

Prey processing in the Siamese fighting fish (*Betta splendens*)

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Abstract We studied prey processing in the Siamese fighting fish (*Betta splendens*), involving slow, easily observed head-bobbing movements, which were compared with prey processing in other aquatic feeding vertebrates. We hypothesized that head-bobbing is a unique prey-processing behaviour, which alternatively could be structurally and functionally analogous with raking in basal teleosts, or with pharyngognathy in neoteleosts. Modulation of head-bobbing was elicited by prey with different motility and toughness. Head-bobbing involved sustained mouth occlusion and pronounced cranial elevation, similar to raking. However, the hyoid and pectoral girdle were protracted, and not retracted as in both raking and pharyngognathy. High-speed videofluoroscopy of hyoid movements confirmed that head-bobbing differs from other known aquatic prey-processing behaviours. Nevertheless, head-bobbing and other prey-processing behaviours converge on a recurrent functional theme in the trophic ecology of aquatic feeding vertebrates; the use of intraoral and

oropharyngeal dentition surfaces to immobilize, reduce and process relatively large, tough or motile prey. Prey processing outside the pharyngeal region has not been described for neoteleosts previously, but morphological evidence suggests that relatives of *Betta* might use similar processing behaviours. Thus, our results suggest that pharyngognathy did not out-compete ancestral prey-processing mechanisms completely during the evolution of neoteleosts.

Keywords Convergence · Kinematics · Oropharyngeal · Videofluoroscopy · Nutritional physiology

Abbreviations

am	Adductor mandibulae muscle
ba _r	Branchial arch remainders
bh	Basihyal
bh _h	Horizontal movement of basihyal
bh _v	Vertical movement of basihyal
bo	Body
cb	Ceratobranchial
cbl	Cleithrobranchial ligament
cv	Craniovertebral joint
gp	Mandibular jaw gape expansion
j	Jaw joint
jp	Jaw protrusion
l	Lower jaw
mnc	Magnitude of cranial elevation
mpg	Magnitude of pectoral girdle protraction
n	Neurocranium
nc	Neurocranial elevation
p	Pectoral girdle
pb	Pharyngobranchial
pg	Pectoral girdle movement
ph	Protractor hyoideus muscle

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ps	Parasphenoid
r	Rostrum
sus	Suspensorium
t_0	Time-zero (cranial elevation onset)
TL	Total length
u	Upper jaw
v	Vomer
vpg	Velocity of pectoral girdle protraction
vnc	Velocity of neurocranial elevation
μ CT	Micro-computed tomography

Introduction

Prey processing is critical for immobilizing prey, preventing its escape, and reducing it prior to digestion. A wide variety of prey-processing behaviours are governed by three different feeding mechanisms in aquatic feeding vertebrates (Table 1): the anterior-most mandibular or oral jaw apparatus governs chewing, which likely is a generalized gnathostome trait (Lauder 1981; Gintof et al. 2010; Konow et al. 2011). Some basal teleosts, including salmonids (Salmoniformes) and bony-tongues (Osteoglossomorpha) have a tongue-bite apparatus, used in raking prey-processing behaviours (Sanford and Lauder 1989, 1990; Konow and Sanford 2008a, b; Konow et al. 2008). Most neoteleosts have a pharyngeal jaw apparatus used for pharyngognath, which includes crushing, grinding and winnowing prey processing (Liem 1973; Aerts et al. 1986; Claes and De Vree 1991; Drucker and Jensen 1991; Grubich 2000, 2003; Wainwright 1989a, 2006; Gidmark et al. 2013). Yet, it is unknown if prey processing occurs in neoteleosts with a different or reduced oropharyngeal dentition, relative to a pharyngeal jaw apparatus.

A popular model Neoteleost is the Siamese fighting fish, *Betta splendens*, [Anabantoidei (Ruber et al. 2006)]. *Betta* has a well-described bony anatomy, is widely used in behavioural biology (Mabee and Trendler 1996), and is one of the most frequently traded aquarium species (Balboa 2008). *Betta* displays a peculiar head-bobbing behaviour after ingesting relatively large, hard and brittle prey, such as commercially available pellets. This behaviour is not displayed during feeding on more delicate and malleable flake food. The slow and obvious movements (see Online Resource 1) suggest that head-bobbing is observed regularly by *Betta* owners feeding pellets to their pet. Still, head-bobbing has not been described or studied in a comparative functional context.

Descriptions of the trophic ecology of *Betta* mainly come from heavily modified rice paddock habitats, suggesting that *Betta* feeds on small, soft-bodied larval insects (Taki 1978; Berra 1981; Rainboth 1996). The idea of a

Table 1 Morphological, kinematics and muscle-activity pattern diagnostics of prey-processing systems in fishes; indicating structural and functional similarities between the intraoral jaw mechanism in *Betta*, and jaw mechanisms seen in other fish taxa

Behaviour	Jaw system	Morphology			Kinematics			Muscle activity	References
		Ventral jaw	Dorsal jaw	Other	Cranial	Pectoral girdle	Mandible		
Head-bobbing in <i>Betta</i>	Paraspheno-pharyngeal	5th ceratobranchial ^a	1st pharyngo-branchial ^a parasphenoid	Edentulous basihyal	Elevation	Protracted	Occluded	Early am, ph ^b	Liem (1963) ^a ; Gosline (1985); Liem and Greenwood (1981) ^b
Raking	Tongue-bite apparatus	Basihyal	Vomer, parasphenoid	CBL with grill-arch processes ^c	Elevation	Retracted	Occluded	Early am, ph	Sanford and Lauder (1989; 1990) ^c ; Sanford (2001a), Sanford (2001b); Hilton (2001) ^c ; Camp et al. (2009); Konow and Sanford (2008a, b); Konow et al. (2008)
Chewing	Mandibular jaws	Mandible	Premaxilla maxilla		Level, slight elevation	Unaltered	Cyclic abduction-adduction	Late am, ph	Lauder (1981); Gintof et al. (2010)
Pharyngeal processing	Pharyngeal jaw apparatus	5th ceratobranchial	3rd pharyngobranchial	Fused gill-arches ^{d,*}	Level ^e , slight (<7°) elevation ^f	Unaltered ^{g,h} retracted ^{e,f}	Depressed passive ^h	N/A ⁱ	Liem (1973) ^e ; Wainwright (1989b); Sibbing (1982) ^f ; Aerts et al. (1986) ^g ; Claes and De Vree (1991) ^h ; Wainwright (2006) ^d

cbl cleithrobranchial ligament; am adductor mandibulae; ph protractor hyoideus

^{a-b} Specific works reporting the state of a given character

ⁱ The pharyngeal jaw apparatus has distinct musculature relative to more anteriorly placed jaw apparatus, and thus an independent muscle-activity pattern, see Wainwright (2006)

nominal micro-invertebrate feeder using prey processing is puzzling. It suggests that head-bobbing may be a display instead of a feeding behaviour, or that the lack of dietary data from undisturbed habitats has led to incorrect trophic niche description for *Betta*.

Functional analyses of morphology and kinematics may help unravel behavioural, ecological and evolutionary links (Wainwright 1991; Losey 1993). Therefore, we aimed at comparing existing morphological data with high-speed video and X-ray motion analyses of feeding apparatus kinematics. Our aim was to determine if *Betta* is capable of capturing and processing large, tough and motile prey.

Behavioural modulation, involving a predictable change in behavioural pattern as a response to changes in biophysical stimuli, has been described for numerous feeding behaviours. One consensus from feeding studies is that behavioural modulation reflects a heightened ecological versatility compared with organisms with a stereotyped, or invariable behavioural response (Liem and Osse 1975; Frost and Sanford 1999). For example, differences in prey hardness or motility lead to modulation of raking prey processing in some basal teleosts, but not in others (Frost and Sanford 1999; Konow et al. 2008). Pharyngognathia is also modulated in some neoteleosts but not in others (Wainwright 1989b; Grubich 2000). We hypothesized that the apparent selective use of the head-bobbing behaviour in *Betta* reflects behavioural modulation.

In some neoteleosts, the intra-oral and intra-pharyngeal distribution of dentition closely resembles a tongue-bite apparatus. This is particularly true in anabantoid and nandid relatives of *Betta* (Liem 1963, 1970; Liem and Greenwood 1981; Lauder and Liem 1983). Prominent dentition on mouth-roofing bones and directly opposing dentition on hyobranchial bones (Fig. 1) closely match the primary diagnostics of a tongue-bite apparatus (Liem 1963; Gosline 1985; Konow and Sanford 2008a, b). However, this dentition pattern has been interpreted as a generalized bony fish trait (Hilton 2001), and alternatively hypothesized to be a novel pharyngeal bite (Gosline 1985). The latter hypothesis of Gosline (1985) would be falsified if structural homologies and functional analogies exist between the intraoral and pharyngeal dentition in *Betta* and the generalized prey-processing mechanism in neoteleosts; the pharyngeal jaw apparatus (Grubich 2003; Wainwright 2006; Wainwright et al. 2012).

In the present study, we therefore aimed to test several hypotheses. Given its nominal micro-invertivore status, we hypothesized that *Betta* would not ingest relatively large, tough and motile prey. To test this hypothesis, we offered guppy fry during feeding trials. If this null hypothesis was falsified, we would test the hypotheses that head-bobbing is modulated when prey with different behavioural and mechanical properties is being processed. To test the

modulation hypothesis, we fed *Betta* individuals with both blackworms and guppy fry, expecting that very different demands would be imposed on the prey-processing behaviour by the contrasting toughness and motility of these prey types.

Finally, we compared prey-processing morphology and kinematics in *Betta* with available data from raking and pharyngognath taxa, using the diagnostics of a tongue-bite apparatus developed earlier (Konow and Sanford 2008a, b) and diagnostics of a pharyngeal jaw apparatus gathered from the literature. We tested the hypotheses that oropharyngeal dentition morphology and prey-processing kinematics in *Betta* were homologous with the tongue-bite apparatus and raking, or with the pharyngeal jaw apparatus and pharyngognathia. Acceptance of the first morphology-kinematics hypothesis would mean that raking prey processing has evolved convergently in a derived neoteleost clade, in addition to the two basal teleost clades. Alternatively, acceptance of the second morphology-kinematics hypothesis would mean that *Betta* processes prey like other neoteleosts. The alternative hypothesis was that prey processing in *Betta* relies on a novel combination of morphology and kinematics.

Methods

Individuals ($N = 5$) of *Betta splendens* (Regan, 1910) measuring 8.0 ± 0.2 mm total length (TL) were obtained commercially, housed individually, and fed a varied diet during acclimation. During experiments, each animal was placed in a narrow arena between the aquarium front window and a millimetre square-grid background (Fig. 2). The grid was used for scaling purposes and to ensure that the body axis of the animal was perpendicular to the camera lens axis during filming. High-speed video was recorded at 250 frames per second using a Photron Fastcam-X 1280 PCI camera with a shutter speed of 1/250 ms, yielding 12 s video sequences that constituted our trials. Two 600 W tungsten floodlights provided illumination. X-ray high-speed video data were obtained from similar-sized individuals ($N = 5$; TL = 8.1 ± 0.2 mm) that were anaesthetized (20 ppm alcoholic Eugenol) and implanted with 0.3 mm radio-opaque markers (Fig. 2) (Aerts et al. 1986). The markers provided reliable quantification of the hyoid and cranial motion via motion analyses (Aerts et al. 1986). X-ray videos were generated using a Philips Optimus M200 X-ray generator and recorded at 250 Hz using a Philips image intensifier retrofitted with a Redlake MotionPro2000 camera.

During feeding trials the two prey types, whole live blackworm (*Lumbriculus variegatus*) and guppy fry (*Poecilia reticulata*), were presented at the water surface (via

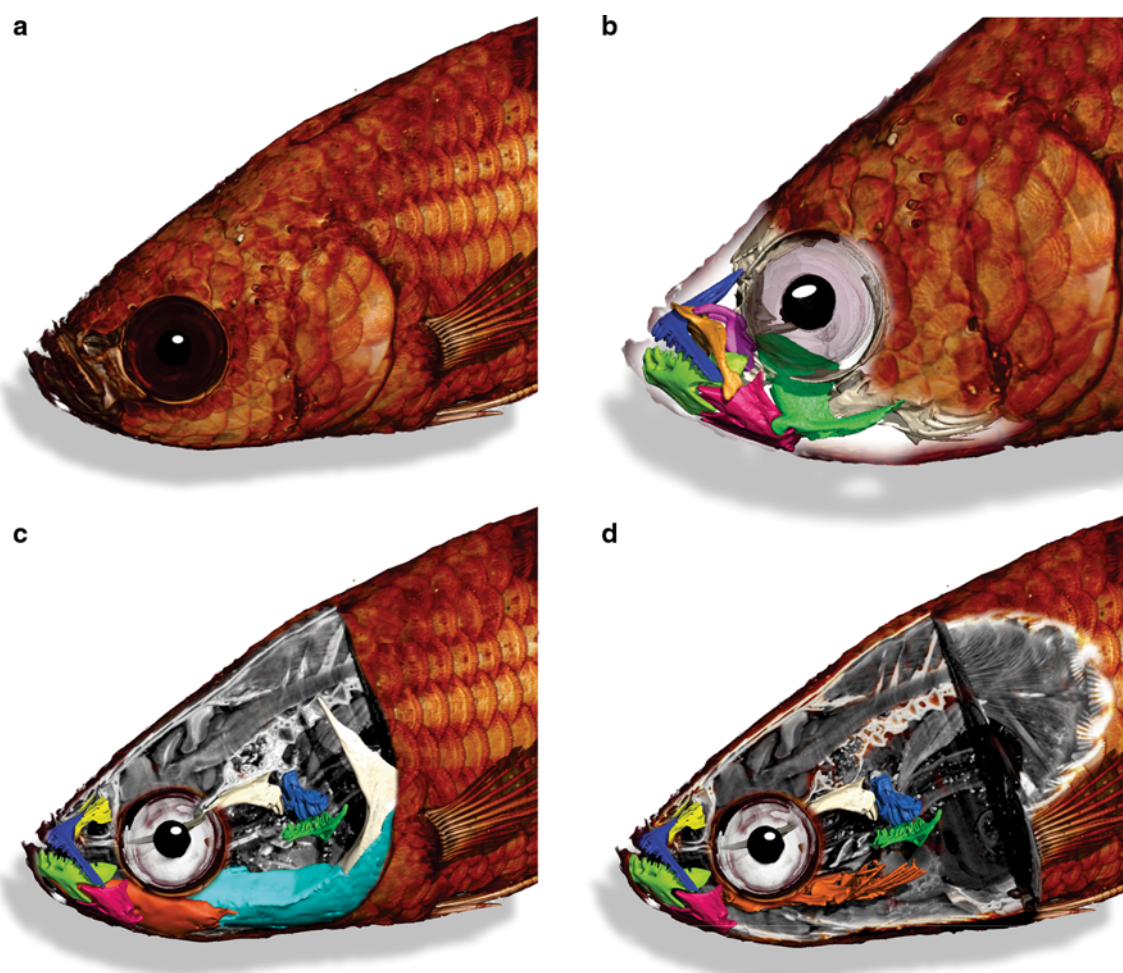


Fig. 1 Feeding apparatus morphology in *Betta splendens*. **a** Lateral view. **b** Detail of mandibular jaw apparatus. Bones rendered: *light green* mandible, *red* articular, *purple* premaxilla, *orange* maxilla, *forest green*, quadrate. **c** Deeper parasagittal slice showing bony elements in the parasphenoid and pharyngeal regions that carry teeth; *off-white* tooth rosette on the pharyngeal process of the parasphenoid,

green tooth plate on ceratobranchial V, *blue* tooth plate on pharyngobranchial I (following Liem 1963). **d** Sagittal slice, showing musculature and distal pharyngeal arch elements in *orange*. Note that the ceratobranchial and pharyngobranchial elements are positioned lateral to the parasphenoid, although they appear posteriorly displaced in this view

manual release from blunt forceps) in a pseudo-randomized manner, while high-speed video was recorded under both illumination-types (light and X-ray). Care was taken to standardize prey-size by using relatively large worms and relatively small fry. Cutting was avoided so as not to influence the natural motility and structural integrity of each prey-type. The body diameter of prey was approximately 3 mm, slightly less than the lateral mouth-width of the *Betta* specimens.

A minimum of five sequences per individual per prey-type were selected from the light and X-ray video datasets (totalling over 100 sequences for analyses) and digitized for frame-by-frame motion analyses in TEMA Motion (Imagesystems AB, Sweden). We digitized seven landmarks in the light videos (Fig. 2a) and six landmarks in the X-ray videos (Fig. 2e) so that the absolute angular excursions could be calculated for the following structures:

elevation of the neurocranium (nc), was measured as the angular increase between the rostrum and the dorsal body point around a point overlying the craniovertebral joint. By measuring neurocranial elevation in both video streams we were able to synchronize internal and external kinematics and produce Fig. 3. Rotation of the pectoral girdle (pg; light video only), was measured as the angular increase between the pectoral girdle and the body axis. Mandibular jaw gape (gp; light video only), was measured as the angular movement of the upper and lower jaws relative to the lower jaw joint. Protrusion of the premaxillary upper jaw (jp; light video only) was measured as the angular increase between the tip of the upper jaw and the rostrum with the lower jaw joint as vertex. Jaw protrusion was relevant in this study (Gosline 1985), but not in previous studies of basal raking taxa, which lack protrisible jaws. Horizontal hyoid movement (bh_h, from X-ray only) was

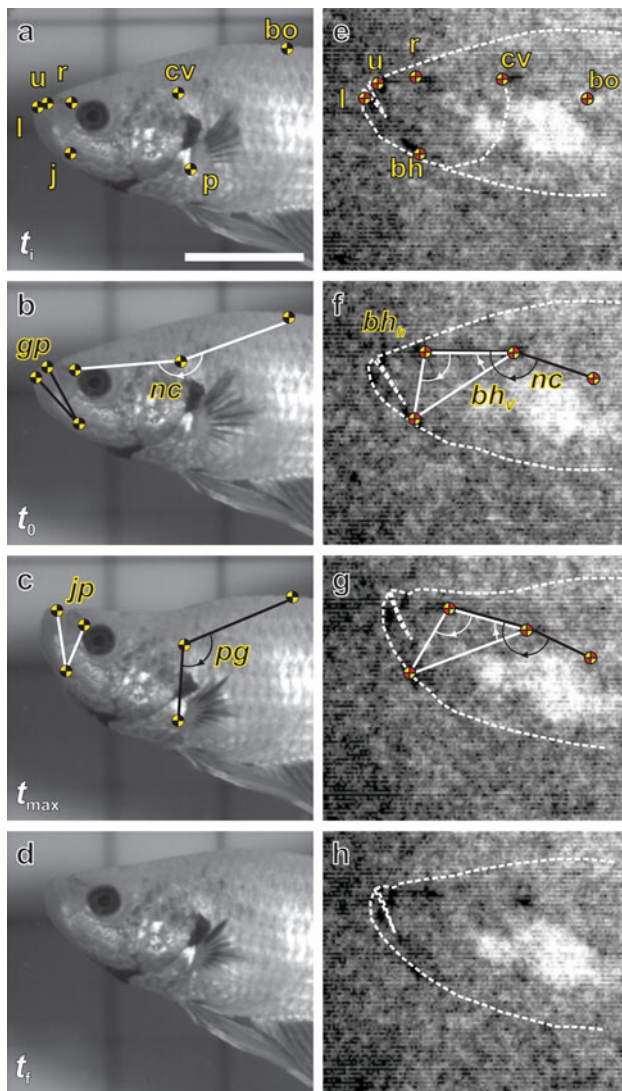


Fig. 2 Lateral high-speed video images, left lateral view (left column; see also Online Resource 1) and X-ray view (right column) at key time-points during prey processing in *Betta*. In **a** and **e** at $t_i = 20$ ms prior to the head-bob, the digitized points are illustrated. t_0 (time zero), the frame prior to onset of rapid cranial elevation, marks the behavioural onset. t_{max} is the behavioural power-stroke maximum, approx. 50 ms into the behaviour, and t_f is conclusion of prey processing bout. Measurements of angular motion kinematics variables are indicated: In **b** neurocranial elevation (nc ; white angle); gape expansion (gp ; black). In **c** jaw protrusion (jp ; white); pectoral girdle movement (pg ; black). In **f–g** vertical hyoid movement (bh_v), horizontal hyoid movement (bh_h). Abbreviations: bo body, bh basihyal, j jaw joint, l lower jaw, p pectoral girdle, r rostrum, u upper jaw, cv craniovertebral joint. Scale bar 15 mm

measured as the angle of a triangle formed by the basihyal and posterior cranial markers with the anterior cranial marker as vertex. Vertical hyoid movement (bh_v , from X-ray only) was measured as the angle of a triangle formed by the anterior cranial and basihyal markers with the posterior cranial marker as vertex.

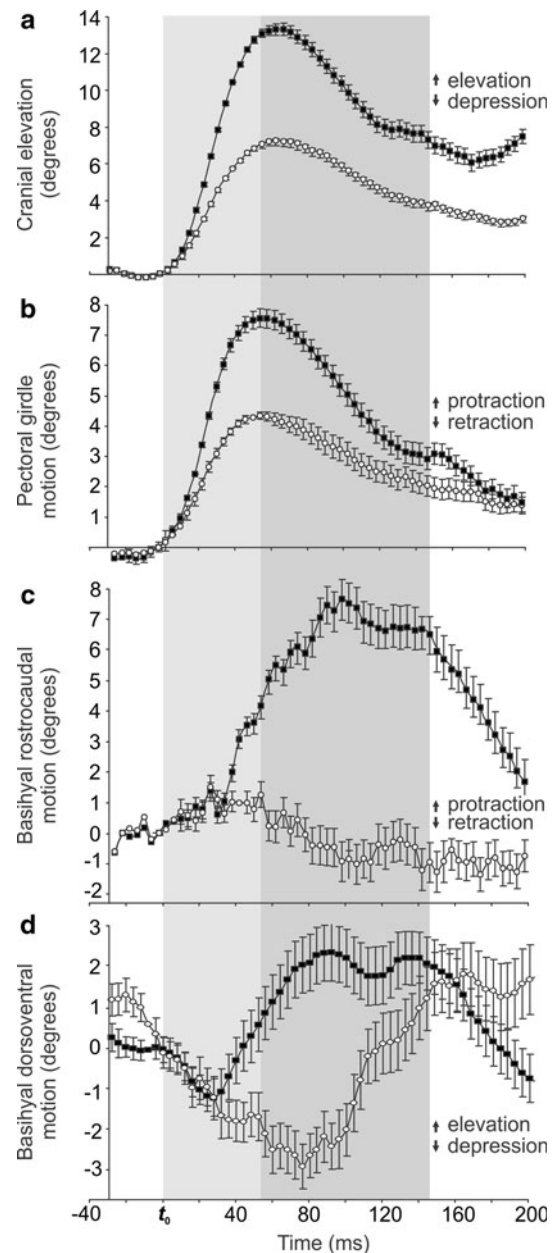


Fig. 3 Motion timing, magnitude and duration of angular excursions (in degrees) from approx. 30 ms prior to t_0 (the video frame prior to onset of cranial elevation) through 200 ms showing **a** cranial elevation, causing movement of the upper oropharyngeal jaw, **b** pectoral girdle protraction, causing movement of the lower hyobranchial jaw (measured from external high-speed video), **c** horizontal motion of the lower hyobranchial jaw relative to the oropharyngeal upper jaw, **d** vertical motion of the hyobranchial lower jaw relative to the oropharyngeal upper jaw. **c, d** Measured from X-ray videos. Filled squares fish prey, open circles worm prey. Whiskers are SEM

Kinematic excursion variables were chosen to permit direct kinematic comparisons of head-bobbing in *Betta* with raking in osteoglossomorph and salmonid fishes (Sanford and Lauder 1990; Konow et al. 2008; Konow and

Sanford 2008a). Pharyngognathy has mainly been studied with a focus on the motion pattern of the jaw elements themselves. Therefore, data on the motion of oral, cranial, pectoral and hyoid elements during pharyngognathy are scarce and restricted to a few basal teleosts, including carp (Sibbing 1982; Gidmark et al. 2013), *Pristolepis*, (Liem 1973), and to neoteleost cichlids (Liem 1973; Aerts et al. 1986; Claes and De Vree 1991).

All variables were expressed in degrees of incremental change from t_0 (cranial elevation onset-time) to peak excursion, in frame-by-frame increments of 4 ms. Mean performance curves were plotted for those kinematic variables (Fig. 3) that had a prey-type effect in the parametric analyses (see below). Motion-analysis data were also used to derive variables of excursion magnitudes, onset-timings, durations, and mean velocities of all angular movements (Table 2). Velocity was calculated by differentiation of the incremental (4 ms) angular change measurement and then smoothing the data using a spline interpolation ($SD = 0.05$). We ran a principal component analysis constrained to four axes on the correlation matrix of the light video kinematic dataset, consisting of 12 kinematic variables. Then, mixed-model ANOVAs on the PC factor scores with prey-type as fixed effect and individual as random effect were used to determine if kinematics variables exhibited a prey-type effect. Hyoid motion

was quantified in different animals and therefore analysed separately, using first a MANOVA to establish the overall presence of variation, followed by ANOVAs on the raw values for each of the three derived hyoid motion variables. In calculations of F statistics for both datasets, we used the interaction term as the denominator following (Zar 1999). All P values were Bonferroni-corrected, due to the potential dependence between kinematic variables (Table 2).

To produce Fig. 1, one *Betta* specimen was euthanized in an overdose of MS222, fixed overnight in a 10 % aqueous formaldehyde solution, rinsed and left for a week in a 70 % ethanol solution. The specimen was then transferred to a 5 % phosphomolybdic acid solution (Metscher 2009) for 10 days prior to scanning on a Phoenix vltomelx L 240-180 μ CT scanner at the AST-RX of the MNHN Paris (France). Scan geometry was set to obtain 8.83 μ m voxels in the three dimensional (3D) image reconstruction, which was done using FDK algorithms of Phoenix solution. 3D images were down-sampled from 16-bit to 8-bit voxels for visualization. 3D rendering was obtained after semi-automatic segmentation of bone and muscle using Avizo 7.1 (VSG, Visualization Sciences Group, France). Direct volume rendering was applied for the body wall, skin and skull, and the iso-surface function was applied for each bone and muscle of interest.

Results

All individuals of *Betta* caught both prey types, and all successful prey-capture events were followed by vigorous head-bobbing, sequenced in trains lasting 6–8 s, which often contained over 10 head-bobbing cycles. Each cycle lasted approx. 100 ms, and successive trains were interrupted by short pauses (0.5–1 s), resulting in a highly conspicuous behaviour. We often heard crushing noises through the aquarium glass and observed prey debris being expelled through the gill-slits, confirming that head-bobbing indeed is a prey-processing behaviour.

Analyses of motion kinematics from high-speed light and X-ray movies revealed that the behaviour was divided into two phases (Fig. 3). In the first *preparatory* phase, the mouth was closed around the prey, the cranium was elevated and the pectoral girdle was protracted. In the second power-stroke phase, the cranium was depressed and the pectoral girdle was retracted, after which the cranium and pectoral girdle returned to their resting positions. X-ray data showed that the hyoid was protracted and elevated during the second phase, especially during processing of guppy fry.

Motion analyses of light and X-ray high-speed videos revealed very limited variation across individuals processing of one type of prey (Fig. 3; Table 2). However,

Table 2 Mean values \pm SEM and ANOVA results for excursion magnitude, peak-timing and velocity of cranial, pectoral girdle and hyobranchial kinematics in *Betta splendens* during processing of two different prey types

Variable	Fish prey	SEM	Worm prey	SEM	P values ^a
Magnitude (°)					
Neurocranium	−14.16	0.13	−8.11	0.12	***
Pectoral girdle	8.18	0.19	4.94	0.10	***
Hyoid horizontal	7.07	0.86	0.25	0.77	*
Hyoid vertical	3.60	0.69	−4.25	0.59	***
Timing from t_0 (ms)					
Neurocranium	68.4	0.17	66.92	0.26	NS
Pectoral girdle	62.88	0.24	58.88	0.25	NS
Hyoid horizontal	105.33	1.60	92.67	1.59	NS
Hyoid vertical	91.2	1.72	79.1	1.60	NS
Velocity (degrees ms ^{−1})					
Neurocranium	0.22	0.06	0.12	0.07	***
Pectoral girdle	0.14	0.07	0.08	0.07	*
Hyoid horizontal	0.07	0.11	−0.02	0.09	***
Hyoid vertical	0.09	0.13	−0.02	0.1	***

NS non-significant

* Significant 0.05 level

*** Significant 0.001 level

^a Bonferroni-corrected ANOVA ($df_{1,24}$); for basihyal traits ($df_{1,12}$)

Table 3 PCA component loadings and summary statistics for the prey-type effect in the prey-processing kinematics of *Betta splendens*

Principal component axis	1	2	3	4
Eigenvalues	3.609	2.538	1.801	1.043
% variation explained	30.076	21.153	15.011	8.693
Neurocranial elevation velocity	0.909^a	−0.015	−0.117	0.011
Pectoral girdle protraction velocity	−0.879^a	−0.148	0.128	0.046
Pectoral girdle protraction magnitude	−0.773^a	0.035	0.449	0.108
Neurocranial elevation magnitude	0.687^a	−0.253	−0.488	−0.201
Gape occlusion timing	0.589	−0.052	0.369	0.154
Gape occlusion velocity	−0.139	0.883	−0.283	−0.170
Gape occlusion magnitude	−0.100	0.836	−0.298	−0.225
Jaw protrusion velocity	0.222	−0.700	0.176	−0.149
Gape protrusion timing	0.432	0.532	0.487	0.118
Neurocranial elevation timing	0.488	0.412	0.554	0.371
Jaw protrusion magnitude	−0.094	−0.077	0.465	−0.668
Jaw protrusion timing	0.285	0.140	0.485	−0.515
ANOVA <i>F</i> statistics ^b	<i>F</i> = 14.3 <i>P</i> < 0.05	<i>F</i> = 1.7 <i>P</i> > 0.05	<i>F</i> = 8.0 <i>P</i> < 0.01	<i>F</i> = 0.6 <i>P</i> > 0.05

^a Eigenvectors for boldfaced values (>0.6) are plotted in Fig. 4

^b Bonferroni-corrected; *df* = 1, 44; random factor *P* > 0.5

there was a clear prey-type effect on cranial, pectoral girdle and hyoid movements (Fig. 3): processing of guppy fry involved faster cranial and pectoral girdle rotation (Fig. 3; Table 2); both cranial and pectoral girdle excursions during processing of guppy fry reached approximately twice the magnitude and velocity of blackworm processing (Table 2).

The MANOVA on kinematics variables was significant (Wilks λ = 0.518; $F_{4,41}$ = 9.548; *P* < 0.001). The strong prey-type effect was driven by inverse depression-elevation hyoid motion and a weaker, yet significant effect of hyoid protraction magnitude. Axes one and three from a principal component analysis on the input kinematic variables of gape, cranial and pectoral girdle rotation were statistically significant. These axes explained 30.1 and 15.0 %, respectively, of the total amount of variation in the dataset (Table 3).

The principal component loadings revealed a clear variation in velocity and magnitude of cranial elevation and pectoral girdle protraction, all scoring significantly higher for guppy fry processing. Eigenvector plots of these PC loadings (Fig. 4.) clearly revealed how kinematics influenced the distribution of guppy fry and blackworm processing events across kinematic space. Although some overlap between prey types existed, guppy fry processing events were distributed across a region of kinematic space characterized by high velocity and magnitude of cranial elevation. In contrast, blackworm processing was characterized by slower velocity and lower magnitude of pectoral girdle protraction. The loading of mouth closure timing and cranial elevation timing approached our conservative

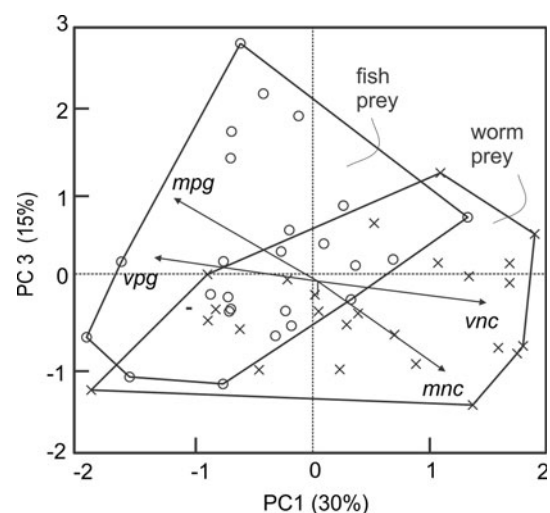


Fig. 4 Scatter plot of the PC axes 1 and 3 that drive significant prey-type differences (see ANOVA results, Table 3). Significant variables with strongly influential component loadings (PCL > 0.6; Table 3) are scaled to PC axis length and plotted as Eigenvectors to depict the role of these variables in segregating the prey-type specific events across the available multivariate kinematic space: *vpg* velocity of pectoral girdle protraction, *mpg* magnitude of pectoral girdle protraction, *vnc* velocity of neurocranial elevation, *mnc* magnitude of neurocranial elevation. Negative results for pectoral girdle velocity and magnitude result from calculations on movement measures (Fig. 3)

arbitrary cut-off value of 0.6 on PC1 and PC3. However, the vector-orientation of these variables, as shown by the factor scores in Table 3, suggested that timing variables had an overall weak effect on prey-type separation (Fig. 4).

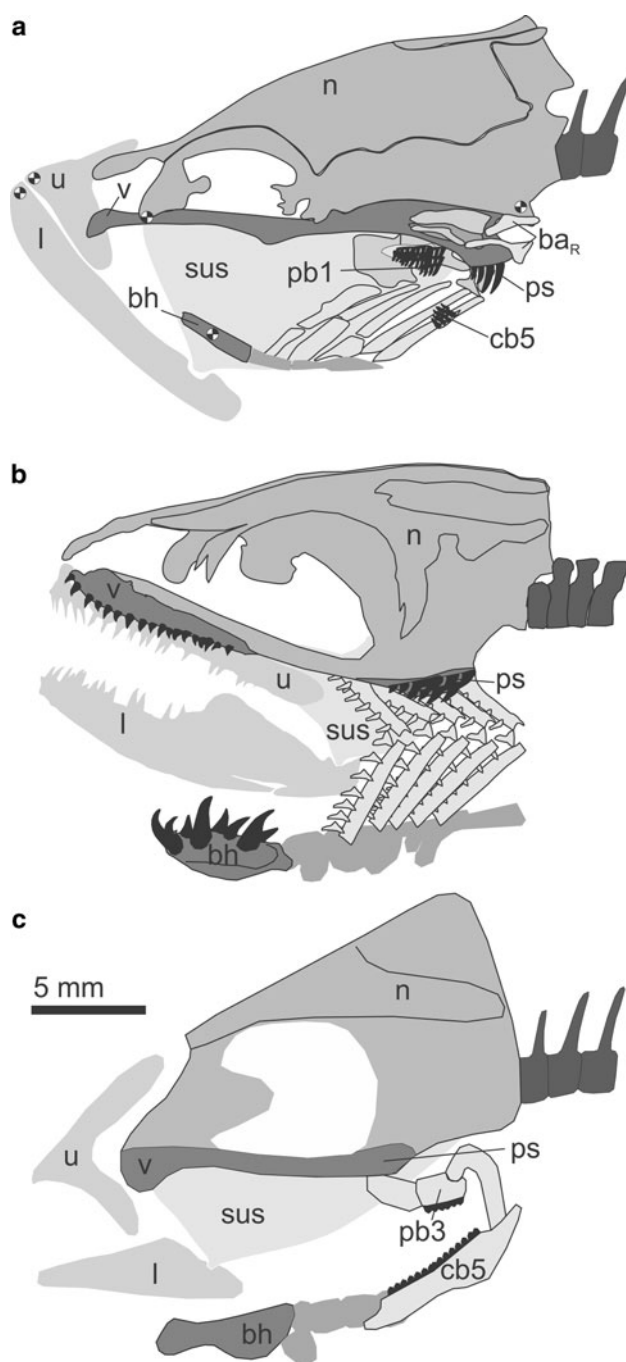


Fig. 5 Comparison of osteology and dentition surfaces. **a** The pharyngo-parasphenoid jaw apparatus (*Betta*), **b** the tongue-bite apparatus (*Salvelinus*) and **c** the pharyngeal jaw apparatus (generalized neoteleost). Left lateral views with anterior facing left and left mandibular jaws and suspensorium (mouth-wall) removed (right side elements are shown in light shading). Left hyoid bar and gill elements removed to expose tooth-bearing bony elements. Circular targets in **a** indicate where radio-opaque markers were implanted for recording of high-speed X-ray video (one marker placed near the dorsal fins is not shown here but seen in Fig. 2). **a** The inter-oral dentition in *Betta* is displaced to a rosette on the posterior parasphenoid, and opposing tooth plates on the 1st and 5th gill arches (in black). **b** Primary tongue-bite apparatus dentition is located on vomer and basihyal, which are edentulous in *Betta*, and on the parasphenoid. **c** Pharyngeal jaw dentition is on the 3rd pharyngobranchial, which are edentulous in *Betta*, and on the 5th ceratobranchial, which also carries teeth in *Betta*. Labels: *ba_r*, remainders of left branchial arches that were removed to exposed elements of interest on animals right, *bh* basihyal, *cb* ceratobranchial, *l* lower jaw, *n* neurocranium, *pb* pharyngobranchial, *ps* parasphenoid, *sus* suspensorium, *u* upper jaw, *v* vomer

variables, including cranial elevation and pectoral girdle retraction, the mechanisms driving the behavioural power-stroke. In terms of mechanical output, the pattern of hyoid motion was also modulated in response to the type of prey offered, with guppy fry eliciting more compressive, more amplified and faster hyoid movements than blackworm.

Below, we discuss the implications of our findings, under the assumptions that prey processing in *Betta* does not result from captivity-induced megalophagy, and the natural trophic niche of *Betta* does include prey that requires processing. Our conclusion is that *Betta* uses a different prey-processing behaviour than those previously described for teleost fishes; head-bobbing involves a unique suite of morphological and kinematics traits, some of which resemble, and some of which differ from raking using a tongue-bite apparatus and pharyngognath using a pharyngeal jaw apparatus.

Morphological comparison with other prey-processing mechanisms

It was hypothesized by Gosline (1985) that *Betta*, and its anabantid sisters, might process prey using a novel ‘pharyngeal bite’. Our data support Gosline’s hypothesis, given that *Betta* has a unique combination of morphological traits. The parasphenoid tooth ‘rosette’ appears homologous with the ancestral tongue-bite apparatus (Liem 1963; Gosline 1985; Mabee and Trendler 1996; Konow and Sanford 2008a). However, *Betta* lacks several other morphological diagnostics of a tongue-bite apparatus (Table 1). The basihyal (tongue bone) and the mouth-roofing anterior vomerine and pterygoid bones are entirely edentulous in *Betta*, but dentition adorned in the ancestral tongue-bite apparatus (Fig. 5). The edentulous state of these bones in *Betta* appears

Discussion

Prey processing in *Betta*

After successful prey-captures, all *Betta* individuals displayed the head-bobbing behaviour, both on relatively large, tough and motile guppy fry, and on soft-bodied and less motile blackworm prey. However, head-bobbing was not observed during ingestion of commercial flake food. Different types of prey elicited modulation of most kinematic

to be a derived character relative to its relatives (e.g. *Anabas*, *Ctenopoma* and *Sandelia*), which have extensive basihyal dentition (Liem 1963). The dentition differences between *Betta* and its anabantid and nandid sister taxa resemble the potentially pedomorphic dentition reduction in coregonid whitefishes, compared with their salmonid sisters (Liem 1963; Sanford and Lauder 1989).

Other dentition-bearing elements in the oropharynx of *Betta* are adjacent to, but not homologous with dentition-bearing elements in the pharyngeal jaw apparatus of neoteleosts; neither in the generalized perciform state, nor in the derived ‘labrid’ state (Wainwright 2006). Overall, these morphological results support our hypothesis that the configuration of the prey-processing mechanism in *Betta* differs from previously known configurations in aquatic feeding vertebrates (Konow and Sanford 2008a, b).

Kinematics comparison with other prey-processing mechanisms

We rejected the hypothesis that head-bobbing in *Betta* is functionally analogous with raking or pharyngognathy (Table 1), despite some clear similarities, in particular with raking (Sanford and Lauder 1990; Sanford 2001a, b; Konow and Sanford 2008a; Konow et al. 2008). During the preparatory phase of raking, the prey is fixed as the mouth is occluded, while the tongue-bite apparatus is primed for the power stroke via protraction and elevation of the basihyal ‘bony tongue’. During the preparatory phase of head-bobbing, we observed a similar mouth closure, along with protraction and elevation of the basihyal. In comparison, during pharyngognathy, the mouth often remains at a passive gape (Sibbing 1982; Claes and De Vree 1991).

The cranium is elevated during the power stroke of raking, resulting in depression and protraction of the upper tongue-bite apparatus jaw, formed by the tooth-bearing parasphenoid and vomerine midline bones of the mouth roof (Konow and Sanford 2008a; Camp et al. 2009). Significant amounts of cranial elevation ($>10^\circ$) often occur during prey-capture in aquatic feeding vertebrates but has previously not been reported for any other prey-processing behaviour than raking (Konow and Sanford 2008a). During head-bobbing in *Betta*, however, cranial elevation reached 11° – 14° , comparable to 11° during raking in the knife fish (*Chitala*) and 13° in the arowana (*Scleropages*). Salmonids, the other raking group, typically elevate their cranium approximately three times more during raking (Sanford 2001a; Camp et al. 2009; Konow et al. 2008). However, during pharyngognathy, cranial elevation is either omitted or only reaches 4° – 7° (Liem 1973; Sibbing 1982; Claes and De Vree 1991).

During the raking power stroke, the basihyal lower jaw is retracted via posterior-directed movement of the pectoral girdle, occurring along with cranial elevation (Konow and Sanford 2008a; Konow et al. 2008). During the power stroke of pharyngognathy, both the dorsal and ventral pharyngeal jaws are moved posteriorly (Aerts et al. 1986; Claes and De Vree 1991; Grubich 2000). However, during the power stroke in *Betta*, the pectoral girdle is protracted. Hyoid protraction during the first phase of head-bobbing may occur passively, as a result of cranial elevation forcing the ventral oropharynx anteriorly. However, hyoid protraction during the second phase likely results from hyoid protractor muscle contraction, meaning that hyoid protractor contraction is delayed relative to its characteristically early activity onset during raking (Sanford and

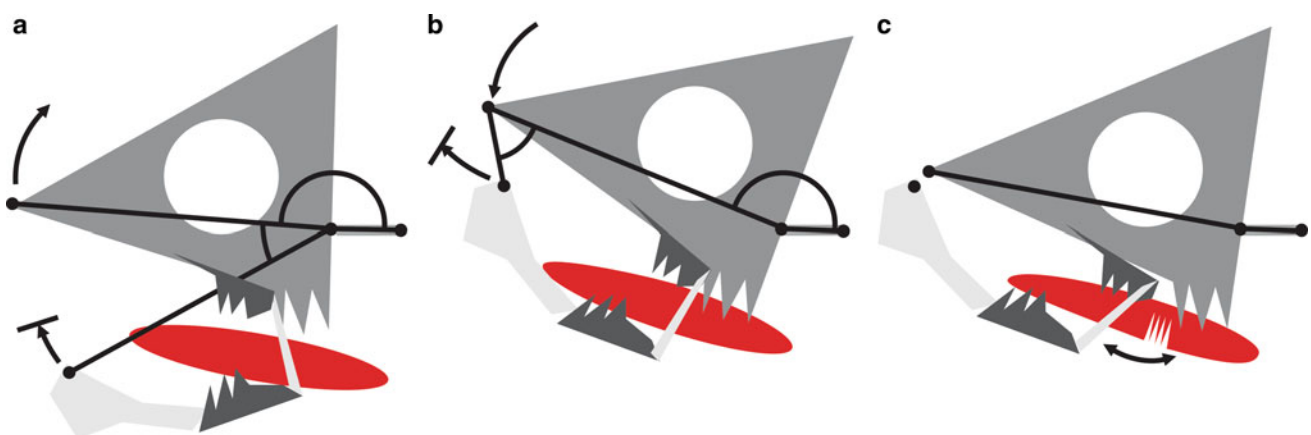


Fig. 6 Hypothesis of prey-processing function in *Betta*. **a** During phase one, the neurocranium is elevated, and the ceratobranchial “lower jaw” is protracted and elevated towards the skull-base (mouth roof). **b** The result is pinching of the prey (red) between the ceratobranchial and pharyngobranchial tooth plates, and pinning of the prey onto the parasphenoid tooth rosette. X-ray data indicates that

the hyoid remains protracted and elevated against the skull during phase two, where the skull is depressed. **c** Prey is torn and crushed between five points of contact; the medial parasphenoid tooth rosette, and the bilateral tooth plates on the ceratobranchial and pharyngobranchial elements

Lauder 1989; Konow and Sanford 2008b). The latter idea unfortunately remains speculation as we were not able to record reliable EMG from the hyoid protractor in *Betta*.

The functional significance of prey processing in *Betta*

The hyoid motion pattern in *Betta* was clearly modulated in response to the type of prey processed. During processing of soft-bodied and relatively immobile blackworm prey, hyoid depression was followed by hyoid elevation and retraction, likely forcing the prey towards the oesophagus. In contrast, processing of tough and motile guppy fry involved hyoid protraction and elevation during both the preparatory and power-stroke phases. Guppy fry also elicited a significantly amplified magnitude of occlusive cranial and hyoid excursions, meaning that this prey type likely was subjected to higher crushing forces.

The rate of cranial depression during power strokes on guppy fry was also significantly higher than on blackworm, possibly meaning that significantly more positive muscle work was produced when tougher and motile prey was processed (Fig. 3). Figure 6 provides a conceptual diagram of how ‘pharyngo-parasphenoid’ prey processing in *Betta* might function: the prey is first pinned onto the parasphenoid tooth rosette, and then grappled by the more lateral ceratobranchial and pharyngobranchial tooth plates.

The question remains: why does *Betta* process soft and relatively immobile prey? There may in theory be an energetic advantage in processing any and all prey. For instance, prey fragmentation may increase digestion rates. Moreover, this behaviour could also be driven by a central pattern generator, triggered by a stimulus. Given our observation of behavioural modulation between the large prey types, combined with no head-bobbing on small and soft flake food, we speculate that the stimulus might be prey-size. Similarly, in raking salmonids, there is evidence of a central pattern generator drive (Konow and Sanford 2008b), but the trigger appears to be a prey-type stimulus (Konow et al. 2008). Regardless of the neural mechanism, the ability to break down large, robust and motile prey may let *Betta* broaden its ecological versatility from a diet exclusively made up by microscopic soft-bodied invertebrates.

Concluding remarks

The small size of *Betta* prevented reliable electromyography recordings in our study. However, an early burst of preparatory activity was recorded from the hyoid protractor musculature in *Anabas testudineus* (Liem and Greenwood 1981), which is a larger relative of *Betta*. The onset of hyoid protractor activity in *Anabas* was simultaneous with

mandible adductor muscle activity. This sequencing is identical to the preparatory muscle-activity pattern in raking (Sanford and Lauder 1989; Konow and Sanford 2008b; Konow et al. 2008). A repeated occurrence of this particular muscle-activity pattern across teleost evolution adds additional evidence to suggest that a significant ecological advantage is conferred by immobilizing and reducing prey through intra-oral processing.

The discovery of a similar muscle-activity pattern in *Anabas* to that in raking taxa is at least circumstantial evidence of other anabantids deploying raking-like hyoid movements during prey processing. Recordings of feeding apparatus motion kinematics and EMG from *Anabas*, *Ctenopoma* and *Sandelia* could yield examples of prey processing that resemble raking more closely than what we describe here for *Betta*. The likelihood of this is underlined by similarities in oropharyngeal tooth-distribution between anabantoid, nandid and basal raking species (Liem 1963; Gosline 1985; Liem and Greenwood 1981; Hilton 2001).

As several earlier studies have highlighted, teleosts often engage in vigorous prey processing (Lauder 1980, 1981; Aerts et al. 1986; Wainwright 1989a; Drucker and Jensen 1991; Grubich 2000; Konow and Sanford 2008a; Gidmark et al. 2013). This realization underscores the ubiquity of prey processing across the jaw-bearing vertebrates (Gintof et al. 2010; Konow et al. 2011). Our study revealed that neoteleost prey processing is not necessarily governed by a pharyngeal jaw apparatus. Future studies of similar behaviours in close sisters of *Betta* will likely provide important insight into the evolution and ubiquity of prey processing, highlighting its impact on the evolution of trophic diversity.

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