

Morphological Variation in Head Shape of Pipefishes and Seahorses in Relation to Snout Length and Developmental Growth

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ABSTRACT The feeding apparatus of Syngnathidae, with its elongate tubular snout and tiny, toothless jaws, is highly specialized for performing fast and powerful pivot feeding. In addition, the prolonged syngnathid parental care probably enables the juveniles to be provided with a feeding apparatus that resembles the one in adults, both in morphology and function. In this study, a landmark-based geometric morphometric analysis was carried out on the head of syngnathid representatives in order to (1) examine to what degree pipefish shape variation is different from that of seahorses; (2) determine whether the high level of specialization reduces the amount of intraspecific morphological variation found within the family; and (3) elucidate whether or not important shape changes occur in the seahorse head during postrelease ontogeny. We found that (1) there is a significant shape difference between head shape of pipefish and seahorse: the main differences concern snout length and height, position and orientation of the pectoral fin base, and height of the head and opercular bone. We hypothesize that this might be related to different prey capture kinematics (long snout with little head rotation versus short snout with large head rotation) and to different body postures (in line with the head versus vertical with a tilted head) in pipefishes and seahorses; (2) both pipefishes and seahorses showed an inverse relation between relative snout length and intraspecific variation and although pipefishes show a large diversity in relative snout elongation, they are more constrained in terms of head shape; and (3) the head of juvenile *Hippocampus reidi* specimens still undergoes gradual shape changes after being expelled from the brood pouch. Ontogenetic changes include lowering of the snout and head but also differences in orientation of the preopercular bone and lowering of the snout tip. *J. Morphol.* 272:1259–1270, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: Syngnathidae; geometric morphometrics; specialization; variation; ontogeny

INTRODUCTION

The family Syngnathidae consists of pipefishes, seadragons, pipehorses, and seahorses, all having a tubular snout with tiny, terminal, toothless jaws. The snout can be extremely elongate, up to a snout length of almost two-thirds of the total head length as in the weedy seadragon (*Phyllopteryx*

taeniolatus). In most animals, jaw morphology, mouth shape, and dentition type can provide a quite accurate idea about the preferred prey type and feeding strategy. The shape of a fish skull, consisting of over 30 moveable bony elements and more than 50 muscles (Lauder, 1983), reflects the individual's diet and prey capture mechanism to a great extent (Gerking, 1994; Delariva and Agostinho, 2001; Ferry-Graham et al., 2001; Palma and Andrade, 2002). Dietary influences on head morphology (or vice versa) are particularly evident in trophic specialists, who exploit a limited dietary breadth with respect to the available prey types in their habitat (Sanderson, 1991). These species have highly specific feeding demands and their head usually is characterized by specific adaptations that make the feeding apparatus well-suited for their diet (e.g., jaw asymmetry and specialized dental morphology in scale-eating cichlids; Liem and Stewart, 1976; Takahashi et al., 2007; Stewart and Albertson, 2010).

The syngnathid feeding apparatus and feeding strategy are highly specialized (e.g., Roos et al., 2009; Leysen et al., 2011). Seahorses and pipefishes approach their prey ventrally, or they sit and wait until a prey passes above the snout. The head rapidly rotates dorsally to position the mouth close to the prey and almost at the same time, a fast buccal expansion creates a water flow that transports the prey into the mouth (Van Wassenbergh et al., 2008). It is shown that a syngnathid suction feeding event is performed with great

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precision and at high speed (Muller and Osse, 1984; Bergert and Wainwright, 1997; de Lussanet and Muller, 2007). The rate of the incoming water is increased by the small diameter of the snout, hence suction velocity is enhanced. Prey intake time is as little as 6 ms, making syngnathids among the fastest feeding teleosts ever recorded (Muller and Osse, 1984; Bergert and Wainwright, 1997; de Lussanet and Muller, 2007; Roos et al., 2009). Due to the tiny mouth aperture and narrow snout, the syngnathid diet consists solely of small prey items (mostly crustaceans; Tipton and Bell, 1988; Teixeira and Musick, 1995; Woods, 2003; Foster and Vincent, 2004; Kendrick and Hyndes, 2005). We already noted the similarities between pipefishes and seahorses (the long and slender snout with small toothless jaws at the tip), but there are also some noticeable differences. Pipefishes (subfamily Syngnathinae) are characterized by a long, cylindrical body and a narrow head without spines, whereas seahorses (subfamily Hippocampinae) have an upright body axis with a prehensile tail and a high, tilted head usually with spines and a corona (i.e., the first nuchal plate with a crown-like process on top). In this study, we are interested in the variation in head shape of syngnathid and we address three different questions. First, we investigated whether pipefishes and seahorses occupy a distinct part of the syngnathid morphospace, or whether their head shape is spread over a continuum of shapes with transitional forms in between the typical pipefish and seahorse morphotypes.

Another interesting aspect is the hypothesis that the trophic apparatus in specialists is well-adapted to the prevailing circumstances. Hence, only a small deviation of one element of the specialized and complexly integrated feeding system may result in reduced functional performance of the whole system (Adriaens and Herrel, 2009). Consequently, it is expected that a specialized organism will show a less versatile feeding system morphology and hence a reduced amount of intraspecific variation. So our second question is whether this can be generalized for syngnathids: does the high level of specialization influences the amount of morphological variation found within the family? Long-snouted species are expected to be able to catch a prey from a greater distance and have shorter prey capture times (Muller and Osse, 1984; Kendrick and Hyndes, 2005; de Lussanet and Muller, 2007). Analysis of the syngnathid stomach content has demonstrated that long-snouted species consume a greater amount of prey items that are relatively more mobile, compared to short-snouted species (Kendrick and Hyndes, 2005). Hence, long-snouted syngnathids can be regarded as trophically more specialized and they are therefore expected to show a reduction in morphological variation with respect to shorter snouted syngnathids.

Thirdly, not only their feeding apparatus and strategy but also their remarkable parental care

makes syngnathids an interesting study object. Normally, newly hatched larval fishes are not fully developed yet; however, their feeding apparatus must be operational by the time the yolk sac is resorbed. Often this critical period is accompanied by a dietary shift during development so that in early stages feeding can occur in a different way and on different prey items than after the transition to juvenile and adult feeding. This can involve intense morphological transformations during ontogeny (e.g., the ventral shift of the mouth in the suckermouth catfish *Ancistrus cf. triradiatus* described by Geerinckx et al., 2005 or the radical transformation from tapetail to whalefish as recently discovered by Johnson et al., 2009, which consists of a major increase in jaw length among other things). In syngnathids, however, this metamorphosis does not take place (at least not after release from the pouch): newly born seahorses are known to be provided with an adult feeding apparatus and prey capture kinematics (Van Wassenbergh et al., 2009). This similarity is facilitated by the prolonged parental care in seahorses and pipefishes; embryos hatch in a special brood area on the male body where they are nourished and protected (Lourie et al., 1999; Carcupino et al., 2002; Foster and Vincent, 2004). Release of the young is delayed until an advanced developmental stage has been reached; likely the investment of seahorse parents in their offspring has extended the developmental phase in the pouch (Foster and Vincent, 2004; Dhanya et al., 2005; Van Wassenbergh et al., 2009; Leyesen et al., 2010). All elements of the external skeleton in juvenile pipefishes and seahorses are present and they are capable of independent feeding at the moment of release from the pouch (Kornienko, 2001; Foster and Vincent, 2004). Therefore, it is plausible that all major morphological transformations will take place during the period in the brood pouch. The only changes that are likely to occur after being expelled from the pouch will be the result of differential relative growth patterns (allometries) as the relative snout length of newly released seahorses is much smaller compared to the one of adults. Traditional morphometric research has demonstrated that there are some gradual ontogenetic changes in relative head and snout dimensions. Choo and Liew (2006) noted a decrease in head length, head height, snout length, and snout height in relation to standard length in *Hippocampus kuda*, with snout length and height increasing relatively to head length and height. Other allometries were observed by Roos et al. (2010) in *Hippocampus reidi*, where snout length increased and snout height decreased relative to head length. However, potential subtle differences in head shape (e.g., position of the corona, orientation of the opercular bone, steepness of the mesethmoidal curvature) rather than changes in head dimension could not be discovered with the applied linear

measurements. Detection of these shape changes within a coordinate system requires a detailed geometric morphometric analysis. The final question of this research is whether or not juvenile seahorses still show a period with substantial morphological transformations after release from the brood pouch.

The previously mentioned questions were addressed by quantifying the morphological variation in the cranium of a broad range of syngnathid representatives. First, we examined to what degree pipefish shape variation is different from that of seahorses. We performed a landmark (LM)-based geometric morphometric analysis on the external head of a large number of syngnathid species and expected it to yield a clear separation of the two subfamilies, due to the prominent differences in head height and tilting among other things. Second, we examined whether syngnathid shape variation is constrained in relation to relative snout length, either at an interspecific or intraspecific level. For this part, our predictions are based on the previously formulated assumption that a high level of specialization is associated with a reduction of morphological variety. If this hypothesis holds true, a long-snouted pipefish or seahorse species, being more specialized than a short-snouted one, will show a reduced level of intraspecific morphological variation. For the same reason, at an interspecific level, we expected pipefishes, which can have extremely elongated snouts and thus are trophically more specialized, to be morphologically more constrained than seahorses. To test this hypothesis at an intraspecific level, a regression analysis between the relative average snout length of a species and the variance of the shape variables of that species as a proxy of intraspecific variation was carried out. For the hypothesis at an interspecific level, we compared both subfamilies in terms of their variance. Finally, we investigated the hypothesis that syngnathids do not experience extensive ontogenetic shape changes from the moment they are released from the pouch because important morphological transitions have likely occurred during the period of parental care.

MATERIAL AND METHODS

In total, 368 specimens of 38 different species were analyzed morphometrically (Table 1). This includes 54 juvenile specimens of *H. reidi*, ranging in age between 1 and 65 days, which were used for the ontogenetic study in combination with the adults of *H. reidi*. Most specimens belong to the collection of the Zoological Museum of Amsterdam or the Royal Museum of Central Africa (all specimens of *Microphis brachyurus aculeatus*) and were wild caught. The specimens stored at the research group were either wild caught (*Syngnathus rostellatus*), bred at the Antwerp Zoo (*H. capensis*), or obtained from commercial trade (all others). The juvenile *H. reidi* are the offspring of seahorses held in a breeding facility at the research group (as described by Roos et al., 2010).

Animals were sacrificed by an overdose of MS 222 (tricaine methanesulfonate) and specimens were fixed in a 10% buffered

TABLE 1. List of all 38 syngnathid species plus the juveniles of *Hippocampus reidi* included in the analysis, the number of specimens of each species used, the mean relative snout length with standard deviation for those species with more than one specimen, and the intraspecific variation for the species represented by over 10 specimens (see text for explanation about the calculation of intraspecific variation)

Species	Number of specimens	Mean (SnL/HL) ± SD	Intraspecific variation
<i>Anarchopterus criniger</i> ^a	1	0.253	–
<i>A. tectus</i> ^a	1	0.250	–
<i>Bryx dunckeri</i> ^a	1	0.265	–
<i>B. randalli</i> ^a	1	0.337	–
<i>Campichthys tricarinatus</i> ^a	1	0.315	–
<i>C. balli</i> ^a	1	0.359	–
<i>C. brachycephalus</i> ^a	1	0.271	–
<i>C. hildebrandi</i> ^a	1	0.328	–
<i>Minyichthys inusitatus</i> ^b	1	0.238	–
<i>Siokunichthys bentuvai</i> ^a	1	0.180	–
<i>Syngnathus parvicarinatus</i> ^c	1	0.278	–
<i>Microphis caudocarinatus</i> ^a	1	0.420	–
<i>Doryrhamphus dactyliophorus</i>	1	0.662	–
<i>Gasterotokeus biaculeatus</i>	1	0.539	–
<i>Hallichthys taeniophorus</i>	1	0.569	–
<i>Doryichthys martensii</i>	2	0.347 ± 0.013	–
<i>Dunckerocampus pessuliferus</i>	2	0.649 ± 0.083	–
<i>D. melanopleura</i>	3	0.438 ± 0.023	–
<i>D. janssi</i>	6	0.572 ± 0.012	–
<i>D. retzii</i>	6	0.372 ± 0.018	–
<i>Corythoichthys intestinalis</i>	7	0.469 ± 0.019	–
<i>S. rostellatus</i>	15	0.458 ± 0.011	1.85E – 03
<i>Siphonostoma typhle</i>	15	0.483 ± 0.038	4.41E – 03
<i>Syngnathoides biaculeatus</i>	18	0.553 ± 0.026	2.01E – 03
<i>M. brachyurus aculeatus</i>	95	0.578 ± 0.012	1.16E – 03
<i>Hippocampus hippocampus</i>	1	0.328	–
<i>H. breviceps</i>	2	0.424 ± 0.014	–
<i>H. barbouri</i>	3	0.502 ± 0.017	–
<i>H. spinosissimus</i>	3	0.375 ± 0.028	–
<i>H. zosteræ</i>	3	0.315 ± 0.135	–
<i>H. capensis</i>	4	0.338 ± 0.022	–
<i>H. abdominalis</i>	5	0.390 ± 0.024	–
<i>H. histrix</i>	5	0.476 ± 0.015	–
<i>H. trimaculatus</i>	5	0.496 ± 0.006	–
<i>H. reidi</i>	12	0.426 ± 0.026	3.92E – 03
<i>H. ramulosus</i>	18	0.354 ± 0.032	9.13E – 03
<i>H. guttulatus</i>	29	0.360 ± 0.029	8.56E – 03
<i>H. kuda</i>	40	0.428 ± 0.036	7.46E – 03
<i>H. reidi</i> juveniles	54	0.442 ± 0.028	–

HL, head length; SnL, snout length.

^aDrawing made by Dawson (Kuitert, 2003).

^bDrawing made by Dawson (Dawson, 1983).

^cDrawing made by Dawson (Dawson, 1978).

and neutralized formalin solution. After anesthetization, the right side of the head of all animals was photographed and a scale bar was included in all images. Pictures of small individuals were captured using a digital camera (ColorView 8, Soft Imaging System, GmbH Münster, Germany) mounted on a stereoscopic microscope (Olympus SZX-9) and driven by the software program analySIS 5.0 (Soft Imaging System). Larger specimens were photographed by means of a macroscopic digital camera (Canon EOS 400D or Nikon D40X, respectively, with a Canon EF-S 18–55 mm f/3.5–5.6 and a 18–55mm f/3.5–5.6GII AF-S DX NIKKOR lens). All specimens were oriented in a plane parallel to the camera, with maximal overlap of paired anatomical structures as to minimize orientational errors.

The geometric morphometric analysis involved the use of the Thin Plates Spline freeware (tps; Rohlf, F.J., State University of Stony Brook, New York). First tpsUtil version 1.46 (Rohlf, 2010a) was used for construction of the tps-files and LMs were digitized on the pictures of the head with tpsDig2 version 2.16

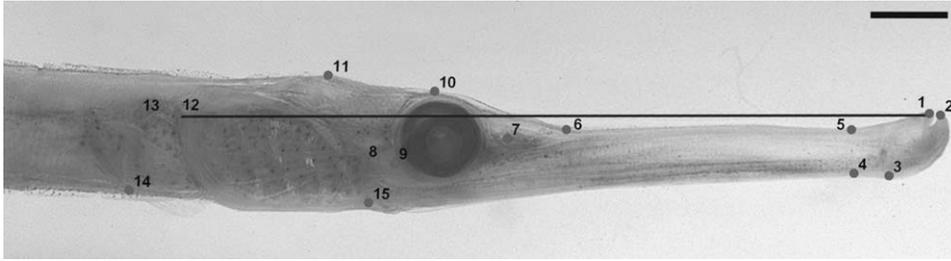


Fig. 1. Right lateral side of the head of a *Microphis brachyurus aculeatus* specimen showing the 15 landmarks used for the geometric morphometric analysis. The thin black line, connecting landmark 1 with landmark 12, measures head length and was used to define landmarks 10 and 11. See text for an anatomical description of the landmarks. Scale bar, 2 mm.

(Rohlf, 2010b). The following 15 homologous LMs were digitized (Fig. 1): (1) dorsorostral tip of premaxillary bone; (2) distal point of dentary bone; (3) ventrocaudal point of lower jaw below suspensorial articulation; (4) ventral point where snout is dorsoventrally at its narrowest; (5) dorsal point where snout is dorsoventrally at its narrowest; (6) base of mesethmoidal curvature; (7) caudal border of nostril; (8) operculo-hyomandibular articulation; (9) ventrorostral tip of sphenotic bone; (10) most dorsal point of skull at the level of the eye with respect to the line connecting LM1 with LM12; (11) most dorsal point of skull at the level of the braincase with respect to the line connecting LM1 with LM12; (12) base of cleithral curvature where the gill slit is situated; (13) dorsal point of pectoral fin base; (14) ventral point of pectoral fin base; and (15) ventrocaudal tip of preopercular bone. For all specimens, head length was calculated as the distance between LM1 and LM12, snout length as the distance between LM1 and LM7, and snout height as the distance between LM4 and LM5. The inter-LM distances were calculated using PAST version 2.03 (Hammer et al., 2001).

To remove all nonshape variations (i.e., variation in position, orientation, and scale), the LMs were submitted to a generalized Procrustes analysis. TpsSmall version 1.20 (Rohlf, 2003) was used to confirm the fitness of the dataset for further statistical analysis. Next, for each specimen partial warps were computed in tpsRelw version 1.49 (Rohlf, 2010c) and submitted to a principal component analysis resulting in relative warps (RWs) that summarize the variation among the specimens. Deformation grids were generated to visualize the shape variation, and RW scores and centroid size were saved for further analyses.

One tps-file comprising all pipefishes and one for the seahorses were loaded in CoordGen6f (Integrated Morphometrics Package Coordinate Generating Utility; <http://www3.canisius.edu/~sheets/morphsoft.html>) and TwoGroup6h (Integrated Morphometrics Package; <http://www3.canisius.edu/~sheets/morphsoft.html>), both being part of the Integrated Morphometrics Package freeware (Sheets, H.D., Canisius College, Buffalo, New York). The (dis)similarity of pipefishes versus seahorses was determined by means of a Goodall's F -test, which examines differences in mean shape between two groups relative to the shape variation found within the groups. It compares the Procrustes distance between the means of two samples to the amount of variation found in the samples. The probability that this F -score could have arisen by chance was tested by a Bootstrap resampling procedure (4,900 Bootstraps).

Finally, tpsRegr version 1.37 (Rohlf, 2009) was used to perform regression analyses between the obtained RW scores on the one hand and centroid size, log head length, or snout length over head length (i.e., relative snout length) on the other hand.

The range of snout lengths in our sample did not adequately reflect the diversity in relative snout length known to exist in the pipefish subfamily; it lacked short-snouted pipefishes. For example, species like *Siokunichthys bentuviai* and *Anarchopterus tectus* have a relative snout length of, respectively, about 0.180 and 0.250 (measurements made on drawings by Dawson

in Kuitert, 2003), which is even shorter than the relative snout length of the seahorse species with the shortest snout in our analysis, *Hippocampus zosterae*. Therefore, drawings of the lateral side of the head of 11 short-snouted species and one long-snouted species (*Microphis caudocarinatus*) made by Dawson (1978; 1983; Kuitert, 2003) were added to the original dataset (Table 1). These drawings did not include a scale bar, hence for analyses relying on size factors (e.g., regression between centroid size and RW scores) the drawings were left out. The use of the dataset without the drawings is clearly mentioned throughout the results.

The position of LM2 (on the distal point of the dentary bone) is expected to be dependent on the gape; in specimens with a depressed lower jaw, LM2 will be located more ventrorostrally compared to its position in fishes with a closed mouth. In order to test whether the use of LM2 confounded the actual shape variation (due to the variation in the gape), a two-block partial least squares (PLS) analysis was performed on the shape data with and that without LM 2. For this, tpsPLS version 1.18 (Rohlf, 2006) was used to calculate correlations between the paired vectors for each shape dataset (resulting from a PLS analysis of the covariation between the partial warp scores of both datasets), and a permutation test was performed to calculate the level of significance (1,000 randomizations).

To compare the morphospace occupied by pipefishes on the one hand to the one of seahorses on the other hand, the eccentricity (E) was calculated as described by Turner et al. (2010). They define the eccentricity as $E = (1 - \lambda_2/\lambda_1)$, with λ_i is the eigenvalue of the i th principal component.

Another tps-file was made, comprising only *H. reidi* specimens, both adult and juvenile. Identical LMs were placed, a PC analysis was carried out and deformation grids were used to visualize shape changes. In order to test whether the observed ontogenetic shape variation in *H. reidi* was the result of size-dependent shape variation, a multivariate regression analysis was performed on all shape variables (i.e., partial warp scores) versus the log of head length as the independent variable (because we did not know the exact age of all specimens head length was used as a proxy, correlation coefficient between age and head length is 0.93).

For those species represented in the analysis by more than 10 specimens, the species variance was calculated using the following formula:

$$\sum_j \frac{d_{ij}^2}{n_i - 1}$$

with d_{ij} the Euclidean distance of all RW scores of the j th specimen in species i to the RW scores of the species consensus, and n_i the number of specimens in species i . The species variance is a measure of how much each specimen within a species deviates from the species consensus; the larger the variance, the more diverse that species is. Hence, it is a reflection of the morphological intraspecific variation of a species.

The same equation was used to determine a measure for interspecific variation for pipefishes and seahorses; only the overall subfamily consensus was used instead of the species consensus and all species variances were summed for each subfamily. The previously mentioned equation is calculated analogous to the partial disparity described by Foote (1993), but it has the number of specimens in a subgroup ($n_i - 1$) in the denominator rather than the total number of specimens of all subgroups combined ($N_i - 1$). In this way, the proxy for intraspecific variation is less susceptible to differences in subgroup size.

RESULTS

Overall Shape Variation

First, the two-block PLS analysis showed that LM2 did not influence the observed shape pattern, as the paired vectors for both datasets (with and without LM2) were highly correlated for the first 21 vector pairs (correlation coefficient between 0.92 and 1.00) of a total of 24 pairs (remaining three showed correlation coefficients as low as 0.59; $P = 0.0001$). As such, the observed patterns based on the inclusion of LM2 can be considered as representative of the biologically relevant head shape variation. In addition, we could prove that simplification of a picture by a drawing did not affect the placing of LMs as the results after adding the drawings were as expected: the short-snouted species all cluster together near the *Doryichthys* species, which are also short snouted, and the long-snouted pipefish *Micropphis caudocarinatus* could be found closer to the other long-snouted species (Fig. 2). As expected, the Goodall's F -test showed that there is a significant difference between pipefishes and seahorses (F score = 713.03; $df = 26.00$, 7800.00; P level < 0.0001; Bootstrap P level = 0.0002).

The first and second RW scores are plotted to visualize the shape differences (Fig. 2). The first axis of the RW plot (RW1), which explains 75.08% of the total variation, separates pipefishes from seahorses. Positive scores of the RW1 (i.e., pipefish morphotype) mainly reflect a vast decrease in head height with respect to the consensus (ventral shift of LM10 and LM11) and a dorsal displacement of the shortened pectoral fin base (dorsal shift of LM13 and LM14). In addition, they reflect a dorsoventral shortening of the opercular bone (shortening of the distance between LM15 and LM8), an increase in snout length (caudal displacement of LM6 and LM7), and a dorsoventral lowering of the snout and lower jaw (dorsal shift of LM2, LM3 and LM4). Specimens at the other extreme (i.e., seahorse morphotype) have a large and very high head (dorsorostral shift of LM10 and dorsocaudal shift of LM11), a dorsoventrally enlarged opercular bone (dorsal shift of LM8 in combination with the ventral displacement of LM15), a relatively short but high snout (convergence of LM1-5 with LM6-7 together with a ventral displacement of LM3, LM4, and LM15) and an elongated and ventrally displaced pectoral fin base (ventrorostral shift of

LM13 and even more so for LM14) compared to the consensus configuration.

The shape changes represented by the second RW axis, which explains 10.02% of the total shape variation, also reflects variation in snout height (dorsoventral shift of LM1 and LM5), snout length (divergence or convergence of LM5 and LM6), and head length (rostrocaudal movement of LM8 and LM15 together with rostrocaudal movement of both LM13 and LM14) but all to a lesser degree compared to RW1. Besides that, RW2 represents variation in a rostrocaudal pectoral fin base displacement (shifts in LM13 and LM14 position along the long axis of the head) and in the orientation of the opercular bone (rostrocaudal movement of LM8 with respect to LM15).

Shape Variation and Snout Dimensions

The regression analysis (of the dataset without the drawings) showed that less than 3.5% of the total shape variation was explained by either centroid size or log head length, indicating that the obtained RW scores are independent of size. On the other hand, 65.32% of the overall variation is explained by differences in relative snout length. Of all RWs, the first is the only one that is correlated with relative snout length (a correlation coefficient of 0.89). Hence, these results confirm that one of the main differences between the pipefish and seahorse morphotype is their relative snout length, as was already evident from the comparison of deformation grids along RW1. Also, the RW scores of the first axis are significantly negatively correlated with relative snout height (a correlation coefficient of -0.92).

Four pipefish and four seahorse species are represented in the analysis by more than 10 specimens and thus their species variance was calculated as a proxy for intraspecific shape variation as described earlier (Table 1). Both pipefish and seahorse subfamilies seemed to show an inverse relation between relative snout length and intraspecific variation, so the morphological variation of long-snouted species is likely to be more constrained than that of short-snouted species (Fig. 3). However, with only four data points in each group, there was no sufficient statistical support for this trend. Another thing that can clearly be noted is that almost each seahorse species shows a higher amount of intraspecific variation than the pipefishes. This was also found on an interspecific level, overall variation for pipefishes was only 62.33% of the variation found in seahorses (5.13×10^{-3} versus 8.23×10^{-3}).

It is obvious from the plot of RW1 versus RW2 scores (Fig. 2) that the seahorse specimens are scattered all around the graph whereas the space occupied by the pipefishes is more stretched along an axis as if not all possible positions in the syngnathid

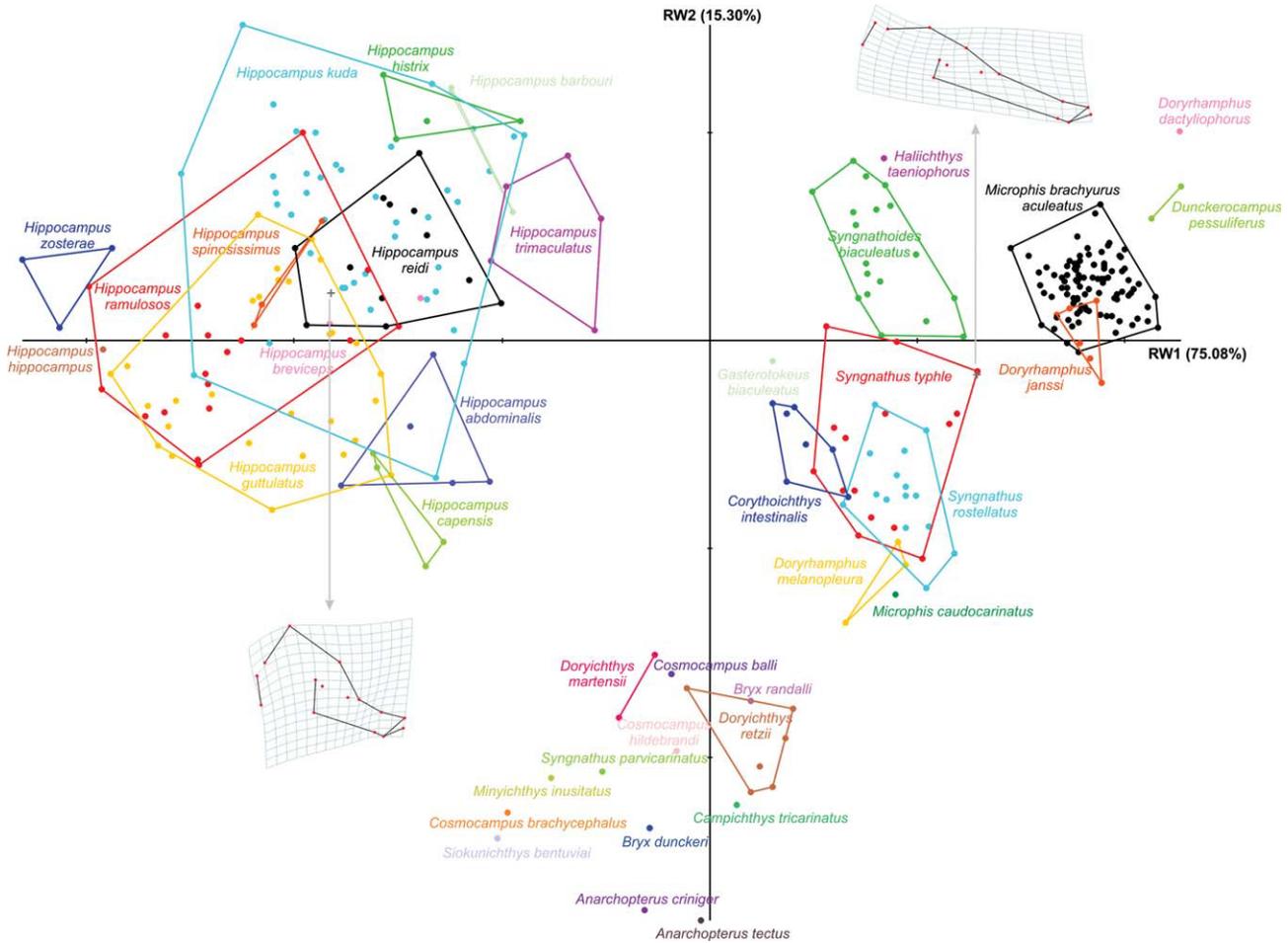


Fig. 2. Scatter plot of relative warps 1 and 2, together explaining 90.38% of the total shape variation. The position of the mean subfamily configuration is depicted by a “+” (right for pipefishes, left for seahorse) and the corresponding deformation grids with respect to the consensus are illustrated.

morphospace could be occupied. This difference was quantified by calculating the eccentricity (E) of both groups. For pipefishes $E = 0.453$ whereas seahorses have an eccentricity $E = 0.0011$, confirming the almost circular distribution of seahorses compared to the more linear relationship of pipefishes. This proves again that seahorses show a higher level of interspecific variation and that pipefishes all follow a similar morphological pattern. The pipefish morphospace is best described as an ellipse with the long axis primarily explaining differences not only in relative snout length, but also in snout height, rostrocaudal position of the pectoral girdle, head height, and opercular height. This trend probably depends greatly on the selection and number of specimens and species in the analysis.

Ontogenetic Scaling

The adult *H. reidi* specimens are well separated from the other age classes by a combination of the first and second RW axis, explaining, 40.25% and

16.75%, respectively, of the total shape variation (Fig. 4). Apparently, even after 65 days, changes in head shape still occur. Ontogenetic shape transi-

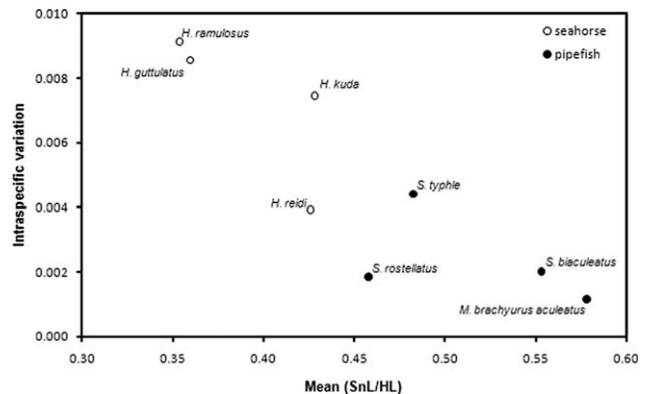


Fig. 3. Scatter plot of the mean relative snout length versus the intraspecific shape variation of four pipefish species (black dots) and four seahorse species (white dots). HL, head length; SnL, snout length.

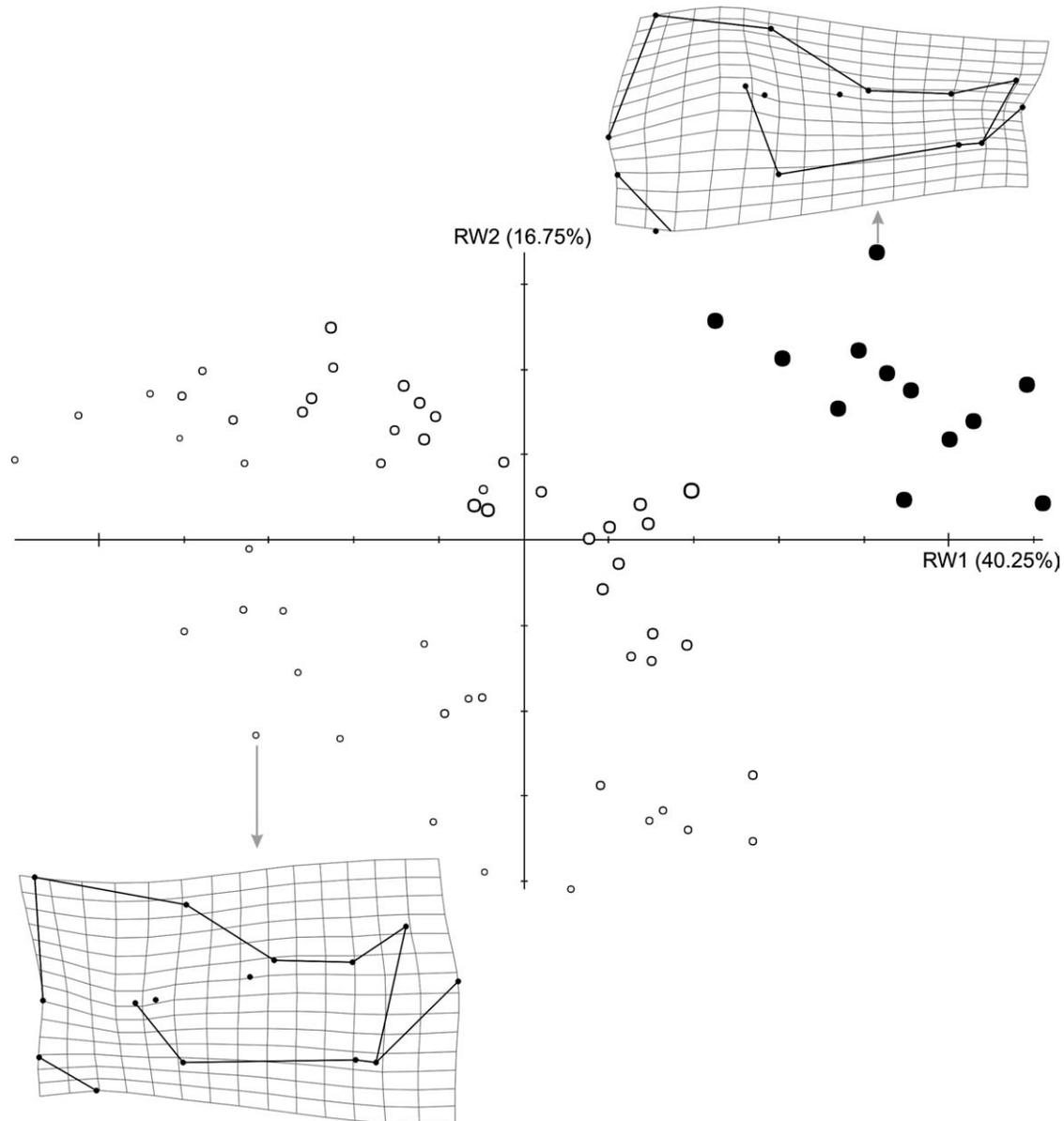


Fig. 4. Scatter plot of relative warps 1 and 2 of the ontogenetic series of *Hippocampus reidi*, together explaining 57% of the total shape variation. Black dots represent adults, white dots are juveniles. White dots are scaled according to specimen head length. The deformation grids of the two encircled specimens, a 1-day-old (below) and an adult (above), are visualized.

tions from a 1-day-old stage to adult involve a reduction of the obtuse angle between the rostral edge of the opercular bone and the snout to an almost right angle (dorsorostral shift of LM8 and to a lesser degree of LM15), dorsoventral lowering of the snout tip (ventral displacement of LM1 with respect to LM5) and tilting of the pectoral fin base (dorsocaudal shift of LM13 and even more of LM14), besides a dorsoventral lowering of the head with respect to its length (ventral displacement of LM10 and LM11) and a dorsoventral compression of the snout (dorsal shift of LM3, LM4, and LM15) (Fig. 4). Although there are changes in

head shape during postrelease development, the regression analysis of head shape versus size showed that only very little of the observed shape variation could be interpreted as size-related shape variation (29.46% of the total variation, $P = 0.0001$).

DISCUSSION

Pipefish Versus Seahorse Shape Variation

Both subfamilies are morphologically clearly separated from each other, suggesting a bimodal distribution in head shape on subfamily level

instead of a morphological continuum (Fig. 2). The main differences between the head shape of pipefish and seahorse are snout length and height, position and orientation of the pectoral fin base, and height of the head and opercular bone. The short-snouted pipefishes cluster at the left hand side of the pipefish morphospace, as expected as the first RW axis, separating pipefishes (+RW1) from seahorses (−RW1), is highly correlated with snout length (Fig. 2). However, they do not occur more to the left in the plot than *H. zosterae*, yet the relative snout length of some of them is smaller. This confirms that RW1 reflects more than just relative snout length, as elaborated on in the results.

The first RW axis is correlated positively with snout length and negatively with snout height. When relative snout length is plotted over relative snout height for all syngnathids studied, linear regression yields a negative slope (slope = −0.3794, $R^2 = 0.81$, P level < 0.0001). Hence an increase in relative snout length results in a decrease of relative snout height and this relation is more pronounced in pipefishes (slope = −0.3134, $R^2 = 0.67$, P level < 0.0001) compared to seahorses (slope = −0.1982, $R^2 = 0.36$, P level < 0.0001). In pipefishes, absolute snout height does not vary much over a wide range of snout lengths, so long-snouted pipefishes tend to have a relatively narrow snout. de Lussanet and Muller (2007) have determined that the optimal snout length of syngnathids is inversely related with snout cross-section. If it can be assumed that natural selection would favor an optimal snout length, our results confirm the hypothesis of de Lussanet and Muller (2007). They stated that a long snout is advantageous as it minimizes the angle over which the head must be turned and hence reduces the time to reach the prey (de Lussanet and Muller, 2007). However, a longer snout implicates a larger striking distance, which may reduce striking success (Van Wassenbergh et al., 2011a). Moreover, an increased snout length implies a higher total moment of inertia, which negatively influences the angular acceleration of head rotation. Reduction of the cross-sectional area of the snout decreases the total moment of inertia and compensates for the longer snout. This is confirmed by our analysis. Muller and Osse (1984) reasoned that the velocity and acceleration of the water relative to the mouth opening increases with increasing snout length, hence faster prey can be caught. Roos et al. (2010) established this experimentally as they found that the snout length in both juvenile and adult *H. reidi* approaches the optimal length for a given width, in order to minimize the time to reach prey. Apparently, long-snouted species only require limited head rotation in comparison with a short-snouted species that requires a large excursion of the head to overcome the same distance. In

addition, the increase in cranial rotation performance by snout elongation is opposed to the associated reduction in suction performance (Roos et al., 2011). Improvement of the efficiency of the two different aspects of pivot feeding, that is, head rotation versus suction performance might be reflected in pipefish versus seahorse morphology. Seahorses have an upright posture and in rest their head is at an acute angle with their body. During head rotation of a feeding strike, the supraoccipital bone slides under the corona (thus producing the characteristic clicking sound; Colson et al., 1998). Pipefishes, on the other hand, have their head in line with their body at the beginning of a prey capture event. Manipulation of cleared and stained specimens (*D. dactyliophorus*, *S. rostellatus*, and *C. intestinalis*) showed that from a certain angle, further head rotation is mechanically impeded. Either the cranio-vertebral joint morphology limits neurocranial elevation or the supraoccipital bone or the epaxial bones (very long and slender ossifications within the tendon of the epaxial muscle in some pipefishes, see Leysen et al., 2011) are pushed against the first nuchal plate (no sliding was observed in pipefishes). Muller and Osse (1984) suggest a mechanical limitation of head rotation by the ligament connecting the supraoccipital bone with the cleithrum (which they refer to as the plate formed by the fused pectoral radials and dorsal vertebral processes). We presume that the seahorse configuration might allow for a greater rotation of the head with respect to the body compared to the pipefish situation. If this hypothesis holds, seahorses do not need to have an extremely elongated snout to overcome the prey–mouth distance as a vast amount of cranial rotation would suffice to reach the prey. A recent study by Van Wassenbergh et al. (2011b) proved that the bent trunk orientation of seahorses improves feeding performance by increasing the strike distance. A typical pipefish body orientation, on the other hand, appears to be beneficial by increasing strike velocity. Another reason to assume improved head rotation performance with an increase in tilting of the head is the large insertion site for the well-developed epaxial muscle, which plays a crucial role in neurocranial elevation, on the high supraoccipital bone in seahorses. Experimental support for the hypothesis is found in kinematical data: neurocranial elevation in adult *H. reidi* reaches 31° (Roos et al., 2009) and even as much as 40° in juvenile *H. reidi* (Van Wassenbergh et al., 2009), versus a rotation of only about 20° in *Syngnathus leptorhynchus* (Van Wassenbergh et al., 2008) and rotation does not exceed 10° in *Doryrhamphus dactyliophorus* or *D. melanopleura* (Van Wassenbergh et al., 2011a). However, Bergert and Wainwright (1997) found a neurocranial elevation of 29° in *S. floridae*, equal to what they recorded for *Hippocampus erectus*, which shows that our hypothesis

cannot be generalized for all syngnathids. A comparative study of the supraoccipital-nuchal plate morphology and mechanics that will help resolve this is called for.

Other morphological differences between pipefish and seahorse heads, that is, ventral position of the pectoral fin base and opercular bone higher than long in the latter, might also be explained as a consequence of the head tilting in seahorses. The pectoral girdle is attached to the vertebral column so the ventral displacement of the pectoral fin base observed in our deformation grids actually represents a shift of the entire body from a caudal position to a ventrocaudal one with respect to the head. At the same time, by tilting the head with the pectoral girdle immovably fixed to the vertebral column, the distance between the caudal margin of the preopercular bone and the rostral tip of the cleithrum is reduced leaving only little space for the gills in rostrocaudal direction. This might have caused the branchial chamber in seahorses to expand more in a dorsoventral direction hence the height of the opercular bone exceeds its length (the opposite is the case in pipefishes).

We demonstrated the existence of several differences in the head shape of pipefishes and seahorses, yet they evolved from the same ancestor. The oldest fossils of the family date 50 Myr back and morphologically resemble modern pipefishes (Teske et al., 2004; Teske and Beheregaray, 2009). It would be interesting to see where in the syngnathid morphospace the most recent common ancestor, from which pipefishes and seahorses diverged from each other, will plot. According to a recent publication by Teske and Beheregaray (2009), pipefishes of the genus *Idiotropiscis* can be regarded as the extant evolutionary link between seahorses and pipefishes. These seahorse-like pipefishes swim horizontally but their head is tilted ventrally over an angle of about 25° with respect to the body (Kuitert, 2004). Based on pictures, the head of *Idiotropiscis* species seems to stand midway between the one of pipefishes and seahorses in terms of snout and head dimension. Hence, it is expected to show other transitional features as well, for instance, in the position of the pectoral fin and the shape of the opercular bone. A future geometric morphometric analysis that includes one of the three *Idiotropiscis* species might yield elucidating results with respect to syngnathid evolution.

Shape Variation and Snout Length

We hypothesized that there would be a greater amount of morphological variation within a short-snouted species versus within a long-snouted one (intraspecifically) and also in seahorses compared to pipefishes (interspecifically). As explained before, the expectation that long-snouted species would be morphologically more constrained follows from the

experimentally based assumption that they are more specialized. Support for syngnathids with a long snout being more specialized was found in the analyses of gut contents. The diet of long-snouted syngnathids consists of a higher number of mobile prey compared to what is ingested by short-snouted species (Franzoi et al., 1993; Kendrick and Hyndes, 2005). This suggests that a long snout enables a species to capture prey from a greater distance and in a shorter time, as argued by Muller and Osse (1984) and de Lussanet and Muller (2007), and was later demonstrated by Van Wassenbergh et al., (2011a). Hence long-snouted syngnathids can be confidently regarded as trophic specialists.

Our preliminary results on intraspecific variation are within our expectations: for both pipefishes and seahorses, there is an indication of a negative relationship between relative snout length and degree of intraspecific variation. In other words, the more specialized a species (longer snout), the more morphologically constrained it is (reduced variation). However, we must be careful when drawing conclusions from these results as we only looked at four different species for each subfamily and also because both the correlation coefficient and the slope in pipefishes are small ($R^2 = 0.27$, slope = -0.013). Adding another species might change the observed relationship between pipefishes and seahorses. For future research, it will be interesting to include other species, both short-snouted ones (for instance *H. zosterae* and *S. bentuviai*) and long-snouted species (*D. dactyliophorus* and *H. barbouri*) to see if the present inverse relation still stands.

On an interspecific level, seahorses show a higher degree of variation compared to pipefishes. However, we must note that the average relative snout length of the four pipefish species is higher than that of the four seahorse species. As we saw that long-snouted species are morphologically more constrained than short-snouted ones, the difference in interspecific variation (i.e., pipefishes having less variation than seahorses) is possibly the result of our selection of long-snouted pipefishes versus shorter snouted seahorses. Nevertheless, if we look at the distribution of all specimens, both long- and short-snouted ones, in the plot of RW1 versus RW2 scores (Fig. 2), we see that the morphospace occupied by seahorses is smaller compared to the pipefish morphospace. Hence, the diversity in relative snout shape is larger for pipefishes than for seahorses. Yet, the fact that the pipefish specimens have an elliptical distribution in the plot suggests that they are more constrained in terms of cranial morphology as all specimens follow a similar allometric pattern. Although some further investigation is needed, for now we can conclude that the level of interspecific variation in seahorses probably is higher than that in pipefishes.

A methodological consideration might be the fact that in these analyses specimens belonging to the

“species” *H. guttulatus*, *H. kuda*, and *H. ramulosus* were used. These species are known to be species complexes and they encompass various morphotypes. Lourie et al. (1999) do not even recognize *H. ramulosus* as a separate species but rather as a synonym of *H. guttulatus*. Currently, there is no alternative to using these species complexes as they have not been revised yet. The use of the invalid species name *H. ramulosus* should be avoided, however, the seahorse identification key (Lourie et al., 2004) often does not yield one unambiguous species name but rather various possible solutions. Hence, here the assignment of specimens to the species complexes is preferred over the use of unidentifiable specimens. Furthermore, this complication of seahorse identification does not take the edge of the results of the geometric morphometric analysis. For the calculation of the RW scores, the variation among specimens (and not groups) is calculated hence whether a specimen belongs to species A or species B will not change its position on the RW1 versus RW2 plot. On an intraspecific level, it might be so that what we consider now as one species in fact are several, which could reduce the amount of intraspecific variation for the separated species compared to what we have found here. However, there is no reason to assume that breaking down the species complex into separate species will affect the intraspecific variation differently for long- versus short-snouted species. Hence, in the absence of taxonomical clarification, we do not consider the use of species complexes an issue.

Shape Variation and Ontogenetic Scaling

No evidence of saltatory development was found that could contradict the hypothesis that in syngnathids complex morphological changes and ontogenetic modifications takes place during the extended period of parental care in the brood pouch and not afterward. In many other fish, freshly hatched embryos possess a yolk sac for endogenous nutrition. The yolk sac will be depleted at the transition to active feeding; a period characterized by the development of the jaw apparatus and hence changes in head shape. In syngnathids, there is no larval period and metamorphosis is absent or occurs in the brood pouch, so the feeding apparatus is already fully developed before emerging (Kornienko, 2001; Choo and Liew, 2006). This is consistent with the findings for *Syngnathoides biaculeatus*, *H. kuda*, and *H. reidi*, where the newborns resemble miniature adults with a completely functional feeding apparatus and a fully absorbed yolk sac (Dhanya et al., 2005; Choo and Liew, 2006; Roos et al., 2011). Another reason to expect little change is the fact that prey capture events in juvenile seahorses are observed to be even faster than in adults, 2.5 ms compared to 5 ms (Roos

et al., 2009; Van Wassenbergh et al., 2009). To ensure the success of the incredibly rapid pivot feeding, the combined movement of all elements involved in the feeding apparatus (lower jaw, hyoid, neurocranium, suspensorium, and operculum) is required. The slightest deviation of one element from its normal path might cause a reduction in performance. So, juvenile seahorses are expected to be highly constrained, not only morphologically but also kinematically.

Still the juveniles differ from the adults; among the main allometric shape changes are not only lowering of the snout and head with respect to snout length but also a different orientation of the preopercular bone and of the snout tip. Roos et al. (2010) noticed a transition from short and broad snouts to long and narrow ones during the development of *H. reidi*. Reynolds number of the juvenile's environment is relatively low and therefore the escape response of the (even smaller) prey items will be limited (Larsen et al., 2008). High-velocity head rotation for a fast approach of the prey is thus not a necessity; on the other hand, powerful suction is required to set the viscous fluid into motion. A feeding apparatus with a short and broad snout is beneficial for optimizing suction performance and is hence typically found in juvenile syngnathids. This supports our findings that the head of juvenile seahorses still undergoes gradual shape changes after release from the brood pouch.

Probably the allometric shifts in buccal cavity shape, changes in the orientation of the opercular bone, and lowering of the snout tip will have an effect on the hydrodynamics of suction feeding. The high efficiency of the feeding mechanism in juvenile *H. reidi* can most likely only be assured if along with these morphological changes the kinematics of prey capture were also altered to a certain degree. Indeed, ontogenetic changes in the snout dimension of *H. reidi* appear to have an influence on suction feeding capacity and on cranial rotation performance (Roos et al., 2011).

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