Kinematic analysis of a novel feeding mechanism in the brook trout *Salvelinus fontinalis* (Teleostei: Salmonidae): behavioral modulation of a functional novelty

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Summary

The tongue-bite apparatus (TBA) of salmonids represents an impressive novel feeding mechanism. The TBA consists of a set of well-developed teeth on the dorsal surface of the anterior hyoid (basihyal) and an opposing set of teeth on the roof of the mouth (vomer). A kinematic analysis of behaviors associated with the TBA in the brook trout *Salvelinus fontinalis* was performed using high-speed video (250 frames s^{-1}). Two distinct behaviors were identified, raking and open-mouth chewing. Univariate analysis demonstrated that these behaviors were significantly different from one another. The power stroke of raking is characterized by significantly greater neurocranial elevation (raking, 36°; open-mouth chewing, 16°) and retraction of the pectoral girdle (raking, 0.85 cm or 21% of head length; open-mouth chewing, 0.41 cm or 10% of head length). Open-mouth chewing is characterized predominantly by dorso-ventral excursions of the anterior hyoid (open-mouth chewing, 0.26 cm; raking, 0.14 cm). Raking is significantly shorter in duration (mean 49 ms) than open-mouth chewing (mean 77 ms). When presented with three different types of prey (crickets, fish or worms), *Salvelinus fontinalis* showed no variation in raking behavior, indicating that raking is highly stereotyped. In contrast, when feeding on worms, *Salvelinus fontinalis* modulated open-mouth chewing behavior with shorter durations to maximum displacement (at least 20 ms shorter than for either fish or cricket), although the magnitude of displacements did not vary. The reasons for the shorter duration of displacement variables while feeding on worms remains unclear. During post-capture processing behaviors in *Salvelinus fontinalis*, the magnitude of displacement variables is highly variable between individuals, but temporal patterns are not. This study characterizes two novel post-capture feeding behaviors and modulation of those behaviors in salmonids.

Key words: tongue-bite apparatus, feeding, kinematics, prey-type effect, modulation, brook trout, *Salvelinus fontinalis*.

Introduction

The diversity of feeding mechanisms in fishes and the phylogenetic position of fishes as basal vertebrates make them an important study group for understanding form/function relationships. Indeed, fish feeding has been a focus of functional morphologists for some time (for a review, see Lauder, 1985) and has provided important insights into broad biological questions regarding the evolution of the vertebrate musculoskeletal system (e.g. how conserved muscle activity patterns are within lower vertebrates) (Wainwright et al., 1989).

Representing a 'primitive' feeding mode, basal fishes such as *Polypterus senegalus* and *Amia calva* have non-protrusible jaws and prey processing is limited (Lauder, 1980). Structural modifications of the head region in higher teleostean fishes are common and have led to a number of novel feeding behaviors; for example, the pharyngeal jaw apparatus (Lauder, 1983; Wainwright, 1989; Grubich, 2000), protrusible upper jaws (Schaeffer and Rosen, 1961; Lauder, 1982) and protrusible lower jaws (Westneat and Wainwright, 1989; Ferry-Graham et al., 2001). These novel behaviors increase the feeding repertoire of fish and potentially expand the range of prey types available to the predator.

Most studies to date have focused on modifications of the pharyngeal jaws or the mandibular jaws and initial prey acquisition to explore form/function relationships (Liem, 1974; Stiassny and Jensen, 1987; Norton and Brainerd, 1993; Ferry-Graham et al., 2001; Parmentier et al., 2000). Although these studies have contributed a great deal to our understanding of teleostean feeding mechanisms, they have generally focused on initial prey acquisition and general patterns of attack in establishing the morphological correlates of feeding performance (see Norton, 1991; Norton and Brainerd, 1993). There are very few studies using motion patterns of the head (kinematics) that address patterns of variation in post-capture behaviors involving the hyoid system in teleostean fishes (see Drucker and Jensen, 1991; Gillis and Lauder, 1995; Sanford, 2001). This is surprising given the importance of post-capture behaviors during feeding (e.g. prey immobilization, transport and prey reduction). Although a number of studies have used motor activity patterns to establish prey type differences in

post-capture behaviors (Sibbing et al., 1986; Wainwright and Turingan, 1993; Grubich, 2000), there have also been almost no studies designed to establish the effect of prey type on post-capture behaviors using kinematic analyses (see Sibbing et al., 1986; Frost and Sanford, 1999). Certainly, part of the reason for this paucity of quantitative kinematic data on post-capture behaviors is the inability to establish (using external landmarks) movement patterns of internal structures with sufficient confidence. Furthermore, in teleostean fishes, there are few modifications of the elements associated with the hyoid system that are well suited for such a study. Kinematic studies of post-capture behaviors are important because they provide direct information on movements that can only be inferred from electromyographic data.

Salmonids, however, possess a highly modified anterior region of the hyoid system, namely the tongue-bite apparatus (TBA), which represents a notable example of a novel feeding mechanism that is well suited for kinematic study. Furthermore, this type of kinematic analysis can lead to hypotheses regarding motor and neural control of behaviors that can then be tested using electromyography.

The TBA of salmonids consists of an extensive modification of the anterior hyoid into a well-developed toothed basihyal plate (Fig. 1) that opposes a set of teeth on the ventral surface of the neurocranium (specifically the vomer) (see Sanford, 2000).

A similar, but non-homologous, TBA has evolved independently in osteoglossomorph fishes (Lauder and Liem, 1983). In osteoglossomorphs, this modified hyoid system has become decoupled from its primitive function of buccal expansion during suction feeding, prey manipulation and lower jaw depression to provide a novel way of immobilizing and macerating prey following prey acquisition (Sanford and Lauder, 1989; Sanford and Lauder, 1990; Frost and Sanford, 1999; Sanford, 2001). The TBA of osteoglossomorphs is associated with two novel behaviors, raking and open-mouth chewing, which have been characterized by Sanford and Lauder (1989, 1990). In osteoglossomorphs, raking behavior is primarily for prey immobilization (Frost and Sanford, 1999) and starts with grasping the prey in the mandibular jaws. Once the prey has been stabilized, well-developed basihyal teeth are 'raked' through the prey by extensive retraction of the pectoral girdle (defined as the power stroke) (see Sanford, 2001). During the power stroke of raking, concomitant neurocranial elevation results in a shearing action between the two sets of

teeth comprising the TBA (Sanford and Lauder, 1989). Open-mouth chewing seems to be more of a prey reduction feature and is also associated with the TBA, but is characterized predominantly by a more dorsoventral excursion of the anterior hyoid. Both raking and open-mouth chewing behaviors are repeated several times before the prey item is swallowed (Sanford and Lauder, 1989).

Lauder and Liem (1980) did study the functional morphology and kinematics of the strike in *Salvelinus fontinalis* but did not really consider any of the post-capture behaviors. The present study is a first step in understanding the role of the TBA in salmonids. The purpose of this study was to use the brook trout *Salvelinus fontinalis* (i) to identify and characterize any post-capture behaviors associated with the TBA, (ii) to establish the distinctness of these post-capture behaviors and (iii) to determine whether variable prey types affect any of these behaviors.

Materials and methods
Specimens

Brook trout [*Salvelinus fontinalis* (Mitchill)] were obtained from Cold Spring Harbor Fish Hatchery. They were maintained in a 1136 l circular holding tank in dechlorinated water at $12\pm0.2\,°C$ with a specially constructed gravity filter on a 12 h:12 h (light:dark) photoperiod. All individuals were fed once a day with goldfish (*Carassius* sp.), Canadian earthworms (*Lumbricus* sp.) or crickets (*Gryllus* sp.) presented in a random sequence to prevent acclimation to any
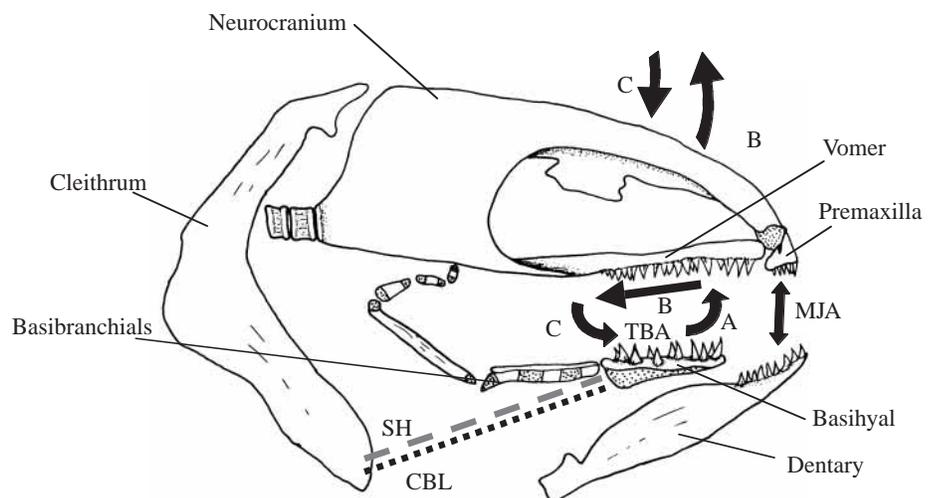

Fig. 1. Schematic lateral view of the skull of a generalized salmonid showing the tongue-bite apparatus (TBA). For illustrative purposes, the suspensorial and most of the gill arch elements have been excluded and the position of the lower jaw has been artificially lowered to allow all the elements of the TBA to be visible. MJA, mandibular jaw apparatus. The proposed movements of cranial elements during raking are overlaid. A, preparatory phase; B, power stroke; C, recovery phase; SH, sternohyoideus muscle; CBL, cleithrobranchial ligament. Retraction of the pectoral girdle (cleithrum) during the power stroke of raking provides a posterior force on the hyoid *via* the sternohyoideus and/or cleithrobranchial ligament. See text for details.

particular prey type. These prey types are found in the natural diet of *Salvelinus fontinalis* (Bryan and Larkin, 1972; Beauchamp et al., 1999) and represent extremes of visual and tactile stimuli. Prey items were approximately 3–4 cm in length, and the initial prey diameter (i.e. before they were presented to the predator) was not significantly different when a random sample of prey was measured using dial calipers (goldfish width 0.7±0.4 cm, N=15; worm diameter 0.6±0.3 cm, N=15; cricket diameter 0.6±0.4 cm, N=15; means ± S.E.M.; analysis of variance P=0.85). This eliminates prey size as a factor contributing to any kinematic differences observed (Ferry-Graham, 1998).

Videography

Four specimens were size-matched and selected for filming. They ranged in standard length from 14.7 to 17.0 cm (mean 16.3±0.9) and head length from 3.6 to 4.1 cm (mean 4.0±0.1) (means ± S.E.M.). The individuals selected for filming were placed in a 114 l experimental tank maintained at 12±0.2 °C, with a clear Plexiglas background grid (for scaling) placed in the center of the tank. The feeding area was 15 cm deep (relative to the camera position, see below), and scaling was calibrated at both the front and rear of the feeding area to verify that there was minimal error due to the distance of the predator from the grid.

The fish were acclimated in the experimental tank for a period of 2 weeks, during which they were fed every day using randomly selected prey types. Following the 2 week acclimation period and 2 days of starvation, high-speed video recordings of *Salvelinus fontinalis* feeding randomly on all three prey types were used for kinematic analysis. Feeding sequences were videotaped in black and white (to maximize contrast) using a NAC HSV-500 recording at 250 frames s^{-1}. Illumination was provided by synchronized strobe, and additional light was provided by two 600 W Testrite QC6 filming lights. Prey capture events were filmed over a period of 2–15 days until five digitizable sequences for each prey type were obtained.

The only feeding sequences digitized were those in which the fish was parallel to the lens of the camera (eliminating correction for parallax) and all processing behaviors associated with the tongue bite were clearly identifiable. The first five sequences that met these conditions for a particular prey type and behavior (see below) were digitized.

Two behaviors associated with the tongue bite in *Salvelinus fontinalis* were readily identified, raking and open-mouth chewing (see also Frost and Sanford, 1999). For the four individuals filmed, five raking and five open-mouth chewing sequences for each prey type were analyzed, so 120 chewing sequences were used for detailed analysis. When a digitizable sequence was selected, it was converted into a movie file (avi format, Microsoft) using a Matrox RT2000 video-capture board and Panasonic (AG-1980) video recorder. From the avi file, individual frames were stacked using Scion Image (beta 3b, based on NIH image modified for Windows by Scion Corporation, National Institutes of
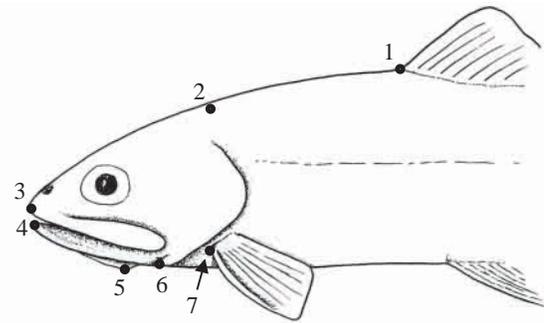

Fig. 2. Digitizing protocol for analysis of high-speed video recordings. The seven points digitized are explained in the text.

Health, Washington, DC, USA). Kinematic variables were digitized frame by frame (every 8 ms) from 80 ms before the onset of each behavior until 200 ms after the onset of the behavior. The 'onset' designated 'time zero' of each behavior was defined as the frame prior to rapid neurocranial elevation. This elevation was a component common to both behaviors and was easily identifiable.

Variables measured

Seven points were used to quantify cranial movements during raking and open-mouth chewing (Fig. 2). These seven points were: (1) the anterior margin of the first dorsal fin (or a spot on the dorsal margin of the back in the same general location, representing a fixed reference point on the animal); (2) a spot dorsal to the operculum (representing a fixed point at the posterior margin of the neurocranium); (3) the anterior tip of the premaxilla (upper jaw); (4) the anterior tip of the dentary (lower jaw); (5) the most ventral point of the hyoid (specifically, the antero-ventral tip of the anterohyal, which was clearly visible as a bony projection); (6) the lower jaw joint (i.e. the articulation of the anguloarticular and the quadrate); and (7) the most anterior point of insertion of the pectoral fin. On each frame, Scion Image was used to measure the following five variables (refer to Fig. 2): (i) gape distance (distance from 3 to 4); (ii) lower jaw angle (the angle defined by 2, 6 and 4, with 6 as the vertex); (iii) hyoid distance (the perpendicular distance of 5 from the lower jaw, defined as the line between 4 and 6); (iv) neurocranial elevation (the angle defined by 1, 2 and 3, with 2 as the vertex); and (v) pectoral girdle distance (distance from 3 to 7). Neurocranial elevation can artificially inflate pectoral girdle distance. Therefore, a second measure of pectoral girdle distance (i.e. point 7 to a point midway along the body posteroventral to the dorsal fin) verified that neurocranial elevation has a negligible effect on this measurement due to the angle of rotation of the neurocranium. Linear distance measurements were calibrated using the centimeter grid at the back of each tank.

From these variables, a set of derived variables was obtained from the kinematic profile of each feeding sequence. These derived variables were measured relative to time zero (the

Table 1. *Summary of mean displacement and mean time to maximum displacement of kinematic variables of two feeding behaviors (raking and open-mouth chewing) and three prey types (cricket, fish and earthworm) in* Salvelinus fontinalis

Variable	Behavior	Prey Type		
		Cricket	Fish	Worm
Maximum gape displacement (cm)	Raking	0.10±0.01	0.13±0.03	0.11±0.02
	OMC	1.18±0.07	0.94±0.07	0.78±0.06
Maximum lower jaw angle (degrees)	Raking	30.5±1.0	31.9±1.2	30.8±1.2
	OMC	11.9±0.9	12.8±1.6	13.4±1.3
Maximum hyoid displacement (cm)	Raking	0.15±0.02	0.14±0.02	0.13±0.02
	OMC	0.27±0.03	0.22±0.03	0.28±0.03
Maximum neurocranial angle (degrees)	Raking	33.7±0.9	38.6±1.1	36.0±0.9
	OMC	16.6±1.4	17.7±1.9	14.6±1.1
Maximum pectoral girdle displacement (cm)	Raking	0.81±0.05	0.83±0.06	0.90±0.03
	OMC	0.46±0.04	0.40±0.04	0.38±0.03
Time to maximum gape displacement (ms)	Raking	32.7±2.6	36.0±3.3	35.7±3.0
	OMC	82.2±6.1	68.6±5.5	44.0±5.9
Time to maximum lower jaw angle (ms)	Raking	46.4±2.0	45.6±1.7	49.6±1.2
	OMC	76.4±6.1	72.4±5.4	51.2±7.1
Time to maximum hyoid displacement (ms)	Raking	36.0±2.7	41.2±3.2	37.9±3.2
	OMC	102.6±6.5	93.6±7.8	70.7±8.7
Time to maximum neurocranial angle (ms)	Raking	47.6±2.2	48.4±1.4	47.4±1.2
	OMC	90.4±6.0	80.2±6.7	59.1±7.2
Time to maximum pectoral girdle displacement (ms)	Raking	49.8±1.7	48.4±2.2	48.4±1.4
	OMC	90.0±6.0	84.0±5.5	56.8±7.3

Values are means ± S.E.M.; *N*=20 for all observations.
OMC, open-mouth chewing.

onset of neurocranial elevation, see above) such that all maximum values were subtracted from the value at time zero, resulting in a measure of displacement. Time to peak displacement was also measured (in ms). This standardization relative to time zero allowed direct statistical comparison across behaviors, prey types and individuals. In total, 10 displacement and temporal variables were generated from the five original variables. Details of this approach can be found in both Frost and Sanford (1999) and Sanford (2001) (refer to Table 1).

Statistical analyses

Basic descriptive statistics were calculated for all 10 derived kinematic variables for both raking and open-mouth chewing; these consisted of means and standard errors and are presented in Table 1. Second, a mixed model three-way analysis of variance (ANOVA) was performed on the derived variables. This ANOVA was designed to test for differences between prey type, behavior and individual. Prey types and behavior were crossed fixed effects, while individuals was a crossed random effect. Although several interaction terms are generated, the only interaction term that was the focus of this study was prey type×behavior. The significance of the prey type×behavior term is designed to test the null hypothesis that any difference in behaviors is consistent across prey types. For example, a significant prey type×behavior interaction might indicate that a difference in behavior present when feeding on fish is absent when feeding on worms. Following

Zar (1999), *F*-ratios used to test the prey type factor used the mean square of the prey type×individual term as the denominator. *F*-ratios used to test the behavior factor were constructed using the mean square of behavior×individual as the denominator. *F*-ratios used to test the individual factor used the error mean square as the denominator. *F*-ratios used to test the prey type×behavior factor were constructed using the mean square of the prey type×behavior×individual interaction term as the denominator. For those tests in which a significant prey type or behavior effect was demonstrated, or an interaction term was significant, *post-hoc* tests using Bonferroni pairwise comparisons were performed to determine which prey type was responsible for the difference.

As the complexity increases dramatically when comparing multiple variables simultaneously, I also performed a multivariate analysis of variance (MANOVA) on the derived variables. This involved a principal components analysis (PCA), which reduces the complexity of comparing multiple variables into a few indices that are linear combinations of the original variables (the number of components used was selected on the basis of eigenvalues greater than 1). These components can be used to produce scatterplots depicting the major axes of variation. Finally, a MANOVA was performed on the factor scores from the PCA to test the overall hypothesis that, within each behavior, there was a prey-type effect when all variables are considered simultaneously.

Results

Overview

In *Salvelinus fontinalis*, I identified two post-capture chewing behaviors: raking and open-mouth chewing. Generally, after taking the prey into the oral cavity, *Salvelinus fontinalis* raked several times followed by a series of repeated open-mouth chews. It is important to note that, when goldfish were drawn into the buccal cavity tail first, they were often expelled and turned around so that they could be swallowed head first. In some cases, a second series of rakes followed open-mouth chewing, although this was rare. Both raking and open-mouth chewing were present in almost all feeding bouts, irrespective of prey type. Visual observation revealed that both raking and open-mouth chewing are characterized primarily by extensive neurocranial elevation and pectoral girdle retraction (Figs 3, 4). A summary of mean maximum displacement and time to maximum displacement variables is given in Table 1 and Figs 5 and 6. Overall, the most characteristic difference between processing behaviors associated with the TBA was greater displacement values (except for gape and hyoid displacements) and shorter durations during raking compared with open-mouth chewing; this holds true for all prey types (Figs 5, 6). Because all derived variables are measured as displacement relative to time zero, any slight differences due to variation in landmark position at time zero (e.g. a slightly open jaw) are eliminated from the analysis.

Raking

Raking is a very rapid movement and involves extensive displacement of the neurocranium and pectoral girdle (the power stroke; see also Sanford, 2001) (Figs 3–6, Table 1). Just prior to the onset (time zero) of the power stroke, the jaw begins to close. During the power stroke, there is only a small change in gape as the jaw becomes fully closed (Table 1, Figs 3–5). Once closed, the mandibular jaws are firmly held shut throughout the entire

behavior. Also, during the power stroke, the neurocranium undergoes extensive neurocranial elevation (mean 36°) and pectoral girdle retraction (mean 0.85 cm), and this occurs very quickly (in approximately 50 ms) irrespective of prey type (Table 1, Fig. 6). Hyoid displacement was small during raking (Table 1, Fig. 5), but it is important to note that initial hyoid displacement during raking was towards the lower jaw, in contrast to open-mouth chewing in which it was away from the lower jaw (Fig. 4). One feature common to feeding on all prey types is that, during raking, the lower jaw consistently reaches its maximum angular value earlier than the peak displacement of the neurocranium (Table 1, Fig. 6). This seems to be the result of *Salvelinus fontinalis* slightly relaxing its mandibular jaws prior to reaching maximum neurocranial elevation and pectoral
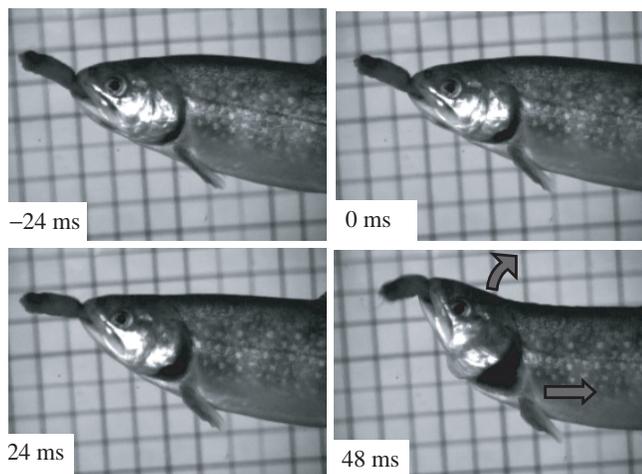

Fig. 3. Representative sequence of raking in *Salvelinus fontinalis* showing the extensive elevation of the neurocranium (curved arrow) and retraction of the pectoral girdle (straight arrow) that is typical of this post-capture behavior. The grid is 1 cm square.
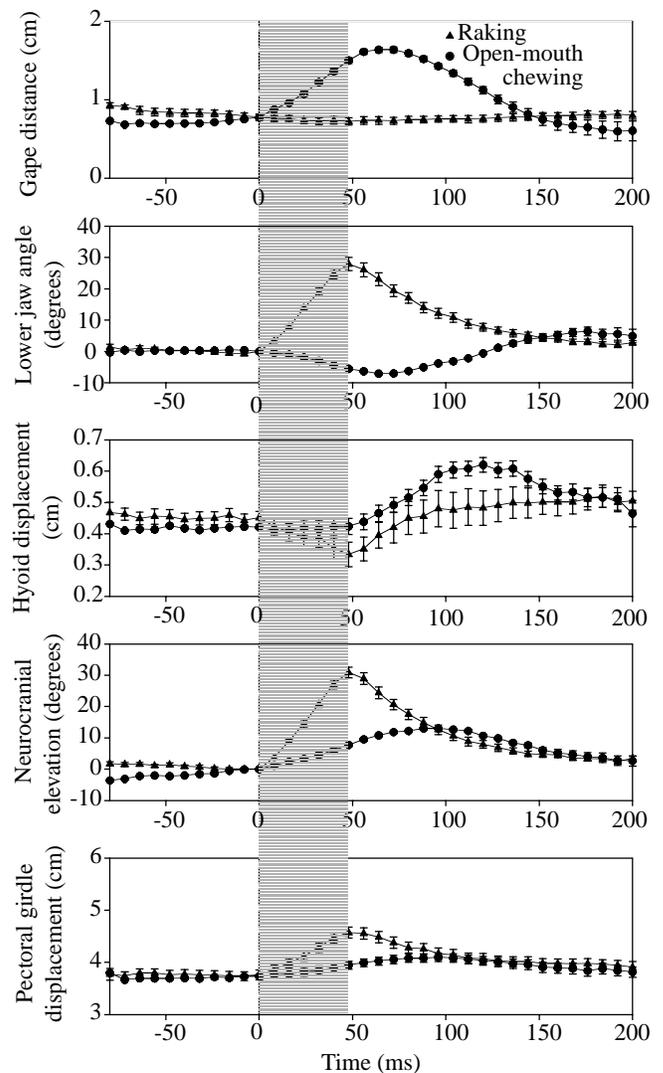

Fig. 4. Mean kinematic profiles of the post-capture behaviors, raking and open-mouth chewing, in *Salvelinus fontinalis* while feeding on crickets. The shaded area represents the power stroke of raking (see text for details). Note that hyoid displacement is towards the lower jaw during raking and away from the lower jaw during open-mouth chewing. Values are means ± S.E.M., $N=20$.

girdle retraction. This interpretation is supported by the earlier time of maximum gape displacement (approximately 10 ms) (Table 1, Fig. 6). Perhaps the most notable feature of raking is the remarkable consistency of values, both displacement and temporal, for all variables irrespective of prey type (Table 1, Figs 5, 6, see below).

Open-mouth chewing

Open-mouth chewing is much longer in duration than raking (Fig. 6). The average time to reach maximum displacement for the key variables (neurocranial elevation, pectoral girdle retraction and gape) was 77 ms in comparison with raking, for which it was approximately 50 ms (Table 1, Fig. 6). The onset of open-mouth chewing is characterized by a prominent opening and closing of the jaws, which involves noticeable neurocranial elevation and lower jaw depression (Table 1, Fig. 5). Peak gape is reached approximately 10 ms prior to maximum neurocranial elevation (Table 1), suggesting that the jaw is starting to close while the head is still being elevated. Maximum hyoid displacement is greater during open-mouth chewing than during raking and reaches a peak at least 10 ms later than the other variables (Table 1, Figs 5, 6). In contrast, most other variables had a magnitude of displacement approximately half that found during raking, notable exceptions being maximum gape and maximum hyoid movement, which were both larger in open-mouth chewing (Table 1, Fig. 5). Differences in the magnitude of displacement showed only small changes according to prey type (Table 1, Fig. 6). However, open-mouth chewing was performed most quickly when feeding on worms (Fig. 6).
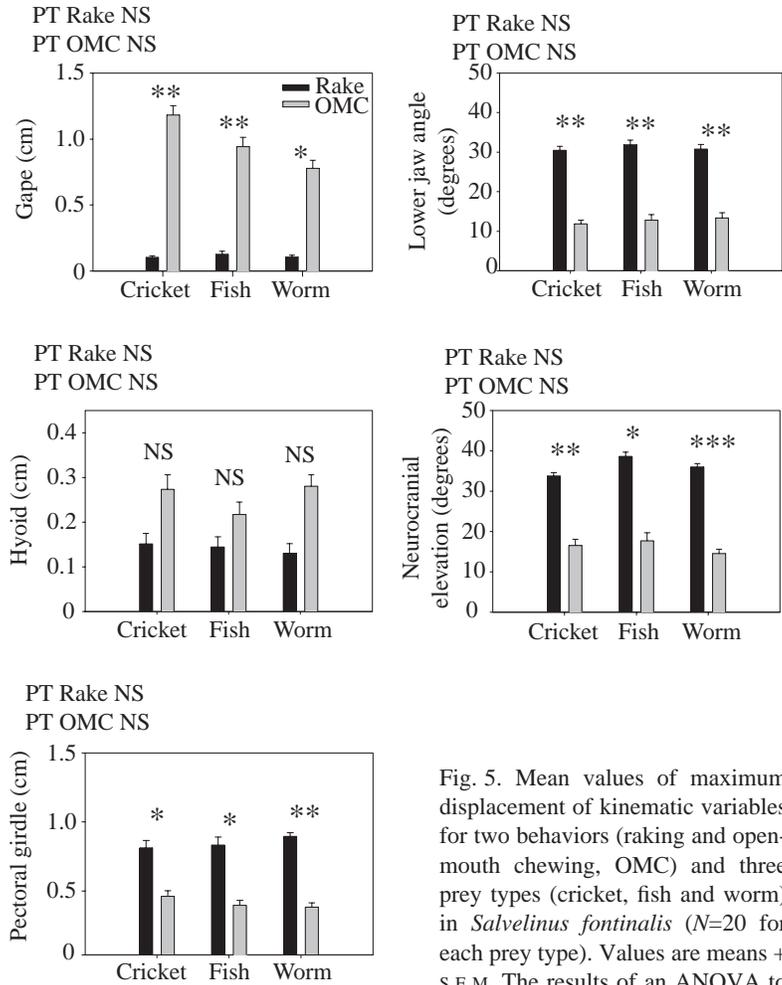
Fig. 5. Mean values of maximum displacement of kinematic variables for two behaviors (raking and open-mouth chewing, OMC) and three prey types (cricket, fish and worm) in *Salvelinus fontinalis* (N=20 for each prey type). Values are means + S.E.M. The results of an ANOVA to determine whether behaviors differ within prey are indicated within the graph. NS, $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$. Above each graph are the results of ANOVA *post-hoc* tests after Bonferroni correction contrasting prey-type differences within behavior. PT Rake, raking; PT OMC, open-mouth chewing.

Table 2. *Summary of univariate three-way ANOVA* F-*ratios for derived kinematic variables of two feeding behaviors (raking and open-mouth chewing) and three prey types (cricket, fish and worm) in* Salvelinus fontinalis

	Effect from ANOVA			
Variable	Prey type, d.f.=2,6	Behavior, d.f.=1,3	Individual, d.f.=3,96	Prey type × behavior, d.f.=2,6
Maximum gape displacement	4.34	94.44**	8.38***	0.13
Maximum lower jaw angle	0.85	780.28***	2.71*	0.86
Maximum hyoid displacement	0.49	13.48*	6.65***	0.66
Maximum neurocranial angle	2.91	64.97**	16.66***	0.54
Maximum pectoral girdle displacement	0.20	48.05**	6.90***	0.27
Time to maximum gape displacement	4.83	117.75**	0.38	29.05**
Time to maximum lower jaw angle	2.79	21.01*	1.05	4.07
Time to maximum hyoid displacement	6.55*	91.42**	0.83	5.16
Time to maximum neurocranial angle	4.20	41.71**	1.87	3.68
Time to maximum pectoral girdle displacement	11.35**	124.15**	0.35	7.48*

*Significant difference at $P<0.05$, **$P<0.01$, ***$P<0.001$ after Bonferroni correction.

PT OMC NS
PT OMC NS

PT OMC NS
PT OMC NS

PT Rake NS
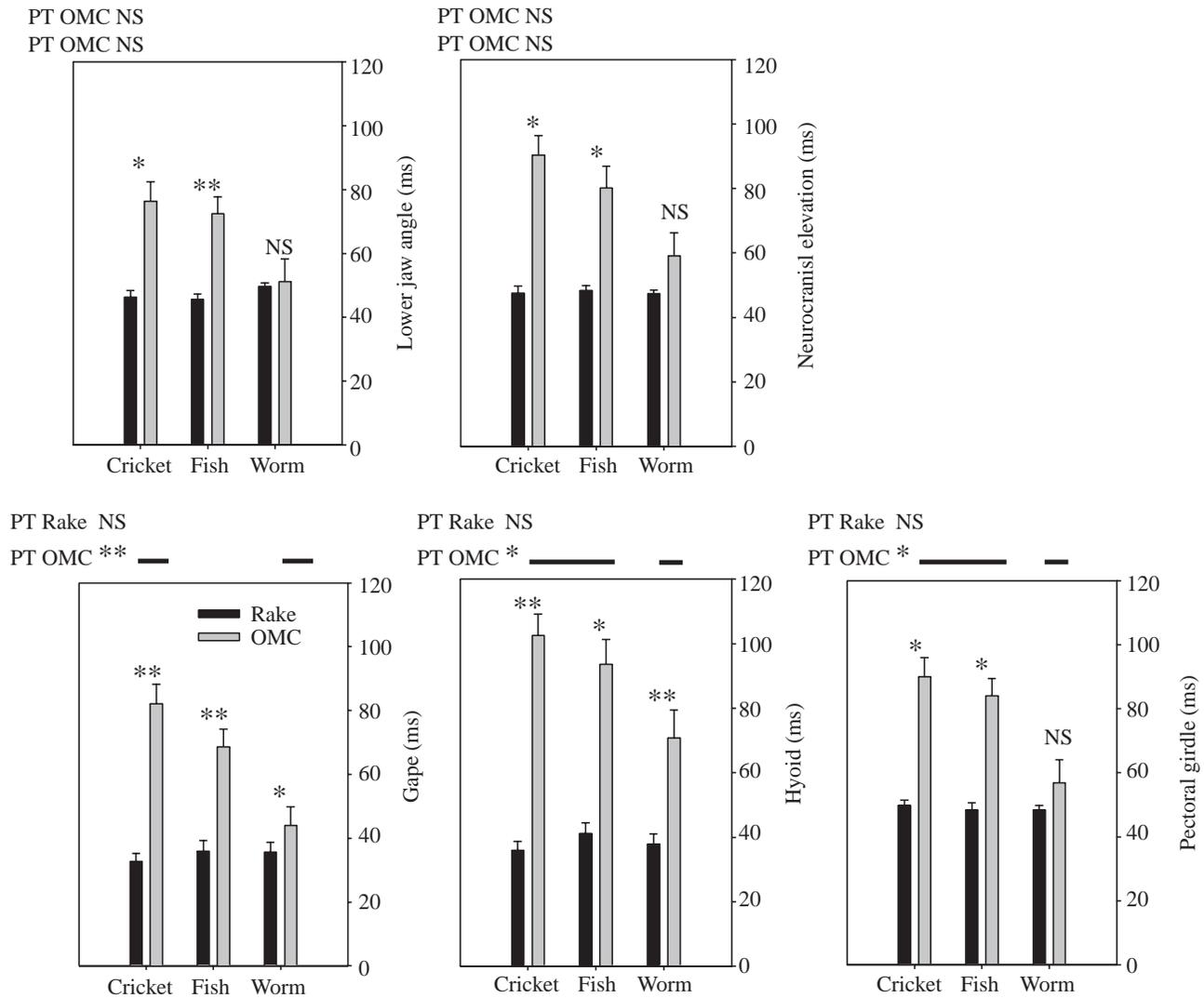PT OMC **

PT Rake NS
PT OMC *

PT Rake NS
PT OMC *

Fig. 6. Mean values of time to maximum displacement of kinematic variables for two behaviors (raking and open-mouth chewing) and three prey types (cricket, fish and worm) in *Salvelinus fontinalis* (N=20 for each prey type). Values are means + S.E.M. The results of an ANOVA to determine whether behaviors differ within prey are indicated within the graph. Above each graph are the results of ANOVA *post-hoc* tests after Bonferroni correction contrasting prey-type differences within behavior (PT Rake, raking; PT OMC, open-mouth chewing). Horizontal bars separate groups that are significantly different. NS, P>0.05; *P<0.05; **P<0.01; ***P<0.001.

Statistical overview

A summary of the univariate three-way ANOVAs is presented in Table 2. The results demonstrated that there was a significant difference in processing behaviors for all 10 variables (Table 2), indicating that, irrespective of prey type, these two behaviors, raking and open-mouth chewing, are significantly different from one another. These differences between raking and open-mouth chewing were evident in spite of significant individual variation (Table 2). In contrast, the ANOVA also demonstrated that there was little effect of prey type on the kinematic variables. The only two variables to demonstrate any prey-type effect were time to maximum hyoid displacement and time to maximum pectoral girdle displacement (Table 2). However, the significance of the prey-type×behavior interaction and *post-hoc* tests for time to

maximum gape do reveal a level of prey type difference during open-mouth chewing that is not present during raking (Fig. 6). Raking, then, is remarkably consistent across prey types (Fig. 5, Fig. 6). The PCA performed on raking behavior supports this view: there was significant overlap of the polygons along both principal components 1 and 2 (Fig. 7), and there was no significant effect of prey along either axis (PC1 P=0.65, d.f.=2,57; PC2 P=0.51, d.f.=2,57). This lack of prey-type effect during raking was further supported by the MANOVA on all principal component scores in which the overall kinematic pattern during raking demonstrated no significant prey-type effect (Wilks' Lambda P=0.65, d.f.=8,108).

In contrast to the conservative nature of raking, open-mouth chewing in *Salvelinus fontinalis* did demonstrate a prey-type
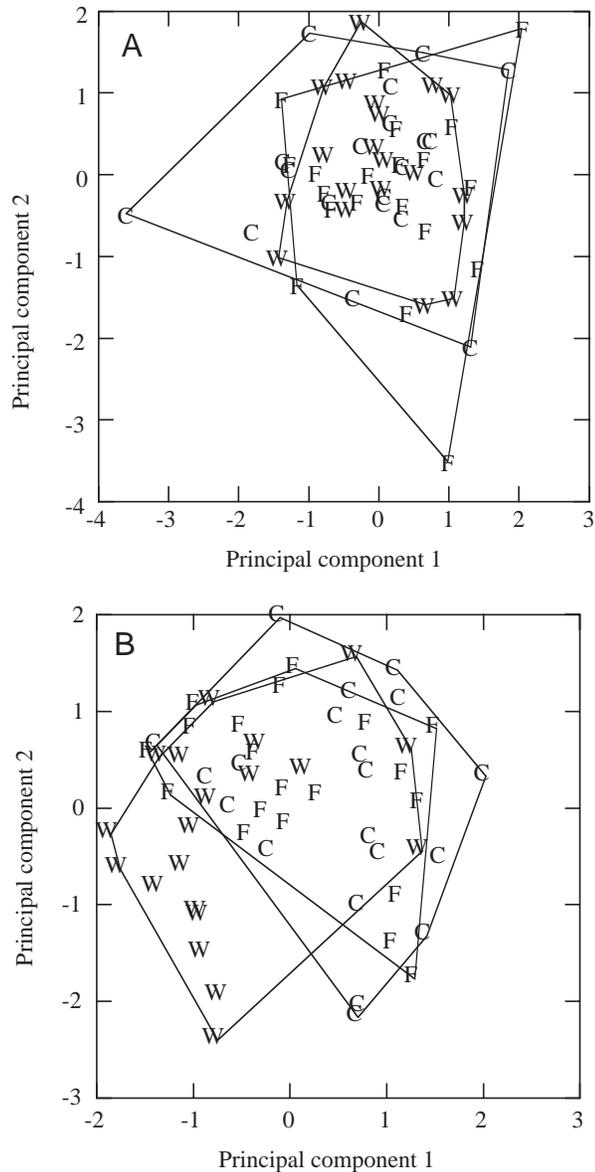
Fig. 7. Principal components analysis of (A) raking and (B) open-mouth chewing behavior in *Salvelinus fontinalis* for 10 derived kinematic (displacement and temporal) variables. Each symbol represents one chewing behavior for one individual: C, cricket; F, goldfish; W, worm. All factor loadings are presented in Table 3. Prey-type polygons are not significantly different for raking (MANOVA, Wilks' Lambda *P*=0.65, d.f.=8,108), but are significantly different for open-mouth chewing (Wilks' Lambda *P*<0.05, d.f.=6,110).

effect. *Salvelinus fontinalis* when open-mouth chewing on worms produced a distinct kinematic pattern when compared with chewing on either fish or crickets, as demonstrated by 50% of the worm feedings falling outside either of the other two prey-type polygons (Fig. 7). Six of the ten variables loaded high for principal component 1 (Table 3), and it was PC1 that separated worm feedings from fish and cricket feedings (Fig. 7). The effect of prey was significant for PC1 (univariate ANOVA *P*<0.01, d.f.=2,57). The six variables that loaded high

for PC1 in open-mouth chewing were maximum gape displacement, maximum neurocranial displacement and the timing variables for maximum gape displacement, maximum hyoid displacement, maximum pectoral girdle retraction and maximum neurocranial angle (Table 3, Fig. 7). High loadings of these variables along PC1 in combination with the *post-hoc* tests (see Table 3, Figs 5, 6) indicate that *Salvelinus fontinalis* when open-mouth chewing on a worm had a smaller change in gape and neurocranial displacement (see Fig. 7) together with a shorter time to maximum gape, maximum hyoid displacement and pectoral girdle movement. Univariate three-way ANOVAs showed a significant effect in only two of these six variables, namely time to maximum hyoid displacement and time to maximum pectoral girdle retraction (Table 2). This lack of significance relative to the PCA could be attributable to the large degree of individual variation in all displacement variables and to the interaction between prey type and behavior in time to maximum gape (Table 2, see above). In support of this assertion, Bonferroni *post-hoc* tests performed on the univariate ANOVAs (see above) revealed that *Salvelinus fontinalis* when open-mouth chewing on a worm had a significantly shorter time to maximum gape than when feeding on crickets (Fig. 6).

Overall, there was a significant effect of prey type during open-mouth chewing in *Salvelinus fontinalis* (MANOVA of the PCA: Wilks' Lambda *P*<0.05, d.f.=6,110). However, this difference is the result of distinct kinematics when *Salvelinus fontinalis* feeds on worms as opposed to feeding on either crickets or fish (Figs 6, 7).

The effect of individual was significant for all displacement variables but not for the timing variables (Table 2), indicating that, while there is significant individual variation in maximum displacements, individuals do not vary the timing of those displacements.

Discussion

The results of this study confirm that the tongue-bite apparatus (TBA) of *Salvelinus fontinalis* has two identifiable post-capture feeding behaviors associated with it, raking and open-mouth chewing. During a feeding bout, these behaviors are common, occurring almost 100% of the time, and are repeated several times before swallowing. Raking and open-mouth chewing are significantly different from one another in the kinematic variables measured (Table 2). In spite of these differences, both behaviors are characterized by movements of the hyoid, neurocranial elevation and pectoral girdle retraction. However, raking seems to involve greater excursions of the neurocranium and pectoral girdle (Table 1, Fig. 5), while movement of the hyoid away from the lower jaw seems to dominate open-mouth chewing. Neurocranial elevation is particularly notable during raking (36° compared with 16° during open-mouth chewing). During the strike of prey capture in *Salvelinus fontinalis*, Lauder and Liem (1980) report a neurocranial elevation of approximately 10°, which is much less than recorded here for either post-capture behavior.

Table 3. *Principal component loadings of derived kinematic variables for two feeding behaviors (raking and open-mouth chewing) in* Salvelinus fontinalis

	Raking				Open-mouth chewing		
Variable	PC1	PC2	PC3	PC4	PC1	PC2	PC3
Maximum gape displacement	0.29	−0.48	−0.22	**0.67**	**0.75**	−0.50	0.02
Maximum lower jaw angle	0.47	−0.56	0.10	−0.34	−0.27	0.24	**0.89**
Maximum hyoid displacement	0.15	−0.59	0.69	0.17	0.29	**−0.78**	0.00
Maximum neurocranial angle	0.47	0.05	−0.41	−0.41	**0.65**	−0.53	0.28
Maximum pectoral girdle displacement	−0.02	0.34	0.29	0.39	0.55	**−0.68**	0.27
Time to maximum gape displacement	0.29	−0.53	−0.58	0.24	**0.86**	0.42	−0.05
Time to maximum lower jaw angle	**0.84**	0.30	0.05	0.11	0.52	**0.64**	0.28
Time to maximum hyoid displacement	0.57	−0.37	0.26	−0.36	**0.85**	0.09	−0.24
Time to maximum neurocranial angle	**0.83**	0.36	−0.02	0.15	**0.91**	0.30	0.05
Time to maximum pectoral girdle displacement	**0.77**	0.39	0.14	0.07	**0.87**	0.39	−0.05
Variance explained (%)	29	18	12	11	48	25	11
Eigenvalues	2.52	1.49	1.10	1.03	4.57	2.47	1.09

Loadings greater than 0.6 are highlighted in bold type.
PC, principal component.

Raking and open-mouth chewing: functional interpretations

Raking always precedes open-mouth chewing and involves closing of the mandibular jaws followed by a rapid elevation of the neurocranium and concomitant retraction of the pectoral girdle (Figs 3, 4). Raking and open-mouth chewing are behaviors that have also been identified in the osteoglossomorph fishes (Sanford and Lauder, 1989), a group that have independently evolved a TBA (Lauder and Liem, 1983; Sanford and Lauder, 1989). I propose that the TBA operates in very much the same way in both groups (compare Fig. 1 here with fig. 2B in Sanford and Lauder, 1989). In *Salvelinus fontinalis*, as in osteoglossomorphs, the basihyal is mechanically linked *via* the sternohyoideus muscle and the cleithrobranchial ligament to the pectoral girdle (Fig. 1). During the power stroke of raking (Fig. 4), I propose that retraction of the pectoral girdle serves to draw the basihyal teeth posteriorly (i.e. causing a decrease in hyoid distance relative to the lower jaw) and, together with neurocranial elevation, causes a shearing action between the opposing sets of teeth comprising the TBA (Fig. 1).

One notable feature of raking is the opening of the jaw prior to peak neurocranial elevation and pectoral girdle retraction. The mechanism of this slight opening is unclear. A regression analysis on the timing of minimum gape with maximum neurocranial elevation and maximum pectoral girdle retraction failed to reveal any significant relationship between the timing of gape and these variables (regression for timing of gape and neurocranial elevation: $r^2=0.004$, $y=0.1161x+29.23$; regression for timing of gape and pectoral girdle retraction $r^2=0$, $y=0.008x+34.36$). However, a regression analysis of the timing of maximum lower jaw angle and pectoral girdle distance did show a significant correlation ($r^2=0.65$, $y=0.643x+15.8048$). It has been suggested previously for osteoglossomorphs (Sanford, 2001) that opening of the jaw prior to the peak of the power stroke in *Salvelinus fontinalis* is

the likely consequence of a postero-ventral force applied to the back of the lower jaw by the pectoral girdle *via* the sternohyoideus–hyoid–mandibular coupling (Lauder, 1985; Liem, 1980). Although the results of the regression analysis on the timing of minimum gape do not seem to support this view (see above), it is important to note that gape can result from both lower jaw depression and neurocranial elevation. Thus, the regression analysis on lower jaw angle (which is measured independently of neurocranial elevation and gape) does support the idea that this sternohyoideus–hyoid–mandibular linkage might be important in opening the jaw during the later stages of the power stroke of raking. However, this relationship might be confounded by the differing nature of the elements making up the sternohyoideus–hyoid–mandibular coupling, together with other jaw-depression mechanisms such as the interopercular-mandibular coupling (Lauder, 1985).

Open-mouth chewing in *Salvelinus fontinalis* is longer in duration than raking, reaching peak displacement values approximately 28 ms later. The predominant features of raking are smaller displacements of most variables, but larger displacements of gape and hyoid (Figs 5, 6).

In *Salvelinus fontinalis*, the temporal pattern of kinematic events during raking and open-mouth chewing does not vary among individuals, indicating that, although both behavioral and prey-type (for open-mouth chewing) differences exist (Fig. 7), there is little variation in the timing of those variables among individuals (Table 2). This lack of individual variability in temporal variables is unusual because individuals tend to be a major source of variation in functional attributes (Wainwright and Lauder, 1986).

Comparison with osteoglossomorphs

In *Salvelinus fontinalis*, the magnitudes of neurocranial elevation and pectoral girdle displacement are much greater for raking than for open-mouth chewing (Table 1, Fig. 5), which

is also a feature common to osteoglossomorphs (Sanford, 2001). Although direct statistical comparisons were not possible because of differences in predator size and the use of different prey types, the magnitudes of displacement values are generally larger in *Salvelinus fontinalis* than in osteoglossomorphs (compare Table 1 with table 5 in Sanford, 2001). For example, peak neurocranial elevation averages 36° in *Salvelinus fontinalis*, greater than that found in osteoglossomorphs, which ranges from 10.9° (*Chitala ornata*) to 34.7° (*Xenomystus nigri*) (Sanford, 2001). More importantly, however, maximum displacement of the neurocranium in *Salvelinus fontinalis* is achieved 10 ms earlier than in the osteoglossomorph *Xenomystus nigri*, which is a much smaller fish and should take less time to reach peak displacements (Richard and Wainwright, 1995). Maximum pectoral girdle retraction in *Salvelinus fontinalis* averages 0.85 cm, while in osteoglossomorphs it ranges from 0.21 cm (*Pantodon buchholzi*) to 0.84 cm (*Xenomystus nigri*). Again, the time it takes to reach maximum pectoral girdle retraction is 10 ms shorter in *Salvelinus fontinalis* than in *Xenomystus nigri* (the smaller fish). It is difficult to draw any conclusions using this comparison given the complex nature of scaling on linear kinematic variables (Richard and Wainwright, 1995). Nevertheless, the angular comparisons, which are not subject to scaling effects, demonstrate that raking in *Salvelinus fontinalis* involves the largest neurocranial elevation of any raking teleost.

In *Salvelinus fontinalis*, the mandibular jaws remain almost closed during raking, although sometimes the prey item is not held firmly between the mandibular teeth as it is in the osteoglossomorph *Chitala ornata* (Sanford and Lauder, 1989). The tension on the lower jaw during the power stroke of raking is likely to be maintained primarily by contraction of the adductor mandibulae muscle. Although no direct observation of muscle activity was made in the present study, Sanford and Lauder (1989) demonstrated that high levels of activity in the adductor mandibulae muscle were characteristic of raking in the osteoglossomorph fish *Chitala ornata*. Investigation of the electromyographic patterns associated with the tongue-bite apparatus in *Salvelinus fontinalis* would be necessary to confirm whether this is also true of salmonids.

It is noteworthy that raking in *Salvelinus fontinalis* is a very fast behavior compared with open-mouth chewing; on average, raking is 28 ms shorter in duration than open-mouth chewing when reaching maximum displacement of the key variables (neurocranial elevation and pectoral girdle retraction) (Table 1, Fig. 6). This is the opposite pattern to that found in osteoglossomorph fishes, in which raking is approximately 31 ms longer in duration than open-mouth chewing (Sanford, 2001). It is unclear why this paradox exists. However, rapid raking behavior in *Salvelinus fontinalis* might explain why this behavior is not modulated (see below).

Modulation of behaviors

Modulation of feeding behavior is a key factor in determining whether predators will adapt successfully to changes in feeding conditions (such as changes in prey availability) (Nemeth, 1997). Furthermore, modulation of motor patterns associated with feeding seems to be widespread in teleost feeding mechanisms (Wainwright, 1989), and functional versatility is generally associated with trophic generalists (Holm, 1985; Sanderson, 1990). The absence of plasticity in feeding behaviors can reflect either a genuine lack of ability to modulate the central pattern generator responsible for the behavior (Dellow, 1976) or the presentation of food types that are not different enough to elicit modulation (Reilly and Lauder, 1989; Ferry-Graham, 1997). How is modulation recognized? I have adopted a classical definition of modulation as any statistically different behavior in response to different stimuli (Nemeth, 1997; Frost and Sanford, 1999).

The present study demonstrates that, in *Salvelinus fontinalis*, post-capture raking behavior is highly stereotyped (not modulated) (Figs 5–7), and yet this species cannot be considered to be a trophic specialist (Mittlebach and Persson, 1998; Beauchamp et al., 1999). There are two lines of evidence to suggest that in *Salvelinus fontinalis* there is a genuine inability to modulate raking behavior. First, open-mouth chewing in *Salvelinus fontinalis*, a behavior also associated with the tongue bite, does demonstrate a prey-type effect (see below; Fig. 7), indicating that morphological factors are not limiting the behavioral response of this functional system. Second, the osteoglossomorph *Chitala ornata* does modulate raking behavior when presented with worms *versus* fish (Frost and Sanford, 1999), the same prey types used in the present study of *Salvelinus fontinalis*. Both the salmonid *Salvelinus fontinalis* and the osteoglossomorph *Chitala ornata* are trophic generalists, and both will encounter and feed on a range of prey types (Beauchamp et al., 1999; Smith, 1945). Therefore, the absence of modulation in *Salvelinus fontinalis* during raking has not limited the range of prey types on which *Salvelinus fontinalis* can feed successfully.

Why does *Chitala ornata* modulate raking while *Salvelinus fontinalis* does not? If raking is a prey-immobilization feature, as suggested by Frost and Sanford (1999), it might be that raking in *Salvelinus fontinalis* is already optimized for prey immobilization. This notion is supported by my finding here that *Salvelinus fontinalis* has the largest and fastest displacement values for neurocranial elevation and pectoral girdle retraction of any raking fish studied (Sanford, 2001) (and see above). It has been demonstrated in sharks that size of prey can elicit modulation of feeding behaviors not evident when presented prey of differing elusivity (Ferry-Graham, 1998). It is possible that *Salvelinus fontinalis* will modulate its raking behavior when presented with different-sized prey. However, this remains to be investigated.

Open-mouth chewing in *Salvelinus fontinalis* does demonstrate a prey-type effect that is the result of differences in timing of the gape, hyoid, neurocranium and pectoral girdle (Fig. 6). Although these differences are not readily apparent from the ANOVA (Table 2) (only two of the 10 variables show a significant difference), the PCA and *post-hoc* tests (Figs 6, 7) (Table 3) reveal that there is a much greater difference in

the kinematics that is masked by behavioral and individual variation. This can be seen by the separation of the PCA polygon for worms relative to the extensive overlap of the cricket and fish polygons (Fig. 7). These differences result from the distinct kinematics when feeding on worms relative to fish and crickets (Figs 6, 7). In general, *Salvelinus fontinalis* performed an open-mouth chew more quickly on a worm than on a fish or a cricket (average time to maximum values for the two key variables: neurocranial elevation and pectoral girdle retraction, worm 59 ms; fish 82 ms; cricket 90 ms). This is similar to the pattern that exists during open-mouth chewing in *Chitala ornata* (worm 87 ms; fish 107 ms) (Frost and Sanford, 1999). In salamanders, shorter durations of kinematic variables during a strike are associated with feeding on elusive prey (Deban, 1997). Frost and Sanford (1999) demonstrated that, during raking behavior in the osteoglossomorph fish *Chitala ornata*, the time to peak displacement of kinematic variables was generally greater in magnitude and shorter in duration for elusive prey (goldfish compared with worms). Thus, raking was proposed to be a prey-immobilization feature, while open-mouth chewing was associated with prey reduction (see Introduction) (see Frost and Sanford, 1999). If this is also true of salmonids, then it follows that in *Salvelinus fontinalis* the only effect of prey type is evident during prey reduction but not during prey immobilization.

It seems paradoxical that shorter durations of variables should exist in response to what probably represents the least elusive prey (worms). Although it is unclear why *Salvelinus fontinalis*, during open-mouth chewing on a worm, demonstrates modulation, one possible explanation for the short duration of variables associated with open-mouth chewing on worms is the softness of the worm. Of the three prey types, worms are the most malleable, and this difference could be responsible for the kinematic differences seen in *Salvelinus fontinalis*. This hypothesis could be tested using a prey type that has the soft outer body of an oligocheate worm but a harder internal body consistency.

Previous studies on the tongue bite in osteoglossomorphs have demonstrated that the motor patterns associated with raking and open-mouth chewing are significantly different from one another (Sanford and Lauder, 1989). Further electromyographic studies are necessary to determine whether these differences are paralleled in salmonids. They would also establish whether the lack of modulation in *Salvelinus fontinalis* during raking kinematics is mirrored in the associated motor patterns. Differences in kinematic patterns that are not matched by differences in motor patterns would provide important clues about the relationship between these two aspects of functional design.

To summarize, in *Salvelinus fontinalis*, the tongue-bite apparatus (a modified anterior hyoid) provides a convergent feeding system that is used in two unique behaviors, raking and open-mouth chewing. This study has demonstrated that raking and open-mouth chewing behaviors are not unique to osteoglossomorph fishes and, like the tongue-bite apparatus itself, have evolved independently in salmonids and

osteoglossomorphs. It is notable that well-developed basihyal teeth (a key component of the tongue-bite apparatus) are present in other teleostean groups such as some osmeroids, argentinoids and galaxiids (Sanford, 2000). However, the pattern and distribution of upper teeth are more complex, with many teleostean groups having evolved palatine, parasphenoid and vomerine teeth or any combination of these. Can the tooth patterns of these other teleosts be considered to be a tongue-bite apparatus? Analysis of post-capture feeding behaviors would be a direct approach to answering this question and could provide important insights into a functional novelty that has evolved independently several times.

A study of the behaviors and morphology associated with this novel functional system (the tongue-bite apparatus) in salmonids and osteoglossomorphs would be an important first step in understanding the convergent evolution of functional design in the musculoskeletal system of vertebrates.

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