



Mating Preference and Parental Investment in Northern Pipefish (*Syngnathus fuscus*)



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Introduction

- Northern pipefish (*Syngnathus fuscus*) are part of the Syngnathidae family which exhibit male pregnancy. Males have a brood pouch into which females transfer eggs.
- The male brood pouch is thought to act as additional nutrient provisioning after egg reserves are diminished.¹
- Previous research suggests that male energetic investment is high in this species², but prior BUÉE student research found that male investment was negligible.
- Size-assortative mating was not found in two other pipefish species³. Mating preference based on size has not been studied in *Syngnathus fuscus*.

Our aim is to analyze parental investment by studying nutrient content of unfertilized eggs and released fry. In addition, we are examining size-assortative mating preference through behavioral trials. These questions can help shed light on the evolutionary advantage of male pregnancy in syngnathids.

Methods

Behavioral Trials

One female and two males were placed in each three-chambered experimental tank. One male was at least 2 cm larger than the other.

Biochemical Analysis

Pregnancy lasted approximately 2.5 weeks. Fry were collected after birth and euthanized. Eggs were stripped from the female.

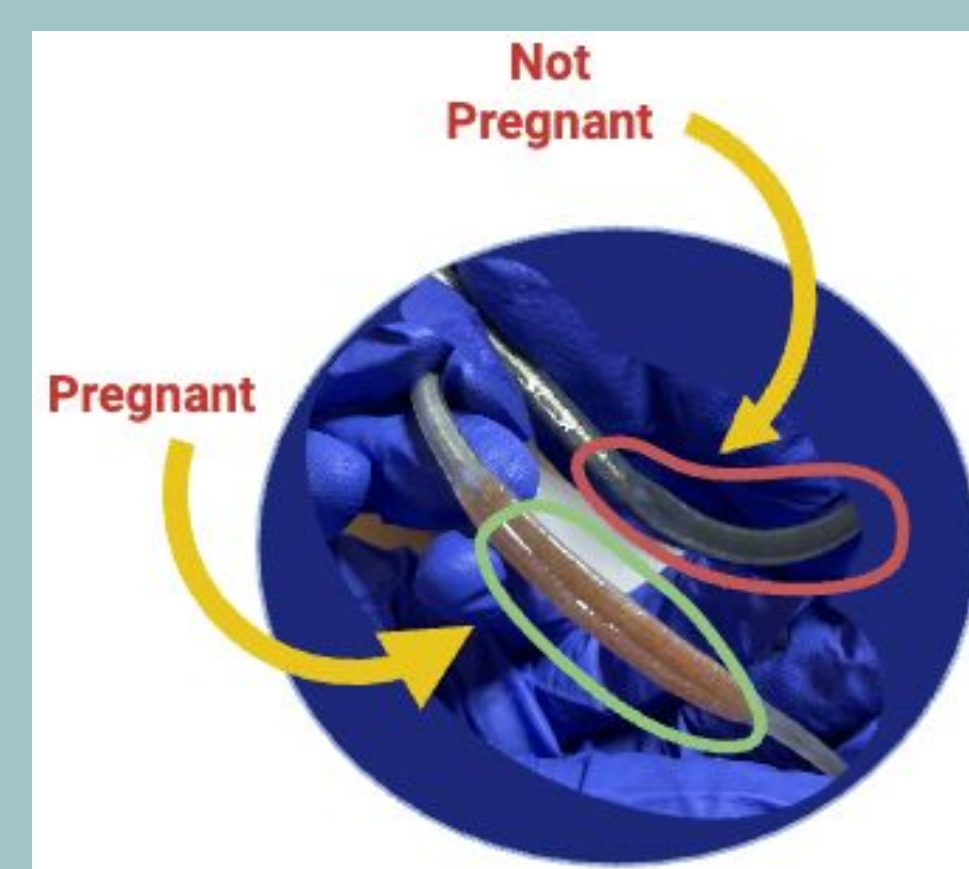


Fig. 2: Difference between brood pouches of pregnant and non-pregnant males

Samples were stored in a freezer at -80C. Nutrient analyses were conducted for each successful mating.

Nutrient analyses were also conducted on the fry of five males that mated in the wild. The fresh analyses were conducted on the day of birth. Aliquots were stored at -80C for approximately 8 days, after which the nutrient analyses were repeated.



Fig. 1: Top-down camera view of experimental tank

The preference trial occurred from 9:30-10:30 AM on day 1 of the three-day trial. Then dividers were removed to allow for mating. Each tank was filmed from two angles to capture video.

Results

Behavior - Mate Preference

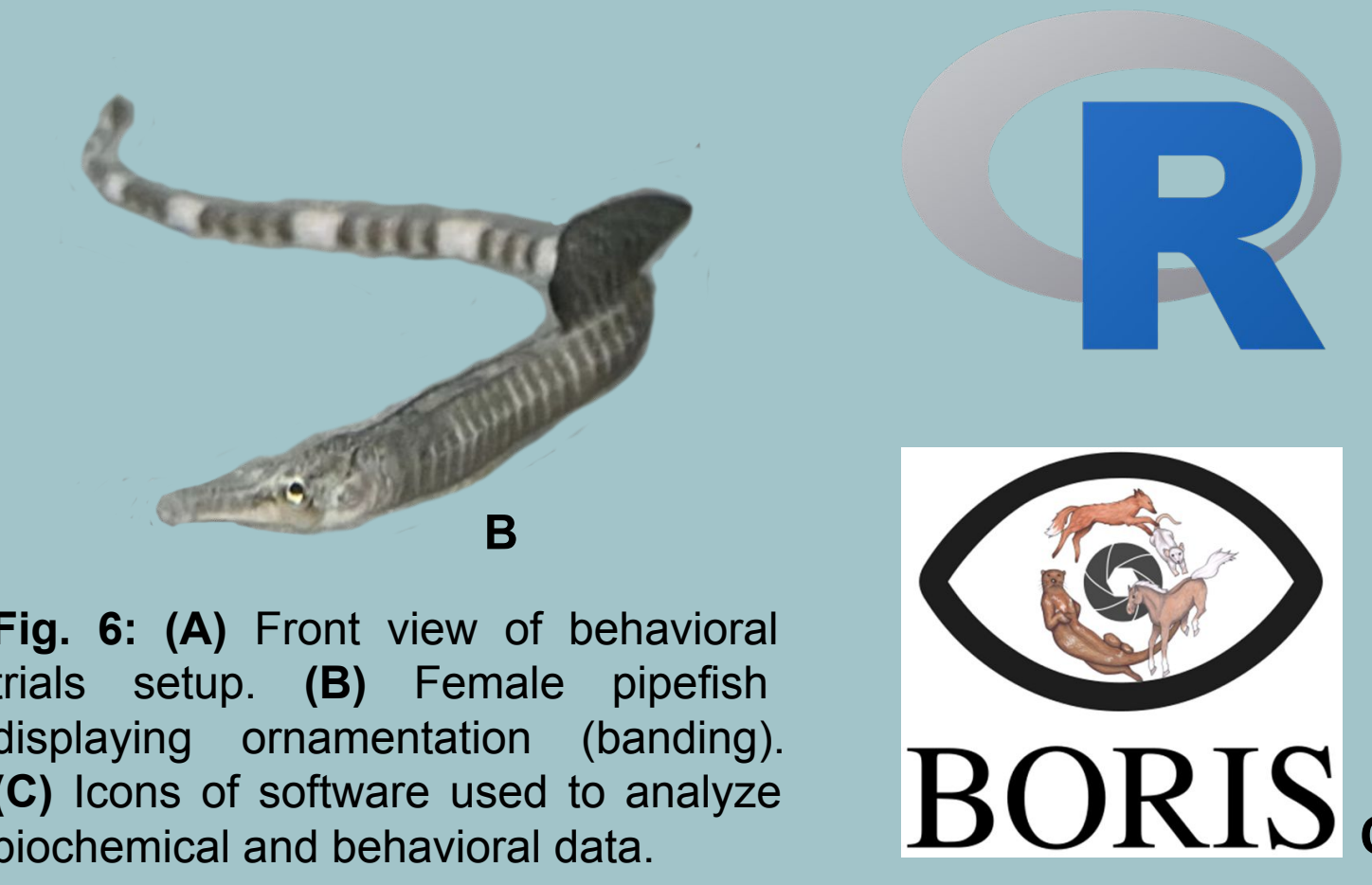


Fig. 6: (A) Front view of behavioral trials setup. (B) Female pipefish displaying ornamentation (banding). (C) Icons of software used to analyze biochemical and behavioral data.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.597	0.745	0.897	0.043	0.891	0.72	0.711	0.043	0.574	0.538	0.517	0.043
B	0.637	0.643	0.647	0.042	0.042	0.043	0.043	0.043	0.043	0.043	0.043	0.043
C	0.46	0.473	0.491	0.042	1.053	0.97	0.846	0.043	0.472	0.466	0.443	0.043
D	0.366	0.399	0.392	0.042	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043
E	0.355	0.334	0.332	0.043	0.939	0.7	0.69	0.043	0.598	0.514	0.548	0.042
F	0.307	0.307	0.312	0.043	0.043	0.043	0.043	0.043	0.043	0.042	0.043	0.042
G	0.277	0.29	0.298	0.043	0.77	0.743	0.648	0.043	0.502	0.508	0.463	0.043
H	0.286	0.289	0.295	0.043	0.043	0.043	0.043	0.043	0.042	0.043	0.042	0.043

Successful mating is defined as a mating that resulted in pregnancy and pregnancy that resulted in ample fry for analysis. Due to extensive mortality among our mating pairs, we had two successful matings.

Fig. 3: (A) Example of well plate absorbance readings. (B) Images of well plates used to read nutrient contents.

Biochemical Analysis

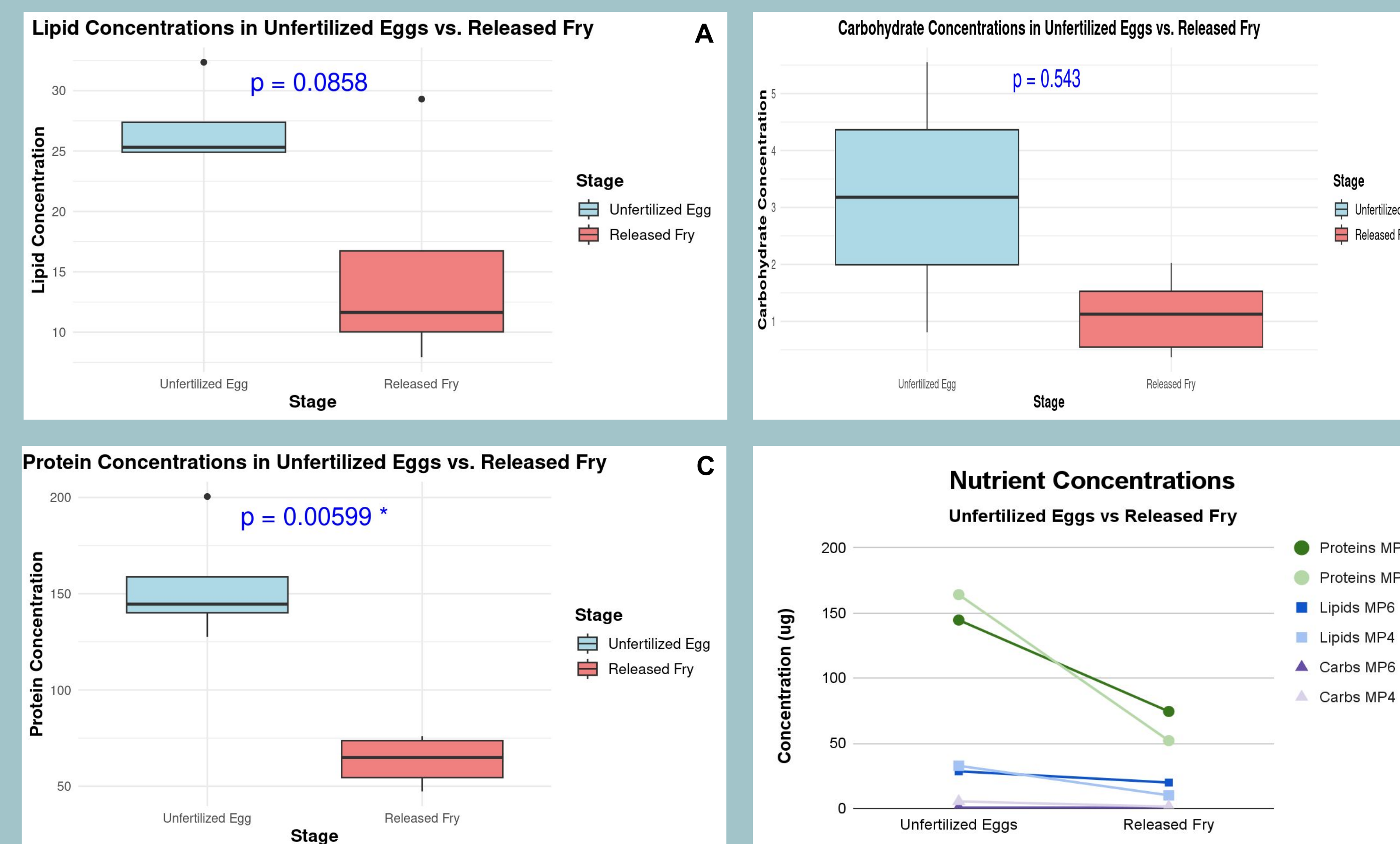


Fig. 4: (A) Average lipid content (ug) in unfertilized eggs and released fry. (B) Average carbohydrate content (ug) in unfertilized eggs and released fry. (C) Average protein content (ug) in unfertilized eggs and released fry. (D) Nutrient content within each mating pair (MP).

Fresh vs. Frozen Nutrient Concentration

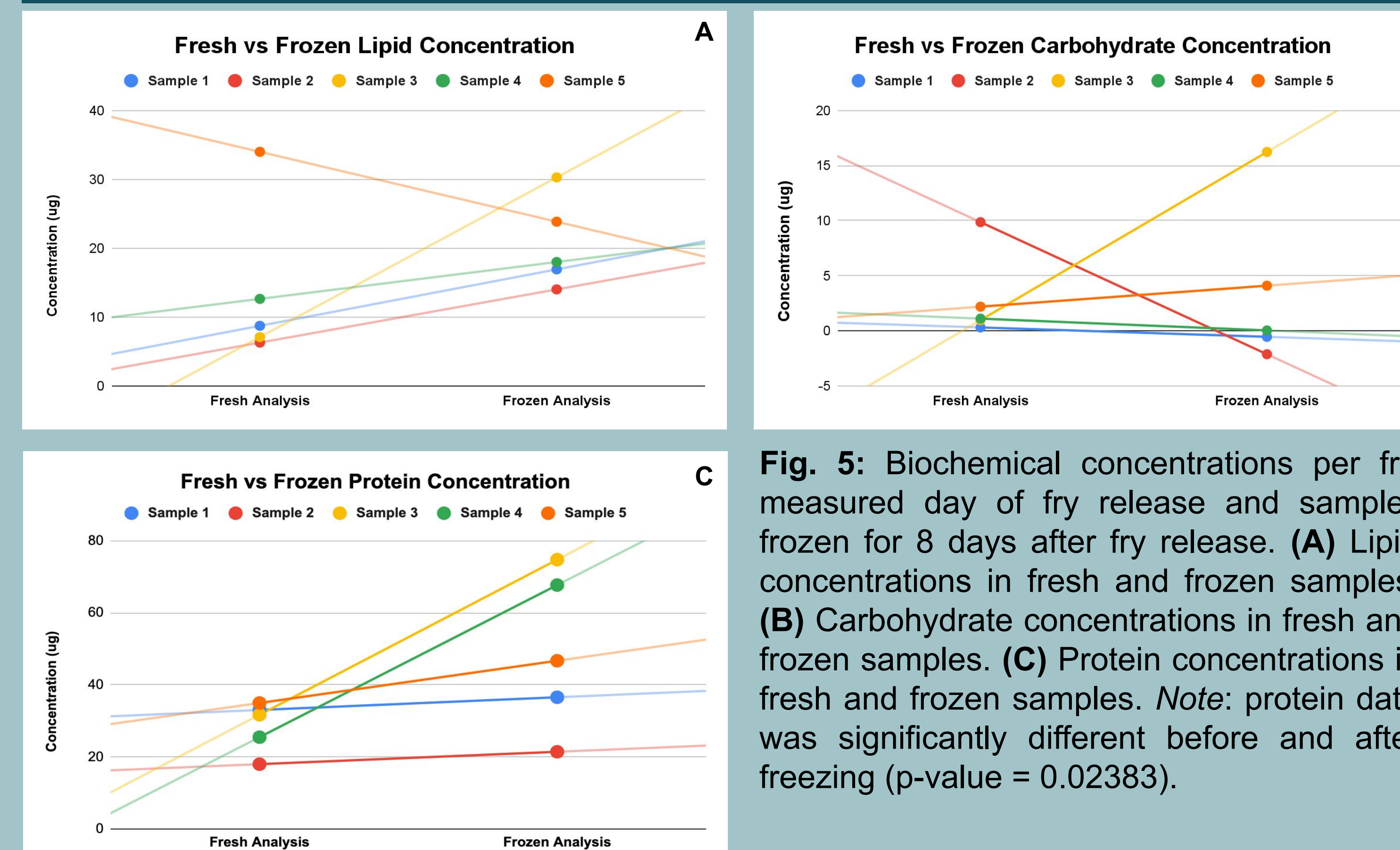


Fig. 5: Biochemical concentrations per fry measured day of fry release and samples frozen for 8 days after fry release. (A) Lipid concentrations in fresh and frozen samples. (B) Carbohydrate concentrations in fresh and frozen samples. (C) Protein concentrations in fresh and frozen samples. Note: protein data was significantly different before and after freezing (p-value = 0.02383).

Mating Pair	Percent time spent with smaller male (%)	Percent time spent with larger male (%)	Preference	Pregnancy
1	3.1	19.7	Larger male	Neither
6	2.3	31.5	Larger male	Both
7	19.4	7.4	Smaller male	Neither
8	6.8	37.5	Larger male	Neither
9	0	0	Neither	Neither
10	47.9	0	Smaller male	Neither

Mating Pair	Percent of visits spent with smaller male (%)	Percent of visits spent with larger male (%)	Preference	Pregnancy
1	2.1	21.7	Larger male	Neither
6	4.4	30.8	Larger male	Both
7	33.3	14.6	Smaller male	Neither
8	15.2	31.3	Larger male	Neither
9	0	0	Neither	Neither
10	43.9	0	Smaller male	Neither

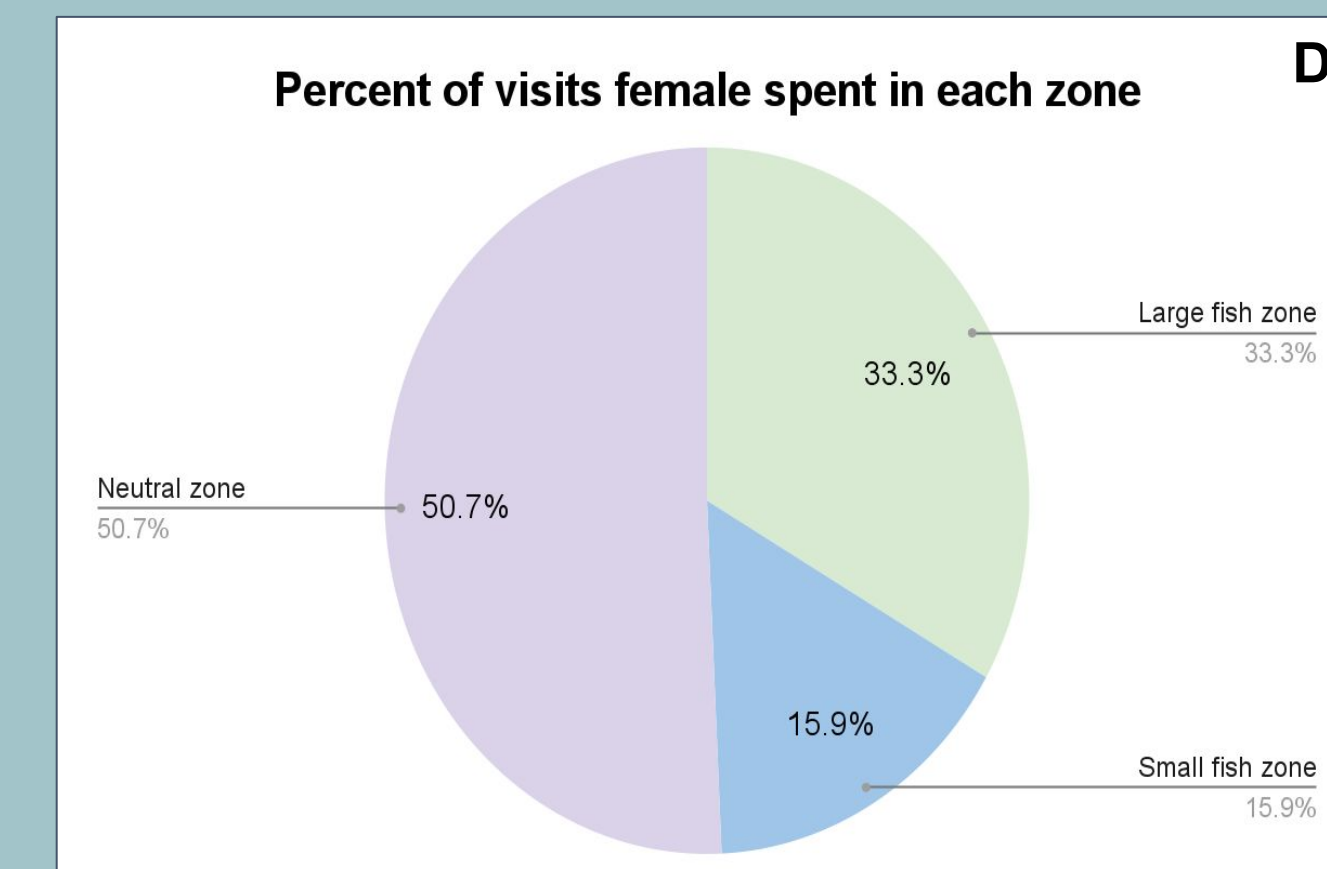
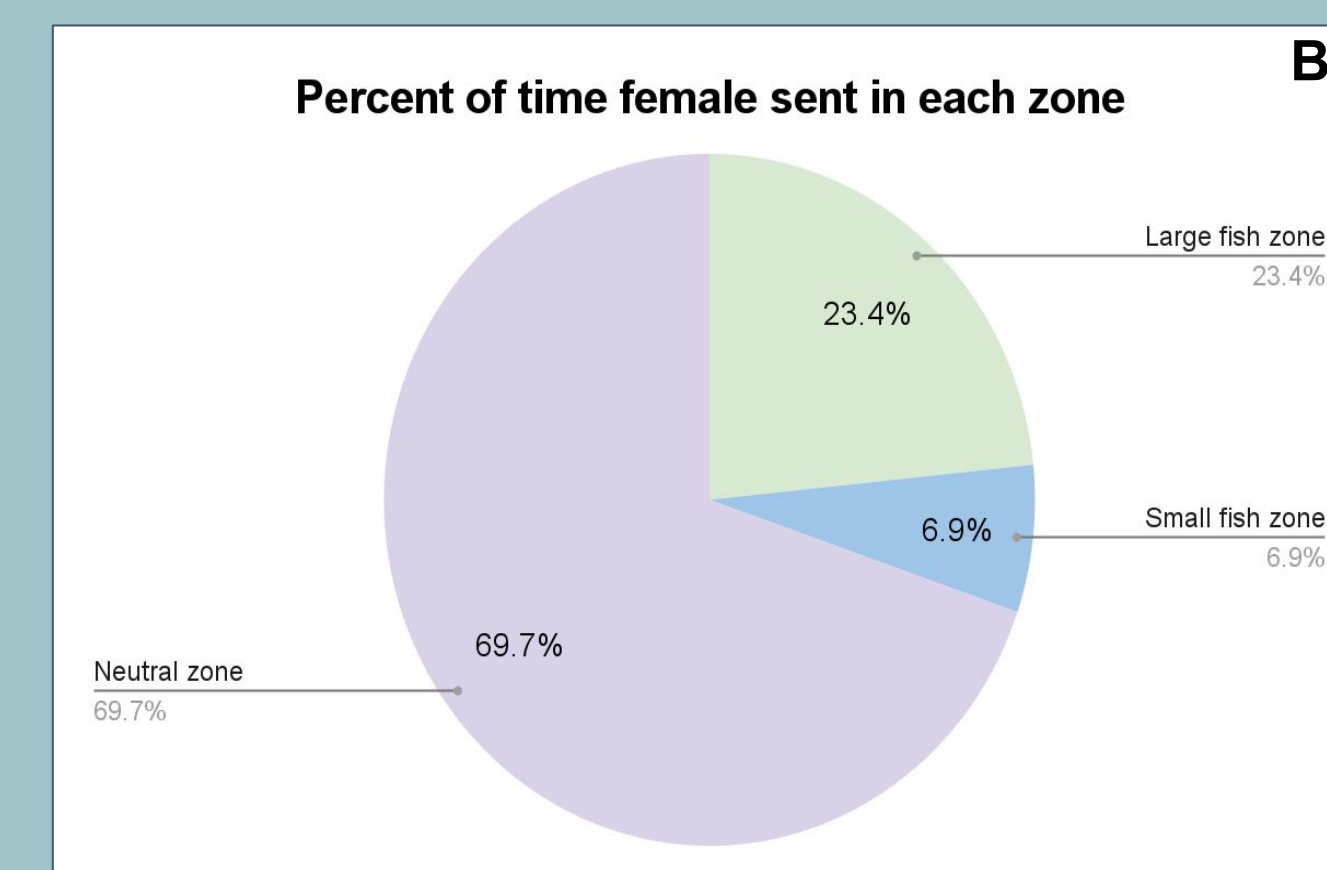


Fig. 7: Behavioral analysis of female focal fish. (A) Percent of time each focal female spent with either sized male. (B) Average percent of time spent with either sized male. (C) Percent of visits by focal female to either sized male. (D) Average percent of visits to either sized male.

Discussion

While sample size is small due to extensive mortality, unfertilized eggs contained higher average lipid, carbohydrate, and protein concentrations than released fry. This data aligns with past BUÉE research findings, suggesting that male nutrient investment is negligible in *S. fuscus*. Proteins were the most abundant nutrient in both unfertilized eggs and released fry and represent a significant difference in protein concentrations across stages. Significant data could be extrapolated through additional protein-specific exploration.

Fresh vs. frozen results revealed slight increases in lipid and carbohydrate content but significant increases in protein content. Additional trials would allow for greater accuracy in determining significance.

Focal female preference did not correlate to successful mating, suggesting that size-assortative mating is negligible in *S. fuscus*. Females spent the most time in the neutral zone in every behavioral trial. However, more time was spent in the zone of the larger male than that of the smaller male when applicable. Because only two pairs were successful, more data is needed to confirm these conclusions.

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