

The Functional Basis of Intraspecific Trophic Diversification in Sunfishes

Peter C. Wainwright, George V. Lauder, Craig W. Osenberg,
and Gary G. Mittelbach

Abstract. Research on the patterns of transformation in the vertebrate feeding mechanism has historically focused on interspecific comparisons. A major generalization that has emerged from this work is that morphological features of the feeding mechanism tend to change frequently during evolution while muscle activity patterns that operate the feeding mechanism during prey capture and prey handling tend to be conserved. As a contrast to these interspecific comparisons, we present a case study of intraspecific trophic divergence in the molluscivorous pumpkinseed sunfish, *Lepomis gibbosus*. We have explored the effect an environmentally determined diet shift has on the morphology and muscle activity patterns associated with snail predation by comparing pumpkinseeds from two lakes. In one lake snails are abundant and comprise the bulk of pumpkinseed diets, while in the other lake snails are rare and pumpkinseed diets are comprised almost entirely of soft-bodied invertebrates. Most pharyngeal jaw muscles and bones were significantly larger in pumpkinseeds inhabiting the snail-rich lake. In contrast, only one of five muscles examined exhibited functional (activity pattern) variation between lakes. This muscle, the pharyngocleithralis externus muscle, showed a major difference in its pattern of use, yet it varied least morphologically between lakes. Thus, patterns of change in muscle morphology are not congruent with changes in the snail crushing motor pattern. The morphological and motor pattern changes underlie differences between populations in the ability to feed on hard-shelled gastropods. These results are consistent with previous interspecific studies of the evolution of the feeding mechanism of fishes, and support the conclusion that, in spite of the highly integrated and complex nature of the feeding mechanism in fishes, morphology and muscle activity patterns frequently change independently of each other.

INTRODUCTION

The evolution of complex biomechanical systems has been an enduring problem in evolutionary biology. Functional units within organisms are often highly complex, tightly integrated networks, and many authors have noted the potential difficulty in modifying these systems during evolution (Darwin, 1859; Dawkins, 1986; Wake & Roth, 1989). Classic examples within vertebrates include the visual system (Darwin, 1887; Levine, 1985), the locomotor system (Goslow et al., 1989; Alexander, 1982), the hearing system (Turner, 1980; Fay & Popper, 1985), and the focus of this paper, the feeding mechanism (Frazetta, 1975; Lauder et al., 1989). These functional systems are all recognized as being

Drs. Wainwright and Lauder are with the Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, USA. Dr. Wainwright's present address is Department of Biological Sciences, Florida State University, Tallahassee, FL 32306, USA. Dr. Osenberg is with the Marine Science Institute, Department of Biological Sciences, University of California, Santa Barbara, CA 93106, USA. Dr. Mittelbach is with the Kellogg Biological Station, Department of Zoology, Michigan State University, Hickory Corners, MI 49060, USA.

tightly integrated and complex, yet vertebrates, in general, are characterized by broad diversity within each system. Thus, any attempt to understand the nature of vertebrate diversity must include an understanding of how complex systems transform during evolution.

One approach to this problem that has proven to be especially fruitful is to separately consider the evolution of different levels of design of the system under evaluation (Liem, 1989; Lauder, 1990; Wainwright & Lauder, in press). For example, the design of the vertebrate feeding mechanism can be rendered into at least three components (Lauder, 1990; Wainwright & Lauder, in press): peripheral morphology (e.g., muscle and bone organization), physiological properties of the peripheral morphology (e.g., contractile properties of feeding muscles), and motor patterns (the patterns of muscle activity that drive feeding behaviors). By analyzing the components of the feeding mechanism separately it is possible to ask how evolutionary transformations at each level of design have contributed to evolutionary alterations in overall feeding performance: is the diversification of feeding habits, so often observed among even fairly closely related taxa, due primarily to alterations in trophic morphology, the physiological capacity of the morphology, or changes in motor pattern, or, are some levels of design more conservative during evolution than others?

Recent comparative research on the evolution of the feeding mechanism in fishes and aquatic salamanders has resulted in the key generalization that trophic diversification is generally associated with morphological transformations and only rarely with changes in the motor pattern used during prey capture and handling. Quantitative comparisons of the motor pattern among members of fairly closely related groups have usually found that fewer than 10% of the motor pattern parameters measured differ significantly among species (Shaffer & Lauder, 1985; Wainwright & Lauder, 1986; Sanderson, 1988; Wainwright, 1989a; Westneat & Wainwright, 1989), though this pattern may break down in very broad phylogenetic comparisons (Wainwright et al., 1989). In contrast, differences in diet and feeding performance are usually associated with morphological changes (Sanderson, 1988; Motta, 1988; Wainwright & Lauder, in press). In some cases even major morphological transformations occur in the face of motor pattern conservatism (e.g., Westneat & Wainwright, 1989).

The data base on the evolution of the feeding mechanism in lower vertebrates is built primarily on interspecific comparative analyses. Few data exist on the process of trophic diversification within species (Liem & Kaufman, 1984; Meyer, 1990). Comparative analyses characterize the diversity present in extant species and, coupled with a well corroborated phylogenetic hypothesis, can give an estimate of the sequence of changes in the feeding system and its components. What these studies do not generally permit, however, is a view of the process of change in the feeding system and the environmental conditions that bring it about.

In this paper we present a summary of a series of investigations that we have conducted on different *populations* of a single species, the pumpkinseed sunfish, *Lepomis gibbosus*. Adult pumpkinseeds are typically trophic specialists on gastropod molluscs that they crush in their pharyngeal jaws. We have taken advantage of natural variation among lakes in the abundance of snails, and documented the influence of an environmentally imposed diet shift on the morphology and the snail crushing motor pattern of the pharyngeal jaw apparatus. The combined morphological and motor pattern analyses permit us to contrast the patterns of change at these two design levels in the same muscles. Do muscles that show the greatest morphological response also tend to show the greatest motor pattern response, or are morphological changes uncorrelated with changes in muscle activity?

Snail crushing in pumpkinseeds offers a particularly promising system to study intra-specific trophic diversification because of extensive previous research on both the func-

tional morphology of snail crushing (Lauder, 1983a, b; Wainwright & Lauder, in press) and the general feeding 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). The existing data strengthen our ability to interpret the functional and ecological consequences of morphological differences and, because the functional morphology of this system is well understood, we are able to infer the mechanistic processes that produced the morphological and motor pattern variation.

The System

The pumpkinseed sunfish, *Lepomis gibbosus*, and its sister species, the redear sunfish, *L. microlophus*, are the only molluscivorous members of the endemic North American freshwater fish family Centrarchidae. Mollusc crushing in these species is one of the few well-documented cases in which the novel trophic habit has involved the evolution of a novel pattern of muscle activity (Lauder, 1983a, b, 1986). Snail crushing is associated with a unique motor pattern not usually found in non-snail crushing sunfishes. In addition to neuromuscular specializations the pumpkinseed and redear also exhibit morphological modifications, including hypertrophy of the pharyngeal jaw muscles, bones, and teeth between which snails are crushed.

Gastropods commonly make up a large fraction of adult pumpkinseed diets in nature (often greater than 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; Mittelbach, 1988). Although pumpkinseeds show considerable specialization for feeding on snails, their prey selection is flexible and responds to changes in resource levels (Werner & Hall, 1979; Osenberg, Mittelbach & Wainwright, in press). In our studies we have used 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 snail crushing motor pattern, pharyngeal jaw morphology, and snail crushing ability. Wintergreen lake has a very dense population of pumpkinseeds and a depauperate snail fauna (Osenberg et al., in press). In nearby Three Lakes II (hereafter simply called Three Lakes), snail abundances are more typical of the region (over an order of magnitude higher than in Wintergreen lake).

METHODS

In this paper we will summarize our comparisons of several aspects of the feeding biology of pumpkinseeds from Wintergreen and Three Lakes: dietary habits, feeding ability, trophic morphology, and the muscle activity patterns used during snail crushing. Below we briefly describe the methodology used in these analyses. More detailed descriptions are presented elsewhere (Wainwright et al., 1991; Osenberg et al., in press).

Fish were collected from the two lakes for dietary analysis on four dates throughout 1988 and 1989. On each sampling date between 30 and 40 pumpkinseeds, ranging in size from 28–130 mm standard length (SL) were collected and the contents of their stomachs were later identified, counted, and measured under a dissecting microscope. Previously determined length-mass regressions were used to convert prey dimensions to dry mass.

The ability of pumpkinseeds from the two lakes to handle and consume snails was compared in laboratory experiments (Osenberg et al., in press). Here we present data for pumpkinseeds feeding on two snail species, *Amnicola limosa*, a relatively thick-shelled

gastropod, and *Physa*, a thinner-shelled snail (laboratory estimates of the force required to crush the snails averaged 10.1 N vs. 2.7 N for *Amnicola* and *Physa* respectively, Osenberg et al., in press). Fish ranging in size from 63 to 125 mm SL were collected from Wintergreen and Three Lakes and housed separately in laboratory aquaria for one week prior to the feeding experiments. Snails were presented to fish individually and the time required to crush each snail was measured. Data were analyzed with ANCOVA using lake as the grouping variable and standard length as the covariate.

A morphological analysis was carried out on 33 fish (40–132 mm SL) collected from Three Lakes and 20 individuals (45–109 mm SL) from Wintergreen lake (Wainwright et al., 1991). Each fish was preserved, ten muscles and five bones were dissected from the pharyngeal jaw region, and each was weighed to the nearest 0.01 mg. Several additional shape variables were measured from two of the bones (the shape analysis is discussed in Wainwright et al., 1991). Nine of the ten muscles function during snail crushing, with the levator posterior being the primary force producing muscle of the system. One muscle, the sternohyoideus, functions during prey capture but not during snail crushing and we used this muscle as a control to ensure that differences between lakes were not simply present in all muscles but were instead specific to muscles that function during snail crushing. Of the five bones, three are directly involved in snail crushing, while the other two were selected as controls. Muscle and bone masses were \log_{10} transformed and regressed against body mass, and comparisons were made between the lake populations with ANCOVA.

Patterns of muscle activity during snail crushing were recorded in electromyographic experiments following methods outlined in detail elsewhere (Wainwright & Lauder, 1986; Wainwright, 1989a). Seven fish collected from Three Lakes (103–120 mm SL) and six fish from Wintergreen lake (99–117 mm SL) were first anesthetized and then fine-wire bipolar electrodes were implanted directly into the belly of five pharyngeal jaw muscles. The five muscles were a subset of the ten muscles examined in the morphological analysis. The five paired electrode wires were then bundled together into a cable that was sutured to the back of the fish. Following recovery from anesthesia, fish were offered snails (*Physa* sp.) that were eagerly accepted. Voltages produced during contractions of the pharyngeal muscles at prey capture and snail crushing were amplified and recorded on a multi-track FM tape recorder. Several feedings were analyzed from each individual for a total of 59 feedings from Three Lakes fish and 51 from Wintergreen fish. The analogue myogram recordings were transformed to digital computer files and 16 variables were measured from each snail crushing event that characterized the overall timing and intensity of muscle activity. A nested ANOVA was used to analyze each EMG variable, with individuals nested within lake.

Diet and Feeding Performance

Pumpkinseeds in Three Lakes show a pronounced diet shift during ontogeny (Mittelbach, 1984; Osenberg et al., in press). Fish smaller than about 45 mm SL have few snails in their stomachs (mean \pm SE percent of diet based on prey biomass: $3.3\% \pm 1.6$) and instead feed on soft-bodied invertebrates (primarily insect larvae such as Chironomidae). Between 45 and 75 mm SL there is a drastic increase in the amount of snails eaten, with fish above 75 mm feeding almost entirely on gastropods ($87.2\% \pm 2.6$). Other studies of pumpkinseeds in midwestern U.S.A. lakes have found patterns similar to those seen in the Three Lakes population (Seaburg & Moyle, 1964; Sadzikowski & Wallace, 1976).

Wintergreen pumpkinseeds show a strikingly different dietary pattern. The classic dietary switch that is seen in Three Lakes fish is absent in the Wintergreen population, with snails comprising only $1.5\% \pm 0.8$ of the diet of pumpkinseeds \geq mm SL (Osenberg

et al., in press). Thus, throughout ontogeny Wintergreen fish feed mostly on insect larvae and other soft-bodied invertebrates.

The difference in dietary patterns between the two populations is reflected in their ability to handle gastropod prey (Osenberg et al., in press). When fed the thick-shelled gastropod *Amnicola* Wintergreen fish required significantly longer to crush the snail shell than fish from the Three Lakes population (Fig 1; ANCOVA lake effect test, $P < 0.01$). In contrast, there was no difference between populations in handling time when fed the thinner-shelled gastropod *Physa* (Fig. 1; ANCOVA lake effect test, $P > 0.05$).

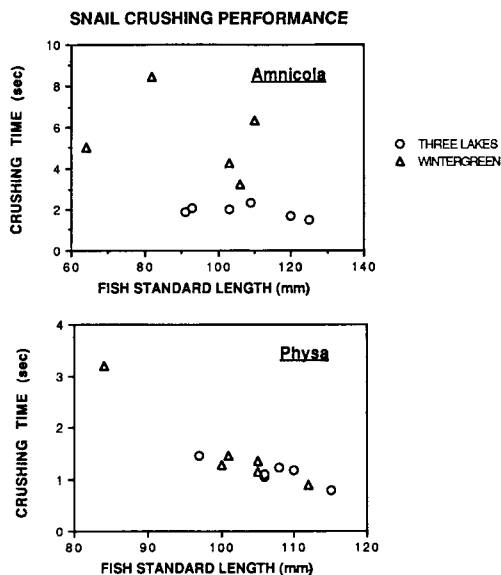


Figure 1. Results of feeding performance experiments conducted with pumpkinseeds from Wintergreen lake and Three Lakes. Crushing time when feeding on the hard-shelled gastropod *Amnicola* is significantly longer in Wintergreen than in Three Lakes fish. There is no difference between populations in the time required to crush the shell of the softer-shelled gastropod *Physa*. See text for statistics.

Mechanism of Snail Crushing

The functional morphology of snail crushing in pumpkinseed sunfish has been discussed in detail elsewhere (Lauder, 1983a; Wainwright, 1989b; Wainwright & Lauder, in press) but a brief account is presented here to provide a context in which to interpret the morphological and motor pattern differences between fish of the two lakes. Snails are first captured by the oral jaws, using suction to draw the prey into the buccal cavity, and are then passed to the pharyngeal jaw apparatus for processing. The key movement in snail crushing behavior is the depressive action of the upper pharyngeal jaw (PB3) against the relatively stationary lower pharyngeal jaw (CB5; see Fig. 2). 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 midventral aspect (Wainwright, 1989b). 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

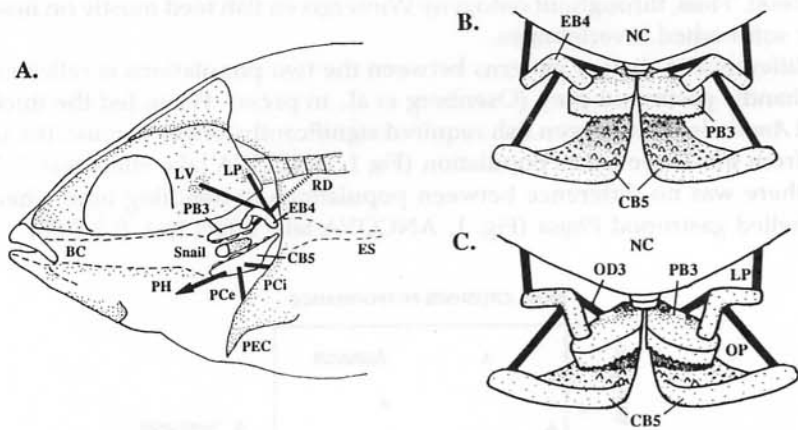


Figure 2. Illustrations of the snail crushing mechanism in the pumpkinseed sunfish. A) Schematic lateral view of the head illustrating the position of the pharyngeal jaw apparatus and the muscles that function during its use. Muscles are shown as thick black lines indicating their attachments. B) Posterior 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. Abbreviations: BC, buccal cavity; CB5, fifth ceratobranchial or lower pharyngeal jaw; EB4, fourth epibranchial; ES, esophagus; LP, levator posterior; LV, fourth levator externus; NC, neurocranium; OD3, third obliquus 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. Figure is from Wainwright et al. (1991).

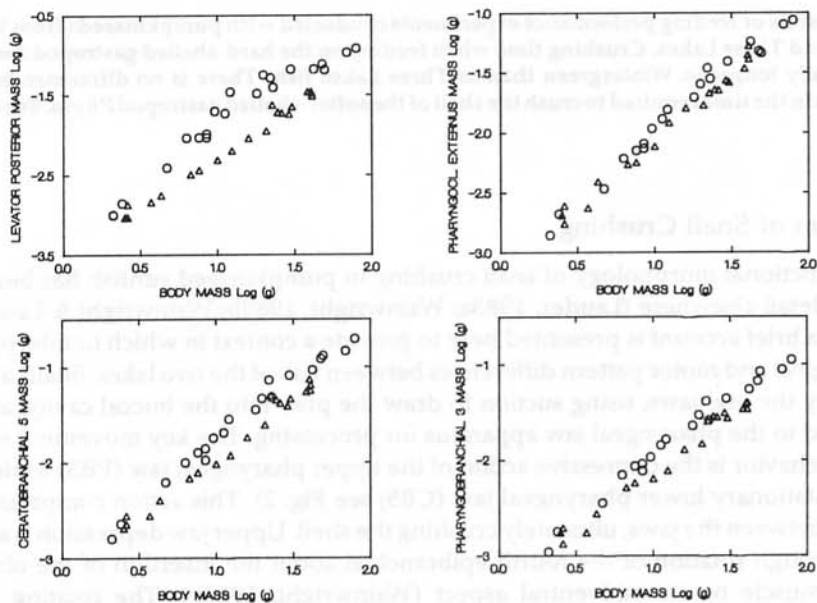


Figure 3. Plots of muscle and bone mass against body mass for pumpkinseeds from Wintergreen lake (triangles) and Three Lakes (circles). The levator posterior and pharyngocleithralis are muscles of the pharyngeal jaws, and the 5th ceratobranchial and 3rd pharyngobranchial are the toothed lower and upper pharyngeal jaw bones respectively. Among the four structures plotted, only the pharyngocleithralis externus muscle is not significantly larger in the Three Lakes population. Data from Wainwright et al. (1991).

Table 1. Results of ANCOVAs for the \log_{10} masses of muscles and bones from the pharyngeal jaw apparatus (PJA) and head of pumpkinseeds from Three Lakes and Wintergreen lake. Body mass was the covariate in each case. Muscles and bones are divided into separate categories depending on whether they are involved in snail crushing function. Data from Wainwright et al. (1991).

Structure	ANCOVA		Ratio of ** adjusted means
	Slopes	Intercept	
muscles:			
PJA muscles:			
levator posterior	NS	109.3*	2.33
levator internus 3	NS	35.1*	1.48
pharyngohyoideus	NS	17.0*	1.39
obliquus dorsalis 3	NS	23.4*	1.37
retractor dorsalis	NS	13.6*	1.31
pharyngocl. internus	NS	16.5*	1.29
levator externus 3/4	NS	6.7	1.19
levator internus 2	NS	5.1	1.14
pharyngocl. externus	NS	4.4	1.10
not PJA muscle:			
sternohyoideus	NS	1.5	1.10
bones:			
elements of the PJA:			
ceratobranchial 5	NS	47.5*	1.52
pharyngobranchial 3	NS	44.9*	1.47
epibranchial 4	NS	41.6*	1.65
not elements of the PJA:			
certobranchial 1	NS	2.4	0.80
epibranchial 1	NS	0.9	0.81

* = for the muscles this is $P < 0.005$, and for the bones this is $P < 0.0083$ (these are Bonferroni corrections of $P < 0.05$ for each data set).

** = using a pooled slope for the two populations, this column lists the ratio of predicted muscle and bone masses for fish of an average body mass from each population. Ratios are predicted values for Three Lakes fish divided by predicted values for Wintergreen fish.

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 redear sunfish, *Lepomis 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.

Pharyngeal Jaw Morphology

Pumpkinseeds in Wintergreen lake eat many fewer snails than fish in Three Lakes and are less proficient at handling gastropod prey. In association with these observations we found extensive differences between the two populations in the morphology of the pharyngeal jaw apparatus. Of the nine pharyngeal jaw muscles that were examined, six were significantly larger in Three Lakes fish, with the other three showing a trend in this direction (Table 1; Fig. 3). The primary crushing muscle, the levator posterior, showed the biggest difference, being over twice as large in Three Lakes fish as in Wintergreen fish. The sternohyoideus, which is not activated during snail crushing (Lauder, 1983a), showed no difference between lakes.

Significant differences were also observed between lakes in the masses of pharyngeal jaw bones. All three pharyngeal jaw bones were significantly heavier in Three Lakes fish than they were in Wintergreen pumpkinseeds (Table 1; Fig. 3). Two branchial arch bones that are not part of the crushing apparatus showed no difference between populations (Table 1). A more detailed analysis of pharyngeal jaw bone morphology, including considerations of tooth and bone shape is presented elsewhere (Wainwright et al., 1991).

Motor Pattern

Electromyographic data were collected from five of the muscles included in the morphological analysis; the levator posterior, levator externus 3/4, the retractor dorsalis, the pharyngocleithralis internus, and the pharyngocleithralis externus (Wainwright et al., ms.) During snail crushing, pumpkinseeds from Three Lakes exhibited muscle activity patterns characterized by long, simultaneous bursts of activity in four of the five muscles (Fig. 4). The pharyngocleithralis externus muscle (PCe) showed a very different pattern of activation from the other four muscles. This muscle exhibited repeated, short bursts of activity between the time the snail was captured and the beginning of crushing. During the crushing event the PCe muscle showed less activity than the other muscles.

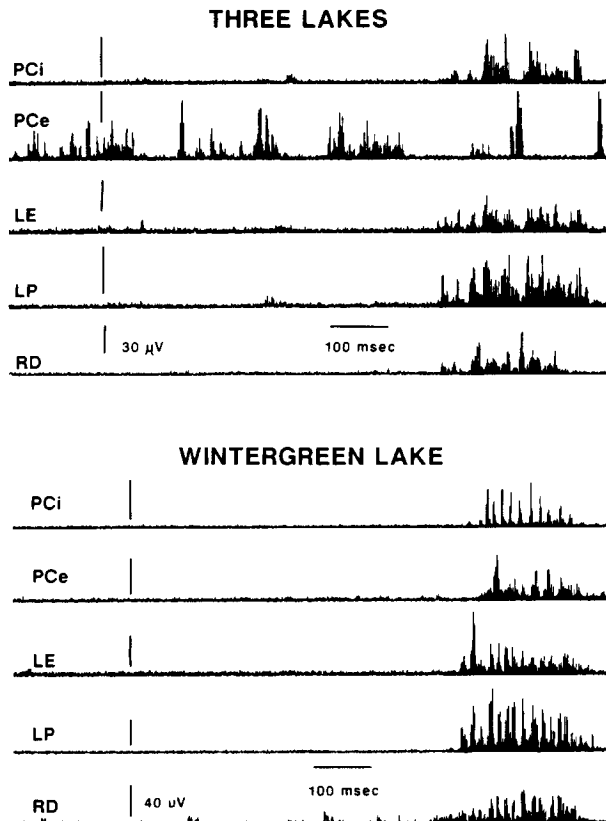


Figure 4. Sample electromyogram from a Three Lakes pumpkinseed and a Wintergreen individual. The myograms have been rectified and the area under each curve is shaded in. The simultaneous bursts of activity that occur in most muscles in each panel correspond to the snail crushing event. Of 16 variables that were measured on myograms during snail crushing only those describing the activity of the PCe muscle were significantly different between populations. Abbreviations: PCi, pharyngocleithralis internus muscle; PCe, pharyngocleithralis externus muscle; LE, levator externus 3/4 muscles; LP, levator posterior muscle; RD, retractor dorsalis muscle. Figure modified from Wainwright, et al. (ms).

Wintergreen pumpkinseeds exhibited a motor pattern that appears very similar to the Three Lakes pattern in four of the five muscles. However, activity of the PCe muscle in Wintergreen fish showed marked differences from that seen in Three Lakes fish (Fig. 4). Activity of this muscle prior to the crushing event was very rare in Wintergreen individuals, and during crushing the PCe was activated in a pattern similar to that seen in the other four muscles.

These differences are born out by statistical analyses. Out of a total of 16 variables that were measured from the electromyographic recordings of each snail crushing event, only 4 varied significantly between lakes (Table 2). Of these four variables, one was the time between the capture of the snail and the onset of the crushing event (significantly longer in Three Lakes fish), while the other three were measures of activity in the PCe muscle. The total integrated area of activity in the PCe prior to crushing was greater in Three Lakes fish. The integrated area and duration of activity of the PCe during crushing were significantly less in Three Lakes pumpkinseeds. Among the other four muscles no integrated area, activity duration, or relative timing variables showed significant lake differences (Table 2).

Table 2. Results of nested ANOVAs (individuals nested within lakes) contrasting 16 electromyographic variables measured from recordings from five pharyngeal jaw muscles made during snail crushing by pumpkinseed sunfish from two lakes. Data analyzed for seven fish from Three Lakes and six fish from Wintergreen lake. Table entries are *F*-ratios from each test. Data are from Wainwright et al. (ms).

Variable	Lake Effect	Individual
Activity Duration:		
PCe1 DUR	28.8**	4.5**
PCi DUR	2.8	2.6**
LE DUR	0.2	4.6**
LP DUR	0.7	1.7
RD DUR	0.7	1.5
Timing:		
STRIKE-LP	7.5*	3.0**
LP-PCe1	2.4	3.0**
LP-PCi	0.8	2.9**
LP-LE	1.0	3.3**
LP-RD	0.7	3.5**
Rectified Integrated Area:		
PCe pre AREA	18.1**	4.9**
PCe AREA	5.4*	7.7**
PCi AREA	0.3	22.5**
LE AREA	1.3	15.4**
LP AREA	1.3	25.9**
RD AREA	2.5	11.6**

Degrees of freedom for most lake effect tests = (1, 11); degrees of freedom for most individual effect tests = (11, 95). For RD DUR, LP-RD, and RD AREA the lake effect degrees of freedom = (1, 9); the individual effect degrees of freedom = (9, 80).

* = $P < 0.05$; ** = $P < 0.01$.

DISCUSSION

Our studies on the pumpkinseeds from these two lake populations have revealed a distinct polymorphism in the functional morphology of the snail crushing pharyngeal jaw apparatus. Both the morphology of the pharyngeal jaw muscles and bones and the motor

pattern used during snail crushing show important differences between populations. Six of the nine pharyngeal jaw muscles were significantly larger in Three Lakes pumpkinseeds, with the other three muscles showing a trend in this direction. Similarly, pharyngeal jaw bones are larger in Three Lakes fish. In the motor pattern analysis, four muscles exhibited the same activity pattern in the two lakes, while the P_{Ce} muscle showed a major, qualitative shift in its activity during snail crushing. In pumpkinseeds from Three Lakes this muscle is repeatedly activated for short, consecutive bursts in the time prior to crushing. In contrast, activity prior to crushing is rare for this muscle in Wintergreen fish, and the P_{Ce} is instead activated during crushing much like the other four muscles. In association with these morphological and neuromuscular differences is a reduction in the ability of pumpkinseeds from Wintergreen lake to handle hard-shelled snail species.

Below we discuss the consequences of the polymorphism for pharyngeal jaw function. This is followed by discussions of the possible causes of the polymorphism, and finally by a comparison of this intraspecific data set with previous interspecific studies of the evolution of the feeding mechanism in aquatic-feeding lower vertebrates.

Consequences of Polymorphism for Pharyngeal Jaw Function

Previous studies of the functional morphology of snail crushing in pumpkinseeds and other perciform fishes (Lauder, 1983a; Wainwright, 1989a, b) have identified the key anatomical and neuromuscular elements of snail crushing and make it possible to infer the consequences of the particular morphological and motor pattern differences that were found.

The muscle mass differences between populations probably result in differences in the force producing capacity of those muscles. Within ontogenetic series muscle mass has been demonstrated to be tightly associated with the force producing capacity of individual muscles (Marsh, 1988; Bennett et al., 1989; Thomason et al., 1990). Similarly, when comparing homologous muscles among closely related species, muscle mass has been found to provide an accurate estimate of relative strength (Powell et al., 1984; Wainwright, 1988). This relationship between muscle size and force producing capability is not unexpected. The maximum tension that a muscle can develop is a combined function of its force producing capacity per unit of cross sectional area of muscle tissue (stress) times the physiological cross sectional area (e.g., Calow & Alexander, 1973). If muscle shape, the degree of fiber pinnation, and stress properties 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, and an increase in the total force that the muscle can develop. Available data for ectothermic vertebrates show that stress is not influenced by body size (Marsh, 1988; Bennett et al., 1989).

Previous studies identify the levator posterior muscle of generalized perciform fishes, like sunfishes, as one of the primary muscles involved in generating the forceful actions used during prey crushing by the pharyngeal jaws (Lauder, 1983a; Wainwright, 1989a, b). Snail crushing performance in fishes has been shown to be limited directly by the amount of force that an individual can exert against the gastropod shell (Wainwright, 1987; Wainwright, 1988; Osenberg & Mittelbach, 1989). Hence, the force capabilities of the levator posterior muscle can be expected to provide a reasonable indicator of the potential crushing force of an individual fish. We estimated the cross sectional area of the levator posterior muscles examined in our morphological analysis and found that the expected force producing capability of this muscle was over twice as large in Three Lakes fish as in Wintergreen fish (Wainwright et al., 1991).

Similarly, the heavier and more robust (Wainwright et al., 1991) pharyngeal jaw bones in Three Lakes fish may be better able to withstand the stress that will be associated with forceful pressing of these elements against gastropod shells. Though we have not measured the mechanical properties of the jaw bones, a correlation between bone robust-

ness and strength has been observed in other systems (e.g., the two Woo et al., 1981 papers). 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. The difference between populations in the ability to feed on hard-shelled snails (Fig. 1) is probably largely due to these differences in crushing strength.

Though the levator posterior can be identified as a key muscle in generating crushing forces, there were no differences between lakes in the motor pattern of this muscle. Hence, the capacity for generating crushing force is greater in Three Lakes fish, yet fish from the two lakes employ the levator posterior muscle in a similar fashion. The only muscle that showed motor pattern differences, the P_{Ce} muscle, functions to retract the lower pharyngeal jaw ventrally, away from the shell of a gastropod that is in position to be crushed (Fig. 2). The repeated bursts of activity that occur in this muscle prior to the crushing event thus reflect repeated cycles of lower pharyngeal jaw depression. We hypothesize that this action of the lower jaw and the water motion that it may create within the buccal cavity assist in manipulating the gastropod shell as the fish prepares for a crushing attempt. Thus, the motor pattern polymorphism seen between lakes does not appear to relate directly to the act of snail crushing, but rather to the manipulation of gastropod prey that occurs prior to crushing.

Pharyngeal Jaw Polymorphism: Plasticity or Evolution?

A key issue in this case study concerns the causal basis of the observed polymorphism. Clearly, the difference between lakes in the abundance of snails ultimately underlies the trophic divergence that we have documented. However, whether this divergence results entirely from developmental plasticity, in response to the abundance of snail prey, or has a genetic basis is an issue that can not be resolved with certainty at this time. The answer to this question is significant because it determines the extent to which the observed transformation in pharyngeal jaw function can be viewed as a model of evolutionary change in functional morphology of the feeding mechanism.

Though the issue remains unresolved until we can address it directly in common environment experiments, two observations suggest that the polymorphism is primarily due to phenotypic plasticity and not due to genetic differences between the populations (a more detailed discussion is presented in Wainwright et al., 1991). The first concerns the history of Wintergreen lake. Available evidence suggests the rarity of snails is a recent phenomena (post 1977) brought about by a change in the Wintergreen fish community (Osenberg et al., in press). The second observation is that all of the changes, morphological and neuromuscular, can be seen as resulting from ontogenetic interactions of the feeding mechanism with prey use. By frequently crushing snails during ontogeny, fish in Three Lakes exert a training effect on their pharyngeal jaw muscles (Chapman & Troup, 1970; Ashton & Singh, 1974). The salient feature of pharyngeal jaw muscle activity in the pumpkinseed is that nearly all muscles are active for long, intense bursts during crushing bouts (Fig. 4; Lauder, 1983a, b). Hence, even muscles that would function alone to abduct the jaws are active during the strong adduction that occurs during snail crushing, presumably to stabilize the jaws during forceful exertion. We have estimated that adult pumpkinseeds in Three Lakes eat over 100 snails per day during the summer months (Osenberg, unpublished data). Forceful exertions of most of the pharyngeal jaw muscles at such a frequency could readily induce the relative hypertrophy of muscles seen in Three Lakes fish compared to their Wintergreen counterparts.

The motor pattern differences may also have a basis in the relative experience of fish in the two lakes. Pumpkinseeds have previously been shown to be able to adjust the motor pattern used during prey capture following continued exposure to a novel prey over a period of several weeks (Wainwright, 1986). This ability to "fine tune" the motor pattern in

response to repeated exposure to specific prey types may occur during the ontogeny of Three Lakes pumpkinseeds. We suggest that Wintergreen pumpkinseeds exhibit the basic snail crushing motor pattern, characteristic of this species (Lauder, 1983a, 1986), and that Three Lakes fish "fine tune" this distinctive motor pattern, specifically by altering the use of the PCe muscle to enhance their ability to manipulate and position snails prior to crushing them. This hypothesis could be tested in the laboratory by maintaining groups of fish from each population on diets of snails or no-snails, and examining the effect that these diets have on the snail crushing motor pattern.

Inter- and Intraspecific Trophic Divergence

One of the most striking results from our combined analysis of muscle morphology and motor pattern is that the pattern of change seen at the morphological level is incongruent with the pattern of change seen in the motor pattern (Fig. 5). Specifically, the muscle that shows the biggest morphological difference between lakes, the levator posterior muscle, shows one of the least indications of a lake effect in the motor pattern. Also, of the five muscles studied electromyographically, only the PCe muscle showed a significant motor pattern lake effect, though this muscle showed the least indication of a morphological lake effect (Fig. 5).

This result has two important implications. First, it suggests that predicting the function of structures entirely from anatomical observations can be a very hazardous undertaking. In the present case one would clearly have been wrong to expect the patterns of divergence in motor pattern to reflect those found in the morphology of the muscles. Directly measuring the function (use) of structures is a crucial component of comprehensive analyses of complex systems that has no replacement.

Secondly, this case study demonstrates the independent nature of these two levels of biological design. In spite of the highly complex, integrated quality of the pharyngeal jaw apparatus, morphology and motor pattern can transform independently. Change at one level of design does not depend on, nor does it necessarily affect, change at the other level.

Our study of the pumpkinseed polymorphism is only the second that we are aware of in which the analysis of an intraspecific trophic polymorphism has included an analysis of muscle activity patterns. Liem and Kaufman (1984) reported that the trophic poly-

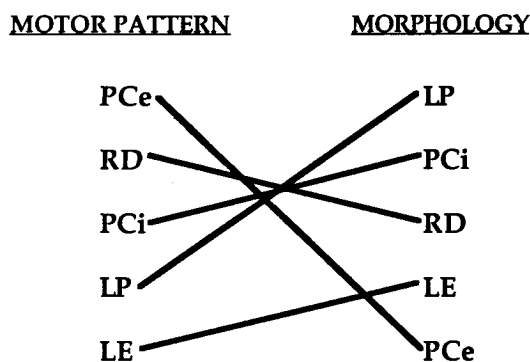


Figure 5. A comparison of rankings of population differences for five pharyngeal jaw muscles in morphological and motor pattern analyses. Within each column the ranking of muscles was determined by averaging the F-ratios from ANOVA significance tests for the lake effect. Thus, the top muscle in each column is the most distinctly different between lakes, based on morphological or motor pattern criteria. The bottom muscle in each column is the least different between lakes. Lines connect the muscles in each list and illustrate the incongruence of morphological and motor pattern divergence in the pharyngeal jaws of the pumpkinseed. Abbreviations are as in Fig. 4.

morphism seen in the Central American cichlid *Cichlasoma minckleyi* involves several morphological modifications of the pharyngeal jaw apparatus, but they found no motor pattern differences during prey capture and manipulating behaviors. With the exception of the P_{Ce} muscle motor pattern, which we found to be activated differently during snail crushing, our results are generally very similar to those of Liem and Kaufman. In addition, our results are largely in agreement with what has been found in interspecific comparative studies of the feeding mechanism in other lower vertebrates. Typically, morphological aspects of the feeding mechanism will vary extensively among closely related species, while the motor pattern exhibited during suction feeding or pharyngeal jaw function tends to be strongly conserved (Shaffer & Lauder, 1985; Wainwright & Lauder, 1986; Sanderson, 1988; Wainwright, 1989a; Westneat & Wainwright, 1989). Hence, we see in comparative studies that: (1) predicting patterns of change in motor pattern from anatomy is difficult, and (2) morphology and muscle activity can evolve independently. One of the concerns over the evolution of complex systems focuses on the potential difficulty in modifying them because of the constraining effects created by their highly integrated, interconnected nature. At least in the case of the feeding mechanism of aquatic feeding lower vertebrates it seems that some levels of design can transform independently of others and they are not constrained by the complex nature of the system.

ACKNOWLEDGMENTS

We thank Marvalee Wake for organizing this symposium and the National Science Foundation for providing traveling expenses. Support for the research described in this paper was provided by NSF grants BSR 87-96261 to G. G. M., BSR 89-05867 to G. G. M. and C. W. O., and BSR 85-20305 and DCB 87-10210 to G. V. L.

LITERATURE CITED

- Alexander, R. M. 1982. *Locomotion of Animals*. Blackie: Glasgow.
- Ashton, T. E. J. & M. Singh. 1974. The effect of training on maximal isometric back-lift strength and mean peak voltage of the erector spinae. Pp. 448-452. In: R. C. Nelson & C. A. Morehouse (eds.), *Biomechanics IV*. University Park Press: Baltimore.
- Bennett, A. F., Garland, T. J. & P. L. Else. 1989. Individual correlation of morphology, muscle mechanics, and locomotion in a salamander. *Am. J. Physiol.* 256:R1200-R1208.
- Calow, L. J. & R. M. Alexander. 1973. A mechanical analysis of a hind leg of a frog (*Rana temporaria*). *J. Zool., Lond.* 171:293-321.
- Chapman, A. E. & J. D. G. Troup. 1970. Prolonged activity of lumbar erector spinae. An electromyographic and dynamometric study of the effect of training. *Ann. Phys. Med.* 10:262-269.
- Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*. Murray: London.
- Darwin, F. 1887. *The Life and Letters of Charles Darwin*. Murray: London.
- Dawkins, R. 1986. *The Blind Watchmaker*. W. W. Norton & Co.: London.
- Fay, R. R. & A. N. Popper. 1985. The octavolateralis system. Pp. 291-316. In: M. Hildebrand, D. M. Bramble, K. F. Liem & D. B. Wake (eds.), *Functional Vertebrate Morphology*. Belknap Press: Cambridge, MA.
- Frazetta, T. H. 1975. *Complex Adaptations in Evolving Populations*. Cambridge University Press: Cambridge.
- Goslow, G. E., Jr., Bennett, A. F., Blickman, D. M., Bramble, D. M., Duncker, H. -R., Fischer, M. S., Hinchliffe, J. R., Jenkins, F. A., Jr., Szekely, G., van Mier, P. & J. J. Videler. 1989. Group report: How are locomotor systems integrated and how have evolutionary innovations been introduced? Pp. 205-218. In: D. B. Wake & G. Roth (eds.), *Complex Organismal Functions: Integration and Evolution in Vertebrates*. John Wiley & Sons: New York.

- Keast, A. 1978. Trophic and spatial interrelationships in the fish species of an Ontario temperate lake. *Env. Biol. Fish.* 3:7-31.
- Lauder, G. V. 1983a. Functional and morphological bases of trophic specialization in sunfishes (Teleostei, Centrarchidae). *J. Morphol.* 178:1-21.
- Lauder, G. V. 1983b. Neuromuscular patterns and the origin of trophic specialization in fishes. *Science* 219:1235-1237.
- Lauder, G. V. 1986. Homology, analogy and the evolution of behavior. Pp. 9-40. In: M. H. Nitecki & J. A. Kitchell (eds.), *Evolution of Animal Behavior*. Oxford University Press: New York.
- Lauder, G. V. 1990. Functional morphology and systematics: studying functional patterns in an historical context. *Ann. Rev. Ecol. Syst.* 21:317-340.
- Lauder, G. V., Crompton, A. W., Gans, C., Hanken, J., Liem, K. F., Maier, W. O., Meyer, A. Preseley, Rieppel, O. C., Roth, G., Schluter, D. & G. A. Zweers. 1989. Group report: how are feeding systems integrated and how have evolutionary innovations been introduced? Pp. 97-115. In: D. B. Wake & G. Roth (eds.), *Complex Organismal Functions: Integration and Evolution in Vertebrates*. John Wiley & Sons: New York.
- Levine, J. S. 1985. The vertebrate eye. Pp. 317-337. In: M. Hildebrand, D. M. Bramble, K. F. Liem & D. B. Wake (eds.), *Functional Vertebrate Morphology*. Belknap Press: Cambridge, MA.
- Liem, K. F. 1989. Respiratory gas bladders in teleosts: functional conservatism and morphological diversity. *Amer. Zool.* 29:333-352.
- Liem, K. F. & L. S. Kaufman. 1984. Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. Pp. 203-215. In: A. A. Echelle & I. Kornfield (eds.), *Evolution of Species Flocks*. University of Maine Press: Orono.
- Marsh, R. L. 1988. Ontogenesis of contractile properties of skeletal muscle and sprint performance in the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* 137:119-139.
- Meyer, A. 1989. Cost of morphological specialization: feeding performance of 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). *Biol. J. Linn. Soc.* 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 zone invertebrates. *Ecology* 69:614-623.
- Motta, P. J. 1988. Functional morphology of the feeding apparatus of ten species of Pacific butterfly fishes (Perciformes: Chaetodontidae): an eco-morphological approach. *Env. Biol. Fish.* 22:39-67.
- Osenberg, C. W. & G. G. Mittelbach. 1989. Effects of body size on the predator-prey interaction between pumpkinseed sunfish and gastropods. *Ecol. Monogr.* 59:405-432.
- Osenberg, C. W. G. G. Mittelbach & P. C. Wainwright. In press. Two-stage life histories in fish: the interaction between juvenile competition and adult performance. *Ecology*.
- Powell, P. L., Roy, R. R., Kanim, P., Bello, M. A. & V. Edgerton. 1984. Predictability of skeletal muscle tension from architectural determination in guinea pig hindlimbs. *J. Appl. Physiol.* 57:1715-1721.
- Sadzikowski, M. R. & D. C. Wallace. 1976. A comparison of the food habits of size classes of three sunfishes (*Lepomis macrochirus*, *L. gibbosus* and *L. cyanellus*). *Amer. Midl. Natur.* 95:220-225.
- Sanderson, S. L. 1988. Variation in neuromuscular activity during prey capture by trophic specialists and generalists (Pisces: Labridae). *Brain, Behav. Evol.* 32:257-268.
- Seaburg, K. C. & J. B. Moyle. 1964. Feeding habits, digestion rates, and growth of some Minnesota warm water fishes. *Trans. Amer. Fish. Soc.* 93:269-285.
- Shaffer, H. B. & G. V. Lauder. 1985. Aquatic prey capture in ambystomatid salamanders: patterns of variation in muscle activity. *J. Morphol.* 183:273-284.
- Thomason, J. J., Russell, A. P. & M. Morgeli. 1990. Forces of biting, body size, and masticatory muscle tension in the opossum *Didelphis virginiana*. *Can. J. Zool.* 68:318-324.
- Turner, R. G. 1980. Physiology and bioacoustics in reptiles. Pp. 117-134. In: A. N. Popper and R. R. Fay (eds.), *Comparative Studies of Hearing in Vertebrates*. Springer-Verlag: New York.
- Wainwright, P. C. 1986. Motor correlates of learning behavior: feeding on novel prey by the pumpkinseed sunfish. *J. Exp. Biol.* 126:237-247.
- Wainwright, P. C. 1987. Biomechanical limits to ecological performance: mollusc-crushing in the Caribbean hogfish, *Lachnolaimus maximus* (Labridae). *J. Zool., Lond.* 213:283-297.
- Wainwright, P. C. 1988. Morphology and ecology: functional basis of feeding constraints in Caribbean labrid fishes. *Ecology* 69:635-645.
- Wainwright, P. C. 1989a. Prey processing in haemulid fishes: patterns of variation in pharyngeal jaw muscle activity. *J. Exp. Biol.* 141:359-375.

- Wainwright, P. C. 1989b. Functional morphology of the pharyngeal jaw apparatus in perciform fishes: an experimental analysis of the Haemulidae. *J. Morphol.* 200:231-245.
- Wainwright, P. C. & G. V. Lauder. 1986. Feeding biology of sunfishes: patterns of variation in the feeding mechanism. *Zool. J. Linn. Soc.* 88:217-228.
- Wainwright, P. C. & G. V. Lauder. In press. The evolution of feeding biology in sunfishes. In: R. L. Mayden (ed.), *Systematics, Historical Ecology, and North American Freshwater Fishes*. Stanford Press: Stanford, CA.
- Wainwright, P. C., Osenberg, C. W. & G. G. Mittelbach. 1991. Trophic polymorphism in the pumpkinseed sunfish (*Lepomis gibbosus*): effects of environment on ontogeny. *Funct. Ecol.* 5:40-55.
- Wainwright, P. C., Sanford, C. J., Reilly, S. M. & G. V. Lauder. 1989. Evolution of motor patterns: aquatic feeding in salamanders and ray-finned fishes. *Brain Behav. Evol.* 34:329-341.
- Wake, D. B. & G. Roth. 1989. Introduction. Pp. 1-5. In: D. B. Wake & G. Roth (eds.), *Complex Organismal Functions: Integration and Evolution in Vertebrates*. John Wiley & Sons: New York.
- Werner, E. E. & D. J. Hall. 1979. Foraging efficiency and habitat switching in competing sunfishes. *Ecology* 60:256-264.
- Westneat, M. & P. C. Wainwright. 1989. The feeding mechanism of the sling-jaw wrasse, *Epibulus insidiator* (Labridae; Teleostei): evolution of a novel functional system. *J. Morphol.* 202:129-150.
- Woo, S. L. -Y., Gomez, M. A., Amiel, D., Cobb, N. G., Hayes, W. C. & W. H. Akeson. 1981. The effects of short and long term training on cortical bone hypertrophy. *Trans. Orth. Res. Soc.* 6:63.
- Woo, S. L. -Y., Kuei, S. C., Amiel, D., Gomez, M. A., Hayes, W. C., White, F. C. & W. H. Akeson. 1981. The effect of prolonged physical training on the properties of long bone: a study of Wolff's law. *J. Bone Jt. Surg.* 63A:780-787.