FEEDING PERFORMANCE AND MUSCULAR CONSTRAINTS IN FISH

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Summary
Analysis of the feeding kinematics of Astatotilapia (a small cichlid fish) suggests the presence of peripheral feedback modulation of the motor pattern, allowing the act of suction to be abbreviated. In this way, the effort spent in suction is minimized. The biological significance of the development of such a modulating feedback system is not immediately obvious from a ‘classical ecological’ point of view. It is postulated that the muscular metabolism itself might constrain the short, transient and strenuous motor output typical of suction feeding. Thus, reducing the suction effort makes sense when successive strenuous head-part movements are immediately required for additional suction, buccal transport or spitting. This hypothesis was tested by in vivo electrical stimulation of muscles important in feeding: the epaxials, which lift the skull and expand the buccal cavity. Reliable stimulation variables for the epaxial muscles were determined from preliminary stimulation experiments and from electromyographic recordings of these muscles in a specimen feeding on crickets. Stimulation trains of variable duration (<150ms) were applied in series of five trains. The intervals between trains were variable as well (<1s). The mechanical output was measured by means of an accelerometer, a force transducer or a magnetoresistive displacement transducer. In the latter case, the time course of the mechanical output could be recorded and analysed. The hypothesis predicts a decrease in the muscular output with increasing effort (long trains) and fast repetition (short intervals). The experimental results show the expected decline in mechanical output from one stimulation train to the next when longer stimulation bursts are imposed in quick succession. Statistical analyses (multiple regression) showed that train length, train rate and train number contribute significantly to the observed variation in mechanical output, supporting the hypothesis. Explanations for the phenomena are discussed.

Introduction
In order to feed, fishes have to generate a flow of food-laden water through their buccopharyngeal cavity. This can be achieved in two ways: (1) by translational movements of the fish with both the mouth and the opercula wide open, leaving the water and prey
relatively unaffected (imparting minimal momentum to the water and prey), or (2) by active suction, resulting from fast expansion of the mouth cavity (inertial suction). In fact, both techniques are combined to a greater or lesser extent (Muller and Osse, 1984). Suction always involves displacements of the fish as it sucks itself forwards or because it is assisted by active swimming. Moreover, advanced fishes protrude the border of their mouth towards the prey during suction, thus improving the flow relative to the mouth (for extreme examples, see Lauder and Liem, 1981, on *Luciocephalus pulcher* and Westneat, 1991, and Westneat and Wainwright, 1989, on *Epibulus insidiator*).

Particularly in cases where translational movements of the fish are minimized, the subsequent expansion of the mouth and opercular cavities for suction proceeds very fast. An entire strike usually lasts less than a few hundredths of a second. Starting with that of Osse (1969), a large number of experimental studies (movement analysis linked to electromyographic recordings) provide insight into the complex mechanism of fast suction feeding (for an extended list of references, see Aerts, 1990, 1991, 1992; Aerts et al. 1987; Lauder, 1983, 1985a,b; Muller and Osse, 1984; Van Leeuwen and Muller, 1983). On the basis of these experimental data, and especially because of the stereotyped motor output of the muscles involved in feeding (Wainwright and Lauder, 1986; Wainwright et al. 1989; Westneat and Wainwright, 1989), it is a common belief that expansion of the mouth for suction occurs as the result of a sequence of muscle activities governed by a central pattern generator that is not subjected to peripheral feedback modifications subsequent to the onset of a specific motor programme. The selection and assemblage of the functional components of the motor programme depend entirely upon peripheral pre-strike information (mainly visual) on the type, size, position and behaviour of the prey. Once started, the motor pattern of the strike cannot be modulated by further feedback control and the suction activity progresses in a preprogrammed fashion (Nyberg, 1971; Liem, 1978, 1979, 1980a,b, 1984; Elshoud-Oldenhave, 1979; Lauder and Liem, 1980, 1981; Lauder 1980, 1981, 1983; Lauder and Norton, 1980; Rand and Lauder, 1981; Grobecker, 1983).

However, Aerts (1990) showed that, despite strictly identical feeding conditions, *Astatotilapia elegans* (a small insectivorous cichlid) does modify its feeding process, apparently triggered by the prey entering the mouth. This modulation is expressed as an inhibition of further rostral expansion of the buccal cavity (the end of the so-called expansion phase; Lauder, 1985a,b; Liem, 1978) and the onset of the compressive phase as soon as the prey is retained in the mouth. In this way, the strike is abbreviated, and no more effort than is strictly required is spent in suction because pumping of superfluous water through the mouth is avoided. Aerts (1990) argued that these adjustments can be controlled by instantaneous in-strike feedback to the central pattern generator. The ability to abbreviate the strike is obviously not exclusive to this species. Other authors have referred to reduced (Grobecker, 1983, in stone fish) or extended (Osse, 1969, in the perch) buccal expansion, without discussing the probability of instantaneous feedback modulation (Aerts, 1990). Grobecker (1983) described it as a distinct feeding mode, implicitly inferring selection of a motor programme different from the usual pattern.

If present, the evolutionary development of strike-abbreviating behaviour and its underlying neuromotor specialisation most obviously aims to optimize the energy
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balance during feeding (i.e. the benefit versus the costs). However, in most cases, the energy spent in suction (i.e. pumping water through the buccal cavity) is only a fraction of the total energy budget when compared to prey searching, pursuit and processing. Moreover, Aerts (1990) deduced that the energy content of the prey is about 1000 times greater than the energy used to create the water flow for prey capture (see also Osse, 1986; Drost and Van Den Boogaart, 1986). Therefore, even with an extremely low digestive efficiency, it does not seem worthwhile economizing on energy expenditure by abbreviating the strike.

Aerts (1990) postulated that the metabolism of the muscle itself (for instance the phosphocreatine store or the Ca\(^{2+}\) flux) might directly constrain the motor output when transient and strenuous activities have to be performed in fast succession, as is typical of the feeding process in fishes. Subsequent bursts of activity may be required for additional suction when the prey is liable to escape, for spitting out undesirable particles and for buccal transport to the pharynx. For instance, in the cichlid *Oreochromis niloticus*, one strike and six successive intensive buccal transport cycles can occur in less than 3s (see Fig. 5 in Claes and De Vree, 1991). Thus, metabolic inefficiency of the expansion musculature immediately after the strike could diminish the feeding performance of the fish. In this context, reduction of the strike ‘effort’ could be beneficial.

This paper aims to verify this hypothesis experimentally by repetitive *in vivo* electrical stimulation of the epaxial muscles, simulating suction feeding activity patterns of different intensity and rate of repetition. Unfortunately, specimens of *Astatotilapia elegans* were no longer available. Therefore, a closely similar species occupying the same trophic niche, *Astatotilapia burtoni*, was used.

**Materials and methods**

Four specimens of *Astatotilapia burtoni* (total length 99–131.5mm) were obtained from the Zoological Institute of the University of Ghent (Belgium). The fishes were anaesthetized with tricaine methanesulphonate (MS222, Federa) and immobilized in a body-shaped corset made of a thermoplastic material (Orthoplast; Johnson and Johnson Orthopaedics). The head was left free, and care was taken not to restrict its movements or the movements of its parts. Teflon-insulated stainless-steel thin wire monopolar electrodes (diameter 0.045mm; Leico Industries, Inc.) were implanted in the occipital region of the epaxial muscles. These muscles power the expansion movements for suction (Aerts, 1991) and their main output, namely skull lifting, can be easily quantified. To avoid skull rotations out of the mid-sagittal plane caused by asymmetrical stimulation of the left and right sides of the body, only one pair of contralateral electrodes was used. The water temperature in the test aquarium was kept at 22°C. Stimulations with a pulse length of 1 or 2ms and a supramaximal voltage of 10 or 12V were given with a Grass S48 muscle stimulator. The stimulation circuit was charge-balanced by a coupling capacitor and a bleed resistor (Loeb and Gans, 1986) to avoid muscle damage and undue fatigue.

Electromyographic (EMG) recordings of the epaxial muscles of one specimen feeding on crickets (N=6) were made prior to the stimulation experiments. Two bipolar thin wire electrodes (like the stimulation electrodes) were inserted at each side of the body. The
signals were passed through Tektronix 26A2 differential preamplifiers and Honeywell Accudata 117DC amplifiers, and simultaneously displayed on a Tektronix R5103N storage oscilloscope and a Gould brush 418 chart recorder; they were stored on a Honeywell medium-bandpass 96 FM 14-channel tape recorder. These EMG recordings were used to estimate the duration of the periods of activity of the epaxial muscles during feeding. This ranged from 60 to 120 ms. According to Altringham and Johnston (1990a) and Johnson and Johnston (1991), maximal power output of fish muscle was attained at a stimulation frequency that gave a fused tetanus. Therefore, preliminary stimulation experiments were performed to determine the fusion frequency, which proved to be in the region of 100 Hz. This stimulation frequency was used in all further experiments.

Physiologically reliable stimulation patterns, simulating repetitive bursts of variable activity, were designed on the basis of these data. The intensity of the simulated feeding act depended on the duration of the stimulation trains. Within a train, the stimulation frequency was always 100 Hz to reach the fused tetanus frequency. Train lengths in the experiments ranged from 1 to 15 pulse strain$^{-1}$, adequately covering the duration of the EMG measurements and simulating a gradient of increasing effort to capture the prey. The rate of activity bursts was adjusted by modifying the frequency of the trains imposed on the test specimen. Train rates of 1, 2.5 and 5 Hz were used to simulate an increasingly faster repetition of activity bursts in the epaxial muscles. Different train rates and train lengths were combined. Each experiment included five successive trains. Intervals between experiments were 10 min for experiments consisting of trains shorter than 10 pulses at a rate lower than 2.5 trains per second. In the other cases, a 15 min interval was used. During the experiments, the fish was kept slightly anaesthetized (as indicated by a slow respiratory rate).

Skull elevation resulting from the electrical stimulation was recorded in three different ways. A miniature monoaxial accelerometer (Endevco 2250A) was attached dorsally on the head of one fish in the orbital region. A force-displacement transducer (Grass FT30C and FT10C) was used for the experiments on two other specimens. A mechanical extension was connected to the transducer with its other end touching the dorsal surface of the head in the orbital region. The position when the head was depressed coincided with the unloaded condition. Finally, a ‘home-made’ displacement transducer, applying a magnetoresistive sensor (Philips KMZ10), was used for a fourth specimen. This method only required a small magnet (2.5 mm × 3.5 mm × 4.5 mm; 0.37 g) to be mounted on the head and is, therefore, assumed to be the best representation of unrestrained skull elevation. All data were recorded on a Gould brush 418 chart recorder.

For readings derived from the accelerometer and the force-displacement transducer, only peak values per train were analyzed, because the significance of the time course of the signal is unknown. The output of the magnetoresistive sensor additionally allowed an analysis of head rotation as a function of time. This required linearization and conversion to degrees of skull rotation of the non-linear output signal. Therefore, the transducer was first calibrated in situ by coupling the signal evoked from manually moving the head of the anaesthetized fish with simultaneous video recordings (Panasonic MS1 camera; Panasonic AG7330 recorder; Sony VP850 printer). The transducer recordings were graphically enlarged and digitized by means of a Hipad (Houston Instruments) linked to
an IBM-compatible PC. Numerical integration of the data sets allowed the angular velocities of skull rotation to be calculated.

A multiple regression analysis (Statistical Package for Social Sciences; SPSS Inc., 1986) was carried out on the two largest data sets. This analysis related peak values of the output to (1) the total number of pulses that the sample had already received (a measure of overall fatigue), (2) the number of pulses per train, (3) the number of trains per second and (4) the serial number of the train within an experiment.

Results

Fig. 1A shows the maximal angle (in degrees) of skull rotation measured by the magnetosensitive sensor. A comparable plot, but representing the maximal elevating force assessed from another specimen using the force-displacement transducer, is depicted in Fig. 1B. Each bar corresponds to the output of one stimulation train. Groups of five successive bars embody a single experiment, combining one specific ‘pulsestrain’/trains s⁻¹, condition (encoded by the hatching of the bar and the labelling on the enclosing arc). The sequence of presentation coincides with the sequence of the stimulation experiments. The numbers on the abscissa refer to the total number of pulses that the sample had received before the onset of the next experiment.

In Fig. 1A, there is an obvious general decrease in the output as the number of pulses received increased. This can probably be attributed to the ‘overall fatigue’ of the sample. However, within some of the experiments (arrowheads in Fig. 1A), maximal output also decreases dramatically from one stimulation train to the next. This occurs under stimulation conditions that simulate a strenuous task for the epaxial muscles by imposing longer trains in quick succession (i.e. at 10 pulsestrain⁻¹ and a rate of 5 trains s⁻¹ and at 15 pulsestrain⁻¹ and 5, 2.5 and 1 trains s⁻¹). In Fig. 1B the same tendencies emerge, although the trend towards a general decrease is less obvious. Note that the stimulation series applied to the specimen in Fig. 1B is carried out in reverse order compared to the series presented in Fig. 1A: i.e. from heavy to light exercise.

A stepwise multiple linear regression was carried out on the entire data set presented in Fig. 1A (60 cases) to express the angle of skull rotation (the dependent variable) as a function of four independent variables. Three of them accord directly with the experimental design: the number of pulses per train, the number of trains per second and the sequential number of the train within each experiment. The fourth variable is the total number of pulses that the sample has already received. Table 1 summarizes the results.

The same analysis was performed on the stimulation series of 10 and 15 pulsestrain⁻¹ in the same data set (30 cases), since only these stimulation conditions show the within-experiment output decrease (see Fig. 1A). The results are given in Table 2. Table 3 shows the results of an identical multiple regression analysis on the data set presented in Fig. 1B (40 cases). In this case, force is the dependent variable.

Fig. 2 gives some additional results obtained from experiments on two other specimens. The peak value attained from each stimulation train (force in Fig. 2A; acceleration in Fig. 2B) is normalized to the maximum within each experiment (of successive trains) and is plotted against time. This is done for a series of experiments
Fig. 1

A

Skull rotation (degrees)

Total number of pulses received

B

Force (N)

Total number of pulses received
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Fig. 1. Peak absolute values of skull rotation (A) and force (B) of two series of stimulation experiments plotted against the total number of pulses that the sample has already received. One experiment consists of five consecutive stimulation trains with a specific interval between the trains. Experiments are grouped by the number of pulses per stimulation train. Detailed information is given in the text. (A) Skull rotation measured by the magnetosensitive transducer. (B) Force measured by the force-displacement transducer. Arrowheads point at the within-experiment decrease in muscular output.

Table 1. Results of multiple regression analysis expressing skull rotation (Fig. 1A) as a function of three independent variables (y=a+bx1+cx2+dx3; see text)

<table>
<thead>
<tr>
<th>Variable</th>
<th>PRC</th>
<th>S.E.</th>
<th>SRC</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP/TR (x1)</td>
<td>-0.205</td>
<td>0.066</td>
<td>-0.559</td>
<td>-3.124</td>
<td>0.0028</td>
</tr>
<tr>
<td>NT/S (x2)</td>
<td>-0.198</td>
<td>0.0765</td>
<td>-0.173</td>
<td>-2.588</td>
<td>0.0123</td>
</tr>
<tr>
<td>TNP (x3)</td>
<td>-4.803e-3</td>
<td>2.357e-3</td>
<td>-0.373</td>
<td>-2.037</td>
<td>0.0464</td>
</tr>
<tr>
<td>Constant</td>
<td>9.811</td>
<td>0.254</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r²</td>
<td>0.84</td>
<td></td>
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</tbody>
</table>

TNWE, not retained in the equation (P>0.05).
PRC, partial regression coefficient; s.e., standard error; SRC, standardized regression coefficient; NP/TR, number of pulses per train; NT/S, number of trains per second; TNP, total number of pulses; TNWE, train number within the experiment.

Table 2. Results of multiple regression analysis expressing skull rotation (10 and 15 pulsestrain⁻¹ experiments in Fig. 1A) as a function of three independent variables (y=a+bx1+cx2+dx3; see text)

<table>
<thead>
<tr>
<th>Variable</th>
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<th>S.E.</th>
<th>SRC</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP/TR (x1)</td>
<td>-0.706</td>
<td>0.102</td>
<td>-1.301</td>
<td>-6.940</td>
<td>0.0000</td>
</tr>
<tr>
<td>TNWE (x2)</td>
<td>-0.494</td>
<td>0.091</td>
<td>-0.538</td>
<td>-5.402</td>
<td>0.0000</td>
</tr>
<tr>
<td>TNP (x3)</td>
<td>9.339e-3</td>
<td>2.348e-3</td>
<td>0.790</td>
<td>3.978</td>
<td>0.0005</td>
</tr>
<tr>
<td>Constant</td>
<td>12.933</td>
<td>0.869</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r²</td>
<td>0.77</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

TN/S, not retained in the equation (P>0.05).
PRC, partial regression coefficient; s.e., standard error; SRC, standardized regression coefficient; NP/TR, number of pulses per train; NT/S, number of trains per second; TNP, total number of pulses; TNWE, train number within the experiment.

Simulating longer bursts of activity for different rates of repetition. Regardless of the recording method (force or acceleration), the 5trains s⁻¹ experiments are characterized by a dramatic drop in muscle output performance over the series of consecutive trains. With an increase of the time interval between the trains (i.e. 2.5trains s⁻¹; 1train s⁻¹), this effect is reduced, but it is still present as a result of the length of the stimulation trains.

Fig. 3A,B shows the time course of head displacements for part of the experimental series presented in Fig. 1A. Only the results for the 10 and 15 pulsestrain⁻¹ experiments are shown, as no within-experiment decrease is observed for the shorter train lengths (see arrowheads in Fig. 1A). In Fig. 3A,B, skull rotation is normalized to the maximal angle found in the respective experiment (see Fig. 1A) and plotted against time. Fig. 3C,D
gives the corresponding estimates of the relative angular velocities (calculated on the basis of the normalized displacements presented in Fig. 3A,B). In feeding fish, the velocity of skull rotation is an important determinant of the suction hydrodynamics (Muller et al. 1982).

The first displacement profile in each set of five in Fig. 3A,B is remarkably constant from one stimulation condition (specific pulsestrain\(^{-1}\) and trains s\(^{-1}\) combination) to another. The head rises rapidly during this first stimulation train (in about 0.03s), but readily depresses again to 40% of the maximum. The degree of skull rotation then increases slightly again. This conspicuous oscillation, which is independent of the experimental conditions, occurs within the stimulation periods (i.e. before 0.1s and 0.15 s for the 10 and 15pulsestrain\(^{-1}\) experiments respectively).

The displacement profiles of the subsequent trains show a tendency towards (1) a decrease in maximal amplitude, (2) an increase in the delay before reaching this maximum, (3) a disappearance of the oscillation (although the time at which it occurs appears to be less affected), and (4) an increase in the magnitude of the initial angle at zero time (because the head is unable to depress entirely when burst intervals are short). These tendencies become more pronounced with an increase in muscular exercise: i.e. when both the duration of the activity increases (more pulsestrain\(^{-1}\)) and the interval between successive activity periods decreases (more trains s\(^{-1}\)). These effects are reflected in the spectacular decrease in the relative angular velocities in successive trains (Fig. 3C,D). This is particularly noticeable for the 15pulsestrain\(^{-1}/5\) trains s\(^{-1}\) condition.

**Discussion**

In a previous paper (Aerts, 1990), arguments were put forward in favour of a simple instantaneous on/off feedback mechanism that rigidly modulates fast inertial suction feeding in fishes. This feedback mechanism allows the duration (or intensity) of the strike to be adjusted according to the all-or-none retention of the prey. If correct, this finding contradicts the previous assumption that the strike is entirely preprogrammed (Nyberg, 1971; Liem, 1978, 1979, 1980a,b, 1984; Elshoud-Oldenhave, 1979; Lauder and Liem, 136 P. AERTS and F. DE VREE

<table>
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<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNWE ((x_1))</td>
<td>-0.010 ((b))</td>
<td>2.200e(^{-3})</td>
<td>-0.473</td>
<td>-4.624</td>
<td>0.0000</td>
</tr>
<tr>
<td>NT/S ((x_2))</td>
<td>-0.010 ((c))</td>
<td>2.234e(^{-3})</td>
<td>-0.441</td>
<td>-4.810</td>
<td>0.0000</td>
</tr>
<tr>
<td>TNP ((x_3))</td>
<td>-3.494e(^{-3}) ((d))</td>
<td>1.411e(^{-4})</td>
<td>-1.013</td>
<td>-2.477</td>
<td>0.0182</td>
</tr>
<tr>
<td>Constant</td>
<td>0.214 ((a))</td>
<td>0.012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(r^2)</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

NP/TR, not retained in the equation \((P>0.05)\).

PRC, partial regression coefficient; s.e., standard error; SRC, standardized regression coefficient; NP/TR, number of pulses per train; NT/S, number of trains per second; TNP, total number of pulses; TNWE, train number within the experiment.
Obviously, this strike-abbreviating behaviour is intended to reduce the 'effort' spent in prey capture, although this apparently cannot be interpreted in terms of energetic efficiency (i.e. the costs of suction versus the benefit). The hypothesis that strike abbreviation economizes muscular output, thus circumventing failure during (potential) successive strenuous activity of the head muscles, is put forward.

To test this hypothesis, in vivo electrical stimulation experiments of the epaxial muscles were carried out. These muscles substantially power buccal expansion movements since neurocranial lifting also causes depression and abduction of the hyoid (and suspensoria; Aerts, 1991; Muller 1987, 1989). The in vivo approach is preferred (instead of in vitro tests on fibre bundles) in order to ensure that morphological and physiological variables that may affect muscle output are as far as possible unaffected. The results obtained using the magnetoresistive sensor are believed to be the closest approximation to unrestrained skull elevation. Initially, efforts were made to perform
Fig. 3
measurements on specimens completely recovered from anaesthesia. However, as 
stimulation evoked undesirable reflexes (struggling), largely obscuring the recordings of 
the phenomenon of interest (skull elevation), the test specimens were kept slightly 
aanaesthetized with tricaine methanesulphonate (opercular rhythm and heart rate slowed 
down; stage 5 in Jolly et al. 1972). This drug, structurally closely related to procaine 
(Ritchie and Cohen, 1975), is thought to block signal conduction and transmission in 
efferent and afferent nerves and does not affect the response of muscle to electrical 
stimulation. In the context of the applied test protocol, this anaesthetic is assumed not to 
influence muscle metabolism, leaving the output mechanics unaffected.

The imposed stimulation conditions are assumed to be biologically relevant. The 
applied stimulation frequency of 100Hz coincides with the frequency at which a 
complete fusion of the tetanus was found. Altringham and Johnston (1990a) found this to 
be the optimal frequency for a stimulus train to maximize power output. Judging from the 
large pressure differences involved in feeding (Liem, 1978; Muller and Osse, 1984; 
Muller et al. 1982), maximized muscle output is likely to be used. For Myoxocephalus 
scorpius (a percomorph, like the study species), the fused tetanus frequency increases 
from 50Hz at 4˚C to 75Hz at 15˚C for fast muscle fibres (Altringham and Johnston, 
1990a; Johnson and Johnston, 1991). Granzier et al. (1983) found a fusion frequency of 
75Hz for carp fast muscle at 20˚C, but force output was maximal at stimulation 
frequencies between 100 and 150Hz. These data support the reliability of our chosen 
frequency. Moreover, an estimation of the neuronal motor frequency from the recorded 
EMG signals also suggests a frequency of about 100Hz. [This is qualitatively deduced on 
the basis of the composition of an EMG signal (Loeb and Gans, 1986). This is a signal of 
high frequency because the electrodes simultaneously sample the action potentials of 
several fibres (belonging to different motor units). These signals are not precisely in 
phase, because of (1) imperfect synchronisation of different motor units, (2) different 
fibre sizes (resulting in different conduction velocities) and (3) different distances 
between the motor end plates and the electrodes. Thus, an EMG record is a superposition 
of action potentials in which some currents are additive, whereas others cancel each other 
out (Loeb and Gans, 1986). Therefore, it is assumed that the large spike patterns 
occuring in the EMG records of feeding Astatotilapia burtoni in a regular, repetitive way 
embody identical superpositions of electrical phenomena and hence represent the 
neuronal stimulation frequency of the muscle.] The length of the stimulation trains is 
established on the basis of the duration of the EMG signals. The direct equivalent of the

Fig. 3. (A) Time course of skull rotation during stimulation bursts at 10pulsestrain−1 (from 
Fig. 1A). The output is normalized to the maximum within each experiment (i.e. each series of 
five trains) and plotted against time within the stimulation burst (zero time coincides with the 
onset of stimulation). The results are grouped per experiment (five consecutive trains). Each 
curve represents the output for one stimulation train. More information is given in the text. 
(B) As in A, but for the 15pulsestrain−1 experiments of Fig. 1A. (C) Estimates of the angular 
velocity of skull rotation based on the normalized output shown in A and represented in a 
comparable way (for more information see text). (D) As in C, but for the 15pulsestrain−1 
experiments. In the 10pulsestrain−1 experiments (A,C) stimulation trains end at 0.1s. For 
15pulsestrain−1 experiments (B,D) this occurs at 0.15s.
activity bursts measured here are train lengths of 6–12 pulses. However, the variation of 1–15 pulses per train actually applied is physiologically relevant, as none of the recorded feeding events was very weak (like those observed during feeding on small bottom particles) or very extreme (like those occurring during failed feeding attempts; Aerts, 1990). The repetition of five trains at a frequency up to 5Hz must simulate repeated strenuous muscle activity. Claes and De Vree (1991; Fig. 5) show kinematic data of the strike and of six successive intense buccal transport cycles in a cichlid. All seven movement cycles are performed in less than 3s, coinciding with a frequency of 2.5Hz. Aerts (1990) reported two successive strikes within 0.3s (about 3.3Hz; see below). Therefore, the train frequency and number of repetitions used here are considered to be appropriate to test the constraints of muscle performance.

The results of the experiments support the present hypothesis. Apart from the general decreasing trend of muscular output with an increase in the total number of electric pulses already imposed on the sample (overall fatigue), Fig. 1A clearly shows that, for those stimulation conditions simulating intense muscle activity, the degree of skull rotation drops significantly from one train to the next within a stimulation experiment (see arrowheads in Fig. 1A). That this cannot be attributed to the overall fatigue of the sample is qualitatively indicated by two independent facts. (1) For the experimental series shown in Fig. 1A, the experiments proceeded from light to strenuous exercise (from 1 to 15 pulsestrain⁻¹). The within-experiment effect first occurs for the 10 pulsestrain⁻¹/strains⁻¹ condition. If this decrease simply represents a fatigue phenomenon directly related to the total number of pulses the sample has already received, then it is difficult to understand why the same effect does not show up in the other 10 pulsestrain⁻¹ experiments (but with longer inter-train intervals), to develop again in all 15 pulsestrain⁻¹ experiments. (2) The results of the experimental series depicted in Fig. 1B were obtained in reverse order with intense exercise first (from 14 to 1 pulsestrain⁻¹). If the total number of pulses (i.e. the overall fatigue) determines the within-experiment decline in the force from one train to the next, then this effect would be expected to be most pronounced in the later experiments. However, the reverse is true (see arrowheads in Fig. 1B).

The data further indicate a clear interaction between the length of the stimulation train and the interval between successive trains: the shorter the delay, the faster the decline in the muscular output within an experiment. This is particularly evident in Fig. 2A,B (force and acceleration), but also from a comparison of the results for specific pulsestrain⁻¹ conditions as presented in Fig. 1A,B (i.e. experiments grouped by arches indicating the longer trains). Again, harder tasks appear to relate negatively to muscular performance.

The qualitative interpretation of the results is strengthened by the statistical analysis. When the maximal angle of skull rotation (plotted in Fig. 1A) is expressed as a function of (1) the total number of pulses received, (2) the number of pulses per train, (3) the number of trains per second and (4) the train number (1–5) within an experiment, all variables except the last contribute significantly ($P<0.05$) to the description of the pattern of skull rotation observed throughout the series of experiments (see Table 1). The total number of pulses probably represents the overall fatigue (throughout the series of experiments), whereas trains s⁻¹ and pulsestrain⁻¹ explain the decrease in the rotation...
angle within an experiment. It is assumed that the effect of the train number (reflected in
the cascade in the decline of the marked experiments in Fig. 1A) is masked by the output
for the 1 and 6pulestrain⁻¹ experiments, which show a positive correlation with pulse
number. This is confirmed by applying the same multiple regression analysis to the 10
and 15pulestrain⁻¹ experiments only (i.e. tests exhibiting the within-experiment
reduction). In this case, the pulse number adds significantly ($P<0.001$) to the description
of the rotation angle of the skull (see Table 2). Logically, the analysis now rejects
trains s⁻¹ from the equation.

Application of the same multiple regression analysis to the data set presented in
Fig. 1B reveals a significant ($P<0.01$) contribution to the description of the force pattern
for all variables except for the number of pulses per train (see Table 3). This might be
related to the fact that even the 1pulestrain⁻¹ experiment shows a tendency for reduced
force output from one train to the next (see Fig. 1B).

In both cases, the statistical analyses indicate that train length and train interval have a
large impact on muscular output in successive bursts of stimulation. The observed shifts
in significance of the various variables between the test series represented in Fig. 1A,B
might be caused by individual variation (i.e. the specimen, electrode locus) and the
difference in test procedure (force versus displacement measurement). It is remarkable
that in the test series presented in Fig. 1B, the 5pulestrain⁻¹ experiment and even the
1pulestrain⁻¹ experiment also show the tendency for a within-experiment decrease in
muscular output. For the first experiment in this series, this decline is much less
conspicuous. This could mean that overall fatigue (expressed as the number of pulses
already received by the sample) interacts with the within-experiment phenomenon.
Because of the reversed order, this effect (if present) should be masked in the test series
shown in Fig. 1A. However, the different experimental procedures can also be an
influencing factor. In contrast to the measurements with the magnetoresistive sensor, the
force measurements are close to isometric conditions, which implies that there is a
reduction in metabolic rate (see, for instance, McMahon, 1984; Woledge et al. 1985). (It
should be pointed out that the series of experiments in Fig. 1B was imposed on the test
specimen after it had been allowed 2h to recover from an intensive run through the
equipment.)

The present data and the statistical analyses support the working hypothesis that the
performance of the head muscles is drastically reduced when vigorous periods of activity
follow each other in fast succession. Assuming that these experimental results can be
generalized to the neuromotor biology, then a reduction in the strike ‘effort’ (in terms of
shortening the activity bursts as observed by Aerts, 1990) can be beneficial for the fish.
Strike abbreviation shifts the muscular activity patterns towards the left-hand side of the
data presented in Fig. 1A, which should allow subsequent efficient activity bursts at a high
repetition rate. This repetitive buccal expansion is frequently required; for example, to
provide additional suction when the prey is liable to escape, for spitting out undesired
particles or for buccal transport to the pharynx.

When the strike is prolonged, there is a shift to the right-hand side of the graph in
Fig. 1A, giving rise to the risk of reduced feeding efficiency when a secondary burst of
activity is needed. According to Aerts (1990), such prolonged strikes often coincide with
mis-feedings or occur in cases where the degree of fixation of the (more or less) sessile prey is unpredictable. As illustrated in Fig. 3, the potential decrease in efficiency should be expressed not only in the amplitude of buccal expansion but also, primarily, in its expansion rate. The latter is directly proportional to the velocity of the generated flow (law of continuity; see Muller et al. 1982; Muller and Osse, 1984; Aerts, 1990), which determines (1) the hydrodynamic forces acting on the prey, and thus (2) the efficiency of prey capture or transport. This interpretation fits well with the single available observation from high-speed film recordings showing Astatotilapia trying to catch a prey within 0.3s after missing it at the first powerful wide-gaping (=prolonged effort; Aerts, 1990) strike. This second trial, however, failed as well, as head-part movements were slow and of low amplitude (Aerts, 1990). As already mentioned, Fig. 5 in Claes and De Vree (1991) showed a strike and six successive buccal transport cycles for Oreochromis niloticus. It is conspicuous that the first three cycles following the strike are of much smaller amplitude than the last three.

These muscular limitations can be an effective driving force for the development of an instantaneous feedback system controlling the duration of the expansion phase during suction feeding, as proposed by Aerts (1990). This ‘development’ could be evolutionary (i.e. genetically programmed), but could also be a gradual modification of the neurological organisation of the feeding performance, sustained by accumulating experiences during the life history of the individual (Bol, 1992). In the first case, it could be a common feature of advanced teleosts. In the latter case, a large degree of individual variability would be expected. Unfortunately, the available information from the literature is too fragmentary to give credible support to either of these concepts. Only two other reports in the literature refer directly to strike length adjustment: Osse (1969) mentioned: ‘In two cases in which the perch missed the prey, the extra abduction of the operculars with wide open gape was such that the opercula acquired a nearly transversal position. This gives the impression of being the last effort to suck up the escaping prey’; Grobecker (1983) described an abbreviated feeding mode of the stone fish when the prey closely approaches the predator’s mouth. In such cases, the expansion movements were reduced compared with the other feeding modes.

According to the definition of Westerblad et al. (1991) and Vøllestad and Sejersted (1988), the within-experiment reduction in muscle output observed in our tests constitutes a type of muscle fatigue (fatigue referring to any decline in force-generating capacity). Westerblad et al. (1991) classify fatigue due to repeated tetanic stimulation as a distinct type. Fig. 3 illustrates how this type is manifested in the epaxial muscles. Most obvious are the reduction in peak output, the increase in rise time and the inability of the muscle to relax completely between two stimulation trains when strenuous bursts of activity follow each other in quick succession. All these factors contribute to the often drastic decrease in angular velocity of the skull. Fig. 3, however, also reveals another conspicuous fact deserving some attention. It concerns the oscillation in muscle output, which vanishes from one train to the next within an experiment. It might be assumed that skull rotation generates feedback to antagonistic muscles, which should then show the same effect of fatigue as the stimulated epaxials themselves (thus explaining the disappearance of the oscillation). However, tricaine blocks nerve function, which makes
this interpretation less plausible. In our opinion, this phenomenon can be attributed to a mechanical effect. The constant frequency of the oscillation (i.e. independent of the drastically changing stimulation conditions) suggests that this is the natural frequency of the moving system (the rotating head plus the small magnet). The time course of the head movements reflects a damped vibration (viscoelastic properties). The changing amplitude of the oscillations from one train to the next probably embodies ‘setting’ of the viscoelastic structure (the entire head) against which the force generator (i.e. the muscle) is working. [Setting phenomena occur in many viscoelastic materials and reflect changing mechanical (viscous) characteristics under a cyclically fluctuating loading regime.]

Although providing a causal explanation for the within-experiment decline in the muscular output is not the initial purpose of the present study, this report would be incomplete without speculation on this point. Judging from the short duration of the strenuous expansive phase of the feeding act, suction can be assumed to be an anaerobic process, running entirely at the expense of the phosphocreatine store in the muscle (Hoppeler and Billeter, 1991). If anaerobic glycolysis (and later aerobic rephosphorylation) cannot cope with the initial energy demands for muscular contraction, activity bursts occurring in quick succession (within a few tenths of a second) can cause a depletion of the phosphocreatine store, given that the instantaneous energy consumption exceeds the directly available energy stores. Thus, phosphocreatine depletion could be related to decreasing muscular output from one stimulation train (or \textit{in vivo} activity burst) to the next. Unfortunately, biochemical and physiological information on fish head muscles is rather scarce. White trunk muscles show high creatine phosphokinase activity, suggesting that a phosphocreatine-dependent energy supply is involved in burst swimming (Johnston, 1981; Altringham and Johnston, 1986; Johnston and Harrison, 1985). Johnston and Harrison (1985) estimated that, in the case of notothenid fishes, the store of phosphocreatine should suffice for a 50–100s period of activity. Dobson and Hochachka (1987), however, showed that the phosphocreatine content of white trunk muscle in trout drops by 45 and 70% after two and four tail flips respectively (bursts of activity). Concomitant with this, a (smaller) decline in ATP levels was observed (10 and 30% respectively; ADP becomes rephosphorylated at the expense of phosphocreatine). The time between the muscular activity and freeze-clamping of the tissue in these experiments was about 30s. Moon \textit{et al.} (1991) working on cod and Johnson \textit{et al.} (1991) working on sculpin carried out biochemical assays on white muscle fibre bundles performing oscillatory work. Glycolytic and aerobic metabolism was blocked in these experiments so that all energy was derived from ATP and phosphocreatine. In these tests, samples were quickly frozen and it was shown that for the cod (at 4°C) after eight cycles (bursts of activity) at a rate of 5 cycles s$^{-1}$ (the train rate in our experiments) and 3 pulses per cycle at 50Hz, phosphocreatine level had only dropped by 20%, whereas no significant change in ATP level could be detected. In the case of the sculpin, the same measurements were performed at 15°C after eight cycles of one stimulus at a cycle rate of 17Hz (i.e. within 0.5s). Again, the drop in phosphocreatine concentration was found to be small (about 20%), whereas the ATP concentration remained virtually unchanged. Therefore, unless there are large chemical differences between \textit{Astatotilapia burtoni} muscle and the white trunk muscle of cod, the direct
energetics of the epaxials of *Astatotilapia burtoni* do not account for the decline in muscle output described in this paper.

Moon *et al.* (1991) also observed a reduction in the maximal force from one cycle to the next for about the first eight cycles. Moreover, the degree of decline was positively related to the cycle rate (Altringham and Johnston, 1990b). At 18Hz (with only 1 pulsetrain$^{-1}$), the profile of the decline in maximal tension was comparable to the present simulations of strenuous activity (long bursts in fast succession: for instance, 5 trains s$^{-1}$, but 10 or 15 pulsestrain$^{-1}$), whereas at 5Hz the decline was present, but less pronounced. At this cycling rate, Altringham and Johnston (1990b) applied only 3 pulsecycle$^{-1}$, so that the inter-burst delay was three times longer than for our 5 trains s$^{-1}$ at 15 pulsestrain$^{-1}$ stimulations. Thus, in general, the data obtained from the *in vitro* experiments on the cod seem to confirm the present findings.

However, according to Moon *et al.* (1991) and Altringham and Johnston (1990b) this decline is not due to ‘fatigue’, because if it were the effect should be greater at low cycling rates when the muscles are performing much more work. Moon *et al.* (1991) and Altringham and Johnston (1990b) use ‘fatigue’ differently from the definition used here (that of Westerblad *et al.* 1991; Vøllestad and Sejersted, 1988: fatigue is any decline in tension). Therefore, these authors attribute the decline in tension to a mechanical rather than to a metabolic mechanism, which should represent a velocity-dependent mechanical deactivation induced by shortening. Moon *et al.* (1991) found that this interpretation was further supported by the observation that an interruption of 4s after 32 cycles in a series of 64 stimulation cycles (at 5Hz) results in a (partial) recovery of the initial force level and an immediate subsequent decline when stimulation starts again. Thus, the decline in tension could represent a kind of mechanical ‘setting’, comparable to (and perhaps incorporated into) the mechanism we have described for reducing the oscillations of the head.

A mechanical velocity-dependent shortening deactivation mechanism should cause an identical decrease in tension from the first stimulation cycle to the second, if the shortening of the muscle were the same (in amplitude and time course) for the first cycles of different experiments. This is not confirmed by the comparison of the 10 and 15 pulsestrain$^{-1}$ experiments at 2.5 trains s$^{-1}$ (see Figs 1A, 3A,B). The amplitude of the first stimulation cycle (Fig. 1A) and the corresponding time–displacement curves (Figs 3A,B) are very comparable, but the decline in tension for the second cycle differs considerably. Moreover, a mechanical velocity-dependent shortening deactivation mechanism should cause a reduced effect in the experiments using the force transducer (Figs 1B, 2A), as under these conditions shortening and velocity are much smaller. Such a reduced decline in tension decline is not found in our experiments (see Figs 1B, 2A). So, taken together, these observations suggest a metabolic rather than a mechanical mechanism.

Recently, Westerblad *et al.* (1991) published a review dealing with the cellular mechanisms of fatigue due to repeated tetanic stimuli. According to these authors, changes in intracellular metabolites, in particular an increase in inorganic phosphate level and a reduction in pH, result in a reduced Ca$^{2+}$ sensitivity of the myofilaments and a reduced Ca$^{2+}$-activated cross-bridge tension, both readily leading to a decline in tension...
and slower contraction, even for the first train of a stimulation series (Le Rumeur et al. 1989). The present experiments, using the magnetoresistive sensor, reveal a reduced force output (smaller amplitude; Fig. 1A) and slower contraction (increased time to peak displacement; Fig. 3). Unfortunately, there are no studies presenting data on intracellular metabolic shifts during the first second of repeated tetanic stimulations, following the pattern applied in our strenuous activity simulations (long bursts and fast repetition). Johnson et al. (1991) present data obtained within 0.5s, but they embody repetitive trains (eight pulses at 17Hz). Moon et al. (1991) obtained biochemical measurements after eight cycles (i.e. after about 1.2s), but with a burst length of only three stimuli per cycle applied at 50Hz. Nevertheless, a significant increase in free creatine concentration was detected in both studies. As the ATP level remained constant, inorganic phosphate levels must have increased. An acidification of the muscle occurs during the repetitive stimulation (Van Waarde et al. 1990; Moon et al. 1991). Le Rumeur et al. (1989) used $^{31}$P nuclear magnetic resonance to study in vivo intramuscular chemical changes in rat gastrocnemius muscle during repetitive tetanic stimulations comparable to our excitation patterns (train length 100ms; stimulation frequency 100Hz; train frequency 0.25–4Hz). Unfortunately, chemical data are presented only after an interval of 3min. Nevertheless, at a train rate of 4Hz, tension is almost abolished after 3min and this is accompanied by a drop in phosphocreatine level and pH and a sharp increase in the level of inorganic phosphate. (Remarkably, a metabolic recovery of ATP, phosphocreatine and pH occurs when stimulation proceeds without restoration of the original force level.)

In conclusion, muscular inefficiency might constrain feeding performance in fishes when bursts of activity in the head muscles follow each other very rapidly. Apparently, burst length and the interval between successive activity periods (and probably the neuronal stimulation frequency as well) interactively influence the mechanical output of the muscles. When bursts of feeding activity are required in quick succession it can be beneficial for the fish to restrict the duration of the first activity burst as much as possible. This might explain the differences in buccal expansion observed in *Astatotilapia elegans* (Aerts, 1990). Intracellular metabolic shifts possibly cause the decrease in mechanical output of the muscles, but a mechanical velocity-dependent shortening deactivation mechanism cannot be ruled out. Future in vitro stimulation experiments linked to biochemical assays with a better time resolution could resolve this problem.

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