

Functional consequences of trophic specialization in pufferfishes

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Summary

1. Trophic breadth may be correlated with behavioural and functional versatility or flexibility (Liem 1984), such that species with limited diets would be predicted to be less versatile or flexible in their feeding capabilities than would trophic generalists. The relationship was examined between the trophic breadth of a species and the degree to which muscle activity is influenced by prey type in two species of pufferfishes: *Chilomycterus schoepfi*, a specialist on hard-shelled prey, and *Sphoeroides nephalus*, a generalist predator. It was predicted that the specialist would show a decreased ability to alter its motor pattern in response to prey type and an increased amount of motor-pattern variability when feeding on different prey.

2. Electromyographic recordings of five feeding muscles were made from five individuals per species feeding on four prey types that differed in their hardness and escape abilities. Muscle-activity duration, relative onset and integrated rectified area were analysed for prey capture and manipulation behaviours for each prey type.

3. Prey-type effects on mean motor-pattern variables for capture were limited to one of 14 variables in each species. Prey effects on buccal manipulation were not significant for any of the 14 variables for *C. schoepfi*, but *S. nephalus* exhibited prey effects for seven out of 14 variables. No differences in motor-pattern variation were found for the strike, but species differed significantly in overall motor-pattern variability during buccal manipulation; *C. schoepfi*, the dietary specialist, exhibited greater motor variability than *S. nephalus*, the dietary generalist.

4. The results therefore support a direct relationship between the trophic breadth of a species and its functional versatility for buccal manipulation. In contrast, prey capture was a relatively stereotyped behaviour for both species, as few prey-type effects were found.

Key-words: Diodontidae, electromyography, generalist, motor pattern, Tetraodontidae

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Introduction

A trophic specialist is a species that utilizes only a subset of the prey available in its environment (Futuyma & Moreno 1988; Sanderson 1988). Because specialists restrict themselves to a limited range of resources, they decrease their potential resource pool. This reduction may evolve, for example, when a predator that 'focuses' its abilities on a particular prey type can competitively exclude other species that are less effective at feeding on that prey in the same habitat. Trophic generalists, in contrast, exploit a wider range of prey types, although they may be unable to exploit any one of these as efficiently as would a specialist (Drummond 1983; Sanderson 1988).

Trophic breadth may be correlated with behavioural and functional versatility or flexibility (Liem 1984),

such that species with limited diets would be predicted to be less versatile or flexible in their feeding capabilities than would trophic generalists. Decreased versatility and flexibility may involve behavioural, morphological or functional modifications (Drummond 1983; Futuyma & Moreno 1988). Behavioural alterations can be changes in foraging or handling behaviours resulting in more efficient search, capture or acquisition of prey (e.g. Beissinger, Donnay & Walton 1994). Morphological specializations may include modifications of feeding structures (e.g. Lauder 1983; Meyer 1989). Functional modifications may include changes in neural control or patterning of movement (e.g. Lauder 1983; Sanderson 1988, 1990, 1991). Although behavioural and morphological specializations have been studied extensively (Lauder 1983; Jackson & Hallas 1986; Meyer 1989; Beissinger

et al. 1994; Turingan 1994), little work has addressed the proposed relationship between functional patterns and trophic specialization (Liem 1978, 1979, 1980; Lauder 1983; Sanderson 1988).

Patterns of muscle contraction serve as the basis of movement and thus are important effectors of prey capture and manipulation behaviours. One would therefore predict a relationship between the dietary breadth of a species and the muscle activation patterns used to capture and consume prey. Specifically, trophic specialists should show reduced flexibility, relative to generalists, in the motor patterns they exhibit during feeding on a range of prey, and they should show specialized motor patterns for use during feeding on preferred prey (Liem 1984; Sanderson 1988). Conversely, trophic generalists should exhibit a greater ability to modify or modulate muscle activity in response to different prey, corresponding to an increase in versatility. In this context, modulation is operationally defined (Liem 1978) as the ability of an organism to alter its muscle-activation patterns in response to changes in feeding conditions and is predicted to be particularly well developed in trophic generalists. In this paper, we use the terms 'versatility' and 'flexibility' to indicate the ability of a species to modulate the timing and intensity of muscle contraction.

To test these predictions, we investigated the motor control of feeding behaviour in two species of pufferfishes, the Southern Puffer, *Sphoeroides nephalus*, and the Striped Burrfish, *Chilomycterus schoepfi*. These species belong to sister families of the teleost order Tetraodontiformes (Winterbottom 1974), *C. schoepfi* to the Diodontidae and *S. nephalus* to the Tetraodontidae. The two species share many novel features associated with the ability to inflate their bodies into a spherical shape (Winterbottom 1974; Tyler 1980; Wainwright, Turingan & Brainerd 1995). Also, both use the oral jaws for prey capture and manipulation and possess beak-like jaws formed by their fused teeth (Winterbottom 1974). More detailed anatomical descriptions are presented by other authors (Turingan 1994; Tyler 1980; Wainwright *et al.* 1995; Winterbottom 1974). In contrast, dietary data from closely related species (Randall 1967) suggested that *C. schoepfi* was a trophic specialist feeding on hard-shelled molluscs, whereas *S. nephalus* was a generalist feeding on a wider variety of prey. We therefore began by evaluating and comparing the dietary profiles of the two species.

Our primary goal was to test the prediction that a narrow trophic breadth is associated with decreased functional flexibility. This relationship was addressed using electromyographic data to compare two aspects of the motor patterns exhibited by these pufferfishes: (1) the ability to modulate mean motor patterns in response to different prey, corresponding to versatility and (2) the variance of motor-pattern data exhibited during feeding on each prey type, corresponding to variability. In our use of the term versatility, we follow

Chu (1989) who stated that 'versatility implies a controlled, consistent adjustment in response to particular feeding situations or prey types' which we looked for in a greater ability to alter average motor patterns. Versatility may be altered independently of variability. It was predicted that the generalist *S. nephalus* would show a greater ability to alter the average motor pattern than would *C. schoepfi*, indicating increased flexibility as compared with the trophic specialist. It was predicted *C. schoepfi* would exhibit a more variable motor pattern when feeding on prey outside its restricted diet, indicating increased variability as the specialist normally feeds on a specific prey type and prey outside this realm represents a 'novel prey' with which it is unfamiliar. In addition, the prediction was tested that the specialized *C. schoepfi* would exhibit a unique motor pattern when eating its preferred prey.

Materials and methods

DIET

Individuals of both species were collected with otter trawls during the month of September 1993 from the seagrass beds off the Florida State Marine Laboratory in the northeastern Gulf of Mexico. Immediately after capture, each fish was killed by overanaesthesia (MS-222; > 1.0 g l⁻¹), the body cavity was opened by a small incision and the fish was placed in 10% formalin. Specimens remained in 10% formalin for about 6 months and were then transferred to 75% ethyl alcohol. For the dietary analysis, the gastrointestinal tract of each preserved fish was removed and its contents examined under a Wild M5 A dissecting microscope (WILD, Heerbrugg). Contents were grouped into categories by class, and their volumes were calculated by water displacement. The Shannon–Wiener index of dietary diversity (H') was used to quantify the degree of specialization in each species (Magurran 1988):

$$H' = -\sum p_i \ln p_i$$

where p_i is the volumetric proportion of a particular prey category for N prey categories. A t -test was performed to compare dietary diversity in the two species (Magurran 1988).

ELECTROMYOGRAPHY

Live fishes were collected from the seagrass beds off the Florida State Marine Laboratory and nearby Dog Island Reef during 1993 and 1994. Individuals were maintained in 100-l laboratory aquaria at 20 ± 2 °C and were fed a mixed diet of squid pieces, live earthworms, live penaeid shrimp and frozen crab legs for approximately 4–6 weeks. Food was withheld from individuals for 3 days prior to experimentation. *Chilomycterus schoepfi* individuals ranged from 153 to 194 mm standard length, and *S. nephalus* individuals ranged from 185 to 220 mm standard length.

Electromyographic recordings were made from the left-side member of five muscle pairs. Experiments were performed on five individuals per species. Muscles were chosen because of their prominence in the feeding mechanism and known function (Wainwright *et al.* 1995; see Fig. 1). The levator operculi (LOP) abducts the lower jaw via the interoperculum and is the primary mouth opener in all pufferfishes. The hyohyoideus abductor (HAB) expands the mouth cavity by flexing the joint between the first branchiostegal ray and the hyoid bar (Wainwright *et al.* 1995). Adductor mandibulae divisions 2α and 2β ($A2\alpha$, $A2\beta$) close the mouth by adducting the lower jaw. The protractor pectoralis (PP) compresses the mouth cavity by rotating the pectoral girdle forward. Methods for electromyography followed those of Wainwright & Turingan (1993). Electrodes were constructed from two 2.7-m pieces of insulated steel alloy wire (0.002 gauge) threaded through either 0.5-inch 26-gauge or 1.5-inch 25-gauge needles. The last 15 cm of the wires was glued together and the insulation removed 0.5 mm from the ends. The ends were then bent back over the needle tip to form a hook to anchor the electrode after implantation in the muscle. Each fish was anaesthetized with a saltwater mixture of anaesthetic (MS-222; 1.0 g l^{-1}) for about 10 min and then placed in a tray with a mixture of 50% saltwater and 50% anaesthetizing solution. Up to eight colour-coded electrodes were implanted in standardized locations in each muscle. After implantation, electrodes were bundled and secured to the fish's head with a suture on the left dorsolateral side posterior to the eye. The bundle was then glued into a common cable, and the fish was returned to its aquarium to recover from anaesthesia. Total implant time was always less than 30 min.

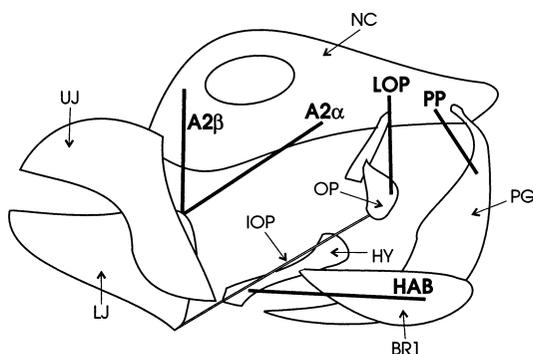


Fig. 1. Schematic diagram of the skull of *C. schoepfi* with the suspensorium removed, illustrating the functional units of the feeding mechanism addressed in this study. Thick lines indicate muscles and their attachments. Most of the suspensorium and branchial apparatus are not shown in this figure. Abbreviations: $A2\alpha$ section 2α of the adductor mandibulae; $A2\beta$ section 2β of the adductor mandibulae; BR1, first branchiostegal ray; HAB, hyohyoideus abductor; HY, hyoid bar; IOP, interoperculum; LJ, lower jaw; LOP, levator operculi; NC, neurocranium; OP, operculum; PG, pectoral girdle; PP, protractor pectoralis; UJ, upper jaw.

Approximately 2 h after recovery, fish were fed a randomized series of four experimental prey that encompassed a broad range of hardness and escape abilities: 3-cm sections of blue crab (*Callinectes sapidus*) walking legs, 5-cm-long live earthworms, 6-cm-long live penaeid shrimp with swimmerets removed and square 2-cm pieces of squid mantle. Crab legs were tough, hard, non-evasive prey requiring powerful and extensive processing. Worms also required extensive processing but were soft and elongate. Shrimp were evasive and often required multiple strikes before capture. Squid pieces were soft, non-evasive prey requiring little effort to capture or process.

Molluscs, the normal prey of *C. schoepfi*, were not used as the hard prey type because *S. nephelus* would not feed on them while implanted with electrodes, but the gastropod *Littorina irrorata* was fed to four *C. schoepfi* individuals, and muscle activity patterns were recorded.

General differences between the pufferfishes in their overall behaviours were noted. *Chilomycterus schoepfi* was much slower, both in approaching and in handling all prey types, than was *S. nephelus*.

Muscle electrical activity was amplified 10 000 times by Grass P511 J preamplifiers (Grass Instruments, Quincy, MA). A bandpass of 100–3000 Hz was used, and the 60-Hz notch filter was always employed. Signals were stored on VHS cassette by a TEAC XR-5000 tape recorder at 9.5 cm s^{-1} and printed for visual inspection at one-quarter record speed by a Western Graphtec Mark-11 thermal array recorder (Western Graphtec, Irvine, CA). Experiments were terminated after a minimum of two feedings per prey type were recorded, fishes were killed by over-anaesthesia and electrode placement was verified.

Feeding events were later digitized by a Keithley analogue-to-digital converter (Keithley Metrabyte, Taunton, MA) at a sampling rate of 8000 Hz and stored on optical disks. A feeding event consisted of prey capture and subsequent prey processing in the oral jaws. For each individual, two feeding sequences per prey type were used for analysis. To quantify patterns of muscle activity, we calculated the integrated rectified area (Basmajian & DeLuca 1985), burst duration and onset of activity relative to the onset of the levator operculi (LOP) for the prey-capture event and up to 10 subsequent cycles of processing activity per feeding event for each muscle (see Fig. 2). Unsuccessful strikes were not analysed. These measurements were made with custom software (Updegraff 1990) and resulted in 14 variables ($A2\alpha$ area, $A2\beta$ area, HAB area, LOP area, PP area, $A2\alpha$ dur, $A2\beta$ dur, HAB dur, LOP dur, PP dur, LOP- $A2\alpha$, LOP- $A2\beta$, LOP-HAB, LOP-PP). If more than 10 processing cycles occurred, only the first 10 were used. The levator operculi was used as a reference muscle because it was consistently active in all cycles of activity and has been used in previous studies

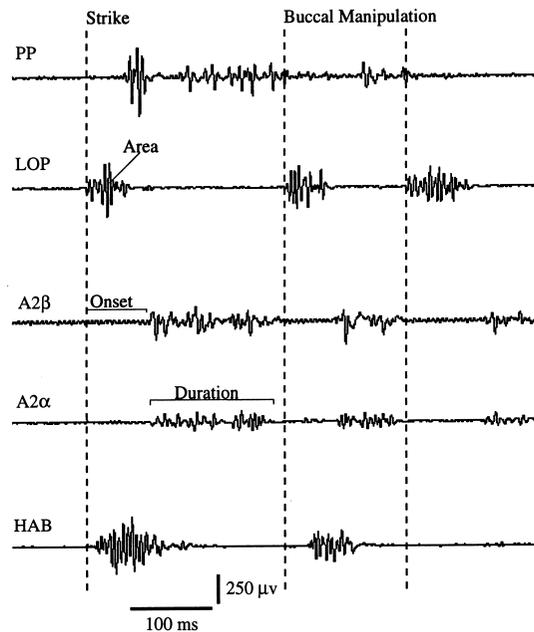


Fig. 2. Sample electromyogram of *S. nephalus* feeding on a shrimp to illustrate the electromyographic variables measured and the two behaviours studied. Variables illustrated are the relative onset and duration of the activity of each muscle. Vertical dashed lines indicate the onset of the levator operculi muscle, which was used as the reference in measuring relative onset times. Two behaviours were used for analysis: the strike, or prey capture, and buccal manipulation. Abbreviations as in Fig. 1.

(Wainwright & Lauder 1986; Turingan & Wainwright 1993). For this study, two distinct behaviours were recognized, the strike and buccal manipulation. The strike was defined as the activity cycle associated with prey capture, whereas buccal manipulation consisted of repetitive cycles of oral-jaw prey reduction and transport that preceded swallowing.

STATISTICAL ANALYSES

Two approaches were used to evaluate motor control of the feeding muscle activity patterns used by the test species. First, we used analyses of variance to compare the ability of each species to modulate its mean motor pattern for the different experimental prey. This approach investigated the capacity of each species to 'fine-tune' its motor patterns for different prey. Second, we quantified the amount of variation exhibited both for each species overall and for each prey type using principal-components analysis and centroid distances. This approach investigated the variability of the motor pattern and addressed whether one species was more variable than the other and whether one experimental prey elicited a more variable response than others. Previous research in this area has used a range of variables as an indicator of variability (e.g. Sanderson 1988; Wainwright & Lauder 1986). While range offers a measure of the extreme possibilities of a variable, variance was used here to

measure the dispersion of the motor pattern for each feeding around the mean. Our intent was to develop a metric that could be used to quantify how tightly clustered feeding sequences were around their mean. The methods employed in these analyses are discussed in the following two paragraphs.

The first approach was used to compare the ability of each species to alter the average motor pattern used to feed on the four experimental prey by applying analyses of variance to each electromyographic (EMG) variable. Two-way analyses of variance (ANOVAs) were used to test for differences in the mean value of each muscle-activity variable for each species in response to the different prey. In these ANOVAs, the individual factor (random effect) was crossed with the prey-type factor (fixed effect), resulting in an individual-by-prey-type interaction term. *F*-ratios for prey-type effects were calculated with the prey mean squares in the numerator and the interaction-term mean squares in the denominator. Total numbers of strike events per individual ranged from 17 to 41 with a mean of 31 for *C. schoepfi* and from 39 to 48 with a mean of 42 for *S. nephalus*. The total number of buccal-manipulation cycles per individual used in the ANOVAs ranged from 67 to 254 with a mean of 155 for *C. schoepfi* and from 115 to 272 with a mean of 211 for *S. nephalus*. Mean values of muscle-activity variables for species and prey type were inserted into cells with missing values (< 5% of the total data set). The number of missing cells did not differ significantly among species or prey type within species. Fourteen EMG variables were analysed from five individuals per species feeding on the four experimental prey common to the two species.

The second approach, using principal-components analysis, compared overall motor-pattern variability between species and tested the impact of prey type on motor variability. Our rationale was twofold. First, taxa may differ in the variance of motor activity they show during feeding, regardless of prey type. Second, prey type may influence not only the average value of EMG variables but the variability or range of motor activity during capture and handling behaviours as well. Principal-components analyses were first performed separately on the strike and buccal-manipulation data from three individuals and three muscles ($A2\alpha$, $A2\beta$, LOP) using a total of eight variables ($A2\alpha$ area, $A2\alpha$ dur, LOP- $A2\alpha$, $A2\beta$ area, $A2\beta$ dur, LOP- $A2\beta$, LOP area, LOP dur) per species. The PCAs resulted in three variables that accounted for about 80% of the variation in the original EMG data set. From these three variables (the principal components) a multivariate centroid was calculated in three-dimensional space for each species and prey-type combination that represented the multivariate mean motor pattern of each species feeding on each prey type. We quantified the 'variability' of the motor pattern by calculating the Euclidean distance between

the multivariate position of each feeding in this behaviour space (the co-ordinates of the three-dimensional position were determined by the factor scores on the principal components) and the appropriate centroid. Thus, each 'centroid distance' was a measure of how different that feeding was from the average for that species and prey type. Average centroid distances were calculated for each species and prey type combination as a basis for comparing the effect of prey type on motor variability. This approach is analogous to measuring the impact of prey type on the variance of each EMG variable, rather than the mean values, but we used this approach to simplify the final interpretation by providing a single, parametric test of significance rather than separate tests for each EMG variable. Furthermore, motor patterns are inherently multivariate phenomena, and this method makes use of the pattern of covariance among the original EMG variables.

To determine whether the two pufferfish species differed in the variability of the motor patterns used for the strike and buccal manipulation behaviours, we performed a two-way nested ANOVA on the centroid distances with species, prey type and individual as the main effects. After the discovery of an overall significant species effect, the species were analysed separately in two-way mixed-model ANOVAs, with prey type (fixed effect), individual (random effect) and an interaction term.

To address whether *C. schoepfi* exhibited a specialized motor pattern during feeding on its preferred prey, the data for this species were reanalysed, including the electromyographic data from buccal manipulations during feeding on the gastropod *L. irrorata*. ANOVAs assessing the effect of prey type on average motor patterns and on centroid distances were run and compared with the same test conducted without the

gastropod data. All statistics were calculated on \log_{10} -transformed data with Systat for Windows version 5 (Wilkinson 1992).

Results

DIET

Molluscs were the largest dietary contributor for *C. schoepfi*, whereas *S. nephalus* fed on a variety of prey (Table 1). Over 90% of the diet of *C. schoepfi* was composed of hard, sessile prey types. In contrast, the diet of *S. nephalus* included hard, soft, evasive and non-evasive prey. The Shannon–Wiener index of dietary diversity values was significantly smaller for *C. schoepfi* (0.5) than for *S. nephalus* (1.4), indicating a narrower diet for *C. schoepfi* ($P < 0.001$).

MOTOR PATTERN VARIATION

Both species showed a large range and variance in motor-pattern variables when feeding on all prey types (Tables 2 and 3), but some trends in average values of the EMG variables were evident. First, during the strike both species tended to show higher values of integrated area, burst duration and relative onset when feeding on shrimp. Second, crab and squid appeared to elicit greater EMG values during buccal manipulation for *C. schoepfi* and *S. nephalus*, respectively.

Most of these trends were not found to be significant in the ANOVAs (Table 4). During the strike, one EMG variable in each species changed in response to prey type. During buccal manipulation, prey type changed no EMG variables in *C. schoepfi*, but *S. nephalus* showed significant prey-type effects in seven of the 14 variables. Using the criteria proposed by Zolman (1993) to test for homogeneity of variance, significant heteroskedacity due to one individual in two out of 14 variables was found. The offending individual was removed from the analysis, the ANOVAs were rerun, and no differences were found in the number of significant variables (LOP area for *C. schoepfi* $P = 0.4$ without individual three, $P = 0.2$ with individual three; $A2\alpha$ dur for *S. nephalus* $P = 0.036$ without individual three, $P = 0.02$ with individual three). If the sequential Bonferroni technique is applied (Rice 1989), the significance level is reduced from $P > 0.05$ to $P > 0.00089$. This conservative experiment-wise error rate results in only one variable showing a significant effect ($A2\beta$ dur for *S. nephalus* buccal manipulation). While the number of significant variables is greatly reduced based on this conservative correction technique, the directionality is still the same. Thus, throughout this paper our discussion is based on the original ANOVA results, but because of the problems associated with interpreting multiple significance tests (Rice 1989), we prefer to interpret ANOVA results as trends rather than as 'significant' or 'non-significant' results (see also Stewart-Oaten 1995).

Table 1. Dietary composition of two species of pufferfishes from their intestinal contents. Table entries are averages of the percentage of volumetric contribution of each prey category

	<i>C. schoepfi</i>	<i>S. nephalus</i>
	95–266 (mm SL) <i>N</i> = 19	80–149 (mm SL) <i>N</i> = 12
Prey category (class)		
Gastropoda, Bivalvia (mollusc)	86.2	5.4
Malacostraca* (crab)	5.8	54.9
Ophiuroidea (brittle star)	0.0	15.9
Sand	0.0	12.9
Osteichthyes† (bony fish)	0.0	6.3
Gymnolaemata (bryozoan)	4.6	0.1
Other‡	3.1	4.3
Malacostraca§ (grass shrimp)	0.3	0.2

* Division Brachyura

† Teleostei.

‡ Includes class Polychaeta, order Isopoda and the seagrass *Syringodium filiforme*.

§ Infraorder Carida.

Table 2. Means (\pm SE of the mean) of 14 EMG variables for both strike and buccal manipulation behaviours for all prey types for *C. schoepfi*. Rectified, integrated areas are given in micro-volts times milliseconds ($\mu\text{V}\cdot\text{ms}$). Burst duration and onset times are given in ms

Variable	Strike				Buccal manipulation			
	Crab	Shrimp	Squid	Worm	Crab	Shrimp	Squid	Worm
A2 α area	4340 \pm 2131	10162 \pm 4352	7226 \pm 3992	2748 \pm 1322	7349 \pm 1506	7175 \pm 1067	4430 \pm 1297	4514 \pm 1205
A2 β area	7382 \pm 1956	15587 \pm 5587	5484 \pm 2417	14762 \pm 4367	14334 \pm 1972	10005 \pm 1268	11783 \pm 2441	7952 \pm 1267
HAB area	1714 \pm 1113	4107 \pm 1919	1249 \pm 632	1673 \pm 767	1316 \pm 438	1937 \pm 276	800 \pm 148	1077 \pm 278
LOP area	1477 \pm 404	1036 \pm 251	1083 \pm 289	1231 \pm 414	1886 \pm 211	1299 \pm 86	1083 \pm 94	1067 \pm 192
PP area	1505 \pm 555	1265 \pm 663	1181 \pm 617	1142 \pm 355	701 \pm 89	736 \pm 138	518 \pm 115	746 \pm 289
A2 α dur	183 \pm 72	269 \pm 56	102 \pm 36	119 \pm 21	299 \pm 19	227 \pm 18	182 \pm 16	166 \pm 11
A2 β dur	164 \pm 42	224 \pm 50	118 \pm 53	310 \pm 77	276 \pm 20	211 \pm 16	132 \pm 17	142 \pm 12
HAB dur	80 \pm 54	142 \pm 41	86 \pm 24	97 \pm 23	56 \pm 10	89 \pm 11	68 \pm 12	95 \pm 21
LOP dur	109 \pm 24	89 \pm 16	110 \pm 23	99 \pm 23	170 \pm 19	101 \pm 6	93 \pm 7	124 \pm 23
PP dur	344 \pm 123	157 \pm 82	346 \pm 197	186 \pm 43	134 \pm 27	72 \pm 9	79 \pm 13	71 \pm 18
LOP-A2 α	727 \pm 350	300 \pm 35	758 \pm 310	878 \pm 262	245 \pm 17	177 \pm 14	236 \pm 26	181 \pm 28
LOP-A2 β	367 \pm 77	379 \pm 84	539 \pm 157	482 \pm 93	239 \pm 16	159 \pm 11	170 \pm 21	144 \pm 18
LOP-HAB	290 \pm 105	379 \pm 134	184 \pm 12	321 \pm 102	85 \pm 23	67 \pm 10	95 \pm 23	146 \pm 48
LOP-PP	377 \pm 221	279 \pm 85	371 \pm 128	357 \pm 64	78 \pm 20	50 \pm 16	45 \pm 12	60 \pm 32

Table 3. Means (\pm SE of the mean) of 14 EMG variables for both strike and buccal manipulation behaviours for all prey types for *S. nephalus*. Rectified, integrated areas are given in $\mu\text{V}\cdot\text{ms}$. Burst duration and onset times are given in ms

Variable	Strike				Buccal manipulation			
	Crab	Shrimp	Squid	Worm	Crab	Shrimp	Squid	Worm
A2 α area	3899 \pm 1237	6048 \pm 1317	2659 \pm 955	1990 \pm 453	8036 \pm 656	5153 \pm 317	4350 \pm 637	4234 \pm 271
A2 β area	6858 \pm 3272	7307 \pm 2732	7111 \pm 2814	3607 \pm 986	9567 \pm 1798	5678 \pm 813	11556 \pm 1251	4753 \pm 548
HAB area	4661 \pm 1242	7784 \pm 2565	4429 \pm 1289	4277 \pm 836	1963 \pm 261	2336 \pm 231	3284 \pm 488	1444 \pm 207
LOP area	8999 \pm 7084	17098 \pm 9164	8319 \pm 6662	16025 \pm 10770	6407 \pm 1453	9072 \pm 1428	22778 \pm 10069	10770 \pm 2551
PP area	1125 \pm 338	3479 \pm 618	2701 \pm 1404	1426 \pm 299	1219 \pm 258	1521 \pm 222	2180 \pm 527	1221 \pm 194
A2 α dur	199 \pm 67	163 \pm 30	120 \pm 36	102 \pm 29	184 \pm 11	123 \pm 6	133 \pm 12	103 \pm 5
A2 β dur	260 \pm 72	229 \pm 65	304 \pm 115	244 \pm 58	226 \pm 13	137 \pm 8	221 \pm 17	158 \pm 9
HAB dur	173 \pm 30	157 \pm 20	151 \pm 19	151 \pm 20	86 \pm 8	63 \pm 4	99 \pm 9	50 \pm 4
LOP dur	89 \pm 20	129 \pm 19	82 \pm 25	83 \pm 14	68 \pm 6	67 \pm 4	117 \pm 25	65 \pm 5
PP dur	144 \pm 31	336 \pm 62	191 \pm 54	95 \pm 31	90 \pm 7	76 \pm 6	113 \pm 21	67 \pm 6
LOP-A2 α	984 \pm 379	1217 \pm 276	303 \pm 98	1291 \pm 563	116 \pm 7	1291 \pm 77	119 \pm 16	87 \pm 5
LOP-A2 β	841 \pm 392	1056 \pm 279	319 \pm 107	1669 \pm 996	93 \pm 8	67 \pm 3	70 \pm 10	44 \pm 8
LOP-HAB	1086 \pm 441	1275 \pm 310	251 \pm 99	1347 \pm 775	48 \pm 5	45 \pm 3	62 \pm 16	38 \pm 5
LOP-PP	1013 \pm 414	1139 \pm 296	267 \pm 153	1254 \pm 707	26 \pm 12	25 \pm 7	65 \pm 27	27 \pm 9

The principal-components analysis (PCA) performed on the eight-strike EMG variables yielded three components with eigen-values greater than 1 which accounted for 78% of the total variance. PC1 explained 38% of the variance with an eigen-value of 3.0. Factor loadings for PC1 were 0.644 (A2 α area), 0.868 (A2 α dur), 0.320 (LOP-A2 α), 0.772 (A2 β area), 0.771 (A2 β dur), -0.169 (LOP-A2 β), 0.435 (LOP area) and 0.567 (LOP dur). PC2 explained 24% of the variance with an eigen-value of 1.9. Factor loadings for PC2 were 0.536 (A2 α area), 0.319 (A2 α dur), -0.482 (LOP-A2 β), 0.370 (A2 β area), -0.001 (A2 β dur), 0.220 (LOP-A2 β), -0.827 (LOP area) and -0.627 (LOP dur). PC3 explained 16% of the variance with an eigen-value of 1.3. Factor loadings for PC3 were 0.119 (A2 α area), 0.002 (A2 α dur), 0.694 (LOP-A2 α), -0.044 (A2 β area), -0.076 (A2 β dur), 0.877 (LOP-A2 β), -0.078 (LOP area) and -0.046 (LOP dur). Plots

of the factor scores (Fig. 3) with 95% confidence intervals suggested no differences between species in the range of motor-pattern variables used during feeding on the four experimental prey because the confidence intervals were approximately equal in size.

The PCA performed on the buccal-manipulation data also yielded three components with eigen-values greater than 1, which accounted for 79% of the total variance. PC1 explained 37% of the variance with an eigen-value of 3.0. Factor loadings for PC1 were 0.665 (A2 α area), 0.870 (A2 α dur), 0.410 (LOP-A2 α), 0.774 (A2 β area), 0.876 (A2 β dur), -0.305 (LOP-A2 β), -0.304 (LOP area) and 0.186 (LOP dur). PC2 explained 24% of the variance with an eigen-value of 1.9. Factor loadings for PC2 were -0.488 (A2 α area), 0.017 (A2 α dur), 0.765 (LOP-A2 α), -0.239 (A2 β area), -0.213 (A2 β dur), 0.826 (LOP-A2 β), -0.063 (LOP area) and 0.519 (LOP dur). PC3

explained 18% of the variance with an eigen-value of 1.5. Factor loadings for PC3 were -0.024 ($A2\alpha$ area), 0.188 ($A2\alpha$ dur), -0.202 (LOP- $A2\alpha$), 0.021 ($A2\beta$ area), 0.121 ($A2\beta$ dur), -0.204 (LOP- $A2\beta$), 0.873 (LOP area) and 0.757 (LOP dur). Representative plots of the factor scores (Fig. 4) with 95% confidence intervals indicated differences in the amount of motor-pattern variability exhibited by the two species. The confidence intervals of *C. schoepfi* are larger than those of *S. nephalus*, suggesting that *C. schoepfi* exhibited a more variable motor pattern.

Table 4. *P* values from ANOVAs testing prey effects on EMG variables for strike and buccal manipulation behaviours for each species. Significant table entries indicate that the species altered that particular EMG variable in response to the four experimental prey types. Degrees of freedom varied owing to incomplete data sets for some prey types and/or individuals (e.g. individual refused to feed on a certain prey, muscle was not correctly implanted, etc.). NS = $P > 0.05$

Variable	Degrees of freedom	Strike		Buccal manipulation	
		<i>C. schoepfi</i>	<i>S. nephalus</i>	<i>C. schoepfi</i>	<i>S. nephalus</i>
A2 α area	3, 12	NS§	NS	NS§	NS
A2 α dur	3, 12	NS†	NS	NS†	0.02
A2 β area	3, 6	NS	NS	NS†	NS†
A2 β dur	3, 6	NS	NS	NS†	0.000†
HAB area	3, 9	NS*	NS	NS*	0.018
HAB dur	3, 9	NS*	NS	NS*	0.035
PP area	3, 12	NS	NS	NS†	NS†
PP dur	3, 12	NS	NS	NS†	0.037†
LOP area	3, 12	NS	0.006	NS	NS
LOP dur	3, 12	NS	NS	NS	NS
LOP-A2 α	3, 12	0.01†	NS	NS†	0.012
LOP-A2 β	3, 6	NS	NS	NS†	NS†
LOP-HAB	3, 9	NS*	NS	NS*	0.048
LOP-PP	3, 9	NS	NS‡	NS	NS

* Degrees of freedom are 3, 3.

† Degrees of freedom are 3, 9.

‡ Degrees of freedom are 3, 12.

§ Degrees of freedom are 3, 6.

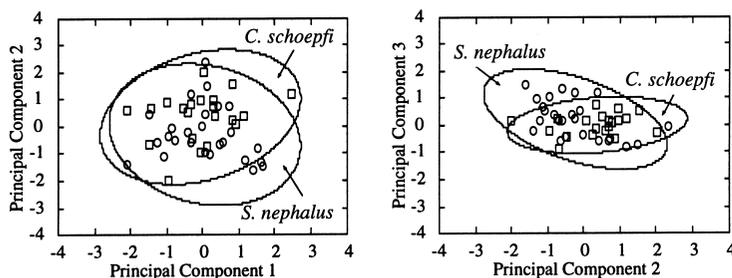


Fig. 3. Representative plots of the factor scores from the first three principal components of a principal-components analysis on three individuals and three muscles for each species exhibiting the strike behaviour. Squares indicate strike events for the four experimental prey types for *C. schoepfi*; circles indicate strike events for the four experimental prey types for *S. nephalus*. Ellipses indicate 95% confidence intervals around the centroids for each species. Note that, for the strike, the areas of the confidence intervals, which correspond to the variability of the motor pattern, are approximately the same. See text for a statistical confirmation of this trend.

In general, the centroid distance data support the qualitative interpretations of the PCA plots. Differences among species for each behaviour were investigated using a two-way ANOVA with species, prey type and an interaction term as the main effects. For the strike, there were no significant differences in the average centroid distances, indicating no differences in the variability of the motor pattern. For buccal manipulation, *C. schoepfi* had a significantly higher average centroid distance, indicating a more variable motor pattern overall. The overall ANOVA for buccal manipulation with both species and all experimental prey showed a significant species ($P_{1, 271} = 0.000$) and prey-type ($P_{3, 271} = 0.007$) effect. Thus, for buccal manipulation the two species had significantly different overall mean centroid distances (*C. schoepfi*, 1.45 vs *S. nephalus*, 1.25) and no prey types had the same mean centroid distances (*C. schoepfi*, 1.66, 1.52, 1.21, 1.39, vs *S. nephalus*, 1.35, 1.23, 1.46, 0.96 for crab, shrimp, squid and worm prey, respectively). Two-way mixed model ANOVAs performed on each species separately for buccal manipulation with prey type, individual and an interaction term as the main effects showed no significant prey-type effects on motor-pattern variability for either species. Significant differences among individuals within species were found (*C. schoepfi*, $P_{2, 112} = 0.002$, and *S. nephalus*, $P_{2, 146} = 0.000$) and are common results in many EMG studies (e.g. Wainwright & Lauder 1986; Sanderson 1988).

Including data for *C. schoepfi*-eating gastropods did not significantly alter the results of the analyses. The two-way ANOVA examining prey-type effects on the motor pattern of *C. schoepfi* showed that *C. schoepfi* altered only one of the 14 EMG variables ($A2\alpha$ onset mean (SEM was 241 ± 28 , $P = 0.029$), but no significant effects of prey type were apparent without the gastropod data. The PCA generated three components with eigen-values greater than 1, which accounted for 80% of the total variance. ANOVAs on the centroid distance data showed a significant species effect ($P < 0.0001$), as in the original analysis. When the ANOVAs were run on *C. schoepfi* separately, no significant prey-type effects were found ($P > 0.1$), with or without the gastropod data. Thus, there is little evidence to suggest that *C. schoepfi* uses a specialized motor pattern when eating its primary prey.

Discussion

On the basis of discussions in the literature that relate trophic breadth to functional versatility and flexibility (Liem 1984; Sanderson 1988) three predictions were identified and tested. We predicted that, when compared with the broad-dieted *S. nephalus*, a species with greater trophic specialization, *C. schoepfi*, would show (1) a reduced ability to alter its feeding motor pattern in response to different prey, (2) increased variance in motor-pattern data and (3) evidence of a

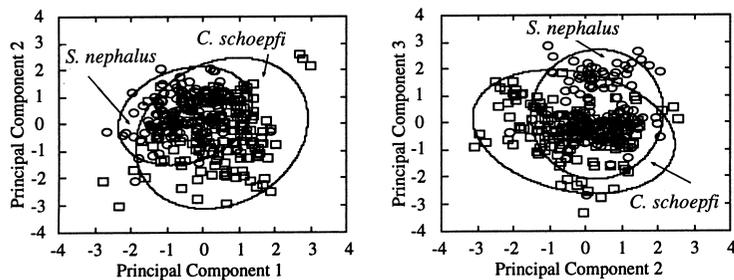


Fig. 4. Representative plots of the factor scores from the first three principal components of a principal-components analysis on three individuals and three muscles for each species exhibiting the buccal-manipulation behaviour. Squares indicate cycles of buccal manipulation for the four experimental prey types for *C. schoepfi*; circles indicate cycles of buccal manipulation for the four experimental prey types for *S. nephalus*. Ellipses indicate 95% confidence intervals around the centroids for each species. Note that, for buccal manipulation, the areas of the confidence intervals, which correspond to the variability of the motor pattern, appear smaller for *S. nephalus*, indicating less variable motor patterns than for *C. schoepfi*. See text for a statistical confirmation of this trend.

specialized motor pattern used while feeding on the prey on which it specializes. Our results for the buccal manipulation behaviour show considerable support for the first and second predictions but are only weakly consistent with the third prediction and our results for the strike behaviour are inconsistent with all the predictions.

DIETARY SPECIALIZATION

The dietary analysis confirmed our classification of *C. schoepfi* as a trophic specialist and *S. nephalus* as a trophic generalist. *Chilomycterus schoepfi* fed almost exclusively on hard, sessile prey items, which it crushed between its jaws, whereas the diet of *S. nephalus* was composed of a variety of prey (fish, crabs, brittle stars, molluscs) representing a range of functional challenges (Table 1). Futuyma & Moreno (1988) concluded that the classification of a species as specialized will be most accurate when 'the specialist's diet is included within that of the generalist's', as was the case here. The H' value for *C. schoepfi*, 0.5, was well below the normal Shannon–Wiener index range of 1.0–3.5, indicating a particularly narrow diet (Magurran 1988).

PREY TYPE AND AVERAGE MOTOR PATTERN

During prey capture, neither *C. schoepfi* nor *S. nephalus* showed a strong response to prey type, as each species altered only 1 EMG variable out of 14 (Table 4). Thus, for both species the strike was characterized by a relatively stereotyped muscle-activity pattern, which was altered little for capture of the four experimental prey. This stereotypy is striking because it occurred in the face of four prey with a broad range of escape abilities. Shrimp typically were pursued around the aquarium for several minutes, eliciting

several unsuccessful strikes prior to capture. It should be pointed out that Tables 2 and 3 do suggest a general trend toward greater muscle activity during capture of shrimp, but only a single EMG variable showed a statistically significant prey-type effect.

During buccal manipulation, *S. nephalus* showed a substantial ability to modify its motor pattern in response to different prey and modulated seven out of 14 EMG variables (Table 4). In general, squid pieces and crab legs elicited longer bursts of muscle activity than the other two prey (Table 3). In contrast, *C. schoepfi* altered no variables, a particularly noteworthy result given the range of hardness encompassed by the experimental prey. Although variation in consistency appeared to influence the buccal-manipulation behaviour of *S. nephalus* strongly, it had no effect on the average values of the EMG variables in *C. schoepfi*.

PREY TYPE AND VARIANCE OF MOTOR PATTERN

In the strike data set, average centroid distances were similar in the two species (Fig. 3), so the species did not differ in variability of EMG activity. Neither species showed an effect of prey type on centroid distance for either strike or buccal manipulation, indicating the variance of the motor pattern did not change with prey. Coupled with the results on the effect of prey on average values of EMG variables (Table 4) these data indicate little effect of prey type on variation in the strike motor pattern.

In the buccal-manipulation data a strong difference was found between the average centroid distances of the two species. *Chilomycterus schoepfi* exhibited greater motor variability than *S. nephalus* (Fig. 4). In general, this result suggests a statistical reason for the large difference between the species in the effect of prey type on the average EMG variables. Higher variances for *C. schoepfi* (Tables 2 and 3) would have the general effect of reducing the likelihood of finding significant differences between the mean values of the EMG variables measured during feeding on different prey. Tables 2 and 3 show a trend in which the standard errors associated with the mean variable values for *C. schoepfi* in the buccal-manipulation data are higher than corresponding values in the *S. nephalus* data set, whereas the mean values of variables show a trend across prey type in *C. schoepfi* similar to that in *S. nephalus*. The centroid distance ANOVAs confirm an overall difference between species in the variability of motor activity.

Even when feeding on gastropod prey, *C. schoepfi* showed only a slight increase in the ability to modulate mean values of the EMG variables. A single variable showed a significant prey-type effect when the gastropod data were included. Thus, our results indicate weak evidence that, when feeding on its preferred prey, *C. schoepfi* uses a specialized muscle-activity pattern. Indeed, given the importance of the adductor mandibulae muscles in prey-crushing behaviour, it is

remarkable that the only variable showing a prey-type effect was the onset time of the A2 α . Thus, it appears that *C. schoepfi* employs a non-distinct, highly variable motor pattern even on the prey type representing over 85% of its diet.

TROPHIC HABITS AND MOTOR FLEXIBILITY

Few studies have addressed the relationship between the trophic habits of fishes and their motor-pattern correlates. Sanderson (1988) found that during prey capture labrid fishes that were trophic specialists 'did not employ distinctive neuromuscular activity patterns relative to trophic generalists' when feeding on the same prey, including prey typically consumed by the specialists. In general, the labrid species studied tended to show restricted motor-pattern repertoires, altering only one to four EMG variables out of nine. African cichlids show a negative association between range of prey and ability to alter muscle-activity patterns and strong evidence of novel motor patterns during feeding on novel prey (Liem 1978, 1979, 1980). Lauder (1983) found that one centrarchid species that specialized on hard prey exhibited a stereotyped motor pattern of the pharyngeal jaw apparatus it used when feeding on different prey types, whereas those species that normally fed on a wide variety of prey were able to modulate their motor patterns.

With respect to buccal manipulation, our results support the proposed relationship between trophic breadth and versatility; an increased dietary range was correlated with an increase in motor modulation. The trophic specialist, *C. schoepfi*, altered no aspect of its motor pattern when performing the buccal-manipulation behaviour, but the trophic generalist, *S. nephalus*, altered multiple motor elements in response to different prey. Also, variation in the buccal-manipulation motor pattern was greater in *C. schoepfi*.

Pufferfishes do not appear to tailor their mode of prey capture to different prey. Both *C. schoepfi* and *S. nephalus* changed only one aspect of their mean muscle-activity patterns when capturing different experimental prey.

MOTOR-PATTERN EVOLUTION

The ability to modulate motor patterns in response to different prey appears to be a general (Wainwright 1989) and primitive (Lauder 1981) characteristic of teleost feeding mechanisms, as has been found in numerous fish taxa (e.g. Liem 1978, 1979, 1980; Lauder 1981, 1983; Wainwright & Lauder 1986; Sanderson 1988, 1990, 1991; Chu 1989; Wainwright 1989; Wainwright & Turingan 1993). Only rarely have tests with teleost fishes failed to find the ability to modulate prey-capture motor patterns (e.g. see results for *Ambloplites* in Wainwright & Lauder 1986). In this respect our results for modulation of the strike appear to be exceptional, suggesting that the

two pufferfishes are unusual in this respect. Clearly the results are not due to a general inability to alter muscle-activity patterns, because *S. nephalus* showed extensive modulation during buccal manipulation, and both species exhibited highly variable patterns of muscle activity in general. The lack of response to prey type in these species may reflect an inability to alter prey-capture performance significantly through motor modifications at the strike.

Limited work with other tetraodontiform taxa may provide insight into the broader phyletic changes in modulatory capabilities. Wainwright & Turingan (1993) studied the ability of the Queen Triggerfish (*Balistes vetula*), a member of a more generalized sister group to the pufferfish lineage (Winterbottom 1974), to modulate motor patterns in response to prey. They found that this triggerfish was able to modulate motor patterns extensively in response to prey type, altering seven EMG variables out of 13 for prey capture and three out of 13 for buccal manipulation. This limited comparative sample does not suggest a strong trend toward less modulation in more derived taxa. Although the pufferfishes showed a sharply limited ability to modulate during the strike, *S. nephalus* appears to have an increased capacity for modulation during buccal manipulation.

MEASURING AND DEFINING MOTOR-PATTERN VARIABILITY

Since Liem (1978) coined the term 'modulatory multiplicity' to describe the ability of predators to alter motor pattern in response to prey type, workers have typically used analysis of variance to compare the average value of EMG variables quantitatively as a test of this capacity (e.g. Sanderson 1988; Wainwright & Lauder 1986). In general, the differences between taxa in motor-pattern variance and the effect of prey type on the variability of motor patterns have not been considered. We suggest that analyses like our comparison of centroid distances provide a different and valuable view of the degree of stereotypy in muscle-activity patterns. The muscle-activity patterns measured during feeding in lower vertebrates are remarkably variable (see review by Wainwright 1989), and quantifying the variance of motor patterns provides a direct measure of how fixed the response of the animal is. The use of centroid distances from a principal-components analysis as in the present study has one important advantage over traditional tests of inequality of variances. Variances for the centroid distance parameter (essentially the variance of the average distance of a feeding from the average feeding) can be calculated, and standard parametric statistics (e.g. ANOVA) can then be used. Our discovery of a strong difference between taxa in centroid distance, and hence in the variance of the motor pattern, provides two new levels of insight. First, it shows an important way in

which the feeding responses of these two species differ, and second, it suggests that the lack of prey-type effect on the mean EMG variables in *C. schoepfi* may be due in part to the significantly higher variance in this species and not to different abilities of the species to alter muscle-activity patterns.

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