Investigating Parental Investment and Reproductive Behavior in Northern Pipefish (*Syngnathus fuscus*)

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Introduction

- Northern pipefish belong to the family Syngnathidae.
- Syngnathids are unique in that males are equipped with a brood pouch, allowing for male pregnancy.
- During reproduction, females transfer eggs to male brood pouch.
- Previous research[1] shows males provide nutrients to embryos in the brood pouch, but investment is not confidently quantified.
- Some species of pipefish mate monogamously in the wild potentially due to ecological constraints[2].

Fig. 1 Sexually mature *S. fuscus* female.

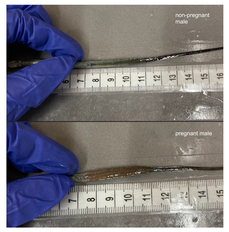


Fig. 2 Comparison between brood pouch in non-pregnant (top) and pregnant (bottom) *S. fuscus* male.

Methods

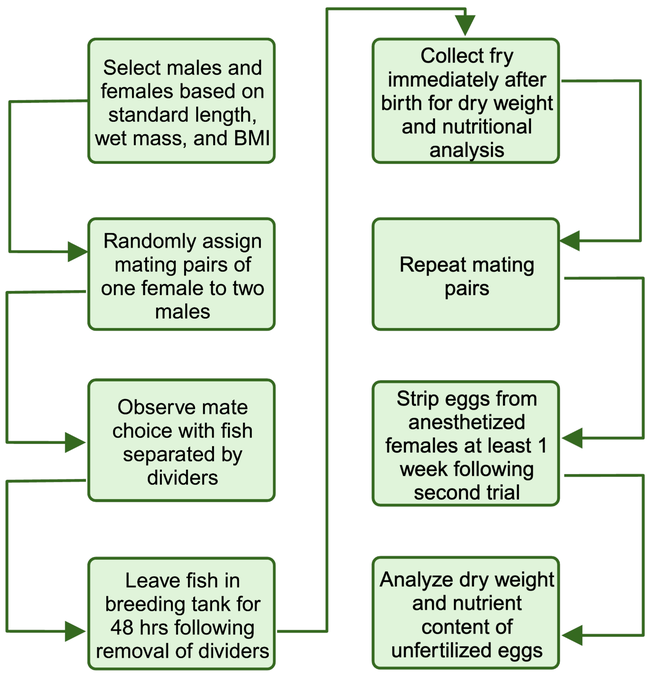


Fig. 3 (A) Fry separated for analysis. **(B)** Zoomed in several week-old fry.

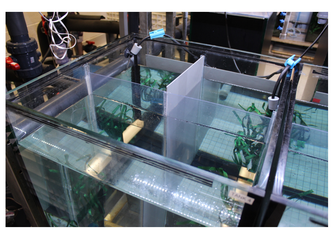
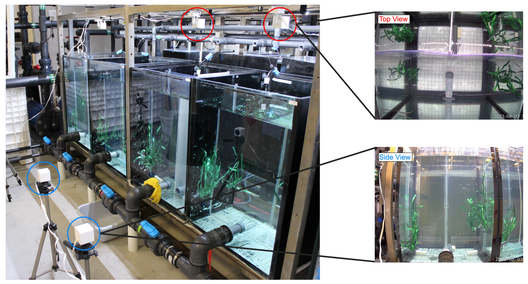


Fig. 4 Mate choice tank set-up with grey divider separating males and clear divider separating female from males.

Results

A



B

Mate Preference in *S. fuscus*

Mating Pair	Time Spent with Smaller Male (s)	Time Spent with Larger Male (s)	Preference	Results
1	1318.811	135.902	Smaller male	Both males pregnant
2	503.865	164.295	Smaller male	Smaller male pregnant
3	255.718	1568.898	Larger male	Smaller male pregnant
4	88.848	1446.805	Larger male	Larger male pregnant
5	542.423	608.159	Larger male*	Both males pregnant
6	110.459	351.618	Larger male*	No pregnancy
7	144.941	335.475	Larger male	Both males pregnant

*preference potentially influenced by neighboring tank interactions or males being on same side at certain point

Fig. 6 (A) Camera view of tank from two angles to observe reproductive behavior. **(B)** Table displaying results from mate choice experiments. Preference is determined by total time in seconds that the female spends in a particular zone and shows courtship display. Mate choice experiments lasted a total of 3,600 seconds (1 hr).

Preliminary Analyses: -80C Storage Provides Results Comparable to Fresh Samples

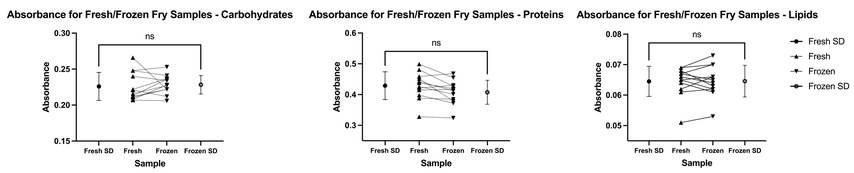


Fig. 7 Standard deviation for the absorbance of fresh and frozen fry samples analyzed for carbohydrates, proteins, and lipids is not significantly different.

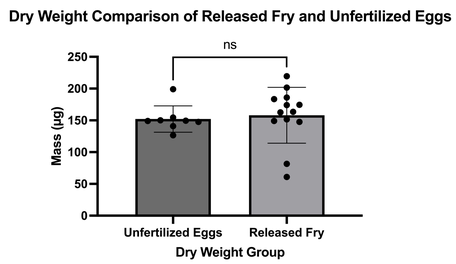


Fig. 8 No significant difference between the dry weights of unfertilized eggs and released fry.

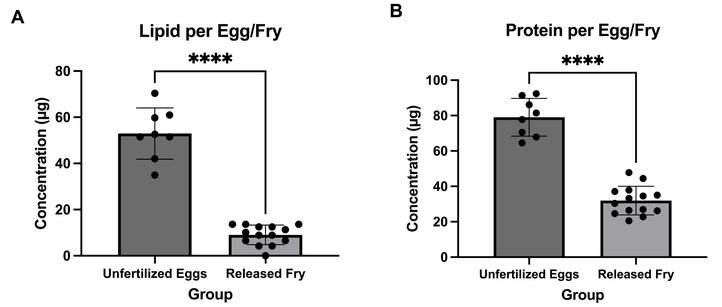


Fig. 9 (A, B, C) Significant difference in concentrations of lipid, protein, and carbohydrate between unfertilized eggs and released fry. **(D)** 96-well plate with samples for protein analysis.

Discussion + Future Directions

- There is no clear trend between preference and successful matings.
- Mating multiply is possible, which is consistent with previous research[3].
- Frozen samples can be used for analysis- fresh samples are not required for accuracy.
- There are no significant differences between dry weight for eggs and fry.
- Unfertilized eggs are significantly more nutrient-rich for lipids, proteins, and carbohydrates than released fry.

- We will continue the second trial of mating experiments and look at individual mating events to identify specific courtship behaviors and their duration.
- Looking for changes in nutrient concentrations in released fry from the second trial can reveal more about energy investment and potentially post-copulatory sexual selection[4].
- Pairwise comparisons of nutrient composition of eggs and fry within the same mating pairs will allow the identification of individual-level variation in mating investment.

Acknowledgements

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