



TITLE

The Evolution of Ecological Specialization Across the Range of a Broadly Distributed Marine Species

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ABSTRACT

Ecological specialization is an important engine of evolutionary change and adaptive radiation, but empirical evidence of local adaptation in marine environments is rare, a pattern

that has been attributed to the high dispersal ability of marine taxa and limited geographic barriers to gene flow. The broad-nosed pipefish, *Syngnathus typhle*, is one of the most broadly distributed syngnathid species, and shows pronounced variation in cranial morphology across its range, a factor that may contribute to its success in colonizing new environments. We quantified variation in cranial morphology across the species range using geometric morphometrics, and tested for evidence of trophic specialization by comparing individual-level dietary composition with the community of prey available at each site. While the diets of juvenile pipefish from each site were qualitatively similar, ontogenetic shifts in dietary composition resulted in adult populations with distinctive diets consistent with their divergent cranial morphology. Morphological differences found in nature are maintained under common garden conditions, indicating that trophic specialization in *S. typhle* is a heritable trait subject to selection. Our data highlight the potential for ecological specialization in response to spatially variable selection pressures in broadly distributed marine species.

KEYWORDS

Adaptation, dietary analysis, functional morphology, geometric morphometrics, *Syngnathus typhle*, trophic ecology

INTRODUCTION

Ecological specialization is an important driver of evolutionary change, and key to adaptive radiation (Streelman and Danley 2003; Gavrillets and Losos 2009; Nosil 2012). Specialization allows organisms to capitalize on unique resources (i.e. food, space, or mates), promoting local coexistence via niche

partitioning (Bellwood et al. 2006; Herder and Freyhof 2006; Belmaker et al. 2012). While specialization is frequently inferred based on differential resource use, understanding the evolutionary origins of the morphological, physiological, and behavioral mechanisms facilitating specialization is key to understanding its adaptive significance (Ferry-Graham et al. 2002). Functional morphology studies the relationship between organismal structure and function, and provides a mechanistic framework for understanding adaptive change.

Trophic partitioning is perhaps the best studied form of ecological specialization (e.g., Smith and Skulason 1996; Rüber et al. 1999; Schluter 2000; von Rintelen et al. 2004), and has been implicated in the well-known adaptive radiations of African cichlid fishes (Burress 2015) and Galapagos finches (Grant and Grant 2011). Trophic specialists are thought to trade off improved feeding efficiency on a preferred food resource against the cost of reduced niche breadth, and may benefit when competition for resources is high (Futuyma and Moreno 1988; Ferry-Graham et al. 2002).

While adaptive radiations highlight the importance of specialization in ecological speciation, there is a growing appreciation that geographically widespread species may exhibit resource specialization at the population level (Fox and Morrow 1981; Shipley et al. 2009; Loxdale et al. 2011). Marine fishes often show complex adaptations for resource acquisition and processing (e.g., Wainwright et al. 2004, 2015; Cooper et al. 2017), creating the potential for trophic partitioning and ecological specialization in spatially heterogeneous environments. Molecular data suggest that ecological specialization has likely contributed to the high diversity of marine groups such as coral reef fishes (Wainwright et al. 2004; e.g., Rocha et al. 2005), but empirical evidence of dietary specialization remains rare (Barnett et al. 2006; Bellwood et al. 2006), making it difficult to link pattern and process in marine systems (Puebla 2009).

Suction feeding is an important mechanism of resource acquisition in aquatic environments, and has led to a variety of functional innovations for enhanced feeding efficiency in ray-finned fishes

(Wainwright et al. 2015). Syngnathid fishes (seahorses, pipefish and seadragons) are specialized suction feeders that use a sit-and-wait predation strategy to target zooplankton, crustaceans, and small fishes in nearshore marine environments. The unique morphology of syngnathids has attracted considerable interest (Consi et al. 2001; Hoffman et al. 2006; Van Wassenbergh et al. 2011b), and the feeding kinematics of the group have been explored in detail (e.g., de Lussanet and Muller 2007; Leysen et al. 2011). High resolution video imaging indicates that increases in syngnathid snout length create explosive capture velocities and the ability to target prey at greater distances, enhancing the capture of highly mobile prey (de Lussanet and Muller 2007; Van Wassenbergh et al. 2011a). However, mathematical modelling indicates that optimal snout length in this group is inversely correlated with snout height, creating a trade-off between capture efficiency and the maximum prey size that can be consumed (de Lussanet and Muller 2007). An interspecific analysis of trophic ecology in syngnathid fishes is consistent with this hypothesis, and indicates that long-snouted species consume significantly more mobile and smaller prey than do species with short snouts (Kendrick and Hyndes 2005). Increases in snout to head length ratios have also been associated with increases in brain size in this group, a relationship that may reflect the importance of enhanced visual information processing when feeding on highly mobile prey (Tsuboi et al. 2017).

The broad-nosed pipefish (*Syngnathus typhle*) is one of the most broadly distributed syngnathid species, and shows pronounced variation in cranial morphology across its range (Kendrick and Hyndes 2005; Leysen et al. 2011). *S. typhle* is distributed from the Baltic Sea in northern Europe and along the Atlantic and Mediterranean coasts through into the Black Sea (Fig. 1), and inhabits a variety of nearshore environments and conditions (Wilson and Eigenmann Veraguth 2010), offering opportunities for trophic specialization to capitalize on locally abundant food resources. As offspring develop within the male brood pouch and are released as free-living juveniles (Rispoli and Wilson 2008), dispersal in *S. typhle* is thought to be limited, but the species is genetically diverse, and exhibits a phylogeographic history consistent with the post-glacial colonization of northern Europe

15-20,000 years ago (Wilson and Eigenmann Veraguth 2010).

Here, we investigate the role of ecological specialization in the evolution of morphologically-divergent populations of *S. typhle*. We first use geometric morphometrics to quantify intraspecific variation in the snout morphology of *S. typhle* from across the species range, and then study associations between prey availability and dietary composition across ontogeny in populations showing pronounced morphological divergence. Finally, we use a common garden experiment to test whether observed morphological differences among populations reflect short-term ecological responses to locally abundant prey, or fixed genetic differences associated with trophic specialization (e.g., Meyer 1987; Warner 1997; Mittelbach et al. 1999). We predict that the presence of long, low-snouted phenotypes, and high, short-snouted phenotypes in natural populations reflects trophic specialization to feed on small, mobile prey, and large, low motility prey, respectively (e.g., Kendrick and Hyndes 2005), and that the relative prevalence of these phenotypes at the population-level reflects local prey availability, consistent with trophic divergence in a spatially heterogeneous marine environment.

MATERIALS AND METHODS

Specimen Collection

Species-level variation in the cranial morphology of *S. typhle* was initially quantified using randomly-selected specimens from twelve populations collected from across the species range (Fig. 1). Detailed information on specimen collections is provided in Wilson and Eigenmann Veraguth (2010), which analyzed genetic diversity and population structure across the species range.

Intrapopulation variation in cranial morphology and dietary composition was quantified for 21 juveniles and an equal number of reproductively mature adults (11 females and 10 males)

collected from the Gullmar Fjord, Sweden (KLU), Ria Formosa, Portugal (RIA) and Venice Lagoon, Italy (VEN) (Table 1). Pipefish specimens were collected at depths of 0.5-2m by beach seine or beam trawl during June and August 2001 (RIA) and 2008 (KLU and VEN). Animals were euthanized with an overdose of MS-222 (Tricaine), reproductive status was identified by the assessment of gonad morphology, and the digestive tract was dissected for dietary analysis. RIA animals included a subset of specimens collected as part of a larger study of commercially important fish species in the Ria Formosa (Erzini et al. 2002). A detailed analysis of the dietary composition of these specimens has been published elsewhere (Oliveira et al. 2007).

Morphological Analyses

Specimens were digitally photographed in standard orientation with closed gape for morphometric analyses using a Nikon D300 camera with 50mm f/1.8D lens, and total length (TL), head length (HL) and snout length (SnL) were measured to the nearest 0.5 mm using a sizing board. Gape size of each specimen was calculated as the average of the height and width of the open mouth measured with digital calipers, following the method of Kendrick & Hyndes (2005).

Morphological landmarks (LM) were selected to capture the major components of cranial variation (Fig. 2b; Table S1). A total of 13 homologous landmarks and four semi-landmarks were recorded for each specimen using TpsDig v2.25 (Rohlf 2016). Semi-landmarks (LM4-7) demarcated the dorsal snout surface, and were equally spaced between LM2 and LM8. Independent scoring of landmarks in the twelve-population sample by AW and ABW showed high repeatability of data (Procrustes ANOVA: $R=0.927$; Arnqvist and Martensson 1998), indicating minimal measurement error of cranial landmarks relative to sample variation.

A Generalized Procrustes fit of landmark data controlling for specimen size, position and orientation was performed in R (R Development Core Team 2016) using the geomorph v3.0.3 package (Adams and Otárola-Castillo 2013), restricting semi-landmarks to slide along tangent vectors to the outline curve using the Procrustes distance criterion (Ivan Perez et al. 2006). Canonical variate analyses (CVA) were carried out using the Morpho v2.7 package (Schlager 2016), and 95% confidence ellipses of population samples were generated using Car v3.0-6 (Fox and Weisberg 2011). Warped outlines summarizing morphological variation along major CVA axes were produced using the shape.predictor function in geomorph, using an outline trace of *S. typhle* prepared in TpsDig.

Global and pairwise tests of variation in cranial morphology were conducted for populations and developmental stages, against the null hypothesis of a lack of morphological variation among groups, using Procrustes ANOVAs, as implemented in the advanced.ProcD.lm function of geomorph, using randomized residual permutation (RRPP; n=10,000 permutations; Collyer et al. 2015). Sexual dimorphism in the cranial shape of KLU, RIA and VEN adults was separately tested using the same procedure to test for statistical differences between male and female morphology at each site. All analyses used Sequential (Type I) Sums of Squares. Sequential Bonferroni correction (Rice 1989) was used to correct for multiple testing.

Allometric trajectories of cranial morphology were compared by linear regression of Procrustes regression scores on the natural logarithm of centroid size (Adams et al. 2013; Klingenberg 2016), with population identity included as an additional factor. A global test of slope homogeneity was performed using ProcD.allometry, and pairwise comparisons of slope angles (in degrees) between populations were conducted using the advanced.ProcD.lm function in geomorph to test for allometric differences across populations. Statistical

significance was estimated using RRPP resampling (n=10,000 permutations). Allometric regressions were plotted separately for each population, along with the common allometric component (CAC), which reflects the expected allometric trajectory in the absence of population structure (Mitteroecker et al. 2004).

Dietary Analysis

Prey items were identified using a stereomicroscope (Wild Heerbrugg M3). An identification key has been prepared for prey items recovered in *S. typhle* from VEN and KLU (Wegmann 2009). Prey items were grouped into seven major categories (Amphipods, Copepods, Decapods, Fish, Isopods, Mysids, and Ostracods), following the methodology of Oliveira *et al.* (2007). Prey that were represented by only a single individual (a decapod postlarva, a polychaete, and a mollusk), and unidentifiable items/detritus were excluded from further analyses. Prey items were digitally photographed, and the length and width of intact prey were recorded. Average prey volume per group and population was estimated from length and width data using the volume proxy equation provided in Steffe *et al.* (1989). As prey width data were unavailable for the RIA population, average prey size for adult and juvenile pipefish at this site was estimated as the mean of the values for KLU and VEN. Similarly, as the isopod prey of VEN juveniles were not intact, the prey size for this group was taken from the intact prey collected from VEN adults.

The relative representation of prey categories for each group was calculated using both simple abundance and the Index of Relative Importance (IRI), a composite measure incorporating numerical abundance, prey volume and frequency of occurrence (Pinkas et al. 1971). The proportion of juveniles and adults recovered with empty stomachs in each population were compared using a 3-sample test for equality of proportions using the `prop.test` function in R.

Prey items were ranked by relative mobility using literature data (amphipods: (Kendrick and Hyndes 2005); copepods: Mauchline (1998); decapod shrimp: Arnott *et al.* (1998); fish: Rollo & Higgs (2008); isopods: Alexander (1988); mysids: Mauchline (1980); ostracods: Davenport (1990)). According to these estimates, amphipods are the slowest of the targeted prey, while copepods, ostracods, mysids, decapod shrimps, isopods and fish are ranked from lowest to highest mobility, respectively.

The relative abundance of zooplankton at each site was derived from survey data from the Gullmar Fjord (KLU), Ria Formosa (RIA), and Venice Lagoon (VEN) (Marques 2006; Riccardi 2010; Tiselius *et al.* 2015). Plankton surveys sampled nearshore surface waters (0-20m), the typical depth range of *S. typhle* (Froese and Pauly 2010), using standard 80-200 μ M mesh nets, recording the taxonomic diversity and numerical composition of nearshore plankton communities at each site. As the methodology of sampling differed somewhat between studies (surface tow for Marques *et al.* 2006; on-deck pump for Riccardi *et al.* 2010; vertical tow for Tiselius *et al.* 2015), the absence of particular prey groups from zooplankton records should not be taken as evidence that these prey are entirely absent from these sites, but rather as an indication that they are numerically rare relative to other prey groups.

Zooplankton community composition across sites was compared against the null hypothesis of no spatial structure using a permutation approach based on Bray-Curtis dissimilarities. Given differences in zooplankton sampling methods across sites, abundance data were first rarefied to a common sample size using `rrarefy`, and rarefied data were randomized by site, maintaining fixed row and column totals (N=10,000 permutations). Standardised z-scores based on the simulated data were used to test if observed dissimilarities were greater than that expected by chance. All analyses were carried out using `vegan v2.5-6` (Oksanen *et al.* 2016).

Comparisons of zooplankton relative abundance and pipefish dietary composition at each site were carried out using a similar approach, excluding prey items not recovered in the pipefish diet. Data were again rarefied for each pairwise comparison, and adult and juvenile dietary compositions were compared to the local zooplankton community using oecosimu. Separate pairwise tests compared the dietary composition of juveniles and adults among sites to test for dietary differences between developmental stages. Comparisons of pipefish dietary composition were also carried out using % IRI, to correct for differences in prey size and frequency of occurrence across each population.

Two-block partial least squares analyses were used to test for an association between cranial morphology and dietary composition at each site using the two.b.PLS function in Geomorph. Dietary data were square-root transformed and mean-centered prior to analysis, and individuals with empty stomachs were excluded. The correlation between the primary PLS axes of shape and diet was tested by permutation (N=10,000 replicates). Shape outlines were prepared to show shape deformation along the line of best-fit, estimated using major-axis regression for each population. As cranial morphology showed evidence of allometric variation (see below), additional PLS analyses were carried out using adjusted Procrustes coordinate data for each population, combining fitted data from an intercept-only model with ontogeny-centered residuals, removing the effect of development.

Common Garden Experiment

To investigate the genetic basis of cranial variation in *S. typhle*, a common garden experiment was carried out using offspring from pregnant males from KLU (N=3) and VEN (N=5).

Pregnant males from KLU were wild-caught, while 2 of the 5 VEN males were mated with VEN females after their arrival in the lab. Broods were raised separately under standard culture conditions (salinity 33-34 ppt, temperature 18-19°C and pH ~8.0), approximating summer breeding conditions at the KLU and VEN sites (Rispoli and Wilson 2008), and were

fed an identical diet consisting of fresh *Artemia* spp. nauplii for the first 10 weeks of development (OK Nature Cysts, INVE Aquaculture), and a combination of live *Artemia* and frozen adult *Artemia* spp. (Gamma Slice, Tropical Marine Centre) until the conclusion of the experiment.

Common-garden animals were raised until they reached the size of field-collected juveniles, which occurred after 129 ± 10.0 days for the VEN offspring and 139 ± 4.0 days for KLU animals. Animals were anesthetized for photographing in a 100 mg/L solution of AQUI-S (10% eugenol; Aquatatics Inc.). A total of 4-5 juveniles per brood were analyzed for VEN, and 5-8 offspring per brood for KLU, to match the sample size of 21 individuals for the wild-caught samples.

Qst – Fst Comparison: Inferring Adaptive Evolution

Comparisons of the geographical partitioning of phenotypic and genotypic variation across populations can be used to test for adaptive evolution in natural populations (Gilbert and Whitlock 2015). We used the breeding design of our common garden experiment to estimate quantitative genetic variation (Q_{st}) along the primary axes of cranial variation from our canonical variate analysis (Figure 3c), and compared the distribution of phenotypic variation to genetic variation of nine neutral microsatellite loci sampled in a previous study of population genetic structure in *S. typhle* (Wilson and Eigenmann Veraguth 2010). Estimates of Q_{st} and F_{st} were calculated following the methodology of Gilbert and Whitlock (2015) using the $Q_{st}F_{st}Comp$ v0.2 package in R (Gilbert and Whitlock 2014). Note that the unique reproductive system of syngnathid fishes creates the opportunity for both maternal and paternal effects that may influence estimates of genetic variation in quantitative traits (Sagebakken et al. 2017). *S. typhle* is polygynous in natural populations (Rispoli and Wilson 2008), and we provide Q_{st} estimates based on half sib ($r=0.25$) and full sib ($r=0.50$) mating designs for comparative purposes. Confidence intervals of both Q_{st} and F_{st} were estimated by parametric bootstrapping ($N=10,000$ permutations) under the null hypothesis of neutrality of phenotypic and

genetic variation. A comparison of the relative magnitude of Q_{st} and F_{st} estimates (permutation test; $N=10,000$ permutations) provided a statistical test of adaptive evolution (i.e., $Q_{st}>F_{st}$).

RESULTS

Intraspecific Variation in Cranial Morphology

Two individuals from each of 12 populations were analyzed in order to visualize species-level variation in the cranial morphology of *S. typhle* (Fig. 2a). Sampled animals ranged from 10.80-27.70 cm TL and differed significantly in relative snout length (SnL:HL range: 0.588-0.660; Table 1), exceeding the level of intraspecific variation observed in a previous study of twelve syngnathid species by Kendrick & Hyndes (2005). Similarly, gape sizes of individuals included in the species sample (range: 1.43-3.75 mm) exceeded that documented by Kendrick & Hyndes (2005), and were positively correlated with body length ($F_{1,11}=8.82$; $p=0.013$) after controlling for source population.

Canonical variates 1 (CV1) and 2 (CV2) of Procrustes-transformed landmark data explain more than 80% of standardized between-group variance in Procrustes coordinate data (Fig. 3a), and partition morphological variation in snout length (CV1: 52.6% of standardized between-group variance) and gape angle (CV2: 28.4%). Populations are clearly discriminated in trophic morphospace, and show strong geographic affinities, with northern populations (ASK, KLU) displaying an intermediate phenotype including a moderate snout length and a slightly angled gape, Atlantic coast populations (GRO, PED, RIA) with the exception of ROS showing a high and short snout with angled gape, and eastern populations (ERD, ODE, TIL, VEN) exhibiting a hyperextended snout and strongly angled gape, almost perpendicular

to the anterior-posterior axis (Fig. 2a). LIV, MUR and ROS display the highest snouts and are distinct in having a protracted mandible, producing a distinctive blunt-ended snout (Fig. 2a).

Interpopulation Analysis of Adults and Juveniles

Three populations were selected for the comparative analysis of cranial morphology and dietary composition, representing the high (RIA), hyperextended (VEN), and intermediate snout (KLU) phenotypes (Fig. 2). Morphological variation in the population sample mirrors that observed in the intraspecific analysis, revealing significant differences in SnL:HL ratios among populations ($F_{2,120}=35.3$; $p<0.001$) and across developmental stages ($F_{1,120}=31.1$; $p<0.001$), with a significant population * ontogeny interaction ($F_{2,120}=7.2$; $p=0.001$). Among adults, VEN specimens have the longest snouts (SnL:HL \pm SD: 0.639 ± 0.01), while KLU and RIA have similar SnL:HL ratios (KLU: 0.621 ± 0.02 ; RIA: 0.628 ± 0.01) (Table 1). Size-standardized gape (Gape size:HL) is greatest in RIA adults (0.089 ± 0.01 mm), intermediate in KLU animals (0.080 ± 0.01 mm) and smallest in the VEN population (0.062 ± 0.01 mm), consistent with a trade-off between snout length and gape size in this species.

These morphological differences dominate geometric morphometric analyses, with RIA pipefish showing a high and angled snout, KLU animals exhibiting an intermediate phenotype, and VEN individuals showing a low and hyperextended snout (Fig. 3b). CV1 explains 62.0% of the standardized between-group variance, and shape changes along this axis parallel those found along CV1 in the broader species-level analysis (Fig. 3a). Variation in gape angle and snout height again dominate CV2 (28.7% of standardized between-group variance), ranging from the concave snout and slightly angled gape of the KLU population to the flat snout and moderately angled gape displayed by RIA pipefish (Fig. 2a).

Comparisons of adult males and females across sampling sites failed to detect a significant signal of sexual dimorphism in cranial morphology (Population: $F_{2,62}=39.34$, $Z=15.00$, $p<0.0001$, Sex: $F_{1,62}=0.92$, $Z=0.87$, $p=0.46$, Population * Sex: $F_{2,62}=0.97$, $Z=0.97$, $p=0.42$).

Subsequent analyses were conducted on the combined adult sample for each site.

Tests of shape differences across the interpopulation sample revealed significant differences across ontogeny and among populations, and the pattern of ontogenetic change differed across populations, as evidenced by a significant population * ontogeny interaction (Population: $F_{2,125}=67.29$, $Z=25.72$, $p<0.0001$, Ontogeny: $F_{1,125}=12.79$, $Z=9.92$, $p<0.0001$, Population * Ontogeny: $F_{2,125}=4.77$, $Z=4.37$, $p<0.0001$). Pairwise comparisons using Procrustes distances indicated highly significant differentiation between all populations ($p<0.0002$ for all comparisons), but within-population differentiation between juveniles and adults was only significant for the RIA population after controlling for multiple testing (sequential Bonferroni correction: $p<0.0001$; Fig. 3b).

An analysis of shape allometry revealed only modest differences in allometric trajectories across populations (Fig. 4; Homogeneity of slopes test: $F_{2,125}=2.26$, $Z=2.03$, $p=0.02$), indicating that morphological differences among populations are already present in juvenile animals. While KLU and RIA exhibited significantly different allometric slopes ($p=0.006$), all other pairwise comparisons were not significant.

Dietary Composition

A total of 1,649 prey items were identified in 56 juvenile pipefish from across the three sites, the bulk of which were calanoid copepods (1,233/1,649: 74.8%). The proportion of individuals with empty stomachs differed significantly among sites ($\chi^2=6.11$, $df=2$, $p=0.047$), and 5 of 21 RIA juveniles (23.8%) carried no identifiable stomach contents (Table 2).

Adult diets at all three sites were dominated by larger prey, with a total of 388 prey items recovered in the adult sample ($n=46$ individuals) (Table 2). Similar to the pattern found in juvenile pipefish, RIA had the highest proportion of adults with empty stomachs (11/21: 52.4%), while 20 of 21 VEN adults (95.2%) carried at least one identifiable prey item. The proportion of individuals with empty stomachs differed significantly across populations ($\chi^2=12.25$, $df=2$, $p=0.002$).

The composition of local zooplankton communities differed significantly across the three survey sites (Fig. 5; average Bray-Curtis dissimilarity: 0.20, z -score: 41.3, $p<0.0001$), but communities at all three locations were dominated by copepods (54.5-82.2%), along with a diversity of groups not included in the pipefish diet (e.g., Mollusca, Cladocera, Tunicata, Ctenophora, Annelida, Cirripedia), comprising up to 43% of available zooplankton at each site (Fig. 5).

Despite qualitative similarities in zooplankton composition across sites, animals exhibited distinctive dietary compositions in each population, and clear ontogenetic shifts in dietary composition with growth (Fig. 5). Both juvenile and adult diets were significantly different from the dominant zooplankton found at each site (pairwise Bray-Curtis dissimilarity: 0.058-1.000, z -scores: 5.26-39.80, $p<0.001$ for all comparisons). The diet of KLU juveniles most closely resembled the local zooplankton community (Fig. 5; Bray-Curtis dissimilarity: 0.058), but remained significantly different even after correction for multiple testing.

Juvenile pipefish from KLU and RIA fed predominantly on copepods (94.2% and 87.8% of total diet by abundance, respectively), while the diet of VEN juveniles was dominated by amphipods (57.8%), along with a large (i.e. >10%) proportion of mysids (23.2%) and copepods (15.8%) (Fig. 5). Dietary composition shifted towards larger items in adults, and adult populations from the three sites were highly distinctive (pairwise Bray-Curtis dissimilarities: 0.643-0.786, z-scores: 3.49-10.98, $p < 0.002$ for all comparisons). Copepods made up 47.2% of the diet of KLU adults in terms of numerical abundance, but the remainder of the diet for this population was dominated by fish (26.4%) and decapods (12.5%), the two largest prey categories (Table 2). The diet of RIA adults shifted dramatically toward larger prey, with decapods and fish comprising 57.1% and 35.7% of total diet, respectively. Most notably, low-snouted adult pipefish from VEN had a diet dominated by amphipods (57.9%), with smaller contributions from mysids (13.6%) and isopods (10.3%), and was qualitatively similar to the juvenile diet at this site (Bray-Curtis dissimilarity: 0.212). Nonetheless, adult diets differed significantly from juveniles at every site (pairwise Bray-Curtis dissimilarities: 0.212-1.000, z-scores: 6.90-8.55, $p < 0.0001$ for all comparisons).

While copepods dominated the diets of KLU and RIA juveniles, the dietary value of this group is likely to be limited given their small size. Dietary composition was therefore also quantified using the Index of Relative Importance, which takes into account prey abundance, frequency of occurrence, and size (Pinkas et al. 1971). Relative prey composition shifted towards larger prey categories after the incorporation of prey size and frequency of occurrence (Fig. S1), but all developmental stages and populations remained highly distinct (Bray Curtis distances: 0.193-0.976, z-scores: 13.34-72.08, $p < 0.0001$ for each pairwise comparison).

Individual-Level Specialization

Intrapopulation variation in resource use is widespread, and may play an important role in niche partitioning and the early stages of adaptive speciation (Bolnick et al. 2003; Araújo et al. 2011). Two-block partial least squares analyses were used to test for an association between cranial morphology and dietary composition at each site (Fig. 6). While all three plots showed significant scatter ($r=0.535-0.657$, $p>0.1058$ for all comparisons), they revealed clear separation in dietary composition with cranial shape (Fig. 6). Small prey (copepods, ostracods, amphipods and mysids) exhibited negative loadings on PLS1 in both KLU and RIA, while large prey (decapods and fish) had positive loadings, indicating that individuals feeding on larger prey items at these sites have a distinctive trophic morphology. The pattern for the VEN population was distinct, with copepods, isopods and decapods showing negative loadings on PLS1, while amphipods, mysids and fish loaded positively on the PLS1 axis. Juveniles and adults were clearly separated along PLS1 in the RIA population (consistent with the significant separation of these two groups in shape space; Fig. 3b), and two-block PLS indicated a highly significant association between cranial morphology and diet in this population after controlling for ontogeny ($r=0.843$, $p=0.001$). Developmental stages overlapped in KLU and VEN, indicating that associations between cranial morphology and diet in these populations do not simply reflect underlying ontogenetic change.

*Morphological Comparison of Wild-Caught and Laboratory-Reared *S. typhle**

Laboratory-reared juveniles from KLU and VEN juveniles raised on a common *Artemia* diet remain morphologically distinct (Fig. 3c), indicating that differences in cranial morphology among populations have an underlying genetic basis. Lab animals overlap with their population of origin in

relative snout height (CV1), but are clearly separated along CV2, which explains 41% of standardized between-group variance in Procrustes coordinate scores in this comparison (Fig. 3c), and partitions morphological variation in gape angle. Both laboratory-reared populations have distinctive blunt-ended snouts (compare with LIV, MUR and ROS populations in Fig. 3a), but are clearly distinguishable by snout height, with the KLU laboratory population retaining a high, flat snout, and the VEN population showing the hyperextended low snout characteristic of the wild population. Lab and field populations were statistically different for all interpopulation comparisons (NPMANOVA, 10,000 permutations, $p < 0.002$ for all comparisons after sequential Bonferroni correction).

While Q_{st} estimates of cranial divergence in common garden animals are high along CV1 (Fig. 3c; Table 3), confidence limits are broad, and a statistical comparison of phenotypic and genetic variance between KLU and VEN failed to reject the null hypothesis of neutral evolution ($P > 0.075$ for both half- and full-sib analyses; Table 3). We discuss the potential limitations of this analysis below.

DISCUSSION

Ecological specialization has played a central role in the majority of adaptive radiations (Streelman and Danley 2003), but is thought to be rare in marine species, given the limited geographic barriers to gene flow (Sotka 2012). While empirical examples of local adaptation in marine systems are limited, phylogenetic reconstructions of some of the most species-rich groups of marine fishes suggest that ecological specialization has been key to their diversification (Wainwright et al. 2004; Rocha et al. 2005), a phenomenon that has been dubbed the “marine speciation paradox” (e.g., Bierne et al. 2003): How can local adaptation and specialization evolve in the face of high levels of gene flow?

Our data suggest that the extensive variation in snout morphology found in *S. typhle* is the product of dietary specialization at the population level. Similar to the classical example of resource partitioning in Darwin’s finches (*Geospiza fortis*), in which beak size reflects the size and hardness of seeds

consumed (Grant et al. 1976; Price 1987; De León et al. 2012), local populations of *S. typhle* have specialized to feed on distinctive subsets of available zooplankton, and juvenile and adult morphotypes reflect the dominant prey categories consumed at each site. Pipefish populations exhibit distinctive prey compositions despite the fact that zooplankton communities at all three localities are qualitatively similar, indicating that dietary composition does not simply reflect relative zooplankton abundance, but is consistent with selective foraging on the most energetically valuable prey available for a given gape size (e.g., Stephens and Krebs 1986). Morphological differences found in natural populations are maintained under common garden conditions, indicating that cranial morphology in *S. typhle* is a heritable trait subject to spatially variable selection.

Based on de Lussanet and Muller's (2007) analysis of the functional morphology of feeding in *Syngnathus*, we predicted that the diets of high-snouted KLU and RIA individuals would be dominated by large prey items, while gape-limited VEN pipefish would feed on smaller prey. Consistent with this hypothesis, adult pipefish from RIA fed predominantly on the two largest prey categories (decapods and fish), and while copepods comprised a significant component of the KLU diet based on numerical abundance (Fig. 5), they contributed a negligible proportion of the total diet after controlling for prey size and frequency of occurrence, which indicated that fish and decapods dominated the diet of KLU adults (Fig. S1). The diet of VEN adults, in contrast, was dominated by amphipods, one of the smallest prey categories. Juvenile diets in all populations were dominated by the smallest prey items (copepods for KLU and RIA; amphipods for VEN), consistent with the important role of gape size in determining dietary composition in this species.

Although snout height was positively correlated with the dominant prey size consumed at each site, snout length did not strongly predict prey mobility, as expected based on the model of trophic

optimization proposed by de Lussanet & Muller (2007), and analyses of interspecific variation in syngnathid fishes (Kendrick and Hyndes 2005). Short-snouted KLU and RIA adults consumed some of the most mobile prey items (decapods and fish), while the diet of long-snouted VEN pipefish was dominated by low mobility amphipods. This observation does not appear to be a sampling artifact, as a longitudinal study of the dietary composition of VEN pipefish found that amphipods dominate the diets of *S. typhle* throughout the summer sampling period (Franzoi et al. 2004).

While the interspecific analysis of Kendrick & Hyndes (2005) revealed a strong positive association between snout length and prey mobility in syngnathids, a closer investigation of the underlying data suggests a possible explanation for the lack of association between these variables in *S. typhle*. *S. typhle* has the longest relative snout length of the syngnathids examined by Kendrick and Hyndes (2005), and its minimum snout length:head length ratio exceeds that of eight of the fourteen species included in their analysis, suggesting that even short-snouted *S. typhle* may retain the ability to capture mobile prey.

The results of our common garden experiment indicate that observed differences in snout morphology among populations have a significant genetic component. Laboratory populations of pipefish raised on a common *Artemia* diet remained morphologically distinct, and showed a cranial morphology consistent with that observed in wild-caught animals (Fig. 3c). At the same time, cranial morphology is also influenced by environmental conditions, as laboratory-specimens can be distinguished from their wild counterparts in having a distinctive gape angle and dorsal snout surface (CV2 in Fig. 3c), suggesting the potential for short-term responses to local conditions via phenotypic plasticity. While the rearing temperature of our common garden experiment was selected to simulate average summer breeding temperatures and developmental rates of KLU and VEN were approximately equal, skeletal development is highly temperature-dependent in fish (Hubbs 1922), and it would be interesting to explore the extent to which changes in ambient temperature influence cranial variation.

While quantitative genetic variation in cranial morphology is pronounced between KLU and VEN, neutral genetic divergence between these populations is high ($F_{st}=0.325$), limiting our ability to infer adaptive evolution in the sample based on comparisons of Q_{st} and F_{st} . As genetic divergence between many pipefish populations showing pronounced morphological divergence (Fig. 3) is modest (F_{st} range: 0.004-0.458; Wilson and Eigenmann Veraguth 2010), quantifying the genetic component of phenotypic divergence between more closely related populations could offer an opportunity to explore adaptive evolution at an earlier stage in the divergence process.

The analysis of quantitative genetic variation based on our common garden experiment should also be considered preliminary, given the modest size of the experiment and unique aspects of the reproductive biology of syngnathid fishes, where both maternal and paternal effects can influence offspring phenotype (Sagebakken et al. 2017). As parental effects can both inflate and depress estimates of Q_{st} , an ideal experiment would be based on a half-sib breeding design investigating variation in the F2 generation (Gilbert and Whitlock 2015). An expanded common garden experiment involving the analysis of several hundred F2 offspring from multiple half-sib families in each of several populations (e.g., MUR, VEN and RIA; Fig. 3a) would offer a robust test of adaptive evolution in this system, and would ideally be coupled by experimental comparisons of feeding performance in pipefish differing in trophic morphology.

Given the lack of evidence of a functional tradeoff between snout height and length in *S. typhle*, what factors could be responsible for the distinctive hyperextended snouts found in Venice pipefish? An answer may be found through the analysis of interspecific competition, which can influence resource availability, driving dietary specialization and trophic divergence (Connell 1983; Schluter 1994; Belmaker et al. 2012). Fish community diversity is significantly higher at southern sites, and Venice *S. typhle* occur together with four congeners (Pihl et al. 2006; Ribeiro et al. 2008; Franzoi et al. 2010), each of which exhibits differences

in foraging behavior and trophic morphology associated with resource specialization (Franzoi et al. 1993, 2004; Teixeira and Musick 1995). The hyperextended snout of *S. typhle* at these sites is an outstanding feature of the species (Hablützel and Wilson 2011), and may allow individuals to access novel resources in the face of strong competition. Experimental tests of feeding performance in *S. typhle* and its congeners, coupled with comparative analyses of dietary preferences, would provide a test of this hypothesis.

CONCLUSIONS

Our data indicate that geographically widespread marine species such as *S. typhle* may exhibit resource specialization at the population level despite a lack of geographic barriers to gene flow, generating the potential for ecological divergence and local adaptation. While a comparison of phenotypic and genetic divergence between common garden populations failed to reject the null hypothesis of neutral evolution, the genetic contribution to morphological variation was high, suggesting that cranial features associated with feeding performance could be subject to spatially-variable selection pressures. We discovered functional associations between morphological variation and dietary composition both within and across populations, an observation that parallels that found in classical models of resource specialization (Lavin and McPhail 1985; Price 1987). Extending functional studies such as this to a variety of temperate and tropical systems will help to clarify the extent to which ecological specialization has influenced broader patterns of marine diversity.

LITERATURE CITED

- Adams, D. C., and E. Otárola-Castillo. 2013. Geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* 4:393–399.
- Adams, D. C., F. J. Rohlf, and D. E. Slice. 2013. A field comes of age: Geometric morphometrics in the 21st century. *Hystrix Ital. J. Mammal.* 24:7–14.
- Alexander, D. E. 1988. Kinematics of swimming in two species of *Idotea* (isopoda: valvifera). *J. Exp. Biol.* 138:37–49.
- Araújo, M. S., D. I. Bolnick, and C. A. Layman. 2011. The ecological causes of individual specialisation. *Ecol. Lett.* 14:948–958.
- Arnott, S. A., D. M. Neil, and A. D. Ansell. 1998. Tail-flip mechanism and size-dependent kinematics of escape swimming in the brown shrimp *Crangon crangon*. *J. Exp. Biol.* 201:1771–1784.
- Arnqvist, G., and T. Martensson. 1998. Measurement error in geometric morphometrics: empirical strategies to assess and reduce its impact on measures of shape. *Acta Zool. Acad. Sci. Hung.* 44:73–96.
- Barnett, A., D. R. Bellwood, and A. S. Hoey. 2006. Trophic ecomorphology of cardinalfish. *Mar. Ecol. Prog. Ser.* 322:249–257.
- Bellwood, D. R., P. C. Wainwright, C. J. Fulton, and A. S. Hoey. 2006. Functional versatility supports coral reef biodiversity. *Proc. R. Soc. B Biol. Sci.* 273:101–107.
- Belmaker, J., C. H. Sekercioglu, and W. Jetz. 2012. Global patterns of specialization and coexistence in bird assemblages. *J. Biogeogr.* 39:193–203.
- Bierne, N., F. Bonhomme, and P. David. 2003. Habitat preference and the marine-speciation paradox. *Proc. R. Soc. B Biol. Sci.* 270:1399–1406.
- Bolnick, D. I., R. Svanbäck, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, M. L. Forister, and A. E. M. A. McPeck. 2003. The ecology of individuals: Incidence and implications of individual specialization. *Am. Nat.* 161:1–28.

- Burress, E. D. 2015. Cichlid fishes as models of ecological diversification: Patterns, mechanisms, and consequences. *Hydrobiologia* 748:7–27.
- Collyer, M. L., D. J. Sekora, and D. C. Adams. 2015. A method for analysis of phenotypic change for phenotypes described by high-dimensional data. *Heredity* 115:357–365.
- Connell, J. H. 1983. On the prevalence and relative importance of interspecific competition: Evidence from field experiments. *Am. Nat.* 122:661–696.
- Consi, T. R., P. A. Seifert, M. S. Triantafyllou, and E. R. Edelman. 2001. The dorsal fin engine of the seahorse (*Hippocampus* sp.). *J. Morphol.* 248:80–97.
- Cooper, W. J., C. B. Carter, A. J. Conith, A. N. Rice, and M. W. Westneat. 2017. The evolution of jaw protrusion mechanics is tightly coupled to benthic-pelagic divergence in damselfishes (Pomacentridae). *J. Exp. Biol.* 220:652–666.
- Davenport, J. 1990. Observations on swimming, posture and buoyancy in the giant oceanic ostracods, *Gigantocypris mulleri* and *Macrocypridina castanea*. *J. Mar. Biol. Assoc. U. K.* 70:43–55.
- De León, L. F., G. Rolshausen, E. Bermingham, J. Podos, and A. P. Hendry. 2012. Individual specialization and the seeds of adaptive radiation in Darwin’s finches. *Evol. Ecol. Res.* 14:365–380.
- de Lussanet, M. H. E., and M. Muller. 2007. The smaller your mouth, the longer your snout: predicting the snout length of *Syngnathus acus*, *Centricus scutatus* and other pipette feeders. *J. R. Soc. Interface* 4:561–573.
- Erzini, K., L. Bentes, R. Coelho, C. Correia, P. G. Lino, P. Monteiro, J. Ribeiro, and J. M. S. Gonçalves. 2002. Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (Southern Portugal).
- Ferry-Graham, L. A., D. I. Bolnick, and P. C. Wainwright. 2002. Using functional morphology to examine the ecology and evolution of specialization. *Integr. Comp. Biol.* 42:265–277.
- Fox, J., and S. Weisberg. 2011. An R companion to applied regression. 2nd ed. Sage, Thousand Oaks, CA.

- Fox, L. R., and P. A. Morrow. 1981. Specialization: Species property or local phenomenon? *Sci. Wash. C* 211:887–893.
- Franzoi, P., A. Franco, and P. Torricelli. 2010. Fish assemblage diversity and dynamics in the Venice lagoon. *Rendiconti Lincei-Sci. Fis. E Nat.* 21:269–281.
- Franzoi, P., R. Maccagnani, R. Rossi, and V. U. Ceccherelli. 1993. Life cycles and feeding habits of *Syngnathus taenionotus* and *S. abaster* (Pisces, Syngnathidae) in a brackish bay of the Po River Delta (Adriatic Sea). *Mar. Ecol. Prog. Ser.* 97:71–81.
- Franzoi, P., F. Riccato, A. Franco, and P. Torricelli. 2004. Dietary differences in three pipefish species (Osteichthyes, Syngnathidae) related to snout morphology. *Biol. Mar. Mediterr.* 11:592–594.
- Froese, R., and D. Pauly. 2010. FishBase.
- Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. *Annu. Rev. Ecol. Syst.* 19:207–233.
- Gavrilets, S., and J. B. Losos. 2009. Adaptive radiation: Contrasting theory with data. *Sci. Wash. C* 323:732–737.
- Gilbert, K. J., and M. C. Whitlock. 2015. Qst–Fst comparisons with unbalanced half-sib designs. *Mol. Ecol. Resour.* 15:262–267.
- Gilbert, K. J., and M. C. Whitlock. 2014. QstFstComp: Qst Fst comparisons with unbalanced half-sib designs. R package version 0.2.
- Grant, P. R., and B. R. Grant. 2011. How and why species multiply: The radiation of Darwin’s finches. Princeton University Press, Princeton, New Jersey.
- Grant, P. R., B. R. Grant, J. N. Smith, I. J. Abbott, and L. K. Abbott. 1976. Darwin’s finches: Population variation and natural selection. *Proc. Natl. Acad. Sci. U. S. A.* 73:257–261.
- Hablützel, P. I., and A. B. Wilson. 2011. Notes on the occurrence of *Syngnathus rostellatus* (Teleostei: Syngnathidae) in the Mediterranean. *Mar. Biodivers. Rec.* 4:e57.

- Herder, F., and J. Freyhof. 2006. Resource partitioning in a tropical stream fish assemblage. *J. Fish Biol.* 69:571–589.
- Hoffman, E. A., K. B. Mobley, and A. G. Jones. 2006. Male pregnancy and the evolution of body segmentation in seahorses and pipefishes. *Evolution* 60:404–410.
- Hubbs, C. L. 1922. Variations in the number of vertebrae and other meristic characters of fishes correlated with the temperature of water during development. *Am. Nat.* 56:360–372.
- Ivan Perez, S., V. Bernal, and P. N. Gonzalez. 2006. Differences between sliding semi-landmark methods in geometric morphometrics, with an application to human craniofacial and dental variation. *J. Anat.* 208:769–784.
- Kendrick, A. J., and G. A. Hyndes. 2005. Variations in the dietary compositions of morphologically diverse syngnathid fishes. *Environ. Biol. Fishes* 72:415–427.
- Klingenberg, C. P. 2016. Size, shape, and form: Concepts of allometry in geometric morphometrics. *Dev. Genes Evol.* 226:113–137.
- Lavin, P. A., and J. D. McPhail. 1985. The evolution of freshwater diversity in the threespine stickleback (*Gasterosteus aculeatus*): Site-specific differentiation of trophic morphology. *Can. J. Zool.* 63:2632–2638.
- Leysen, H., G. Roos, and D. Adriaens. 2011. Morphological variation in head shape of pipefishes and seahorses in relation to snout length and developmental growth. *J. Morphol.* 272:1259–1270.
- Loxdale, H. D., G. Lushai, and J. A. Harvey. 2011. The evolutionary improbability of ‘generalism’ in nature, with special reference to insects. *Biol. J. Linn. Soc.* 103:1–18.
- Marques, A. J. S. 2006. Distribution of the plankton community in the Ria Formosa, a coastal lagoon in South Eastern Portugal. University of Wales, Bangor, Wales.
- Mauchline, J. (ed). 1998. The biology of calanoid copepods. Academic Press, London.
- Mauchline, J. 1980. The biology of mysids and euphausiids. Academic Press, London.
- Meyer, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* 41:1357–1369.

- Mittelbach, G. G., C. W. Osenberg, and P. C. Wainwright. 1999. Variation in feeding morphology between pumpkinseed populations: Phenotypic plasticity or evolution? *Evol. Ecol. Res.* 1:111–128.
- Mitteroecker, P., P. Gunz, M. Bernhard, K. Schaefer, and F. L. Bookstein. 2004. Comparison of cranial ontogenetic trajectories among great apes and humans. *J. Hum. Evol.* 46:679–698.
- Nosil, P. 2012. *Ecological speciation*. Oxford University Press, Oxford, England.
- Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlenn, P. R. Minchin, R. B. O’Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2016. *vegan: Community Ecology Package*.
- Oliveira, F., K. Erzini, and J. M. S. Goncalves. 2007. Feeding habits of the deep-snouted pipefish *Syngnathus typhle* in a temperate coastal lagoon. *Estuar. Coast. Shelf Sci.* 72:337–347.
- Pihl, L., S. Baden, N. Kautsky, P. Ronnback, T. Soderqvist, M. Troell, and H. Wennhage. 2006. Shift in fish assemblage structure due to loss of seagrass *Zostera marina* habitats in Sweden. *Estuar. Coast. Shelf Sci.* 67:123–132.
- Pinkas, L., M. S. Oliphant, and I. L. K. Iverson. 1971. Food habits of albacore, blue fin tuna, and bonito in California waters. Department of Fish and Game, California.
- Price, T. 1987. Diet variation in a population of Darwin’s finches. *Ecology* 68:1015–1028.
- Puebla, O. 2009. Ecological speciation in marine v. freshwater fishes. *J. Fish Biol.* 75:960–996.
- R Development Core Team. 2016. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rauther, M. 1925. Die Syngnathiden des Golfes von Neapel. *Stazione Zoologica di Napoli*, Naples.
- Ribeiro, J., C. C. Monteiro, P. Monteiro, L. Bentes, R. Coelho, J. M. S. Gonçalves, P. G. Lino, and K. Erzini. 2008. Long-term changes in fish communities of the Ria Formosa coastal lagoon (southern Portugal) based on two studies made 20 years apart. *Estuar. Coast. Shelf Sci.* 76:57–68.

- Riccardi, N. 2010. Selectivity of plankton nets over mesozooplankton taxa: Implications for abundance, biomass and diversity estimation. *J. Limnol.* 69:287–296.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- Rispoli, V. F., and A. B. Wilson. 2008. Sexual size dimorphism predicts the frequency of multiple mating in the sex-role reversed pipefish *Syngnathus typhle*. *J. Evol. Biol.* 21:30–38.
- Rocha, L. A., D. R. Robertson, J. Roman, and B. W. Bowen. 2005. Ecological speciation in tropical reef fishes. *Proc. R. Soc. Lond. B Biol. Sci.* 272:573–579.
- Rohlf, F. J. 2016. tpsDIG: Digitize landmarks and outlines. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY.
- Rollo, A., and D. Higgs. 2008. Differential acoustic response specificity and directionality in the round goby, *Neogobius melanostomus*. *Anim. Behav.* 75:1903–1912.
- Rüber, L., E. Verheyen, and A. Meyer. 1999. Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. *Proc. Natl. Acad. Sci. U. S. A.* 96:10230–10235.
- Sagebakken, G., C. Kvarnemo, and I. Ahnesjö. 2017. Nutritional state: A survival kit for brooding pipefish fathers. *Biol. J. Linn. Soc.* 121:312–318.
- Schlager, S. 2016. Morpho: Calculations and visualisations related to geometric morphometrics.
- Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Sci. Wash. C* 266:798–801.
- Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford.
- Shipley, L. A., J. S. Forbey, and B. D. Moore. 2009. Revisiting the dietary niche: When is a mammalian herbivore a specialist? *Integr. Comp. Biol.* 49:274–290.
- Smith, T. B., and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.* 27:111–133.
- Sotka, E. E. 2012. Natural selection, larval dispersal, and the geography of phenotype in the sea. *Integr. Comp. Biol.* 52:538–545.

- Steffe, A. S., M. Westoby, and J. D. Bell. 1989. Habitat selection and diet in two species of pipefish from seagrass: Sex differences. *Mar. Ecol.-Prog. Ser.* 55:23–30.
- Stephens, D. W., and J. R. Krebs. 1986. *Foraging Theory*. Princeton University Press, Princeton, New Jersey.
- Streelman, J. T., and P. D. Danley. 2003. The stages of vertebrate evolutionary radiation. *Trends Ecol. Evol.* 18:126–131.
- Teixeira, R. L., and J. A. Musick. 1995. Trophic ecology of two congeneric pipefishes (Syngnathidae) of the Lower York River, Virginia. *Environ. Biol. Fishes* 43:295–309.
- Tiselius, P., A. Belgrano, L. Andersson, and O. Lindahl. 2015. Primary productivity in a coastal ecosystem: A trophic perspective on a long-term time series. *J. Plankton Res.* fbv094.
- Tsuboi, M., A. C. O. Lim, B. L. Ooi, M. Y. Yip, V. C. Chong, I. Ahnesjö, and N. Kolm. 2017. Brain size evolution in pipefishes and seahorses: The role of feeding ecology, life history and sexual selection. *J. Evol. Biol.* 30:150–160.
- Van Wassenbergh, S., G. Roos, P. Aerts, A. Herrel, and D. Adriaens. 2011a. Why the long face? A comparative study of feeding kinematics of two pipefishes with different snout lengths. *J. Fish Biol.* 78:1786–1798.
- Van Wassenbergh, S., G. Roos, and L. Ferry. 2011b. An adaptive explanation for the horse-like shape of seahorses. *Nat. Commun.* 2:164.
- Van Wassenbergh, S., G. Roos, A. Genbrugge, H. Leysen, P. Aerts, D. Adriaens, and A. Herrel. 2009. Suction is kid's play: Extremely fast suction in newborn seahorses. *Biol. Lett.* 5:200–203.
- von Rintelen, T., A. B. Wilson, A. Meyer, and M. Glaubrecht. 2004. Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. *Proc. R. Soc. B Biol. Sci.* 271:2541–2549.
- Wainwright, P. C., D. R. Bellwood, M. W. Westneat, J. R. Grubich, and A. S. Hoey. 2004. A functional morphospace for the skull of labrid fishes: Patterns of diversity in a complex biomechanical system. *Biol. J. Linn. Soc.* 82:1–25.

Wainwright, P. C., M. D. McGee, S. J. Longo, and L. Patricia Hernandez. 2015. Origins, innovations, and diversification of suction feeding in vertebrates. *Integr. Comp. Biol.* 55:134–145.

Warner, R. R. 1997. Evolutionary ecology: How to reconcile pelagic dispersal with local adaptation. *Coral Reefs* 16:S115–S120.

Wegmann, A. 2009. Geographic variation in the trophic morphology of a syngnathid pipefish. University of Zurich, Zurich, Switzerland.

Wilson, A. B., and I. Eigenmann Veraguth. 2010. The impact of Pleistocene glaciation across the range of a widespread European coastal species. *Mol. Ecol.* 19:4535–4553.

Figure Legends:

Fig. 1. Geographic distribution of sampled populations of *S. typhle*, highlighting the three focal localities (KLU, RIA, VEN).

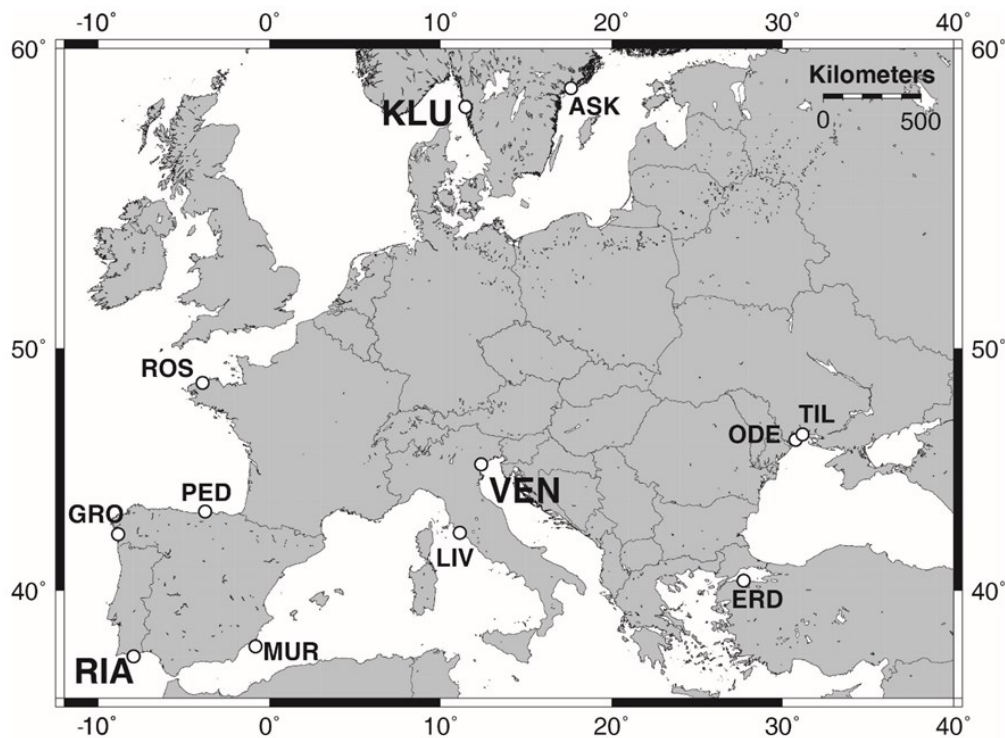


Fig. 2. (a) Intraspecific variation of cranial morphology across populations of *S. typhle* (scale bar: 1cm) (see Fig. 1 for population locations); (b) 13 homologous landmarks and 4 semi-landmarks (LM4-7) were used to quantify head morphology using geometric morphometrics (skeletal diagram modified after Rauther (1925)).



Fig. 3. Canonical variate analysis showing major axes of variation in trophic morphology across (a) *S. typhle* species range (see Fig.1 for population locations); (b) adults and juveniles in three focal populations; and (c) wild-caught animals and captive-bred individuals raised in a common environment. 95% confidence ellipses delineate intrasample variation.

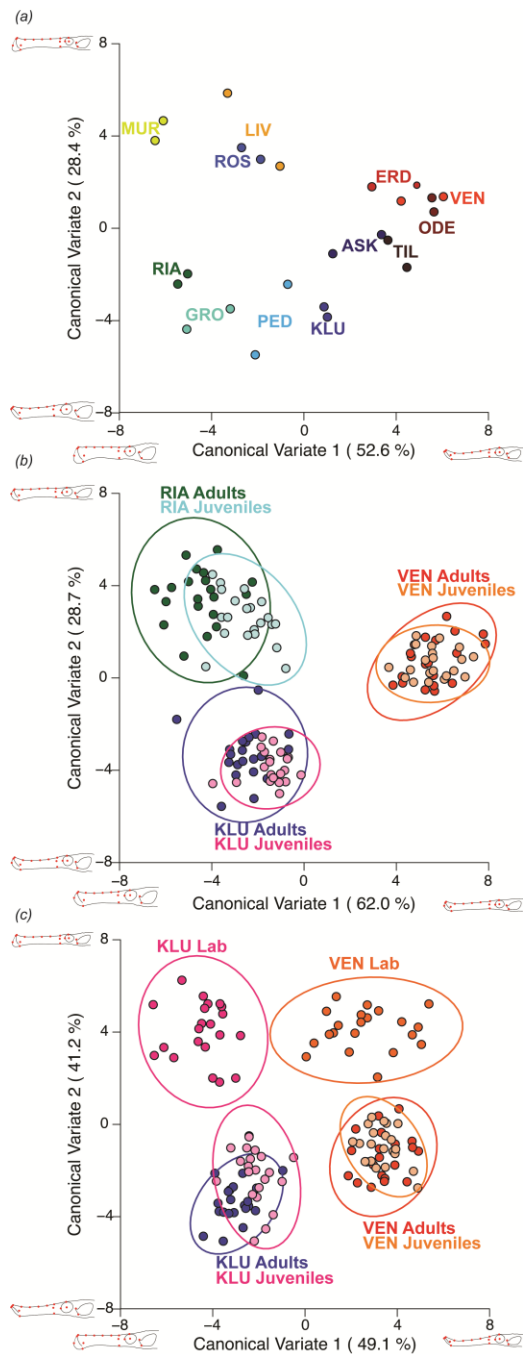


Fig. 4. Allometric trajectories of juvenile and adult populations from KLU (blue), RIA (green) and VEN (red), along with the common allometric component, showing the predicted allometric relationship in the absence of population structure (black). Shape outlines show major changes in cranial morphology estimated from CAC regression (Mitteroecker et al. 2004) .

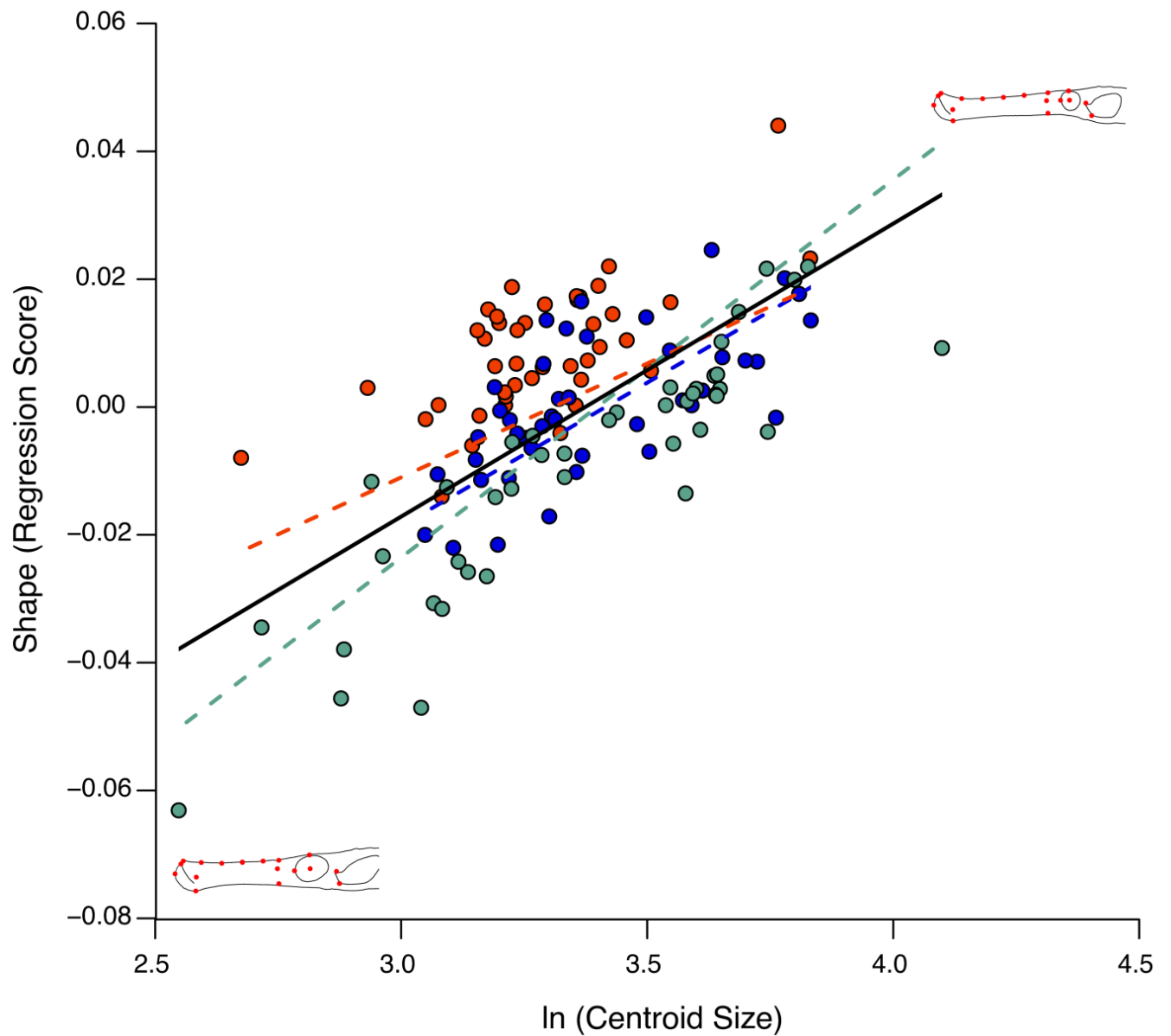


Fig. 5. Relative representation of free-living zooplankton communities and dietary composition of adults and juveniles at each of the three study sites.

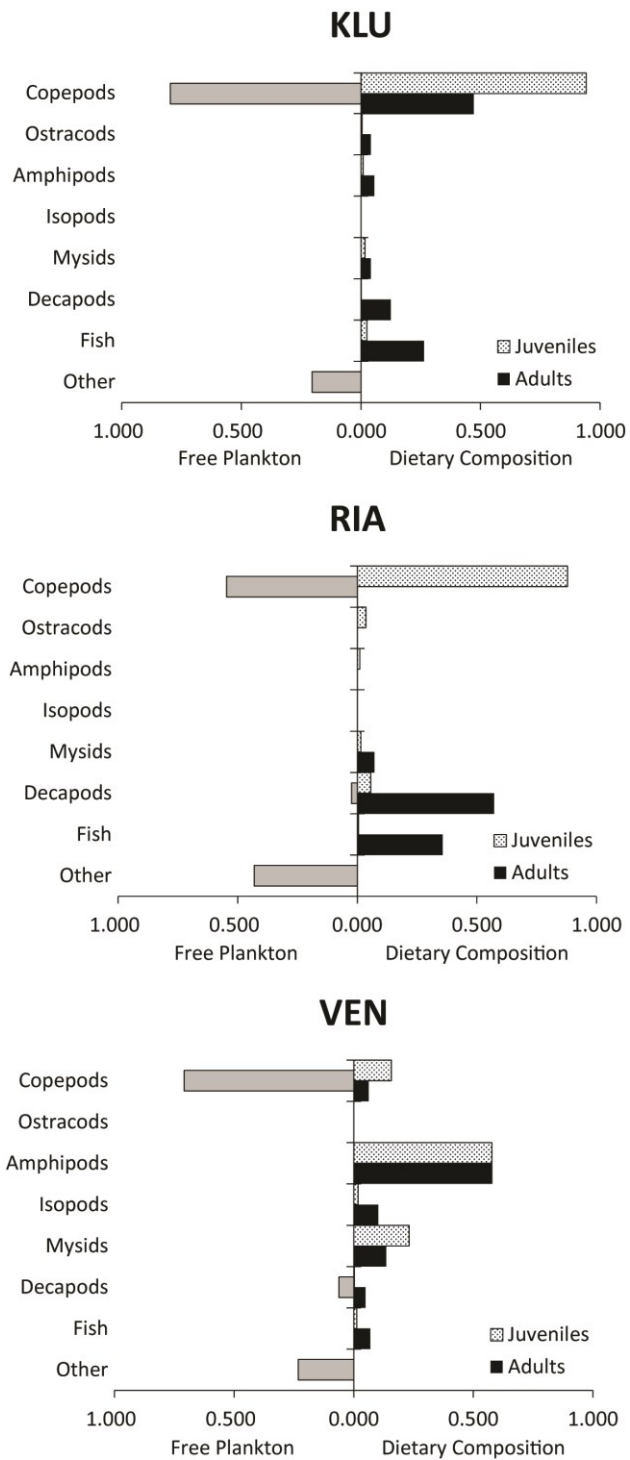


Fig. 6. Primary axes of a two-block partial least squares analysis of pipefish shape and dietary composition for each site. Dashed line indicates the line of best fit between PLS1 axes for shape and diet. Prey items positioned along dietary axis according to relative loadings on PLS1. Juveniles (light points) and adults (dark points) separately shaded for clarity.

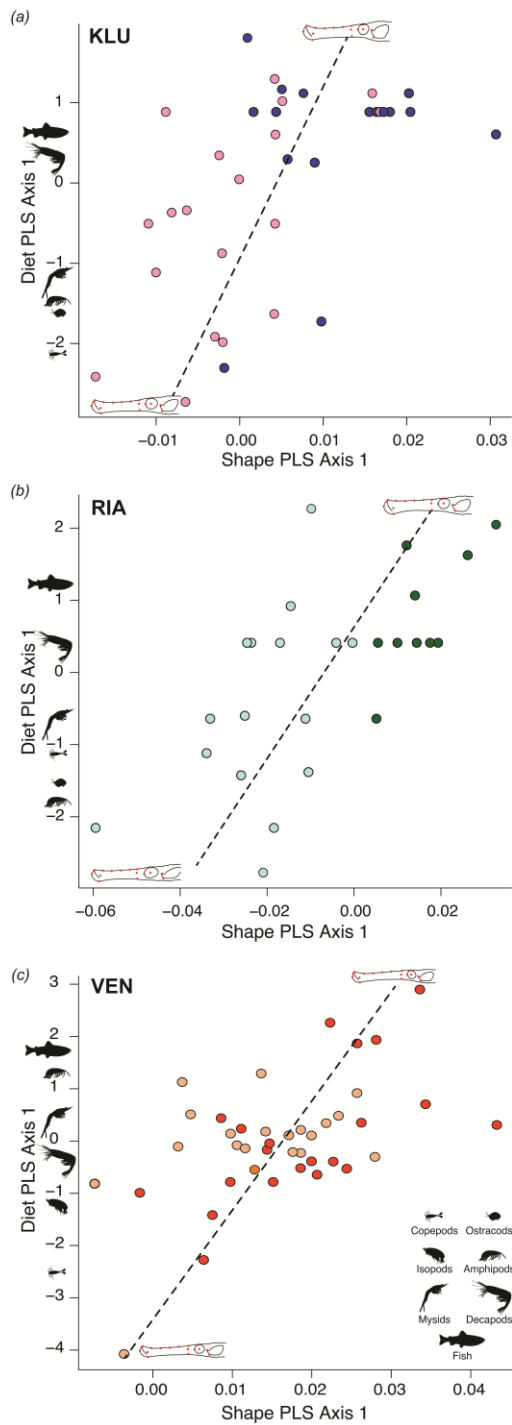


Table 1. Descriptive statistics for sampled populations

Pop	Maturity	#	Total Length \pm SD (cm)	Head Length \pm SD (mm)	Snout Length \pm SD (mm)	SnoutLength: Head Length	Gape Size \pm SD (mm)
<i>Intraspecific sample</i>							
ASK	Adu	2	16.90 \pm 3.25	29.78 \pm 7.21	18.43 \pm 4.48	0.619 \pm 0.00	2.35 \pm 0.64
KLU	Adu	2	16.10 \pm 3.39	28.35 \pm 4.91	17.48 \pm 2.88	0.617 \pm 0.01	2.50 \pm 0.78
ROS	Adu	2	25.20 \pm 0.42	42.61 \pm 0.63	27.00 \pm 0.28	0.633 \pm 0.00	3.53 \pm 0.32
PED	Adu	2	17.05 \pm 3.75	28.59 \pm 7.45	17.43 \pm 5.26	0.606 \pm 0.03	2.23 \pm 0.25
GRO	Juv	2	11.20 \pm 0.57	20.89 \pm 1.03	12.44 \pm 0.62	0.595 \pm 0.00	1.93 \pm 0.04
RIA	Adu	2	18.60 \pm 0.42	31.88 \pm 0.06	19.81 \pm 0.49	0.621 \pm 0.01	3.01 \pm 0.23
MUR	Adu	2	24.70 \pm 0.99	42.70 \pm 0.25	27.26 \pm 1.20	0.638 \pm 0.02	3.10 \pm 0.18
LIV	Adu	2	25.85 \pm 2.62	46.84 \pm 1.34	30.17 \pm 0.91	0.644 \pm 0.00	3.50 \pm 0.14
VEN	Adu	2	13.95 \pm 1.63	26.97 \pm 3.15	17.51 \pm 2.49	0.648 \pm 0.02	1.57 \pm 0.19
ERD	Adu	2	20.45 \pm 1.34	41.01 \pm 0.87	26.34 \pm 0.55	0.642 \pm 0.00	3.52 \pm 0.13
ODE	Adu	2	21.80 \pm 0.57	41.15 \pm 2.54	25.86 \pm 1.50	0.629 \pm 0.00	2.96 \pm 0.02
TIL	Adu	2	18.25 \pm 1.20	32.99 \pm 0.83	21.04 \pm 1.06	0.638 \pm 0.02	2.64 \pm 0.41
Range (min-max)		24	10.80–27.70	20.16–47.79	12.00–30.81	0.588–0.660	1.43–3.75
<i>Wild-caught specimens</i>							
KLU	Juv	21	13.05 \pm 1.14	22.91 \pm 2.05	14.05 \pm 1.43	0.613 \pm 0.01	1.54 \pm 0.12
	Adu	21	18.20 \pm 3.15	31.58 \pm 5.39	19.63 \pm 3.58	0.621 \pm 0.02	2.56 \pm 0.71
RIA	Juv	21	11.33 \pm 2.03	20.12 \pm 3.84	12.19 \pm 2.53	0.604 \pm 0.02	1.37 \pm 0.32
	Adu	21	20.08 \pm 3.32	34.64 \pm 5.12	21.78 \pm 3.34	0.628 \pm 0.01	3.10 \pm 0.65
VEN	Juv	21	10.73 \pm 1.41	20.43 \pm 2.50	12.96 \pm 1.67	0.634 \pm 0.01	1.15 \pm 0.18
	Adu	21	14.21 \pm 3.11	26.42 \pm 4.73	16.88 \pm 3.06	0.639 \pm 0.01	1.64 \pm 0.38
<i>Common-garden experiment</i>							
KLU	Juv	21	11.25 \pm 1.66	20.74 \pm 2.95	12.24 \pm 2.07	0.588 \pm 0.02	n.a.
VEN	Juv	21	11.99 \pm 1.78	23.92 \pm 3.36	15.27 \pm 2.63	0.636 \pm 0.03	n.a.

Table 2: Stomach contents of juvenile and adult *S. typhle* listed by prey group, along with average prey size (length (L) x width (W) in mm). Prey sizes for RIA estimated as mean of VEN and KLU measures. Isopod length and width for VEN juveniles based on measured size for adults

Pop	Maturity	#	Total prey	Copepods			Ostracods			Isopods			Amphipods			Mysids			Decapod shrimp			Fish		
				#	L	W	#	L	W	#	L	W	#	L	W	#	L	W	#	L	W	#	L	W
KLU	Juv	19	1,059	998	0.74	0.30	5	0.26	0.14	0	-	-	10	2.75	0.49	18	4.42	0.81	2	7.04	0.81	26	12.86	1.68
	Adu	16	72	34	0.50	0.25	3	0.58	0.26	0	-	-	4	2.91	0.66	3	4.81	1.27	9	9.12	3.27	19	18.95	2.93
RIA	Juv	16	197	173	0.69	0.29	7	0.26	0.14	0	-	-	2	2.91	0.53	3	4.21	0.81	11	6.83	1.46	1	16.88	1.34
	Adu	10	14	0	-	-	0	-	-	0	-	-	0	-	-	1	5.66	1.21	8	8.23	2.74	5	18.37	2.06
VEN	Juv	21	393	62	0.64	0.28	0	-	-	7	2.23	0.53	227	3.06	0.56	91	3.99	0.80	1	6.62	2.11	5	20.89	1.00
	Adu	20	302	19	0.96	0.47	0	-	-	31	2.23	0.53	175	2.57	0.71	41	6.51	1.14	15	7.34	2.21	21	17.78	1.19

Table 3: Comparison of genetic (F_{st}) and phenotypic (Q_{st}) variation between KLU and VEN. F_{st} estimates derived from the analysis of nine microsatellite loci (Wilson and Eigenmann-Veraguth 2010), and Q_{st} estimates inferred from patterns of cranial variation in captive-reared animals measured along the two primary axes of the canonical variate analysis (CV1 and CV2; Fig. 3b). Q_{st} estimates calculated for half-sib and full-sib mating designs provided for comparative purposes. 95% confidence limits of parameter estimates estimated by parametric bootstrapping (N=10,000 permutations)

Parameter	Estimate (95% CI)	$Q_{st}-F_{st}$	P-value ($Q_{st}>F_{st}$)
Half-Sibs (r=0.25)			
F_{st}	0.325 (0.188, 0.431)		
Q_{st} CV1	0.848 (-0.046, 1.089)	0.523 (-0.495, 0.958)	0.093
→ CV2	0.074 (-0.651, 0.660)	-0.252 (-2.303, 1.911)	0.599
Full-Sibs (r=0.50)			
F_{st}	0.325 (0.188, 0.431)		
Q_{st} CV1	0.918 (-0.090, 1.055)	0.593 (-0.593, 0.970)	0.075
→ CV2	0.137 (-1.255, 1.253)	-0.188 (-2.345, 2.240)	0.499

Table S1. Description of cranial landmarks

Landmark ID	Landmark/Semi-landmark?	Description
LM1	Landmark	Anterior point of the upper snout (premaxilla)
LM2	Landmark	Highest point of the anterior part of the upper snout at the premaxilla
LM3	Landmark	Most posterior point of the maxilla, at maximal curvature
LM4	Semilandmark	Semilandmarks spaced equally along dorsal snout between LM2 and LM8
LM5	Semilandmark	Semilandmarks spaced equally along dorsal snout between LM2 and LM8
LM6	Semilandmark	Semilandmarks spaced equally along dorsal snout between LM2 and LM8
LM7	Semilandmark	Semilandmarks spaced equally along dorsal snout between LM2 and LM8
LM8	Landmark	Midpoint between the nostrils at the dorsal surface, where the mesethmoidal bone meets the frontal bone
LM9	Landmark	Anterior end of anterior nostril
LM10	Landmark	Posterior ventral corner, at maximum curvature, of the lateral ethmoidal bone
LM11	Landmark	Center of the eye (triangulation of three landmarks around orbit of eye)
LM12	Landmark	Dorsal surface of the head at the midpoint of the eye (LM11)
LM13	Landmark	Anterior point of the operculum
LM14	Landmark	Ventral posterior edge of the preopercular bone where it intersects the operculum
LM15	Landmark	Ventral surface at the height of the anterior nostril (LM9)
LM16	Landmark	The most ventral point at the intersection of the mandible and quadrate bone
LM17	Landmark	Anterior point of the lower snout (mandible)