

FUNCTIONAL ANATOMY OF FEEDING IN THE
BLUEGILL SUNFISH, *LEPOMIS MACROCHIRUS*:
IN VIVO MEASUREMENT OF BONE STRAIN

BY G. V. LAUDER, JR.* AND L. E. LANYON†

* *The Museum of Comparative Zoology, Harvard University, Cambridge, Mass. 02138 U.S.A.*, † *Department of Anatomy, University of Bristol School of Veterinary Science, Park Row, Bristol B21 5LS England*

(Received 19 April 1979)

SUMMARY

The suction feeding mechanism of the bluegill sunfish, *Lepomis macrochirus*, was studied in unrestrained fishes by the simultaneous recording of cranial muscle electromyograms, opercular cavity pressures, and opercular bone strain patterns. The electromyographic profile was similar to that of other advanced teleosts and consisted of preparatory, expansive, and compressive phases. The pectoral girdle remained nearly stationary during the strike. Opercular cavity pressures showed peak negative values of 145 cm H₂O followed by a positive afterpressure of up to 50 cm H₂O as water flowed out of the opercular chamber. Characteristic 'coughing' patterns showed an initial positive phase, followed by a negative phase, and then a final positive pressure pulse. Bone strain on the operculum was measured with rosette and single element strain gauges. Peak principal compression during the strike was 1800 $\mu\epsilon$ and the peak strain rate was $-615 \times 10^3 \mu\epsilon/s$, more than ten times that previously recorded in vertebrate bone during normal activity. Opercular bone strain results from the rapid reduction of pressure in the opercular cavity which causes the operculum to deform medially and does not result from muscle activity. The observed strain pattern is consistent with a stress regime of bending and twisting moments applied to the operculum during feeding. Two prominent orthogonal bony struts on the medial opercular surface are hypothesized to resist these bending and twisting moments.

INTRODUCTION

In the last ten years the mechanics of feeding in fishes has been the subject of a large number of investigations. Beginning with Osse (1969) who first used electromyography as a means of ascertaining cranial muscle activity during feeding, electromyography and high-speed cinematography have been used with increasing frequency to study the mechanics of feeding in primitive teleost fishes (Ballintijn, van den Berg & Egberink, 1972; Lauder, 1979; Lauder & Liem, 1979) and in advanced teleost fishes (Elshoud-Oldenhavé & Osse, 1976; Liem, 1973, 1978; Liem & Osse, 1975).

Despite these experimental studies, very few direct measurements exist of the pressures generated in the mouth cavity during feeding. Alexander (1969, 1970) provided the first measurements of pressures in the mouth cavity of fishes by training fish to take food which was slipped over the end of a pressure transducer tube. He found that different species varied widely in the peak negative pressures generated during feeding (from -80 cm H₂O in *Ictalurus* to -400 in *Pterophyllum*) and that the mean duration of the negative pressure pulse could be as short as 30 ms. Only recently, however, have *in vivo* pressures been measured in unrestrained fishes capturing elusive prey (Liem, 1978). No studies have examined pressure changes in the opercular cavity during feeding.

One technique, potentially an extremely useful tool for analysing the mechanics of feeding and the relationship between form and function of the fish musculoskeletal system, has yet to be applied to non-mammalian vertebrates: the use of strain gauges *in vivo* to determine the patterns of bone strain occurring during normal functional activity. Strain gauge analysis has been used most extensively to study mammal locomotion (Cochran, 1972; Lanyon, 1974; Lanyon & Baggott, 1976; Lanyon & Smith, 1970; Turner, Mills & Gabel, 1975) although recently Hylander (1977, 1979) and Weijs & DeJongh (1977) have used strain gauge techniques to analyse mammalian mastication.

The aims of this study are (1) to investigate experimentally the suction feeding mechanisms of an advanced teleost fish *Lepomis macrochirus*, the bluegill sunfish (Family Centrarchidae), with particular reference to the role of the opercular bone in suction feeding, (2) to test the applicability of strain gauge techniques to the analysis of form-function relationships in fishes, and (3) to examine strain-related parameters with regard to the significance of the characteristic acellular bone structure in advanced teleost fishes.

MATERIALS AND METHODS

Fish

Five large specimens of *Lepomis macrochirus* were chosen for experimental study from a sample seined from local streams and ponds around Boston, Massachusetts. The fish were acclimated to laboratory water and temperature (25 °C) and were each confined in separate 80 l tanks. The size of the fish ranged from 15 to 20 cm total length; the larger individuals were used for EMG, opercular cavity pressure, and strain gauge experiments while the smaller ones were used exclusively for electromyographic studies. The fish were fed a mixed diet of commercially prepared 'flake food', live meal worms, pieces of dead smelt (*Osmerus*) and live goldfish (*Carassius auratus*), all of which they ate readily. Fish were deprived of food for up to a week before an experiment.

Electromyography

Bipolar fine-wire electrodes were implanted in the cranial muscles using the method of Basmajian & Stecko (1962). Up to ten pairs of electrodes were implanted simultaneously. The bipolar wires were colour coded, glued together, and attached to a lightweight plastic clamp which was fastened to a wire passing through the

epaxial muscles just anterior to the dorsal fin. The electrodes were left implanted for up to 2 weeks, during which time multiple sets of experiments were conducted on different (but overlapping) sets of muscles. No deterioration of signal quality was noticed over the experimental period. Over 150 feeding sequences were recorded in this fashion.

During recording sessions the fine-wire leads were connected to a freely rotating slip-ring connector which was attached to a Honeywell 5600 tape recorder, Gould-Brush high-gain biomedical couplers, and a Brush 260 chart recorder. Signals were recorded at 37.5 cm/s and played back at 4.7 cm/s to facilitate analysis. High-speed cinematography (200 frames per second) was also used to determine the main pattern of bone movements during the strike. A Photosonics 16 mm 1 PL camera and Kodak 4X reversal film was used in conjunction with three 600 W Smith-Victor filming lights.

Pressure recordings

A cannula (Intramedic polyethylene tubing, 0.86 mm i.d. and 1.52 mm o.d.) was implanted in the opercular cavity by drilling a hole through the cleithrum and passing a short piece (2 cm) of tubing (1.55 mm i.d.) through the hole. This was flanged on both ends to secure it in place. A 40 cm length of the thinner tubing was then passed through the larger tubing and was flanged on the end facing into the opercular cavity, pulled flush with the anterior surface of the cleithrum, and glued to the wider tubing. This technique, a slight modification of that used by Ballintijn (1969*b*) and Liem (1978), prevents crimping of the cannula in its passage through the bone. The tubing was then passed through a hole in a small plastic clamp, glued in place, and the clamp then attached to a wire through the epaxial muscles. This technique allowed very little movement of the tip of the cannula. Cannulae were flushed regularly with fresh water to ensure patency although some clogging was experienced in one series of experiments two weeks after implantation.

A Statham Model P23 Db pressure transducer was attached to the cannula to record opercular cavity pressures. A three-way stopcock was used (Hughes & Adeney, 1977) to allow filling of the cannula and transducer with water, and a Gould Transducer Coupler/Preamplifier (Model 11 4307 04) processed the signals.

Dynamic calibration of the transducer was conducted according to the procedure outlined in Gabe (1972). Step changes in pressure ranging in size from 50 to 600 cm H₂O were applied to the transducer and the resonant frequency calculated to be 70 Hz (using water). Transient tests were performed with the apparatus used during *in vivo* pressure recording and thus included the 40 cm long cannula. Transducer oscillations following the step change in pressure had the form of a damped sinusoid (damping factor $\beta = 0.138$) and the transducer-cannula system could thus be regarded as a single degree of freedom system (Gabe, 1972).

Damping was increased for certain experiments by increasing the viscosity of the fluid in the transducer and cannula following the procedure of Alexander (1969). At maximum damping $\beta = 0.524$. Similar ranges of pressure magnitudes and patterns of pressure change were obtained with the damped transducer system.

The time to 90% response for the transducer and cannula over step changes of

50 to 600 cm H₂O was 5 ms or less. Since this greatly exceeds the rapidity of the expansive phase of feeding and since the frequency of fish feeding ranges from about 5 Hz to a maximum of 16 Hz (expansive phase = $\frac{1}{2}$ cycle) the transducer was considered capable of accurately recording *in vivo* pressures.

A series of control pressure recordings were made without electromyograms or strain gauges but no differences could be detected after the addition of electrodes or a strain gauge.

Static calibration of the transducer over a range of water pressures greater than that encountered during the experiments was routinely performed. The transducer was linear over this range.

Strain-gauge technique

Rosette (3 element) foil strain gauges were attached to the lateral surface of the opercular bone using the technique of Lanyon (1976) and Lanyon & Baggott (1976). The gauges used (TML FRA-2, Techni Measure, U.K.) consisted of three single element 120 ohm foil grids mounted at 45° angles to each other (the gauge grids are visible in Fig. 12) and bonded to a thin epoxy resin base. Before gauge implantation, 0.08 mm Teflon-insulated copper wires were soldered to the end tabs of the three gauge elements, and the gauge surface was insulated with a pliable epoxy resin (Lanyon, 1976). These fine copper wires were then soldered to thicker (0.12 mm) Teflon-insulated copper wires which led to the recording apparatus. The joint between the wires was insulated with a small block of epoxy resin which had a hole in the centre. During implantation, a wire through the epaxial muscles was passed through this hole and allowed the gauge and wires to move freely with the operculum.

Gauges were implanted on fish anaesthetized with a standard dose of tricaine methanesulphonate, a procedure also followed for electrode and cannula implantation. The lateral surface of the operculum in the area between the prominent horizontal and vertical bone struts and the ventral joint of the operculum to the suboperculum (Fig. 12) was scraped clean of scales and periosteum, dried with a low pressure air jet, and degreased with ether. During this procedure and during gauge implantation the operculum was held out of the water. When a clean dry surface had been obtained the gauge was immediately bonded to the operculum with isobutyl 2-cyanoacrylate monomer.

Three separate gauge attachments were made on two different individuals and experiments were conducted over the following 3 weeks. In no case was there any evidence that the bond between the gauge and the bone had loosened.

In one set of experiments a single element foil strain gauge was also bonded to the medial surface of the operculum in addition to the lateral rosette gauge.

Bone strain was recorded with a Vishay Strain Gauge Conditioner coupled to a 14-channel Bell and Howell CPR-4010 tape recorder. Grass P-5 series preamplifiers with high impedance probes were used for synchronous electromyograms. Radiographs of the head and operculum allowed precise measurements of principle strain angle relative to various skull elements and landmarks.

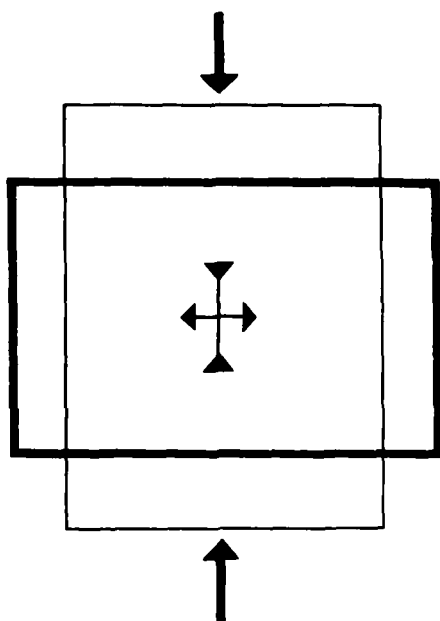


Fig. 1. A schematic diagram illustrating the effect of a uniaxial compressive load on a block of material (original dimensions in thin lines, final dimensions in thick lines). The pattern of principal compressive and tensile strain (indicated by the arrows pointing together and apart respectively) is indicated in the centre. Principal compressive and tensile strains are at right angles to each other.

Strain-data analysis

Strain is the change in length of a given element divided by the original length of that element. A strain of 1 therefore involves a doubling of any dimension and is a very large strain. A more convenient unit is the micro-strain ($\mu\epsilon$), which is strain $\times 10^6$. In vertebrate bone, strain results from muscle contraction and externally imposed loads normally occurring in activities such as mastication and locomotion. *In vivo* strain gauges, if they are to reflect bone strain patterns accurately, must be bonded firmly to the bone surface so as to deform with it. Independent calibration has shown that gauges bonded to mammalian bone *in vivo* are capable of giving absolute strain data and that the bond does not deteriorate over a 3-week period (Baggott & Lanyon, 1977). Although a strain gauge can only give information on strain at the bone surface and in the small area to which it is attached, the relevant surface of the operculum is quite small relative to the area covered by the strain gauge and the measured strain patterns will reflect those over the bone surface.

Compression in one direction of a solid such as bone (Fig. 1) will cause a compressive strain along this direction and a concomitant tensile strain at right angles to it. These are the directions of the principal tensile and compressive strains. In other directions, there will be a principal strain component and a shear strain component. A *principal strain* direction is thus one in which the shear component is zero. Shear strain reaches a maximum at 45° to the principal strain directions.

A single-element gauge is maximally sensitive only in the direction of gauge alignment and thus cannot distinguish between a change in strain magnitude and a change in strain angle. This deficiency can be overcome by using rosette strain gauges, which allow the independent measurement of strain in three directions simultaneously. Then, no matter what the gauge alignment relative to the principal strain directions, the principal strain magnitude and angle can be calculated (Dally & Riley, 1965; Lanyon, 1976). In this study the magnitude of the principal compressive and tensile strains as well as the angle of principal compression was calculated for each strike at the prey and for each 'coughing sequence' at 0.002 s intervals. Synchronously recorded bone strain, opercular cavity pressure, and electrical activity in five cranial muscles were obtained for over 75 feeding sequences.

RESULTS

Electromyographic profile of feeding

Prey capture may be divided into a preparatory phase followed by expansive and compressive phases. The preparatory phase is not consistently present but when it does occur (see Fig. 3), activity in the adductor mandibulae and geniohyoideus is seen prior to mouth opening.

Mouth opening (expansive phase) is initiated by activity in the sternohyoideus and levator operculi muscles (Figs. 2, 3: LO, SH). The levator operculi acts to depress the lower jaw by rotating the opercular series caudodorsally and transmitting a posterodorsal force to the retroarticular process of the lower jaw via the interoperculo-mandibular ligament (Fig. 2). The sternohyoideus, consistently active 5 ms after the onset of activity in the levator operculi, also acts to depress the lower jaw. Posterodorsal hyoid movement is transmitted to the interopercular bone by the interoperculo-hyoid ligament. The expansive phase usually lasts about 35 ms. Other expansive phase muscles, characterized by short high-amplitude bursts of activity, include the epaxial muscles, hypaxial muscles, obliquus superioris (a division of the hypaxialis), levator arcus palatini, and protractor pectoralis (Figs. 2, 3). The epaxial muscles are active exactly synchronously with the levator operculi and act to increase the gape by lifting the head and also to protrude the upper jaw (Liem, 1979). The dilator operculi (Fig. 3: DO) is active throughout the expansive phase and is often initially active up to 5 ms before the levator operculi. The adductor operculi (Fig. 3: AOP) is variable in time of onset of activity (from 10 ms before the levator operculi to 5 ms after) and holds the operculum against the side of the head during the early part of the expansive phase.

The activity pattern in the expansive phase muscles attaching to the pectoral girdle and measurements of pectoral girdle movement indicate that the pectoral girdle is stabilized during the strike by the simultaneous activity of antagonistic muscle complexes attaching to both its dorsal and ventral aspects. Dorsally, the protractor pectoralis (Fig. 3: PP) acts to protract the pectoral girdle and is active simultaneously with the obliquus superioris (Figs. 2, 3: OBS) which aids in pectoral girdle retraction. Ventrally, the hypaxial muscles act to stabilize the pectoral girdle and allow the antagonistic sternohyoideus to exert its primary effect upon the hyoid apparatus (Figs. 2, 3: SH, HY).

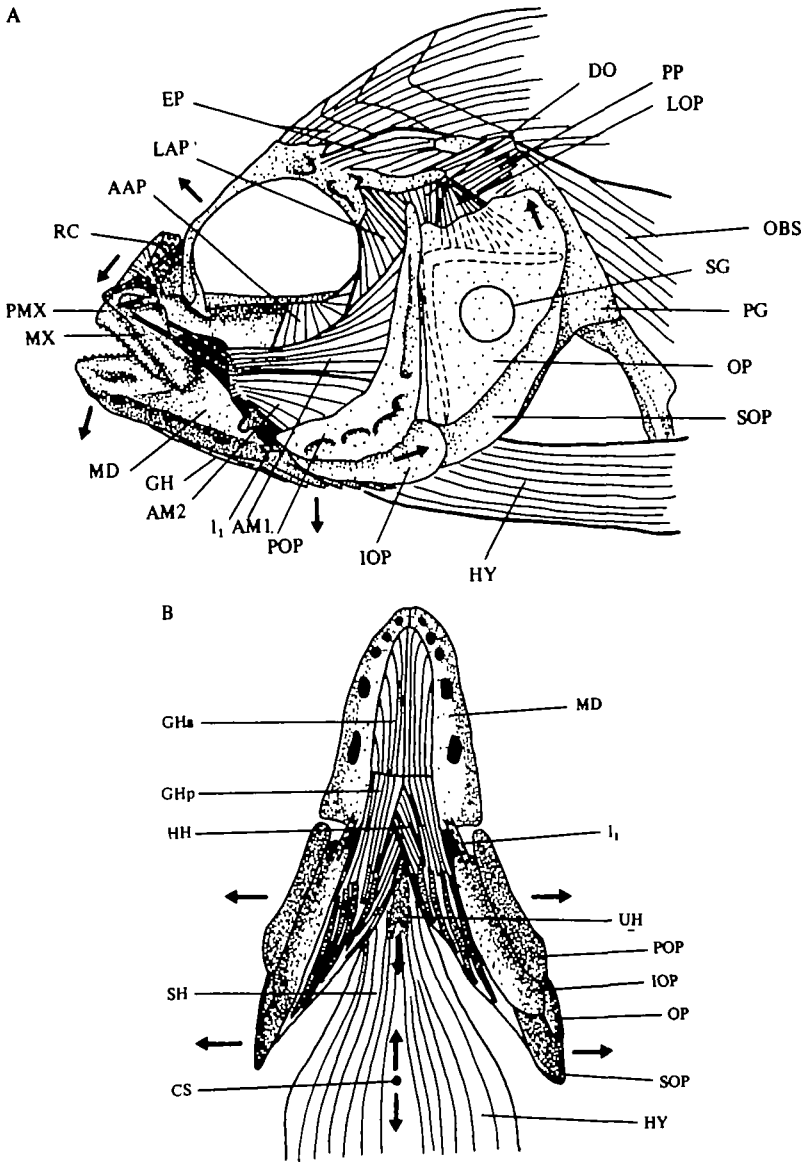


Fig. 2. Lateral view (A) and ventral view (B) of the cranial anatomy of *Lepomis macrochirus*, the bluegill sunfish. The eye, infraorbital bones and pectoral fin have been removed. Arrows indicate the major movements of the bony elements during prey capture. Note that the pectoral girdle (CS, PG) remains nearly stationary due to the synchronous activity of dorsal and ventral muscle complexes. Dashed lines on the operculum indicate the position of the bony struts on the medial opercular surface. Abbreviations: AAP, adductor arcus palatini; AM₁, division one of the adductors of the mandibulae; AM₂, division two of the adductors of the mandibulae; CS, position of the cleithral symphysis; DO, dilator operculi; EP, epaxial muscles; GHa, p, anterior and posterior divisions of the geniohyoideus muscle; HH, hyohyoideus abductores; HY, hypaxial muscles; IOP, interopercular bone; I₁, interoperculo-mandibular ligament; LAP, levator arcus palatini muscle; LOP, levator operculi; MD, mandible; MX, maxilla; OBS, obliquus superioris muscle; OP, operculum; PG, pectoral girdle; PMX, premaxilla; POP, preoperculum; PP, protractor pectoralis; RC, rostral cartilage; SG, approximate location of the rosette strain gauges (see text); SH, sternohyoideus muscle; SOP, suboperculum; UH, urohyal.

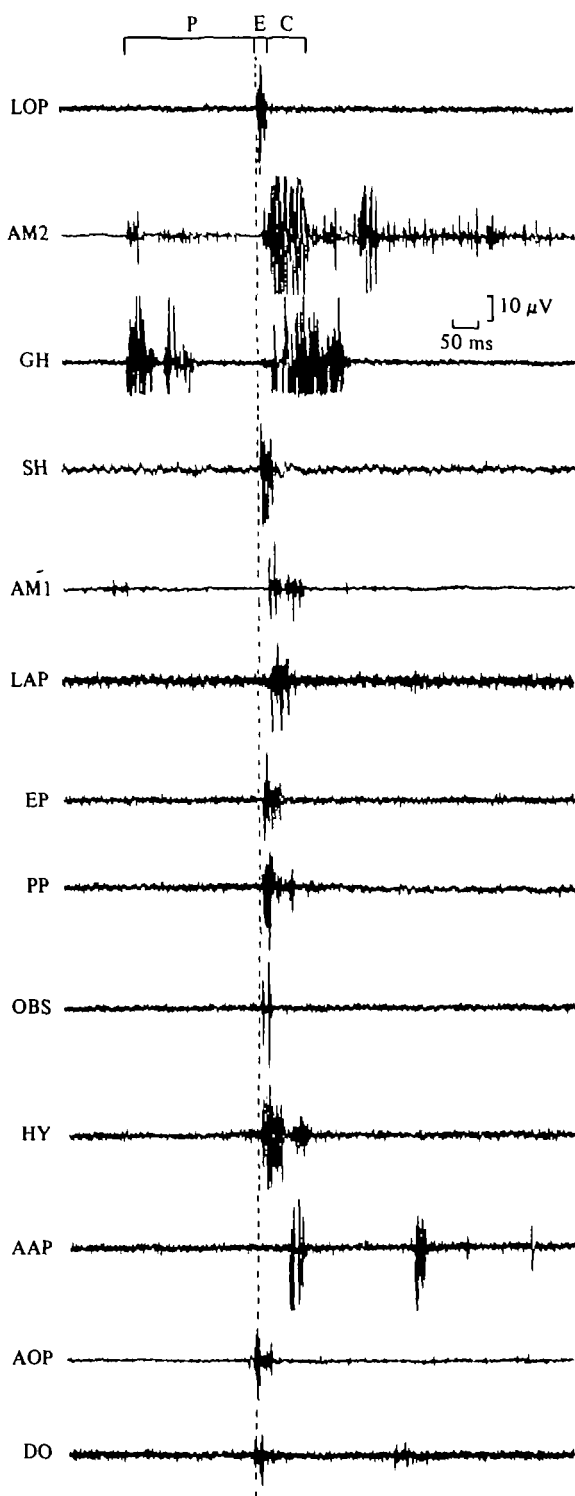


Fig. 3. Electromyographic profile (composite of recordings selected from 150 feeding sequences) of electrical activity in thirteen cranial muscles during the strike at a prey item. Not all muscles were recorded simultaneously. P, Preparatory phase; E, expansive phase; C, compressive phase. The dashed line indicates the onset of electrical activity in the levator operculi muscle. AAP, adductor arcus palatini; AOP, adductor operculi; other abbreviations as in Fig. 2.

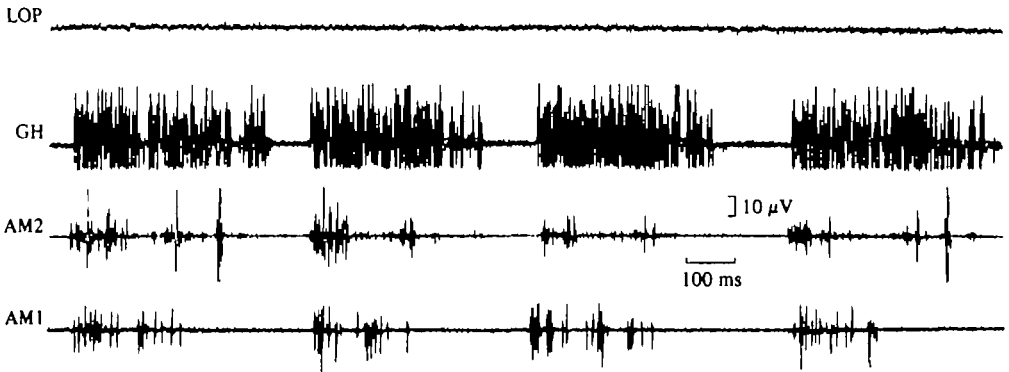


Fig. 4. Electromyographic pattern in four cranial muscles during chewing and manipulation of prey in the buccal cavity prior to deglutition. Abbreviations as in Fig. 2.

Activity in the levator arcus palatini muscle (Figs. 2, 3: LAP) is delayed 15–20 ms after the onset of levator operculi activity and overlaps greatly with muscles that are active during the compressive phase. This pattern is consistent with that found in other teleosts (Lauder, 1979; Liem, 1970) in which the suspensory apparatus only reaches its maximum point of abduction late in the strike well after the mouth has begun to close.

The expansive phase of the strike thus consists of a rapid initial mouth opening followed by hyoid depression and lateral expansion of the head by the levator arcus palatini muscle. All these events contribute to a rapid reduction in pressure in the buccal cavity which creates a flow of water into the mouth, drawing the prey in. The water then passes out over the gills in the compressive phase and out to the exterior between the operculum and the pectoral girdle.

The compressive phase of the strike is initiated by activity in the jaw adductors (Figs. 2, 3: AM2, AM1). Divisions one and two of the adductor mandibulae complex commence activity within 10 ms of each other and both exhibit prolonged (100–150 ms) levels of high activity. During strikes at slower moving prey, adductor activity commences 20–25 ms after activity in the levator operculi. Elusive, rapidly moving prey elicit nearly synchronous activity in the levator operculi and adductor mandibulae. The geniohyoideus muscle (Figs. 2, 3: GH) is generally active 25–30 ms after the onset of electrical activity in the adductor mandibulae complex. More variability was found, however, in the onset and duration of electrical activity in this muscle than in any other cranial muscle. Activity could begin nearly synchronously with activity in the adductor mandibulae or could be delayed for up to 300 ms, when chewing and manipulation of the prey usually commenced. At no time was the geniohyoideus active during the expansive phase.

Activity in the adductor arcus palatini (Fig. 3: AAP) occurs near the end of the compressive phase, 50–75 ms after the start of mouth opening. Multiple bursts of the adductor arcus palatini may occur immediately following prey capture.

Nearly all studies on the functional anatomy of fish feeding have concentrated on events occurring during the strike at the prey. However, the pattern of jaw muscle

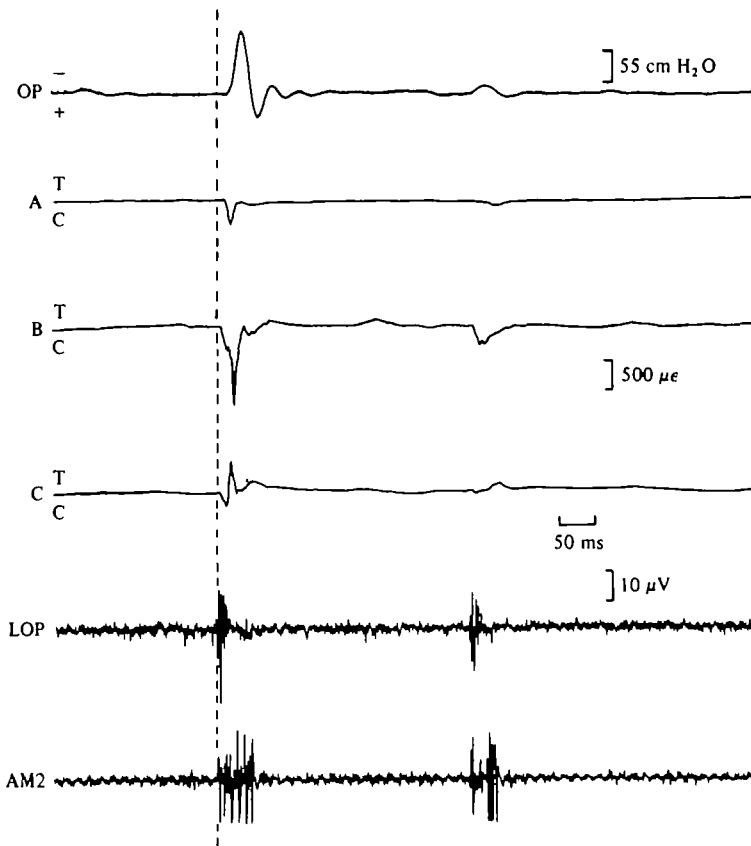


Fig. 5. Simultaneous recordings of opercular cavity pressure (OP), opercular bone strain (A, B, C: three gauges of the rosette strain gauge), and cranial muscle electromyograms (for abbreviations see Fig. 2) during a strike at the prey. —, negative pressure; +, positive pressure; T, tensile strain; C, compressive strain in the strain recordings. Note the second burst of electrical activity subsequent to the initial strike and the relatively little bone strain and opercular cavity pressure change. Pressure recordings are delayed 15–20 ms due to transducer delay.

activity during manipulation and deglutition may deviate dramatically from the strike (Fig. 4).

In the bluegill, chewing and manipulation of the prey following capture involves a remarkable pattern of muscle activity (Fig. 4). The geniohyoideus exhibits long regular uninterrupted bursts of activity usually lasting about 350 ms. These bursts are generally accompanied by synchronous activity in the adductor complex. Adductor activity is less well defined but the second adductor division usually exhibits strong activity at the start and end of each geniohyoideus burst with intermittent low level activity in between, while division one activity is usually confined to a large burst occurring synchronously with the onset of geniohyoideus activity followed by occasional low level bursts. No electrical activity is seen in any expansive phase muscle.

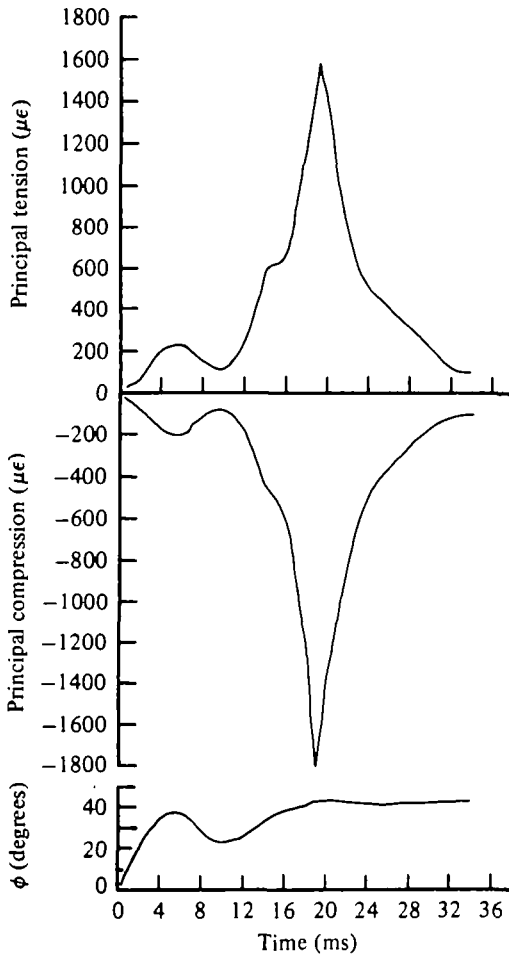


Fig. 6. Principal tensile and compressive strains and strain angle during a strike at the prey. The principal strains have been calculated from a typical pattern of opercular bone strain during a strike at a goldfish. Tensile strains are positive and ϕ is the angle of principal compressive strain with respect to gauge B (see text).

Bone strain and opercular cavity pressures

A typical opercular bone strain pattern recorded with rosette strain gauges is shown in Fig. 5 with the synchronous opercular cavity pressure record and two EMG traces for reference. The pattern of deformation was consistent in all experiments and showed no significant variations between experiments conducted during the two to three week experimental period with each gauge. Patterns of deformation (Fig. 5) were correlated with variations in the magnitude and shape of opercular cavity pressures.

Bone strain, which was temporally correlated with cranial muscle electromyograms, commenced 5 ms after the onset of electrical activity in the levator operculi. Similar EMG patterns may, however, be associated with very different strain regimes (Fig. 5).

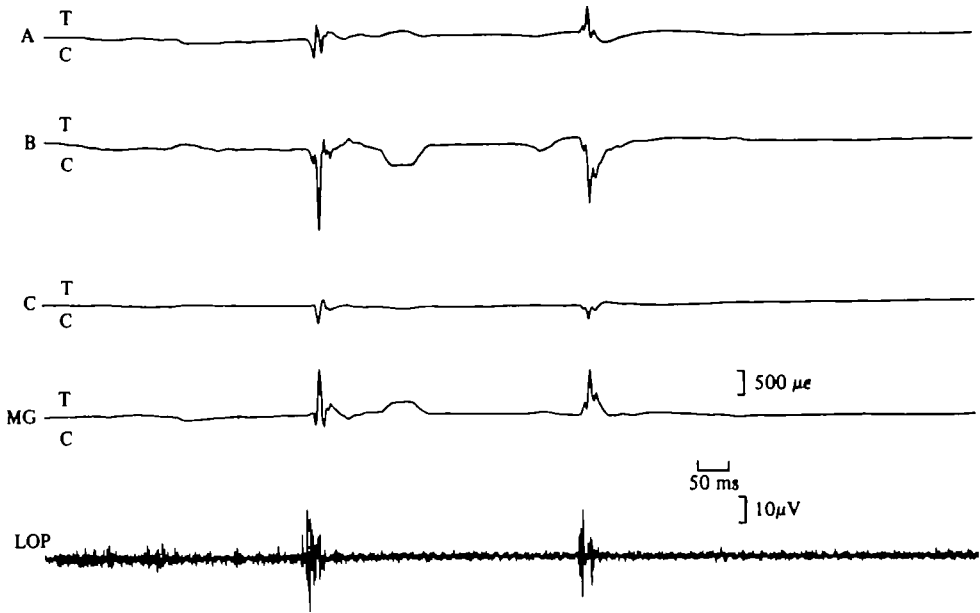


Fig. 7. Opercular bone strain recorded from a rosette gauge on the lateral opercular surface (recordings A, B and C) and simultaneously from a single element gauge (MG: medial gauge) attached to the medial opercular surface. The medial gauge was aligned along the direction of principal compressive strain on the lateral opercular surface and mirrors this compressive strain (see gauge B) in tension. Electrical activity in the levator operculi is shown for reference. Two successive strikes occurred at the prey in rapid succession.

Almost all the bone deformation occurred between the onset of the negative pressure pulse and the peak negative pressure, i.e. during the expansive phase.

Opercular cavity pressures during the strike at the prey also showed a consistent pattern: an initial negative pressure followed by a positive pressure pulse (Fig. 5). Peak negative pressures ranged from 10 to 145 cm H₂O depending on the prey item. Positive pressure pulses ranged from 5 to 50 cm H₂O and were usually smaller than the preceding negative pressure but occasionally were nearly equal in size.

The principal strains (Fig. 6) varied in magnitude depending on the type of prey but the pattern of strain change with time remained constant. Peak principal tension was nearly equal to peak principal compression during rapid strikes at the prey which generated high strain values (1200–1800 $\mu\epsilon$), but was consistently less than principal compression in less vigorous strikes (principal tension averaging one-third principal compression). Strain angle (Fig. 6) varied considerably during the early phases of the strike but remained relatively constant during peak deformation.

Peak strain rate also varied with the type of prey. The maximum strain rate recorded was $615 \times 10^3 \mu\epsilon/s$ during capture of an elusive prey requiring an extremely rapid strike. Peak strain rates during strikes at more sluggish prey varied between 15×10^3 and $110 \times 10^3 \mu\epsilon/s$.

One possible hypothesis of opercular deformation based on the convex lateral shape of the operculum is that the operculum was bending medially, in effect flattening itself against the side of the head as a result of the large negative pressures generated

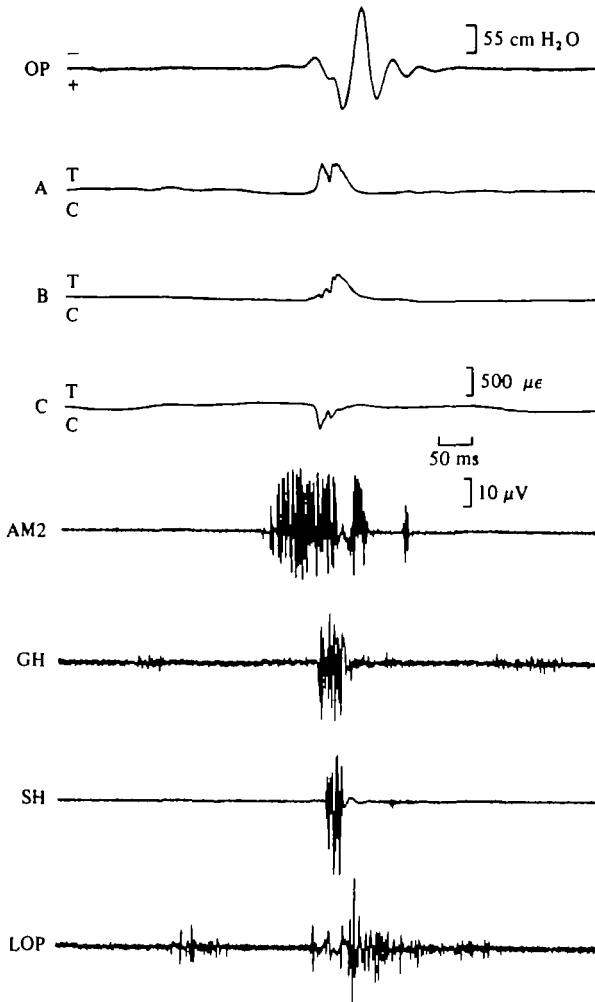


Fig. 8. Simultaneous recordings of opercular cavity pressure (OP), opercular bone strain (A, B, C: three gauges of the rosette strain gauge), and cranial muscle electromyograms (for abbreviations see Fig. 2) during 'spitting out' or 'forward cough' behaviour. Note the positive-negative-positive pressure pattern, the occurrence of tension in gauge A, and the reverse sequence of muscle activity from that seen during the strike. T, tension; C, compression.

during the strike. This hypothesis was tested by implantation of a single element strain gauge on the medial opercular surface. The medial gauge was aligned parallel to the angle of principal compression on the lateral surface. Bending was confirmed as the medial gauge registered almost exactly in tension the principal compressive strain pattern recorded on the opposite surface, approximated most closely by gauge B of the rosette (Fig. 7).

The pattern of electromyographic activity, opercular bone strain, and opercular cavity pressures changed greatly during various 'spitting out' or 'forward coughing' (Hughes & Adeney, 1977) movements which occurred during chewing and maceration

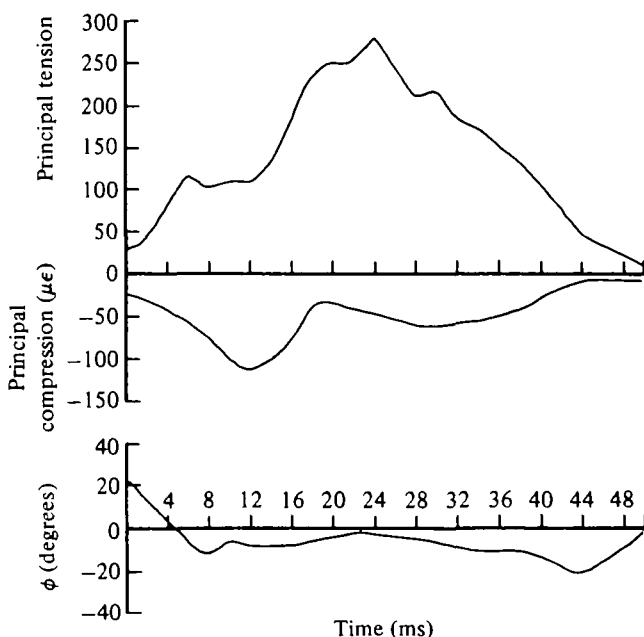


Fig. 9. Principal tensile and compressive strains and strain angle, ϕ , during 'spitting out' behaviour. Note that peak principal tension now exceeds principal compressive strain.

of prey following capture. Often scales or other undesirable materials such as small pebbles ingested with prey captured on the bottom were ejected following prey capture. The characteristic pressure profile obtained during these events (Fig. 8) consisted of an initial positive pulse, followed by a negative pulse, and then a final positive phase. Complicated mixtures of negative pressures followed by positive pulses (characteristic of the strike or efforts to swallow the prey) and the positive-negative-positive pattern (seen during spitting out) were often recorded during manipulation of the prey following capture. These sequences presumably reflect efforts to position the prey in the buccal cavity during chewing or prior to deglutition.

The pattern of electromyographic activity during these spitting out movements was the reverse of that seen at the strike (Fig. 8). The geniohyoideus was active synchronously with the adductor mandibulae (Fig. 8: GH, AM₂) to generate the first positive pressure pulse by compression of the buccal cavity. Activity then commenced in the levator operculi and the mouth opened allowing the forward flow of water to carry the unwanted material out of the mouth. A final low level burst of activity in the adductor mandibulae resulted in jaw closing. The final positive pressure pulse is presumably the result of jaw closure and opercular adduction.

The bone strain pattern in 'spitting out' movements showed interesting differences from patterns seen during the strike (compare Fig. 8 to Fig. 5). Gauge A of the rosette was in tension during 'spitting out' movements while it consistently registered compression during the strike at the prey. This same result was obtained for every occurrence of a positive-negative-positive pressure pattern as was the reversed electromyographic sequence.

Principal tensile and compressive components and principal strain angle during

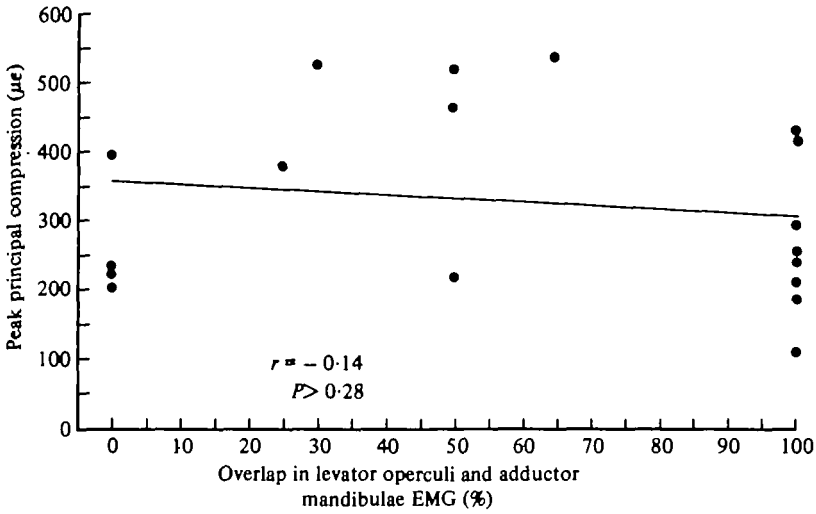


Fig. 10. The relationship between peak principal compression and the percentage overlap in levator operculi and adductor mandibulae electromyograms during strikes at prey items. Each point represents the peak principal compression and the percentage overlap during a particular strike. Electromyograms and strain were recorded simultaneously. The equation of the line fitted by least squares regression is $y = 0.474x + 356$; $r = 0.142$, $P > 0.28$.

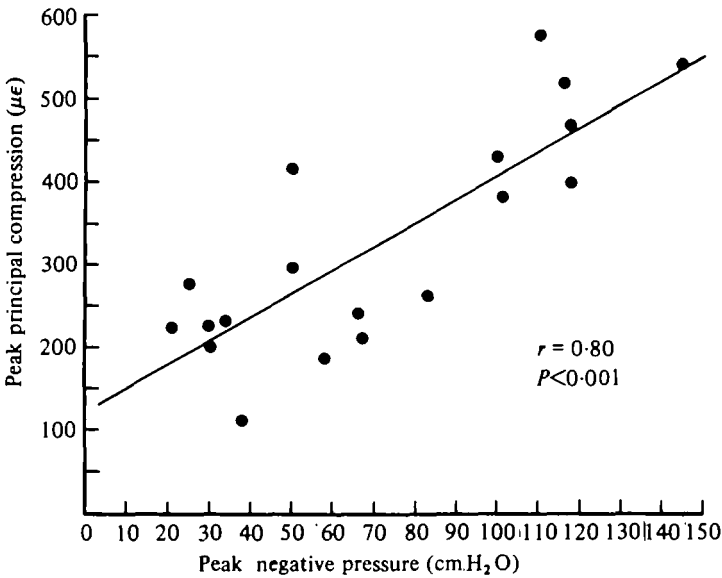


Fig. 11. The relationship between principal compression and peak negative pressure during strikes at prey items. Pressures and strain data for each point were recorded simultaneously. The equation of the line fitted by least squares regression is $y = 2.85x + 123$; $r = 0.80$, $P < 0.001$.

'spitting out' (Fig. 9) varied significantly from patterns during the strike (Fig. 6). Peak principal tension averaged twice peak principal compression while the principal strain angle changed relatively little. The relationship of principal compressive to tensile strain is the reverse of that found during the strike.

The cause of the normal pattern of bone deformation during the strike could be one (or a combination) of two factors: (1) muscle contraction directly resulting in tensile and/or compressive bone strains or (2) the pressure differential on either side of the operculum as a result of buccal expansion.

The greater the overlap in activity of the two antagonistic muscles acting on opposite ends of the operculum, the levator operculi and adductor mandibulae (Fig. 2: LO, AM₁, 2), the greater the resulting strain should be if muscle activity is the cause of bone strain. In addition, isolated bursts of activity in the adductor mandibulae and levator operculi should correlate with observed bone strain. Neither of these predictions are corroborated. High levels of electromyographic activity in the levator operculi and adductor mandibulae often failed to correlate with the presence of bone strain (e.g. Fig. 5). An explicit test of the hypothesis that greater temporal overlap in levator operculi and adductor mandibulae EMG should result in greater strain (Fig. 10) in fact showed a slight negative correlation ($r = -0.142$; $P > 0.28$).

In sharp contrast, the 'pressure hypothesis' predicts a positive correlation between peak negative pressure in the opercular cavity and peak principal compression on the lateral opercular surface. A test of this hypothesis (Fig. 11) showed a highly significant correlation ($r = 0.80$; $P < 0.001$) between principal compression and negative pressure. In simultaneous recording of opercular cavity pressures, bone strain, and electromyographic activity, a change in bone strain was never seen without a corresponding pressure reduction while it was often observed in the absence of electrical activity in the levator operculi. In these cases, the pressure drop was achieved by muscles not attaching to the operculum: the hypaxial and sternohyoideus muscles. Direct electrical stimulation of the levator operculi and adductor mandibulae (part 2) in anaesthetized fish also failed to produce any significant bone strain.

Principal strain angle varied considerably with the type of food eaten but the mean angle of peak principal compression for all foods aligns nearly exactly with the medial horizontal strut on the operculum (Fig. 12). The mean angle of principal tension aligns with the vertical strut.

Strikes at elusive prey (live goldfish) shifted the angle of peak principal tension toward the vertical strut while strikes on 'flake food' and meal worms resulted in peak principal tension angles shifted away from the strut. The total range of angular swing was within 30° on either side of the mean strain angle.

DISCUSSION

Electromyographic profile

The electromyographic profile of cranial muscle activity during the strike at the prey (Fig. 3) represents a summary of over 150 feeding sequences using a wide variety of foods. The range of foods tested (see Materials and Methods) encompasses

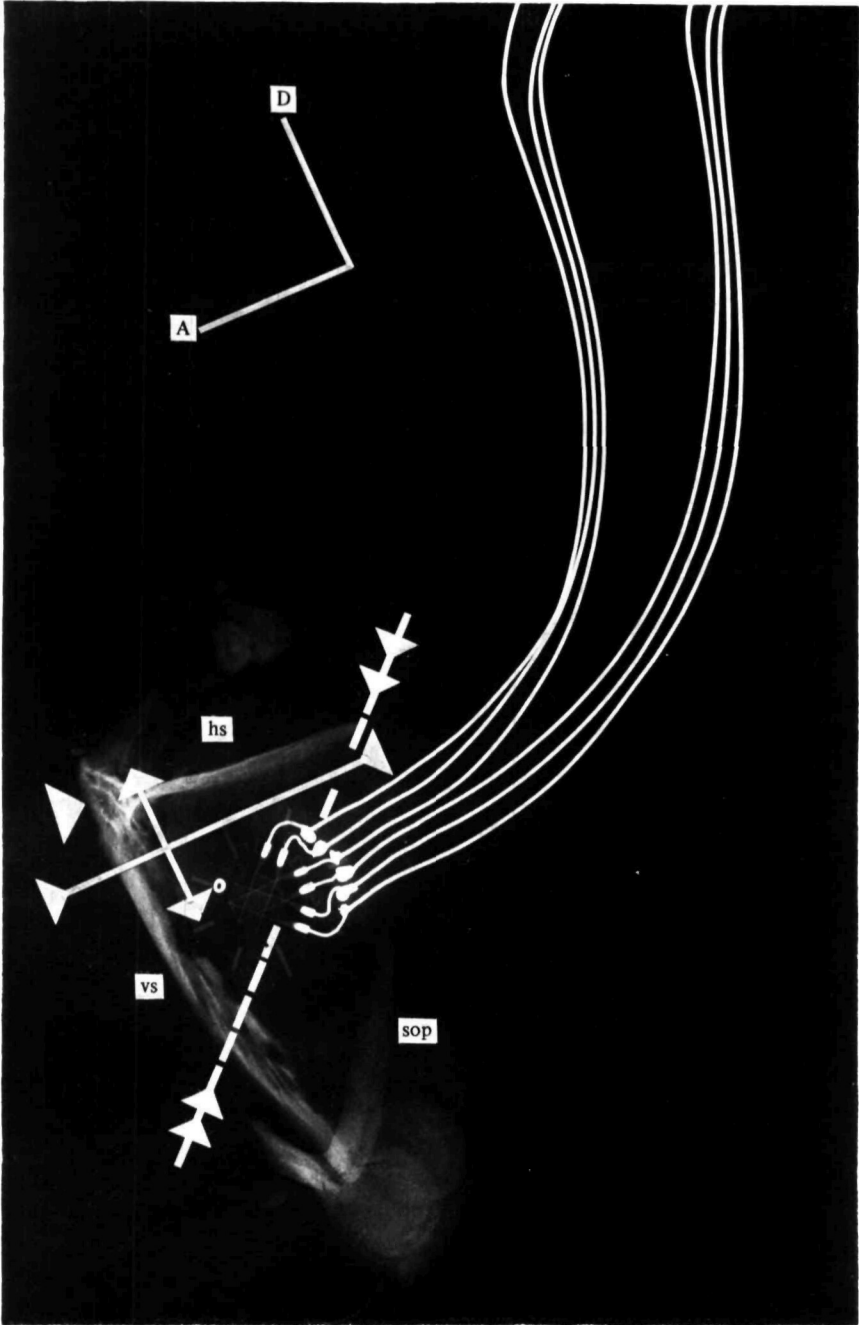


Fig. 12. Radiograph of the left operculum of the bluegill sunfish showing the position of the rosette strain gauge (the alignment of gauge B has been marked with a small piece of solder) the prominent horizontal (hs) and vertical (vs) struts, and the posteroventral joint with the subopercular bone (sop). Mean principal compressive strain ($\blacktriangleright\text{---}\blacktriangleleft$) is parallel to the horizontal strut while the mean principal tensile strain ($\blacktriangleleft\text{---}\blacktriangleright$) is parallel to the vertical strut. The length of the lines is proportional to the relative mean magnitudes of principal peak compressive and tensile strains during strikes at moderately elusive prey. These data actually reflect strains measured at the gauge site. The triangle indicates the articulation of the hyomandibula with the operculum. The dashed line represents the proposed axis of twisting; the direction of twisting follows the right-hand rule. A, anterior; D, dorsal.

the known food of bluegills in their natural habitat (see Keast, 1965, 1978*a, b*; Keast & Webb, 1966) and was designed to elicit a wide range of behavioural feeding responses. No consistent differences were observed in the movement pattern or electromyograms which could be correlated specifically with food type. Strikes at prey located on the surface ('flake food'), prey sinking slowly through the water column (pieces of smelt), or meal worms located on the bottom, did tend, however, to be longer in duration and show less overlap in antagonistic muscle complexes than the more rapid strikes at elusive prey (live fish). The more rapid the strike (determined from the start of mouth opening to complete closure of the jaws) the more temporal overlap in the levator operculi and adductor mandibulae EMG occurred. This seemingly paradoxical situation has also been found by Liem (1978) in cichlid fishes and may be related to the occurrence of lengthening contractions in the adductor mandibulae during rapid strikes. A stretching of the jaw adductors by the action of the sternohyoideus and levator operculi muscles during mouth opening may allow a much more rapid closing of the mouth than would otherwise be possible (Hill, 1953, 1970). Preliminary investigations on other fishes have revealed that the phenomenon of greater electromyographic overlap in antagonistic muscle complexes with increasing rapidity of the strike may be of widespread occurrence among fishes.

The characteristic activity pattern for the strike (Fig. 3) may not always occur during very low amplitude strikes at stationary prey. In such cases electrical activity in the levator operculi and adductor mandibulae is recorded, but no EMG from the hypaxial muscles, sternohyoideus, or geniohyoideus.

The function of the geniohyoideus muscle has been the subject of a great deal of controversy (see Ballintijn (1969*a*) for a review). Opinions have generally been divided between two views: that the geniohyoideus only protracts the hyoid arch and is active when the lower jaw is fixed in position by the jaw adductors, or that the geniohyoideus can either protract the hyoid or abduct the mandible depending on the presence of antagonistic activity in the adductor mandibulae and/or synchronous activity in the sternohyoideus. Ballintijn (1969*a*) has claimed that the geniohyoideus functions only as a protractor of the hyoid in the carp and that a similar situation occurs in the trout (Ballintijn & Hughes, 1965; Hughes & Shelton, 1962).

The conclusions for the trout were based on studies of respiration in restrained fishes, however, and Lauder & Liem (1979) have shown that while the geniohyoideus does indeed function to protract the hyoid during the initial strike at the prey, it may also act synchronously with the sternohyoideus and hypaxial muscles to cause mandibular abduction during manipulation of the prey following initial capture.

In the sunfish, no such plasticity in activity periods was observed and the geniohyoideus seems to function primarily to protract the hyoid as in the carp.

The unique pattern of muscle activity, especially of the compressive phase muscles, observed during 'chewing' movements following prey capture (Fig. 4), indicate that functional analyses of fish anatomy must include events following prey capture and not just those occurring at the strike. The pattern of muscle activity seen here may function to fragment the prey into smaller pieces or position the prey prior to deglutition. The prolonged and regular intervals of geniohyoideus activity are probably also associated with movements of the pharyngeal jaws during deglutition.

The traditional hypothesis of pectoral girdle movement during feeding in fishes stems from Tchernavin (1953), who proposed that the pectoral girdle undergoes large posteroventral movements during mouth opening and thus contributes to expansion of the orobranchial cavity. Lauder & Liem (1979) using electromyography and a movement analysis based on high-speed X-ray movies during feeding have shown that the pectoral girdle in *Salvelinus* is actually protracted or remains nearly stationary when the mouth is opened during feeding. The more complete electromyographic data presented here corroborate this finding in the sunfish and suggest that the pectoral girdle is held nearly stationary by pairs of antagonistic muscle complexes attached to its dorsal (protractor pectoralis-obliquus superioris) and ventral (sternohyoideus-hypaxial muscles) aspects.

A preparatory phase of muscle activity has been observed by Liem & Osse (1975) and by Liem (1978) in cichlid fishes in which the geniohyoideus, adductor mandibulae, and adductor arcus palatini are active just prior to mouth opening. These muscles compress the buccal cavity so as to minimize its volume prior to explosive expansion at the strike. A small positive pressure pulse in the buccal cavity associated with this muscle activity has also been found (Liem, 1978).

A preparatory phase is clearly present during feeding in the bluegill (Fig. 3), although it does not always occur. No relationship could be found between the presence of a preparatory phase and the type of prey. Activation of the preparatory phase muscles prior to the expansive phase may be correlated with the volume in the buccal cavity as the prey is approached. An initially large volume could result in the presence of a preparatory phase. A preparatory phase has not been reported for non-acanthopterygian fishes.

Possible functional correlates of the preparatory phase include an increased velocity of water flow into the mouth, an increase in the duration of fluid flow (assuming a constant rate of expansion), and an increase in the absolute volume of water drawn into the buccal cavity.

Pressure profile

Recordings of opercular cavity pressures during feeding have not been previously reported and only a few buccal cavity pressures (Alexander, 1969, 1970; Liem, 1978) are available for comparison. The opercular cavity pressure patterns presented here most resemble the buccal pressure records of Alexander (1969, 1970). An initial sharp negative pressure pulse occurs followed by a positive pressure pulse. Alexander measured buccal cavity pressures and thus observed a much smaller positive pulse (1–9 cm H₂O) while our opercular cavity records show a much larger pulse (10–60 cm H₂O) as water is expelled through the opercular cavity. Liem (1978) found an initial preparatory positive pressure pulse in the buccal cavity (not observed by Alexander (1970) and only occasionally seen here) prior to the large negative pressure and found no positive after-pressure. A systematic investigation of the difference between opercular and buccal pressure patterns during feeding has yet to be conducted.

Hughes & Adeney (1977) have comprehensively studied the different patterns of coughing in lightly anaesthetized rainbow trout and have defined a number of different coughing patterns which overlap one another. The positive-negative-positive 'spitting

out' pattern commonly seen in the sunfish during manipulation of prey (Fig. 8) most closely resembles one of the 'forward coughs' of Hughes & Adeney (1977: fig. 7G) although differences do exist which probably reflect the different experimental conditions.

Bone strain

The most common view of the operculum is as a link in a mechanism functioning to transfer the pull of the levator operculi muscle to the lower jaw (e.g. Osse, 1969). Although its role in the opercular suction pump during respiratory movements has been emphasized (Ballintijn & Hughes, 1965) the importance of the opercular bone in the inertial suction feeding mechanism has not been explicitly analysed. The data presented here suggest that the form of the operculum is also determined by its role in the suction feeding mechanism and that overall opercular design must be viewed as a compromise between factors relating to suction feeding and those governing the use of the operculum as a mechanical link in the jaw-opening mechanism.

The pattern of bone strain observed on the operculum during the strike at the prey is consistent with a hypothesis of bending and twisting moments on the operculum. The relative proportion of twisting moment to bending moment increases with more rapid strikes.

Bending is the result of the rapid reduction in pressure in the buccal and opercular cavities which causes the operculum to flatten against the side of the head (the operculum is laterally convex in shape). Bending occurs around a vertical plane through the centre of the operculum, parallel to the mean axis of principal tension in Fig. 12. Bending is demonstrated by the simultaneous recording of strain on the medial and lateral opercular surface (Fig. 7) and by the ratios of principal compressive to principal tensile strains. During slow and moderately fast strikes at the prey the ratio of principal compressive to tensile strain is 1.5:1.

Twisting of the operculum is proposed to occur about an axis passing anteroventrally from the posterodorsal corner of the operculum (Fig. 12). The anterodorsal margin of the operculum is twisted anteromedially by the levator and adductor operculi muscles while the ventral margin is twisted medially by the resistance of the lower jaw and adductor mandibulae to jaw opening. Further deformation may also be induced by a change in buccal pressure due to the irregular shape of the opercular bone. This pattern of twisting would result in the nearly equal principal compressive and tensile strains observed and would shift the line of peak principal tension towards the anterodorsal margin of the operculum. This is exactly the pattern obtained during rapid strikes at the prey where the ratio of principal tensile to compressive strains is nearly 1:1 (Fig. 6) and the line of principal tension (Fig. 12) is shifted toward the anterodorsal opercular margin.

The observed pattern of strain change could be reproduced *in vitro* by twisting the isolated operculum about an axis passing anteroventrally from the posterodorsal margin of the operculum (Fig. 12).

Only a limited set of comparative data are available with which to compare the bone strain data presented here, since all previously published work with strain gauges *in vivo* has been conducted on mammals (Hylander, 1977, 1979; Lanyon, 1973,

1974; Lanyon *et al.* 1975; Turner *et al.* 1975; Weijs & DeJongh, 1977). The peak principal strain of $-1800 \mu\epsilon$ reported here during rapid strikes at live fish is consistent with previous reports of strain from recordings in mammals during normal functional activity. Strain in mandibular alveolar bone during mastication in rabbits averages about $350 \mu\epsilon$ (Weijs & DeJongh, 1977) while during walking compressive strains of some $-1200 \mu\epsilon$ occur in the sheep's radius rising to $-1700 \mu\epsilon$ at a fast trot (Lanyon & Baggott, 1976). On the caudal aspect of the radius in horses during pacing, Turner *et al.* (1975) have recorded $-2600 \mu\epsilon$.

The peak strain rate on the opercular bone was $615 \times 10^3 \mu\epsilon/s$. Lanyon & Baggott (1976) and Lanyon & Bourn (1979) reported peak imposed strain rates in sheep radii and tibiae of 28×10^3 and $49 \times 10^3 \mu\epsilon/s$ during fast locomotion. The opercular bone strain rates reported in this study are thus more than ten times those previously reported for vertebrate bone. This high strain rate reflects the explosive rapidity of the strike at the prey in a suction-feeding fish and represents a normal level of repetitive functional activity.

Robertson & Smith (1978) have demonstrated that porcine mandibular bone behaves essentially as a brittle material at strain rates higher than $2.4 \times 10^3 \mu\epsilon/s$ and Carter, Spegler & Frankel (1977) have shown evidence of fatigue damage in bovine bone after only 2300 cycles loaded to $3000 \mu\epsilon$ at $10 \times 10^3 \mu\epsilon/s$. It might be expected that teleost bones subjected to comparable strains and higher strain rates than those in mammals would be liable to microfracture damage and that skeletal remodelling in this type of bone would be continuously necessary to effect internal repair. While areas of high strain and high internal remodelling appear to coincide in mammalian bone (Lanyon & Baggott, 1976; Lanyon, Magee & Baggott, 1979) it is paradoxical that advanced teleost bone (such as that in the sunfish) possesses an acellular structure (Moss, 1961*a, b*, 1962, 1963) which apparently has a limited ability to repair gross fractures, essentially undergoes no internal remodelling, and thus has neither the ability for internal repair nor a calcium reserve which can be effectively mobilized for metabolic use (Simmons, 1971).

Dullemeijer (1974, p. 114) has suggested that teleost bone is acellular because primary tensile forces act upon it. This hypothesis is not compatible with the data presented here. The lateral surface of the operculum has been shown to be subjected to large and rapidly induced compressive strains which during the strike exceed tensile strains.

Morphology and evolution of the operculum

The presence of two prominent raised struts nearly at right angles to each other on the medial opercular surface has been noted by several authors (e.g. Rosen & Patterson, 1969) and these struts have been hypothesized as functioning to strengthen the operculum against the pull of the levator operculi muscle (Gijsen & Chardon, 1976). The pattern of bone strain recorded in the sunfish suggests an alternative hypothesis: that the struts are aligned to resist twisting moments about the articulation of the hyomandibula with the operculum.

The two bony struts (Fig. 12) are positioned at a 45° angle to the axis of twisting and will resist the twisting moments proposed above. Each strut will also resist

bending around a plane perpendicular to the long axis of the strut. The arrangement of these struts in line with the principal strain directions ensures that the operculum is bent in the fewest planes possible.

Several additional aspects of opercular function during the strike may now be considered. During the onset of the expansive phase, the operculum is held against the side of the head by the adductor operculi which is active synchronously with the levator operculi (Fig. 3). Opercular levation, however, is accomplished against relatively little resistance since the lifting force is acting perpendicularly to the medial force holding the operculum against the side of the head (generated by the adductor operculi and negative pressure in the buccal and opercular cavities). In the compressive phase, as positive pressure is reached in the opercular cavity, the operculum pivots laterally, allowing water to pass out. The dilator operculi and levator arcus palatini produce the lateral opercular movement during this phase.

The evolution of the opercular bone may also be considered in the light of functional data presented here. The occurrence of an operculum in the early ray-finned fishes (Actinopterygii) is probably linked to both protection of the gills and the presence of an opercular suction pump in the respiratory cycle. These fishes did not possess a suction feeding mechanism and primarily used forward body velocity to overtake and capture prey. A true suction feeding mechanism makes its first appearance in the halecostome fishes (Liem & Lauder, 1980) and the presence of opercular thickenings in some early teleost fishes (though not to the extent found in the sunfish) corroborates other features of the feeding mechanism identified as functional correlates of an inertial suction strategy of prey capture (a free maxillary bone in the jaw, a coronoid process on the mandible, and the presence of an interopercular bone). While thickenings on the medial surface of the operculum may presumably also occur as a response to constant low level strain patterns imposed during respiration (low level (10–20 $\mu\epsilon$) strains occurring in a cyclical pattern were recorded on the operculum during respiration in unanaesthetized fish), the greater demands imposed during feeding may be expected to exert the primary influence on bone structure.

The well developed opercular struts in many advanced teleost fishes may be correlated with the ability to develop large negative pressures in the buccal and opercular cavities during prey capture.

This study was supported by an NIH Predoctoral Fellowship in musculoskeletal biology (5T32GM077117) to G.L. The experiments were carried out while L.E.L. was an Associate in Zoology at the Museum of Comparative Zoology.

We thank Uri Oron, Sheila Bourn, W. Fink, Vern Liebman, Andy Biewener, and especially David Kraus for assistance. Professor K. F. Liem provided many helpful comments on the manuscript and we thank Dr W. Hylander for many discussions and for suggesting the 'twisting hypothesis'. We are grateful to Professors F. A. Jenkins, Jr and A. W. Crompton for the loan of equipment and funds to purchase the strain gauge conditioner.

REFERENCES

- ALEXANDER, R. McN. (1969). Mechanics of the feeding action of a cyprinid fish. *J. Zool., Lond.* **159**, 1-15.
- ALEXANDER, R. McN. (1970). Mechanics of the feeding action of various teleost fishes. *J. Zool., Lond.* **162**, 145-156.
- BAGGOTT, D. G. & LANYON, L. E. (1977). An independent 'post-mortem' calibration of electrical resistance strain gauges bonded to bone surfaces 'in vivo'. *J. Biomech.* **10**, 615-622.
- BALLINTIJN, C. M. (1969a). Functional anatomy and movement co-ordination of the respiratory pump of the carp (*Cyprinus carpio* L.). *J. exp. Biol.* **50**, 547-567.
- BALLINTIJN, C. M. (1969b). Muscle co-ordination of the respiratory pump of the carp (*Cyprinus carpio* L.). *J. exp. Biol.* **50**, 569-591.
- BALLINTIJN, C. M., VAN DEN BERG, A. & EGBERINK, B. P. (1972). An electromyographic study of the adductor mandibulae complex of a free-swimming carp (*Cyprinus carpio* L.) during feeding. *J. exp. Biol.* **57**, 261-283.
- BALLINTIJN, C. M. & HUGHES, G. M. (1965). The muscular basis of the respiratory pumps in the trout. *J. exp. Biol.* **43**, 349-362.
- BASMAJIAN, J. V. & STECKO, G. (1962). A new bipolar electrode for electromyography. *J. appl. Physiol.* **17**, 849.
- CARTER, D. R., SPENGLER, D. M. & FRANKEL, B. H. (1977). Bone fatigue in uniaxial loading at physiologic strain rates. IRCS Medical Science: *Biomedical Technology, Connective Tissue, Skin & Bone Surgery & Transplantation* **5**, 592.
- COCHRAN, G. V. B. (1972). Implantation of strain gauges on bone *in vivo*. *J. Biomech.* **5**, 119-123.
- DALLY, J. W. & RILEY, W. F. (1965). *Experimental Stress Analysis*. New York: McGraw Hill.
- DULLEMEIJER, P. (1974). *Concepts and Approaches in Animal Morphology*. The Netherlands: Van Gorcum.
- ELSHOUD-OLDENHAVE, M. J. W. & OSSE, J. W. M. (1976). Functional morphology of the feeding system in the ruff - *Gymnocephalus cernua* (L. 1758) - (Teleostei, Percidae). *J. Morph.* **150**, 399-422.
- GABE, I. T. (1972). Pressure measurement in experimental physiology. In *Cardiovascular Fluid Dynamics* (ed. D. H. Bergel). London: Academic Press.
- GIJSEN, L. & CHARDON, M. (1976). Muscles et ligaments céphaliques, splanchnocrâne et quelques possibilités de mouvements dans la tête d'*Hoplerythrinus unitaeniatus* (Spix) Teleostei Ostariophysii (Characoidei). *Ann. Sci. Nat. Zool.* **18**, 251-274.
- HILL, A. V. (1953). The mechanics of active muscle. *Proc. R. Soc. Lond. B* **141**, 104-117.
- HILL, A. V. (1970). *First and Last Experiments in Muscle Mechanics*. Cambridge: Cambridge University Press.
- HUGHES, G. M. & ADENY, R. J. (1977). Variations in the pattern of coughing in rainbow trout. *J. exp. Biol.* **68**, 109-122.
- HUGHES, G. M. & SHELTON, G. (1962). Respiratory mechanisms and their nervous control in fish. *Adv. comp. Physiol. Biochem.* **1**, 275-364.
- HYLANDER, W. L. (1977). *In vivo* bone strain in the mandible of *Galago crassicaudatus*. *Am. J. phys. Anthrop.* **46**, 309-326.
- HYLANDER, W. L. (1979). Mandibular function in *Galago crassicaudatus* and *Macaca fascicularis*: an *in vivo* approach to stress analysis of the mandible. *J. Morph.* **159**, 253-296.
- KEAST, A. (1965). Resource subdivision amongst cohabiting fish species in a bay, Lake Opinicon, Ontario. Great Lakes Division, University of Michigan, publ. no. 13.
- KEAST, A. (1978a). Feeding interactions between age-groups of pumpkinseed (*Lepomis gibbosus*) and comparisons with bluegill (*L. macrochirus*). *J. Fish. Res. Bd Can.* **35**, 12-27.
- KEAST, A. (1978b). Trophic and spatial interrelationships in the fish species of an Ontario temperate lake. *Env. Biol. Fish.* **3**, 7-31.
- KEAST, A. & WEBB, D. (1966). Mouth and body form relative to feeding ecology in the fish fauna of a small lake, Lake Opinicon, Ontario. *J. Fish. Res. Bd Can.* **23**, 1845-1874.
- LANYON, L. E. (1973). Analysis of surface bone strain in the calcaneus of sheep during normal locomotion. *J. Biomech.* **6**, 41-49.
- LANYON, L. E. (1974). Experimental support for the trajectorial theory of bone structure. *J. Bone Jt Surg.* **56-B**, 160-166.
- LANYON, L. E. (1976). The measurement of bone strain *in vivo*. *Acta orthop. belg.* **42** (Suppl. 1), 98-108.
- LANYON, L. E. & BAGGOTT, D. G. (1976). Mechanical function as an influence of the structure and form of bone. *J. Bone Jt. Surg.* **58-B**, 436-443.
- LANYON, L. E. & BOURN, S. (1979). The influence of mechanical function on the development and remodelling of the tibia. An experimental study in sheep. *J. Bone Jt Surg.* (In the press.)
- LANYON, L. E., HAMPSON, W. G. J., GOODSHIP, A. E. & SHAH, J. S. (1975). Bone deformation recorded *in vivo* from strain gauges attached to the human tibial shaft. *Acta orthop. scand.* **46**, 256-268.

- LANYON, L. E., MAGEE, P. T. & BAGGOTT, D. G. (1979). The relationship of functional stress and strain to the process of bone remodelling. An experimental study on the sheep radius. *J. Biomech.* (In the press.)
- LANYON, L. E. & SMITH, R. N. (1970). Bone strain in the tibia during normal quadrupedal locomotion. *Acta orthop. scand.* **41**, 238-248.
- LAUDER, G. V. (1979). Feeding mechanisms in primitive teleosts and in the halecomorph fish *Amia calva*. *J. Zool., Lond.* **187**, 543-578.
- LAUDER, G. V. & LIEM, K. F. (1979). The feeding mechanism and cephalic myology of *Salvelinus fontinalis*: form, function, and evolutionary significance. Chapter 10. In: *Charrs: Salmonid Fishes of the Genus Salvelinus* (ed. E. K. Balon). The Netherlands: Junk. (In the Press.)
- LIEM, K. F. (1970). Comparative functional anatomy of the Nandidae (Pisces: Teleostei). *Fieldiana Zoology* **56**, 1-166.
- LIEM, K. F. (1973). Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Syst. Zool.* **22**, 425-441.
- LIEM, K. F. (1978). Modulatory multiplicity in the functional repertoire of the feeding mechanism in cichlid fishes. I. Piscivores. *J. Morph.* **158**, 323-360.
- LIEM, K. F. (1979). Modulatory multiplicity in the functional repertoire of the feeding mechanism in cichlid fishes: invertebrate pickers of Lake Tanganyika. *J. Zool., Lond.* (In the Press.)
- LIEM, K. F. & LAUDER, G. V. (1980). The evolution and interrelationships of the actinopterygian fishes. In *Fish Neurobiology and Behavior*. University of Michigan Press. (In the Press.)
- LIEM, K. F. & OSSE, J. W. M. (1975). Biological versatility, evolution, and food resource exploitation in African cichlid fishes. *Am. Zool.* **15**, 427-454.
- MOSS, M. L. (1961a). Osteogenesis of acellular teleost bone. *Amer. J. Anat.* **108**, 99-110.
- MOSS, M. L. (1961b). Studies of the acellular bone of teleost fish. I. Morphological and systematic variations. *Acta anat.* **46**, 343-362.
- MOSS, M. L. (1962). Studies of the acellular bone of teleost fish. II. Response to fracture under normal and acalcemic conditions. *Acta anat.* **48**, 46-60.
- MOSS, M. L. (1963). The biology of acellular teleost bone. *Ann. N.Y. Acad. Sci.* **109**, 337-350.
- OSSE, J. W. M. (1969). Functional morphology of the head of the perch (*Perca fluviatilis* L.): an electromyographic study. *Neth. J. Zool.* **19**, 289-392.
- ROBERTSON, B. M. & SMITH, D. C. (1978). Compressive strength of mandibular bone as a function of microstructure and strain rate. *J. Biomech.* **11**, 455-471.
- ROSEN, D. E. & PATTERSON, C. (1969). The structure and relationships of the paracanthopterygian fishes. *Bull. Am. Mus. nat. Hist.* **141**, 357-474.
- SIMMONS, D. J. (1971). Calcium and skeletal tissue physiology in teleost fishes. *Clin. Orthop.* **76**, 244-280.
- TCHERNAVIN, V. V. (1953). The feeding mechanisms of deepsea fish *Chauliodus sloani* Schneider. British Museum, N.H., London.
- TURNER, A. S., MILLS, E. J. & GABEL, A. A. (1975). *In vivo* measurement of bone strain in the horse. *Am. J. vet. Res.* **36**, 1573-1579.
- WEIJS, W. A. & DEJONGH, H. (1977). Strain in mandibular alveolar bone during mastication in the rabbit. *Arch. oral. Biol.* **22**, 667-675.