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The sex-role reversed pipefish Syngnathus typhle is a member of the Syngnathidae, a family of fishes in which males brood embryos on their body surface. As in most ectotherms, embryonic development is highly temperature-dependent in syngnathids and male brooding periods are extended when water temperatures are reduced. The influence of temperature on reproduction is expected to effectively truncate the breeding season and reduce fecundity in cold waters, potentially enhancing the opportunity for both fecundity and sexual selection. We studied spatial variation in the morphology and reproductive biology of S. typhle in five European populations which vary in latitude and water temperature. Microsatellite analyses indicated that the average number of male mates per population ranged between 1.3 and 3.7. The frequency of multiple mating by males was negatively correlated with the degree of sexual size dimorphism in each population, suggesting that disproportionate increases in female fecundity may be able to compensate for increased male brood pouch capacity. Both sexes were larger and males had an increased brood size where water temperatures during the breeding season were lower. Morphological variation among populations may be mediated by differences in fecundity selection associated with different optimal reproductive strategies in cold and warm water environments.

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# Sexual size dimorphism predicts the frequency of multiple mating in the sex-role reversed pipefish *Syngnathus typhle*

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## Abstract

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*Keywords:* Bergmann's Rule
genetic mating system;
life history variation;
sex-role reversal;
sexual size dimorphism

## Introduction

Molecular methods have revolutionized the study of reproductive biology, providing direct estimates of genetic mating and overturning established paradigms on the frequency of multiple mating in the wild (Griffith *et al.*, 2002). Despite the importance of these studies, most of this work has been limited to single populations at a single point in time, potentially obscuring both spatial and temporal variation in reproductive biology. Reproductive variation within a species may influence the opportunity for sexual selection and is thought to be critical to the evolution of mating systems (Panhuis *et al.*, 2001).

Local environmental conditions may strongly influence both morphological and life history traits. Bergmann's Rule (1847), the positive correlation between latitude and organismal body size, was originally postulated for endothermic animals and thought to be caused by adaptations for thermal regulation in cold environments. This pattern has also been found in many ectothermic organisms (Ray, 1960; Atkinson, 1994, Angilletta & Dunham, 2003). Thermal optimization cannot explain the presence of latitudinal size gradients in ectotherms, and other factors must be responsible for the existence of this pattern in ectothermic species. Fecundity is strongly correlated with organismal body size in a diversity of taxa (Roff, 2002) and fecundity selection for large body size may produce latitudinal body size clines: Low temperatures at higher latitudes may lead to increased developmental times, leading to the evolution of large individuals with higher fecundity per reproductive event. As temperature clearly influences both development and reproduction, it is likely that selection at both of these life history stages has a strong effect on the evolution of body size.

One model used to predict the direction and the intensity of the competition for access to mates is based on the potential reproductive rate, the number of offspring an organism is able to produce per unit time (PRR; Kvarnemo & Ahnesjö, 1996). PRR is often higher in males than in females due to the fact that females of most species have extended intervals between

reproductive episodes. When temperatures are low, sexual differences in PRR may increase, as female interbrood intervals are extended. This may lead to a male-based operational sex ratio (OSR, the proportion of reproductively active males and females), increasing male-male competition for access to females and favouring the evolution of secondary sexual characteristics in males through mating competition and female mate choice (Darwin, 1871).

Sexual selection theory predicts that when the PRR of males falls below that of females, the OSR may become female-biased. Under these conditions, a reversal in the direction of sexual selection may occur, such that competition for access to mates can be observed in females; a phenomenon known as sex-role reversal (Vincent *et al.,* 1992). As syngnathid fishes (seahorses and pipefishes) exhibit both conventional sex-roles and sex-role reversal, they are ideal candidates to test theories related to the evolution of sex differences and their influence on sexual selection (Williams, 1975; Wilson *et al.*, 2003). All members of this family display paternal brood care, with males brooding embryos in brooding pouches or patches on their body. During mating, the female transfers her eggs to the male's brood pouch, where they are fertilized. In the broad-nosed pipefish *Syngnathus typhle*, sex-roles are reversed, and competition for access to mates can be observed among females (Berglund *et al.*, 1986a). The wide distribution of *S. typhle* along the European coast (Fig. 1) makes this species ideal to study environmentally-mediated reproductive variation. Recent investigations indicate that the genetic diversity of *S. typhle* in northern Europe is greatly reduced compared to southern populations and suggest that the Mediterranean is likely the origin of this species (Eigenmann, 2007).

In *S. typhle,* males brood eggs much longer in cold water (58 days at 10°C) than in warm water (35 days at 15°C) and consequently have a reduced PRR at lower temperatures (Ahnesjö, 1995). Conversely, female PRR is not significantly influenced by temperature (Ahnesjö, 1995). This scenario likely leads to an increased sexual difference in the PRR in

colder environments, and is expected to intensify sex-role reversal. A study of a Swedish population of *S. typhle* found that 90% of males mate multiply, with an average of 3.1 females contributing to each brood (Jones *et al.*, 1999). If the ripe eggs available for transfer by a single female are insufficient to fill a males' pouch, multiple mating may allow male pipefish to increase their PRR. Given the increased brooding times and the truncated breeding seasons in colder environments, male body size is expected to be under fecundity selection in cold water environments and, if multiple mating is necessarily to completely fill a males pouch, polygyny is expected to be more frequent in males from northern latitudes. Alternatively, larger females may be capable of producing enough eggs to complete fill a male's brood pouch, allowing the maximization of male reproductive output without the necessity of multiple mating. Sexual selection on female body size might therefore be intensified in northern latitudes through a combination of male preferences for large partners and female-female competition for mates.

Recent work on a North American pipefish species (*S. leptorhynchus*) supports these predictions (Wilson, unpublished data). Both male and female body size increases with latitude in this species, consistent with Bergmann's Rule. Fecundity of both sexes increases with body size, but increased female fecundity at higher latitudes is unable to fully compensate for increased male brood pouch capacity. As a consequence, the frequency of polygyny in this species also increases with latitude. Here, we test the generality of the pattern observed in *S. leptorhynchus* through the investigation of reproductive variation in populations of the European pipefish *S. typhle* across a broad latitudinal scale.

Five European *S. typhle* populations differing in latitude and ambient water temperature were analyzed morphologically and genetically in order to explore spatial variation in the reproductive biology of the species. Our results highlight significant differences in genetic mating behaviour in populations of *S. typhle* associated with sexual size dimorphism, and

indicate that reproductive differences are strongly correlated with local water temperatures. Disproportionate increases in female body size and fecundity relative to males allow the maximization of male brood capacity without the necessity of increased multiple mating. Morphological variation among sites may be influenced by differences in fecundity selection associated with different reproductive strategies in cold and warm water environments.

## Materials and Methods

### Collections / Sample preparation

Pipefish were sampled from five populations along the European coast between June and August 2006 (Fig.1). Monthly surface water temperature data and data on the breeding season of *S. typhle* were provided by local researchers or obtained through the literature (Fig. 2). Local salinity was measured with a refractometer (VITAL SINE, SR-6).

At Askö Island (ASK) and Fiskebäckskil, Sweden (KLU), Roscoff, France (ROS) and Venice, Italy (VEN), pipefish were found in eelgrass (*Zostera marina*) meadows. Additional *S. typhle* samples were provided by researchers from the University of Algarve from Ria Formosa, Portugal (RIA), a habitat dominated by *Cymodocea nodosa*. *S. typhle* were collected with a beach seine (mesh size between 2 and 3.2 mm) at all sampling locations with the exception of KLU, where specimens were sampled with a boat-drawn trawl with a mesh-size of 4 mm.

Male pipefish were considered reproductively mature when they had a completely developed brood pouch. Females were considered mature when their ovaries contained mature eggs. Size-calibrated digital photos were taken of each pipefish before preservation in 97% ethanol.

Standard body length SL (the distance between the snout and the caudal peduncle) of each fish was measured from digital images. Female fecundity was estimated by counting the

number of mature eggs in both ovaries. All females collected in ROS were transported alive to the laboratory in Zurich, where their SL was measured. These animals were kept in a closed recirculating system at a water temperature of 20°C and a salinity of $33^{o}/_{oo.}$ ROS females were dissected after 4-6 months using the same procedure as females from the other populations in order to investigate egg development.

Male fecundity was calculated as the number of fertilized eggs carried in the brood pouch. Embryos were removed and transferred to a 96-well plate in the order that they were found in the brood pouch and were classified as one of six developmental stages ranging from A (undeveloped egg) to F (completely developed offspring ready to be released). The classification of embryonic stages followed Boisseau's (1967) work on European seahorses. For maternity analysis, 5-10 males were randomly chosen from each population (Table 1).

**Maternity analyses**

A minimum of every fifth egg from each male was genetically analyzed for the assessment of maternity (10-69 eggs per individual). DNA of embryos of developmental stages C to F was extracted following the DNA-extraction method of Gloor and Engels (1992), providing moderate DNA yields (average: 48 ng/µl). Preliminary analyses indicated DNA yields from early stage embryos were low (4 ng/µl) and these embryos were subsequently extracted using a DNeasy® 96 Tissue Kit (Qiagen). The procedure was conducted according to manufacturer's recommendation, with the exception of the final step, where DNA was diluted in 50 µl of elution buffer. Every fifth egg was genetically analyzed for the assessment of maternity for a total of 10 to 69 eggs per individual.

Three highly variable microsatellite loci (Styph04, Styph12, and Styph18) were employed for maternity analyses. Primers were designed for *S. typhle* by Jones *et al.* (1999) except for primer Styph18.2R (AAG TGG TCC AAT GAG GGC), which was redesigned

from the sequence of the original clone provided by A. Jones. The 5' end of each primer was fluorescently labelled for visualization.

Microsatellites were PCR-amplified (DNA Engine Tetrad® 2, Peltier Thermal Cycler, MJ Research) in 11.1 µl volumes containing 1 µM dNTP (Roche), 4.3 µl $ddH_20$, 1 X ThermoPol Buffer (New England Bio Labs Inc.), 0.2 µM of each primer (Applied Biosystems and Bio Labs Inc.), 1 U Taq Polymerase (Bio Labs Inc.) and 20-200 ng of extracted DNA. Cycling parameters consisted of an initial denaturation step at 94°C for 2 min, followed by 30 cycles at 94°C for 1 min, an annealing temperature at 55°C for 1.5 min and at 72°C for 1 min. A final extension at 72°C for 30 min concluded the cycling.

For genotyping, amplified DNA of embryos at stages A and B was not diluted after amplification. Amplified DNA of embryos at stages C and D was diluted 1:20 in $ddH_2O$ while DNA from embryos at stages E and F was diluted 1:60. 2 µl of the amplification product was mixed with 5 µl $ddH_2O$, 9.95 µl deionized Formamide (Minimum 99.5%, Applied Biosystems and Sigma) and 0.06 µl of LIZ size standard (Gene Scan ™ 500 LIZ ™, Applied Biosystems). Mixtures were denatured at 95°C for 5 min (DNA Engine Tetrad® 2, Peltier Thermal Cycler, MJ Research) and placed immediately on ice. Capillary electrophoresis was conducted on an automated sequencer (ABI 3730, Applied Biosystems).

Genotyping analysis was performed with GENEMAPPER® Software, Version 4 (Applied Biosystems). Maternity analysis was conducted with the software program GERUD 2.0 (Jones, 2005). This program also calculates expected exclusion probabilities to determine the statistical power of the markers for parentage assessment. Population allele frequency data was incorporated, as parentage estimation incorporating this information has been shown to be more accurate (Jones 2005). Population allele frequencies were provided by I. Eigenmann (2007).

### Statistical analyses

The relationship between male and female body size, collection population, local water temperature, multiple maternity, female fecundity and male brood size were investigated by linear and exponential regression analyses. Analysis of variance (ANOVA) and t-tests for independent samples were conducted in order to investigate morphological and reproductive differences between populations and between the sexes of each population. F-tests were constructed by taking the ratio of the between-group variation to the within-group variation. Regression and General Linear Model Analyses were performed with SPSS V13.0 (© SPSS Inc., 2004, Chicago, IL, www.spss.com).

## Results

### Breeding seasons

A compilation of direct estimates of reproductive activity highlighted significant differences in breeding seasons among the five study sites. The breeding season lasts approximately four months from May until the end of August in KLU (Berglund, 1986b; Ahnesjö, pers.comm.) and ASK (Duncker, 1908). In ROS, the breeding season starts in April and lasts until the end of September (Duncker, 1908). *S. typhle* from VEN breed from April until the end of October (Riccato *et al.*, 2003) (Fig. 2). In RIA, pregnant males of *S. typhle* can be found from February until the end of October (Freire, 2004). The breeding season is thus longest in the most southern population (RIA; 9 months), followed by VEN (7 months), ROS (6 months) and ASK and KLU (4 months) (Fig. 2).

### Morphology and fecundity

The number of sexually mature *S. typhle* collected at each site was 56 (M:F sex ratio: 1:1.2) at RIA, 23 (1:0.2) at VEN, 16 (1:0.3) at ROS, 81 (1:0.5) at KLU and 31 (1:0.4) at ASK.

Significant differences in male body size were found among populations ($F_{5,128}$= 41.46, p< 0.001). Mean SL ± SE of males from ROS was highest (23.4 cm ± 0.5), followed by RIA (16.5 cm ± 0.6), KLU (15.9 cm ± 0.4), VEN (14.2 cm ± 0.5) and ASK (12.4 cm ± 0.2) (Fig. 3). Female body sizes also differed significantly among populations ($F_{5,69}$= 13.57, p< 0.001). ROS females has the highest mean SL (27.8 cm ± 1.5 SE) followed by VEN (21.1 cm ± 1.8 SE), RIA (17.0 cm ± 0.5 SE), KLU (16.6 cm ± 0.6 SE) and ASK (16.3 cm ± 1.0 SE) (Fig. 3). Females were larger than males in all five populations (Fig. 3). Sexes differed significantly by size at VEN ($t_{21}$= 5.00, p< 0.001), ROS ($t_{14}$= 3.47, p= 0.004) and ASK ($t_{29}$= 3.73, p= 0.006) while no significant SSD was detected in either KLU or RIA (KLU: $t_{79}$ = 1.07, p= 0.288; RIA: $t_{54}$= 0.61, p= 0.543).

While latitude and average annual water temperature did not correlate with body size, fecundity or number of mates per male, water temperature during the breeding season was significantly negatively correlated with body size in males (y = -0.41x + 23.22, $r^2$= 0.09, $F_{1,131}$= 12.2, p=0.001) and showed similar trend in females: y = -0.36x + 24.30, $r^2$= 0.05, $F_{1,72}$= 5.83, p= 0.054). Water temperature during the breeding season and male fecundity showed a significant negative correlation (y = -19.79x + 483.16, $r^2$= 0.52, $F_{1,54}$= 44.57, p< 0.001).

Within each pouch, embryos were all at the same stage of development (Table 1) as previously documented by Jones *et al.* (1999). Populations differed significantly in the number of fertilized eggs carried by pregnant males ($F_{4,52}$= 34.27, p< 0.001). ROS males had the highest number of fertilized eggs (Mean= 268.5 ± 26.5 SE, N= 10) followed by KLU (Mean= 115.1 ± 4.3 SE, N= 22), VEN (Mean= 81.0 ± 19.5 SE, N= 8), RIA (Mean= 67.6 ± 9.1 SE, N= 11) and ASK (Mean= 64.6 ± 7.6 SE, N= 5).

Populations did not differ significantly in the number of mature eggs carried by females ($F_{3,24}$=1.85, p= 0.167). As all females sampled in ROS were kept in aquaria for several

months prior to dissection, fecundity could have been affected. ROS females were therefore excluded from this analysis, but their fecundity is shown in the descriptive graph (Fig. 4b). Mean ± SE number of mature eggs is highest in females from ROS (Mean= 174.5 ± 63.8, N= 4), followed by VEN (Mean= 87.0 ± 26.9, N= 4), ASK (Mean= 61.0 ± 9.4, N= 7), KLU (Mean= 59.3 ± 14.5, N= 7) and RIA (Mean= 47.0 ± 11.3, N= 9).

Male body size and fecundity exhibit a significantly positive linear (y= 20.92x - 250.19, $r^2$= 0.81, $F_{1,54}$= 232.76, p< 0.001) and exponential (y= $6.80e^{0.1522x}$, $r^2$= 0.85, $F_{1,54}$= 306.97, p<0.001) relationship (Fig. 4a). While most males had only two or three rows of eggs within the pouch, larger males often had up to five rows of eggs lying next to each other and several layers lying atop one another (Table 1). This may explain the linear and exponential relationships evident in the dataset. Female body size and the number of mature eggs in ovaries was also positive and significant for both a linear (y= 6.93x - 64.13, $r^2$= 0.53, $F_{1,25}$= 28.70, p< 0.001) and exponential relationship (y= $5.63e^{0.122x}$, $r^2$= 0.57, $F_{1,25}$= 32.75, p< 0.001) (Fig. 4b). Because of the three-dimensional structure of ovaries, an exponential relationship between female SL and fecundity is expected to best predict this relationship. Once again, ROS females were excluded from the regression analysis but are included in the graph (Fig. 4b).

**Maternity analyses**

The intrapopulation variability of the microsatellite markers was high (10-40 alleles per locus). Consequently, the combined exclusion probability of the three microsatellite loci for the investigation of an embryo's maternal genotype with known paternal genotype was greater than 0.989 for all populations, indicating that the power of the three loci is adequate for parentage assessment. Maternity analysis was not possible for embryos from ASK, as the low DNA yields from early stage embryos were inadequate for PCR amplification. Similarly, in

the population from KLU, where all broods were at the earliest developmental stage (Table 1), repeated DNA extractions were necessary in order to get reliable DNA yields for 10 broods. Populations differed significantly in the average number of mates per male ($F_{3,34}$= 10.79, p< 0.001). The highest mean ± SE of number of mates per males was found in KLU (Mean= 3.7 ± 0.2, N= 10) followed by RIA (Mean= 2.9 ± 0.3, N= 10), ROS (2.7 ± 0.4, N= 10) and finally VEN (1.3 ± 0.3, N= 8) (Fig. 5), the only population where the mean number of mature eggs carried by females exceeded male clutch size (Table 1). The average number of mates per male decreased with increasing population SSD (y = -0.29x + 3.55, $r^2$= 0.371, $F_{1,36}$= 21.26, p< 0.001) (Fig. 5). No correlation was found between the average water temperature during breeding season and the number of mates per male (y = 0.098x – 4.47, $r^2$= 0.08, $F_{1,36}$= 3.13, p= 0.086).

Identical maternal genotypes were found in broods of two males from RIA, two males from ROS and three males from VEN, providing direct evidence of female multiple mating in these populations. The degree of female multiple mating is likely underestimated due to the fact that only a small fraction of the males in each population were sampled. Clearly, the average number of mates for males and females must be identical when the adult sex ratio is equal (Shuster & Wade 2003). It is thus straightforward to calculate the average number of mates per female as the frequency of multiple mating by males multiplied by the adult sex ratio. Thus, the average number of mates per female is 9.0 in ROS, 7.4 in KLU, 6.5 in VEN and 2.4 in RIA. These inferred estimates imply that many of the females who contributed eggs to analyzed males likely mated with additional males not sampled here.

## Discussion

Genetic analyses indicated that 76% of the analyzed *S. typhle* males mated multiply with as many as five females. While the frequency of multiple mating in the KLU population (100%

polygyny, 3.7 mates per male) is similar to previously reported data from this population (90% polygyny, 3.1 mates per male; Jones *et al.*, 1999), mating behaviour varied significantly among populations, and in the most extreme case (VEN), almost all males (7 of 8) had mated monogamously. The extreme variation in mating behaviour observed here highlights the importance of spatial sampling in assessments of genetic mating systems.

The relationship between fecundity and body size in both sexes found here are consistent with previous work on Swedish populations of *S. typhle* which found a positive correlation between body size and fecundity in both males and females (Berglund *et al.*, 1986b). Recent investigations of *Syngnathus leptorhynchus* (Wilson, unpublished data) and *S. floridae* (Mobley & Jones, 2007), have also found positive correlations between male body size and fecundity across a broad geographical scale.

As colder temperatures during the breeding season increase the duration of embryo incubation in *S. typhle* (Ahnesjö, 1995), pipefish are likely under selective pressure for increased body size to maximize reproductive output per breeding episode. Consistent with this prediction, body size and the number of eggs males carried in their pouch both correlated negatively with the average water temperature during the breeding season.

When females produce fewer eggs than males can brood, males must mate multiply in order to fill their pouch. The only population where the fecundity of females exceeded that of males was VEN. Males from this population were almost all monogamous (88%), indicating that the difference in male and female body size may be a critical determinant of multiple mating in this species. This prediction is supported by the fact that the number of mates per male was negatively correlated with the sexual size difference in all populations (Fig. 5). A study of a population of Swedish *S. typhle* found that while male growth is negligible during brooding, females continue to grow while producing eggs (Svensson, 1988). As a result, continued female growth during the breeding season results in an increase of sexual size

dimorphism over the course of the breeding season (Svensson, 1988). Based on the results presented here, we predict a decline of the frequency of multiple mating over the breeding season, as the increased fecundity of larger females later in the season may be adequate to completely fill a males' pouch. The sampling design used here explicitly restricted sampling to a two month time-frame to minimize temporal variation in reproduction at the study sites. While this approach controls for time, the breeding season in southern populations is considerably more advanced than that found in northern sites at the same time of year, a pattern which likely explains the different stages of egg development in the study populations (Table 1). VEN, the population with the lowest number of matings per male (1.3) was sampled during the middle of its breeding season. If the predictions outlined here are correct, the average number of mates per male in this population would likely be even further reduced towards the end of the season, possibly reaching strict monogamy. This pattern might also explain why the number of mates per male was so high in KLU, where pipefish were sampled near the beginning of the breeding season when SSD was negligible. Rensch's Rule (1960) suggests that female-biased SSD may be reduced in large-bodied populations. If this pattern also holds in *S. typhle*, morphological responses to fecundity selection may be constrained in large-bodied populations.

While sexual size dimorphism appears to explain how increases in fecundity of male *S. typhle* may be possible without concurrent increases in polygyny, this pattern contrasts strikingly with that observed in North American populations of pipefish (*S. leptorhynchus*), where male fecundity is positively correlated with the frequency of multiple mating (Wilson, unpublished data). Interestingly, there is no consistent pattern of sexual size dimorphism in *S. leptorhynchus* across either spatial (Wilson, unpublished data) or temporal (Wilson, Harber & de Graaf, unpublished data) scales. While this may explain the contrasting results observed in *S. typhle* and *S. leptorhynchus*, it raises the larger question: What factors contribute to

apparent growth trajectory differences between these two species? Various studies have documented that SSD can vary and may even be reversed within a single genus (reviewed in Fairbairn 1997). Clarifying the causes of differences in male and female body size in *Syngnathus* spp. will be critical to understand the interaction between morphology and reproduction in this group.

The number of reproductive episodes per breeding season in syngnathid fishes depends on two key factors: the development time of embryos and the length of the breeding season. Two divergent life history strategies could be envisioned to make most efficient use of the breeding season: male pipefish could start to reproduce early in the breeding season in order to increase their potential for multiple broods or could reproduce later in the season in order to maximize body size prior to first reproduction, increasing reproductive output per breeding episode. As female growth in *S. typhle* does not appear to be significantly influenced by reproductive activity (Svensson, 1988), divergent interests of male and female pipefish could result in ongoing conflicts over reproductive timing in the two sexes.

Reproductive biology is highly complex and is likely influenced by additional factors not considered here. Water temperature clearly has an effect on reproduction of *S. typhle* (Ahnesjö, 1995), but temperature also influences other factors which affect mating behaviour, such as mating encounter (Berglund, 1995), predation risk (Berglund, 1993), mate quality (Jones *et al.*, 2000) and parasites (Rosenqvist & Johansson, 1995). These studies suggest that it may be very difficult to isolate the effect of a single factor on wild populations of *S. typhle*. In order to isolate the impact of water temperature on the mating system, one must study animals under controlled experimental conditions in order to exclude complicating environmental factors. By studying *S. typhle* from different populations in a standardized lab environment, the genetic component of observed reproductive differences could also be studied.

Due to the important influence of temperature on syngnathid reproduction, steadily increasing temperatures of our oceans are likely already influencing the mating system of *S. typhle*, a pattern recently documented in a related species of pipefish (*Entelurus aequarous*) (Kirby *et al.* 2006) . Studying natural variation in reproductive dynamics may provide insights into the expected impacts of long term climate warming on reproductive biology of nearshore fishes.

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## References

Ahnesjö, I. 1995. Temperature affects male and female potential reproductive rates differently in the sex role reversed pipefish, *Syngnathus typhle*. *Behav. Ecol.* **6**: 229-233.

Angiletta, M. J., & Dunham, A. E. 2003. The temperature-size rule in ectotherms: Simple evolutionary explanations may not be general. *Am. Nat.* **162**: 332-342.

Atkinson, D. 1994. Temperature and organism size - A biological law for ectotherms? *Adv. Ecol. Res.* **25**: 1-58.

Berglund, A. 1993. Risky sex: male pipefishes mate at random in the presence of a predator. *Anim. Behav.* **46**: 169-175.

Berglund, A. 1995. Many mates make male pipefishes choosy. *Behaviour* **132**: 213-218.

Berglund, A., Rosenqvist, G. & Svensson, I. 1986a. Reversed sex-roles and parental energy investment in zygotes of two pipefish (Syngnathidae) species. *Mar. Ecol. Prog. Ser.* **29**: 209-215.

Berglund, A., Rosenqvist, G. & Svensson, I. 1986b. Mate choice, fecundity and sexual dimorphism in two pipefish species (Syngnathidae). *Behav. Ecol. Sociobiol*. **19**: 301-307.

Bergmann, C. 1847. Ueber die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Gottinger Studien*. **3**: 595-708.

Boisseau, J. P. 1967. Les regulations hormonales et l' incubation chez un vertebré male: Recherches sur la reproduction de l' Hippocampe. PhD Thesis. Universite de Bordeaux.

Darwin, C. 1871. The descent of man and selection in relation to sex. Murray, London.

Dawson, C.E. 1986. Syngnathidae. In: *Fishes of the North-eastern Atlantic and the Mediterranean* (P.J.P. Whitehead, M.L. Bauchot, J.C. Hereau, J. Nielsen & E. Tortonese, eds.). pp. 628-639. UNESCO, Paris.

Duncker, G. 1908. Syngnathiden-Studien. I. Variation und modifikation bei *Siphonostoma typhle* L. *Mitteil. Naturhist. Mus. Hamburg.* **25**: 1-115.

Eigenmann, I. 2007. Southern diversity and northern uniformity. Phylogeography and population genetics of the European pipefish *Syngnathus typhle*. MSc thesis, Zoological Museum, University of Zurich, Zurich.

Fairbairn, D.J. 1997. Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annu. Rev. Ecol. Syst.* **28:** 659-687.

Freire, T. 2004. Reproductive biology of *Syngnathus typhle*, Linnaeus, 1758, (Pisces, Syngnathidae), from the Ria Formosa. MSc thesis, Coastal Fisheries Research Group, University of Algarve.

Gibbons, J. W., Greene, J. L. & Patterson, K. K. 1982. Variation in reproductive characteristics of aquatic turtles. *Copeia* **1982**: 776-784.

Gloor, G. & Engels, W. 1992. Single-fly DNA preps for PCR. *Dros. Inf. Serv.* **71**: 148-149.

Griffith, S.C., Owens, I.P.F. & Thuman, K.A. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol. Ecol.* **11:** 2195-2212.

Jones, A. G. 2005. GERUD2.0: A computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. *Mol. Ecol. Notes* **5**: 708-711.

Jones, A. G., Rosenqvist, G., Berglund, A. & Avise, J. C. 1999. The genetic mating system of a sex role-reversed pipefish (*Syngnathus typhle*): a molecular inquiry. *Behav. Ecol. Sociobiol*. **46**: 357-365.

Jones, A. G., Rosenqvist, G., Berglund, A. & Avise, J. C. 2000. Mate quality influences multiple maternity in the sex-role reversed pipefish *Syngnathus typhle*. *Oikos* **90**: 321-326.

Kirby, R.R., Johns, D.G. & Lindley, J.A. 2006. Fathers in hot water: Rising sea temperatures and a Northeastern Atlantic pipefish baby boom. *Biol. Lett.* **2**: 597-600.

Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends Ecol. Evol.* **11**: 404-408.

Larson, U. 2006. http://www2.ecology.su.se/dbhfj/index.htm. [Accessed 11 July 2007].

MacArthur, R. & Wilson, E. O. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton.

Mobley, K.B. & Jones, A.G. 2007. Geographical variation in the mating system of the dusky pipefish (*Syngnathus floridae*). *Mol. Ecol.* **16**: 2596-2606.

Panhuis, T.M., Butlin, R., Zuk, M., Tregenza, T. 2001. Sexual selection and speciation. *Trends Ecol. Evol.* **16**: 364-371.

Ray, C. 1960. The application of Bergmann's and Allen's rules to the poikilotherms. *J. Morphol.* **106**: 85-108.

Rensch, B. 1960. Evolution above the species level. Columbia University Press, New York.

Rexs, B. 2007. http://tmbl.gu.se:16080/BertilsBrygga/. [Accessed 11 July 2007].

Riccato, F., Fiorin, R., Franco, A., Franzoi, P., Libertini, A., Pranovi, F. & Torricelli, P. 2003. Population Structure and reproduction of three pipefish species (Pisces, Syngnathidae) in a sea grass meadow of the Venice lagoon. *Biol. Mar. Medit.* **10**: 138-145.

Roff, D.A. 2002. Life History Evolution. Sinauer Associates, Sunderland.

Rosenqvist, G. & Johansson, K. 1995. Male avoidance of parasitized females explained by direct benefits in a pipefish. *Anim. Behav.* **49**: 1039-1045.

Sautour, B. 2007. Service d'Observation en Milieu Littoral, INSU-CNRS, Station Estacade. http://www.domino.u-bordeaux.fr/somlit_national/. [Accessed 25 July 2007].

Shuster, S. M. & Wade, M. J. 2003. Mating systems and strategies. Princeton University Press, Princeton.

Sprung, M. 2001. Larval abundance and recruitment of *Carcinus maenas* L. close to its southern geographic limit: a case of match and mismatch. *Hydrobiologia* **449:** 153-158.

Svensson, I. 1988. Reproductive costs in two sex role reversed pipefish species (Syngnathidae). *J. Anim. Ecol*. **57**: 929-942.

Vincent, A., Ahnesjö, I., Berglund, A. & Rosenqvist, G. 1992. Pipefishes and seahorses: Are they all sex role reversed? *Trends Ecol. Evol.* **7**: 237-241.

Williams, G. C. 1975. Sex and evolution. Princeton University Press, Princeton.

Wilson, A.B., Ahnesjö, I., Vincent, A.C.J. & Meyer, A. 2003. The dynamics of male brooding, mating patterns, and sex roles in pipefishes and seahorses (family Syngnathidae). *Evolution*. **57**: 1274-1386.

**Figure Legends**

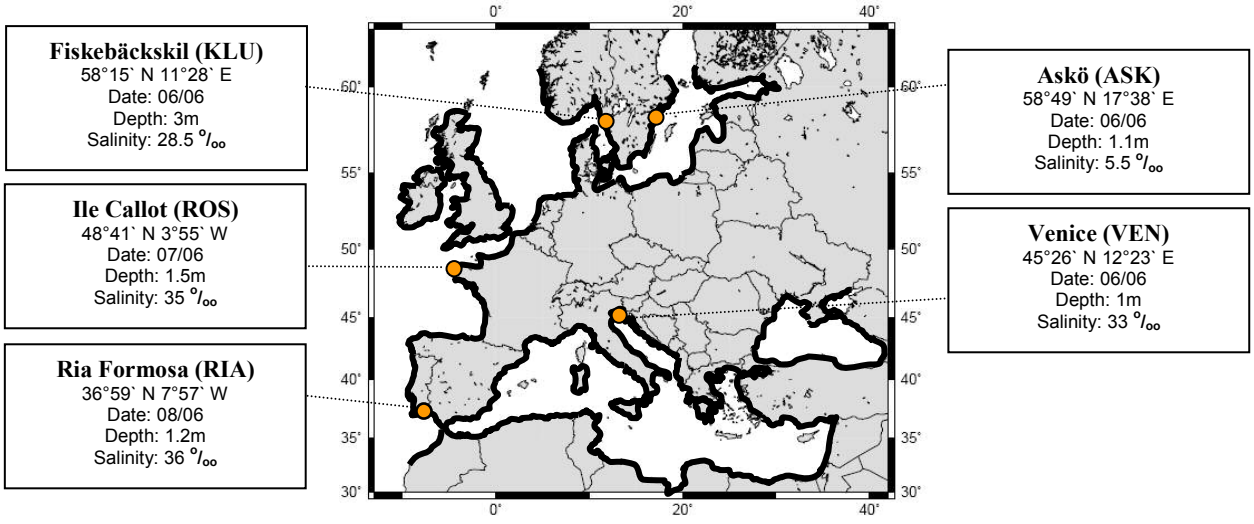
**Figure 1:** Geographical sampling and physical parameters of the study sites. The distribution of *S. typhle* is indicated in bold (Dawson 1986).

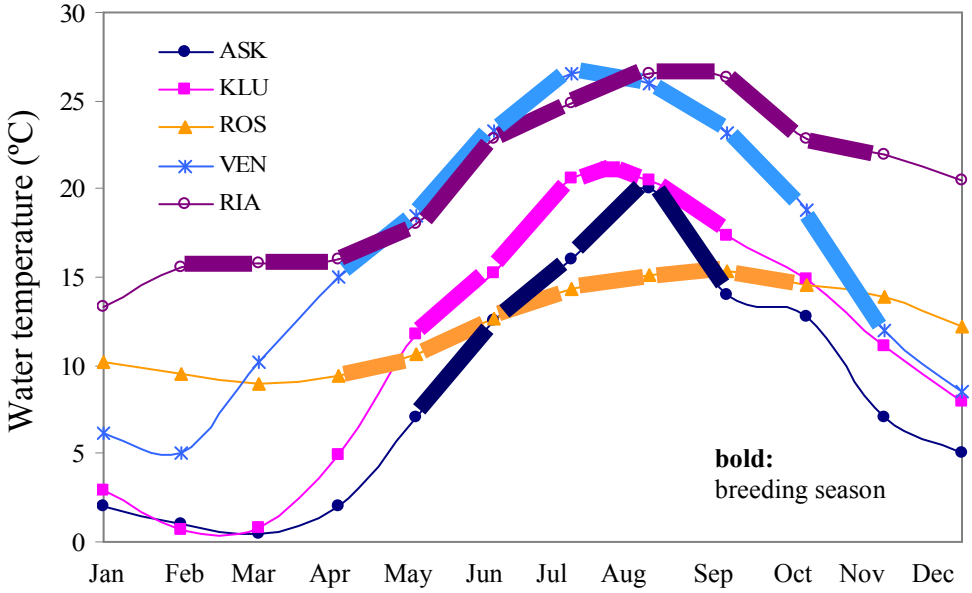
**Figure 2:** Monthly water temperatures (ºC) for each sampling location. The breeding season is marked in bold. References: RIA: Sprung (2001); Freire (2004), VEN: Riccato *et al.*(2003); F. Riccato, pers. comm., ROS: Sautour (2007); Duncker (1908); KLU: Rexs (2006), ASK: Duncker (1908); Larson (2006).

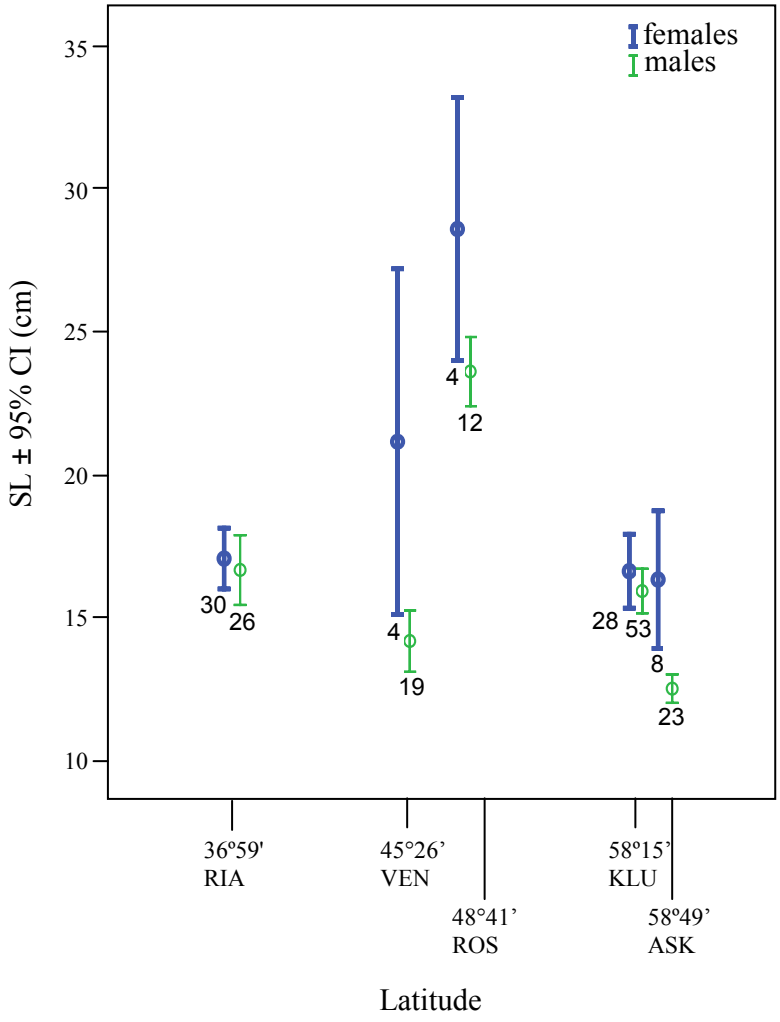
**Figure 3:** Mean ± SE of male and female body size in each population with the respective sample size. Females have a higher average SL than males in each population. Differences of standard length (SL) between males and females are significant for VEN, ROS and KLU ($p < 0.05$).

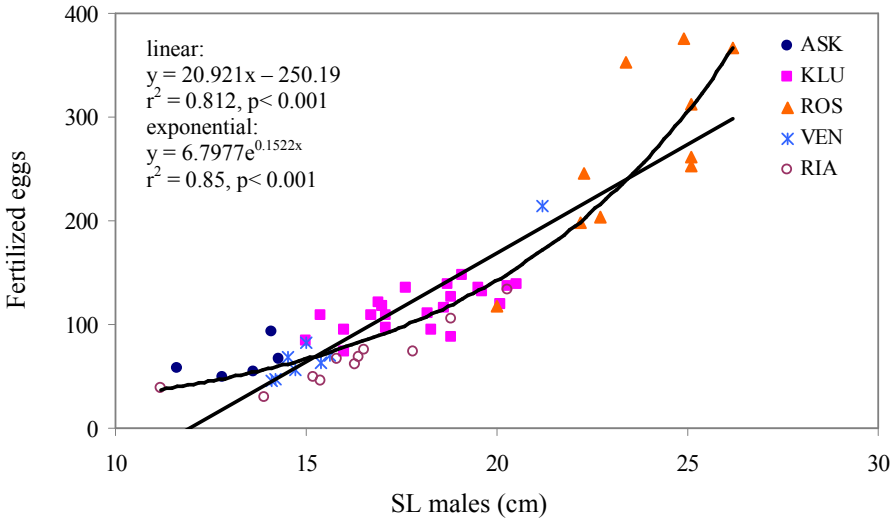
**Figure 4:** Regression analysis for a linear and an exponential relationship of (a) male and (b) female body size and fecundity in each population. Females from ROS were not included in the regression analysis.

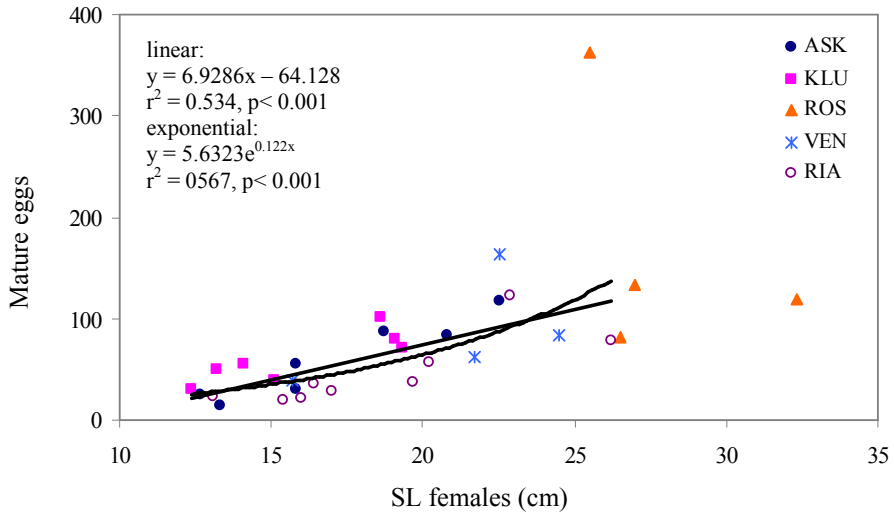
**Figure 5:** The relationship between number of mates per male and the sexual difference in SL in each population. Sample sizes are indicated.


Fiskebäckskil (KLU)
58°15` N 11°28` E
Date: 06/06
Depth: 3m
Salinity: 28.5 °/oo
Ile Callot (ROS)
48°41` N 3°55` W
Date: 07/06
Depth: 1.5m
Salinity: 35 °/oo
Ria Formosa (RIA)
36°59` N 7°57` W
Date: 08/06
Depth: 1.2m
Salinity: 36 °/oo
Askö (ASK)
58°49` N 17°38` E
Date: 06/06
Depth: 1.1m
Salinity: 5.5 °/oo
Venice (VEN)
45°26` N 12°23` E
Date: 06/06
Depth: 1m
Salinity: 33 °/oo
0°
20°
40°
60°
55°
50°
45°
40°
35°
30°


Water temperature (ºC)
30
25
20
15
10
5
0
ASK
KLU
ROS
VEN
RIA
bold:
breeding season
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec


females
males
SL ± 95% CI (cm)
35
30
25
20
15
10
30
26
4
19
4
12
28
53
8
23
36°59'
RIA
45°26'
VEN
48°41'
ROS
58°15'
KLU
58°49'
ASK
Latitude


linear:
y = 20.921x – 250.19
r2 = 0.812, p< 0.001
exponential:
y = 6.7977e0.1522x
r2 = 0.85, p< 0.001
ASK
KLU
ROS
VEN
RIA
Fertilized eggs
SL males (cm)
0
100
200
300
400
10
15
20
25
30


linear:
y = 6.9286x – 64.128
r2 = 0.534, p< 0.001
exponential:
y = 5.6323e0.122x
r2 = 0567, p< 0.001
ASK
KLU
ROS
VEN
RIA
Mature eggs
SL females (cm)
0
100
200
300
400
10
15
20
25
30
35

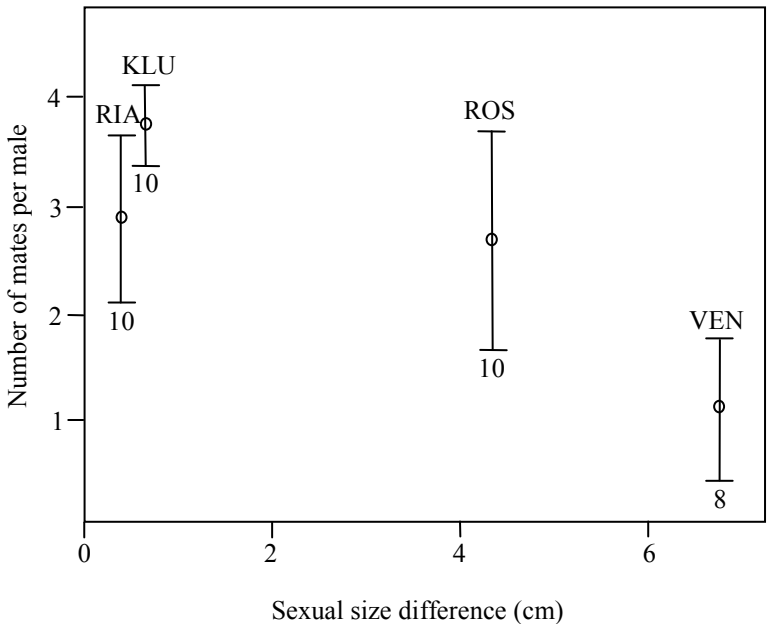

KLU
RIA
ROS
VEN
10
10
10
8
Number of mates per male
1
2
3
4
0
2
4
6
Sexual size difference (cm)

**Table 1** Genetic mating behaviour of pipefish males. 5-10 males were randomly chosen from each population for analysis.

| Individual | Body size (SL) (cm) | Number of Fertilized eggs | Developmental stage of embryos | Matings |
|---|---|---|---|---|
| RIA 1 | 16.3 | 61 | E | 2 |
| RIA 2 | 15.2 | 49 | E | 2 |
| RIA 3 | 18.8 | 105* | C | 5 |
| RIA 4 | 15.4 | 45 | F | 3 |
| RIA 5 | 16.5 | 75 | E | 4 |
| RIA 6 | 17.8 | 73 | C | 3 |
| RIA 7 | 16.4 | 68 | F | 3 |
| RIA 8 | 13.9 | 29 | C | 2 |
| RIA 9 | 15.8 | 67 | B | 2 |
| RIA 10 | 20.3 | 134* | E | 3 |
| **average** | **16.64** | **70.6** | **D** | **2.9** |
| VEN 1 | 14.2 | 47 | A | 1 |
| VEN 2 | 14.5 | 68 | A | 1 |
| VEN 3 | 15 | 82 | C | 1 |
| VEN 4 | 14.1 | 45 | B | 1 |
| VEN 5 | 15.6 | 71 | C | 1 |
| VEN 6 | 15.4 | 64 | B | 1 |
| VEN 7 | 21.2 | 214* | A | 3 |
| VEN 8 | 14.7 | 57 | B | 1 |
| **average** | **15.59** | **81** | **B** | **1.25** |
| ROS 1 | 24.9 | 376* | D | 2 |
| ROS 2 | 22.7 | 203* | F | 1 |
| ROS 3 | 25.1 | 312* | D | 2 |
| ROS 4 | 22.2 | 198* | D | 1 |
| ROS 5 | 25.1 | 252* | C | 4 |
| ROS 6 | 23.4 | 353* | C | 4 |
| ROS 7 | 25.1 | 262* | D | 3 |
| ROS 8 | 26.2 | 366* | E | 4 |
| ROS 9 | 20 | 117 | F | 2 |
| ROS 10 | 22.3 | 246* | D | 4 |
| **average** | **23.7** | **268.5** | **D** | **2.7** |
| KLU 1 | 18.2 | 111 | A | 4 |
| KLU 2 | 16.7 | 108 | A | 4 |
| KLU 3 | 18.8 | 97 | A | 4 |
| KLU 4 | 17.1 | 94 | A | 3 |
| KLU 5 | 19.6 | 132 | A | 3 |
| KLU 6 | 18.6 | 116 | A | 3 |
| KLU 7 | 18.3 | 94 | A | 4 |
| KLU 8 | 19.5 | 135 | A | 4 |
| KLU 9 | 20.5 | 137 | A | 4 |
| KLU 10 | 20.3 | 135 | A | 4 |
| **average** | **18.76** | **115.9** | **A** | **3.7** |
| ASK 1 | 11.6 | 58 | A | n/a |
| ASK 2 | 13.6 | 55 | A | n/a |
| ASK 3 | 14.1 | 93 | A | n/a |
| ASK 4 | 12.8 | 50 | A | n/a |
| ASK 5 | 14.3 | 67 | A | n/a |
| **average** | **13.28** | **64.6** | **A** | **n/a** |

* eggs stored in several layers within the brood pouch.