

Comparing mating systems and the strength of sexual selection across three congeneric species of pipefish spanning the continuum of sexual dimorphism

Authors:

Nicole M. Tosto¹ (ORCID: 0000-0001-9858-7046)

Emily Rose² (ORCID: [0000-0002-8794-1887](https://orcid.org/0000-0002-8794-1887))

Heather D. Mason³ (ORCID: 0000-0001-7171-2919)

Sarah P. Flanagan^{1*} (ORCID: 0000-0002-2226-4213)

Affiliations:

1 - School of Biological Sciences, University of Canterbury, Christchurch New Zealand

2 - Division of Natural Sciences, New College of Florida, Sarasota, Florida, USA

3 - Department of Biology, University of Tampa, Tampa, Florida, USA

* - corresponding author, sarah.flanagan@canterbury.ac.nz

Data Availability

The microsatellite genotyping data, morphological measurements, and reproductive success for *Syngnathus fuscus* are archived on Zenodo (<https://zenodo.org/records/14053624>). Publicly accessible data was used for *S. floridae* (<https://zenodo.org/records/10558631>) and *S. scovelli* (<http://dx.doi.org/10.5061/dryad.bk03m>). Photographs taken of pipefish prior to entering the mesocosms for all three species have been archived on Zenodo (<https://doi.org/10.5281/zenodo.22906552>).

All code used for the analyses is on GitHub (<https://github.com/flanagan-lab/syngnathus-selection-comp>), the version of the repository at the time of submission is archived on Zenodo (<https://doi.org/10.5281/zenodo.21330225>).

Author contributions

NMT and SPF designed the project. NMT conducted all field and laboratory work, supported by ER and HDM. NMT analyzed and visualized data and wrote the first draft. SPF provided substantive edits with additional review from ER and HDM. SPF and HDM provided funding for the research.

Funding

This work was supported by the Marsden Fund Council from government funding, managed by Royal Society Te Apārangi (grant UOC1904) and support from the University of Canterbury and University of Tampa.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

While the experimental breeding populations were not conducted in New Zealand, we acknowledge Ngāi Tūāhuriri upon whose lands we conducted the consequent analysis. We thank Mary Amato, Will Love, Sam Johnson, Ben Share, James Heiser, Jada Brown, Ashley Govig, Kristina Townsend, Sarah Sexton, Tyler Pollert, Patrick O'Brien, Isabella Lesinski, Celina Kostich-Nilsen, Wyatt Larrinaga, Alex Butler, Shane Kullen, Geoffrey Beane, Griffin Baughn, Katie Serba, and Tamar Smith at the University of Tampa and Anthony Rose for their assistance with collecting and caring for pipefish. Adam Jones provided feedback on an early version of the manuscript. Bob Wong and Judith Mank guided early thinking about this project. Emily Beasley and other members of the Flanagan lab contributed helpful feedback throughout the project. We thank three anonymous reviewers and Tim Janicke for constructive feedback on the manuscript.

Ethics Statement

All *Syngnathus fuscus* were collected in the southern part of Chesapeake Bay under scientific collection permit #21-038 granted by the State of Virginia. All animal-use protocols outlined below were approved under the University of Tampa AUP #2020-01.

Key words: Bateman Gradient; Selection Differential; Jones Index; Syngnathus; selection trade-off; polygynandry

ABSTRACT

The strength of sexual selection is often linked to genetic mating system and is thought to be a driving force in the evolution of sexual dimorphism. Here, we compared selection between males and females across three closely related species: the dusky (*Syngnathus floridae*), Northern (*S. fuscus*), and Gulf (*S. scovelli*) pipefishes, using new and previously published datasets. These three species exhibit male pregnancy but vary in mating systems and sexual dimorphism, enabling us to test if (i) the species differ in their opportunities for pre- and post-copulatory selection, (ii) females demonstrate a steeper Bateman gradient in polyandrous species, and (iii) sexually dimorphic traits are targets of selection. We quantified pre- and post-copulatory selection using a variance decomposition of the opportunity of selection, Bateman gradients, and selection differentials on snout-vent length. Pre-copulatory fitness was estimated with genetic mating success and post-copulatory fitness with the number of eggs stored and proportion of developed eggs. We found polygynandrous species had higher opportunities for selection in both sexes than the polyandrous species, *S. scovelli*. Despite *S. scovelli* showing large sex differences in the opportunity for selection and selection differentials, Bateman gradients varied little between sexes and across species. Pre-copulatory selection accounted for the largest opportunity for selection across all three species; however, we identified potential sexually antagonistic selection acting on body size in *S. floridae* and *S. fuscus*, and a trade-off between pre- and post-copulatory selection for female *S. fuscus* and *S. scovelli*. Overall, the

largest differences emerged between polygynandrous and polyandrous species, with potential trade-offs across episodes of selection.

INTRODUCTION

Sexual selection is an important driver of the evolution of secondary sexual characteristics, especially ornaments and armaments. Sexually selected traits often differ between the sexes, a phenomenon known as sexual dimorphism, and are presumed to evolve due to selection favoring different trait values in each sex (Hedrick & Temeles, 1989; Mori et al., 2017; Punzalan & Hosken, 2010; Tosto et al., 2023). The intensity of sexual selection and magnitude of sexual dimorphism are thought to be closely related to an organism's genetic mating system (Björklund, 1991; Jones & Avise, 2001; Shuster, 2009; Wiegmann & Nguyen, 2006). Sexual selection theory predicts strong selection arising when reproductive success is highly variable, with a few individuals achieving high mating success and others failing to mate or producing few offspring (Jones, 2009; Jones & Ratterman, 2009). Following this prediction, strong sexual selection in one sex and extreme sexual dimorphism may be linked to the extent of multiple mating, whereas monogamous mating systems or lower variation in mating success may result in lower selection strengths and minor differences in selection strengths between males and females (Shuster, 2009). Therefore, uncovering the dynamics of animal mating systems is vital for developing a complete understanding of sexual selection strengths in any system.

Quantifying sexual selection and its effects on trait evolution has been a central component of mating system research (Anthes et al., 2017; Henshaw et al., 2018; Jones, 2009; Krakauer et al., 2011). A powerful set of inferential tools for the study of sexual selection, with a strong focus on pre-copulatory sexual selection, has been produced (reviewed in Henshaw et al., 2018). One commonly used approach involves the incorporation of three metrics based on Bateman's principles (Bateman, 1948), including the opportunity for selection (Crow, 1958), the opportunity

for sexual selection (Wade, 1979), and the Bateman gradient (Arnold & Duvall, 1994). The opportunity for selection (I) and the opportunity for sexual selection (I_S) are defined as the variance in relative reproductive success (total number of offspring produced by an individual divided by the population mean) and relative mating success (number of successful mates an individual acquires within a set period divided by the population mean), respectively. The Bateman gradient (β_{SS}) relates relative reproductive success to relative mating success, commonly estimated as a least-squares regression of reproductive success on mating success (Arnold & Duvall, 1994). A shallow slope in the Bateman gradient implies a low fitness gain with each additional mating event, and therefore weak or no sexual selection acting on traits related to mating success, while a steeper Bateman gradient shows the reverse (Bateman, 1948; Jones, 2009). The Jones Index (s'_{max}) incorporates the Bateman gradient and the opportunity for sexual selection and has been shown to be the leading metric for identifying sex differences in the strength of sexual selection (Henshaw et al., 2016; Jones, 2009).

These approaches for quantifying sexual selection have been predominantly used to measure pre-copulatory sexual selection, i.e., selection related to mate competition and mate choice (Jones, 2009). However, behavioral, morphological, and physiological traits can be targets of sexual selection after mating through mechanisms including sperm competition and cryptic female choice (Birkhead, 2010). Therefore, a full understanding of sexual selection requires its decomposition into multiple episodes, at least one of which incorporates the influence of post-copulatory sexual selection (Evans & Garcia-Gonzalez, 2016). To do this, an individual's total reproductive success can be divided into distinct fitness components (Arnold & Wade, 1984b, 1984a). With this framework, previous research has demonstrated the different contributions of pre- and post-copulatory selection in a variety of organisms (hermaphroditic plants: Barbot et al., 2025; brown anoles: Bhave et al., 2024; red junglefowl: Collet et al., 2012; guppies: Devigili et al., 2015; snails: Janicke et al., 2015; flatworms: Marie-Orleach et al., 2016; fruit fly: Morimoto et al., 2016; snails: Pélissié et al., 2014; fruit fly: Pischedda & Rice, 2012;

pipefish: Rose et al., 2013a; crickets: Turnell & Shaw, 2015; flour beetles: Winkler et al., 2023). For example, in the transparent flatworm (*Macrostomum lignano*), male reproductive success was most limited through their ability to convert achieved matings into successful fertilization events (post-copulatory selection), rather than the number of copulations they could obtain (pre-copulatory selection; Marie-Orleach et al., 2016). In contrast, in brown anoles (*Anolis sagrei*), larger males obtained more mates, resulting in pre-copulatory selection driving reproductive success (Bhave et al., 2024). The relative importance of pre- and post-copulatory selection differing across species emphasizes the importance of quantifying both.

Males typically experience stronger sexual selection compared to females, but this is not always the case (Fromonteil et al., 2023). Within the fish family Syngnathidae (seahorses, pipefish, and seadragons), male parental care in the form of male pregnancy exists and can limit females' access to receptive mates, resulting in increased sexual selection on females, the evolution of female sexual signals (particularly ornamentation), and sex-role-reversal (Berglund & Rosenqvist, 2003; Rosenqvist & Berglund, 2011). Sex-role-reversal is defined here as species with steeper sexual selection gradients and higher opportunities for sexual selection in females compared to males (Jones et al., 2000), as opposed to a definition dependent on stereotyped behaviors (Ah-King & Ahnesjö, 2013; Fritzsche et al., 2021). Syngnathid mating systems range from monogamous to polyandrous and polygynandrous (Jones & Avise, 2001; Rosenqvist & Berglund, 2011), presenting the opportunity to compare selection strengths across closely related species that span a continuum of sexual dimorphism and mating systems.

In this study we investigated the relationship between sexual selection, sexual dimorphism in body size, and mating systems across three species of pipefish from one genus: the dusky pipefish (*Syngnathus floridae*), the Northern pipefish (*Syngnathus fuscus*), and the Gulf pipefish (*Syngnathus scovelli*). The species used in this study fall in the western-Atlantic clade with *S. fuscus* and *S. scovelli* being the closest in relation (Hamilton et al., 2017; Stiller et al., 2022; Wilson & Orr, 2011). In the wild, all three species have shown evidence of multiple matings in at

least one sex, with the highest reported rates of multiple mating in both sexes present in *S. floridae* (Jones & Avise, 1997b; Paczolt et al., 2016). The three species additionally range in degree of ornamentation, from the more monomorphic *S. floridae* to the highly dimorphic *S. scovelli*, which possess a permanent, sexually selected female ornament that is absent in males (Flanagan et al., 2014; Jones et al., 2001). *S. scovelli* is the only species to be previously documented as polyandrous (Jones et al., 2001), while the other two are polygynandrous (Jones & Avise, 1997b; Paczolt et al., 2016). With these three species, we set out to answer several questions: (1) How do opportunities for pre- and post-copulatory sexual selection differ among species; will certain episodes be more prominent for the different species? (2) In a group of species with obligate male parental care and female secondary sexual traits, will females demonstrate a steeper Bateman gradient compared to males, and will sex differences in the gradient reflect the mating system? (3) Can we identify traits that may be a target of pre- and post-copulatory sexual selection?

To answer these questions, we established experimental breeding populations of male and female pipefish and used molecular parentage analysis to determine mating and reproductive success for *S. fuscus*, combining the results with publicly accessible data on mating and reproductive success for *S. scovelli* and *S. floridae* (Rose et al., 2013a; Tosto et al., 2025). We compared the relative strength of sexual selection in males and females within each species using Bateman gradients. Selection was further quantified by partitioning the opportunity for selection into variance arising from genetic mating success, the number of eggs stored per mate, and the proportion of stored eggs that developed. While the definition of sexual selection is subject to debate (Janicke, 2024, 2026; Shuker & Kvarnemo, 2021, 2025; and other commentaries), regardless of which definition is used, these episodes of fitness likely reflect both natural and sexual selection in pipefish (Table 1). For clarity, in this manuscript we have adopted the approach recommended by Simmons (2025), namely that we will refer to 'narrow-sense sexual selection' to specify competition over gametes alone and 'broad-sense sexual

selection' when we cannot differentiate between fitness benefits arising from gametes or other resources provided by the mating partner. In the case of pipefishes, broad-sense sexual selection would include competition for nutrient provisioning of offspring, either transferred along with gametes (in the case of females) or provided during male parental care (Kvarnemo et al., 2011; Ripley & Foran, 2006a). As the limiting resource is likely the space in male brood pouches rather than sperm, pre-copulatory selection on females would be broad-sense sexual selection but narrow-sense for males (Janicke, 2024; Shuker & Kvarnemo, 2021; Simmons, 2025; Table 1). We also measured selection differentials on male and female body size in terms of snout-vent length, partitioning these differentials into the same pre- and post-copulatory components. The combination of the different quantification techniques across males and females from three species of pipefish allows for a more holistic view on the relationship that exists between sexual selection, genetic mating systems, and sexual dimorphism.

Table 1. A summary of the decomposition of selection used in this manuscript, with some guidance on the biological interpretations of each episode for females and males in the three focal pipefish species for this study. Note that mechanistic data is lacking so many of these interpretations are hypothetical possibilities. W_1 = relative mating success; W_1W_2 = relative number of eggs stored; $W_1W_2W_3$ = relative number of developed eggs (relative values were calculated by dividing by the average value).

Partitioned Fitness estimate	Quantitative definition	Types of selection in female pipefish	Types of selection in male pipefish
Genetic mating success	$w_1 = W_1$	Pre-copulatory broad-sense sexual selection (competition for access to mates, including male choice), but does not reflect any individuals that mated but did not produce zygotes, which means that it could include some elements of post-copulatory selection.	Pre-copulatory narrow-sense sexual selection (competition for access to mates, including female choice), but does not reflect any individuals that mated but did not produce zygotes, which means that it could include some elements of post-copulatory selection.
Number of eggs stored per mate	$w_2 = \frac{W_1 W_2}{W_1}$	Fecundity selection and sexual selection (either narrow- or broad-sense) arising from copulation duration or other factors such as male choice or female-female competition.	Natural selection on body size and/or brood pouch size, along with fecundity selection, if sperm production is a limiting factor. Could also reflect narrow-sense sexual selection arising from copulation duration or other factors such as female choice
Proportion of eggs developed	$w_3 = \frac{W_1 W_2 W_3}{W_1 W_2}$	Sexual selection (either narrow or broad) arising from male provisioning of the female's eggs, mediated through female manipulation and/or cryptic male choice. Could also capture natural selection arising from genetic incompatibilities, congenital diseases, or other factors that impact offspring survivorship.	Selection on male provisioning of embryos. Could reflect provisioning of eggs by females and/or transfer of compounds to manipulate provisioning of other females' eggs, which would likely capture broad-sense sexual selection. Could also capture natural selection arising from genetic incompatibilities, congenital diseases, or other factors that impact offspring survivorship.

MATERIAL AND METHODS

Collection of specimens

Adult *Syngnathus fuscus* were collected using 1.3m wide push-nets with a 6mm mesh size at two timepoints, in late June and mid-July 2021, from a single continuous seagrass bed in Cape Charles, Virginia, United States of America (Figure 1). To ensure sexual maturity, only pregnant males were collected and only females larger than 125mm standard length (Figure S1A) were retained (Ripley & Foran, 2006b). Pipefish were transported to the University of Tampa in 150L aerated coolers and sustained with food caught during collection until arrival in the lab. Once in the laboratory, all pipefish received a freshwater bath lasting three minutes to remove external parasites and were acclimated to 38L sex-specific holding tanks held at conditions to mimic their natural environment (24°C and 25 parts per thousand) until males gave birth. Pouches were visually inspected to ensure absence of eggs after birth.

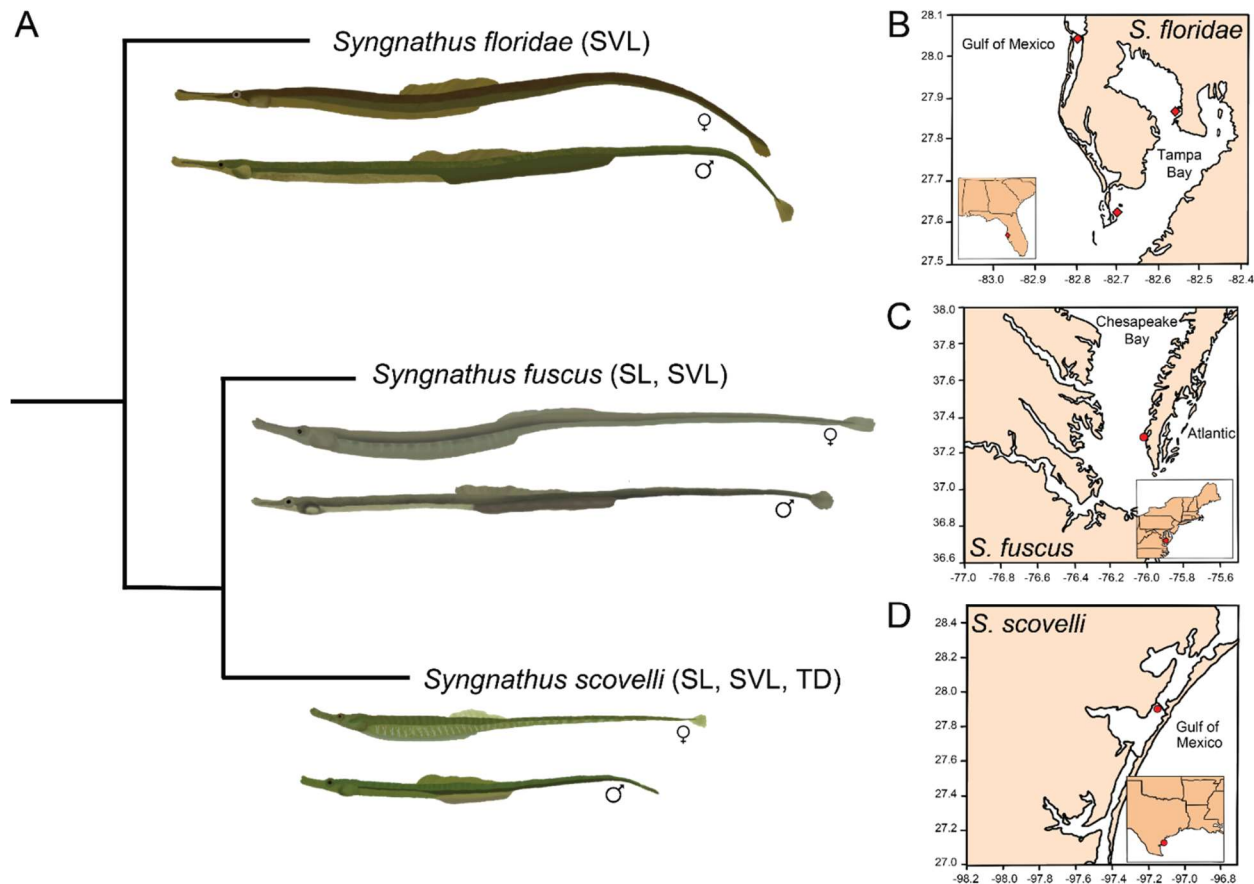


Figure 1. Description of the three *Syngnathus* spp. and the corresponding collection locations. (A) Shown here is the phylogenetic relationship between the three species of *Syngnathus* (Adapted from Hamilton et al., 2017 and Stiller et al., 2022) and examples of female (top) and male (bottom) pipefish from each species. The images are scaled to represent accurate proportional differences between both sexes and species using averages for standard length (SL), SVL, torso depth (TD), and pouch length. Traits that differed significantly between females and males are listed in parentheses following the species name. (B) *S. floridae* were collected in three locations, including the Saint Joseph Sound (28.0516324, -82.7921436), the southern region of Old Tampa Bay (27.8730210, -82.5397645), and off Fort De Soto (27.6273507, -82.7002240). (C) *S. fuscus* were collected off the coast of Cherrystone Family Camping Resort in Cape Charles, VA (37.3927330, -76.0139170), (D) *S. scovelli* were collected off Aransas Pass in Port Aransas, TX (27.8941861111, -97.131025).

Alt text: In A, a phylogenetic tree shows *Syngnathus scovelli* and *S. fuscus* as most closely related, with *S. floridae* as a close outgroup. Drawings of male and female individuals of each species are pictured, scaled by actual size – showing *S. floridae* is the largest and *S. scovelli* is the smallest species. The image also shows that *S. floridae* has a sex difference in SVL, *S. fuscus* shows sexual dimorphism in standard length and SVL, and *S. scovelli* is sexually dimorphic in standard length, SVL, and torso depth. Panel B shows a map with the collection location of *S. floridae* in Tampa Bay, C shows the collection location of *S. fuscus* in the Chesapeake Bay, and D shows the collection location of *S. scovelli* in the Gulf of Mexico on the Texas coastline.

Experimental breeding populations with *Syngnathus fuscus*

The experimental protocol outlined here mirrored a similar experiment conducted with Gulf pipefish, *Syngnathus scovelli* (Rose et al., 2013a). To individually identify individuals, all pipefish were anesthetized with a 0.01% clove oil solution for one minute and marked with three visible implant fluorescent tags ([Northwest Marine Technology](#)) along the left side of their dorsal fins. Protocols for marking followed those outlined in Woods & Martin-Smith, (2004). Two colors were used in a combination of six unique patterns which contained at least one mark of each color on each individual to limit mating preference based on tag color. Photographs were taken immediately after marking the fish before placing them back in their original sex-specific holding tanks and then again following the three-day recovery period prior to the start of trials.

Sixteen replicates were completed, involving a total of 192 *S. fuscus*. Each replicate included six sexually mature females and six nonpregnant males housed together in a 140-L tank (77cm x 30.5cm x 56cm) for 6 weeks. Tanks were equipped with three sources of biological filtration and activated charcoal with 10% water changes performed daily. Pipefish were sustained with a mixed diet of day-old artemia, 14-day-old artemia, and various grass shrimp, zooplankton, and copepods collected from the local seagrass beds. Males were checked daily for pregnancies. Once eyespots had developed in at least two-thirds of the visible eggs, the pregnant male was euthanized in a 0.05% clove oil solution until ventilation rates dropped to zero. At this stage in the male's pregnancy, any eggs that failed to develop can be easily distinguished from those which were successfully developing (Paczolt & Jones, 2010). At the end of the trial period all remaining male and female pipefish were euthanized. Pipefish were stored in 70% ETOH at either 4°C or -20°C. Twelve of the 16 trials resulted in at least one successful mating and were used in the analyses outlined below.

Morphological analyses

Photos taken of *S. fuscus* prior to entering the trials were analyzed in ImageJ v1.53t (Schneider et al., 2012) to measure standard length, dorso-ventral torso depth, and snout-vent length (SVL) for each individual (Figure S1). Raw measurements were analyzed as follows using R v 4.4.2 (R Core Team 2025). Torso depth was adjusted by SVL (depth/SVL) prior to analysis. Morphometric data were first analyzed for normality and equal variances and then compared between males and females via a two-sample t-test to identify significant differences between the sexes (i.e., degree of sexual dimorphism). If assumptions were not met, a Wilcoxon rank sum test was used instead.

Molecular parentage analysis

Parentage analysis was conducted on the pregnant *S. fuscus* males resulting from the mesocosm trials following protocols outlined in Jones & Avise (2001) and Tosto et al. (2025). Briefly, each male's pouch was dissected into 14 roughly equal sections with three randomly sampled developed eggs per section and all remaining eggs were counted and stored separately (Figure S2). When a brood pouch was not full (~25% of pregnant males), only the sections containing eggs were sampled, resulting in fewer embryos being genotyped, but often a higher proportion of the embryos sampled (Table S1, Figure S2). Embryo and adult pipefish DNA were extracted using a Chelex-based method and the Qiagen Puregene extraction kit, respectively (Tosto et al., 2025). Three polymorphic microsatellite loci (*micro25.10*, *micro25.22*, and *micro22.3*; Table S2) were used to genotype all sexually mature adults and embryos (Jones & Avise, 1997a, 1997b). The markers were amplified using polymerase chain reaction (PCR) conditions modified from Jones & Avise (1997a), with the primer concentration doubled for *micro25.22* (See supplemental methods). Primers were labeled with fluorescent dyes and analyzed on a 3130xl Genetic Analyzer at the University of Canterbury, Christchurch, New

Zealand, using the GeneScan 500 LIZ (Applied Biosystems) as the size standard. Alleles were called and sized with the Geneious Prime microsatellite plugin (v1.4.7) using the automatic bins. Alleles sizes were exported from Geneious, and maternity was assigned using Cervus v3.0.7 (Kalinowski et al., 2007).

Cervus computed the number of alleles, observed and expected heterozygosity, and the likelihood ratio (LOD scores) between the offspring and candidate parents by comparing their genotypes. LOD scores necessary to assign parentage with either 90% or 95% confidence were determined by simulating maternity assignments for 100,000 offspring with the following parameters: the number of candidate females in each experimental breeding population (6), 100% of the candidate females sampled, and the default 1% of loci mistyped. The proportion of typed loci for the simulation was 66.9%. Known fathers were included in the analysis, since paternity was known from the dissections. Cervus assignments were evaluated using trio LOD scores which considered potential genotyping errors and the genotypes of the known fathers when assigning maternity. All offspring were required to have a minimum of two typed loci to be included in the analysis.

Previous studies have shown that eggs are spatially segregated by maternity in the brood pouches of *S. fuscus* (Paczolt et al., 2016). Therefore, if only one maternal genotype was found for the three embryos from a section, all eggs counted in that section were allocated to that female. In the sections in which multiple mothers were detected, one-third and two-thirds of the total counted eggs were assigned to each mother depending upon the proportion of genotyped embryos that were assigned genetically to each mother. Female reproductive contribution was then calculated by summing all eggs assigned to each mother across all the sections and all the fathers. Note that we are using these estimates of genetic mating success for all our analyses, rather than observations of mating rates. We were unable to assign parentage for two males due to lower rates of heterozygosity, therefore, while they were

included in the analysis of selection in males, they were omitted in the calculation of selection in females (as we cannot identify the mother).

*Analyzing publicly accessible data for *Syngnathus scovelli* and *Syngnathus floridae**

Previous studies have quantified sexual selection strengths in male and female *Syngnathus scovelli* and *Syngnathus floridae* using similar set-ups to those outlined here (Flanagan et al., 2014; Rose et al., 2013a; Tosto et al., 2025), including the estimation of maternity using subsets of offspring. The datasets used in Rose et al. 2013a and Tosto et al. 2025 were included here to assess patterns across species with different extents of sexual dimorphism. Following Rose et al. 2013a, two male *S. scovelli* who died during their pregnancy and one female *S. scovelli* whose only mate died were removed prior to analysis. Additionally, two males experienced complete brood loss throughout the trials. While they were included in the calculation of opportunity for selection in males, due to the inability to genotype any of the offspring, they were omitted in the calculation of opportunity for selection in females. The resulting *S. scovelli* dataset included a total of 55 females and 54 males across seven experimental breeding populations. For the *S. floridae* dataset (Tosto et al., 2025), only trials with at least one successful pregnancy were included, resulting in a total of 56 males and 56 females across seven experimental breeding populations. All the following analyses were conducted in R v 4.4.2 (R core team 2025), using packages magick (Ooms, 2021) and spfTools (Flanagan, 2026) for visualization. In our analyses, mating success was measured as the number of genetic mates. Because we partitioned post-copulatory episodes into two components (Table 1), two possible measures of reproductive success are available. We will use 'reproductive success' to refer to the number of eggs developed, which is the final episode of selection captured in this study.

Estimating the relative strength of sexual selection in males and females using Bateman gradients

We calculated the Bateman gradient (β_{SS}) for males and females separately as the slope of a linear mixed effects model of relative reproductive success (total number of developed eggs stored/mean number of developed eggs) on relative mating success (number of mates/mean number of mates), with the trial number included as a random effect. The model was run using the R package lme4 (Bates et al., 2015). Because trials were independent, relative reproductive and mating success were calculated within trials. To compare Bateman gradients across groups (i.e., sex or species), the group was added as an interaction term in the linear mixed effects model. Non-mated individuals were included in both the calculation of relative fitness (i.e., included in the mean) and the calculation of the Bateman gradient (as zeroes). To further investigate fitness gain or loss associated with obtaining more than one mate without the influence of the fitness gain corresponding to obtaining a single mate, the analysis was done with and without the males and females who secured no mates. In addition, we performed Wilcoxon rank sum tests with continuity correction to compare the proportion of eggs developed between singly mated and multiply mated females within each species.

Decomposing the opportunity for selection in males and females

We calculated opportunity for selection (I) as the variance in relative reproductive fitness (number of eggs that developed during the male's pregnancy) and decomposed this calculation into the same selection episodes outlined above for the selection differentials using equations 14 – 16 outlined in Arnold and Wade (1984b). For the specific fitness components, we first set relative mating success as W_1 , relative number of eggs stored as W_1W_2 , and relative number of developed eggs as $W_1W_2W_3$ (similar to the data in Table 1 of Arnold and Wade 1984a). Those initial fitness components were then used to generate the variables employed in equations 14 –

16 and A1 – A11 (w_i) for the decomposition of the opportunity for selection and calculating the co-variances (Arnold and Wade, 1984b). The final fitness components were calculated as 1) Relative mating success, $w_1 = W_1$; 2) Relative number of eggs stored per mate, $w_2 = \frac{w_1 w_2}{w_1}$; 3) Relative proportion of eggs developed, $w_3 = \frac{w_1 w_2 w_3}{w_1 w_2}$ (Table 1). As each trial provides an independent “population” (i.e., pipefish from one trial could not mate with pipefish from another trial) fitness decompositions were calculated separately for each trial and then averaged across all trials to get the final measures of opportunity for selection.

Calculating selection differentials on snout-vent length in males and females

For all three species, the absolute and standardized selection differentials (s and s') were calculated as the covariance between SVL (Figure S1C) and relative fitness (Lande & Arnold, 1983). We decomposed the total selection differential into three successive, multiplicative episodes (Arnold & Wade, 1984b, 1984a). In this study, the episodes included pre-copulatory selection (number of genetic mates, s_1 and s'_1) and two episodes of post-copulatory selection (number of eggs stored per mate, s_2 and s'_2 , and the proportion of eggs developed within the brood pouch during mid-pregnancy, s_3 and s'_3). Individuals who did not mate were included in the calculations for pre-copulatory selection (the first episode) but not for the following episodes of post-copulatory selection.

Although we aimed to capture sexual selection primarily, sexual selection in either the narrow- or broad-sense definitions (Simmons, 2025) cannot be fully differentiated from natural selection in all episodes (Table 1). The first episode of selection, although mostly reflecting pre-copulatory sexual selection, does not reflect any individuals that mated but did not produce zygotes, which means that it could include some elements of post-copulatory selection. The second episode of selection likely captures fecundity selection in females and selection on brood pouch size, as well as some elements of sexual selection related to choice in mating

duration and/or post-copulatory male choice. The 'maternity share' of the population (the proportion of eggs stored per female per male brood) is very similar to this estimate, and in this experimental design is equivalent to the relative number of eggs stored. The final post-copulatory episode of selection also likely includes a mix of natural and sexual selection, as embryos might not develop due to congenital defects (natural selection), although pipefish males have been demonstrated to absorb nutrients from embryos (Ripley & Foran, 2006a; Sagebakken et al., 2010) and exhibit post-copulatory choice (Paczolt & Jones, 2010), which could reflect sexual selection.

Finally, we estimated the maximum strength of selection a trait may experience related to that trait's involvement in obtaining a mate by calculating the maximum standardized sexual selection differential (s'_{max} or the Jones Index; Jones, 2009). This metric is the product of the Bateman gradient and the square root of the opportunity for sexual selection (I_s or I_1). We used the averaged I_1 across all trials for this calculation.

Generating 95% confidence intervals for the selection differentials and opportunity for sexual selection

We estimated confidence intervals for the opportunity for selection and selection differentials using a bootstrapping approach. Specifically, we randomly sub-sampled each trial to retain 5 individuals. From this sub-sample, we re-calculated the metrics for the opportunity for sexual selection partitions and the decomposed selection differentials, as described above. We repeated this sub-sampling 1000 times and used those 1000 estimates to calculate the mean for each metric and estimated the 95% confidence intervals as the values corresponding to the 2.5% and 97.5% quantiles. If the confidence intervals for the same metric from males and females did not overlap, we concluded that the estimates of those metrics were significantly

different between the sexes. Similarly, selection differentials with confidence intervals not overlapping zero were considered significant.

RESULTS

Variation in genetic mating success and sexual dimorphism across Syngnathus

In *S. fuscus*, an average of 30.6% of males mated in each trial with the number of pregnant males per trial ranging from 1 – 3 of the six potential males. Only one male sired offspring with two females (Figure S2). While male *S. floridae* sired offspring with more females per brood (Table 2, Figure S2) compared to *S. fuscus*, the average brood size for pregnant males was about the same (253 ± 28.4 and 252 ± 34.9 respectively) between the two species.

Species	Mating Success		Eggs stored per Mate		Percent of Eggs Developed	
	Male	Female	Male	Female	Male	Female
<i>S. floridae</i> Males = 24 Females = 21	1.25 ± 0.09	1.42 ± 0.15	215 ± 24.1	202 ± 24.0	$84.9 \% \pm 4.31$	$82.0 \% \pm 4.95$
<i>S. fuscus</i> Males = 22 Females = 19	1.05 ± 0.05	1.10 ± 0.07	249 ± 35.6	244 ± 36.5	$92.4 \% \pm 2.36$	$93.8 \% \pm 1.72$
<i>S. scovelli</i> Males = 49 Females = 29	1.00 ± 0.00	1.62 ± 0.14	28.5 ± 1.42	30.1 ± 1.78	$87.3 \% \pm 3.29$	$92.8 \% \pm 1.98$

Table 2. Summary of genetic mating success and two components of reproductive success (eggs stored per mate and proportion of eggs developed) for the three different species of *Syngnathus*. Reported are the means $\pm$ standard error. All averages were calculated within the successfully mated individuals (omitting individuals who did not mate).

An average of 26.4% of the *S. fuscus* females mated at least once per trial, with a range of 1 – 3 of the six females per trial and two females siring offspring with two males (Figure S2). In *S. floridae*, six of the broods had multiple mothers, with two females siring offspring with three males (Figure S2). Female *S. scovelli* had higher mating success than the other species but had

the lowest number of eggs stored per mate (Table 2). The lower egg quantity in *S. scovelli* is likely linked to the differences in body and egg size between the three species (Figure 1A).

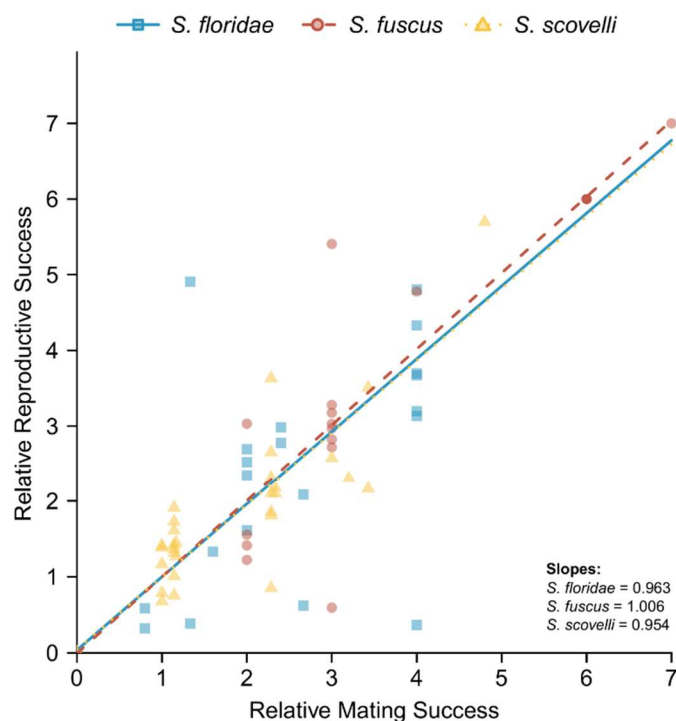
The least sexual dimorphism was found in *Syngnathus floridae* (Figure 1A, Table S3), which demonstrated no obvious ornamental differences between the sexes, no significant difference in standard length (Wilcoxon rank sum: $W = 3930$, $p = 0.478$, $n_{\text{Male}} = 86$, $n_{\text{Female}} = 86$) or adjusted torso depth (Two sample t-test: $t_{169} = 1.37$, $p = 0.173$), but a larger female SVL than males (Welch two sample t-test: $t_{149.6} = 3.86$, $p < 0.001$). We found a slightly greater degree of sexual dimorphism in *S. fuscus* (Figure 1A, Table S3), which showed a previously undocumented temporary female ornament: an increased contrast of their torsos to show alternating light and dark bands (Figure S3), only displayed during courtship. Female *S. fuscus* also had a significantly larger standard length (Wilcoxon rank sum: $W = 5828$, $p < 0.001$, $n_{\text{Male}} = 92$, $n_{\text{Female}} = 91$) and SVL (Two sample t-test: $t_{181} = 10.6$, $p < 0.001$) than males, but no difference in adjusted torso depth (Wilcoxon rank sum: $W = 4382$, $p = 0.585$, $n_{\text{Male}} = 92$, $n_{\text{Female}} = 91$). *Syngnathus scovelli* was the most sexually dimorphic as females possess a permanent, sexually selected, iridescent ornament that is fully absent in the males (Flanagan et al., 2014), and were significantly larger than males in terms of standard length (Wilcoxon rank sum: $W = 2531$, $p < 0.001$, $n_{\text{Male}} = 54$, $n_{\text{Female}} = 55$), SVL (Wilcoxon rank sum: $W = 2801$, $p < 0.001$, $n_{\text{Male}} = 54$, $n_{\text{Female}} = 55$), and adjusted torso depth (Welch two sample t-test: $t_{94.5} = 14.983$, $p < 0.001$).

Differences in selection across the three species of pipefish - β_{SS} and I

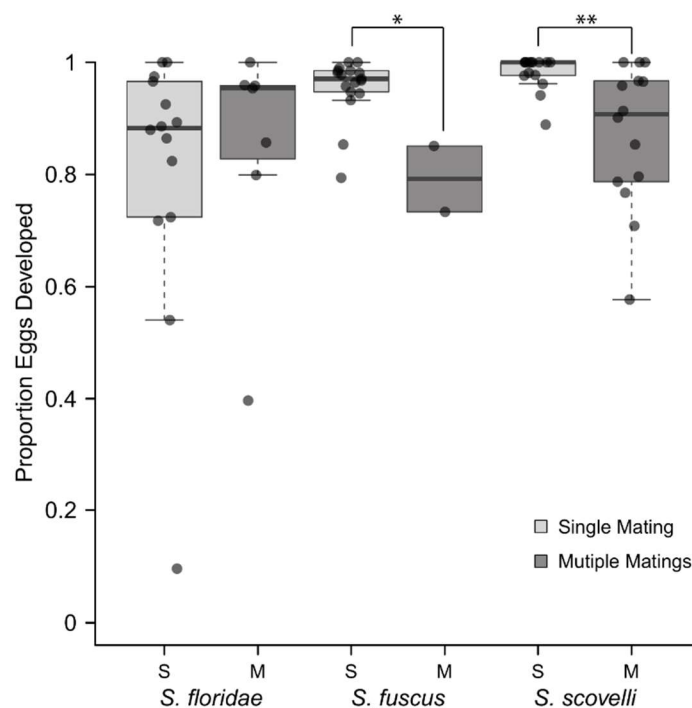
Female pipefish from all three species exhibited a significant positive relationship between relative number of eggs stored and relative mating success, resulting in significantly positive slopes of Bateman gradients (β_{SS}) (*S. floridae* $\beta = 0.96 \pm 0.086$, $t_{56} = 11.2$, $p < 0.0001$; *S. fuscus* $\beta = 1.00 \pm 0.028$, $t_{74} = 35.8$, $p < 0.0001$; *S. scovelli* $\beta = 0.95 \pm 0.049$, $t_{55} = 19.4$, $p < 0.0001$; Figure 2A). The three species have very similar female Bateman gradients. When non-

456 mated individuals were removed from the analysis, we compared male and female Bateman
 457 gradients within *S. fuscus* and *S. floridae* and found that only female *S. fuscus* had a
 458 significantly positive slope (*S. floridae* $\beta = 0.69 \pm 0.40$, $t_{21} = 1.78$, $p = 0.10$; *S. fuscus* $\beta = 1.77 \pm$
 459 0.67 , $t_{19} = 2.64$, $p = 0.017$, Figure S4). While there was no significant difference in β_{SS} between
 460 male and female *S. floridae* ($\beta = -0.44 \pm 0.61$, $t_{45} = -0.72$, $p = 0.47$), we found a slight significant
 461 difference in β_{SS} between male and female *S. fuscus* ($\beta = -2.06 \pm 1.03$, $t_{39} = -2.00$, $p = 0.05$).
 462 Further investigation into the impact of additional mates on female fitness (Figure 2B) showed
 463 no significant difference in the proportion of eggs developed between singly and multiply mated
 464 females in *S. floridae* (Wilcoxon rank sum: $W = 53$, $p = 0.794$, $n_{\text{Single}} = 14$, $n_{\text{Multiple}} = 7$). However,
 465 singly mated females had a significantly higher proportion of eggs developed compared to
 466 multiply mated females in *S. fuscus* (Wilcoxon rank sum: $W = 1$, $p = 0.0394$, $n_{\text{Single}} = 17$, $n_{\text{Multiple}} =$
 467 2) and *S. scovelli* (Wilcoxon rank sum: $W = 41.5$, $p = 0.004$, $n_{\text{Single}} = 15$, $n_{\text{Multiple}} = 14$).

A



B



469 **Figure 2.** Females with increased genetic mating success had more developed eggs in all three species,
 470 but multiply mated females had a lower proportion of eggs developed in *S. fuscus* and *S. scovelli*. (A)

Bateman gradients were similar for female *S. floridae* (blue), *S. fuscus* (red), and *S. scovelli* (yellow). Relative fitness was calculated as the individual's fitness level (either total number of developed eggs or number of mates) divided by the average fitness across all individuals within each trial. Bateman gradients are derived from a linear mixed model with trial number as a random effect for each species. The slopes for all three species were significantly greater than zero, however there was little difference between the species. (B) Comparing the proportion of eggs developed in singly (S) mated (light grey) versus multiply (M) mated (dark grey) females shows slightly lower embryo survival rates for *S. fuscus* and *S. scovelli* females who mated more than once.

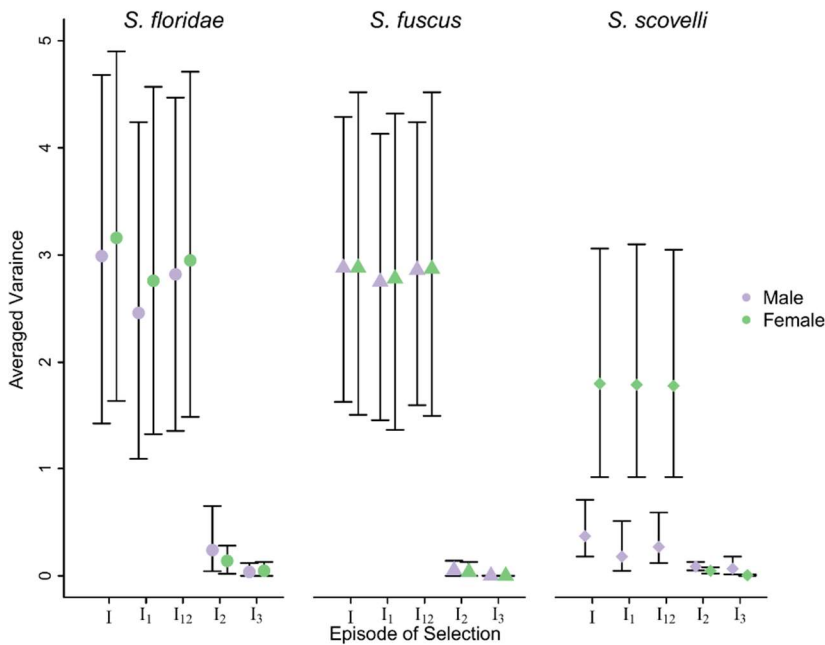
Alt text: In A, the x-axis is "Relative Mating Success", ranging from 0 to 7, and the y-axis is "Relative reproductive success" ranging from 0 to 7. In blue are points for *S. floridae*, ranging from 0 to 5 on the x-axis and 0 to 5.5 on the y-axis. The line for *S. floridae* is labelled as having a slope of 0.963. In red are points for *S. fuscus*, ranging from 0 to 7 on both axes. The line for *S. fuscus* is labelled as having a slope of 1.006. In yellow are points for *S. scovelli*, which range from 0 to 5 on both axes. The line for *S. scovelli* is labelled as having a slope of 0.954. In B, the x-axis is labelled with the three species names and "S" or "M" for single or multiple matings. The y-axis shows the "Proportion Eggs Developed", ranging from 0 to 1. The boxplot for single-mated *S. floridae* has a median around 0.9 and the interquartile range (IQR) from about 0.75 to 0.95, with raw points mostly in the box and whiskers (from 0.5 to 1), with one outlier at 0.1. The multiply mated *S. floridae* box has a median around 0.95 and an IQR from 0.85 to 0.95. The raw data points are mostly on the box and whiskers (which extend to 0.8 and 1) but one point is at 0.4. The single mated *S. fuscus* box has a median around 0.95 and an IQR from about 0.9 to 0.97 and most raw data points are within the box and whiskers (from 0.9 to 1). Two points are below, between 0.8 and 0.9. The multiple mated *S. fuscus* box has a median of 0.8 and an IQR of 0.7 to 0.85, with only two raw data points at each of those edges. The single mated *S. scovelli* box has a median close to 1, with the IQR from about 0.95 to 1. Only two points fall below the bottom whisker (near 0.9), and both are above 0.85. The multiple mated *S. scovelli* box has a median around 0.9 with an IQR from about 0.8 to 0.95 and whiskers from about 0.6 to 1. The raw data points are all within the range of the whiskers.

The total opportunity for selection (I) was not significantly different between males and females for *S. floridae* or *S. fuscus* (Table 3; Figure 3A), regardless of the episode of selection. However, female *S. scovelli* experienced a significantly higher total opportunity for selection compared to male *S. scovelli* (Table 3; Figure 3A), especially in the pre-copulatory episode. The variance in fitness due to differences in mating success (I_1 or I_5) and its corresponding influence on variance in number of eggs stored (I_{12}) was the most important contributor for the total opportunity for selection for both sexes in all three species (Figure 3B; Table 3, S4). The lesser role of I_1 in male *S. scovelli* is balanced by a larger proportion of selection arising from post-copulatory episodes related to more variance in the number of eggs stored per mate (I_2) and selection related to proportion of eggs developed (I_3). For female *S. scovelli*, and male and female *S. fuscus* and *S. floridae*, the next most important contributor was also I_2 followed by I_3 , but both episodes made up a much lower percentage of the total opportunity for selection compared to male *S. scovelli* (Table 3, S4). The highest opportunity for selection overall was

511 found in male and female *S. floridae*, followed very closely, and with overlapping 95%
512 confidence intervals, by *S. fuscus*. The overall patterns described here were consistent if non-
513 mated individuals were excluded from calculations after the first episode of selection, although
514 the estimates of *I* were of lower magnitudes (Table S5).

ORIGINAL UNEDITED MANUSCRIPT

A



B

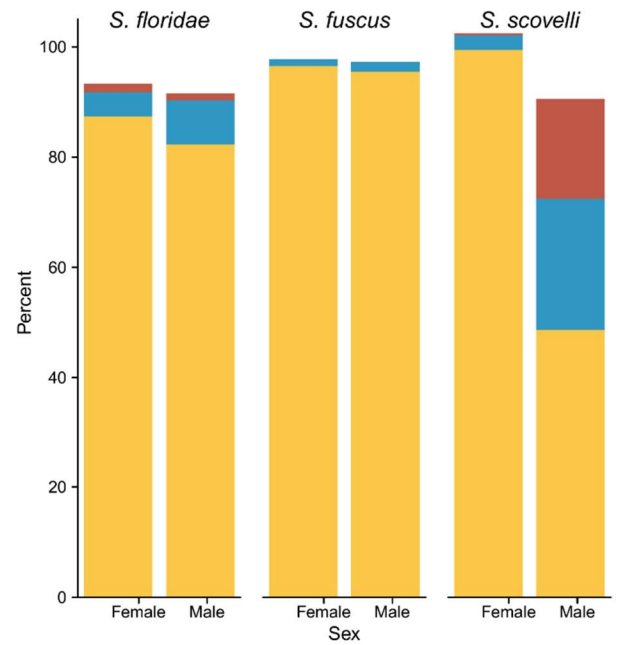


Figure 3. The partitioning of the opportunity for selection across the three species of *Syngnathus* by selection episode. (A) We calculated the partitioning for each trial individually, shown here is the average value for each selection episode across all trials for males (purple) and females (green) in *S. floridae* (circle), *S. fuscus* (triangle), and *S. scovelli* (diamond). Average values represented here were derived from bootstrapping. Error bars around the point represent percentile based 95% confidence intervals derived from bootstrapping (actual means and standard deviations are in Table S4). (B) The percentage of the total opportunity for selection (I) that can be attributed to each selection episode for males versus females in the three species of pipefish. Note that the contributions of each decomposed episode of I do not sum to 100% because the covariances are not shown; please see Table 3 for the complete decomposition.

Alt text: In A, the x-axis is labelled "Episode of selection", with three separated x-axes with repeated labels of "I", "I₁", "I₁₂", "I₂", and "I₃". The y-axis is labelled "Average fitness value" and ranges from 0 to 5. The three separated x-axes have labels "*S. floridae*", "*S. fuscus*", and "*S. scovelli*". For each of the x-axis labels, there is a Male and a Female point for each species, with confidence intervals extending above and below each point. The estimates and confidence intervals can be found in Table 3. In B, the x-axis is labelled "Sex" and has three separate axes, each with "Female" and "Male", with the separate axes labelled "*S. floridae*", "*S. fuscus*", and "*S. scovelli*". The y-axis reads "Percent" and goes from 0 to 100. Each sex and species has stacked bar charts showing the percent of I that comes from I₁, I₂, and I₃ (shown in yellow, blue, and red, respectively). The precise percentages are found in Table 3. The stacked bar plot show the following percentages: Female *S. floridae* – I₁ = 87%, I₂ = 4%, I₃ = 1.5%; male *S. floridae* – I₁ = 82%, I₂ = 8%, I₃ = 1.24; Female *S. fuscus* – I₁ = 97%, I₂ = 1%, I₃ = 0%; male *S. fuscus* – I₁ = 96%, I₂ = 2%, I₃ = 0%; female *S. scovelli* – I₁ = 99%, I₂ = 3%, I₃ = 0%; male *S. scovelli* – I₁ = 49%, I₂ = 24%, I₃ = 18%.

536 **Table 3.** The opportunity for sexual selection split across mating phases is similar in male and female *Syngnathus floridae*, *Syngnathus fuscus*, and
537 *Syngnathus scovelli*. The values shown here are the average opportunity for selection across trials alongside percentile-based confidence intervals
538 in parentheses which were both derived from bootstrapping. The percentages are calculated from the means shown in this table and reflect the fact
539 that most of the variation in fitness arises from premating selection across all three species. Numbers in bold indicate values which are significantly
540 different between males and females (i.e., the confidence intervals do not overlap).

ORIGINAL UNEDITED MANUSCRIPT

Source of Variation in Fitness	Symbol	<i>Syngnathus floridae</i>				<i>Syngnathus fuscus</i>				<i>Syngnathus scovelli</i>			
		Male (95% CI)	M%	Female (95% CI)	F%	Male (95% CI)	M%	Female (95% CI)	F%	Male (95% CI)	M%	Female (95% CI)	F%
Pre-copulatory selection (mating success, w_1)	I_1	2.46 (1.09, 4.24)	82.3	2.76 (1.32, 4.47)	87.3	2.75 (1.46, 4.14)	95.5	2.78 (1.36, 4.32)	96.5	0.18 (0.047, 0.51)	48.6	1.79 (0.92, 3.10)	99.4
Post-copulatory selection arising from eggs stored per mate (w_2)	I_2	0.24 (0.043, 0.65)	8.03	0.14 (0.020, 0.28)	4.43	0.052 (0.00086, 0.14)	1.80	0.036 (0.00020, 0.12)	1.25	0.088 (0.052, 0.13)	23.8	0.048 (0.022, 0.080)	2.67
Covariance between w_1 and w_2 : Unweighted	$COI(1, 2)$	0.57 (0.36, 0.73)	19.1	0.65 (0.45, 0.81)	20.6	0.64 (0.43, 0.83)	22.2	0.64 (0.40, 0.85)	22.2	0.10 (0.041, 0.19)	27.0	0.51 (0.35, 0.66)	28.3
Weighted	$COI(1, 2 1)$	-0.050 (-0.19, 0.088)	-1.67	-0.04 (-0.17, 0.072)	-	-0.012 (-0.046, 0.00)	-0.42	0.0073 (0.00, 0.024)	0.25	0.00 (0.00, 0.00)	0.0	-0.050 (-0.12, 0.0060)	-2.77
Change in covariance between number of eggs stored ($w_1 w_2$) and eggs stored per mate (w_2) caused by pre-copulatory selection	$COI(12, 2 1) - COI(12, 2)$	-0.41 (-0.67, -0.018)	-13.7	-0.57 (-0.76, -0.32)	-	-0.57 (-0.80, -0.31)	-19.8	-0.59 (-0.83, -0.34)	-20.5	-0.094 (-0.16, -0.036)	-	-0.52 (-0.67, -0.36)	-28.9
Variance in number of eggs stored ($w_1 w_2$)	Subtotal: I_{12}	2.82 (1.35, 4.47)	94.3	2.95 (1.49, 4.71)	93.4	2.86 (1.60, 4.24)	99.3	2.87 (1.50, 4.42)	99.6	0.27 (0.12, 0.59)	73.0	1.78 (0.92, 3.05)	98.9
Post-copulatory selection arising from proportion of eggs developed (w_3)	I_3	0.037 (0.0014, 0.12)	1.24	0.048 (0.0012, 0.13)	1.51	0.0013 (0.00, 0.0036)	0.045	0.000300 (0.00, 0.00067)	0.010	0.067 (0.014, 0.18)	18.1	0.0063 (0.0014, 0.013)	0.35
Covariance between number of eggs stored ($w_1 w_2$) and proportion of eggs developed (w_3): Unweighted	$COI(12, 3)$	0.59 (0.39, 0.75)	19.7	0.66 (0.47, 0.82)	20.9	0.64 (0.44, 0.84)	22.2	0.64 (0.40, 0.85)	22.2	0.12 (0.048, 0.20)	32.4	0.52 (0.37, 0.67)	28.9
Weighted by number of eggs stored	$COI(12, 3 2)$	0.04 (-0.053, 0.17)	1.33	0.062 (-0.041, 0.20)	1.96	0.0075 (-0.001, 0.022)	0.26	0.0026 (-0.0017, 0.0074)	0.090	0.015 (-0.002, 0.039)	4.05	0.0050 (-0.024, 0.040)	0.28
Change in covariance between number developed eggs ($w_1 w_2 w_3$) and proportion of eggs developed (w_3) caused by the previous episodes of selection	$COI(123, 3 2) - COI(123, 3)$	-0.50 (-0.73, -0.19)	-16.7	-0.55 (-0.78, -0.24)	-	-0.64 (-0.83, -0.43)	-22.2	-0.63 (-0.85, -0.40)	-21.9	-0.10 (-0.18, -0.038)	-	-0.51 (-0.66, -0.36)	28.3

Total selection (number of developed eggs, $w_1w_2w_3$)	<i>I</i>	2.99 (1.42, 4.68)	100	3.16 (1.64, 4.90)	100	2.88 (1.63, 4.24)	100	2.88 (1.51, 4.42)	100	0.37 (0.18, 0.71)	100	1.80 (0.92, 3.06)	100
---	----------	----------------------	-----	----------------------	-----	----------------------	-----	----------------------	-----	--------------------------------	-----	-----------------------------	-----

ORIGINAL UNEDITED MANUSCRIPT

Selection on snout-vent length and the maximum sexual selection differential (s'_{max})

While I remained largely similar between the sexes, our results for the absolute selection differentials (s) and the standardized selection differentials (s') showed that males and females experienced different patterns of selection on body size in all three species of pipefish (Table 4, Figure 4). We found non-zero selection on female SVL for *S. fuscus* and *S. scovelli*, but not female *S. floridae*. Male *S. floridae* had significant selection differentials whereas *S. fuscus* and *S. scovelli* males did not. Our calculations of s and s' demonstrated that smaller males had higher fitness overall in *S. floridae*, with both s and s' being significantly negative in the total selection differential. However, of the male *S. floridae* that mated, larger males had a higher fitness in terms of the number of eggs stored per mate (post-copulatory selection; s'_2 was significantly positive). We did not observe any significant selection differentials for female body size in *S. floridae*, but the magnitude of the estimates was always opposite the males.

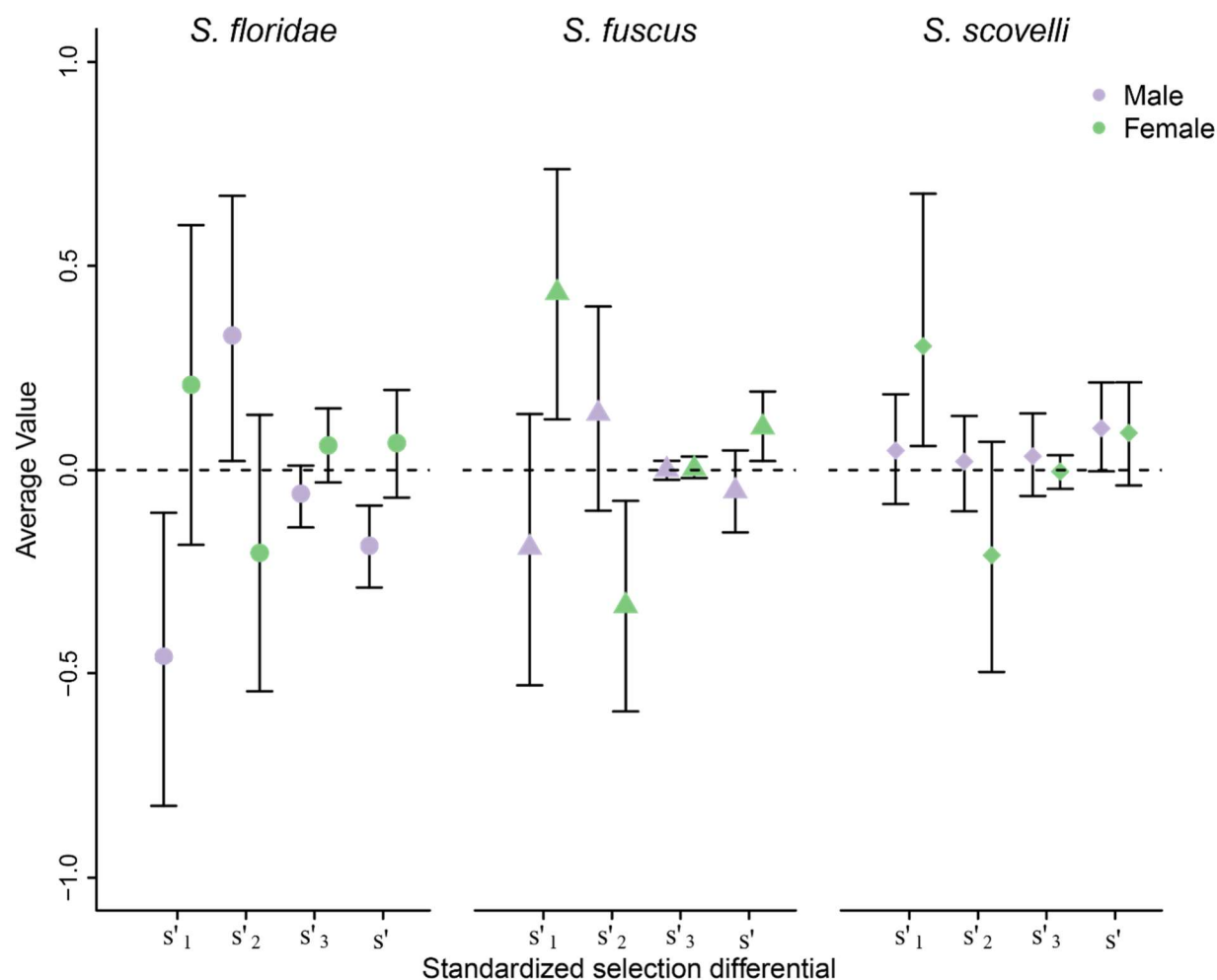
In *S. scovelli* females we calculated significantly positive s' on SVL in the pre-copulatory selection (s'_1) with a larger SVL favored. Similarly, large female *S. fuscus* had higher fitness in the pre-copulatory phase (s_1 and s'_1), but they also had a significantly negative post-copulatory selection differential associated with number of eggs stored per mate (s_2 and s'_2). However, overall SVL had a positive selection differential in female *S. fuscus* (Table 4, Figure 4). The maximum standardized sexual selection differential (s'_{max}) was largest for *S. fuscus* males and females and smallest for *S. scovelli* males (Table 4). When comparing s'_1 to s'_{max} we found males and females experienced 3 – 9X lower strengths of sexual selection on SVL compared to the maximum strength of selection that trait could experience. This difference was the most dramatic for *S. scovelli* males and most similar for *S. floridae* males (Table 4). Note again that the reported mean selection differentials were derived from the bootstrapping procedure used to generate the 95% confidence intervals; actual means and standard deviations from the trials are included in Table S6.

567 **Table 4.** Decomposition of selection differentials for male and female *Syngnathus floridae*, *S. fuscus*, and *S. scovelli*. Shown are the contributions
568 of pre-copulatory selection and post-copulatory selection to the total selection differential for snout-vent length. Decomposition was done separately
569 for each trial; therefore, we are reporting the means across all trials and percentile-based 95% confidence intervals. Both the means and confidence
570 intervals were derived via bootstrapping. The selection differential is shown as the absolute selection differential (s), in millimeters, and as the
571 standardized selection differential (s'), in units of phenotypic standard deviations. A bolded selection differential represents significant selection in
572 that direction. The maximum strength of selection snout-vent length may experience is shown with the maximum sexual selection differential (s'_{max}).

573

ORIGINAL UNEDITED MANUSCRIPT

	<i>Syngnathus floridae</i>		<i>Syngnathus fuscus</i>		<i>Syngnathus scovelli</i>	
	Male (95% CI)	Female (95% CI)	Male (95% CI)	Female (95% CI)	Male (95% CI)	Female (95% CI)
Absolute selection differential						
Pre-copulatory selection (Mating success, s_1)	-2.69 (-5.09, -0.13)	2.13 (-1.73, 6.33)	-0.61 (-2.21, 0.93)	2.14 (0.61, 3.68)	0.0067 (-0.37, 0.32)	0.86 (-0.060, 1.87)
Post-copulatory selection (Number of eggs per mate, s_2)	1.93 (-0.28, 4.07)	-2.14 (-5.30, 1.46)	0.42 (-0.82, 1.65)	-1.58 (-2.88, -0.34)	0.13 (-0.17, 0.45)	-0.63 (-1.44, 0.13)
Post-copulatory selection (Proportion of eggs developed, s_3)	-0.42 (-1.07, 0.098)	0.70 (-0.34, 1.81)	0.011 (-0.052, 0.087)	-0.012 (-0.14, 0.10)	0.051 (-0.25, 0.37)	0.010 (-0.09, 0.12)
Total selection differential (s)	-1.18 (-1.89, -0.47)	0.69 (-0.66, 2.07)	-0.18 (-0.63, 0.29)	0.54 (0.084, 1.01)	0.18 (-0.14, 0.53)	0.24 (-0.056, 0.55)
Standardized selection differential						
Pre-copulatory selection (Mating success, s'_1)	-0.46 (-0.82, -0.10)	0.20 (-0.18, 0.60)	-0.19 (-0.52, 0.14)	0.43 (0.12, 0.74)	0.048 (-0.08, 0.18)	0.30 (0.059, 0.67)
Post-copulatory selection (Number of eggs per mate, s'_2)	0.33 (0.022, 0.67)	-0.20 (-0.54, 0.13)	0.14 (-0.10, 0.40)	-0.33 (-0.59, -0.076)	0.021 (-0.10, 0.13)	-0.21 (-0.50, 0.069)
Post-copulatory selection (Proportion of eggs developed, s'_3)	-0.058 (-0.14, 0.011)	0.060 (-0.030, 0.15)	0.00056 (-0.024, 0.023)	0.0023 (-0.019, 0.033)	0.033 (-0.063, 0.14)	-0.0037 (-0.046, 0.036)
Total selection differential (s')	-0.18 (-0.29, -0.087)	0.066 (-0.067, 0.20)	-0.051 (-0.15, 0.048)	0.10 (0.022, 0.19)	0.10 (-0.003, 0.21)	0.091 (-0.038, 0.21)
Max sexual selection differential						
s'_{max}	1.53	1.60	1.64	1.68	0.424	1.28



575

576 **Figure 4.** The decomposition of standardized selection differentials for males (purple) and females (green)
 577 from three species of pipefish, *S. floridae* (circle), *S. fuscus* (triangle), and *S. scovelli* (diamond). Because
 578 the decompositions were performed separately for each trial, the points here represent the average
 579 selection differential for that sex across all trials within a species. Error bars surrounding the points show
 580 the percentile based 95% confidence intervals. Both the averages and confidence intervals were derived
 581 from bootstrapping. Total selection differential for snout-vent length (s') was decomposed to show
 582 contributions of mating success (s'_1), the number of eggs stored per mate (s'_2), and proportion of eggs
 583 developed (s'_3).

584 **Alt text:** The x-axis is "Standardized selection differential", with three separate axes, each with four labels:
 585 s'_1 , s'_2 , s'_3 , and s' . The three axes are labelled *S. floridae*, *S. fuscus*, and *S. scovelli*. The y-axis is labelled
 586 "Average value" and ranges from -1 to 1, with a dashed line at 0. Each of the x-axis ticks has two
 587 corresponding points, one for male and one for female values, each with a confidence interval extending
 588 above and below it. The precise values and confidence intervals are in Table 4. The estimates whose
 589 confidence intervals do not cross the zero line are: *S. floridae* male s'_1 , which is -0.46; *S. floridae* male s'_2 ,
 590 which is 0.33; *S. floridae* male s' , which is -0.18; *S. fuscus* female s'_1 , which is 0.43; *S. fuscus* female s'_2 ,
 591 which is -0.33; *S. fuscus* female s' , which is 0.10; *S. scovelli* female s'_1 , which is 0.3.

592

DISCUSSION

Across three closely related species of pipefish, with different mating systems and a spectrum of sexual dimorphism, pre-copulatory episodes contributed the most to selection in both sexes – with one exception: in the only group that mated singly, male *S. scovelli*, post-copulatory selection contributed similarly to pre-copulatory episodes. Female Bateman gradients (β_{SS}) did not differ across the species, whether or not unmated individuals were excluded. Selection differentials for snout-vent length in males and females were opposing for all selection episodes in *S. floridae* and *S. fuscus*. This result could reflect sexually antagonistic selection, but more work is needed. Further, we found a potential tradeoff between pre- and post-copulatory selection in female *S. scovelli* and *S. fuscus* – larger females that mated multiply had lower fitness in the post-copulatory phases of selection. Intriguingly, an opposite tradeoff appears to exist for *S. floridae* males.

An important caveat regarding the interpretation of our results is the number of unmated individuals in the *S. fuscus* and *S. floridae* datasets, especially compared to *S. scovelli*. These unmated individuals could reflect biological properties of these species relative to *S. scovelli*, such as increased mutual mate choice resulting in increased refusals to mate (e.g., Sandvik et al., 2000). All males were capable of mating, as only pregnant males were brought into the laboratory for the experiments, but these two species could have a longer refractory period between matings than *S. scovelli*. The females were all of reproductive size, but it is possible that some were not actually mature or had depleted their mature eggs prior to use in the experiment. Alternatively, the increased numbers of unmated individuals could be due to laboratory artifacts. Unfortunately, these unmated individuals influence all the selection metrics we have used (Klug et al., 2010); as always, extrapolating from laboratory populations to the wild should only be done with extreme caution.

618 *No sex differences in the relative strength of sexual selection*

619 We calculated Bateman gradients, which can be used to compare the relative strength of
620 sexual selection across sexes, environments (Mobley & Jones, 2007, 2009), and species
621 (Fromonteil et al., 2023; Gerlach et al., 2012; Janicke et al., 2016; Janicke & Morrow, 2018;
622 Lopez-Nava et al., 2026), to compare sexual selection in males and females of three pipefish
623 species and found few differences. Across all groups, we found significantly positive slopes
624 (β_{SS}), which reflect a significant increase in the number of developed eggs through the addition
625 of at least one mate, in line with a previous study on wild populations of *S. floridae* (Henshaw et
626 al., 2018; Mobley & Jones, 2013). Bateman gradients calculated without experimentally
627 manipulating mating success, including in our study, cannot fully imply a causal relationship
628 between mating success and reproductive success, as the data is correlational and could
629 include unmeasured confounding variables influencing reproductive success (Anthes et al.,
630 2017; Fromonteil et al., 2023). Additionally, selection is known to be sensitive to varying
631 environmental conditions (Mobley & Jones, 2007, 2009; Monteiro & Lyons, 2012), including
632 predation or infection risk, which may contribute to sexual selection (Taylor et al., 2014) and
633 cannot be included in studies like ours. Thus, Bateman gradients measured under laboratory
634 conditions may not necessarily translate to natural populations. In our case, the unmated
635 individuals are particularly difficult to interpret, as they could reflect aspects of a laboratory setup
636 or seasonality rather than sexual selection. As such, additional data from multiply mated
637 individuals is required to confidently interpret Bateman gradients for these species.

638
639 *Differences in the opportunity for selection between sexes reflects polyandry*

640 Our study provides the opportunity to explore variation in selection across mating
641 systems with a spectrum of multiple mating, instead of comparing monogamous species to
642 multiple-mating species, as has been done previously (Weber & Fisher, 2023). An important

caveat to our descriptions of the mating system is that it is estimated using genetic parentage, which does not fully capture all potential mating attempts. Our results are consistent with previous studies, with many *S. floridae* males and females mating multiply within a reproductive cycle (Jones & Avise, 1997b; Mobley et al., 2010; Mobley & Jones, 2007; this study), low rates of multiple matings in male (Paczolt et al., 2016; this study) and female *S. fuscus* (this study), and *S. scovelli* displaying polyandry (Jones et al., 2001; Jones & Avise, 1997a; Rose et al., 2013a). Only in the polyandrous *S. scovelli* did males and females differ in their opportunity for selection, although the largest values for opportunity for selection were observed in the two polygynandrous species.

Our estimates for the opportunity for selection (I) differ from prior estimates from wild populations in several ways. First, wild populations of *S. floridae* had I estimates in females to be fourfold those found in males (Mobley & Jones, 2013), although significant differences in the opportunity for selection across populations have also been observed (Mobley & Jones, 2009). Additionally, the calculated values for I in this study were considerably larger than those reported from wild studies of *S. floridae* for males (0.24 compared to our 2.99) and females (1.19 compared to our 3.16; Mobley & Jones, 2013). The high values of I are a result of the high variance in mating and reproductive success in our study, driven by the large number of unmated individuals and the relatively few pipefish who mated more than once. When we removed non-mated individuals prior to estimating the opportunity for selection, the values generated for *S. floridae* were closer to what has been found in the wild, which only included mated individuals ($I = 0.56$ for males and 0.51 for females, Table S5) (Mobley & Jones, 2007, 2009, 2013).

We expected the two species where males were able to mate multiply (*S. fuscus* and *S. floridae*) to have a higher contribution of post-copulatory selection due to the potential for egg competition and cryptic male choice, in line with the expectation that high remating rates lead to more post-copulatory selection (Birkhead, 2010; Birkhead & Pizzari, 2002). However, we found

that post-copulatory selection, measured here as the variance in the number of eggs stored per mate and the proportion of those eggs that developed, contributed little to the overall opportunity for selection except for in the polyandrous *S. scovelli*. Because of the higher proportions of non-matings in *S. fuscus* and *S. floridae* compared to *S. scovelli*, we may simply not have the power to detect post-copulatory selection as the majority of variance present is a result of whether the pipefish was able to secure a mate. Alternatively, post-copulatory selection in male *S. scovelli*, which has been documented previously (Paczolt & Jones, 2010), could indeed be an important episode of selection that is less important in the polygynandrous species. The importance of this episode in *S. scovelli* could reflect variation in male care ability, for example variation in the size of the male brood pouch and thus the number of eggs he can care for, which means that this episode would be more indicative of natural selection than sexual selection. Uncovering the mechanisms driving variance in the proportion of eggs developing in *S. scovelli* is a rich area for further study.

Some aspects of our experimental design could have biased us towards detecting pre-copulatory selection over post-copulatory selection. First, our experimental breeding populations had equal sex ratios. Previous work on other pipefish species found stronger selection for both sexes when they were the sex in excess (i.e., females in female-biased trials; Aronsen et al., 2013). While *S. floridae* are commonly found in equal sex-ratios in the wild (Mobley & Jones, 2007, 2009), *S. fuscus* have been discovered in extremely female-biased populations (Ripley & Foran, 2006b), meaning our populations may not have mimicked the selective environment faced by wild populations of this pipefish. The length of the experimental breeding populations also spanned only the length of a single male pregnancy, whereas in the wild male pipefish acquire multiple pregnancies over a prolonged breeding season. This means we were only able to capture a snapshot of selection, rather than an estimate of lifetime reproductive success, where we would potentially identify trade-offs associated with each additional pregnancy in males. Lastly, using genetic mating success instead of behavioral observations prevents us

from accounting for copulations resulting in complete brood reduction and/or multiple copulations with the same partner, therefore obscuring a potentially key component of both pre- and post-copulatory sexual selection (Anthes et al., 2017). Future studies aiming to compare selection decompositions across species would benefit from the inclusion of more than three species, the incorporation of both behavioral and genetic mating success, and extending the study to across the entire breeding season, where possible.

Selection on body size is insufficient to explain variance in mating success

Our estimates of the absolute selection differentials on snout-vent length provide valuable insight into how selection might affect the sexes differently and highlight important differences between the species. In each species, significant selection differentials were only found in one sex. Interestingly, in the species with lower multiple mating (*S. scovelli* and *S. fuscus*), the selection was on females, whereas in *S. floridae* selection was detected on males. Although many selection strengths were non-significant, opposite trends were detected in males and females in most phases (i.e., positive in females and negative in males, or vice versa) in *S. floridae* and *S. fuscus*. If these trends are biologically accurate, antagonistic selection could be acting on body size in male and female pipefishes in these two species. The insignificant selection estimates could reflect the lack of, or weak, directional selection on snout-vent length, or could reflect limited statistical power. Alternatively, we could be under-estimating potential sexual selection due to the experimental setup; sexual selection on female length was reduced at high densities in *S. typhle*, and our experimental setups kept pipefish at high densities (max of ~1.6 pipefish/m² in the wild compared to our 28 – 37 pipefish/m²; Masonjones et al., 2010; Ripley & Foran, 2006b).

Compared to the Jones Index (s'_{max}), which represents the maximal value for pre-copulatory selection, all estimates of s'_1 were an order of magnitude smaller – including the

significant s'_1 estimates – suggesting other traits are likely to be the targets of pre-copulatory selection. As *S. scovelli* is the only species from this study that possesses a permanent ornament, which develops at sexual maturity and is estrogen-regulated (Anderson et al., 2020), we focused on comparing phases of selection for body size for the three species. However, a prior study using the same *S. scovelli* dataset found a larger s'_1 for ornament traits (number of iridescent bands and mean band area), compared to standard length (Flanagan et al., 2014). Although *S. fuscus* only displays a temporary ornament (Figure S3), similar temporary displays in *S. scovelli* influence male preference (Partridge et al., 2013), so it is likely that female ornaments, whether permanent or temporary, contribute to s'_{max} in these species. Other unmeasured traits could reflect fecundity, such as brood pouch volume in males (which differs among species; Table S3) or torso depth in females (e.g., Mobley et al., 2018).

An initially surprising result was the significant total selection differential favoring small male *S. floridae* SVL. This finding was unexpected because this species is often categorized as monomorphic (although sexual size dimorphism varies geographically; Mobley & Jones, 2007, 2009), with no evidence of size assortative mating (Mobley et al., 2014), and in wild populations larger males had significantly higher mating and reproductive success (Jones & Avise, 1997b; Mobley & Jones, 2007, 2009). This difference between our work and previous reports on wild populations could reflect a true pattern for this population of *S. floridae*, or it may be an artifact resulting from our experimental breeding populations. Because a component of the courtship rituals for these species involves rising together in the water column (personal observation, similar to the vertical swimming identified as a component of *S. scovelli* courtship; Anderson & Jones, 2019; Partridge et al., 2013), the height of the tanks (56cm) may have inhibited large males from succeeding. However, some of the larger males successfully mated (*S. floridae*: mated males standard length: 124 – 203mm, all males standard length: 124 – 226mm). Alternatively, previous studies have highlighted the potential for larger males to require more

mating events to fill their brood pouches to capacity (Mobley & Jones, 2007, 2013), so female reproductive output coupled with the length of the trials could have also influenced our estimates of selection if we are underestimating mating, and therefore, reproductive success. However, wild populations of *S. floridae* had similar values to our study in average number of eggs stored (range of 180 – 505 for *S. floridae*; Jones & Avise, 1997b; Mobley & Jones, 2007, 2009) and average male genetic mating success (~1.5 for *S. floridae*; Mobley & Jones, 2007, 2009). Therefore, although laboratory artifacts could have influenced our results, our findings are still relevant to the biology of this species.

Pre- versus post-copulatory episodes of selection

We found females who mated multiply had a significantly lower proportion of eggs developed compared to singly mated females for *S. scovelli* and *S. fuscus*, in line with previous analyses of the *S. scovelli* dataset (Rose et al., 2013a). Additionally, all three pipefish species had positive s_1 and s'_1 with negative s_2 and s'_2 , even if these values were not statistically different from zero in all species (Figure 4). Female *S. floridae* also obtained multiple matings, but we did not find evidence for this same difference between multiply and singly mated females. This suggests that depleting egg stores, and subsequently depositing fewer mature eggs into subsequent mates, is not the best explanation. Instead, this pattern could be linked to a resource tradeoff experienced by females between the cost of ornamentation, both permanent and temporary, and egg production in *S. scovelli* and *S. fuscus* (Figure S3). Most studies of such tradeoffs in fishes focused on carotenoid-based traits (Nordeide et al., 2013), which differs from the iridescent or temporary ornaments in *S. scovelli* and *S. fuscus*. Within pipefish, female ornaments are associated with increased mating and reproductive success (Monteiro et al., 2017) and increased fecundity (Cunha et al., 2017; Mobley et al., 2018). Similar to trade-offs in males of many taxa between traits that increase their chance in gaining matings (armaments and ornaments) and traits that ensure successful fertilizations (such as testis size, sperm

velocity and number, etc.; Durrant et al., 2016; Katsuki & Lewis, 2015; Pitcher et al., 2009; Simmons & Emlen, 2006; Yamane et al., 2010, 2010), a trade-off between investment in pre-copulatory displays and egg quality could exist in female pipefish. This trade-off could be particularly pronounced, since eggs are considered more energetically costly to produce compared to sperm. This trade-off could also be related to circulating hormone levels, as female *S. scovelli* who were exposed to low levels of 17α -ethinylestradiol had increased numbers of eggs transferred and embryo survivorship compared to unexposed females (Rose et al., 2013b). However, despite the potential costs of multiple mating experienced by these females, each additional mate resulted in a higher fitness increase compared to any fitness that may have been lost due to reductions in number of eggs stored per mate or embryo survivorship.

CONCLUSION

Our results support the prediction that large differences in selection between males and females are often associated with a polyandrous mating system rather than a polygynandrous system. However, it was within those polygynandrous mating systems where we found the highest overall opportunities for selection. We also highlighted the potential for ongoing sex-specific selection on the body sizes in male and female pipefish and showed that females who secured multiple mates had lower fitness in post-copulatory episodes of selection. Additionally, we found nuances in the different patterns of selection across species that have at least one sex which mate multiply, adding to the growing knowledge of the relationship between mating systems, sexual selection, and sexually dimorphic ornamentation. Our study sets the stage for future research to perform a truly comparative analysis, reducing potential confounding factors and increasing the total number of species sampled.

REFERENCES

- Ah-King, M., & Ahnesjö, I. (2013). The “sex role” concept: An overview and evaluation. *Evolutionary Biology*, 40(4), 461–470. <https://doi.org/10.1007/s11692-013-9226-7>
- Anderson, A. P., Rose, E., Flanagan, S. P., & Jones, A. G. (2020). The estrogen-responsive transcriptome of female secondary sexual traits in the Gulf pipefish. *Journal of Heredity*, 111(3), 294–306. <https://doi.org/10.1093/jhered/esaa008>
- Anthes, N., Häderer, I. K., Michiels, N. K., & Janicke, T. (2017). Measuring and interpreting sexual selection metrics: Evaluation and guidelines. *Methods in Ecology and Evolution*, 8(8), 918–931. <https://doi.org/10.1111/2041-210X.12707>
- Arnold, S. J., & Duvall, D. (1994). Animal mating systems: A synthesis based on selection theory. *The American Naturalist*, 143(2), 317–348. <https://doi.org/10.1086/285606>
- Arnold, S. J., & Wade, M. J. (1984a). On the measurement of natural and sexual selection: Applications. *Evolution*, 38(4), 720–734.
- Arnold, S. J., & Wade, M. J. (1984b). On the measurement of natural and sexual selection: Theory. *Evolution*, 38(4), 709–719. <https://doi.org/10.2307/2408383>
- Aronsen, T., Berglund, A., Mobley, K. B., Ratikainen, & Rosenqvist, G. (2013). Sex ratio and density affect sexual selection in a sex-role reversed fish. *Evolution*, 67(11), 3243–3257. <https://doi.org/10.1111/evo.12201>
- Barbot, E., Dufay, M., Janicke, T., Lapeyre, B., Orcel, D., Rousset, F., & Tonnabel, J. (2025). Pollinator abundance shapes sexual selection in an angiosperm (p. 2025.12.10.693606). bioRxiv. <https://doi.org/10.64898/2025.12.10.693606>
- Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity*, 2(3), 349–368.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Berglund, A., & Rosenqvist, G. (2003). Sex role reversal in pipefish. *Advances in the Study of Behavior*, 32(32), 131–167.

820 Bhave, R. S., Seears, H. A., Reedy, A. M., Wittman, T. N., Robinson, C. D., & Cox, R. M.
 821 (2024). Behavioral estimates of mating success corroborate genetic evidence for pre-
 822 copulatory selection. *Behavioral Ecology*, 35(1), arad099.
 823 <https://doi.org/10.1093/beheco/arad099>
 824 Birkhead, T. R. (2010). How stupid not to have thought of that: Post-copulatory sexual selection.
 825 *Journal of Zoology*, 281(2), 78–93. <https://doi.org/10.1111/j.1469-7998.2010.00701.x>
 826 Birkhead, T. R., & Pizzari, T. (2002). Postcopulatory sexual selection. *Nature Reviews Genetics*,
 827 3(4), 262–273. <https://doi.org/10.1038/nrg774>
 828 Björklund, M. (1991). Evolution, phylogeny, sexual dimorphism and mating system in the
 829 grackles (*Quiscalus* spp.: Icterinae). *Evolution*, 45(3), 608–621.
 830 <https://doi.org/10.1111/j.1558-5646.1991.tb04332.x>
 831 Collet, J., Richardson, D. S., Worley, K., & Pizzari, T. (2012). Sexual selection and the
 832 differential effect of polyandry. *Proceedings of the National Academy of Sciences*,
 833 109(22), 8641–8645. <https://doi.org/10.1073/pnas.1200219109>
 834 Crow, J. F. (1958). Some possibilities for measuring selection intensities in man. *Human*
 835 *Biology*, 30, 1–13.
 836 Cunha, M. A. B., Berglund, A., & Monteiro, N. M. (2017). Female ornaments signal own and
 837 offspring quality in a sex-role-reversed fish with extreme male parental care. *Marine*
 838 *Ecology*, 38(5), e12461. <https://doi.org/10.1111/maec.12461>
 839 Devigili, A., Evans, J. P., Di Nisio, A., & Pilastro, A. (2015). Multivariate selection drives
 840 concordant patterns of pre- and postcopulatory sexual selection in a livebearing fish.
 841 *Nature Communications*, 6(1), 8291. <https://doi.org/10.1038/ncomms9291>
 842 Durrant, K. L., Skicko, I. M., Sturrock, C., & Mowles, S. L. (2016). Comparative morphological
 843 trade-offs between pre- and post-copulatory sexual selection in Giant hissing
 844 cockroaches (Tribe: Gromphadorhini). *Scientific Reports*, 6(1), 36755.
 845 <https://doi.org/10.1038/srep36755>

- Evans, J. P., & Garcia-Gonzalez, F. (2016). The total opportunity for sexual selection and the integration of pre- and post-mating episodes of sexual selection in a complex world. *Journal of Evolutionary Biology*, 29(12), 2338–2361. <https://doi.org/10.1111/jeb.12960>
- Flanagan, S. P. (2026). *Spflanagan/spfTools* [R]. <https://github.com/spflanagan/spfTools>
- Flanagan, S. P., Johnson, J. B., Rose, E., & Jones, A. G. (2014). Sexual selection on female ornaments in the sex-role-reversed Gulf pipefish (*Syngnathus scovelli*). *Journal of Evolutionary Biology*, 27(11), 2457–2467. <https://doi.org/10.1111/jeb.12487>
- Fritzsche, K., Henshaw, J. M., Johnson, B. D., & Jones, A. G. (2021). The 150th anniversary of The Descent of Man: Darwin and the impact of sex-role reversal on sexual selection research. *Biological Journal of the Linnean Society*, 134(3), 525–540. <https://doi.org/10.1093/biolinnean/blab091>
- Fromonteil, S., Marie-Orleach, L., Winkler, L., & Janicke, T. (2023). Sexual selection in females and the evolution of polyandry. *PLOS Biology*, 21(1), e3001916. <https://doi.org/10.1371/journal.pbio.3001916>
- Gerlach, N. M., McGlothlin, J. W., Parker, P. G., & Ketterson, E. D. (2012). Reinterpreting Bateman gradients: Multiple mating and selection in both sexes of a songbird species. *Behavioral Ecology*, 23(5), 1078–1088. <https://doi.org/10.1093/beheco/ars077>
- Hamilton, H., Saarman, N., Short, G., Sellas, A. B., Moore, B., Hoang, T., Grace, C. L., Gomon, M., Crow, K., & Brian Simison, W. (2017). Molecular phylogeny and patterns of diversification in syngnathid fishes. *Molecular Phylogenetics and Evolution*, 107, 388–403. <https://doi.org/10.1016/j.ympev.2016.10.003>
- Hedrick, A. V., & Temeles, E. J. (1989). The evolution of sexual dimorphism in animals: Hypotheses and tests. *Trends in Ecology & Evolution*, 4(5), 136–138. [https://doi.org/10.1016/0169-5347\(89\)90212-7](https://doi.org/10.1016/0169-5347(89)90212-7)

- Henshaw, J. M., Jennions, M. D., & Kruuk, L. E. B. (2018). How to quantify (the response to) sexual selection on traits. *Evolution*, 72(9), 1904–1917.
<https://doi.org/10.1111/evo.13554>
- Henshaw, J. M., Kahn, A. T., & Fritzsche, K. (2016). A rigorous comparison of sexual selection indexes via simulations of diverse mating systems. *Proceedings of the National Academy of Sciences*, 113(3), E300–E308. <https://doi.org/10.1073/pnas.1518067113>
- Janicke, T. (2024). Sexual selection: Competition for resources provided by mating partners. *Behavioral Ecology*, 35(3), arae016. <https://doi.org/10.1093/beheco/arae016>
- Janicke, T. (2026). On the definition of sexual selection: A response to comments on Janicke (2024). *Behavioral Ecology*, 37(1), araf118. <https://doi.org/10.1093/beheco/araf118>
- Janicke, T., David, P., & Chapuis, E. (2015). Environment-dependent sexual selection: Bateman's parameters under varying levels of food availability. *The American Naturalist*, 185(6), 756–768. <https://doi.org/10.1086/681128>
- Janicke, T., Häderer, I. K., Lajeunesse, M. J., & Anthes, N. (2016). Darwinian sex roles confirmed across the animal kingdom. *Science Advances*, 2(2), e1500983.
<https://doi.org/10.1126/sciadv.1500983>
- Janicke, T., & Morrow, E. H. (2018). Operational sex ratio predicts the opportunity and direction of sexual selection across animals. *Ecology Letters*, 21(3), 384–391.
<https://doi.org/10.1111/ele.12907>
- Jones, A. G. (2009). On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. *Evolution*, 63(7), 1673–1684.
- Jones, A. G., & Avise, J. C. (1997a). Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal. *Molecular Ecology*, 6(3), 203–213. <https://doi.org/10.1046/j.1365-294X.1997.00173.x>

895 Jones, A. G., & Avise, J. C. (1997b). Polygynandry in the dusky pipefish *Syngnathus floridae*
896 revealed by microsatellite DNA markers. *Evolution*, 51(5), 1611–1622.
897 <https://doi.org/10.1111/j.1558-5646.1997.tb01484.x>

898 Jones, A. G., & Avise, J. C. (2001). Mating systems and sexual selection in male-pregnant
899 pipefishes and seahorses: Insights from microsatellite-based studies of maternity.
900 *Journal of Heredity*, 92(2), 150–158. <https://doi.org/10.1093/jhered/92.2.150>

901 Jones, A. G., & Ratterman, N. L. (2009). Mate choice and sexual selection: What have we
902 learned since Darwin? *Proceedings of the National Academy of Sciences USA*,
903 106(Supplement 1), 10001–10008.

904 Jones, A. G., Rosenqvist, G., Berglund, A., Arnold, S. J., & Avise, J. C. (2000). The Bateman
905 gradient and the cause of sexual selection in a sex-role-reversed pipefish. *Proceedings*
906 *of the Royal Society of London. Series B: Biological Sciences*, 267(1444), 677–680.
907 <https://doi.org/10.1098/rspb.2000.1055>

908 Jones, A. G., Walker, D., & Avise, J. C. (2001). Genetic evidence for extreme polyandry and
909 extraordinary sex-role reversal in a pipefish. *Proceedings of the Royal Society B-*
910 *Biological Sciences*, 268(1485), 2531–2535. <https://doi.org/10.1098/rspb.2001.1841>

911 Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program
912 cervus accommodates genotyping error increases success in paternity assignment.
913 *Molecular Ecology*, 16(5), 1099–1106. <https://doi.org/10.1111/j.1365-294X.2007.03089.x>

914 Katsuki, M., & Lewis, Z. (2015). A trade-off between pre- and post-copulatory sexual selection in
915 a bean beetle. *Behavioral Ecology and Sociobiology*, 69(10), 1597–1602.
916 <https://doi.org/10.1007/s00265-015-1971-4>

917 Klug, H., Lindström, K., & Kokko, H. (2010). Who to include in measures of sexual selection is
918 no trivial matter. *Ecology Letters*, 13(9), 1094–1102. [https://doi.org/10.1111/j.1461-](https://doi.org/10.1111/j.1461-0248.2010.01495.x)
919 [0248.2010.01495.x](https://doi.org/10.1111/j.1461-0248.2010.01495.x)

- 920 Krakauer, A. H., Webster, M. S., Duval, E. H., Jones, A. G., & Shuster, S. M. (2011). The
921 opportunity for sexual selection: Not mismeasured, just misunderstood: Opportunity for
922 selection. *Journal of Evolutionary Biology*, 24(9), 2064–2071.
923 <https://doi.org/10.1111/j.1420-9101.2011.02317.x>
- 924 Kvarnemo, C., Mobley, K. B., Partridge, C., Jones, A. G., & Ahnesjö, I. (2011). Evidence of
925 paternal nutrient provisioning to embryos in broad-nosed pipefish *Syngnathus typhle*.
926 *Journal of Fish Biology*, 78(6), 1725–1737. [https://doi.org/10.1111/j.1095-](https://doi.org/10.1111/j.1095-8649.2011.02989.x)
927 [8649.2011.02989.x](https://doi.org/10.1111/j.1095-8649.2011.02989.x)
- 928 Lopez-Nava, G., Safari, I., Küpper, C., & Goymann, W. (2026). Sex-role reversal and the
929 Bateman gradient in coucals—Females benefit from mating with multiple partners.
930 *Proceedings of the Royal Society B: Biological Sciences*, 293(2064), 20252860.
931 <https://doi.org/10.1098/rspb.2025.2860>
- 932 Marie-Orleach, L., Janicke, T., Vizoso, D. B., David, P., & Schärer, L. (2016). Quantifying
933 episodes of sexual selection: Insights from a transparent worm with fluorescent sperm.
934 *Evolution*, 70(2), 314–328. <https://doi.org/10.1111/evo.12861>
- 935 Masonjones, H. D., Rose, E., McRae, L. B., & Dixon, D. L. (2010). An examination of the
936 population dynamics of syngnathid fishes within Tampa Bay, Florida, USA. *Current*
937 *Zoology*, 56(1), 118–133. <https://doi.org/10.1093/czoolo/56.1.118>
- 938 Mobley, K. B., Abou Chakra, M., & Jones, A. G. (2014). No evidence for size-assortative mating
939 in the wild despite mutual mate choice in sex-role-reversed pipefishes. *Ecology and*
940 *Evolution*, 4(1), 67–78. <https://doi.org/10.1002/ece3.907>
- 941 Mobley, K. B., & Jones, A. G. (2007). Geographical variation in the mating system of the dusky
942 pipefish (*Syngnathus floridae*). *Molecular Ecology*, 16(12), 2596–2606.
943 <https://doi.org/10.1111/j.1365-294X.2007.03337.x>
- 944 Mobley, K. B., & Jones, A. G. (2009). Environmental, demographic, and genetic mating system
945 variation among five geographically distinct dusky pipefish (*Syngnathus floridae*)

- populations. *Molecular Ecology*, 18(7), 1476–1490. <https://doi.org/10.1111/j.1365-294X.2009.04104.x>
- Mobley, K. B., & Jones, A. G. (2013). Overcoming statistical bias to estimate genetic mating systems in open populations: A comparison of Bateman's principles between the sexes in a sex-role-reversed pipefish. *Evolution*, 67(3), 646–660. <https://doi.org/10.1111/j.1558-5646.2012.01819.x>
- Mobley, K. B., Morrongiello, J. R., Warr, M., Bray, D. J., & Wong, B. B. M. (2018). Female ornamentation and the fecundity trade-off in a sex-role reversed pipefish. *Ecology and Evolution*, 8(18), 9516–9525. <https://doi.org/doi:10.1002/ece3.4459>
- Mobley, K. B., Small, C. M., Jue, N. K., & Jones, A. G. (2010). Population structure of the dusky pipefish (*Syngnathus floridae*) from the Atlantic and Gulf of Mexico, as revealed by mitochondrial DNA and microsatellite analyses. *Journal of Biogeography*, 37(7), 1363–1377. <https://doi.org/10.1111/j.1365-2699.2010.02288.x>
- Monteiro, N. M., Carneiro, D., Antunes, A., Queiroz, N., Vieira, M. N., & Jones, A. G. (2017). The lek mating system of the worm pipefish (*Nerophis lumbriciformis*): A molecular maternity analysis and test of the phenotype-linked fertility hypothesis. *Molecular Ecology*, 26(5), 1371–1385. <https://doi.org/10.1111/mec.13931>
- Monteiro, N. M., & Lyons, D. O. (2012). Stronger sexual selection in warmer waters: The case of a sex role reversed pipefish. *Plos One*, 7(8), e44251. <https://doi.org/10.1371/journal.pone.0044251>
- Mori, E., Mazza, G., & Lovari, S. (2017). Sexual Dimorphism. In J. Vonk & T. Shackelford (Eds.), *Encyclopedia of Animal Cognition and Behavior* (pp. 1–7). Springer International Publishing. https://doi.org/10.1007/978-3-319-47829-6_433-1
- Morimoto, J., Pizzari, T., & Wigby, S. (2016). Developmental environment effects on sexual selection in male and female *Drosophila melanogaster*. *PLOS ONE*, 11(5), e0154468. <https://doi.org/10.1371/journal.pone.0154468>

- 972 Nordeide, J. T., Kekäläinen, J., Janhunen, M., & Kortet, R. (2013). Female ornaments revisited
973 – are they correlated with offspring quality? *Journal of Animal Ecology*, 82(1), 26–38.
974 <https://doi.org/10.1111/1365-2656.12021>
- 975 Ooms, J. (2021). *magick: Advanced Graphics and Image-Processing in R* (Version 2.7.3)
976 [Computer software]. <https://CRAN.R-project.org/package=magick>
- 977 Paczolt, K. A., & Jones, A. G. (2010). Post-copulatory sexual selection and sexual conflict in the
978 evolution of male pregnancy. *Nature*, 464(7287), 401–404.
979 <https://doi.org/10.1038/nature08861>
- 980 Paczolt, K. A., Martin, W. E., Ratterman, N. L., & Jones, A. G. (2016). A low rate of multiple
981 maternity for pregnant male northern pipefish *Syngnathus fuscus*. *Journal of Fish*
982 *Biology*, 88(4), 1614–1619. <https://doi.org/10.1111/jfb.12905>
- 983 Partridge, C., Boettcher, A., & Jones, A. G. (2013). The role of courtship behavior and size in
984 mate preference in the sex-role-reversed Gulf pipefish, *Syngnathus scovelli*. *Ethology*,
985 119(8), 692–701. <https://doi.org/10.1111/eth.12110>
- 986 Péliissié, B., Jarne, P., Sarda, V., & David, P. (2014). Disentangling precopulatory and
987 postcopulatory sexual selection in polyandrous species. *Evolution*, 68.5, 1320–1331.
988 <https://doi.org/10.1111/evo.12353>
- 989 Pischedda, A., & Rice, W. R. (2012). Partitioning sexual selection into its mating success and
990 fertilization success components. *Proceedings of the National Academy of Sciences*,
991 109(6), 2049–2053. <https://doi.org/10.1073/pnas.1110841109>
- 992 Pitcher, T. E., Doucet, S. M., Beausoleil, J. -M. J., & Hanley, D. (2009). Secondary sexual
993 characters and sperm traits in coho salmon *Oncorhynchus kisutch*. *Journal of Fish*
994 *Biology*, 74(7), 1450–1461. <https://doi.org/10.1111/j.1095-8649.2009.02210.x>
- 995 Punzalan, D., & Hosken, D. J. (2010). Sexual dimorphism: Why the sexes are (and are not)
996 different. *Current Biology*, 20(22), R972–R973. <https://doi.org/10.1016/j.cub.2010.09.067>

- 997 Ripley, J. L., & Foran, C. M. (2006a). Differential parental nutrient allocation in two congeneric
998 pipefish species (Syngnathidae: Syngnathus spp.). *Journal of Experimental Biology*,
999 209(6), 1112–1121. <https://doi.org/10.1242/jeb.02119>
- 1000 Ripley, J. L., & Foran, C. M. (2006b). Population structure, growth rates, and seasonal
1001 abundance of two *Syngnathus* pipefish species. *Estuaries and Coasts*, 29(6), 1161–
1002 1171. <https://doi.org/10.1007/BF02781817>
- 1003 Rose, E., Paczolt, K. A., & Jones, A. G. (2013a). The contributions of premating and postmating
1004 selection episodes to total selection in sex-role-reversed Gulf pipefish. *The American*
1005 *Naturalist*, 182(3), 410–420. <https://doi.org/10.1086/671233>
- 1006 Rose, E., Paczolt, K. A., & Jones, A. G. (2013b). The effects of synthetic estrogen exposure on
1007 premating and postmating episodes of selection in sex-role-reversed Gulf pipefish.
1008 *Evolutionary Applications*, 6(8), 1160–1170. <https://doi.org/10.1111/eva.12093>
- 1009 Rosenqvist, G., & Berglund, A. (2011). Sexual signals and mating patterns in Syngnathidae.
1010 *Journal of Fish Biology*, 78(6), 1647–1661.
- 1011 Sagebakken, G., Ahnesjo, I., Mobley, K. B., Braga Goncalves, I., & Kvarnemo, C. (2010).
1012 Brooding fathers, not siblings, take up nutrients from embryos. *Proceedings of the Royal*
1013 *Society B-Biological Sciences*, 277(1683), 971–977.
1014 <https://doi.org/10.1098/rspb.2009.1767>
- 1015 Sandvik, M., Rosenqvist, G., & Berglund, A. (2000). Male and female mate choice affects
1016 offspring quality in a sex-role-reversed pipefish. *Proceedings of the Royal Society of*
1017 *London. Series B: Biological Sciences*, 267(1458), 2151–2155.
1018 <https://doi.org/10.1098/rspb.2000.1262>
- 1019 Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of
1020 image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
- 1021 Shuker, D. M., & Kvarnemo, C. (2021). The definition of sexual selection. *Behavioral Ecology*,
1022 32(5), 781–794. <https://doi.org/10.1093/beheco/arab055>

- 1023 Shuker, D. M., & Kvarnemo, C. (2025). Gametes best discriminate natural and sexual selection:
1024 A comment on Janicke 2024. *Behavioral Ecology*, 36(5), araf089.
1025 <https://doi.org/10.1093/beheco/araf089>
- 1026 Shuster, S. M. (2009). Sexual selection and mating systems. *Proceedings of the National*
1027 *Academy of Sciences*, 106(supplement_1), 10009–10016.
1028 <https://doi.org/10.1073/pnas.0901132106>
- 1029 Simmons, L. W. (2025). Gametic and non-gametic parental resources and the definition of
1030 sexual selection: A comment on Janicke. *Behavioral Ecology*, 36(4), araf065.
1031 <https://doi.org/10.1093/beheco/araf065>
- 1032 Simmons, L. W., & Emlen, D. J. (2006). Evolutionary trade-off between weapons and testes.
1033 *Proceedings of the National Academy of Sciences*, 103(44), 16346–16351.
1034 <https://doi.org/10.1073/pnas.0603474103>
- 1035 Stiller, J., Short, G., Hamilton, H., Saarman, N., Longo, S., Wainwright, P., Rouse, G. W., &
1036 Simison, W. B. (2022). Phylogenomic analysis of Syngnathidae reveals novel
1037 relationships, origins of endemic diversity and variable diversification rates. *BMC*
1038 *Biology*, 20(1), 75. <https://doi.org/10.1186/s12915-022-01271-w>
- 1039 Taylor, M. L., Price, T. A. R., & Wedell, N. (2014). Polyandry in nature: A global analysis. *Trends*
1040 *in Ecology & Evolution*, 29(7), 376–383. <https://doi.org/10.1016/j.tree.2014.04.005>
- 1041 Tosto, N. M., Beasley, E. R., Wong, B. B. M., Mank, J. E., & Flanagan, S. P. (2023). The roles
1042 of sexual selection and sexual conflict in shaping patterns of genome and transcriptome
1043 variation. *Nature Ecology & Evolution*, 7, 981–993. [https://doi.org/10.1038/s41559-023-](https://doi.org/10.1038/s41559-023-02019-7)
1044 [02019-7](https://doi.org/10.1038/s41559-023-02019-7)
- 1045 Tosto, N. M., Rose, E., Mason, H. D., Mank, J. E., & Flanagan, S. P. (2025). Sexual selection
1046 on non-ornamental traits is underpinned by evidence of genetic constraints on sex-
1047 biased expression in dusky pipefish. *Molecular Ecology*, 34(15), e17550.
1048 <https://doi.org/10.1111/mec.17550>

- 1049 Turnell, B. R., & Shaw, K. L. (2015). High opportunity for postcopulatory sexual selection under
1050 field conditions. *Evolution*, 69(8), 2094–2104. <https://doi.org/10.1111/evo.12721>
- 1051 Wade, M. J. (1979). Sexual selection and variance in reproductive success. *The American*
1052 *Naturalist*, 114(5), 742–747. <https://doi.org/10.1086/283520>
- 1053 Weber, W. D., & Fisher, H. S. (2023). Sexual selection drives the coevolution of male and
1054 female reproductive traits in *Peromyscus* mice. *Journal of Evolutionary Biology*, 36(1),
1055 67–81. <https://doi.org/10.1111/jeb.14126>
- 1056 Wiegmann, D. D., & Nguyen, T. (2006). Mating system and demographic constraints on the
1057 opportunity for sexual selection. *Theoretical Population Biology*, 69(1), 34–47.
1058 <https://doi.org/10.1016/j.tpb.2005.08.001>
- 1059 Wilson, A. B., & Orr, J. W. (2011). The evolutionary origins of Syngnathidae: Pipefishes and
1060 seahorses. *Journal of Fish Biology*, 78(6), 1603–1623. [https://doi.org/10.1111/j.1095-](https://doi.org/10.1111/j.1095-8649.2011.02988.x)
1061 [8649.2011.02988.x](https://doi.org/10.1111/j.1095-8649.2011.02988.x)
- 1062 Winkler, L., Eilhardt, R., & Janicke, T. (2023). Population density affects sexual selection in an
1063 insect model. *Functional Ecology*, 37(10), 2734–2747. [https://doi.org/10.1111/1365-](https://doi.org/10.1111/1365-2435.14410)
1064 [2435.14410](https://doi.org/10.1111/1365-2435.14410)
- 1065 Woods, C. M. C., & Martin-Smith, K. M. (2004). Visible implant fluorescent elastomer tagging of
1066 the big-bellied seahorse, *Hippocampus abdominalis*. *Fisheries Research*, 66(2), 363–
1067 371. [https://doi.org/10.1016/S0165-7836\(03\)00183-8](https://doi.org/10.1016/S0165-7836(03)00183-8)
- 1068 Yamane, T., Okada, K., Nakayama, S., & Miyatake, T. (2010). Dispersal and ejaculatory
1069 strategies associated with exaggeration of weapon in an armed beetle. *Proceedings of*
1070 *the Royal Society B: Biological Sciences*, 277(1688), 1705–1710.
1071 <https://doi.org/10.1098/rspb.2009.2017>
- 1072

FIGURE CAPTIONS AND ALT TEXT

Figure 5. Description of the three *Syngnathus* spp. and the corresponding collection locations.

(A) Shown here is the phylogenetic relationship between the three species of *Syngnathus* (Adapted from Hamilton et al., 2017 and Stiller et al., 2022) and examples of female (top) and male (bottom) pipefish from each species. The images are scaled to represent accurate proportional differences between both sexes and species using averages for standard length (SL), SVL, torso depth (TD), and pouch length. Traits that differed significantly between females and males are listed in parentheses following the species name. (B) *S. floridae* were collected in three locations, including the Saint Joseph Sound (28.0516324, -82.7921436), the southern region of Old Tampa Bay (27.8730210, -82.5397645), and off Fort De Soto (27.6273507, -82.7002240). (C) *S. fuscus* were collected off the coast of Cherrystone Family Camping Resort in Cape Charles, VA (37.3927330, -76.0139170). (D) *S. scovelli* were collected off Aransas Pass in Port Aransas, TX (27.8941861111, -97.131025).

Alt text: In A, a phylogenetic tree shows *Syngnathus scovelli* and *S. fuscus* as most closely related, with *S. floridae* as a close outgroup. Drawings of male and female individuals of each species are pictured, scaled by actual size – showing *S. floridae* is the largest and *S. scovelli* is the smallest species. The image also shows that *S. floridae* has a sex difference in SVL, *S. fuscus* shows sexual dimorphism in standard length and SVL, and *S. scovelli* is sexually dimorphic in standard length, SVL, and torso depth. Panel B shows a map with the collection location of *S. floridae* in Tampa Bay, C shows the collection location of *S. fuscus* in the Chesapeake Bay, and D shows the collection location of *S. scovelli* in the Gulf of Mexico on the Texas coastline.

Figure 6. Females with increased genetic mating success had more developed eggs in all three species, but multiply mated females had a lower proportion of eggs developed in *S. fuscus* and *S. scovelli*. (A) Bateman gradients were similar for female *S. floridae* (blue), *S. fuscus* (red), and

S. scovelli (yellow). Relative fitness was calculated as the individual's fitness level (either total number of developed eggs or number of mates) divided by the average fitness across all individuals within each trial. Bateman gradients are derived from a linear mixed model with trial number as a random effect for each species. The slopes for all three species were significantly greater than zero, however there was little difference between the species. (B) Comparing the proportion of eggs developed in singly (S) mated (light grey) versus multiply (M) mated (dark grey) females shows slightly lower embryo survival rates for *S. fuscus* and *S. scovelli* females who mated more than once.

Alt text: In A, the x-axis is "Relative Mating Success", ranging from 0 to 7, and the y-axis is "Relative reproductive success" ranging from 0 to 7. In blue are points for *S. floridae*, ranging from 0 to 5 on the x-axis and 0 to 5.5 on the y-axis. The line for *S. floridae* is labelled as having a slope of 0.963. In red are points for *S. fuscus*, ranging from 0 to 7 on both axes. The line for *S. fuscus* is labelled as having a slope of 1.006. In yellow are points for *S. scovelli*, which range from 0 to 5 on both axes. The line for *S. scovelli* is labelled as having a slope of 0.954. In B, the x-axis is labelled with the three species names and "S" or "M" for single or multiple matings. The y-axis shows the "Proportion Eggs Developed", ranging from 0 to 1. The boxplot for single-mated *S. floridae* has a median around 0.9 and the interquartile range (IQR) from about 0.75 to 0.95, with raw points mostly in the box and whiskers (from 0.5 to 1), with one outlier at 0.1. The multiply mated *S. floridae* box has a median around 0.95 and an IQR from 0.85 to 0.95. The raw data points are mostly on the box and whiskers (which extend to 0.8 and 1) but one point is at 0.4. The single mated *S. fuscus* box has a median around 0.95 and an IQR from about 0.9 to 0.97 and most raw data points are within the box and whiskers (from 0.9 to 1). Two points are below, between 0.8 and 0.9. The multiple mated *S. fuscus* box has a median of 0.8 and an IQR of 0.7 to 0.85, with only two raw data points at each of those edges. The single mated *S. scovelli* box has a median close to 1, with the IQR from about 0.95 to 1. Only two points fall

below the bottom whisker (near 0.9), and both are above 0.85. The multiple mated *S. scovelli* box has a median around 0.9 with an IQR from about 0.8 to 0.95 and whiskers from about 0.6 to 1. The raw data points are all within the range of the whiskers.

Figure 3. The partitioning of the opportunity for selection across the three species of *Syngnathus* by selection episode. (A) We calculated the partitioning for each trial individually, shown here is the average value for each selection episode across all trials for males (purple) and females (green) in *S. floridae* (circle), *S. fuscus* (triangle), and *S. scovelli* (diamond). Average values represented here were derived from bootstrapping. Error bars around the point represent percentile based 95% confidence intervals derived from bootstrapping (actual means and standard deviations are in Table S4). (B) The percentage of the total opportunity for selection (I) that can be attributed to each selection episode for males versus females in the three species of pipefish. Note that the contributions of each decomposed episode of I do not sum to 100% because the covariances are not shown; please see Table 3 for the complete decomposition.

Alt text: In A, the x-axis is labelled “Episode of selection”, with three separated x-axes with repeated labels of “ I ”, “ I_1 ”, “ I_{12} ”, “ I_2 ”, and “ I_3 ”. The y-axis is labelled “Average fitness value” and ranges from 0 to 5. The three separated x-axes have labels “*S. floridae*”, “*S. fuscus*”, and “*S. scovelli*”. For each of the x-axis labels, there is a Male and a Female point for each species, with confidence intervals extending above and below each point. The estimates and confidence intervals can be found in Table 3. In B, the x-axis is labelled “Sex” and has three separate axes, each with “Female” and “Male”, with the separate axes labelled “*S. floridae*”, “*S. fuscus*”, and “*S. scovelli*”. The y-axis reads “Percent” and goes from 0 to 100. Each sex and species has stacked bar charts showing the percent of I that comes from I_1 , I_2 , and I_3 (shown in yellow, blue, and red, respectively). The precise percentages are found in Table 3. The stacked bar plot show the following percentages: Female *S. floridae* - I_1 = 87%, I_2 = 4%, I_3 = 1.5%; male *S. floridae* - I_1 = 82%, I_2 = 8%, I_3 = 1.24; Female *S. fuscus* - I_1 = 97%, I_2 = 1%, I_3 = 0%; male *S. fuscus* - I_1 = 96%,

1148 $l_2 = 2\%$, $l_3 = 0\%$; female *S. scovelli* – $l_1 = 99\%$, $l_2 = 3\%$, $l_3 = 0\%$; male *S. scovelli* – $l_1 = 49\%$, $l_2 =$
 1149 24% , $l_3 = 18\%$.

1150 **Figure 4.** The decomposition of standardized selection differentials for males (purple) and
 1151 females (green) from three species of pipefish, *S. floridae* (circle), *S. fuscus* (triangle), and *S.*
 1152 *scovelli* (diamond). Because the decompositions were performed separately for each trial, the
 1153 points here represent the average selection differential for that sex across all trials within a
 1154 species. Error bars surrounding the points show the percentile based 95% confidence intervals.
 1155 Both the averages and confidence intervals were derived from bootstrapping. Total selection
 1156 differential for snout-vent length (s') was decomposed to show contributions of mating success
 1157 (s'_1), the number of eggs stored per mate (s'_2), and proportion of eggs developed (s'_3).

1158 **Alt text:** The x-axis is “Standardized selection differential”, with three separate axes, each with
 1159 four labels: s'_1 , s'_2 , s'_3 , and s' . The three axes are labelled *S. floridae*, *S. fuscus*, and *S. scovelli*.
 1160 The y-axis is labelled “Average value” and ranges from -1 to 1, with a dashed line at 0. Each of
 1161 the x-axis ticks has two corresponding points, one for male and one for female values, each with
 1162 a confidence interval extending above and below it. The precise values and confidence intervals
 1163 are in Table 4. The estimates whose confidence intervals do not cross the zero line are: *S. floridae*
 1164 male s'_1 , which is -0.46; *S. floridae* male s'_2 , which is 0.33; *S. floridae* male s' , which is -0.18; *S.*
 1165 *fuscus* female s'_1 , which is 0.43; *S. fuscus* female s'_2 , which is -0.33, *S. fuscus* female s' , which is
 1166 0.10; *S. scovelli* female s'_1 , which is 0.3.

1167