A functional morphological approach to the scaling of the feeding system in the African catfish, *Clarias gariepinus*

Anthony Herrel[1,*], Sam Van Wassenbergh[1], Sarah Wouters[1], Dominique Adriaens[2] and
Peter Aerts[1]

[1]*Dept Biology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium* and [2]*Institute of Zoology,
University of Ghent, K.L. Ledeganckstraat 35, B-9000, Ghent, Belgium*

*Author for correspondence (e-mail: anthony.herrel@ua.ac.be)

Summary

Effects of size are pervasive and affect nearly all aspects of the biology of animals and plants. Theoretical scaling models have been developed to predict the effects of size on the functioning of musculo-skeletal systems. Although numerous experimental studies have investigated the effects of size on the movements of skeletal elements during locomotion and feeding in vertebrates, relatively little is known about the scaling of the muscles and bones responsible for the actual movements. Here, we examine the scaling of external morphology, skeletal elements of the feeding system, and a number of cranial muscles to understand how this may affect the movements observed during suction feeding in the African catfish, *Clarias gariepinus*. The results show that neither the head nor the cranial elements themselves scale according to geometric similarity models. Relative to head size, distinct changes in the mass and configuration of the feeding structures takes place. Unexpectedly, different cranial muscles show different scaling patterns that ultimately all lead to a positive allometry of muscle cross-sectional area relative to fish head size. This suggests that (1) the scaling of the cranial elements cannot be predicted *a priori* based on the scaling of external head dimensions and (2) the scaling of the feeding system is optimised towards high force output in the larger animals. An analysis of the consequences of the observed changes in morphology with size on performance traits, including bite force and jaw closing velocity, suggests a tight link between the scaling of the feeding system and the natural diet of these fish. Whereas for smaller size classes the system is tuned towards high bite forces, for animals with cranial lengths greater than 65 mm the scaling of the feeding system appears to be dictated by the hydrodynamic constraints on suction feeding.

Key words: scaling, fish, feeding, muscle, bone, cross-sectional area, fibre length.

Introduction

The fact that effects of size are pervasive and affect nearly all aspects of the biology of animals and plants has been emphasised repeatedly in the past few decades (for example, see Schmidt-Nielsen, 1984; Brown and West, 2000). To understand the limitations and constraints imposed by physical laws upon the functioning of organisms as they grow, theoretical scaling models have been developed (Hill, 1950; McMahon, 1973; West et al., 2003). Besides models trying to explain physiological processes (e.g. West et al., 2003), scaling models aiming to predict the effects of size on the functioning of musculo-skeletal systems have been put forward (e.g. Hill, 1950; McMahon, 1975; Richard and Wainwright, 1995). The reliability and predictive ability of such models is of paramount importance as they are often used to account for size effects in comparative studies (e.g. Herrel et al., 1998, 2002) and to estimate functional properties and locomotor modes in extinct animals (e.g. Hutchinson and Garcia, 2002).

Although numerous experimental studies have attempted to test these models by investigating the effects of size on the movements of skeletal elements during locomotion and feeding (for example, see O'Reilly et al., 1993; Reilly, 1995; Richard and Wainwright, 1995; Quillin, 1999, 2000; Nauen and Shadwick, 1999, 2001; Meyers et al., 2002; Toro et al., 2003), relatively little is known about the scaling of the functional properties of muscles and bones causing the actual movements. Yet, this is important because the scaling of muscles and bones relates back to the basic premises and assumptions of many of these scaling models. For example, in many models (Hill, 1950; Richard and Wainwright, 1995) geometric similarity is assumed whereby the capacity to generate force increases to the second power (i.e. proportional to the cross-sectional area of muscles), but segmental masses increase to the third power of linear dimensions. Hill's predictions for the scaling of movements (Hill, 1950), for example, are based on the discrepancy between the available muscle force [proportional to body length $(L)^2$] and the force

needed to accelerate a given segmental mass (proportional to L^3) that arises as a consequence of geometric similarity. Consequently, any departure from geometric similarity during growth in muscle properties or segmental masses will probably have an impact on the expected scaling relationships. Because ectotherms typically display indeterminate growth, they are assumed to confer to the assumptions of geometric similarity (O'Reilly et al., 1993; Meyers et al., 2002). However, this is not always the case as subtle changes in shape may take place (e.g. Birch, 1999). Also, during the early ontogeny of fish and amphibians, distinct shape changes in the cranial system take place (e.g. Osse, 1990; Reilly, 1995; Hernandez, 2000; Hunt von Herbing, 2001). Such shape changes are important as they are often related to changes in functional properties (e.g. Birch, 1999).

Despite the large body of work dealing with the effects of size on the functioning of musculo-skeletal systems, no consensus has been reached on which scaling patterns to expect. Studies investigating suction feeding in aquatic vertebrates are exemplary in this regard as different scaling relationships were observed in nearly every single study (see Richard and Wainwright, 1995; Reilly, 1995; Deban and O'Reilly, 1996; Wainwright and Shaw, 1999; Hernandez, 2000; Robinson and Motta, 2002). Although this may be a real phenomenon, it must be clear that an understanding of the scaling of the underlying musculo-skeletal system is crucial to be able to explain the observed diversity in scaling relationships. Surprisingly, relatively little is known of how the actual muscles and muscle properties (e.g. fibre length, cross-sectional area, intrinsic shortening velocity) scale as a function of animal size during ontogeny. Despite it being crucial to our understanding of musculo-skeletal function, only a few studies have examined these issues (e.g. Richard and Wainwright, 1995; Hernandez and Motta, 1997; James et al., 1998; Robinson and Motta, 2002). Given that no or little changes in the muscular activation pattern appear to take place with increasing size (Wainwright and Richard, 1995; but see Wakeling et al., 1999) and that sarcomere length does not change with size in fish (James et al., 1998), studies investigating the scaling of muscle properties such as cross-sectional area and fibre length may be especially insightful and could potentially allow us to fine-tune existing scaling models and make more precise predictions regarding the scaling of movements.

In the present study, we investigate the scaling of external morphology, the dimensions of skeletal elements involved in suction feeding and some functional attributes of a number of cranial muscles in the catfish *Clarias gariepinus* to understand how these scaling patterns may affect the movements observed during suction feeding in animals of different size. Additionally, we use previously published biomechanical models (Herrel et al., 2002; Van Wassenbergh et al., 2005a) to investigate how the observed changes in morphology may affect aspects of feeding function (i.e. bite force and jaw closing velocity). Finally, we compare the scaling of bite force and jaw closing velocity to previously published dietary data for a size series of wild-caught animals (see Bruton, 1979).

Materials and methods

Animals

Twenty-four fishes of the African catfish, *Clarias gariepinus* (Burchell 1822), obtained from the Laboratory for Ecology and Aquaculture (Catholic University of Leuven, Belgium) and commercial breeders (Fleuren & Nooijen BV, Someren, The Netherlands), were used to study the scaling of the feeding apparatus. We chose *Clarias gariepinus* as our model organism as these animals grow to over 1.5 m in total length (Teugels, 1986) and show no distinct changes in the shape of the cranial apparatus during the non-larval growth period (i.e. starting at a standard length of 127 mm; see Adriaens and Verraes, 1998). Moreover, the animals are commercially reared and can easily be obtained at different sizes for study. The fishes used in this study ranged in size from 10.9 to 96.0 cm in total length and spanned three orders of magnitude in mass. A subset of 17 of these individuals was used to study the scaling of feeding kinematics (Van Wassenbergh et al., 2005b). Animals were killed by an overdose of MS-222 (Sigma Chemical Company, St Louis, MO, USA), preserved in a 10% aqueous formaldehyde solution, rinsed and transferred to a 70% aqueous ethanol solution.

Morphological data

External body and head dimensions were quantified using a ruler and digital callipers (Absolute Digimatic; Mitutoyo, Sakato, Japan). External measurements included head length (measured from the tip of the premaxilla to the posterior aspect of the supraoccipital spine), head width (measured both at the level of the eyes and at the level of the pectoral fins) and head depth (measured at the highest point of the head, just posterior to the eyes). The total length (the distance from the tip of the head to the tip of the caudal fin) and standard length (the distance from the tip of the head to the base of the caudal fin) of the fish were quantified by stretching out a piece of rope along the ventral midline of the fish. The rope was then measured using a ruler.

Muscles and bones of interest were exposed by dissecting away the skin, the bony elements covering the jaw adductors and some superficial musculature. Muscles were detached at their origin and insertion and stored in a 70% aqueous ethanol solution. The hypaxial muscles were cut at the level of the pectoral fins in all fish. Given that the exact portion of the hypaxial muscles involved in suction feeding is not known, we considered this approach valid as it provided us with a homologous measurement across all fish. Three fishes had been partly dissected previously and no longer had the hypaxial muscles and m. sternohyoideus (hyoid retractor). Muscles and bones were weighed using electronic scales (AND type FX 3200; ±0.01 g used for the larger fish; Mettler MT 5; ±0.0001 g used for the smaller fish).

Next, muscles were submersed in a 30% aqueous nitric acid solution for 24 h. After 24 h, the nitric acid was removed and replaced by a 50% aqueous glycerol solution. Muscle fibres were teased apart using blunt-tipped glass needles, and at least 20 fibres were selected randomly and drawn using a Wild

Heerbrugg (M3Z) binocular scope with *camera lucida*. A scale bar was included on each drawing. Drawings were scanned, and fibre lengths calculated using NIH image. Muscle volume was obtained by dividing the mass by $1050\ kg\ m^{-3}$, and physiological cross-sectional area was obtained by multiplying muscle volume by the cosine of the pennation angle (where relevant) and dividing this by the mean fibre length.

Digital pictures were taken during all stages of the dissection using a Nikon Coolpix digital camera. These pictures were used to estimate the pennation angle of the A_2A_3'. However, as pennation angles varied widely within a single muscle (see Herrel et al., 2002), an average angle was calculated and used to scale muscle physiological cross-sectional area. As pennation angle did not change with fish size, the same average angle of 30° (see also Herrel et al., 2002) was used for the A_2A_3' in all fish. Additionally, X-ray pictures were taken of the lower jaw, hyoid and cleithrum *in situ* using a Philips Optimus X-ray system coupled to a Redlake Motion Pro high-resolution camera. These pictures were used to measure the angle made by the bones of the left and right sides. The in-lever for jaw opening and in-lever for jaw closing were determined using digital callipers (Mitutoyo; ±0.01 mm) as described in Richard and Wainwright (1995). A number of linear dimensions of skeletal elements were determined using digital callipers (Mitutoyo; ±0.01 mm; see Fig. 1).

Performance estimates

Bite force was estimated for each fish using the static bite force model presented in Herrel et al. (2002). As input for the model, the three-dimensional coordinates of origin and insertion of the jaw closer muscles, the centre of rotation and a bite point located anteriorly on the lower jaw are used. Additionally, the physiological cross-sectional area of the jaw adductors is used to calculate muscle force, given a fixed conversion factor of $25\ N\ cm^{-2}$ (see Herrel et al., 2002 for further information). Simulations were run for a standard gape angle of 10°. As the orientation of the food reaction forces is unknown, a range of orientations was used as input in the model. As a conservative estimate of bite force, we use the forces calculated for food reaction forces perpendicular to the lower jaw.

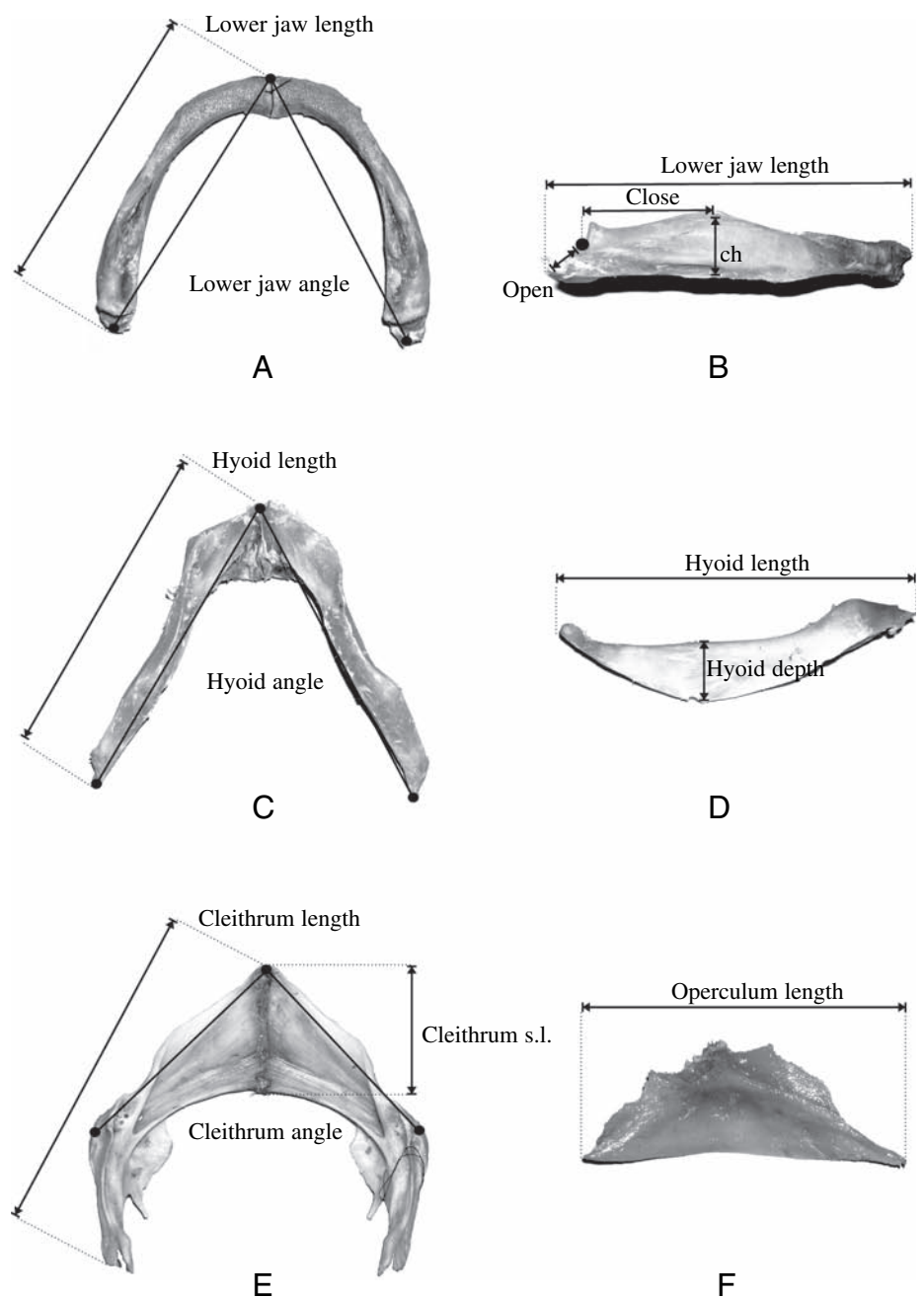Jaw closing velocity was calculated for each fish using the

Fig. 1. Linear dimensions determined on the skeletal elements of the feeding apparatus in *Clarias gariepinus*. (A) Dorsal view of the lower jaw, illustrating the measurement of lower jaw length and angle. (B) Lateral view of the lower jaw, illustrating the measurement of lower jaw length, coronoid height (ch), the in-lever for jaw closing (close) and the in-lever for jaw opening (open). (C) Ventral view of the hyoid, illustrating the measurement of hyoid length and angle. (D) Lateral view of the hyoid, illustrating the measurement of hyoid length and depth. (E) Dorsal view on the cleithrum, illustrating the measurement of cleithrum symhysis length (s.i.) and cleithrum angle. (F) Medial view of the operculum, illustrating the measurement of operculum length. Pictures are not to scale.
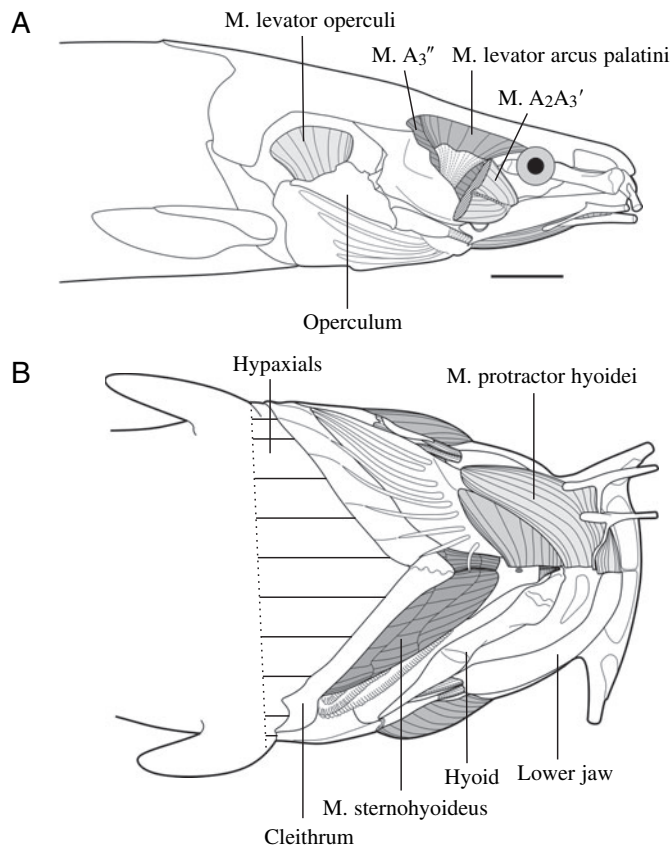
A

B

Fig. 2. (A) Lateral view of the head of a juvenile *Clarias gariepinus*. Indicated are the muscles and bony elements used in this study. Note that the m. A_2A_3' has been partially cut to expose the underlying m. levator arcus palatini and the m. A_3''. (B) Ventral view on the hyoid musculature of the same juvenile *Clarias gariepinus*. Note that the bottom of the fish has been dissected to expose the lower jaw, hyoid, cleithrum and m. sternohyoideus. Scale bar, 5 mm.

dynamic jaw closing model of Van Wassenbergh et al. (2005a), where lower jaw adduction is modelled as the rotation of a half-elliptic surface. As input for the model, the length and half-width of the lower jaw, the length of the in-lever for jaw closing, the angle between the line of action of the muscle and the in-lever, the muscle length, the physiological cross-sectional area, the pennation angle and the fibre length of the jaw closers (A_2A_3' and A_3'') are needed. For a detailed description of the model, we refer to Van Wassenbergh et al. (2005a). Simulations were run for a jaw closure starting at a gape angle of $-32°$ and ending at $-11.8°$ when the jaws typically impact on the prey (values based on actual kinematics of jaw closure in *C. gariepinus*; see Van Wassenbergh et al., 2004, 2005a). Peak mouth closing velocity was calculated for each individual and used as input for our regression analysis. To calculate mouth closing velocities, we made the following assumptions: (1) we set the activation rise time to 20 ms in the smallest individual and scaled it with cranial length to the power of 0.31 in accordance with James et al. (1998); (2) we set V_{max} (muscle lengths s^{-1}) to 10 for the smallest individual

and scaled it with cranial length to the power of -0.34 (see James et al., 1998).

Analyses

All data were $\log_{10}$ transformed before analysis. Kolmogorov–Smirnov tests were used to test the normality of the distributions. Reduced Major Axes (RMA) regressions were used to determine the slopes and intercepts of the regressions of (1) external head and body dimensions *vs* standard length; (2) dimensions of skeletal elements *vs* cranial length and (3) muscle properties *vs* cranial length. Since error levels are similar on *X*- and *Y*-axes for morphological analyses, we prefer to use RMA regressions. However, Ordinary Least Squares (OLS) regressions were used to regress performance estimates *vs* cranial length, as error levels are probably much greater for our performance estimates.

To test if the observed slopes differed from the theoretical predictions of a geometric scaling model, the 95% confidence limits were calculated. If the predicted slope fell outside the calculated limits, the slope was considered significantly different from the predictions. All analyses were performed using SPSS (v.11.5).

Results

First, a brief description of the muscles of the feeding apparatus included in the present study is given (see also Fig. 2). For a more detailed description, see Adriaens and Verraes (1996, 1997).

The adductor mandibulae complex

The adductor mandibulae complex consists of the A_2A_3' and A_3'' muscles. This muscle complex connects the suspensorium with the mandibula. Caudally, the muscles A_2A_3' and A_3'' are separated from each other by the musculus levator arcus palatini (Fig. 2A). The function of the adductor complex is mouth closure (Adriaens and Verraes, 1996; Adriaens et al., 2001).

The hyoid muscles

The m. protractor hyoidei is formed by the fusion of the posterior part of the m. intermandibularis and the anterior part of the m. interhyoideus. The protractor hyoidei connects the hyoid bars to the mandibula and the mandibular barbels (Fig. 2B). The function of the protractor hyoidei is mouth opening, elevation of the hyoid bars and displacement of the mandibular barbels (Adriaens and Verraes, 1997; Adriaens et al., 2001).

The m. sternohyoideus connects the pectoral girdle to the hyoid bars. The sternohyoideus originates at the rostral aspect of the cleithrum and inserts on the parurohyale (Fig. 2B). The m. sternohyoideus contributes to mouth opening, the abduction of the suspensoria and the depression of the hyoid (Adriaens and Verraes, 1997; Adriaens et al., 2001).

The m. levator arcus palatini connects the lateral ethmoid, the infraorbitale IV and the frontale to the suspensorium

(Fig. 2A) and is responsible for the abduction of the lateral sides of the orobranchial cavity (Adriaens and Verraes, 1997; Adriaens et al., 2001).

The m. levator operculi connects the dorsal part of the operculare to the neurocranium (Fig. 2A) and presumably causes the depression of the mandibula by the opercular four-bar system.

The hypaxial muscles attach at the posterior margin of the cleithrum (Fig. 2B) and cause the retraction of the pectoral girdle.

Scaling of external head dimensions

Whereas all head dimensions scaled with significant negative allometry (Fig. 3), the slopes of the regression of total length on standard length did not deviate from the predictions of geometric similarity (Table 1).

Scaling of skeletal elements

Linear dimensions

The linear dimensions of the skeletal elements generally scale in proportion to skull length in accordance with the predictions of the geometric similarity model (Table 2; Fig. 4). Notable exceptions are the length of the hyoid and cleithrum, which scale with small, but significant, positive allometry (Table 2; Fig. 4).

Angles

In contrast to the predictions of the geometric similarity model, all slopes differed significantly from 0. Whereas the angle of the lower jaw and hyoid display small, but significant, positive allometry, the angle of the cleithrum decreased marginally with overall fish size (Table 2; Fig. 4).

Masses

The masses of all skeletal elements examined increased with a slope significantly greater than the predicted slope of 3. Thus, in large fish, the masses of the segments that need to be moved are relatively larger (Table 2; Fig. 4).

Scaling of muscles

When examining the scaling of muscle properties, it becomes apparent that different scaling relationships can be recognised for different muscles (see Table 3).

Muscle masses

Whereas the increase in the mass of the jaw adductors with cranial size did not deviate from isometry, the mass of all other muscles examined scaled with significant positive allometry (Table 3; Figs 5, 6).

Fibre lengths

The length of the muscle fibres of the hypaxial muscles, the m. levator operculi, the m. levator arcus palatini and the m. sternohyoideus increased with a slope not significantly different from 1 relative to fish cranial length. For all other
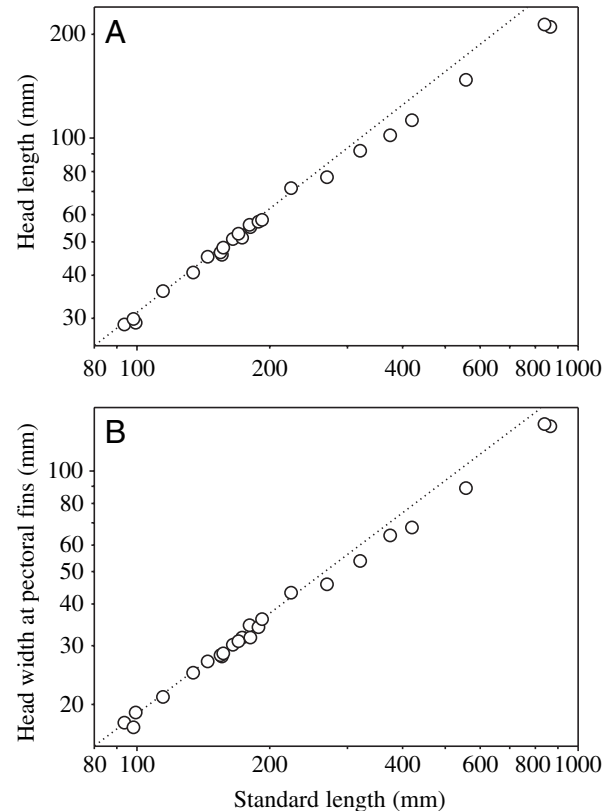

Fig. 3. Graphical representation of the scaling of external head dimensions *versus* fish standard length in *C. gariepinus*. Both head length (A) and head width (B) scale with significant negative allometry (see Table 1). The broken line represents the expected slope of 1 under a model of geometric similarity.

muscles examined, muscle fibre lengths increased with significant negative allometry (Table 3; Figs 5, 6).

Physiological cross-sectional areas

Because of the scaling of muscle mass and fibre lengths, the physiological cross-sectional areas in all muscles increased with significant positive allometry with slopes being close to or larger than 3 (compare to the predicted slope of 2; see Table 3; Figs 5, 6). Thus, larger fish have relatively stronger muscles than smaller fish.

Performance

Our analyses show that bite force, as estimated by the static bite force model (see Herrel et al., 2002), increases with cranial length with a slope significantly steeper than the expected slope of 2 (R^2=0.96; slope=3.0; intercept=–4.54; see Fig. 7A). However, a closer inspection of the relationship of bite force to cranial length suggests the presence of a breakpoint at a cranial length of ~65 mm (Fig. 7B). For fish with cranial lengths greater than 65 mm only, bite force increases with a slope of 2.58 (R^2=0.96; intercept=–3.72). Below the breakpoint, bite force increases more rapidly with fish cranial

Table 1. *Regressions of external cranial dimensions against fish standard length*

Variable	R^2	Intercept	Slope	Confidence limits	
Head length (mm)	0.996	−0.30	**0.90**	0.87	0.93
Head width (eyes) (mm)	0.992	−0.53	**0.87**	0.83	0.91
Head width (fins) (mm)	0.996	−0.57	**0.92**	0.91	0.94
Head depth (mm)	0.968	−0.80	**0.95**	0.89	0.99
Total length (mm)	0.998	0.09	0.98	0.95	1.01

Slopes differing significantly from predictions of geometric similarity are indicated in bold.

Table 2. *Regressions of the dimensions of the most important skeletal elements involved in feeding against fish cranial length*

Variable	R^2	Intercept	Slope	Confidence limits	
Lower jaw mass (g)	0.994	−6.23	**3.25**	3.15	3.35
Coronoid height (mm)	0.984	−1.29	1.04	0.97	1.11
Lower jaw length (mm)	0.992	−0.44	1.02	0.97	1.07
Lower jaw angle (deg.)	0.079	1.43	**0.14**	0.10	0.19
Closing in-lever (mm)	0.970	−0.79	0.99	0.91	1.07
Opening in-lever (mm)	0.972	−1.08	0.91	0.83	1.00
Hyoid mass (g)	0.996	−6.31	**3.31**	3.24	3.37
Hyoid depth (mm)	0.984	−1.19	1.03	0.96	1.10
Hyoid length (mm)	0.996	−0.49	**1.07**	1.04	1.10
Hyoid angle (deg.)	0.093	1.28	**0.21**	0.14	0.29
Cleithrum mass (g)	0.993	−5.81	**3.22**	3.12	3.32
Cleithrum symphysis length (mm)	0.994	−0.63	1.02	0.98	1.06
Cleithrum length (mm)	0.998	−0.35	**1.07**	1.05	1.09
Cleithrum angle (deg.)	0.034	2.05	**−0.08**	−0.11	−0.04
Operculum mass (g)	0.984	−6.92	**3.21**	3.05	3.36
Operculum length (mm)	0.990	−0.63	0.99	0.93	1.04

Slopes differing significantly from predictions of geometric similarity are indicated in bold.

size with a slope of 3.92 (R^2=0.92; intercept=−6.05; see Fig. 7B).

The output of the dynamic jaw closing model (see Van Wassenbergh et al., 2005a) indicates that, across all fish, angular jaw closing velocity decreases significantly with fish cranial length (R^2=0.79; slope=−0.66; intercept=3.85; see Fig. 7C). When data are analysed separately for fish smaller than 65 mm, no relationship of mouth closing velocity with fish cranial length is apparent (P=0.15; see Fig. 7D). However, for fish larger than 65 mm cranial length, mouth closing velocity decreases significantly with increasing cranial length (R^2=0.77; slope=−0.79; intercept=4.10; see Fig. 7D).

Discussion

Our results show that the growth of both the head and the individual components of the feeding apparatus deviate significantly from expectations for geometrically growing systems (see Tables 1, 2). Although shape changes and allometries of cranial elements are common during early ontogeny and metamorphosis (Cook, 1996; Hernandez, 2000), our results are unexpected given that, at first sight, no substantial shape changes of the cranial system occur during post-metamorphic growth in *C. gariepinus* (Adriaens and Verraes, 1998). In contrast to our results, in largemouth bass (Richard and Wainwright, 1995) and nurse sharks (Robinson and Motta, 2002), the feeding system itself scaled generally isometrically with fish size. The only other study that found allometries in the growth of cranial muscles in fish was that of Hernandez and Motta (1997). There, muscle cross-sectional areas and masses increased with slopes significantly greater than 2 and 3, respectively.

The shape of the hyoid, the lower jaw and cleithrum also changed with increasing size for the fish included in our study. Specifically, whereas the angle between the two hyoid bars and lower jaw elements increased with increasing fish size, the angle between the two halves of the cleithrum decreased. Given that the starting position of the hyoid is crucial in determining suction performance in cichlid fish (see De Visser and Barel, 1996, 1998), this suggests that smaller fish may be optimising their feeding system towards suction feeding (i.e. more adducted, and thus more potential for expansion). However, in contrast to cichlids, a large part of the expansion of the buccal cavity in *C. gariepinus* is due to ventral rotation of the hyoid rather than to the abduction of the hyoid bars (Van Wassenbergh et al., 2004). Moreover, as the angle between the

Fig. 4. Graphical representation of the scaling of skeletal dimensions of the cleithrum in *Clarias gariepinus*. The mass (A) and length (B) of the cleithrum both scale with significant positive allometry (slope different from 3 and 1, respectively; see Table 2). The angle between the two halves of the cleithrum (C) scales with marginal but significant negative allometry (Table 2). The broken lines represent the expected slope under a model of geometric similarity (mass=3, length=1, angle=0). (D) A series of representative cleithra, illustrating the difference in shape in different individuals. The different filled symbols on the graphs correspond to the respective cleithra illustrated. Scale bar, 50 mm.

two halves of the lower jaw also increases in larger fish, the relatively small change in hyoid angle with size may just be part of an overall change in shape of the cranial system in *C. gariepinus*. The change in angle of the cleithrum, although significant (Table 2), is marginal and may reflect individual differences rather than scale effects. Note, for instance, the distinct difference in shape of the cleithra depicted in Fig. 4, with one of the intermediately sized animals having the smallest angle.

Unexpectedly, the masses of the skeletal elements examined increased with positive allometry relative to fish cranial length. As there is no *a priori* reason to expect relative increases in segmental masses, this suggests that the bones get more robust to be able to cope with the increased muscular forces (see also Hernandez and Motta, 1997). Histological sections of, for example, the hyoid bars show an increasing formation of bony apolamellae, perpendicular to the outer surface during ontogeny, thus also increasing hyoid rigidity (D.A., personal observation).

In contrast to data for other aquatic vertebrates (Richard and Wainwright, 1995; Hernandez and Motta, 1997; Robinson and Motta, 2002), the scaling of muscle masses in *C. gariepinus* differed for different cranial muscles. Whereas the increase of the mass of the jaw closers did not deviate from isometry, the masses of all other muscles increased with significant positive allometry. This is not unique to the fish studied here, as different scaling patterns were also observed for different muscles in toads (Birch, 1999). Apparently, in aquatic vertebrates, no uniform scaling patterns of jaw muscle mass are observed, as isometry (Richard and Wainwright, 1995; Robinson and Motta, 2002), positive allometry (Hernandez and Motta, 1997) and mixed scaling patterns (present study) have all been documented. Thus, predictions of the scaling of
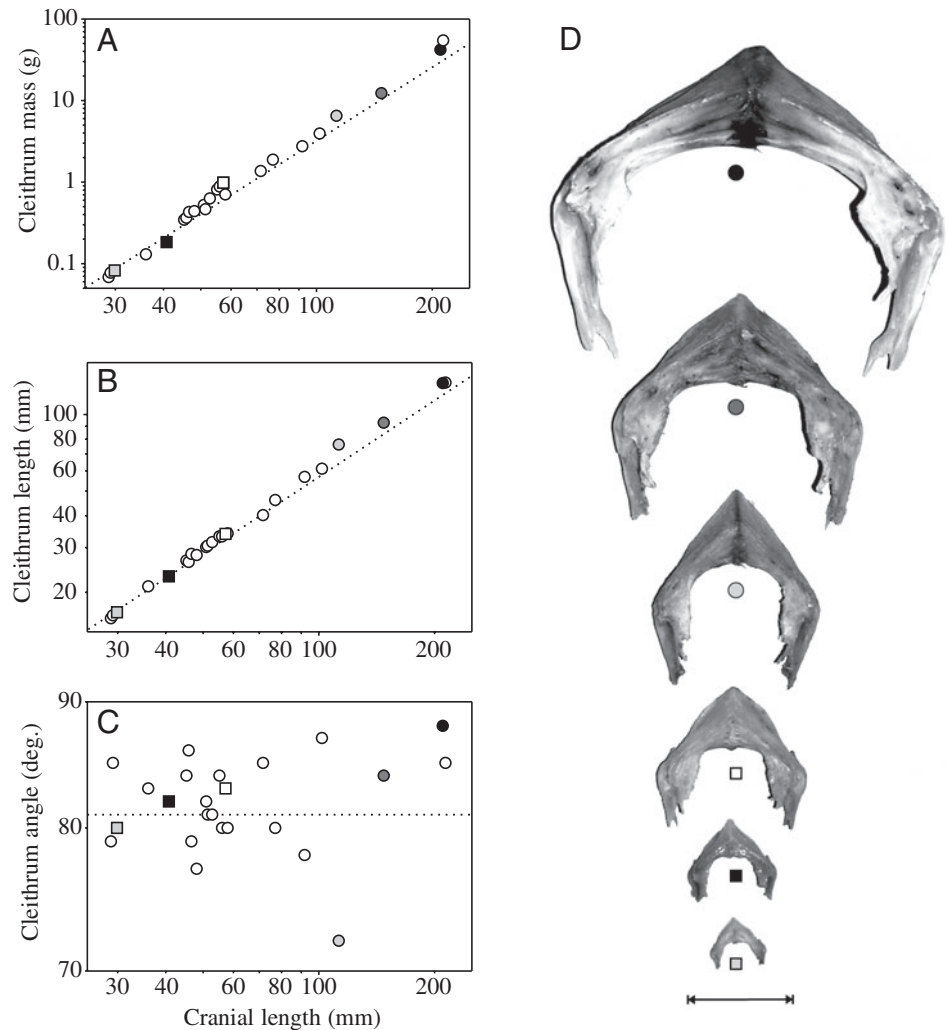
movements based on general models seem at first sight problematic given the already large amount of variation observed in the scaling of the underlying muscle mass.

Similarly to muscle masses, muscle fibre lengths also showed mixed scaling patterns, with both negative allometric scaling (jaw closers, m. protractor hyoidei) and isometry (other muscles) being present in *C. gariepinus* (Table 3). Although we are unaware of other studies examining the scaling of muscle fibre lengths, sarcomere lengths do not appear to change with body size in fish (James et al., 1998). If sarcomere lengths remain constant, then this implies that larger fish have relatively fewer sarcomeres in series in some of the important compressive (m. protractor hyoidei, jaw adductors) muscles. This in turn suggests that the velocity of jaw closure would increase with fish size but get *relatively* slower in the largest fish (i.e. slope less than one). However, the estimates of angular jaw closing velocity based on the dynamic jaw closing model suggest that velocity actually decreases with size (see Fig. 7). Moreover, kinematic data on linear jaw closing velocities show that linear velocities are independent of fish size (Van Wassenbergh et al., 2005b). These observations thus suggest that the actual velocity of movement cannot be extrapolated from changes in muscle fibre lengths.

Table 3. *Regressions of masses, fibre lengths and physiological cross-sections of the most important feeding muscles against fish cranial length*

Variable	R^2	Intercept	Slope	Confidence limits	
A$_2$A$_3'$ mass (g)	0.966	−6.20	3.31	2.97	3.64
A$_2$A$_3'$ fibre length (mm)	0.148	−0.19	**0.53**	0.31	0.75
A$_2$A$_3'$ phys. XS (cm^2)	0.950	−5.63	**3.11**	2.70	3.52
A$_3''$ mass (g)	0.948	−6.41	3.40	2.98	3.82
A$_3''$ fibre length (mm)	0.523	−0.20	**0.55**	0.39	0.72
A$_3''$ phys. XS (cm^2)	0.946	−5.57	**3.04**	2.68	3.40
Hypaxials mass (g)	0.977	−5.93	**3.40**	3.17	3.64
Hypaxials fibre length (mm)	0.088	−0.52	0.73	0.34	1.11
Hypaxials phys. XS (cm^2)	0.972	−5.47	**3.26**	3.08	3.44
LAP mass (g)	0.960	−8.29	**3.72**	3.30	4.14
LAP fibre length (mm)	0.201	−0.95	0.75	0.49	1.00
LAP phys. XS (cm^2)	0.957	−6.99	**3.33**	2.97	3.69
LO mass (g)	0.940	−8.20	**3.68**	3.39	3.97
LO fibre length (mm)	0.594	−0.82	0.77	0.40	1.14
LO phys. XS (cm^2)	0.848	−6.83	**3.15**	2.52	3.77
PH mass (g)	0.976	−6.17	**3.36**	3.14	3.58
PH fibre length (mm)	0.744	−0.47	**0.71**	0.55	0.87
PH phys. XS (cm^2)	0.955	−5.03	**2.82**	2.41	3.23
SH mass (g)	0.975	−5.93	**3.33**	3.08	3.59
SH fibre length (mm)	0.100	−0.64	0.75	0.29	1.20
SH phys. XS (cm^2)	0.969	−5.31	**3.16**	2.91	3.40

Slopes differing significantly from predictions of geometric similarity are indicated in bold. LAP, m. levator arcus palatini; LO, m. levator operculi; PH, m. protractor hyoidei; phys. XS, physiological cross section; SH, m. sternohyoideus.

The muscle cross-sectional area, however, increased with significant positive allometry in all muscles examined, with slopes roughly equal to three (Table 3). This is due to the positive allometry or isometry in muscle mass combined with the negative allometry or isometry in fibre length. The substantial positive allometry observed for the muscle cross-sectional area, in turn suggests that the jaw system in large fish is geared towards high force output. Indeed, our bite force estimates indicate that static bite forces increase much faster with fish cranial size than expected for simple geometrically growing systems (Fig. 7A,B). The increase in physiological cross-sectional area of the muscles may theoretically also increase the velocity of the feeding event (see Van Wassenbergh et al., 2005a) although this is not fully supported by the results of the dynamic jaw closing model (Fig. 7C,D). The positive allometries of physiological cross-sectional areas observed for *C. gariepinus* do not appear to be a general feature of the scaling in fish cranial systems. Despite the fact that a positive allometry of cross-sectional area (of the jaw adductors) was observed in the study by Hernandez and Motta (1997), in other suction feeders such as the nurse shark, muscle cross-sectional area increased isometrically with increases in linear dimensions (slope=2; see Robinson and Motta, 2002). Thus, no general trend appears to be present despite the similarity in feeding mechanism (all suction feeders) of these animals.

Effects of changes in morphology on performance

Our model calculations suggest that the observed changes in morphology have a significant effect on two potentially important performance traits: bite force and jaw closing velocity. Bite forces increase rapidly with fish cranial size, especially in the smallest individuals (Fig. 7B). Interestingly enough, published diet accounts (see Bruton, 1979) suggest that, for smaller fish (roughly up to a total length of 300 mm), crustaceans, insects and larval fish make up the bulk of the diet. At a size of 300 mm, the catfish switches to a diet composed primarily of adult fish (Bruton, 1979). The size at which this dietary switch occurs (300 mm total length; 75 mm cranial length) corresponds closely to the size at which the break point appears in the relationship between cranial length and bite force (±65 mm cranial length). This suggests that the demand for high bite forces in smaller fish is tightly linked to their diet of predominantly hard prey (i.e. crustaceans). At the point where the *C. gariepinus* switches to evasive prey that are swallowed whole, the increase in bite force with cranial length decreases dramatically (see Fig. 7B). Thus, the growth of the cranial system appears geared towards high bite forces for smaller life history stages and is reflected in the significant positive allometry of the physiological cross-sectional area of the jaw closer muscles (Fig. 5).

Given the switch towards more evasive prey in the diet at a size of ~75 mm cranial length, we could expect the feeding
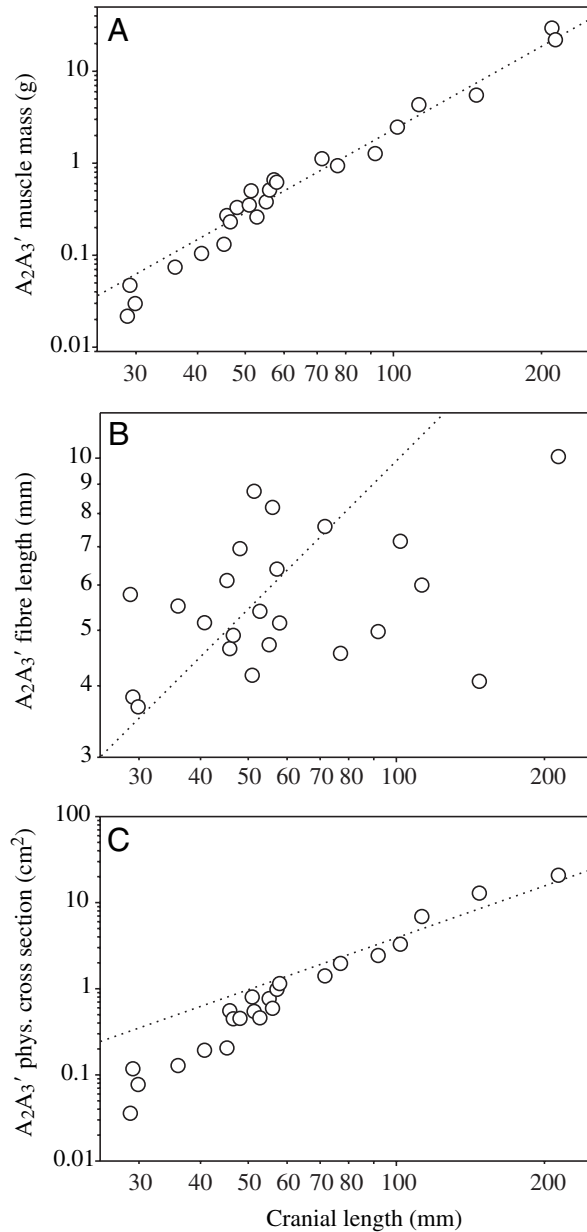
Fig. 5. Graphical representation of the scaling of a jaw closing muscle in *Clarias gariepinus*. Although the mass of the muscle (A) scales with the expected slope of 3, the fibre length (B) scales with significant negative allometry (Table 3). Thus, the physiological cross-section (C) scales with significant positive allometry. The broken lines represent the expected slope under a model of geometric similarity (mass=3, length=1, cross-section=2).
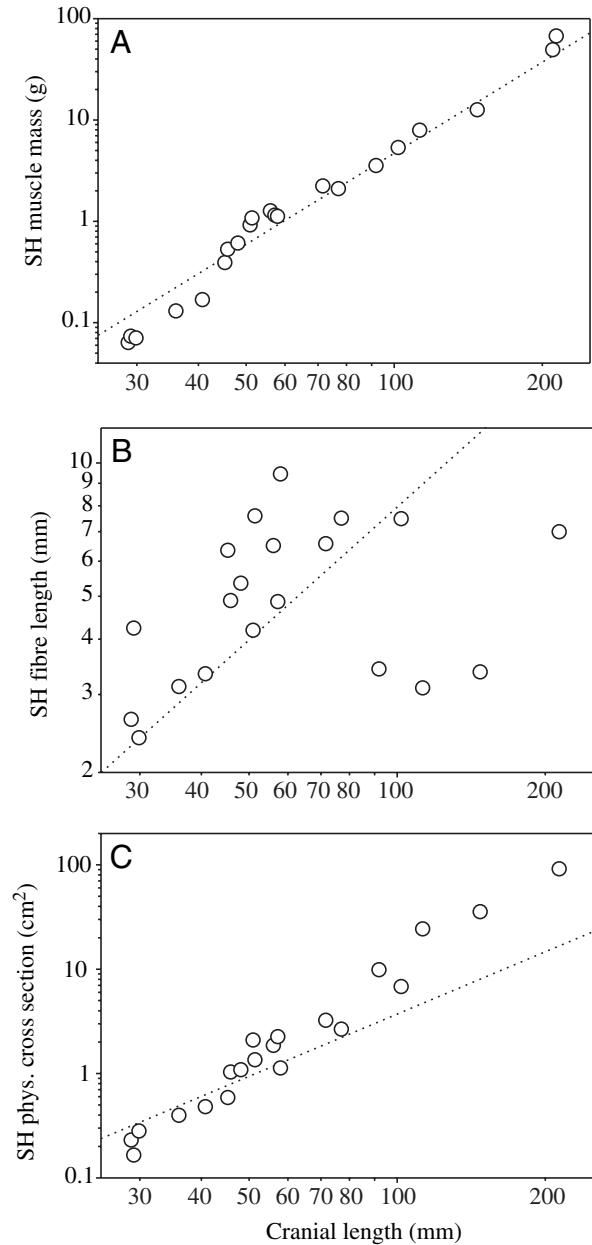
Fig. 6. Graphical representation of the scaling of an expansive muscle in *Clarias gariepinus*. The mass (A) scales with significant positive allometry. Fibre lengths (B) increase with fish cranial length in accordance with the predictions of geometric similarity (slope not different from 1; see Table 3). The physiological cross-sectional area (C) also increases with significant positive allometry. The broken lines represent the expected slope under a model of geometric similarity (mass=3, length=1, cross-section=2). SH = m. sternohyoideus.

system to be geared towards high jaw closing velocities. This appears, however, not to be the case, as suggested by our model output. Rather, angular jaw closing velocity decreases with cranial size across all fish. When analyzing data for small and large fish separately, it becomes clear that jaw closing velocity is independent of size in the smallest individuals but decreases rapidly (slope of –0.78) for fish larger than 65 mm cranial length. At first sight, this appears rather paradoxical as it is the larger fish that appear to be specializing on evasive prey (and

for which high jaw closing velocities would probably be relevant). By contrast, the smaller fish seem to be exploiting the cranial system in such a way that they benefit from a rapid increase in bite force without a concomitant drop in jaw closing velocity. This should not be surprising as previous model calculations have already suggested that a hypertrophy of the jaw adductors has a positive effect on jaw closing velocity as well as static bite force (Van Wassenbergh et al., 2005a).
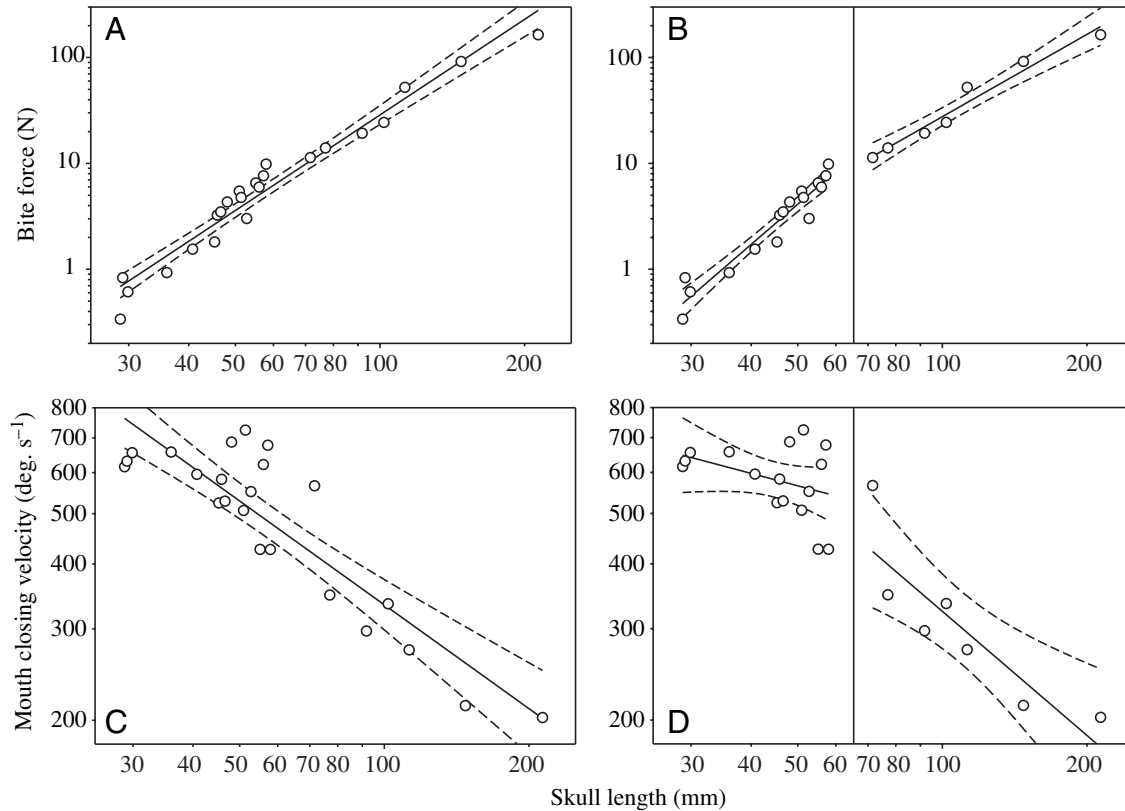
Fig. 7. Graphical representation of the scaling of bite force (A,B) and jaw closing velocity (C,D) *versus* cranial length in *C. gariepinus*. Bite force increases with cranial length with a slope of 3 (A), and jaw closing velocity decreases with cranial length with a slope of –0.66 (C). Upon detailed inspection of the graphs, a break point appears to be present at a cranial length of ~65 mm (B,D). Analysis of the scaling patterns for the two groups separately indicates a rapid increase in bite force (B; slope=3.92) but no change in jaw closing velocity (note that the trend line in D to the left of the break point is not significantly different from 0) for fish under 65 mm cranial length. Above 65 mm cranial length, bite force increases with a slope of 2.58 and jaw closing velocity decreases with a slope of –0.79.

Although our data on jaw closing velocity might initially suggest some constraint on the further development of the cranial musculature, preventing the larger fish from optimising their feeding system towards improved suction performance, this is not necessarily so. Previous models have demonstrated that, when *C. gariepinus* becomes larger, the expansive phase of suction is prolonged, resulting in a longer sustained flow of water into the mouth (Van Wassenbergh et al., in press). This flow also reaches further in front of the mouth, roughly proportional to head size. Moreover, forward dynamic simulations indicate that the absolute distance from which a given prey can be sucked into the mouth, as well as the maximum potential prey diameter, increases substantially with increasing head size (Van Wassenbergh et al., in press) despite the decrease in angular mouth closing velocity, as estimated here. Consequently, the range of potential prey that can be captured through suction feeding continues to increase during growth in *C. gariepinus*, despite the observed decrease in jaw closing velocity.

Can suction-feeding kinematics be predicted based on scaling of morphology?

Previous models, such as the ones proposed by Hill (1950) and Richard and Wainwright (1995), appear inadequate in explaining the movements of structures, as the scaling of the underlying morphology and function often deviates from the assumptions of geometric similarity. However, if the scaling of morphology is known, as in the present case, can the scaling of movements be predicted?

If the inertia of the system dominates the dynamics, acceleration capacity, at least, should be predictable based on knowledge of the scaling of muscle cross-sectional area and segmental mass (O'Reilly et al., 1993). In geometrically similar systems, this would imply a decrease in the capacity to accelerate a load because of the discrepancy of the scaling of mass *vs* cross-sectional area (O'Reilly et al., 1993). In *C. gariepinus*, the increase in cross-sectional area of the muscles is roughly proportional to the increase in segmental mass, and thus the acceleration of the cranial elements should be identical across fishes of different sizes. Whereas in terrestrial systems (e.g. Meyers et al., 2002) accelerations can indeed be predicted if the scaling of forces is known, in the present case this does not hold as peak acceleration decreases linearly with fish head size (Van Wassenbergh et al., 2005b). Additionally, our data for the scaling of fibre length in *C. gariepinus* suggest that mouth opening velocity should increase isometrically relative to cranial length. Thus, the time needed to execute a

proportionally similar displacement should be independent of cranial length (e.g. mouth opening). Even though the magnitude of linear displacements is indeed proportional to size, the time needed to execute a given displacement increases with positive allometry (see Van Wassenbergh et al., 2005b). Thus, our predictive ability appears limited even when the scaling of morphology and functional properties is known.

However, it can be questioned whether inertial forces dominate in aquatic systems and especially during rapid and explosive events such as suction feeding. Previous workers (Muller et al., 1982; Aerts et al., 1987), for instance, concluded that the hydrodynamic power requirements, rather than inertial forces, are the predominant constraint acting on the feeding system during the generation of suction. Indeed, also for fish fast-starts, it was deduced that body-mass-specific hydrodynamic power requirements increase with fish size and that, consequently, size-dependent increases in muscle power output are needed and observed (Wakeling et al., 1999). If hydrodynamic power requirements are indeed driving the scaling of the muscle properties during explosive events such as suction feeding then the predictive ability based on morphology alone may be intrinsically limited. This suggests that more complex mechanical models that take into account the physical constraints of generating negative pressures are needed to better understand the scaling of power requirements during suction feeding (Van Wassenbergh et al., 2005b). Moreover, such models may allow us to make more accurate predictions of the scaling of feeding kinematics in aquatic vertebrates.

Conclusions

The data on the scaling of the feeding system in the African catfish, *Clarias gariepinus*, show that neither the head nor the cranial elements themselves scale according to geometric similarity models. Moreover, relative to head size, distinct changes in the mass and configuration of the feeding structures (muscles and bones) take place that affect feeding function. Unexpectedly, different cranial muscles show different scaling patterns, which ultimately all lead to a positive allometry of muscle cross-sectional area relative to fish head size. Our data thus show that (1) the scaling of the cranial elements cannot be predicted *a priori*, even when the scaling of external head dimensions is known, (2) the scaling patterns may differ for different muscles within a single functional system and (3) the feeding system is optimised towards high bite force in the smaller size classes, which is correlated to the natural diet of these animals. The scaling of the feeding system in the largest fish (over 65 mm cranial length) appears to be dictated by the need to produce high power outputs while generating negative pressure in the buccal cavity during suction feeding.

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