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Litter, Color Variation, and Sex Effects on Feeding and Anti-Predator Behavior in Individual Thamnophis sirtalis

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Litter, Color Variation, and Sex Effects on Feeding and Anti-Predator Behavior in Individual *Thamnophis sirtalis*

Jennifer F. Porter and Gordon M. Burghardt

Abstract

There are stark coloration differences within single populations of *Thamnophis sirtalis* such as the Isle Royale population. While these red color patterns cause the snakes to stand out, it is unsure if it is for some defensive purpose such as aposematic coloring. To see if this or other genetic factors, sex, and relatedness with litters, could influence behavior anti-predator and feeding tests were performed on 38 captive-born neonate T. sirtalis. The feeding experiment recorded the latency of feeding from placement of the piece of night crawler to food capture. The anti-predator experiment recorded reactions to a probe touching each snake 20 times with three seconds in between. The feeding results indicated that there is a connection between that behavior and litter but not coloration or sex. This held true for the anti-predator behaviors of directional reversals and freezing but none of the groups showed any connection to total active anti-predator behaviors. There were also no correlations between the anti-predator and feeding behaviors. Litter seems to be the strongest indicator of behavior.

Introduction

The shy-bold continuum is a relatively simple metric of personality that has been studied across taxa. Fish are quite popular but other studied taxa include birds, squamates, felines, and humans (Wilson et al., 1993; Riesch et al., 2009; Thompson et al., 2012; Carere & van Ores, 2004;

Lopez et al., 2005; Lowe & Bradshaw, 2001; Kagan, 1997). All of these animals express behaviors that can be used to place individuals somewhere on this continuum to provide an indication of personality. This variation could be due to a number of factors including genetic. A genetic cause would allow a variation of expression from a young age and without specific environmental cues. Especially in species with limited to no parental care, these innate behaviors can provide survival skills immediately or soon after birth.

Snakes, generally, do not provide post-natal parental care and *Thamnophis sirtalis sirtalis* is no exception. *T. sirtalis* gives live birth to litters of multiple individuals which are then left to forage and defend themselves against a myriad of predators such as birds, other snakes, raccoons, and almost any carnivorous animal larger than the snake itself. Anti-predator as well as foraging behaviors would be largely innate at this stage.

Color variation is present within populations of *T. sirtalis* including Isle Royale from which the subjects' mothers were captured (Mooi et al. 2011). In the Isle Royale, MI, population this ranges from a dusty brown with black markings to many shades of red instead of brown all the way up to melanistic animals. Unpublished observations note that there may be a link between red coloration and aggressiveness (Brown et al., unpublished observation). Many anti-predator studies have focused on development of anti-predator responses in the *Thamnophis* genus and while sex has sometimes been used to examine possible correlations to behavior, color pattern and litter have been absent in anti-predator analysis (Herzog & Burghardt 1986, Herzog et al. 1989, Herzog et al. 1992, Bowers et al., 1993).

Although previous studies such as Krause and Burghardt 2001 indicate that feeding latency is most strongly correlated with type of prey, there may be a genetic correlation as well. When studying feeding behavior of *T. sirtalis*, litter had a stronger effect on growth than diet (Lyman-Henley, 1995).

This experiment attempts to link this variation of feeding and anti-predator behavior along the shy-bold continuum in neonate *T. sirtalis* to a genetic factor such as something linked to a phenotypic expression (red score or sex) or a more immediate genetic influence (relatedness as measured by litter).

Methods

Thirty-eight subjects were selected from the 83 neonate *T. sirtalis* born August, 2011 in the lab to 16 females captured on Isle Royale, Michigan while gravid. The individuals selected were good feeders which is defined by the following criteria: the individual was able to survive hibernation, and had fewer than three feedings in which the individual did not eat the piece of night crawler. Litters with fewer than three individuals that met criteria were then eliminated. The remaining subjects were balanced for a roughly equal number of males and females as well individuals with red scores of 0 and 2, resulting in 21 females and 18 males, of which 23 are red score 2 and 16 red score 0, and individuals from litters IR-102, IR-104, IR-109, IR-16, IR-17, IR-91, and IR-98, all of which can be seen in Table 1. Red scores were determined using a numbered coding system outlined in Mooi et al. (2011). Examples of animals in this experiment that were scored 0 and 2 can be seen in Figure 1.

The testing apparatus used for both feeding and anti-predator tests was a glass box measuring 52 by 61 by 24 cm in which Astroturf was used to line the enclosure. A camera, Sony model HDR-XR160 was mounted on a stand about a one meter above this box and was used to video tape both the anti-predator and feeding experiments.

Anti-predator Testing

The method was based off of Mori and Burghardt's anti-predator experiments with *Rhabdophis tigrinus* (2000). Between a week and ten days after birth, each neonate was placed in the testing box in a room which was 24 degrees Celsius and allowed to acclimate to the environment for

60 seconds. After this time, the snake was touched with a probe, a large pair of forceps connected and padded with duct tape on the end, on the tail, body, and neck for less than a second every three seconds for a full minute or at least 20 touches. Behavior of each touch was recorded so scores of each behavior could range 0 to 20. Table 2 is an ethogram of each behavior observed.

Feeding Testing

Up to six individuals were placed in the testing apparatus with cardboard dividers between them to reduce visual distraction during the experiment. The neonates are kept in their enclosures which are transparent gel boxes measuring 13 by 18 by 5 cm. These boxes are lined with commercial tan-colored cage liners and contain a small plastic water dish and a shelter is made of a piece of the liner paper measuring 5.5 by 13 cm that is folded down the middle to create a tent-like shelter for the neonate. The room was kept between 23 and 25 degrees Celsius.

The subjects were allowed at least 60 seconds to acclimate to their environment before the camera was turned on. At this time, the food was loaded into each cage, one dish at a time. The food was a piece of night crawler, about one cm, on a small petri dish. After each snake had a dish of food placed in its cage, the camera recorded for at least 600 seconds after the last dish was placed.

Analysis

The videos were converted from AVIHD to WMV using a converter program for size and the ability to play on multiple computers due to format. These videos were then watched and scored using Noldus Observer XT. For the feeding experiment, the moment when the dish was placed, the snake left the shelter, if it started in there, approached the edge of the dish so that its head was over the lip, approached the piece of worm and not just moving near it, and when the snake bit onto the piece of night crawler. For the defensive testing, each behavior was coded and marked when occurred. The behaviors were tail over the probe, tail thrashing, tail wagging, whole body thrashing, striking, directional change, and freezing as described in Table 2.

The latency of feeding from dish placement to food capture of the four trials was averaged for each subject. For the defensive tests, all behaviors except freezing and direction reversal were classified as "active behaviors". A three-way ANOVA was performed using the statistical software JMP 9.

Results

The latency of feeding from dish placement to food capture from the feeding experiment and the frequency of active behaviors, directional changes, and freezes from the anti-predator experiment were all recorded using the Observer software. This data can be found in Table 1 below.

Feeding

For each group of variables (litter, red score, sex), the average latency for each subject was averaged together to get the value for the group. A one-way ANOVA was run and the results displayed in a graph which included the error bars and the p-value. Figures 2, 3, and 4 display the results for the feeding trials when all four trials are included. A one-way ANOVA does not control for the effects between variables but these figures provide the averages of behaviors within groups.

The p-values for the feeding experiment obtained from a three-way ANOVA indicated that sex ($p=0.2359$) and red score ($p=0.2363$) likely have no bearing on this behavior, but the p-value for litter ($p=0.0785$) indicates that there may be a connection of this genetic relatedness to behavior.

Anti-predator

For each group of variables (litter, red score, sex), the average frequency for each set of behaviors for each subject was averaged together to the value for that group. A one-way ANOVA was performed using these values and the results displayed in a graph which includes the error bars and the p-value. Figures 5, 6, and 7 display the frequency of active behaviors. Figures 8, 9, and 10display the frequency of directional changes. Figures 11, 12, and 13 display the frequency of freezes. A one-way ANOVA does not control for the effects between variables but these figures provide the averages of behaviors within groups.

The p-values for the anti-predator experiment obtained from a three-way ANOVA indicated that there was no link with active anti-predator behavior frequency with any group in the three variables. This was also the case with frequency of directional reversals and freezing behaviors in red score ($p=0.2322$ and $p=0.4333$) and sex ($p=0.8481$ and $p=0.8883$). Litter, however, demonstrated a possible link as the p-values were less than 0.10 with $p=0.0721$ for frequency of directional reversals and p=0.0.646 for frequency of freezing behaviors.

Discussion

The three-way ANOVA results indicated no statistically significant link between litter, red score, or sex and any of the results of the behaviors from the feeding and anti-predator experiments. There were, however, lower p-values ($0.05<p<0.10$) for litter in feeding latency as well frequency of directional reversals and freezing behaviors. This would lead to the suggestion that litter is a likely indicator of behavior: littermates will act alike.

There was also a very low correlation coefficient (< 0.10) between feeding latency and any anti-predator behavior indicating that there is no correlation between these behaviors either positive or negative as seen in Figure 14.

As litter is the only genetic factor examined that had a significant correlation with behavior, this would suggest a diversity of feeding and anti-predatory behavior in this population and possibly others. It also would suggest that these litters' feeding and anti-predator behavior are completely separate traits on the shy-bold continuum and this measure can only be used when looking at a singular behavioral trait.

It is possible that coloration and sex can have an influence on behavior but not at this early stage in life or without certain environmental triggers. The subjects were neonates and as the anti-predator experiment occurred in the first two weeks of birth and the feeding experiment within six and seven months of birth, there may be some plasticity as the snakes age. Although Herzog and Burghardt's anti-predator experiment (1986) demonstrated that striking behavior was similar between neonates and adults of *T. sirtalis*, this was using different sets of subjects instead of possibly tracking changes with the same snakes over time. Also, Brown et al. (unpublished observations, 2012) cites a possibility of snakes with a higher red score, 2 and above, as being more aggressive, but these observations were from field observations and did not indicate the age of the possibly aggressive snakes. With these subjects, the added factors of environment and age could have an impact on this differentiation of behavior.

Increasing the number of subjects would provide a more accurate examination of these possible behavioral links. There was an abundance of females with red score 2 which may have skewed some of the results. The larger sample size might reduce this effect.

With the indication of a behavioral link within litters, another study that could further investigate this effect would be testing the mothers of the neonates in the same manner. A correlation of mother's behavior with neonates' behavior would indicate a stronger link in a genetic basis of behavior.

Acknowledgements

I would like to thank my thesis advisor, Dr. Gordon Burghardt, for his guidance and support from designing this experiment through reading my countless drafts. I would also like to thank Catherine Smith for helping me with the logistical aspect of managing my subjects and my fellow undergrads in the lab for assisting with the care of the neonates.

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Table 1- A table of all the means of feeding latency for each subject and the frequencies of anti-predator behavior

Subject Information and Trial Data							
Subject Name	Red Score	Sex	Latency of Feeding Trial for All Trials	Latency of Feeding Trials Only When Snake Fed	Active Behaviors	Direction Reversals	Freezes
IR-102-A	2	F	91.92	91.92	10	0	10
IR-102-B	2	M	177.13	36.17	12	2	6
IR-102-C	2	F	83.7	83.7	13	4	3
IR-102-D	0	F	102.41	102.41	3	0	17
IR-102-E	2	F	233.61	111.47	2	2	16
IR-102-F	0	M	77.65	77.65	8	2	11
IR-102-G	2	F	182.67	182.67	13	2	5
IR-102-H	2	F	58.77	58.77	12	2	6
IR-104-A	0	M	203.2	70.93	15	5	0
IR-104-E	2	M	195.58	60.77	10	2	10
IR-104-F	2	M	110.08	110.08	4	3	13
IR-109-A	0	F	108.05	108.05	1	5	20
IR-109-B	0	M	37.96	37.96	7	6	14
IR-109-D	0	M	47.74	47.74	12	2	8
IR-109-E	2	M	39.38	39.38	2	0	19
IR-109-F	2	M	62.13	62.13	2	1	18
IR-109-I	0	M	35.71	35.71	5	1	18
IR-16-A	0	M	90.82	90.82	18	0	3
IR-16-B	0	F	57.3	57.3	7	0	13
IR-16-C	2	M	50.17	50.17	4	2	14
IR-16-D	0	F	109.26	109.26	4	2	14
IR-16-E	0	M	85.04	85.04	7	2	13
IR-16-F	0	F	75.1	75.1	16	2	3
IR-17-A	0	M	77.19	77.19	7	1	12
IR-17-B	2	F	63.74	63.74	13	0	7
IR-17-D	0	M	41.24	41.24	15	0	5
IR-17-E	2	F	45.2	45.2	14	1	7
IR-17-F	2	F	215.74	87.66	8	0	12
IR-91-B	2	F	233.29	233.29	8	2	12
IR-91-G	2	M	91.33	91.33	8	1	12
IR-91-I	0	F	18.92	18.92	13	2	7
IR-91-M	2	F	209.95	79.93	13	1	7
IR-98-A	2	M	82.97	82.97	8	4	9
IR-98-E	2	F	100.03	100.03	15	2	3
IR-98-F	2	F	60.42	60.42	13	3	6
IR-98-G	2	F	70.19	70.19	6	2	13
IR-98-H	2	M	51.02	51.02	12	3	6
IR-98-I	2	F	121.16	121.16	12	1	7

Table 2- an ethogram of all the behaviors observed and scored in the anti-predator testing

Ethogram of Anti-predator Behaviors	
Behavior Name	**Description of Behavior**
General Behaviors	
Directional change	The snake changes the direction in which it was moving before the stimulus was introduced.
Freeze	The snake ceases movement.
Whole body thrashing	The snake's body on both sides of the probe move in a random fashion not only laterally but vertically as well.
Strike	The snake strikes at the probe or in the direction of the probe with its mouth as if to bite it.
Tail Specific Behaviors	
Over the probe	The snake places its tail over the probe.
Tail thrashing	The snake's tail moves in a random fashion not only laterally but vertically as well.
Tail wagging	The snake's tail moves laterally.

Table 3-A table of the results of the three-way ANOVAs testing for significant of each group, litter, red score, and sex, with feeding latency and the anti-predator behaviors. The highlighted yellow indicates that those p-values are suggestive of a connection.

Group	Test	df	F	P
Litter	Feeding Latency	6	2.1424	**0.0785**
	Total Active Anti-Predator Behaviors	6	1.4123	0.2437
	Directional Reversals	6	2.1975	**0.0721**
	Freezing Behavior	6	2.2638	**0.0646**
Red Score	Feeding Latency	1	1.4626	0.2363
	Total Active Anti-Predator Behaviors	1	0.9026	0.3499
	Directional Reversals	1	1.4893	0.2322
	Freezing Behavior	1	0.6313	0.4333
Sex	Feeding Latency	1	1.4648	0.2359
	Total Active Anti-Predator Behaviors	1	0.0004	0.9851
	Directional Reversals	1	0.0373	0.8481
	Freezing Behavior	1	0.0201	0.8883

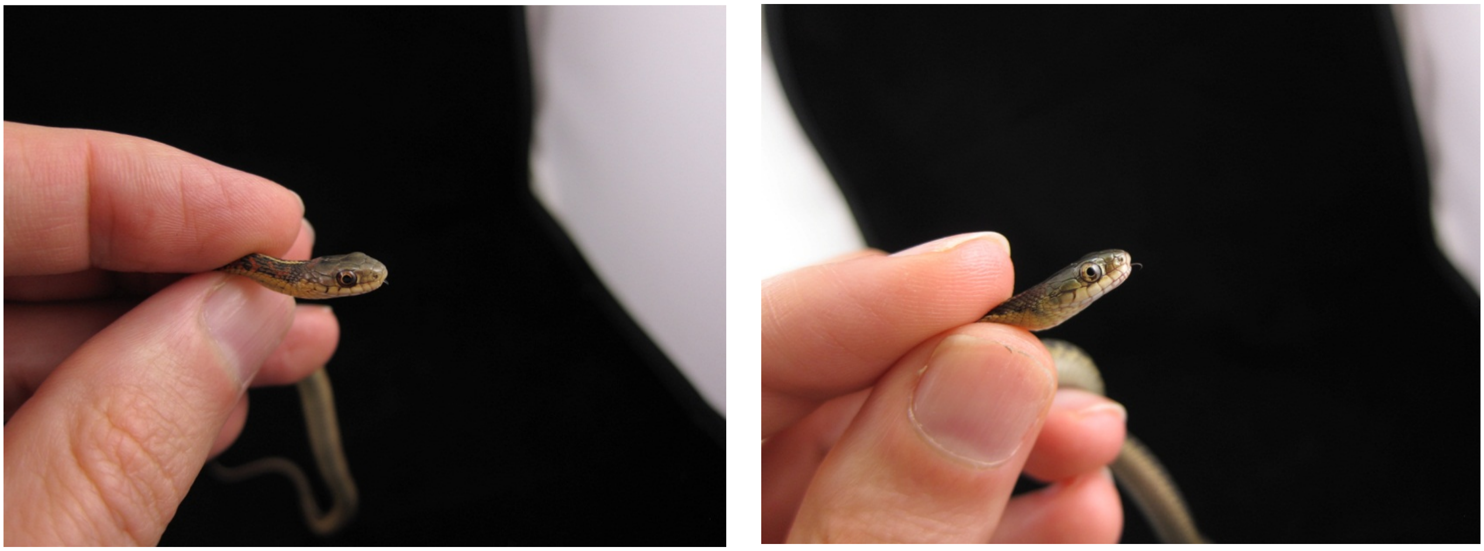

Figure 1- The image on the left is of IR-16-C demonstrating a red score of 2, meaning “significant/prominent red on lateral interscale skin, sometimes extending onto lateral stripe scale” (Mooi et al. 2011) and the image on the right is of IR-16-A demonstrating a red score of 0 meaning no red.

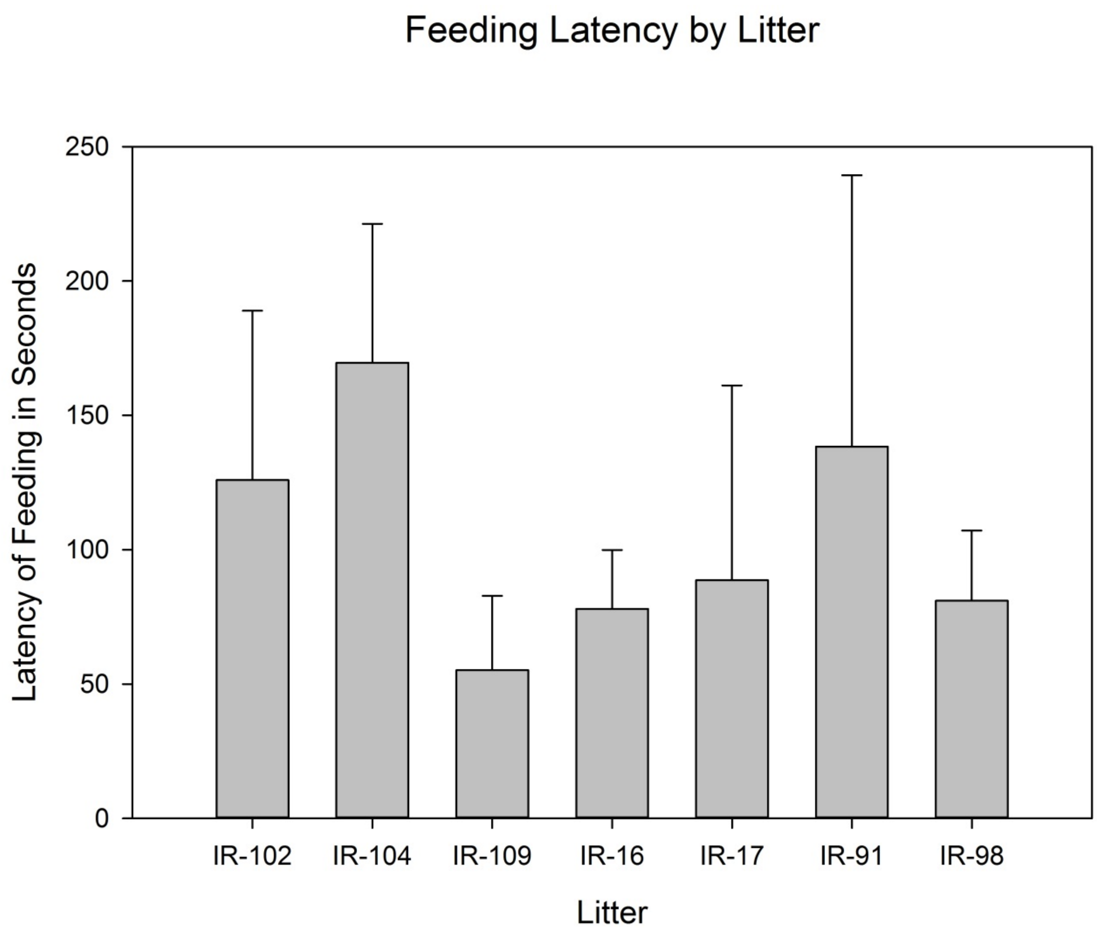

Figure 2- Mean, plus or minus one standard deviation, of feeding latency of neonates by litter. Total n=38, IR-102 N=8, IR-104 n=3, IR 109=6, IR-16=6, IR-17=5, IR-91=4, and IR-98=6.

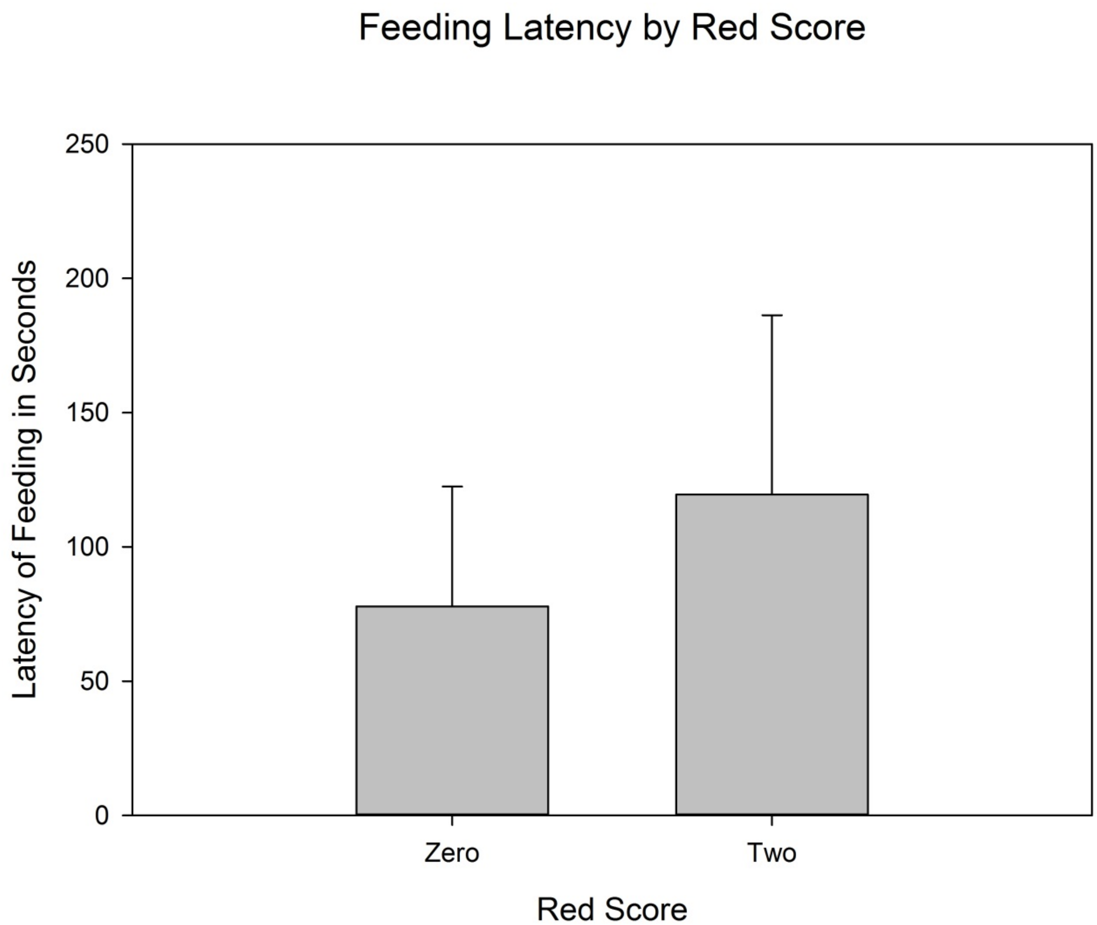

Figure 3-Mean, plus or minus one standard deviation, of feeding latency of neonates by red score. Total n=38, Zero n=15, Two n=22.

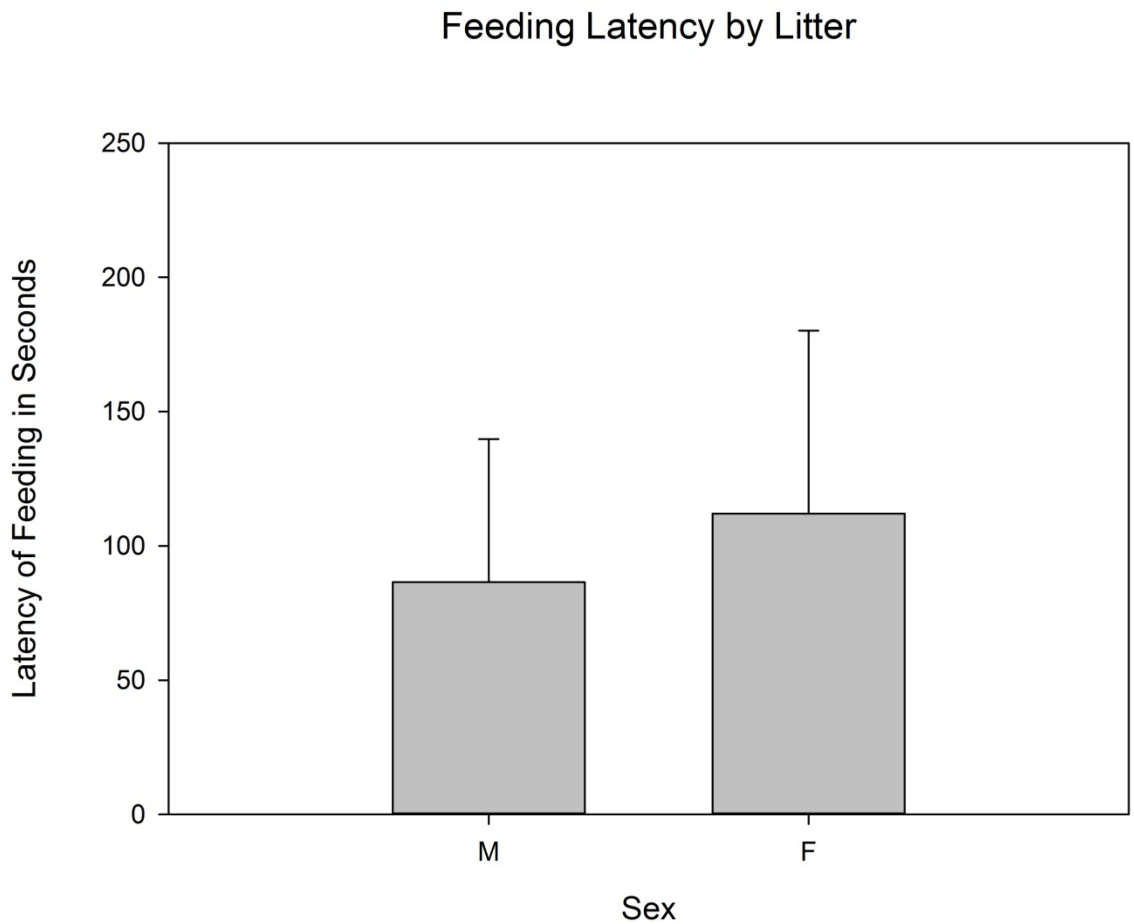

Figure 4-Mean, plus or minus one standard deviation, of feeding latency of neonates by sex. Total n=38, M, males, n=18, F, females, n=20.

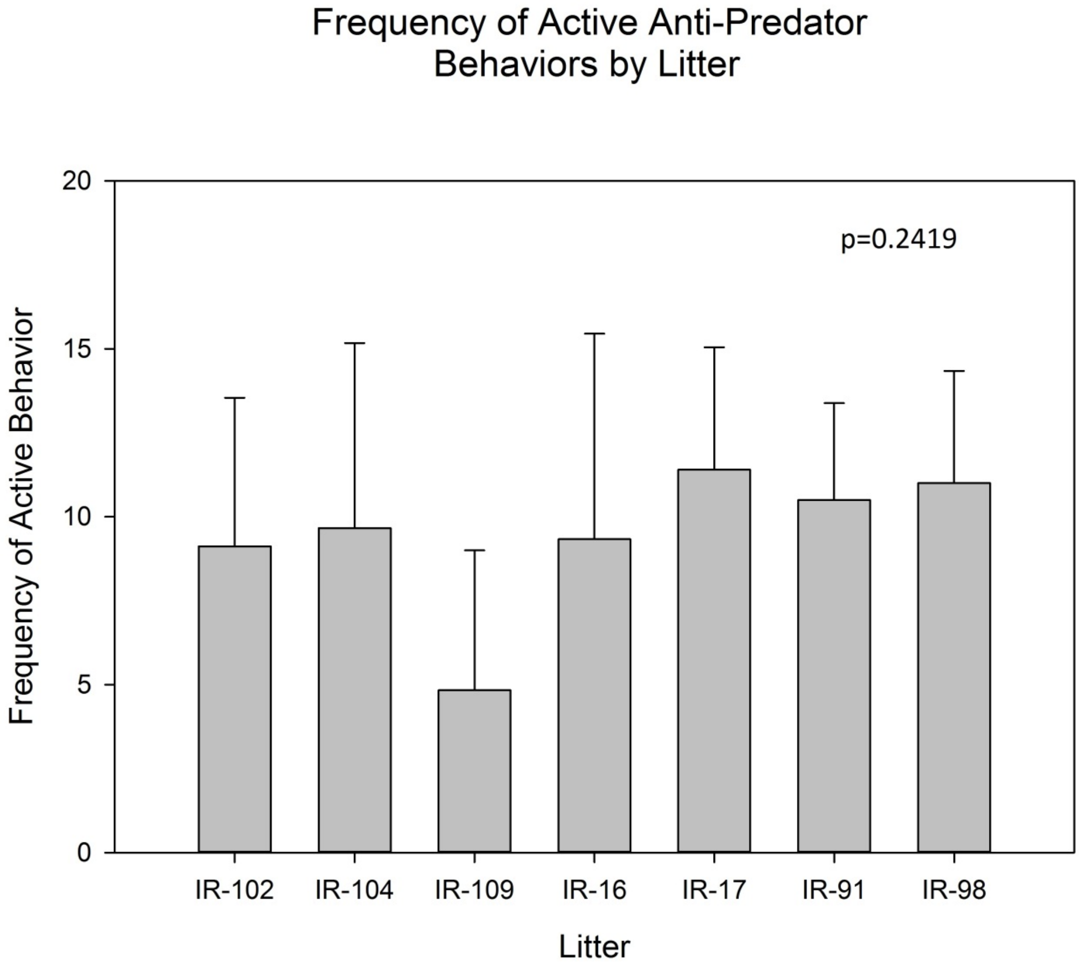

Figure 5-Mean, plus or minus one standard deviation, of frequency of active anti-predator behaviors of neonates by litter. Total n=38, IR-102 N=8, IR-104 n=3, IR 109=6, IR-16=6, IR-17=5, IR-91=4, and IR-98=6.

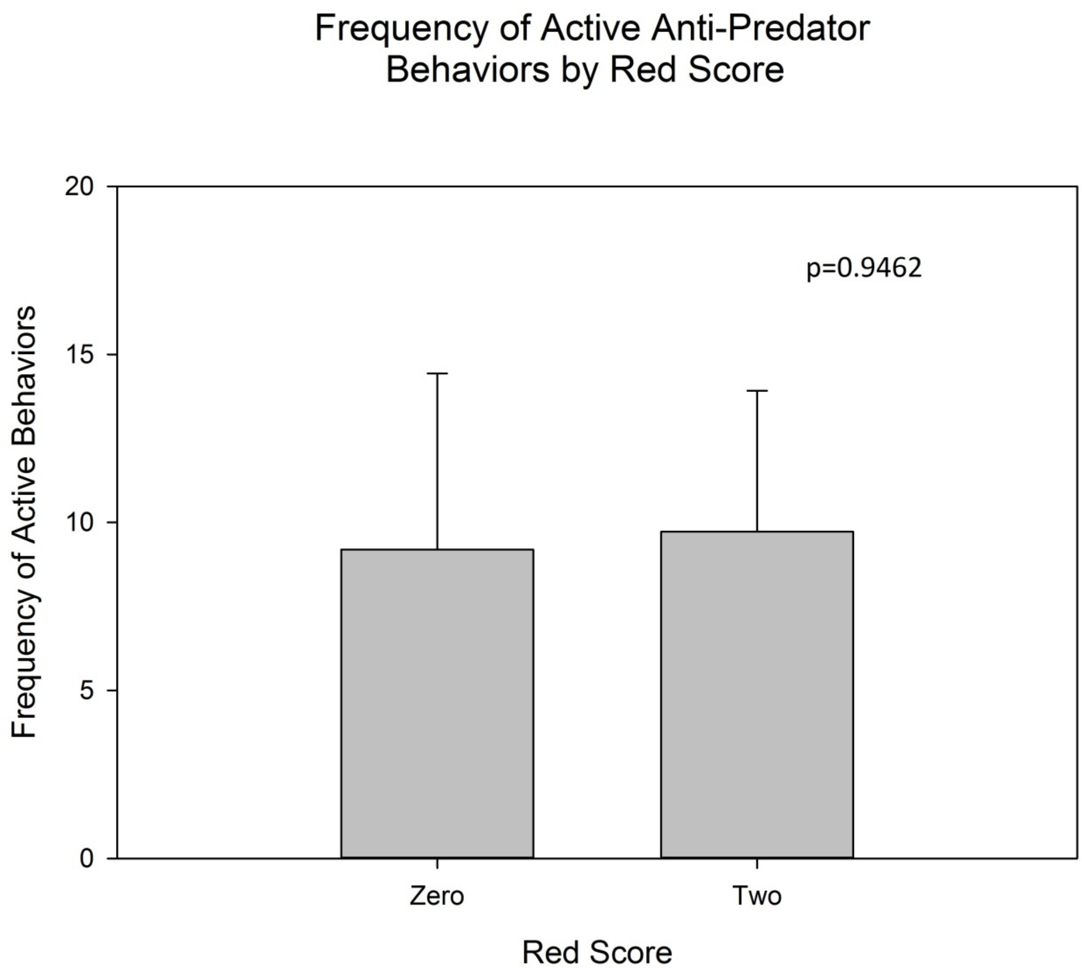

Figure 6-Mean, plus or minus one standard deviation, of frequency of active anti-predator behaviors of neonates by red score. Total n=38, Zero n=15, Two n=22.

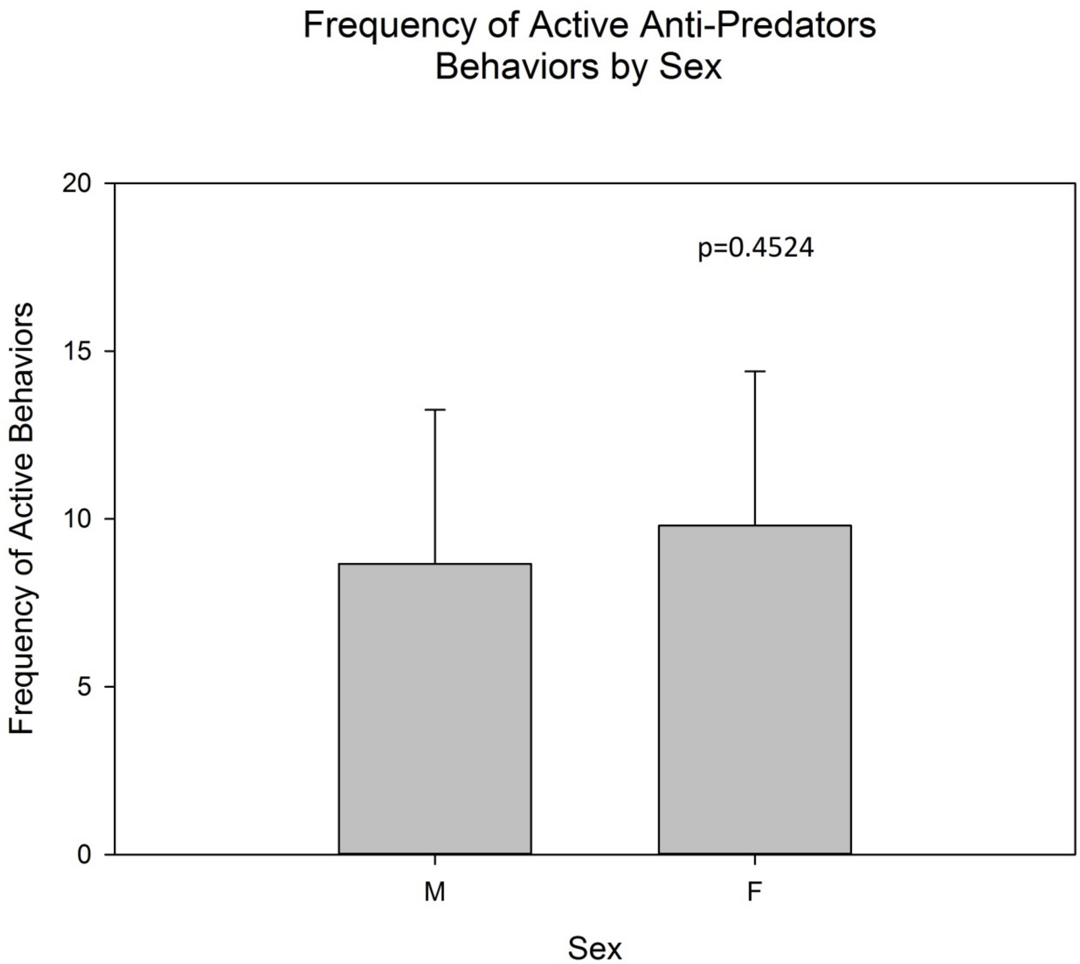

Figure7-Mean, plus or minus one standard deviation, of frequency of anti-predator behaviors of neonates by sex. Total n=38, M, males, n=18, F, females, n=20.

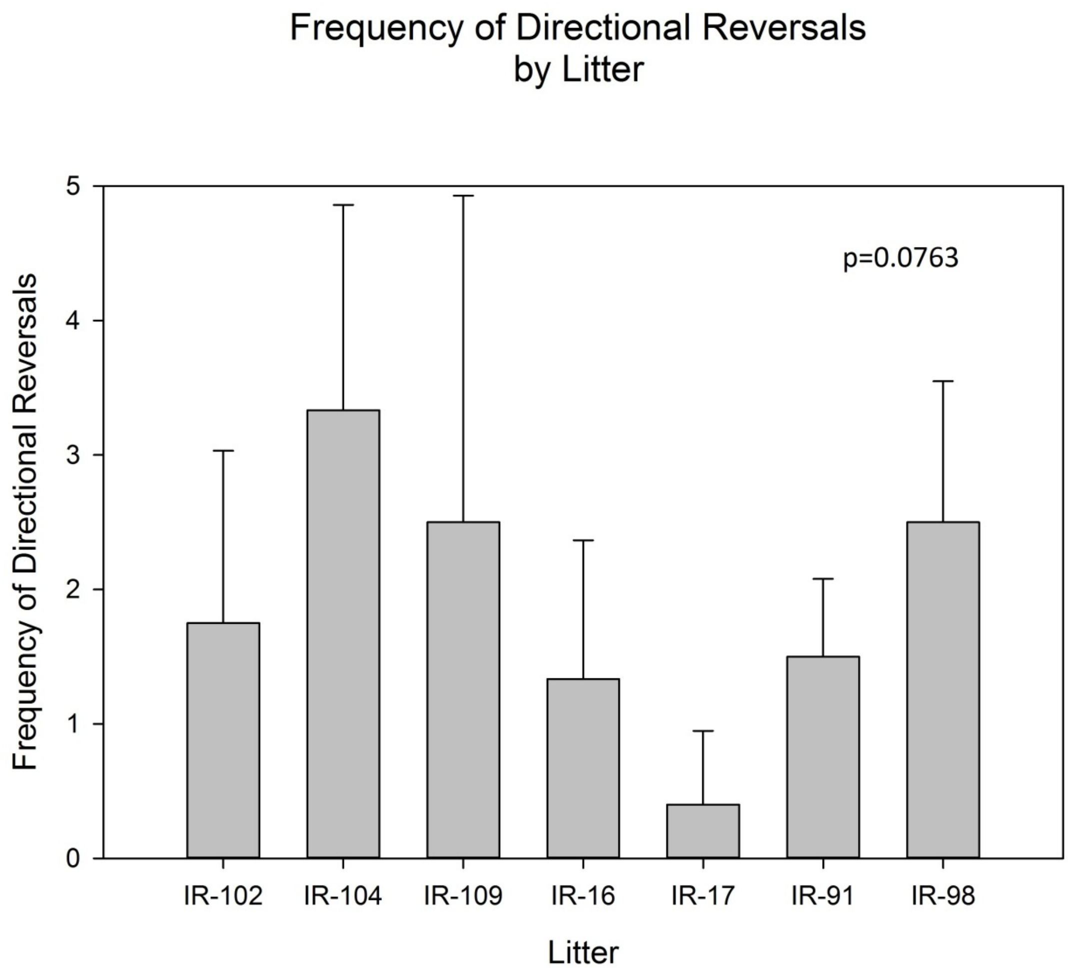

Figure 8- Mean, plus or minus one standard deviation, of frequency of directional reversals of neonates by litter. Total n=38, IR-102 N=8, IR-104 n=3, IR 109=6, IR-16=6, IR-17=5, IR-91=4, and IR-98=6.

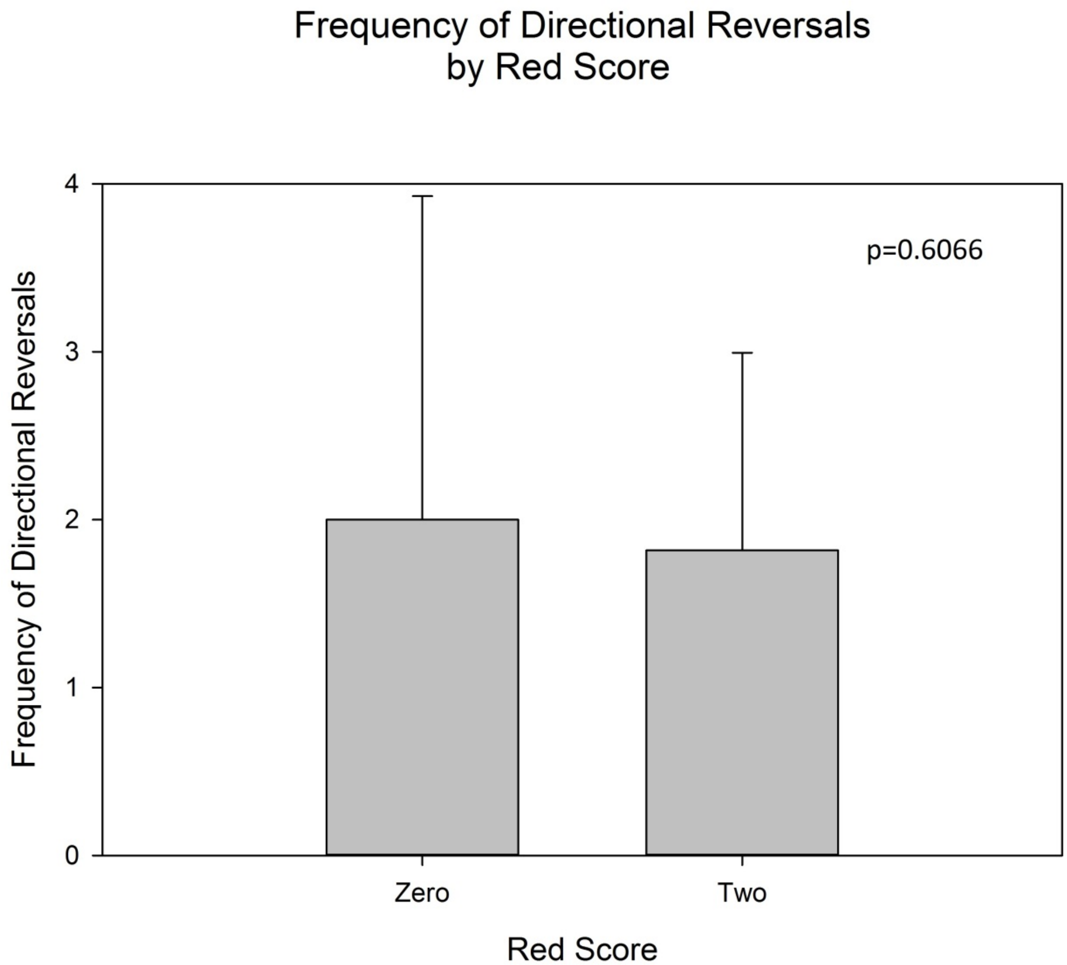

Figure 9-Mean, plus or minus one standard deviation, of directional reversals of neonates by red score. Total n=38, Zero n=15, Two n=22.

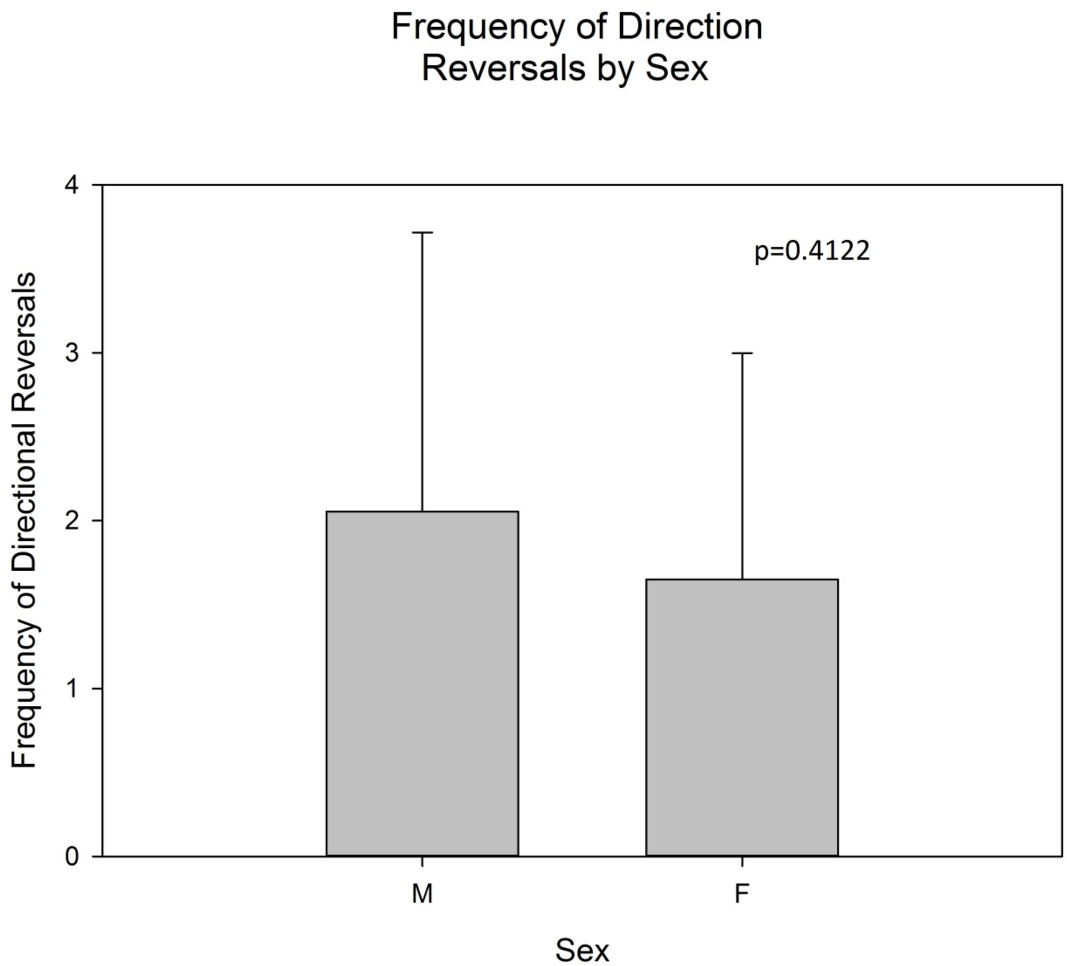

Figure 10-Mean, plus or minus one standard deviation, of frequency of directional reversals of neonates by sex. Total n=38, M, males, n=18, F, females, n=20.

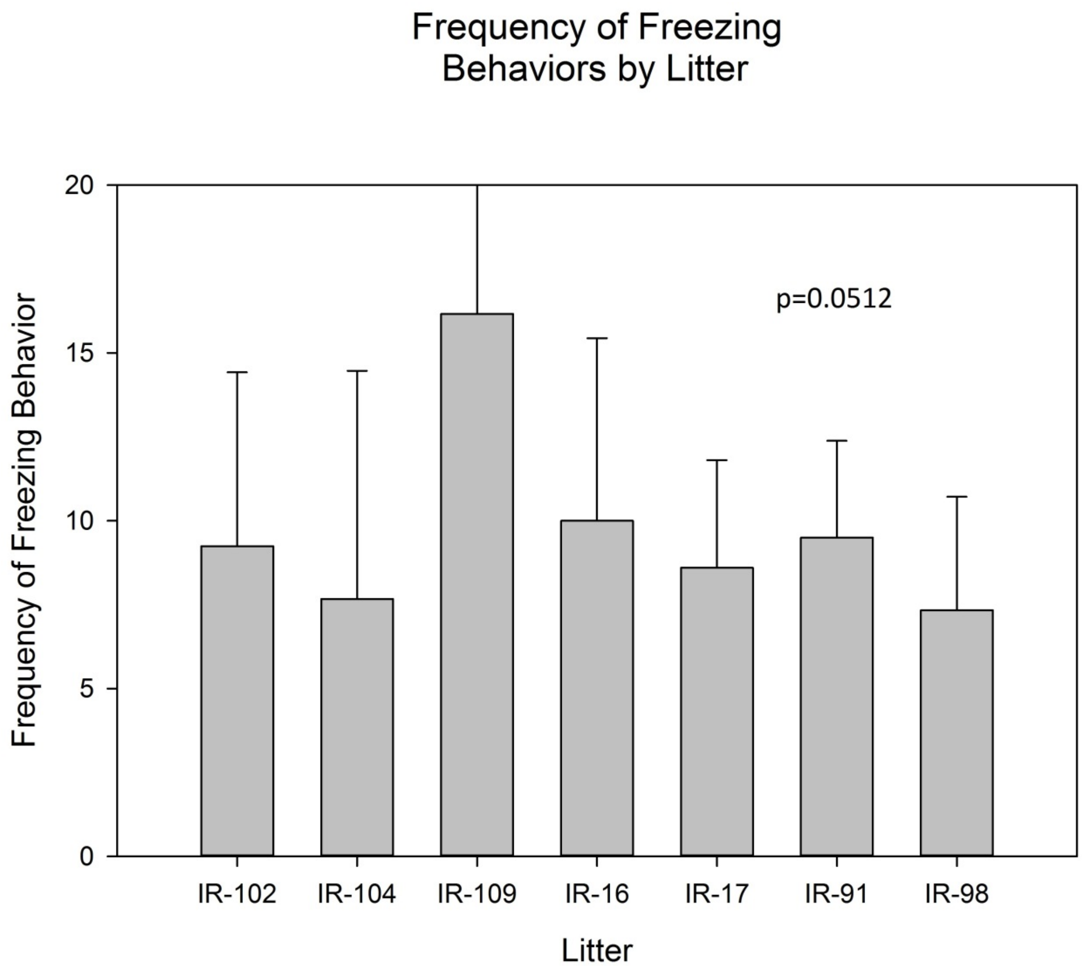

Figure 11- Mean, plus or minus one standard deviation, of frequency of freezing behaviors of neonates by litter. Total n=38, IR-102 N=8, IR-104 n=3, IR 109=6, IR-16=6, IR-17=5, IR-91=4, and IR-98=6.

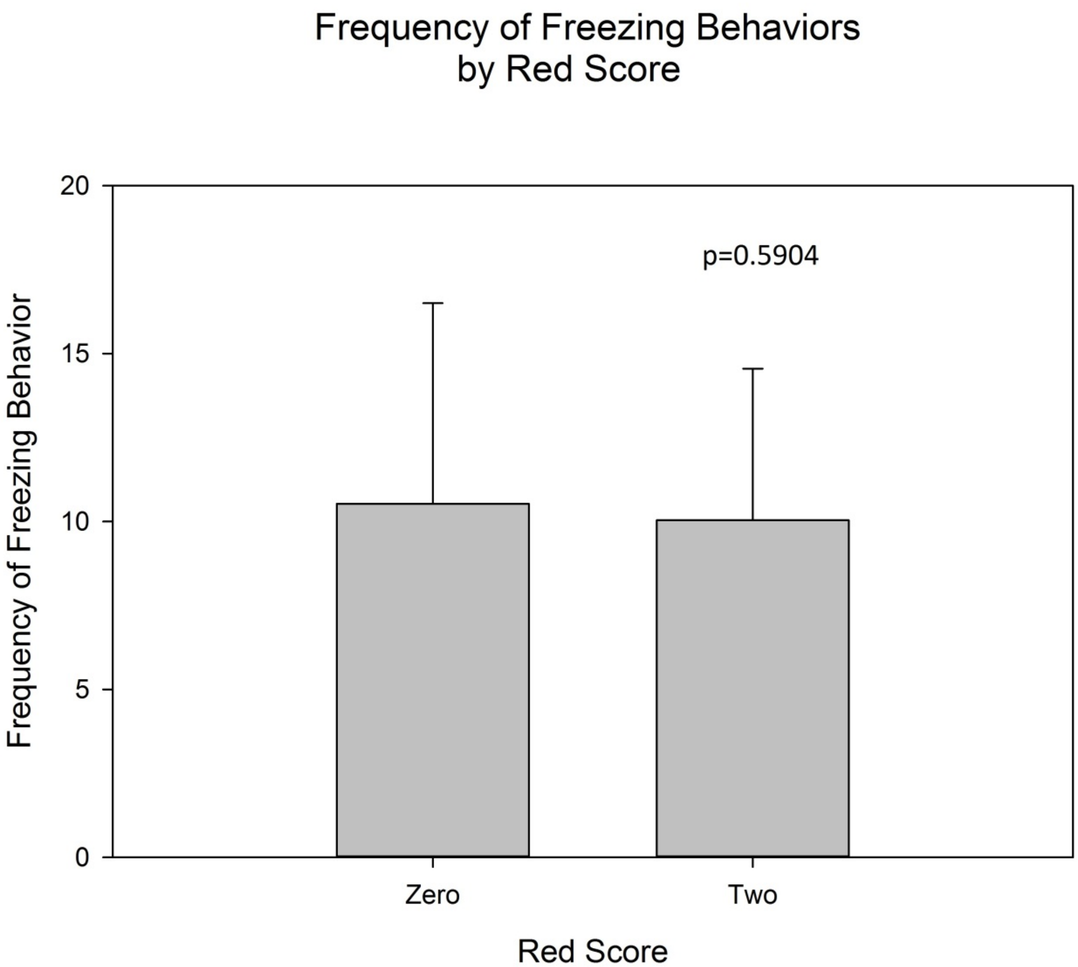

Figure 12-Mean, plus or minus one standard deviation, of frequency of freezing behaviors of neonates by red score. Total n=38, Zero n=15, Two n=22.

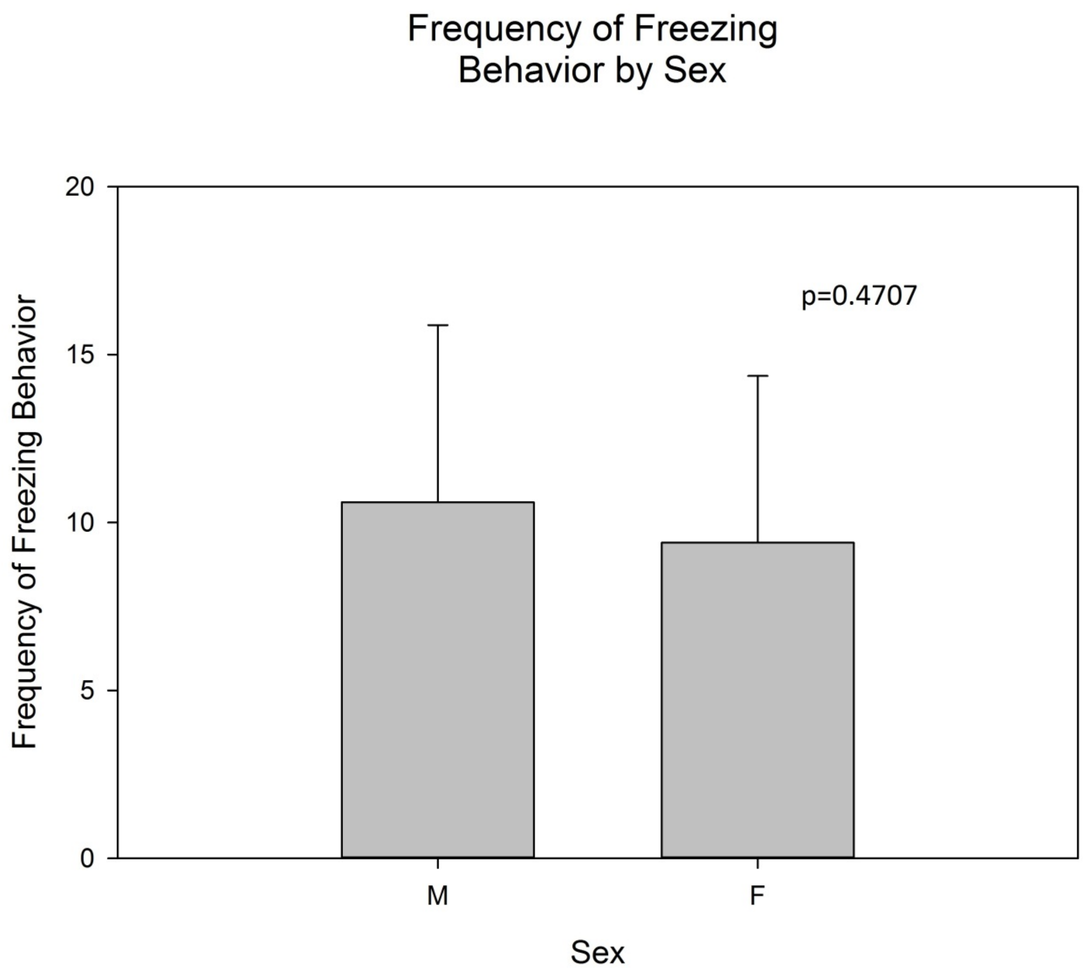

Figure 13-Mean, plus or minus one standard deviation, of frequency of freezing behaviors of neonates by sex. Total n=38, M, males, n=18, F, females, n=20.

Figure 14- The correlation analysis of the feeding latency plotted against the anti-predator behaviors.

Multivariate

Correlations

	Feeding	Active Behaviors	Directional Changes	Freezes
Feeding	1.0000	0.0027	0.0568	-0.0904
Active Behaviors	0.0027	1.0000	-0.0412	-0.9539
Directional Changes	0.0568	-0.0412	1.0000	-0.1000
Freezes	-0.0904	-0.9539	-0.1000	1.0000

Scatterplot Matrix