the corrected critical probability value was $0.05/10 = 0.005$. This is a conservative measure that reduces the chance of finding a significant lake effect when there is none.

Bivariate plots of the levator posterior and retractor dorsalis muscle mass against body mass suggested that these relationships were not linear on a log-log scale for the Three Lakes dataset (also see Results). This was explored further with a continuous two-phase regression method (Nicker-son, Facey & Grossman, 1989; Yeager & Ultsch, 1989) by progressing along the body mass axis, repeatedly dividing the Three Lakes dataset into two continuous groups and calculating regression parameters for the two segments of data. The continuous pair of segments that minimized the combined residual sums of squares was determined and used to estimate the scaling of muscle mass with body size within each size range. The point of intersection of the two scaling trajectories was calculated to determine the critical point, an estimate of the body size at which the rate of muscle growth changes.

An overall comparison of pharyngeal jaw morphology between the two lakes was made on the 24 variables measured on 20 fish from each lake (10 muscle masses, five bone masses and nine bone shape variables). Principle components analysis was used first to reduce the dimensionality of the original dataset to four new variables that accounted for most of the original variance. A discriminant analysis was then performed on the four new variables to examine the distinctiveness of pharyngeal jaw morphology in fishes from the two lakes. All statistical calculations were carried out using Systat version 4.0 (Wilkinson, 1986) on a microcomputer.

Results

Mechanism of snail crushing

The functional morphology of snail crushing in

pumpkinseed sunfish has been discussed in detail elsewhere (Lauder, 1983a; Wainwright, 1989a; Wainwright & Lauder, 1991) but a brief account is presented here to provide a context in which to interpret the morphological differences between the two lakes. Snails are first captured by the oral jaws, using suction feeding, and are then passed to the pharyngeal jaw apparatus for processing. The key movement in snail-crushing behaviour is the depressive action of the upper pharyngeal jaw (PB3) against the relatively stationary lower pharyngeal jaw (CB5; see Fig. 1). This action compresses the snail shell between the jaws, ultimately crushing the shell. Upper jaw depression is accomplished through rotation of the fourth epibranchial about the insertion of the obliquus posterior muscle on its mid-ventral aspect (Wainwright, 1989a). The rotating fourth epibranchial articulates with the dorsal surface of the upper pharyngeal jaw and presses it ventrally. Several muscles, principally the levator posterior, fourth levator externus and third obliquus dorsalis function to depress the upper jaws. At the same time that the upper jaw is depressed it is also retracted posteriorly by the retractor dorsalis muscle. During crushing the lower jaws are situated such that the posterior region is more dorsal than the anterior region. The snail is thus held against this surface while the upper tooth plates press ventrally and posteriorly on the snail.

Electromyographic studies of the patterns of muscle activity exhibited during snail crushing (Lauder, 1983a,b) have revealed a novel motor pattern in the pumpkinseed and its snail-crushing sister species, the redear sunfish, *L. microlophus*. During crushing all pharyngeal jaw muscles are active simultaneously in intense bursts. Thus, in addition to the upper jaw depressors many antagonistic muscles are active during crushing, presumably to stabilize the jaws during the forceful exertion of the crushing action.

Muscle mass

The relationship between standard length and body mass was not different for fish from the two populations (Table 1). The MANCOVA on the 10 muscle masses revealed a highly significant overall difference between the two lakes (MANCOVA test of lake by mass interaction, Wilks' $\lambda = 0.679$, $F_{10,40} = 1.898$, $P = 0.075$; test for lake effect, Wilks' $\lambda = 0.223$, $F_{10,41} = 14.3$, $P < 0.001$). In the univariate ANCOVAs no differences were found between lakes in the slopes associated with any muscle (Table 1; but see below). With the Bonferroni corrected

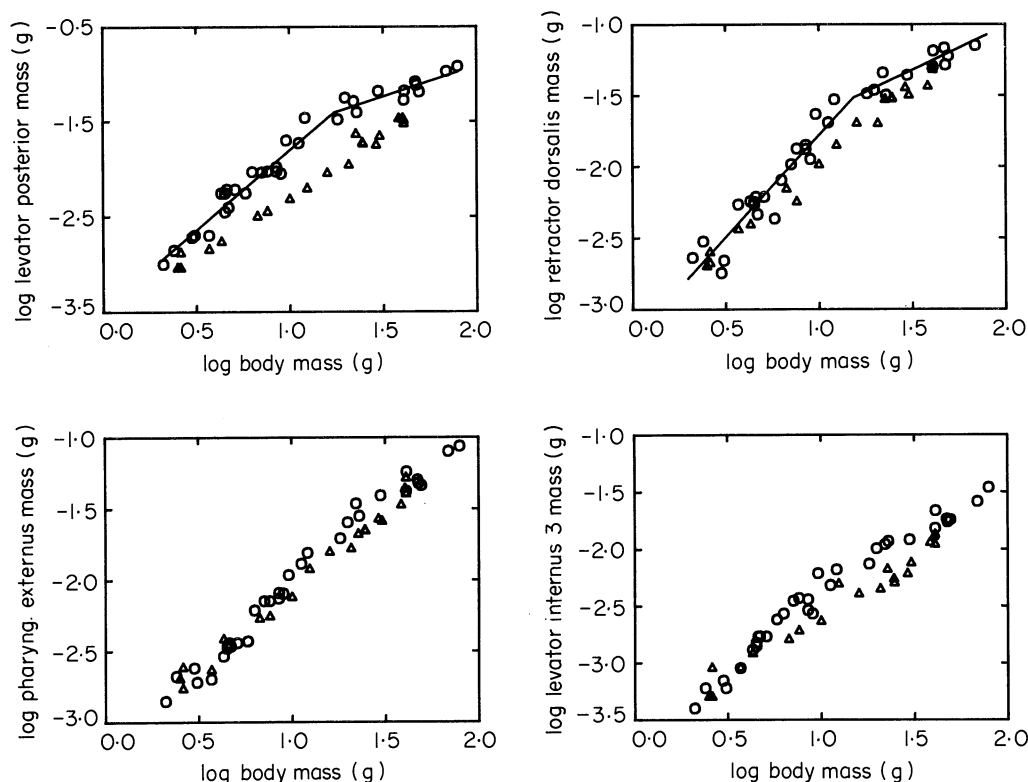


Fig. 3. Comparisons of muscle mass scaling with body mass in two populations of pumpkinseed sunfish. Circles indicate fish from Three Lakes, triangles indicate fish from Wintergreen lake. All muscles plotted are part of the pharyngeal jaw snail-crushing apparatus. Snails, the normal prey of pumpkinseeds, are rare in Wintergreen lake and make up a very small part of the diet compared to fish from Three Lakes whose diets are dominated by snails. The levator posterior, retractor dorsalis and levator internus 3 showed significant lake effects, while the pharyngocleithralis externus did not. Line segments on levator posterior and retractor dorsalis plots indicate two-phase regressions fitted to the Three Lakes data. See Table 1 and text for details of statistical analyses.

probability value six of the nine pharyngeal muscles were significantly larger in fish from Three Lakes (Table 1 and Fig. 3), while the remaining three muscles exhibited a trend toward larger muscles in Three Lakes fish. These three muscles, the levator externus 3/4, pharyngocleithralis externus, and levator internus 2 all show significant lake effects at an uncorrected probability value of $P = 0.05$, though not at the Bonferroni corrected significance level. As expected, the only muscle not involved in crushing, the sternohyoideus, showed no indication of a lake effect (Table 1). The ratios of adjusted means (Table 1) give an indication of the magnitude of the differences between lake populations in muscle masses. The greatest difference between lakes is seen in the mass of the levator posterior muscle, which is 2.32 times as massive in Three Lakes fish as in Wintergreen fish. The least difference is seen in the sternohyoideus muscle, which is 1.07 times as large in Three Lakes fish. Thus, the lake differences appear to be most pronounced in muscles

that are active during snail crushing (most notably the levator posterior) and do not occur in the sternohyoideus, which is not active during snail crushing (Lauder, 1983a).

The physiological cross-sectional area of the levator posterior scaled with positive allometry in the samples from both lakes (slope > 0.67). In the ANCOVA results the allometric coefficients did not differ between populations but the intercepts did (Table 1). The magnitude of the difference in intercepts indicates that Three Lakes fish can generate slightly more than twice as much tension with this muscle as can fish of similar size from Wintergreen lake.

Most muscles scaled with body mass either isometrically (Table 1; slope = 1.0) or with slight positive allometry (Table 1; slope > 1.0). The strongest positive allometry was found in the levator posterior muscle whose allometric coefficient was about 1.3 in both lake samples. Thus, in both lakes several of the pharyngeal jaw muscles (levator posterior, levator externus 3/4, pharyn-

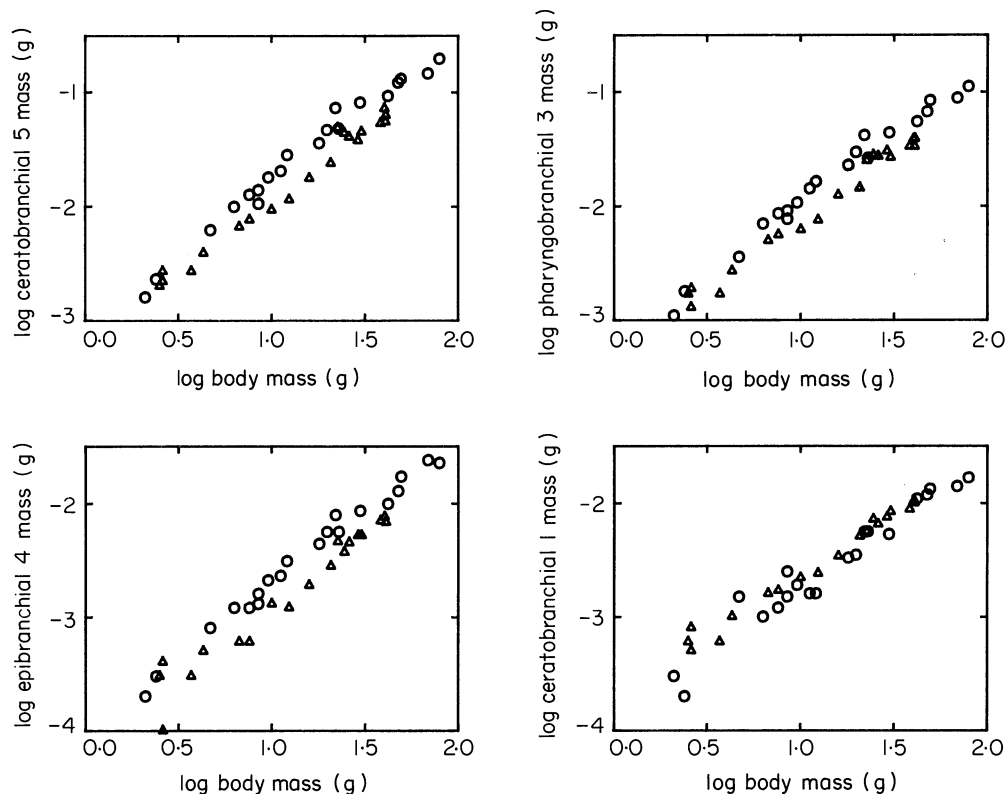


Fig. 4. Comparisons of scaling of bone masses with body mass in two populations of pumpkinseed sunfish. Circles indicate fish from Three Lakes, triangles indicate fish from Wintergreen lake. Ceratobranchial 5, pharyngobranchial 3 and epibranchial 4 are load-bearing elements of the snail-crushing pharyngeal jaw apparatus. Ceratobranchial 1 plays no role in snail crushing. The former three bones show a significant lake effect while ceratobranchial 1 does not. See Table 2 and text for statistical analyses.

gocleithralis externus, pharyngocleithralis internus, levator internus 3) grow relatively faster than overall body mass of pumpkinseeds.

Visually the levator posterior and retractor dorsalis muscles from Three Lakes fish appear to exhibit two growth stages, with a change just above 15 g body mass (Fig. 3). For the levator posterior muscle the two-phase regression analysis revealed that a critical break point for this muscle occurs at 17.4 g body mass (83 mm SL), with muscle mass scaling to body mass in fish smaller than this with a slope of 1.68 (SE = 0.115) and 0.644 (SE = 0.09) for body sizes above the critical point. The slopes of these lines are significantly different from each other (ANCOVA comparison of slopes: $F_{1,29} = 46.3$, $P < 0.001$), and together the two lines explain significantly more variance in the dataset than a simple linear model (residual variance of the two-line model = 0.253, residual variance of the linear model = 0.677; $F_{1,25} = 10.5$, $P < 0.005$). Similarly, the critical point for the retractor dorsalis muscle was determined to occur at 15.4 g body

mass (79.4 mm SL). Retractor dorsalis mass scaled to body mass with an exponent of 1.43 (SE = 0.13) in fish smaller than this critical size, and an exponent of 0.62 (SE = 0.071) in fish larger than this. These slopes are significantly different (ANCOVA comparison of slopes: $F_{1,29} = 25.4$, $P < 0.001$), and together the two segments account for significantly more variance than a linear model fit to the entire dataset (residual variance of two-line model = 0.284, residual variance of the linear model = 0.534; $F_{1,25} = 5.02$, $P < 0.05$). Within the Three Lakes dataset no other muscles or bones showed a significant heterogeneity in growth rates, and in Wintergreen lake fish no structures exhibited the effect.

Bone mass

The MANCOVA on the five jaw bone masses showed a highly significant overall difference between lakes (MANCOVA test of lake by mass interaction, Wilks' $\lambda = 0.795$, $F_{5,31} = 1.332$, $P = 0.27$; test for

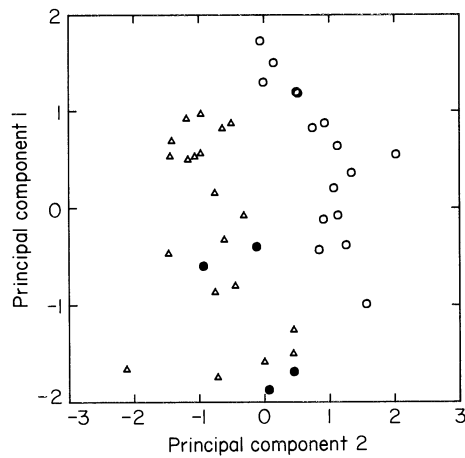


Fig. 5. Plot of the factor scores on the first two principal components from a principal components analysis of 24 size and shape variables of the pharyngeal jaw muscles and bones of pumpkinseed sunfish from two lakes. Circles indicate fish from Three Lakes, triangles indicate fish from Wintergreen lake. PC1 was interpreted as a general size vector, and PC2 shows statistically significant differences in the average score of fish from the two lakes. A discriminant function analysis on the factor scores of the first four principal components accurately identified 36 of the 40 fish. Only the four Three Lakes fish indicated by the filled circles were incorrectly classified.

lake effect, Wilks' $\lambda = 0.405$, $F_{5,32} = 7.824$, $P = 0.001$). In separate ANCOVAs the three bones involved in the crushing mechanism (ceratobranchial 5, pharyngobranchial 3 and epibranchial 4) each showed a highly significant lake effect, and a ratio of adjusted means of around 1.5, indicating that these bones are 50% more massive in Three Lakes fish. Neither the first ceratobranchial nor the first epibranchial (which are not used in snail crushing) showed any indication of a lake effect (Table 2 and Fig. 4).

Positive allometry was found for the same three bones that showed a significant lake effect, as well as for the first epibranchial, while the first ceratobranchial scaled isometrically (Table 2). As with the muscle masses, no cases of negative allometry were found.

Bone shape

The MANCOVA on shape measurements of the three bones involved in exerting crushing forces demonstrated a highly significant overall lake effect (MANCOVA on lake by mass interaction, Wilks' $\lambda = 0.68$, $F_{8,29} = 1.89$, $P = 0.85$; test of lake effect, Wilks' $\lambda = 0.225$, $F_{8,30} = 12.9$, $P < 0.001$). Six of the nine variables showed a significant lake effect at the Bonferroni corrected probability value of $P = 0.0056$. Only the lengths of the fifth ceratobranchial, the third pharyngobranchial and tooth diameter did not show a significant lake effect.

The greatest difference was seen in the area of the articulating surface of epibranchial 4, which is 1.64 times as large in Three Lakes fish as in Wintergreen fish. Scaling of the bone shape variables produced a pattern where the bone width and area variables scaled to body mass with positive allometry (Table 3; for linear measurements slope > 0.33 , for areal measurements slope > 0.67), while the bone length and tooth measurements scaled isometrically.

Discriminant analysis

The first four principal components accounted for 98.2% of the variance in the original 24 variables. The first principal component accounted for 93.9% of the variance and was highly correlated with all 24 variables. All variables had a correlation of greater than 0.96 with PC1. Because the correlations of all variables with PC1 were high and positive this component can be interpreted as a generalized size variable (Bookstein *et al.*, 1985). The second principal component accounted for 2.46% of the total variance. Only three out of the original 24 variables had correlations with PC2 greater than 0.2. The variable most highly correlated with PC2 was levator posterior muscle mass (0.44), followed by tooth length on the fifth ceratobranchial (-0.24) and pharyngobranchial 3 mass (0.21). Principal component 3 accounted for 1.26% of the total variance and was most highly correlated with mass of the first epibranchial (-0.44) and length of the teeth on the fifth ceratobranchial (0.27). The fourth principal component explained 0.57% of the total variance and no variable had a correlation with it above 0.19.

The discriminant function analysis on the first four principal component factor scores for 40 fish from the two lakes found a highly significant overall difference in pharyngeal jaw morphology between the two lakes (MANOVA, Wilks' $\lambda = 0.324$, $F_{4,35} = 18.26$, $P < 0.0001$). When broken down by each of the four principal components only the second principal component showed a significant univariate difference between the two lakes (ANOVA, $F_{1,38} = 44.32$, $P < 0.0001$). This relationship was reflected in the correlations between the principal components and the canonical factor: PC1 = 0.14, PC2 = 0.75, PC3 = 0.01, PC4 = 0.23. The discriminant function correctly classified 36 out of the 40 fish. The four fish that were incorrectly classified were Three Lakes fish and were among the smallest individuals from that lake (Fig. 5).

Table 2. Analyses of covariance results comparing the \log_{10} transformed masses of upper and lower pharyngeal jaw bones in pumpkinseed sunfish from two lakes. Body mass was the covariate in each case. Bones were divided into two categories depending on whether they were an element of the pharyngeal jaws or branchial structures not involved in snail crushing.

Bone	Three Lakes			Wintergreen lake			Ratio of adjusted means TL/WG†	ANCOVA tests	
								(entries are <i>F</i> -ratios)	
	Slope	SE slope	Intercept	Slope	SE slope	Intercept		Slope	Intercept
Elements of the pharyngeal jaws:									
Pharyngobranchial 3	1.23	0.040	-3.20	1.13	0.044	-3.27	1.47	2.92	44.89*
Epibranchial 4	1.30	0.038	-4.00	1.22	0.054	-4.01	1.65	2.32	41.58*
Ceratobranchial 5	1.30	0.044	-3.08	1.19	0.044	-3.15	1.52	2.89	47.54*
Other branchial elements:									
Epibranchial 1	1.21	0.072	-4.31	1.31	0.079	-4.38	0.81	0.84	0.92
Ceratobranchial 1	0.98	0.062	-3.65	1.04	0.030	-3.68	0.80	1.00	2.36

Sample sizes: $n = 20$ for each lake.

ANCOVA tests degrees of freedom: slope = 1, 36; intercept = 1, 37.

* Significant at $P < 0.0083$ (the Bonferroni correction of $P = 0.05$).

† Using a pooled slope for the two populations, this column lists the ratio of predicted bone masses for fish of the same body mass from each population. Ratios are predicted value for Three Lakes fish divided by predicted value for Wintergreen fish.

Table 3. Analyses of covariance results comparing the \log_{10} transformed shapes of upper and lower pharyngeal jaw bones in pumpkinseeds from two lakes. Body mass is the covariate in each case.

Bone shape variable	Three Lakes			Wintergreen lake			Ratio of adjusted means TL/WG†	ANCOVA tests	
								(entries are <i>F</i> -ratios)	
	Slope	SE slope	Intercept	Slope	SE slope	Intercept		Slope	Intercept
Pharyngobranchial 3 length	0.37	0.015	0.30	0.37	0.017	0.28	1.06	0.04	6.77
Pharyngobranchial 3 width	0.42	0.019	0.02	0.43	0.013	-0.06	1.19	0.19	52.86*
Pharyngobranchial 3 area	0.78	0.027	0.21	0.76	0.023	0.17	1.16	0.26	17.41*
Epibranchial 4 area	0.84	0.032	-0.80	0.76	0.035	-0.88	1.48	2.63	64.18*
Ceratobranchial 5 length	0.39	0.012	0.49	0.34	0.013	0.53	1.04	1.84	5.32
Ceratobranchial 5 width	0.43	0.026	-0.02	0.42	0.017	-0.11	1.26	0.13	55.90*
Ceratobranchial 5 area	0.77	0.030	0.39	0.75	0.033	0.29	1.33	0.32	44.21*
Ceratobranchial 5 tooth area	0.33	0.062	-0.63	0.32	0.035	-0.53	0.81	0.01	9.00*
Ceratobranchial 5 tooth diameter	0.37	0.033	-0.67	0.36	0.028	-0.71	1.03	0.42	2.13

Sample sizes: $n = 20$ for each lake.

ANCOVA tests degrees of freedom: slope = 1, 36; intercept = 1, 37.

* Significant at $P < 0.0056$, the Bonferroni corrected $P = 0.05$.

† Using a pooled slope for the two populations, this column lists the ratio of predicted bone dimensions for fish of the same body mass from the two populations.

Ratios are predicted value for Three Lakes fish divided by predicted value for Wintergreen fish.

Discussion

Pumpkinseed sunfish from the two study lakes showed a distinct polymorphism in the anatomy of the pharyngeal jaw apparatus. In comparison to Three Lakes, fish from snail-poor Wintergreen lake exhibited an overall reduction in the size of jaw structures; several pharyngeal jaw muscles were smaller and the three skeletal elements of the jaws involved in snail-crushing actions were smaller, both in mass and in linear and areal aspects of bone dimensions. These reductions were coincident with the rarity of snails in Wintergreen lake and suggest that infrequent snail crushing in Wintergreen fish has resulted in a general atrophy of the crushing apparatus.

Important evidence in support of this interpretation is provided by the absence of polymorphism in the muscles and bones that are not involved in the snail-crushing behaviour. Standard length, sternohyoideus muscle mass, epibranchial 1 mass and ceratobranchial 1 mass all scaled similarly with body mass in the two populations. This is significant because anatomical differences between lakes could be brought about by other effects of the two environments. The different diets and reduced adult growth rates of fish in Wintergreen lake (C.W. Osenberg, G.G. Mittelbach & P.C. Wainwright, unpublished observations) could conceivably result in general reductions of anatomical structures. For example, because Wintergreen fish rarely eat snails, limited calcium availability could retard bone mineralization. In fact, of the numerous morphological features that were measured in addition to aspects of the crushing apparatus none showed a significant lake effect. Only those components known to play a central role in snail crushing varied between lakes. Previous studies of the functional morphology of snail crushing in pumpkinseeds and other perciform fishes (Lauder, 1983a; Wainwright, 1989a) identified the key skeletal and muscular elements of snail crushing and make it possible to infer the consequences and probable causes of the particular morphological differences that were found. Below we discuss the specific anatomical differences between fish in the lakes, their causes and their consequences for pharyngeal jaw function.

Muscle morphology

The view that a reduction in the frequency of snail crushing could lead to the observed morphological differences is supported by previous research on the functional morphology of snail crushing in

sunfishes (Lauder, 1983a) and pharyngeal jaw mechanisms in other generalized perciform fishes (Wainwright, 1989a,b). The larger pharyngeal jaw muscles of Three Lakes fish can be tied to their increased use during snail crushing. Electromyographic studies of snail crushing in pumpkinseeds and the other known snail-eating centrarchid, *L. microlophus*, have shown that this behaviour involves a phylogenetically derived pattern of muscle activity in which all pharyngeal jaw muscles exhibit simultaneous, long, intense bursts of activity (Lauder, 1983a). The more generalized pharyngeal transport behaviour that is used by pumpkinseeds and other sunfishes when handling other types of prey involves a different temporal pattern of muscle firing, with smaller, less intense bursts of activity. Our field observations on the feeding rates of pumpkinseeds in Three Lakes suggest that adult fish consume more than 200 snails in a 14-h summer day (C.W. Osenberg, unpublished observations). The intense activity of all pharyngeal jaw muscles during these frequent crushing events may exert a training effect (Chapman & Troup, 1970; Ashton & Singh, 1974) on these muscles through ontogeny that results in hypertrophy relative to the condition seen in the Wintergreen fish that only rarely crush snails.

To understand why some muscles that function to abduct the pharyngeal jaws showed a lake effect (i.e. pharyngocleithralis internus and levator internus 3), in addition to muscles that exert crushing forces, it is important to emphasize that during snail crushing *all* pharyngeal jaw muscles are intensely active (Lauder, 1983a) and, hence, all muscles are subject to any training effects of crushing activity. The role of the abducting muscles during snail crushing is presumably to stabilize structures during the forceful adduction of the jaws.

The maximum tension that a muscle can develop is a combined function of its physiological cross-sectional area and the force-producing capability of the muscle tissue per unit of cross-sectional area (e.g. Calow & Alexander, 1973). If muscle shape and the degree of fibre pinnation do not change during the growth of a muscle, then an increase in muscle mass will result in an increase in the physiological cross-sectional area of the muscle. In general, when comparing homologous muscles among individuals or closely related species, muscle mass has been found to provide an accurate estimate of relative muscle strength (Powell *et al.*, 1984; Wainwright, 1988), even if slight changes occur in

muscle fibre angle (Lauder & Reilly, 1991). This conclusion is further supported by our data for the levator posterior, a primary crushing muscle, as both mass and physiological cross-sectional area were approximately twice as large in Three Lakes fish relative to Wintergreen fish. Thus, this muscle is about twice as strong in Three Lakes fish, and this differentiation was predicted based on muscle mass. In general, then, the broad-scale differences between lakes in the masses of all pharyngeal muscles indicate that Three Lakes fish have substantially stronger pharyngeal jaws than fish from Wintergreen lake.

Bone morphology

Previous work on the functional morphology of snail crushing has indicated that the fifth ceratobranchial, third pharyngobranchial and fourth epibranchial are the primary force-bearing elements during snail crushing in pumpkinseeds and other perciform fishes (Lauder, 1983a; Wainwright, 1989a). These elements showed the greatest lake effect in the present study (Tables 2 and 3). Frequent crushing actions against resistant snail shells can be expected to cause these skeletal elements to experience intensified stress-strain regimes. Vertebrate bone is well known to respond to mechanical stresses through increased growth and remodelling (Goodship, Lanyon & McFie, 1979; Bouvier & Hylander, 1981; Rubin & Lanyon, 1981) and, thus, the more robust bones found in Three Lakes fish probably reflect such a response.

One result that does not fully conform to the expectations of previous work concerns the response seen in the teeth of the lower jaw. Though the large molariform teeth in Three Lakes fish were shorter than teeth in Wintergreen fish there were no differences in their thickness (Table 3). The simplest explanation for the difference in tooth length is that the shorter teeth in Three Lakes fish are a result of repeated snail-crushing actions wearing down the teeth. This result contrasts with work on African and South American fishes in the family Cichlidae that has repeatedly found that pharyngeal teeth exhibit strong plasticity in length and width, often in response to diet (Liem & Kaufman, 1984; Hoogerhoud, 1986; Meyer, 1990). Cichlid teeth can vary between papilliform and molariform within a single species (e.g. Hoogerhoud, 1986). It appears that the morphology of pumpkinseed teeth is not as readily altered, except by wear.

Plasticity or evolution?

Do the smaller anatomical features seen in pumpkinseeds that occur in the snail-poor environment of Wintergreen lake simply represent the result of ontogenetic plasticity or has there been genetic divergence between these two populations? Though examples of polymorphic animal species are abundant in the literature (e.g. Bernays, 1986; Liveley, 1986; Ehlinger & Wilson, 1988), most cases do not distinguish between these two potential causes of phenotypic separation (for exceptions see Turner *et al.*, 1984; Lavin & McPhail, 1985; Grant, 1986; Trexler & Travis, 1990). In the present case the answer to this question is significant in that it determines the extent to which the observed changes can be viewed as a model for evolutionary change in functional morphology of the feeding mechanism.

Distinctly polymorphic cichlid species that vary in pharyngeal jaw morphology are known from Africa (Greenwood, 1965; Hoogerhoud, 1986) and Central America (Liem & Kaufman, 1984; Meyer, 1989, 1990). Laboratory experiments show that trophic morphology can be altered by rearing fish on different diets (Greenwood, 1965; Hoogerhoud, 1986; Meyer, 1987) and it appears that these effects can sometimes be reversed during ontogeny by a second experimental manipulation of diet (Meyer, 1987). Though the issue of how morphological types are determined has not been entirely determined for any cichlid species, there is much evidence that phenotypic plasticity plays an important role (Greenwood, 1965; Hoogerhoud, 1986; Meyer, 1987) and relatively less evidence that morphological types are genetically determined (e.g. Sage & Selander, 1975; Kornfield *et al.*, 1982).

We are unable to definitely determine the relative importance of phenotypic plasticity and genetic divergence in causing the trophic polymorphism in pumpkinseeds. Evidence regarding the contributions of the two factors could be obtained in common garden experiments (e.g. Trexler & Travis, 1990) in which cohorts from each population could be reared in two groups, one going into a snail-rich environment and the other into a snail-poor environment. If fish from the two populations develop similar morphological patterns growing in each environment then the differences seen between lakes were due to developmental plasticity. Genetic divergence would be implicated if fish from the two lakes developed differently in the common environments.

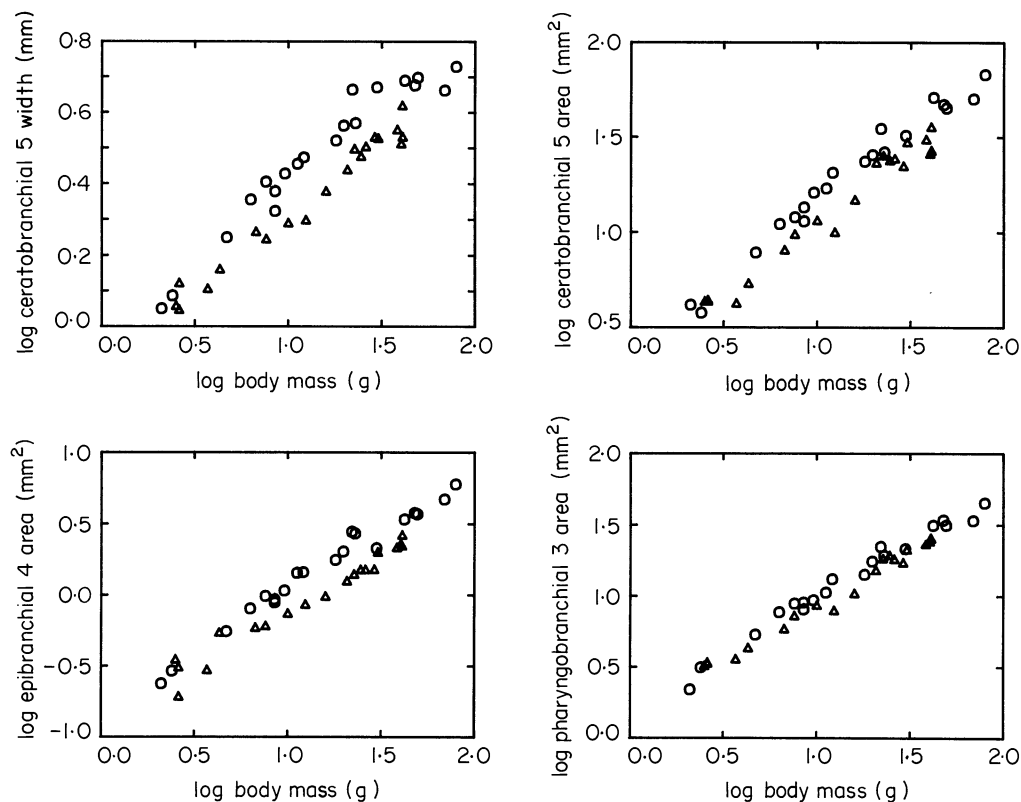


Fig. 6. Scaling of pharyngeal jaw bone shape with body mass in two populations of pumpkinseed sunfish. Circles indicate fish from Three Lakes, triangles indicate fish from Wintergreen lake. All four variables shown exhibited a significant lake effect. See Materials and methods for descriptions of variables. See Table 3 and text for statistical analyses.

Available evidence suggests that the observed polymorphism may be a very recent phenomenon, resulting from a recent perturbation to the Wintergreen ecosystem. During the winters of 1976 and 1977 Wintergreen lake experienced selective fish kills as a result of heavy snowfall and extreme oxygen depletion. These winterkills eliminated the pumpkinseed's major competitor (the bluegill sunfish, *L. macrochirus*) and predator (the largemouth bass, *Micropterus salmoides*) from the lake (Hall & Ehlinger, 1989), allowing for a large increase in the pumpkinseed population. We have shown in caging experiments, that the present high density of pumpkinseeds in Wintergreen lake is capable of maintaining summertime snail densities at their low, ambient levels, and that in the absence of pumpkinseeds snail abundances increase dramatically (C.W. Osenberg, G.G. Mittelbach & P.C. Wainwright, unpublished observations). Thus, we suggest that the extreme rarity of snails in Wintergreen lake is a recent phenomenon, brought about by a large increase in the pumpkinseed population, and that while snails make up a small fraction of the diets of

individual pumpkinseeds the population as a whole is able to maintain snails at their current low levels. If this is correct then the differences in pharyngeal jaw anatomy observed between the two lakes most likely reflect recent divergence and are probably best explained by developmental plasticity rather than a rapid genetic divergence.

Our morphological results support the idea that the polymorphism reflects phenotypic plasticity. Pumpkinseeds in Three Lakes feed on small insect larvae and other littoral zone invertebrates until they reach about 45 mm SL, at which time their diet *begins* to switch to snails (Mittelbach, 1984). If the polymorphism observed between the two lakes reflects the effect of diet on the ontogeny of the feeding apparatus then one expects that (1) pumpkinseeds in the two lakes would show the same anatomy below 45 mm, and (2) the scaling relationships of variables (slopes of regressions on body size) would diverge during growth above 45 mm SL. Our dataset included only one fish from the two lakes under 45 mm (two fish in the muscle analysis). The four individuals that the discriminant analysis misclassified were all Three Lakes

fish and were four of the five smallest fish examined from that lake (Fig. 5; 40, 45, 64 and 65 mm). This poor multivariate discrimination of pharyngeal jaw structures in small fish supports the view that small fish in the two populations are similar and only diverge during the period of ontogeny when snails are being eaten. However, we note that when they were considered separately, none of the morphological variables showed heterogeneity of slopes between lakes. Though the bivariate plots visually suggest greater similarity at small body sizes (Figs. 3, 4 and 6) only the multivariate analysis indicated that this was a significant trend that obscures discrimination of fish that are less than about 65 mm. Below, we provide an additional argument, based on the non-linear scaling of two muscles, that further corroborates our interpretation that divergence is coincident with the diet shift to snails.

Differences between lakes in the ontogenetic scaling patterns of the levator posterior and retractor dorsalis muscles suggest that the pattern of growth in these muscles is related to the inclusion of snails in the diet. A significant change in the rate of growth of both muscles was found in fish from Three Lakes but not in fish from Wintergreen lake (Fig. 3). The change in the rate of levator posterior growth occurred at 17.4 g body mass (83 mm SL). The scaling exponent of this muscle in fish smaller than this was 1.68, and 0.64 in fish larger than 83 mm. Thus, levator posterior growth rate slows by about two-thirds across this critical body size; a striking and significant change. As discussed above, previous work has shown that pumpkinseeds in Three Lakes begin including snails in their diet at about 45 mm SL, about the minimum size examined in this study, and increase the average amount of snails in the diet until this is nearly the only prey type eaten at about 80 mm SL (Mittelbach, 1984; Osenberg, 1988). Thus, there is a strong coincidence in the body size at which pumpkinseeds achieve a diet of nearly 100% snails and when the growth rates of the levator posterior and retractor dorsalis slows. Furthermore, it appears that by the time a pumpkinseed reaches 80 mm it is capable of feeding on most, if not all of the available snails (Osenberg & Mittelbach, 1989). The mean size of snails in the diets of pumpkinseeds increases much between 45 and 80 mm SL, but exhibits little change after this transition (Mittelbach, 1984; Osenberg & Mittelbach, 1989). We suggest that the high growth rates of the levator posterior and retractor dorsalis muscles below about 80 mm SL reflect the period of ontogeny when the diet is changing. As more

and larger snails are added to the diet the intensity of any training effect on the crushing muscles increases but once pumpkinseeds achieve a diet dominated by snails and are able to crush most available snails, the rate of growth in these muscles slows tremendously.

The pharyngeal jaw polymorphism reported here for pumpkinseeds seems to be a direct result of differences in patterns of use of the pharyngeal jaws during prey handling behaviour. While the need to invoke a genetic basis for this polymorphism is in doubt, this system provides a model for the process of evolutionary change in a complex functional network. We observed no morphological differences that did not appear to be directly linked to changes in the use of the pharyngeal jaws. Thus, we found no evidence of other, indirect changes in the skeletal structures of the feeding apparatus not used during snail crushing. The response of the feeding apparatus in the Wintergreen population has been quite specific, with no evidence of complex, indirect effects of the diet shift (i.e. no lake effects were seen in structures not involved in snail crushing). However, we emphasize that our quantitative observations on morphology are mostly limited to several skeletal elements and muscles of the pharyngeal jaw apparatus. It is conceivable that changes have occurred in other parts of the head, as has been suggested for some cichlids (Barel, 1983). If present, however, these changes must be small as none were apparent in our observations of dissected fish. In general, the extent of morphological variation seen among populations was not as extensive as has been found in cichlids (Hoogers, 1986; Meyer, 1989, 1990), perhaps indicating a fundamental difference in the capacity for anatomical plasticity between the phylogenetic groups.

Our results parallel the findings of comparative studies of the functional basis of dietary diversification among species within various fish groups. Recent analyses have almost always found morphological transformations of the feeding apparatus underlying trophic switches (Barel, 1983; Motta, 1988; Wainwright, 1988). Another level of design of the feeding mechanism that is often contrasted among species differing in diet is patterns of muscle activity used during prey capture or prey handling (Liem, 1979; Lauder, 1983a; Wainwright, 1989b). Motor patterns have repeatedly been found to be much more conservative than morphology during the evolution of trophic habits (e.g. Sanderson, 1988; Wainwright & Lauder, 1991). A key goal in future work with the

pumpkinseed polymorphism will be to test the generality of motor pattern conservatism at this level of comparison among populations.

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References

- Ashton, T.E.J. & Singh, M. (1974) The effect of training on maximal isometric back-lift strength and mean peak voltage of the erector spinae. In *Biomechanics IV* (eds. R.C. Nelson & C.A. Morehouse), pp. 448–452. University Park Press, Baltimore.
- Barel, C.D.N. (1983) Towards a constructional morphology of cichlid fishes (Teleostei, Perciformes). *Netherlands Journal of Zoology*, **33**, 357–424.
- Bernays, E.A. (1986) Diet-induced head allometry among foliage-chewing insects and its importance for gramivores. *Science*, **231**, 495–497.
- Bookstein, F., Chernoff, B., Elder, R., Humphries, J., Smith, G. & Strauss, R. (1985) *Morphometrics in Evolutionary Biology*. Special Publication number 15 of the Academy of Natural Sciences of Philadelphia.
- Bouvier, M. & Hylander, W.L. (1981) Effects of bone strain on cortical bone structure in macaques. *Journal of Morphology*, **167**, 1–12.
- Calow, L.J. & Alexander, R.M. (1973) A mechanical analysis of a hind leg of a frog (*Rana temporaria*). *Journal of Zoology*, **171**, 293–321.
- Carl, L.M. & Healey, M.C. (1984) Differences in enzyme frequency and body morphology among three juvenile life history types of the chinook salmon (*Oncorhynchus tshawytscha*) in the Nanaimo river, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, **41**, 1070–1077.
- Chapman, A.E. & Troup, J.D.G. (1970) Prolonged activity of lumbar erector spinae. An electromyographic and dynamometric study of the effect of training. *Annals of Physiological Medicine*, **10**, 262–269.
- Collins, J.P. & Cheek, J.E. (1983) Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. *American Zoologist*, **23**, 77–84.
- Dingerkus, G. & Uhler, L.D. (1977) Enzyme clearing of alcian blue stained whole vertebrates for demonstration of cartilage. *Stain Technology*, **52**, 229–232.
- Ehlinger, T.J. & Wilson, D.S. (1988) A complex foraging polymorphism in bluegill sunfish. *Proceedings of the National Academy of Sciences*, **85**, 1878–1882.
- Goodship, A.E., Lanyon, L.E. & McFie, H. (1979) Functional adaptation of bone to increased stress. *Journal of Bone and Joint Surgery*, **61A**, 539–546.
- Grant, P.R. (1986) *Ecology and Evolution of Darwin's Finches*. Princeton University Press, Princeton.
- Greenwood, P.H. (1965) Environmental effects on the pharyngeal mill of a cichlid fish, *Astatoreochromis alluadi*, and their taxonomic implications. *Proceedings of the Linnean Society of London*, **176**, 1–10.
- Hall, D.J. & Ehlinger, T.J. (1989) Perturbation, planktivory and pelagic community structure: the consequence of winterkill in a small lake. *Canadian Journal of Fisheries and Aquatic Sciences*, **46**, 2203–2209.
- Hoogerhoud, R.J.C. (1986) The ecological and taxonomic aspects of morphological plasticity in molluscivorous haplochromines. *Annales. Musée Royal de l'Afrique Centrale, Zoologie*, **251**, 131–134.
- Keast, A. (1978) Trophic and spatial interrelationships in the fish species of an Ontario temperate lake. *Environmental Biology of Fishes*, **3**, 7–31.
- Kornfield, I.L., Smith, C.D., Gagnon, P.S. & Taylor, J.N. (1982) The cichlid fishes of Cuatro Ciénegas, Mexico: direct evidence of conspecificity among distinct morphs. *Evolution*, **36**, 658–664.
- Lauder, G.V. (1983a) Functional and morphological bases of trophic specialization in sunfishes (Teleostei, Centrarchidae). *Journal of Morphology*, **178**, 1–21.
- Lauder, G.V. (1983b) Neuromuscular patterns and the origin of trophic specialization in fishes. *Science*, **219**, 1235–1237.
- Lauder, G.V. (1985) Aquatic feeding in lower vertebrates. In *Functional Vertebrate Morphology* (eds. M. Hildebrand, D.M. Bramble, K.F. Liem & D.B. Wake), pp. 210–229. The Belknap Press, Cambridge.
- Lauder, G.V. (1986) Homology, analogy and the evolution of behavior. In *Evolution of Animal Behavior* (eds. M.H. Nitecki & J.A. Kitchell), pp. 9–40. Oxford University Press, New York.
- Lauder, G.V. & Reilly, S.M. (1991) Metamorphosis of the feeding mechanism in tiger salamanders (*Ambystoma tigrinum*). *Journal of Zoology*, in press.
- Lavin, P.A. & McPhail, J.D. (1985) The evolution of freshwater diversity in the threespine stickleback (*Gasterosteus aculeatus*). *Canadian Journal of Zoology*, **63**, 2632–2638.
- Liem, K.F. (1979) Modulatory multiplicity in the feeding mechanism of the cichlids, as exemplified by the invertebrate pickers of Lake Tanganyika. *Journal of Zoology*, **189**, 93–125.
- Liem, K.F. & Kaufman, L.S. (1984) Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. In *Evolution of Species Flocks* (eds. A.A. Echelle & I. Kornfield), pp. 203–215. University of Maine Press, Orono.
- Lindsey, C.C. (1981) Stocks are chameleons: plasticity in gill rakes of coregonid fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1497–1506.
- Liveley, C.M. (1986) Predator-induced shell dimorphism in the acorn barnacle. *Evolution*, **40**, 232–242.
- Lowndes, A.G. (1955) Density of fishes. Some notes on the swimming of fish to be correlated with density, sinking factor and the load carried. *Annals Magazine of Natural History*, **8**, 241–256.
- Magnan, P. (1988) Interactions between brook char, *Salvelinus fontinalis*, and nonsalmonid species: ecological shift, morphological shift, and their impact on zooplankton communities. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 999–1009.
- Meyer, A. (1987) Phenotypic plasticity and heterochrony

- in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution*, **41**, 1357–1369.
- Meyer, A. (1989) Cost of morphological specialization: feeding performance of the two morphs in the trophically polymorphic cichlid fish, *Cichlasoma citrinellum*. *Oecologia*, **80**, 431–436.
- Meyer, A. (1990) Ecological and evolutionary aspects of the trophic polymorphism in *Cichlasoma citrinellum* (Pisces, Cichlidae). *Biological Journal of the Linnean Society*, **39**, 279–299.
- Mittelbach, G.G. (1984) Predation and resource partitioning in two sunfishes (Centrarchidae). *Ecology*, **65**, 499–513.
- Mittelbach, G.G. (1988) Competition among refuging sunfishes and effects of fish density on littoral zones invertebrates. *Ecology*, **69**, 614–623.
- Motta, P.J. (1988) Functional morphology of feeding in butterfly fishes (perciformes, Chaetodontidae): an eco-morphological approach. *Environmental Biology of Fishes*, **22**, 39–67.
- Nickerson, D.M., Facey, D.E. & Grossman, G.D. (1989) Estimating physiological thresholds with continuous two-phase regression. *Physiological Zoology*, **62**, 866–887.
- Osenberg, C.W. (1988) *Body size and the interaction of fish predation and food limitation in a freshwater snail community*. PhD dissertation, Michigan State University, East Lansing, Michigan, USA.
- Osenberg, C.W. & Mittelbach, G.G. (1989) Effects of body size on the predator–prey interaction between pumpkinseed sunfish and gastropods. *Ecological Monographs*, **59**, 405–432.
- Powell, P.L., Roy, R.R., Kanim, P., Bello, M.A. & Edger-ton, V. (1984) Predictability of skeletal muscle tension from architectural determinations in guinea pig hindlimbs. *Journal of Applied Physiology*, **57**, 1715–1721.
- Rubin, C.T. & Lanyon, L.E. (1981) Bone remodelling in response to applied dynamic loads. *Journal of Bone and Joint Surgery*, **5**, 237–238.
- Sadzikowski, M.R. & Wallace, D.C. (1976) A comparison of the food habits of size classes of three sunfishes (*Lepomis macrochirus*, *L. gibbosus* and *L. cyanellus*). *American Midland Naturalist*, **95**, 220–225.
- Sage, R.D. & Selander, R.K. (1975) Trophic radiation through polymorphism in cichlid fishes. *Proceedings of the National Academy of Sciences*, **72**, 4669–4673.
- Sanderson, S.L. (1988) Variation in neuromuscular activity during prey capture by trophic specialists and generalists (Pisces: Labridae). *Brain, Behavior, and Evolution*, **32**, 257–268.
- Snedecor, G.W. & Cochran, W.G. (1980) *Statistical Methods*, 7th edn. Iowa State University Press, Ames, Iowa.
- Trexler, J.C. & Travis, J. (1990) Phenotypic plasticity in the sailfin molly, *Poecilia latipinna* (Pisces: Poeciliidae). I. Field experiments. *Evolution*, **44**, 143–156.
- Turner, B.J., Grudzien, T.A., Adkisson, K.P. & White, M.M. (1984) Evolutionary genetics of trophic differentiation in Goodied fishes of the genus *Ilyodon*. *Environmental Biology of Fishes*, **9**, 159–172.
- Wainwright, P.C. (1988) Morphology and ecology: functional basis of feeding constraints in Caribbean labrid fishes. *Ecology*, **69**, 635–645.
- Wainwright, P.C. (1989a) Functional morphology of the pharyngeal jaw apparatus in perciform fishes: an experimental analysis of the Haemulidae. *Journal of Morphology*, **200**, 231–245.
- Wainwright, P.C. (1989b) Prey processing in haemulid fishes: patterns of variation in pharyngeal jaw muscle activity. *Journal of Experimental Biology*, **141**, 359–375.
- Wainwright, P.C. & Lauder, G.V. (1991) The evolution of feeding biology in sunfishes. In *Systematics, Historical Ecology, and North American Freshwater Fishes* (ed. R.L. Mayden). Stanford Press, Stanford, in press.
- Werner, E.E. & Hall, D.J. (1979) Foraging efficiency and habitat switching in competing sunfishes. *Ecology*, **60**, 256–264.
- Wilkinson, L. (1986) *Systat: The System for Statistics*. Systat Inc., Evanston.
- Yeager, D.P. & Ultsch, G.R. (1989) Physiological regulation and conformation: a BASIC program for the determination of critical points. *Physiological Zoology*, **62**, 888–907.

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