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Sex-specific responses to fecundity selection in the broad-nosed pipefish

Jasmin D. Winkler · Kai N. Stölting · Anthony B. Wilson

Abstract Fecundity selection, acting on traits enhancing reproductive output, is an important determinant of organismal body size. Due to a unique mode of reproduction, mating success and fecundity are positively correlated with body size in both sexes of male-pregnant *Syngnathus* pipefish. As male pipefish brood eggs on their tail and egg production in females occurs in their ovaries (located in the trunk region), fecundity selection is expected to affect both sexes in this species, and is predicted to act differently on body proportions of males and females during their development. Based on this hypothesis, we investigated sexual size dimorphism in body size allometry and vertebral numbers across populations of the widespread European pipefish *Syngnathus typhle*. Despite the absence of sex-specific differences in overall and region-specific vertebral counts, male and female pipefish differ significantly in the relative lengths of their trunk and tail regions, consistent with region-specific selection pressures in the two sexes. Male pipefish show significant growth allometry, with disproportionate growth in the brooding tail region relative to the trunk, resulting in increasingly skewed region-specific sexual size dimorphism with increasing body size, a pattern consistent across five study populations. Sex-specific differences in patterns of growth in *S. typhle* support the hypothesis that fecundity selection can contribute to the evolution of sexual size dimorphism.

Keywords Allometric growth · Life history evolution · Pleomerism · Sexual selection · Sexual-size dimorphism

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Introduction

The study of intraspecific variation in body size can provide insights into how historical selective forces have influenced morphological evolution. Natural selection, often called viability or survival selection, is believed to act upon traits associated with survival, whereas fecundity and sexual selective pressures affect traits that enhance reproductive output and reproductive success (Darwin 1871; Mayr 1972; Andersson 1994). In addition to selection, phenotypic plasticity and developmental constraints can also influence morphological evolution, adding a layer of complexity to the study of natural variation.

Species which show sexual dimorphism in body size and vertebral counts offer relatively simple systems in which questions related to the relative importance of selective forces in generating morphological variation can be addressed (Shine 2000), as body size is under selection in most organisms (Blanckenhorn 2000), and vertebral number is often positively correlated with body size (Lindsey 1975). Pleomerism, the positive correlation between maximum body length and vertebral number, has been found across different hierarchical levels in many fishes: within suborders, families, genera, and species (Lindsey 1975). Intraspecific variation in vertebral number is also correlated with sexual size dimorphism (SSD) in several fish species (Springer 1971; Lindsey 1975), and region-specific body size changes have been shown to correspond to changes in regional vertebral counts across several lineages of actinopterygians (Ward and Brainerd 2007), supporting the tight link between vertebral and body size evolution in this group. As vertebral number is fixed early in ontogeny, the presence of pleomerism in a species illustrates how adult body size and proportions can be influenced by factors fixed during early development.

A second important prerequisite for studies investigating the role of selection in generating and maintaining body size and vertebral number variation is the existence of axial regionalisation in the body (Romer 1970; Grande and Bemis 1998), as region-specific changes are the strongest evidence of direct selection. Modules—units of covarying morphological traits that are relatively independent of other such units (Klingenberg 2005)—are seen as important intrinsic factors influencing the direction and rate of evolution (Gould and Lewontin 1979; Gould 2002). Plethodontid salamanders (Wake 1966), snakes (Polly et al. 2001) and teleost fishes (Asano 1977; Ward and Brainerd 2007) all show region-specific changes in vertebral numbers correlated with body elongation, indicating the existence of relatively independent modules along the vertebral column, corresponding to the pre-anal abdominal region, or trunk, and the post-anal caudal region, or tail.

In sex-role reversed pipefishes with exclusive male parental care, selection is thought to act differently upon the body plan of males and females (Hoffman et al. 2006). Similar to other members of the family Syngnathidae (seahorses, pipefishes and seadragons), female pipefish transfer their eggs into specialised brooding structures located on the males' tail or abdomen, where eggs are fertilised and embryos develop (Breder and Rosen 1966; Wilson et al. 2001). The location of these brooding structures is fixed in the major lineages of syngnathid fishes, and brood pouch diversification is linked to a major evolutionary radiation of the group (Wilson et al. 2001, 2003). Predicting that 'the placement of the embryos during pregnancy could provide a selective pressure on body proportions', Hoffman et al. (2006) tested whether phenotypic variation in trunk and tail vertebral counts is heritable in the tail-brooding pipefish *Syngnathus scovelli*, using quantitative genetic analysis. The authors found that both of these traits have a significant additive genetic component, suggesting that both pre- and post-anal body regions of *Syngnathus* pipefishes are able to respond to selective pressures. A lack of genetic and phenotypic correlations

between the number of trunk and tail vertebrae in *S. scovelli* suggests that these body regions are able to evolve independently, and a family-wide comparative analysis detected significant differences in regional vertebral counts in trunk and tail brooders in the Syngnathidae (Hoffman et al. 2006). The genetic independence, or modularity, of pre- and post-anal body segments in syngnathid pipefishes suggests that these regions may be able to respond independently to selective pressures, providing a high degree of flexibility in body size evolution in this group.

In the broad-nosed pipefish *Syngnathus typhle*, natural, fecundity and sexual selection all likely influence body size. Laboratory experiments have demonstrated that large-bodied juvenile pipefish are less prone to predation when compared to smaller-sized conspecifics, and natural selection is thought to have favoured the evolution of large juvenile body size and rapid growth rates (Ahnesjö 1992a). Body weight and size of newly-hatched pipefish correlate positively with egg size (Ahnesjö 1992b), and the ability to produce large eggs of high quality thus offers clear fitness advantages for female pipefish. Larger females produce larger eggs (Berglund 1991), which are typically carried by large-bodied males (Berglund et al. 1986).

Male brood pouch capacity and female egg production both increase with increasing body size in *S. typhle* (Ahnesjö 1992b, 1995; Rispoli and Wilson 2008). Consequently, fecundity selection in syngnathid fishes is predicted to favour the evolution of large-bodied males and females. As eggs develop in the ovaries (located in the trunk-region of the female), and male *S. typhle* brood eggs on their tail (Fig. 1), antagonistic selective forces acting on male and female body size might be expected to limit the potential for allometric growth in this species. Alternatively, the decoupling of male and female growth would offer increased morphological flexibility, potentially leading to differences in the relative proportions of trunk and tail regions in the two sexes as a result of sex-specific fecundity selection.

As *Syngnathus* pipefishes are sex-role reversed and male mate choice dominates in this group (Berglund and Rosenqvist 2003), female body size is also influenced by sexual selection. Both field and laboratory studies indicate that large-bodied females are preferred as mating partners (Berglund et al. 1986; Ahnesjö 1992b), and have higher success in intrasexual competition for mating opportunities (Vincent et al. 1995, Berglund and Rosenqvist 2001), reflecting the competitive benefits of large body size in female pipefish, and suggesting that overall body size may be under strong selection in females.

Assuming a positive relationship between body size and vertebral number in *S. typhle*, sex-specific differences in fecundity selection in this species are predicted to lead to sexual
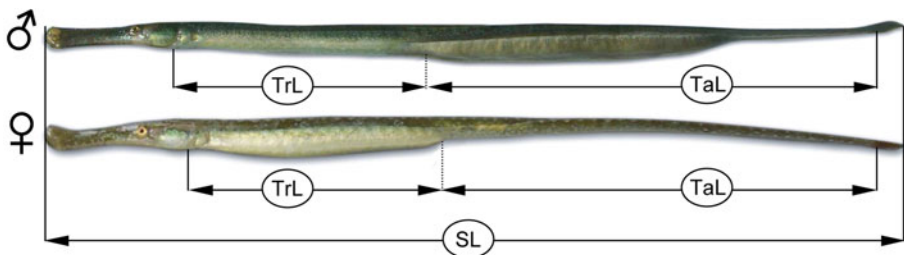

Fig. 1 Adult pregnant male (*top*) and female (*bottom*) *Syngnathus typhle*, lines indicate length measurements recorded for this study. *TaL* Tail length, *TrL* Trunk length, *SL* standard length. Note the presence of external armour rings on the body of *S. typhle*, which correspond to underlying vertebrae (Hoffman et al. 2006)

dimorphism in growth and vertebral development. We expect female pipefish to have relatively longer trunks with more vertebral elements for a given body size, and males to have relatively longer tails with a higher number of tail vertebrae. We tested this hypothesis in five widely distributed European populations of *S. typhle* in an effort to determine how fecundity selective pressures influence body size variation in this species.

Materials and methods

Field sampling

The broad-nosed pipefish *Syngnathus typhle* occurs over a wide geographical range between 71°N–30°N and 11°W–42°E, inhabiting eelgrass beds (*Zostera* sp., *Posidonia* sp., *Cymodocea* sp.) in the Atlantic, Mediterranean, North-, Baltic- and Black Seas (Herald 1941; Hart 1973; Dawson 1986; Wilson and Eigenmann Veraguth 2010). Pipefish populations were sampled in eelgrass meadows from five localities across Europe (Askö, Sweden (ASK); Fiskebäckskil, Sweden (KLU); Ile Callot, France (ROS); Ria Formosa, Portugal (RIA); and Venice, Italy (VEN)) between 2006 and 2008 (Fig. 2), using either a hand-drawn beach seine with a mesh size of 2–3.2 mm (ASK, RIA, ROS, VEN), or a boat-drawn trawl with a mesh size of 4 mm (KLU). GPS coordinates, collecting dates, salinity, temperature, and water depths at collection localities are provided in Fig. 2. Specimens used in this study are archived in the Institute of Evolutionary Biology and Environmental Studies at the University of Zürich, Switzerland.
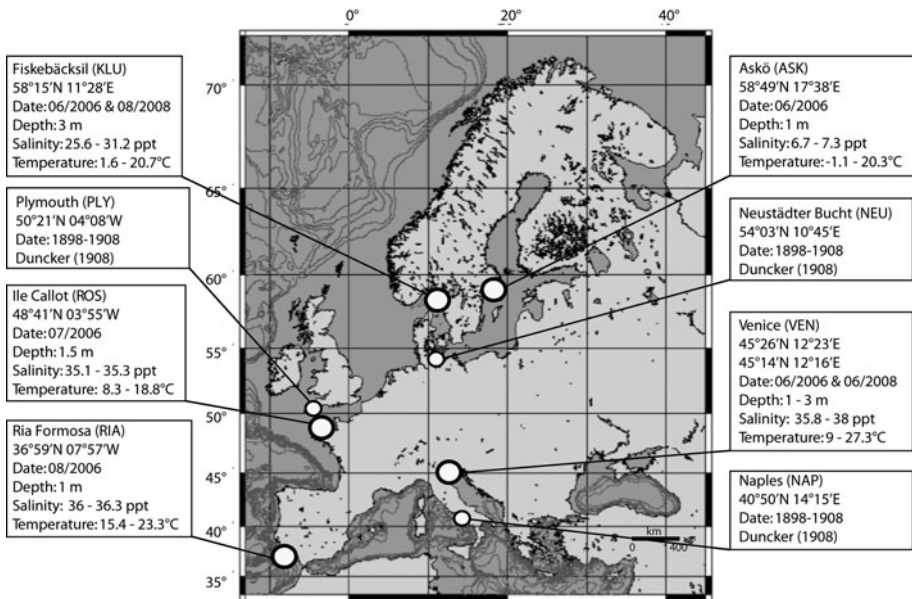
Fig. 2 Sampling localities of *Syngnathus typhle* (small markers), along with historical sampling localities from Duncker (1908) (large markers), with physical and geographical information for each site. Temperature and salinity range data indicate minima and maxima values recorded during the years of collection (www.incofish.org)

Length determination and vertebral counts

Interpopulation variation and sexual size dimorphism in *S. typhle* were investigated using two methods: length measurements and counts of vertebral elements (Fig. 1). As external body armour rings (bony plates arranged in symmetric rings along the postcranium) correspond to individual vertebral elements in syngnathid fishes (Hoffman et al. 2006), the vertebral number of individuals can be readily scored (Duncker 1908). Trunk and tail rings, standard length, and the length of the trunk and tail were scored for individuals from each population. Body rings were counted using a stereo microscope, and length measurements were collected with a manual caliper to the nearest 0.01 mm. Only reproductively mature specimens were included in our analysis, and all characters were coded for both males and females. Sexual maturity of female specimens was assessed by the presence of mature eggs in the ovaries, and specimens containing solely unripe eggs were excluded. Males were considered mature when brood pouch tissue was fully developed.

Standard length was measured from the dorsal anterior end of the premaxillary bone to the distal end of the caudal peduncle (Fig. 1), following the procedure outlined in Rispoli and Wilson (2008). Trunk length was determined as the distance between the anterior end of the anteriormost ventral bony plates (*scuta lateralia inferioria*) and the posterior end of the anal opening, and tail length was measured as the distance between the posterior end of the anal opening and the posterior end of the caudal peduncle (Fig. 1). The number of vertebral elements was counted as follows: trunk rings were counted from the body ring adjacent to the pectoral fin-bearing element to the body ring surrounding the posterior end of the anal opening. As the first pectoral fin-bearing element includes three fused vertebrae that cannot be seen externally as individual body plates, the number of trunk vertebrae was calculated as the number of trunk rings counted plus three, following Duncker (1908). The number of vertebral elements in the tail was counted by defining the body ring adjacent to the anal opening as the first, and the caudal peduncle as the last, vertebral element.

Pleomerism

Pleomerism is a population measure defined as the positive relationship between average vertebral counts and maximum body size (Lindsey 1975). While pleomerism has been found across the family Syngnathidae and at the genus-level in *Syngnathus* (Lindsey 1975), it is unknown whether this relationship is also found at the population-level in *Syngnathus typhle*. As fishes continue to grow throughout their lives, the investigation of pleomerism in wild-caught samples is prone to error, as a single population sample may not represent the full range of body sizes found at a sampling locality. *S. typhle* are thought to live 2–3 years, and most individuals reach reproductive maturity during their first year of life (Berglund and Rosenqvist 2003). Maximum body sizes for each of the populations sampled here were compared with reported data, and ASK was excluded from this analysis, as the largest animals from this population were smaller than reports from other populations in the Baltic (Duncker 1908; Jasmin Winkler, unpublished data). Analyses conducted with the inclusion of this population produced consistent results (data not shown). Populations used in this analysis were supplemented with data from Duncker (1908), who collected large samples of pipefish from Neustädter Bucht, Germany (NEU), Plymouth, England (PLY) and Naples, Italy (NAP) (Fig. 2). A general linear model (GLM) was used to test for an association between maximum body length and average vertebral count for both males and females from each of these seven populations.

Sex-specific differences in region-specific vertebral counts could be present even in the absence of any differences in the total number of vertebrae, and could be produced via a spatial shift of abdominal and caudal regions along the vertebral axis (e.g. Müller et al. 2010). As data on trunk and tail length are not provided in Duncker (1908), we investigated the hypothesis of region-specific pleomerism in *S. typhle* using KLU, ROS, RIA and VEN. The association between maximum tail length and number of vertebrae in the tail, and maximum trunk length and number of vertebrae in the trunk in male and female *S. typhle*, was tested using a GLM.

Sexual size dimorphism and body size allometry

Sex- and population-differences in morphological variables were tested using multivariate ANOVAs for trunk, tail and overall body length. We tested for the presence of sexual dimorphism in body size and allometric growth in male and female *S. typhle*, calculating allometric growth coefficients for trunk and tail length relative to body size. In order to determine how both of these variables scale relative to body size, the implementation of standardized major axis regression in the R package '*smatr* v2.1' (Warton and Olmerod 2005) was used, in order to account for measurement error in both predictor and response variables (Warton et al. 2006). A full general linear model using standard linear regression provided results consistent with those presented here (data not shown). Trunk length, tail length, and standard length were all log10-transformed prior to analysis.

A second set of analyses tested for sex-differences in body size allometry, studying the relationship between standard length and the ratio of trunk length and tail length (TrL:TaL) for males and females. Here again, standard length was log10-transformed prior to analysis and standardized major axis regression was used. All statistical methods used here were implemented in R v2.9.1 (R Development Core Team 2010).

Results

Pleomerism

While vertebral counts differed significantly among populations ($F_{6,5} = 111.983$, $P < 0.001$, Table 1), males and females did not differ in their number of vertebrae (Males: Females: $F_{1,5} = 0.395$, $P = 0.557$) and there was no association between maximum body size and average vertebral count ($F_{1,5} = 0.226$, $P = 0.655$), indicating a lack of pleomerism in *Syngnathus typhle*. There was also no evidence of sexual dimorphism in vertebral counts in trunk and tail regions. No general association was found between maximum tail length and the number of tail vertebrae ($F_{1,2} = 0.078$, $P = 0.806$), or maximum trunk length and the number of trunk vertebrae ($F_{1,2} = 2.055$, $P = 0.288$), and, despite significant differences in the number of tail and trunk vertebrae among populations (Tail: $F_{3,2} = 61.664$, $P = 0.016$; Trunk: $F_{3,2} = 28.495$, $P = 0.034$), no region-specific sexual dimorphism in vertebral counts was detected (Tail: $F_{1,2} = 0.725$, $P = 0.484$; Trunk: $F_{1,2} = 3.488$, $P = 0.203$).

Sexual dimorphism in body proportions

Female and male *S. typhle* differed in overall body size (Females (Mean $\pm$ SD): 18.4 $\pm$ 4.5 cm, Males: 16.4 $\pm$ 3.9 cm, $F_{1,216} = 21.340$, $P < 0.001$), and males were consistently

Table 1 Body size and vertebral count variation in reproductively mature S. typhle from natural European populations

Pop	Sex	n	Standard length (cm)	Trunk length (cm)	Tail length (cm)	Vertebral number (Total)	Vertebral number (Trunk)	Vertebral number (Tail)
ASK	F	10	15.1 (11.9–18.9)	5 (3.9–6.2)	7.6 (5.9–9.9)	56 (53–58)	17.1 (16–18)	35.9 (34–37)
	M	21	12.3 (10.8–14.1)	3.9 (3.3–4.3)	6.4 (5.4–7.4)	54.9 (53–56)	17 (16–18)	34.9 (33–36)
KLU	F	39	18.6 (12.3–28.2)	5.9 (4.0–9.0)	9.7 (6.2–15.3)	55.4 (54–57)	16.8 (16–18)	35.6 (34–37)
	M	37	17 (12.2–21.9)	5.1 (3.6–6.8)	9.2 (6.2–12.1)	55.6 (54–57)	16.9 (16–19)	35.7 (34–38)
ROS	F	10	26.2 (20.8–31.7)	8.5 (6.3–12.1)	13.7 (11.1–16.6)	57.4 (57–58)	17.3 (17–18)	37.1 (36–38)
	M	11	23.1 (19.7–25.5)	6.7 (6.0–7.4)	12.7 (10.8–14.2)	57.2 (56–59)	17.3 (17–18)	36.9 (36–38)
VEN	F	24	16.8 (11.1–25.8)	5.9 (3.8–9.2)	8 (5.3–12.3)	54.9 (53–57)	18.1 (17–19)	33.8 (32–35)
	M	28	16 (12.4–25.2)	5.3 (4.5–8.5)	8 (5.9–13.2)	54.7 (54–56)	17.8 (17–19)	33.9 (33–35)
RIA	F	22	18 (12.9–26.1)	5.8 (4.1–8.7)	9.2 (6.5–13.4)	56.4 (55–58)	17.4 (17–18)	36 (34–38)
	M	20	16.6 (11.4–23.0)	4.9 (3.4–7.0)	8.9 (6.0–12.4)	56.9 (55–59)	17.3 (16–18)	36.6 (35–38)
Overall	F	105	18.4 (11.1–31.7)	6.0 (3.8–12.1)	9.4 (5.3–16.6)	55.8 (53–58)	17.3 (16–19)	35.5 (32–38)
	M	117	16.4 (10.8–25.5)	5.1 (3.3–8.5)	8.7 (5.4–14.2)	55.6 (53–59)	17.2 (16–19)	35.4 (33–38)
NEU[a]	F	82	20.7 (17–27)	NA	NA	52.8 (51–55)	17.2 (16–18)	35.6 (33–38)
	M	65	15.9 (12–20.5)	NA	NA	53.1 (51–55)	17.1 (16–18)	35.9 (34–39)
PLY[a]	F	160	23.6 (20.5–29)	NA	NA	55.5 (54–58)	18.1 (17–19)	37.4 (36–39)
	M	141	22.3 (18.5–29)	NA	NA	55.4 (54–58)	18 (17–19)	37.5 (36–39)
NAP[a]	F	120	23.4 (19–31.5)	NA	NA	54.8 (51–57)	19.1 (18–20)	35.7 (29–37)
	M	102	22.5 (13–35.5)	NA	NA	54.7 (50–57)	19.1 (18–20)	35.6 (19–38)

Average values are indicated in bold along with sample ranges (in brackets)

[a] Duncker (1908)

smaller than females across all sites ($F_{4,216} = 37.490$, $P < 0.001$, Table 1). As trunk and tail length are size dependent, average trunk and tail length of females exceeds that of males (Table 1).

Females and males differed in trunk length (Females: 6.0 ± 1.5 cm, Males: 5.1 ± 1.1 cm, $F_{1,216} = 43.962$, $P < 0.001$), and average female trunk length exceeded that of males collected from all study populations ($F_{4,216} = 27.671$, $P < 0.001$, Table 1). Allometric slope coefficients for the trunk region were steeper for females than for males: with increasing body size, the female trunk grows more rapidly than that of the male (Allometric slope coefficient: M: 0.950, F: 1.017, $\chi_1^2 = 5.290$, $P = 0.02$, Fig. 3; Table 2). Sexual dimorphism in allometry was also detected at the population-level in ROS (M: 0.932, F: 1.481, $\chi_1^2 = 8.111$, $P = 0.004$) and RIA (M: 0.923, F: 1.144, $\chi_1^2 = 9.559$, $P = 0.002$).

Tail length also differed between the sexes (Females: 9.4 ± 2.4 cm, Males: 8.7 ± 2.3 cm, $F_{1,216} = 8.668$, $P = 0.004$) across all collection localities ($F_{4,216} = 44.830$, $P < 0.001$, Table 1). While positive allometry of the tail region was observed in both sexes, male tail allometry significantly exceeded that of females (Allometric slope coefficient: M: 1.107, F: 1.063, $\chi_1^2 = 4.482$, $P = 0.03$, Fig. 3, Table 2). Again, while allometric growth in the tail region was always higher in males (Table 2), this relationship was only significant for RIA (M: 1.101, F: 1.021, $\chi_1^2 = 4.176$, $P = 0.04$) and VEN (M: 1.076, F: 0.987, $\chi_1^2 = 10.733$, $P = 0.001$).

All populations of males showed negative allometry in trunk length relative to tail length, such that large-bodied males had disproportionately larger tails relative to their trunks (Fig. 4a). The pattern observed in females was more complicated (Fig. 4b), with southern populations showing the predicted pattern of positive allometry in TrL:TaL relative to body size (ROS, RIA and VEN), and northern populations (ASK and KLU) exhibiting negative allometry, suggesting that selective factors influencing female body size may vary across environments. While the overall relationship between TrL:TaL and standard length was negative for females (Fig. 4b), significant slope differences among populations complicate the interpretation of this relationship. A single ROS female (likely a 3-year old individual) had an exceptionally large trunk (Fig. 3) as well as a smaller-than-expected vertebral count; the removal of this individual did not influence the results of this analysis (Fig. 4b).

Discussion

Male and female body regions, i.e. trunk and tail, are sexually dimorphic in the European pipefish *Syngnathus typhle*: males have a longer tail than do females for a given body size, suggesting that the body regions of both sexes are genetically independent and able to respond independently to sex-specific selective pressures. This pattern is consistent across several *S. typhle* populations and argues strongly for the existence of sex-specific selective forces in this species. Females, however, do not exhibit a consistent pattern of allometric growth, contrary to our expectation that the female trunk region should grow disproportionately with body size due to fecundity selection. *S. typhle* not only show region-specific dimorphism, but also dimorphism in overall body size: females are on average larger than males across the range of the species (Rispoli and Wilson 2008; Table 1), suggesting the presence of sex-specific selective pressures on overall body size in this species.
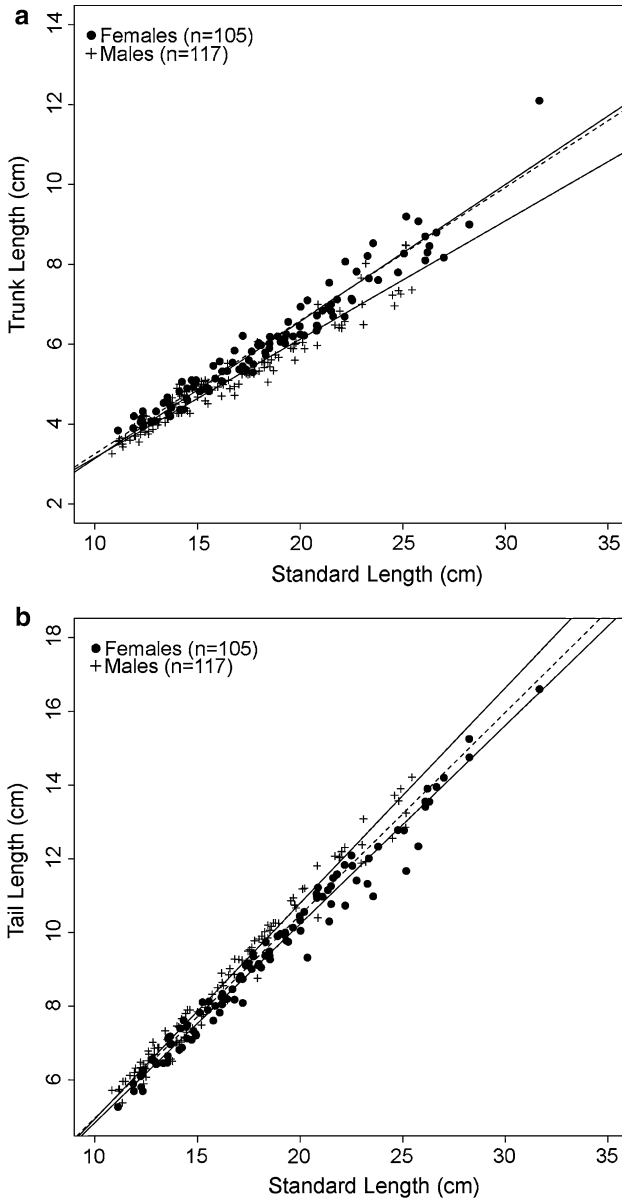
Fig. 3 **a** Trunk and **b** tail length allometry in sexually-mature *Syngnathus typhle*. Regressions of combined male and female data are indicated as *dashed lines* for illustrative purposes. *Asterisks* indicate a ROS female with an exceptionally large body size and trunk. The removal of this outlier individual had no effect on results (data not shown)

Maximum body size and average vertebral counts are uncorrelated in *S. typhle*, indicating a lack of pleomerism in this species. Sexual size dimorphism in body regions is also not due to sex-specific differences in vertebral counts: longer body regions do not contain higher numbers of vertebrae. Below we interpret our results in light of the relative

Table 2 Male pipefish exhibit allometric growth in body proportions

| Population	Sex	TrunkL	95% CI	TailL	95% CI
ASK	M	**0.977**	(0.839–1.139)	*1.164*	(1.036–1.307)
	F	*1.040*	(0.985–1.100)	**1.051**	(0.989–1.117)
KLU	M	**1.014**	(0.975–1.053)	*1.066*	(1.035–1.098)
	F	*1.019*	(0.986–1.054)	**1.033**	(1.004–1.062)
ROS	M	**0.932***	(0.762–1.140)	*1.007*	(0.790–1.284)
	F	*1.481*	(1.167–1.879)	**0.991**	(0.915–1.073)
RIA	M	**0.923***	(0.839–1.015)	*1.101***	(1.058–1.146)
	F	*1.144*	(1.040–1.257)	**1.021**	(0.959–1.087)
VEN	M	**1.042**	(0.992–1.095)	*1.076***	(1.035–1.119)
	F	*1.064*	(1.032–1.096)	**0.987**	(0.955–1.020)
Overall	M	**0.950***	(0.910–0.992)	*1.107***	(1.078–1.136)
	F	*1.017*	(0.978–1.057)	**1.063**	(1.034–1.092)

Allometric slope coefficients (standardized major axis regression) for trunk (TrunkL) and tail (TailL) length relative to overall body size (log-transformed data) in five wild populations of *S. typhle*. Sex differences in allometry are indicated (*italic/bold*), along with instances in which males and females differ significantly in their pattern of growth (*$P < 0.05$)

importance of the selective forces in the evolution of body size in *S. typhle*, and discuss developmental mechanisms which might be responsible for the region-specific size dimorphism observed in this species.

Selection

Region-specific allometry provides strong evidence for direct selection (Romer 1970; Grande and Bemis 1998), and male brooding of embryos has been suggested to influence relative body proportions in syngnathid fishes (Hoffman et al. 2006). Our data show that male *S. typhle* grow disproportionately faster in their tail: with increasing body size, the relative length of the male trunk becomes shorter while tail length becomes longer (Fig. 4). The relative elongation of the tail in males, but not in females, suggests that fecundity selection on the male brood pouch region is a major evolutionary force shaping the male phenotype in this species. Region-specific sexual size dimorphism in *S. typhle* supports the differential fecundity selection hypothesis proposed by Hoffman et al. (2006).

Female-biased sexual size dimorphism in *S. typhle*, a species with female competition for access to mates (Berglund et al. 1986; Vincent et al. 1995), suggests that sex-specific selective forces also influence overall body size in this species (Rispoli and Wilson 2008). Male preference for large-bodied females (Berglund et al. 1986), as well as the higher competitive performance of large bodied females in mating trials (Berglund and Rosenqvist 2001), might help to explain the observation of female-biased sexual size dimorphism in *S. typhle*. It should be noted that differential mortality rates or migration patterns of males and females could also potentially contribute to female-biased SSD. While female ornamental displays in *S. typhle* reduce crypsis and increase the possibility of predation (Bernet et al. 1998), large and dominant females spend less time on risky matings than do small females (Berglund and Rosenqvist 2001), possibly offsetting the increased risk of predation due to large body size. Overall, our data suggest that female *S. typhle*
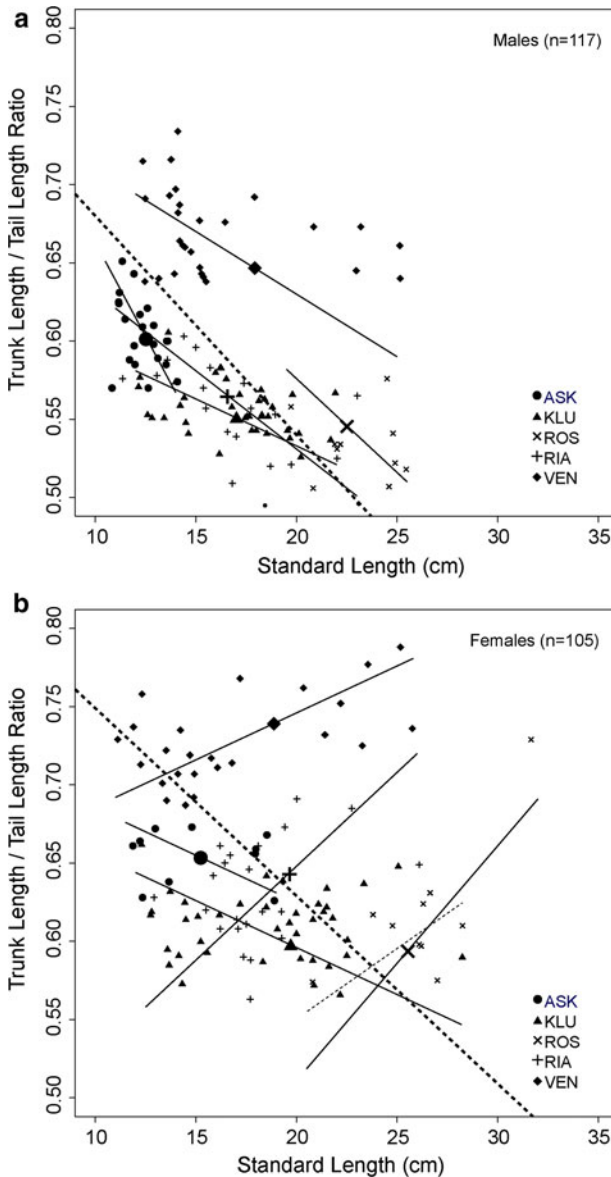
Fig. 4 Ratios of trunk length to tail length (TrL:TaL) plotted against standard length in **a** male and **b** female *Syngnathus typhle*. Population (*solid lines*) and total (*dashed line*) regressions are indicated. *Asterisk* indicates a ROS female with an exceptionally large body size and trunk. Analyses with (*solid line*) and without (*light dashed line*) the inclusion of this outlier individual are indicated for comparative purposes

body size is likely under sexual selection, while region-specific size variation in male *S. typhle* reflects the effects of fecundity selection for increased male brood pouch capacity.

The degree of sexual size dimorphism (Table 1) as well as body proportions (Fig. 4) differ among populations, indicating that local environmental conditions may influence the intensity of sexual and fecundity selection in this species. Two of the most important

environmental factors known to affect body size and fecundity in ectotherms are temperature and salinity (Lankford and Targett 1994; Madsen and Shine 1994), and both factors vary across the distributional range of *S. typhle*. Experimental studies have shown that male *S. typhle* reproductive output is severely constrained by temperature (Ahnesjö 1995), and maximum body size is also expected to be constrained under extreme salinity regimes (Deane and Woo 2009), demonstrating how these two variables might influence patterns of growth in *S. typhle*. While our data do not provide the basis for a robust test of the effects of temperature and salinity on the evolution of body size and proportions in *S. typhle*, spatial variation in the pattern of allometry and SSD indicate that the pipefish system may be ideally suited for such work.

While analyses of intraspecific variation in natural populations are often the only feasible approaches to investigate how selection influences evolution in many long-lived organisms, studies such as this highlight just some of the challenges that arise when comparing morphological variation in field-caught samples collected from different environments. While the morphological differences quantified here are likely biologically significant, it remains difficult to assess whether this size variation reflects differences in growth and/or mortality rates in the absence of a reliable measure of age. Even when vertebral counts offer a means to predict maximum adult body size, differences in resource availability and survival rates among sites may mean that individuals may often fail to reach their potential maximum body size in many natural populations. In order to fully understand the selective forces that influence body size variation in *S. typhle*, investigations in natural variation in body size in this species would benefit from complementary studies of morphological change through time in experimental populations.

Developmental mechanisms

Axial elongation can be achieved through an increase in vertebral numbers (i.e. pleomerism), an increase in the distance between individual vertebrae (changes in vertebral centra length), or a combination of both mechanisms (Wake 1966; Lindsey 1975; Ward and Brainerd 2007). Although a positive correlation between number of vertebrae and maximum body size is pervasive in species with indeterminate growth, there are several notable exceptions to this pattern, including some of the largest known snakes and smallest plethodontid salamanders (Wake 1966; Parra-Olea and Wake 2001; Head and Polly 2007). Vertebral number and body size are uncorrelated in *Syngnathus typhle*, indicating that somitogenesis and maximum body size are decoupled in this species, despite the presence of pleomerism at both the family- and genus-level in syngnathid fishes (Lindsey 1975). Hoffman et al. (2006) found that vertebral numbers in the tail and trunk of syngnathid fishes vary across genera depending on whether males brood eggs on their trunk of their tail, and proposed that fecundity selective pressures could act differentially in trunk- and tail-brooding species. Quantitative genetic analysis supports their proposition that the trunk and tail are separate modules able to respond independently to selective pressures. Although our findings also support the existence of modules along the vertebral axis of *S. typhle*, they indicate that the length of these modules within species can change independently of vertebral number.

Elongation can occur globally along the entire vertebral axis, or can be specific to a particular body-region, involving either the trunk or the tail. A comparative study of region-specific body size variation in actinopterygian fishes suggests that that changes in vertebral centra length (leading to a longer body for the same number of vertebrae) are typically involved in elongation of the entire body, while changes in vertebral number are

associated with region-specific body size variation (Ward and Brainerd 2007). Based on these findings, Ward and Brainerd (2007) concluded that a single developmental module controls vertebral length along the body axis in most species, while vertebral numbers are determined by two modules acting independently in the trunk and the tail. In contrast to this hypothesis, region-specific length variation in *S. typhle* is not associated with changes in vertebral numbers, but rather with changes in vertebral centra length, suggesting that Ward and Brainerd's (2007) interspecific model of vertebral development may not be appropriate for this species. As the mechanisms underlying body size evolution have typically been investigated above the species level (e.g. Ward and Brainerd 2007), the lack of relationship between vertebral numbers and region-specific length variation in *S. typhle* could well be a common pattern at the intraspecific level. Interestingly, pleomerism has rarely been documented at the intraspecific level in fishes despite pervasive evidence of sexual size dimorphism in this group (Lindsey 1975), suggesting that SSD in many species may be achieved via sex-specific differences in vertebral centra length and not through differences in vertebral counts (e.g. Bergmann et al. 2006). Further studies on the developmental mechanisms involved in the evolution of body size within species, and comparisons between the pattern of change at the intra- and interspecific level would be a particularly fruitful area of future research.

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