Why the long face? A comparative study of feeding kinematics of two pipefish with different snout lengths

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Syngnathids are known as specialised feeders which use rapid head rotation to quickly bridge the mouth-prey distance. Despite this specialized feeding mode, syngnathids show remarkable differences in snout size. This study showed that the mouth of *D. dactyliophorus*, a species with a relatively long snout, travels a greater distance compared to *D. melanopleura*, a species with a considerably shorter snout, allowing it to strike at prey that are farther away from the mouth. The long-snouted species also tends to reach significantly higher linear velocities of the mouth approaching the prey. On the other hand, *D. melanopleura* needed less time to capture its prey. A striking difference in prey-capture success was observed between species: *D. melanopleura* and *D. dactyliophorus* had a prey-capture success of 91% and 31%, respectively. The small prey size and the relatively large distance between eyes and prey are potential reasons why directing the mouth accurately to the prey is difficult in *D. dactyliophorus*, hence possibly explaining the lower prey capture success in this long-snouted species.

Key Words: pipefish; kinematics; prey-capture; feeding; snout length.

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INTRODUCTION

The syngnathid family (seahorses, pipefish, pipehorses and seadragons) is known to encompass species with the shortest prey capture times among fishes (5 – 7 ms). Their cranial system is therefore assumed to be highly specialized for rapid prey capture (Bergert & Wainwright, 1997; de Lussanet & Muller, 2007; Van Wassenbergh *et al.*, 2008; Roos *et al.*, 2009a; Van Wassenbergh *et al.*, 2011) and their head morphology is characterized by a long and tubular snout with minute jaws at its end. More detailed morphological studies (Branch, 1966; Roos *et al.*, 2009a; Leysen *et al.*, 2010) show subtle modifications of cranial structures that suggest that these morphological specializations are related to feeding function and performance.

The feeding strategy of syngnathids differs substantially from general suction-feeding fish. In the latter, the feeding strike is initiated by the opening of the mouth, followed by expansion of the buccal cavity through jaw depression, hyoid retraction and depression, neurocranial elevation, suspensorium abduction, and opercular abduction (e.g. Lauder, 1985). The movement of these bony structures results in a rostro-caudal expansion wave, which generates a flow of water from the environment into the mouth. In syngnathids, on the other hand, prey capture is initiated by retraction of the hyoid, followed by rapid cranial rotation and mouth opening. Once the mouth is near the prey, the suspensorium starts to abduct (Bergert & Wainwright, 1997, Roos *et al.*, 2009a, b). Not only the timing of the events is different, but also their function. As hyoid depression and cranial rotation are not directly used to expand the buccal cavity. In syngnathid fishes, hyoid rotation appears to be involved in an integrated power-amplifying system (Muller, 1987; de Lussanet & Muller, 2007; Van Wassenbergh *et al.*, 2008) and cranial rotation is used to bring the entire jaw apparatus close to the prey (de Lussanet & Muller, 2007; Roos *et al.*, 2010). This feeding strategy is referred to as pivot feeding (de Lussanet & Muller, 2007).

Because of their very short prey capture times, their highly specialized cranial morphology, and the peculiar feeding strategy, it is expected that syngnathids are trophic specialists. Indeed, dietary studies show that they mainly prey on small crustaceans (Tipton & Bell, 1988; Teixeira & Musick, 1995; Woods, 2002; Kendrick & Hyndes, 2005). However, despite that all syngnathids tend to feed on similar prey types, a large variation in relative snout length (i.e. the proportion of the snout length relative to the entire head length and the snout length is approximated by the distance from the snout tip to the anterior of the eye) is present. A recent biomechanical model hypothesized that a longer snout is an adaptation to reach the prey as fast as possible (de Lussanet & Muller, 2007). This is ideal to capture agile prey such as small shrimps. The model by de Lussanet & Muller (2007) elegantly showed that species bearing a relatively long snout have shorter prey-reach times compared to species with relatively shorter snouts. These theoretical predictions seemed supported by a study on the dietary composition of eight syngnathid species with different relative snout lengths, which showed that species with a longer snout tend to consume more elusive prey (Kendrick & Hyndes, 2005).

However, increasing snout length possibly comes with some disadvantages. First, as syngnathids are visual predators (James & Heck, 1994; Curtis & Vincent, 2005, Mosk *et al.*, 2007), a longer snout length will increase the distance between the eyes and the mouth and potentially the distance between the prey and the mouth. Consequently, accurate positioning of the mouth relative to the prey could be problematic in species with longer snouts. Second, the model by de Lussanet & Muller (2007) predicts that the cross-sectional area of the snout must decrease with increasing snout length to reduce the angular inertia of the snout. This could limit prey size and potentially narrow the dietary range in species with longer snouts. Furthermore, a relatively small mouth could add to the difficulty of accurately aiming the mouth at the prey in species with a relatively long, narrow snout compared to species with a relatively large mouth and short snout.

A pivot feeder can use two distinct strategies to accurately move the mouth close to the prey by rapid cranial rotation. A first strategy is that the predator assures that the initial position of the mouth relative to the prey is always identical. In this way, a stereotypical pattern of prey capture kinematics can be successful, and there is no need for kinematical flexibility in the feeding system (Nauwelaerts, *et al.*, 2008). However, in this case any deviation of the initial mouth position relative to the prey or unanticipated movement of the prey relative to the predator will result in a decrease in strike success. A second, alternative strategy is that the predator is able to deal with different prey positions relative to the mouth through feed-forward or feed-back information before or during the prey-capture action (Elshoud-Oldenhave & Osse, 1976; Liem, 1980; Lauder, 1981; Van Wassenbergh *et al.*, 2006; Van Wassenbergh & De Rechter, 2011). This means that the predator can estimate the position of the prey relative to its mouth and adjusts its feeding kinematics accordingly. The success of the latter strategy depends on the animal’s capability of modulating its prey-capture kinematics.

In this study a comparison of the feeding kinematics is made of two closely related species of pipefish, the bluestripe pipefish, *Doryrhamphus melanopleura* (Bleeker, 1858), and the ringed pipefish *D. dactyliophorus* (Bleeker, 1853), which are characterized by a different snout length. In *D. melanopleura*, the ratio of snout length relative to the head length is approximately 0.5, while in *D. dactyliophorus* this proportion reaches up to 0.7 (Fig. 1). Both species live in the Indo-pacific region and their body shape is very similar in having a long and slender body with a relatively large caudal fin (Kuiter, 2003). Here, three main questions are addressed: (1) is the species with the relatively longer snout able to generate a higher linear velocity of the mouth compared to that of the species with the relatively shorter snout, as suggested by mathematical modelling (de Lussanet & Muller, 2007) and a dietary study (Kendrick & Hyndes, 2005)? (2) Is the accuracy of moving the mouth close to the prey and the following prey capture reduced in the species with the longer snout? And (3) Are both species capable of positional modulation or does their prey-capture success depends on the position of the mouth relative to the prey just prior to the start of cranial rotation?

MATERIALS AND METHODS

STUDY SPECIES

For each pipefish species, *Doryrhamphus dactyliophorus* and *D. melanopleura* (not CITES listed), two individuals were obtained through the commercial aquarium trade (Bassleer Biofish nv, Westmeerbeek, Belgium, BTW BE 0454833097). In *D. dactyliophorus*, the standard lengths were 143.15 mm and 143.80 mm, the total head lengths (measured from the tip of the snout to the caudal tip of the operculum) were 21.35 mm and 23.20 mm and the maximal anatomical mouth sizes (maximal distance between the tip of the lower and upper jaw) were 1.49 mm and 1.47 mm, respectively. The L_s of *D. melanopleura* were 77.84 mm and 83.40 mm, the head lengths were 9.45 mm and 9.79 mm and the maximal anatomical mouth sizes were 1.44 mm and 1.41 mm, respectively. Species were kept separately in a large aquarium (200 l) at a constant temperature of 24°C, constant salinity of 35, natural photoperiod and were fed defrosted copepods (*Cyclops sp.*) daily. For filming sessions, each individual was transferred separately to a smaller aquarium (30 l), which contained a narrow section. The pipefish were trained to capture their prey (slowly sinking defrosted *Cyclops sp.*, with a length of 0.62 ± 0.05 mm and a height of 0.39 ± 0.05 mm, mean ± S.D., $N = 5$) in this narrow section to increase the chance of capturing a video with the lateral side of the fish's head perpendicular to the camera. Note that only two individuals per species were used in this study. For the purpose of this study, this could suffice since little intraspecific variation exists in feeding kinematics of syngnathid fish (Van Wassenbergh *et al.*, 2008; Flammang *et al.*, 2009; Roos *et al.*, 2009a).

HIGH-SPEED VIDEO RECORDINGS

During filming sessions, four arrays of red LEDs provided the required illumination. Feeding events were captured using a digital high-speed camera (Redlake Motionscope M3, Redlake Inc., Tallahassee, FL, USA, www.redlake.com) at 2000 Hz and a shutter time of 0.2 ms. Only video recordings, in which the lateral side of the head was perpendicular to the camera lens axis during filming, were retained for further analysis. Consequently, for each individual of each pipefish species the first 10 good lateral-view feeding events were analysed (40 in total). For *Doryrhamphus* *dactyliophorus*, six of the twenty analyzed feeding events results in successful prey capture (three for each individual), while nineteen of the twenty analyzed feeding events were successful in *D. melanopleura*. Note that the prey capture success for all the recorded feeding events, including those not suited for proper analysis, was 91% and 31% for *D. melanopleura* and *D. dactyliophorus*, respectively.

The start of each feeding event (time = 0 ms) was defined as the image prior the first visible movement, i.e. hyoid rotation. A sequence length of 10 ms was selected for further analysis, because previous studies showed that during this time interval pipefish usually capture their prey (Muller & Osse, 1984; Bergert & Wainwright, 1997; de Lussanet & Muller, 2007; Van Wassenbergh *et al.*, 2008).

KINEMATIC ANALYSIS

Each of the 40 retained feeding events was digitized frame by frame using Didge (version 2.2.0, A. Cullum, Creighton University, Omaha, NE). Seven landmarks were digitized on each frame (Fig. 2): the dorsal and ventral side of the snout tip (landmarks 1 and 2), the dorsal and ventral side of the operculum (landmarks 3 and 4), the dorsal and ventral side of the body at a distance of approximately one head length, starting at the distal end of the operculum (landmarks 5 and 6) and the middle of the prey's body (landmark 7). The head axis was determined as the middle between landmark 1 and 2 connected to the middle between landmark 3 and 4. In analogy, the body axis was determined as the middle between landmark 3 and 4 connected to the middle between landmark 5 and 6.

Ten time-dependent kinematical profiles were calculated: a) head rotation (angle between the head axis and the horizontal minus initial angle), b) angular velocity of head rotation, c) body rotation (angle between the body axis and the horizontal minus initial angle), d) angular velocity of body rotation, e) mouth path length (total distance travelled by the mouth opening, which is the middle of landmark 1 and 2 starting from time 0 as measured between consecutive frames), f) linear velocity of the mouth, g) prey path length (total distance travelled by landmark 7 starting from time 0 as measured between consecutive frames), h) linear velocity of the prey, i) prey distance (distance of the middle between landmark 1 and 2 and the initial position of the prey) and j) prey angle (angle between the head axis and the distance between the snout tip and the initial position of the prey). Because the reference frames of the head and body angle (a and b) were earth-bound, their respective rotations are expressed as the angle at time *t* minus the initial angle. The displacement profiles were filtered using a fourth order low-pass zero phase shift Butterworth filter with a cut-off frequency of 500 Hz to reduce digitization noise. Velocities were calculated through numerical differentiation of the smoothed profiles.

Five distances, four angles, four maximal velocities and nine timing variables (22 in total) were extracted from the kinematic profiles. The distance variables included i) initial prey distance, ii) prey distance when the head reaches maximal excursion, iii) maximum prey path length (i.e. the maximal distance travelled by the prey between two consecutive frames), iv) total distance traveled by the prey and v) total mouth path length. The four angular variables included i) initial prey angle, ii) prey angle when the head reaches maximal excursion, iii) total head rotation and iv) total body rotation. The four maximum velocities were i) linear velocity of the prey, ii) angular velocity of the head, iii) angular velocity of the body and iv) linear velocity of the mouth. The nine timing variables were i) time to maximum prey displacement, ii) time to maximum prey velocity, iii) prey capture time, iv) time to maximum head rotation, v) time to maximum velocity of head rotation, vi) time to maximum body rotation, vii) time to maximum velocity of body rotation, viii) time to maximum mouth displacement and ix) time to maximum mouth velocity. The distance and linear velocity variables were scaled to the smallest head length (excluding the snout) of the four individuals (i.e. 4.98 mm) to eliminate differences caused by absolute head size.

Before further analysis of the data, the angular head velocity of one individual of each species was plotted to trial number to investigate the effect of satiation (Sass & Motta, 2002). In neither species, this relation was significant ($R^2 = 0.09$, $p = 0.41$ and $R^2 = 0.04$, $p = 0.56$ for *D. melanopleura* and *D. dactyliophorus*, respectively), thus the data could be further analyzed without correction for this factor.

DETERMINATION OF THE CENTRE OF ROTATION

An important assumption of the theoretical model by de Lussanet & Muller (2007) for predicting shorter prey capture times in syngnathid species with relatively longer snouts is that the larger turning radius is caused by the longer snout. The position of the centre of head rotation relative to the head (*CR*) must be assessed to calculate the distance between the tip of the snout and *CR*, i.e. head rotation radius.

The position of *CR* was determined for each of the 40 recorded feeding events. The head and body were treated as two rigid elements and *CR* was calculated for a pre-defined time interval for simplification. This fixed time interval started at the beginning of the feeding event (time = 0 ms) and ended when the head was near maximal rotation (time = 3 ms) (see also Van Wassenbergh *et al.*, 2008). Two landmarks, namely the snout tip and the eye, were traced at the start and end of the fixed time interval of each recording. The intersection between the mid-normals of the lines connecting the same respective landmarks at the start and end of the time interval was determined as the common angular displacement centre, i.e. *CR* (Van

Wassenbergh *et al.*, 2008; Roos *et al.*, 2010). This procedure only holds when the forward translation of the pipefish during this time interval is negligibly small. Both pipefish species approached their prey at velocities lower than 0.05 m s^{-1}, which means that the forward translation never exceeds 0.15 mm during the first 3 ms, and *CR* could thus safely be calculated as described above. The radius of head rotation was calculated as the distance between *CR* and the snout tip.

The position of *CR* was expressed in the pipefish-bound frame of reference, with the head axis (see above) defined as the x-axis. The origin was defined at the level of the operculum (the middle between landmark 3 and 4, Fig. 2) and the y-axis was perpendicular to the x-axis and pointed to the dorsal side of the pipefish. The x- and y-coordinates of *CR* and the radius were made dimensionless, through division of the coordinates by their respective head lengths (head length measured from the anterior tip of the eye to the posterior tip of the operculum).

STATISTICS

The kinematic variables described above, plus the x- and y-coordinates of the centre of rotation (with respect to the head axis), the head-rotation radius and the snout length were subjected to a principal component analysis (PCA). Six prey-related variables (maximum prey path length, time to maximum prey path length, maximal prey velocity, time to maximum prey velocity, total prey path length and prey capture time) were not used in this analysis, because these variables had missing values for unsuccessful strikes. The purpose of the PCA was to condense the large number of (probably interrelated) variables into an amenable number of new composite variables that could then be compared between species. Analyses were performed on the correlation matrix. Variation in the scores of the individual observations on the first three principal component axes was examined using ANOVAs with species as the fixed factor and individual as a random factor. Variation in the six prey-related variables was examined with the same type of ANOVA. Yet, due to the unequal number of observations for each individual, the degrees of freedom for error were calculated using Satterthwaite's method in this case. All statistics were performed using Statistica 8.0 (Statsoft, Inc).

RESULTS

INTER-SPECIFIC COMPARISON

Both species initiated the feeding event by a ventral rotation of the hyoid, quickly followed by an upward rotation of the entire head and the opening of the mouth. The mouth parts did not protrude during feeding. The centre of head rotation was located in the vicinity of the eye. Consequently, the posterior end of the head moved ventrally, and the anterior part of the body rotated in the opposite sense compared to the head. Prey were sucked into the snout just after head rotation was finalised.

The principle component analysis (PCA) showed that the first three axes jointly explained 59.1% of the total variation (see Table S1 for variable loadings). According to the broken stick rule, the fourth principal component did not explain enough of the variation to be included in the analysis (variance explained = 7.0% < broken stick distribution = 8.8%). The first axis accounted for 31.2% of the total variation and correlated strongly positively with snout length, prey distance at the start and end of the feeding event, total mouth path length, maximum mouth velocity, time to maximum head and mouth velocity, x- and y-coordinates of the centre of head rotation and head rotation radius (Table SI). Mean scores on this first axis differed between species (ANOVA, $F_{1,2} = 84$, $P < 0.05$) (Fig. 3). There were no significant differences between individuals within species (ANOVA, $F_{2,36}$ = 2.9, $P > 0.05$). *Doryrhamphus dactyliophorus* scored higher on this first axis than *D. melanopleura* (Fig. 3).

The interspecific differences along this first principal component axis are reflected in the mean differences of the original variables that correlated highly with this axis (Table SI, SII). The prey distances at the start and end of cranial rotation tend to be higher in the long-snouted species: these distances in *D. dactyliophorus* were respectively 34% (2.5 ± 0.5 mm *vs.* 1.8 ± 0.4 mm; mean ± S.D.) and 120% (1.3 ± 0.6 mm vs. 0.6 ± 0.3 mm) greater than those in *D. melanopleura*. The time to peak head velocity was on average 43% higher in *D. dactyliophorus* (2.2 ± 0.5 ms *vs.* 1.5 ± 0.3 ms). Total mouth path length (2.5 ± 0.4 mm *vs.* 1.7 ± 0.3 mm), maximal mouth velocity (0.9 ± 0.2 m s^{-1} *vs.* 0.7 ± 0.1 m s^{-1}) and the time to peak mouth velocity (2.5 ± 0.4 ms *vs.* 1.7 ± 0.3 ms) were respectively 46%, 25% and 40% higher in *D. dactyliophorus*. The x-coordinate (0.80 ± 0.13 mm *vs.* 0.60 ± 0.07 mm) and y-coordinate of the centre of head rotation (0.24 ± 0.03 mm *vs.* 0.20 ± 0.02 mm) and the radius of head rotation (2.5 ± 0.2 mm *vs.* 1.29 ± 0.09 mm) were respectively 34%, 20% and 91% higher in *D. dactyliophorus*. With two exceptions (initial prey distance and max. mouth velocity), these variables also individually showed statistical significant differences between the species (ANOVA, $F_{1,2} > 18$; $P < 0.05$).

The second axis explained 15.9% of the total variation and correlated strongly negatively with total head rotation, maximum head velocity and maximum mouth velocity (Table SI). Scores on this axis showed no significant differences between species (ANOVA, $F_{1,2}$ = 0.65, $P > 0.05$). The kinematic results are in line with these findings, as the profiles of head and body rotation and maximal mouth velocity show a similar pattern (Table SII). Finally, the third axis accounted for a further 11.8% of the variation and was influenced mainly by prey angle and the start, and prey angle and distance at the end of the feeding trial (Table SI). Again, scores on this third axis did not differ between species (ANOVA, $F_{1,2} = 0.06$, $P > 0.05$).

Next, it was tested whether differences in the movement of the prey existed between the two species studied. No difference in maximal prey path length was observed for both species (ANOVA, $F_{1,16.8} = 3.8$, $P > 0.05$). However, the time to peak prey path length differed between species (ANOVA, $F_{1,5.5} = 69$, $P < 0.001$) and was 82% higher in *D. dactyliophorus* (5.5 ms ± 0.6 *vs.* 3.0 ± 0.8 ms; mean ± S.D.). Maximal prey velocity was significantly higher in *D. dactyliophorus* (ANOVA, $F_{1,19.2} = 23$, $P < 0.001$). Also the time to peak prey velocity was statistically different and was 90% higher in *D. dactyliophorus* (ANOVA, $F_{1,3.2} = 30$, $P < 0.01$) (4.0 ± 0.6 ms *vs.* 2.1 ± 0.7 ms). No differences between individuals of the same species were found (P always > 0.28). During successful feeding strikes, the prey travelled the same distance in both species (ANOVA, $F_{1,5.1} = 0.39$, $P > 0.05$) (Table SII). But prey capture time in *D. dactyliophorus* was 70% higher (5.5 ± 0.6 ms *vs.* 3.2 ± 0.9 ms) and was significantly different from that of *D. melanopleura* (ANOVA, $F_{1,21.3} = 109$, $P < 0.001$).

Finally, it was tested whether strike-to-strike variability differed between the species. To do so, the coefficients of variation (standard deviation of a trait divided by the mean of that trait) of the kinematic variables were calculated per individual (Wainwright *et al.*, 2008). The averages of all variables (± S.E.) were 0.12 ± 0.05 and 0.17 ± 0.04 for the *D. dactyliophorus* individuals, and twice 0.14 ± 0.04 for the *D. melanopleura* individuals. An analysis of variance showed no difference in the mean coefficient of variation between species (ANOVA, $F_{1,1} = 0.02$, $P > 0.05$).

POSITIONAL MODULATION

To test whether either species is capable of altering its head rotation in accordance with the initial position of the prey relative to the mouth, a correlation analysis was performed. No significant correlations were observed between the maximum head rotation and the initial prey angle [$R^2 = 0.02$, $P = 0.52$ and $R^2 = 0.01$, $P > 0.05$, for *D. dactyliophorus* and *D. melanopleura*, respectively, Fig. 4(a)] or between the maximum head rotation and the initial prey distance [$R^2 = 0.10$, $P = 0.18$ and $R^2 = 0.06$, $P > 0.05$, for *D. dactyliophorus* and *D. melanopleura*, respectively, Fig. 4(b)].

DISCUSSION

In this study, the kinematics of pivot feeding of two closely related pipefish species were compared to examine the effect of snout length, which is considerably higher in *Doryrhamphus dactyliophorus* compared to *D. melanopleura* (Fig. 1). First, the basic assumption behind the theoretical model that predicts kinematic effects of snout length during prey capture in syngnathids is evaluated (de Lussanet & Muller, 2007): the mouth of species with longer snouts is rotated towards the prey with a larger radius. To verify this, the centre of rotation of the head during prey capture was determined, and used to calculate the average turning radius of the mouth in both species. Despite a slightly more anterior position of its centre of head rotation, the turning radius of the mouth in *D. dactyliophorus* was indeed considerably larger than that of *D. melanopleura*. Consequently, the two study species can be used to test the hypotheses from the biomechanical model (de Lussanet & Muller, 2007).

A first hypothesis was that syngnathid species with longer snouts can generate higher linear velocities of the mouth during pivot feeding. This hypothesis was confirmed by the experimental data: *D. dactyliophorus* showed peak mouth velocities on average approximately 60% higher than *D. melanopleura*. Consequently, a longer snout could be considered advantageous in striking at prey capable of showing quick escape responses (Muller & Osse, 1984; de Lussanet & Muller, 2007). As mentioned in the introduction, a diet study by Kendrick & Hyndes (2005) seems to confirm that this effect can be observed in nature. However, this does not necessarily mean that species with relatively longer snouts capture their prey in a smaller time interval, since *D. dactyliophorus* needed approximately 5.5 ms while *D. melanopleura* only needed approximately 3.1 ms to reach its prey.

A second difference is that the mouth of *D. dactyliophorus* travels a greater distance compared to *D. melanopleura*. Therefore, this data suggests that a relatively longer snout provides the possibility to strike at the prey that are farther away from the mouth. Higher mouth-prey distances are indeed observed in the data for *D. dactyliophorus* compared to *D. melanopleura* (Table SII). Despite the lack of direct evidence for this, a higher mouth-prey distance could decrease the chance of the prey noticing the approaching predator. Therefore, this might positively influence prey-capture success.

It is hypothesized that the accuracy of moving the mouth close to the prey and the subsequent prey capture reduced in the species with the longer snout. This appears to be confirmed as an apparent discrepancy in prey capture success exists between both species. Feeding strikes in *D. melanopleura* were successful in 91% of the feeding events, while in *D. dactyliophorus* this was only 31%. No clear difference was observed between the kinematic profiles of the successful and failed feeding strikes in *D. dactyliophorus*, which suggests a lack of feedback control during feeding (Nauwelaerts *et al.*, 2008). This appears plausible because the mouth of the pipefish is positioned near the prey in less than 5 ms, while typical reaction latencies to respond kinematically to an external stimulus during feeding are considerably higher (e.g. 18 ms in the cyprinid *Aspius aspius* (L., 1758); Van Wassenbergh & De Rechter, 2011). Consequently, it is very unlikely that a movement as fast as pivot feeding in Syngnathidae can be combined with reflexive neural feedback control.

The relatively stereotypical feeding kinematics already suggested a limited flexibility of the feeding system in the pipefish species. Furthermore, no difference in the mean coefficient of variation between species was observed. This indicates that the extent of stereotypy is not influenced by snout length as both species show a similar, rather low value of the coefficient of variation. Further analysis highlighted the limited capacity to modulate the feeding strike by visual, pre-strike feed-forward control in these pipefish since no correlation between the maximum head rotation and the initial prey distance or the initial prey angle was observed (Fig. 4). This means that when accurate positioning of the mouth prior to the strike does not take place, pipefish cannot adjust their mouth movement. As mentioned earlier, this suggests that there is no feedback control by *a priory* choice of different motor programs or by reflex control during the strike, which may be a characteristic common to fish species with a highly specialized morphology and function of the feeding apparatus (Ralston & Wainwright, 1997; Ferry-Graham *et al.*, 2002; Matott *et al.*, 2005; Adriaens & Herrel, 2009).

Given that syngnathids are visual predators (James & Heck, 1994; Curtis & Vincent, 2005, Mosk *et al.*, 2007) and that the distance between the eye and the jaw apparatus in long snouted species such as *D. dactyliophorus* is fairly large, one can imagine that capturing a very small prey is potentially difficult. Attacking larger prey could increase the accuracy of the strike. However, this seems problematic since the mouth opening of *D. dactyliophorus* is relatively small and therefore prey size is limited. A previous study reported that *Syngnathus acus* (L., 1758), used a forceful head rotation to partition a large shrimp by contact with the anterior part of the snout, followed by capture of each piece (de Lussanet & Muller, 2007). This reported event shows alternative prey capture strategies are possible when long-snouted pipefish feed on large prey. In the current sample of species, it seems possible that the long-snouted species uses head rotation and higher linear mouth velocity to quickly capture relatively large prey clinching it between their jaws and further manipulate and transport it with a second feeding strike. Larger prey are easier to locate which could compensate for their low accuracy, and they are less likely to be displaced by bow waves generated by the predator (Van Wassenbergh *et al.*, 2010).

A recent dietary study on 12 different syngnathid species showed that species with a relatively long snout (with the proportion of snout length relative to total head length ≥ 0.6) had specialized diets (Kendrick & Hyndes, 2005). Gut contents of these species showed that a specific prey type like mysids in *Mitotichthys meraculus* (Whitley, 1948), *Phyllopteryx taeniolatus* (Lacepède, 1804) and *Vanacampus poecilolaemus* (Peters, 1868) and copepods in *Stigmatopora argus* (Richardson, 1840) and *S. nigra* (Kaup, 1856), dominated the entire diet (at least 68.7% of the total gut content). Species with relatively short snouts (snout-head proportion of ≤ 0.6) showed no or little difference in diet and presented a wider range of prey types. In the same study they found that the size of the prey items for species with relatively long snouts was at least half of the gape size of the syngnathid. These findings suggest that syngnathid species with long snout are trophic specialists that prey on relatively large and elusive prey. This observation is in accordance with the results of the present study that show a lower feeding success on small prey in a species with a longer snout.

In conclusion, the kinematic data support the hypothesis that pipefish with relatively long snouts can generate higher angular velocities of head rotation compared to a congeneric with a relatively short snout. Furthermore, a longer snout is advantageous to the pipefish in striking its prey at a larger distance. However, these benefits do not necessarily guarantee prey capture success, since the species with a long snout included in this study was able to capture its prey in only 31% of the prey capture events recorded, while the species with a short snout was successful in 91% of the prey capture events. Both pipefish species show very similar, stereotyped feeding kinematics and the capability of adjusting its head rotation kinematics in function of variation in prey position relative to the mouth could not be demonstrated. These findings suggest that the initial position of the mouth relative to the prey is very important to assure successful feeding. The small prey size and the relatively large distance between the eyes and the mouth in *D. dactyliophorus* might explain its low prey capture success.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

TABLE SI. Variable loadings on the three principal component axes used in the analysis.

TABLE SII. Kinematic variables (mean ± S.E) of pivot feeding in *Doryrhamphus dactyliophorus* and *D. melanopleura*.

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Figure captions

Figure 1: Lateral view pictures of the two studied pipefish species *Doryrhamphus melanopleura* (top) and *D. dactyliophorus* (bottom). The head length (the distance between the anterior side of the eye and the posteriormost point on the operculum) of *D. melanopleura* was scaled to the head length of *D. doryrhamphus* to clearly illustrate the difference in snout length. Scale bar, 5 mm.

Figure 2: Schematic illustration of the seven landmarks digitized on each frame of the high-speed videos. The landmarks include the dorsal and ventral side of the snout tip (landmarks 1-2), the dorsal and ventral side of the operculum (landmarks 3-4), the dorsal and ventral side of the body, at a distance of approximately one head length starting at the end of the operculum (landmarks 5-6) and the middle of the prey's body (landmark 7). Landmarks 1 to 4 were used to define the head axis (full gray line) and landmarks 3 to 6 were used to define the body axis (dashed gray line).

Figure 3: Principle component analysis (PCA) results showing the differences between the two pipefish species (filled symbols, *Doryrhamphus melanopleura*, open symbols, *D. dactyliophorus*) on the first principal component (PC1). For each group the 95% confidence ellipse of the mean is presented. See Table SI for variable loadings and the text for more details.

Figure 4: The results of the regression analysis between the maximum head rotation and the initial prey angle (a) and between the maximum head rotation and the initial prey distance (b) for *Doryrhamphus dactyliophorus* (filled circles) and *D. melanopleura* (open circles).

Figure 1

Figure 2

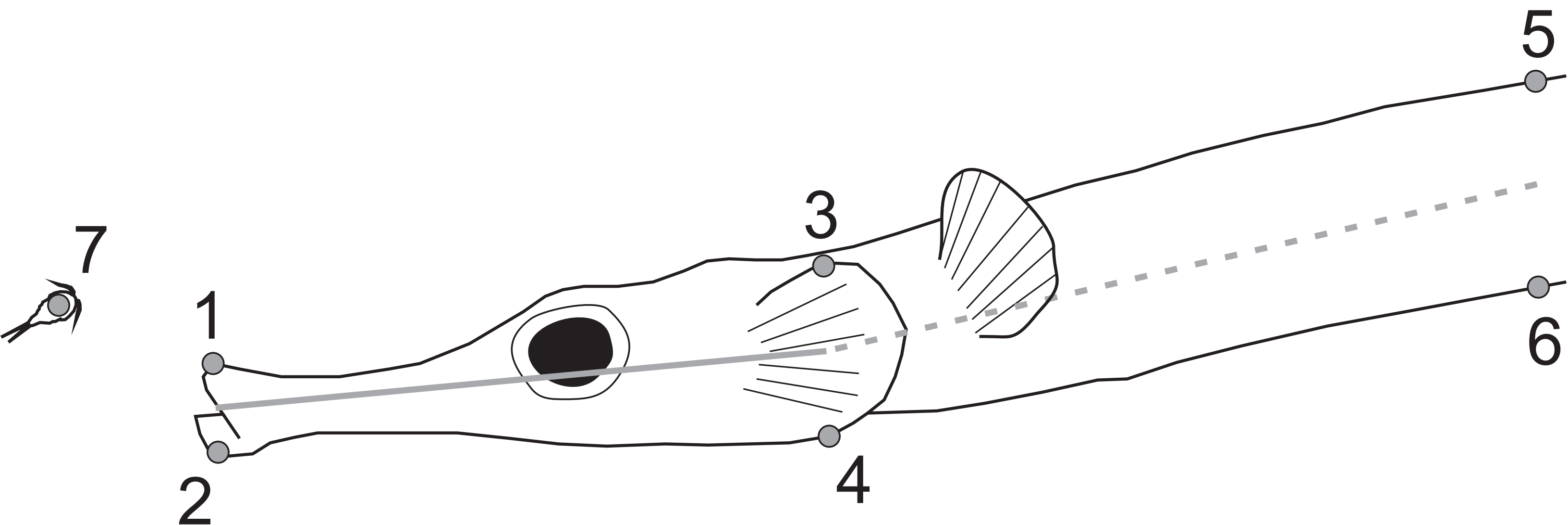
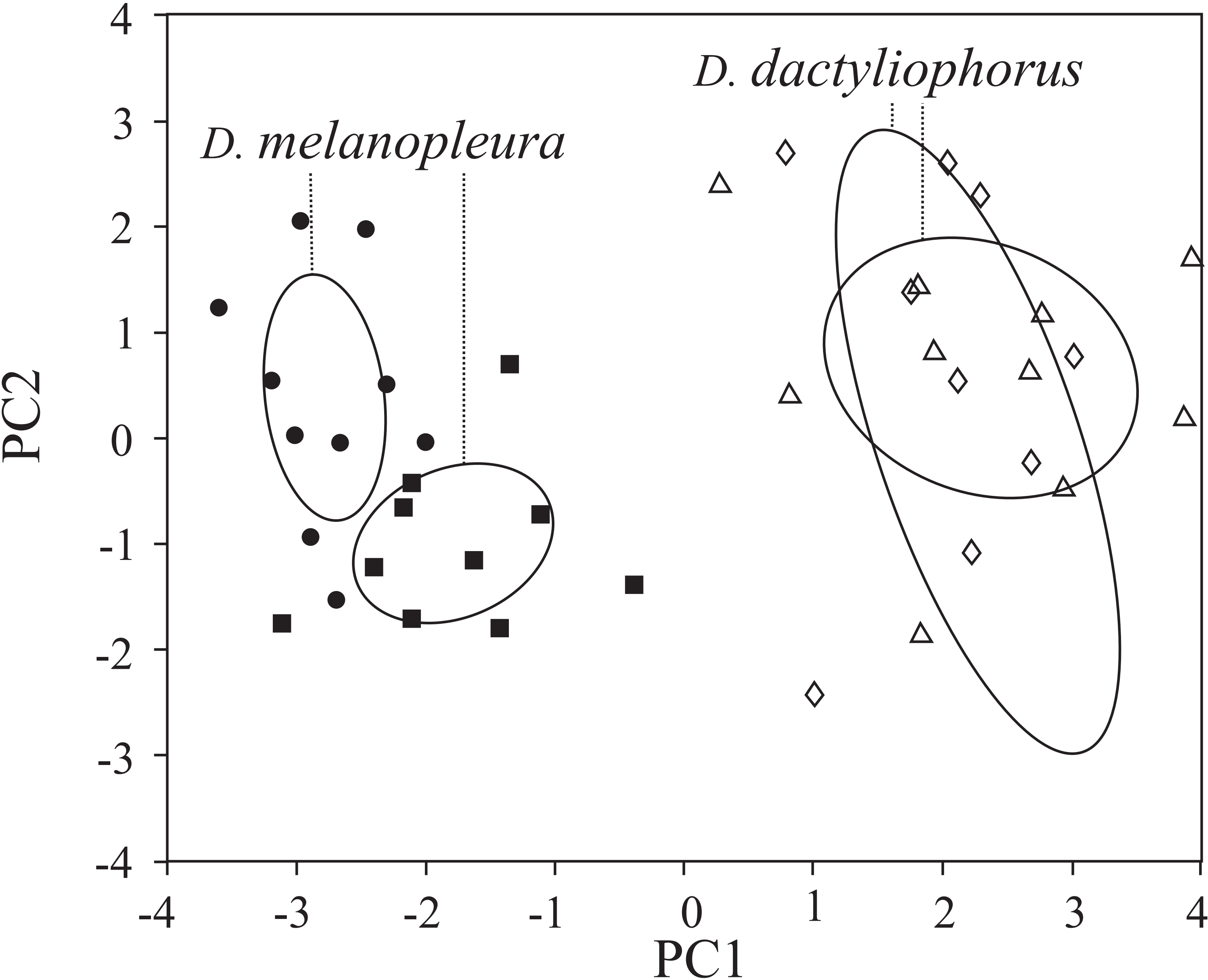


Figure 3



D. melanopleura
D. dactyliophorus
PC2
PC1
4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4

Figure 4

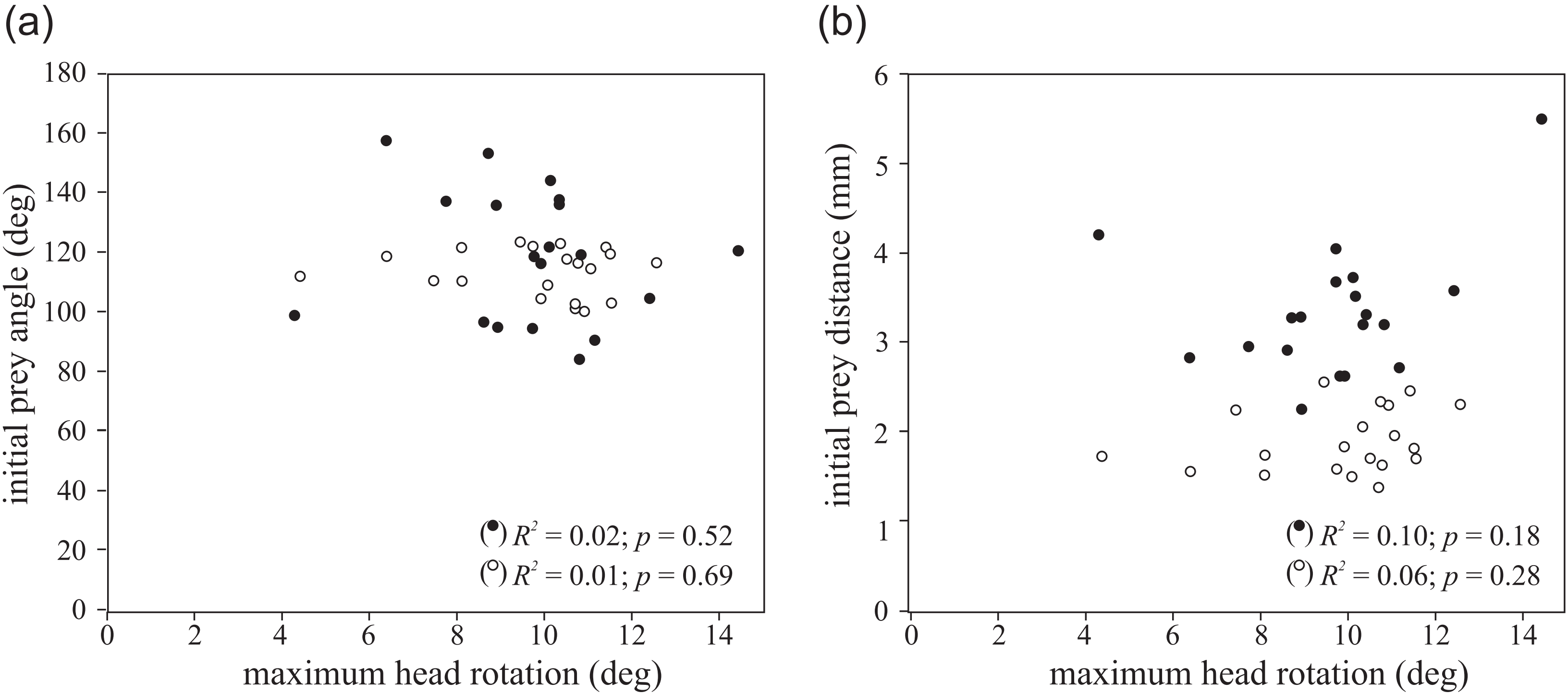

(a)
initial prey angle (deg)
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
maximum head rotation (deg)
(●) $R^2 = 0.02$; $p = 0.52$
(○) $R^2 = 0.01$; $p = 0.69$
(b)
initial prey distance (mm)
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
maximum head rotation (deg)
(●) $R^2 = 0.10$; $p = 0.18$
(○) $R^2 = 0.06$; $p = 0.28$

Table SI

Supporting Information for Van Wassenbergh *et al.* (2011) J. Fish Biol.

Table SI : Variable loadings on the principal component axes.

	Component		
	Axis 1	Axis 2	Axis 3
Initial eigenvalue	6.25	3.19	2.38
% of variance	31.23	15.93	11.87
Variable			
Snout length	**0.93**	0.17	0.02
Initial prey distance	**0.70**	-0.37	-0.03
Prey distance at end	**0.69**	-0.00	-0.58
Initial prey angle	0.16	-0.03	-0.94
Prey angle at end	0.34	0.00	-0.76
Total head rotation	0.15	-0.72	0.21
Time Total head rotation	-0.14	0.00	0.30
Max. head velocity	-0.11	-0.77	0.00
Time max. head velocity	**0.67**	0.47	0.34
Total body rotation	-0.26	0.50	0.09
Time Total body rotation	-0.03	0.14	0.10
Max. body velocity	-0.16	0.59	-0.30
Time max. body velocity	0.32	0.46	-0.01
Total mouth path length	**0.86**	-0.35	0.17
Time Total mouth path length	0.19	0.22	0.07
Max. mouth velocity	**0.59**	-0.71	-0.01
Time max. mouth velocity	**0.77**	0.32	0.26
X-coordinates *CR*	**0.69**	0.07	0.34
Y-coordinates *CR*	**0.77**	0.13	-0.00
Head rotation radius	**0.91**	0.18	-0.01

Loadings >0.50 on the axis that shows significant inter-specific differences (PC1) are marked in bold. *CR* = centre of head rotation.

Table SII

Supporting Information for Van Wassenbergh *et al.* (2011) J. Fish Biol.

Table SII: Kinematic variables (mean ± S.E) of pivot feeding.

Variable	*Doryrhamphus dactyliophorus*		*Doryrhamphus melanopleura*	
	Ind. 1 ($N = 10$)	Ind. 2 ($N = 10$)	Ind. 1 ($N = 10$)	Ind. 2 ($N = 10$)
Initial prey distance (mm)	2.60 ± 0.19	2.32 ± 0.16	2.16 ± 0.09	1.51 ± 0.04
Prey distance at end (mm)	1.36 ± 0.16	1.3 ± 0.2	0.74 ± 0.12	0.45 ± 0.06
Initial prey angle (deg)	116.1 ± 7.3	118.75 ± 7.14	111.7 ± 2.9	114.7 ± 2.1
Prey angle at end (deg)	174.3 ± 10.8	180.4 ± 11.1	157.8 ± 3.9	163.3 ± 7.2
Maximum prey path length(mm) *	0.23 ± 0.03	0.21 ± 0.01	0.17 ± 0.02	0.19 ± 0.03
Time max. prey path length (ms) *	5.50 ± 0.16	5.5 ± 0.3	3.2 ± 0.3	2.9 ± 0.3
Max. prey velocity (ms^{-1}) *	0.19 ± 0.03	0.20 ± 0.01	0.13 ± 0.02	0.13 ± 0.01
Time max. prey velocity (ms) *	4.33 ± 0.18	3.67 ± 0.18	2.3 ± 0.3	1.95 ± 0.16
Total prey path length (mm) *	0.58 ± 0.06	0.44 ± 0.02	0.44 ± 0.07	0.48 ± 0.05
Prey capture time (ms) *	5.50 ± 0.16	5.5 ± 0.3	3.2 ± 0.3	3.3 ± 0.3
Total head rotation (deg)	9.5 ± 0.8	9.8 ± 0.5	10.6 ± 0.4	9.0 ± 0.7
Time total head rotation (ms)	6.4 ± 0.7	6.9 ± 0.5	6.7 ± 0.4	6.3 ± 0.7
Max. head velocity (10^3 $degs^{-1}$)	4.2 ± 0.5	4.3 ± 0.3	4.8 ± 0.3	4.6 ± 0.3
Time max. head velocity (ms)	2.10 ± 0.16	2.25 ± 0.13	1.50 ± 0.13	1.55 ± 0.05
Total body rotation (deg)	4.0 ± 0.3	3.5 ± 0.3	3.9 ± 0.2	3.4 ± 0.2
Time total body rotation (ms)	4.0 ± 0.2	4.35 ± 0.17	4.7 ± 0.8	4.2 ± 0.8
Max. body velocity (10^3 deg)	2.1 ± 0.2	1.9 ± 0.3	2.10 ± 0.18	1.75 ± 0.16
Time max. body velocity (ms)	1.90 ± 0.07	2.00 ± 0.07	1.80 ± 0.08	1.80 ± 0.11
Total mouth path length (mm)	2.40 ± 0.15	2.50 ± 0.11	1.87 ± 0.10	1.50 ± 0.03
Time total mouth path length (ms)	9.7 ± 0.3	9.8 ± 0.3	9.1 ± 0.5	9.6 ± 0.4
Max. linear mouth velocity (ms^{-1})	0.93 ± 0.10	0.84 ± 0.04	0.77 ± 0.02	0.64 ± 0.02
Time max. mouth velocity (ms)	2.35 ± 0.13	2.55 ± 0.12	1.80 ± 0.08	1.70 ± 0.08

Note that all distances and linear velocities are scaled to a head length (excluding the snout length) of 4.98 mm.

* $N = 3$ for both *D. dactyliophorus* individuals, and $N = 9$ and $N = 10$ for *D. melanopleura* individual 1 and 2, respectively.