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Natural Variation in Fertility and GnRH Neurons in a Wild, Natural Population of White-Footed Mice, Peromyscus leucopus

Melissa Proffitt
College of William & Mary - Arts & Sciences

Natural Variation in Fertility and GnRH Neurons in a Wild, Natural Population of White-Footed Mice, *Peromyscus leucopus*

Melissa Proffitt

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Committee Chair
Professor Paul Heideman, Biology
The College of William & Mary

Professor Daniel Cristol, Biology
The College of William & Mary

Professor Eric Bradley, Biology
The College of William & Mary

ABSTRACT

Gonadotropin releasing hormone (GnRH) is considered the master hormone of reproduction because of its regulatory role in reproductive pathways, but little is known about variation within GnRH regulation in natural populations. In natural populations of many rodent species, a high percentage of individuals may undergo a seasonal reproductive repression, but others do not. It is possible that individual variation in GnRH neurons within these seasonally reproductive rodent species is a factor. Therefore, this study will examine if there is a correlation between GnRH neuron number and reproductive status in wild mice. This study was conducted in a wild population near Williamsburg (VA, USA), since it is unknown what the relationship might be in any wild population. Immunocytochemistry was performed to count the number of immunoreactive GnRH neurons present to compare both subsets of the wild population. We predicted a difference in GnRH neuron number between the seasonally repressed mice and the mice that remain reproductively active. In this wild, natural population, a negative correlation between male reproductive organ mass and stained immunoreactive GnRH neurons was identified. Therefore, individuals with lower testes mass and seminal vesicles mass have more stained immunoreactive- GnRH neurons on average than the reproductively active individuals. There was no statistically significant difference between animals collected in long days and animals collected in short days with respect to reproductive organ mass, GnRH neuron number, or weight gain after time in the laboratory. However, the numbers of immunoreactive GnRH neurons, body masses, and male reproductive organ masses were very similar between the laboratory and the wild populations.

COMPLIANCE PAGE

Research approved by

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LIST OF FIGURES

1- INTRODUCTION

GnRH is the master hormone of reproduction. GnRH controls hormone cascades associated with reproduction. This review will focus on some of the highlights of this regulatory hormone system. GnRH is secreted in pulses from the hypothalamus and is released into capillaries to a blood portal system, then to the anterior pituitary (Charlton et al., 2008 and Carmel et al., 1976). In the anterior pituitary, GnRH causes the release of Luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH) (Heitman and Ijzerman, 2008). The pulse frequency of GnRH differentially controls the release of LH and FSH; therefore, different amounts of LH and FSH can be released from the anterior pituitary (Burger et al., 2002, Vizcarra et al., 1997, and Kaiser et al., 1997). It has been demonstrated that the pulse frequency of GnRH is more important than the amount of secreted GnRH (Burger et al., 2002). When the proper GnRH pulse frequency is received by the anterior pituitary, LH and FSH are then released into the bloodstream and are carried to the gonads (Heideman and Pittman, 2009). LH and FSH have different effects in male and female mammals. In males, LH acts on leydig cells in the testes and stimulates the release of testosterone (Cigorraga et al., 1978). As mice mature, the initial release of sex steroids causes increases in expression of LH and FSH receptors. Also in males, FSH in the testes is essential for the production of sperm. In mammalian females, LH stimulates estrogen secretion and a surge of LH induces ovulation and initiates production of progesterone by the corpus luteum (Bingel and Schwartz, 1969). In mammalian females, FSH and accompanying sex steroids stimulate growth of

the follicle, in which maturation of the oocyte to ovum takes place (Hunter, 2000). LH and FSH are necessary to maintain sex organs and their function(s) in both males and females: therefore, an adequate frequency of pulsatile GnRH is essential to maintain reproduction.

Effect of various environmental inputs on the reproductive system:

a- The mammalian biological clock: photoperiodism

GnRH release is affected by various signals, including signals which indicate the photoperiod. In mammals, a photoperiod is defined as the number of hours of light vs. darkness: nearly all mammals in the temperate zone have a biological calendar that is reset by a photoperiod. One signal associated with the detection of photoperiod is the concentration of the hormone melatonin. Melatonin is a hormone that is released from the pineal gland only at night (Stehle et al., 2003; Reiter et al., 2009).The number of hours of darkness provides the biological clock with a mechanism to determine seasons. Therefore, there is an increase in the duration of melatonin signal, because of longer winter nights. One effect of melatonin from a winter night in long-day breeders (such as rodents) is inhibition of the release of gonadatrophin releasing hormone (GnRH) (Roy, 2001, Bartness et al, 1993).

b- Reproduction under the effects of Melatonin:

The GnRH regulatory pathway is a major regulator of reproduction, and is required for fertility (Mintz et al., 2007). During winter, subsets of natural populations may still remain reproductively active; they maintain the reproductive pathway through which GnRH is released, maintaining the release of FSH and LH, and preventing the gonads from regressing. However, other individuals in the winter respond to longer durations of melatonin release with repression of GnRH. Because GnRH is suppressed, a low amount of LH and FSH are released, causing the gonads to regress. Individuals that respond to the photoperiod (longer durations of melatonin) (Claustrat et al., 2005) and undergo this gonadal regression, becoming reproductively inactive, are referred to as photoperiod responsive individuals. Conversely, individuals that do not respond to the longer durations of melatonin signal and maintain their gonads, remaining reproductively active (Gorman and Zucker, 1997), are referred to as non-responsive individuals.

c- Gonadotrophin Inhibiting Hormone (GnIH):

It was once thought that GnRH was unique, because of the lack of a direct neuropeptide antagonist (Tsutsui et al., 2010). However, in the year 2000, Gonadotropin Inhibiting Hormone (GnIH) was discovered to be directly involved in the regulation of GnRH (Tsutsui et al., 2010). GnIH is a hypothalamic hormone which appears to have analogs across all vertebrate species (Tsutsui et al., 2012). In the avian brain, melatonin stimulates the release of GnIH, which inhibits the secretion of GnRH (Ubuka et al., 2004: Bently et al., 2006). Current studies suggest that mammals which breed during long days react similarly; melatonin stimulates the release of GnIH (Bently et al., 2006: Tsutsui et al., 2012).

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The Mouse Model and Life History Traits

a- Peromyscus as a model organism

As a mammal, *Peromyscus leucopus* uses the same sets of neurons and hormones in the regulation of reproduction used by most mammals, including humans. Consequently, *P. leucopus* are used as a model for many experiments (La Marca and Volpe, 2006). *P. leucopus* have a wide geographical distribution, as they can be commonly found in most of the eastern and central United States, southern Canada, and eastern Mexico (Linzey, 1998). *P. leucopus* are common throughout the state of Virginia (Linzey, 1998). In addition to being abundant, *P. leucopus* are easily captured and easily kept in lab. Since *P. leucopus* are small mammals, laboratory colonies of *P. leucopus* are often cheaper to maintain than many other mammal colonies (Heideman, 2004). Presumably, laboratory conditions are a different environment from the field. When a population for a laboratory is founded, it can be a genetic bottleneck, and because laboratory populations are traditionally smaller than natural populations, genetic drift can presumably occur faster (Wade et al., 2002: Heath, 2003: Artamonova and Makhrov, 2006: Athrey et al. 2007). In addition to these factors, genetic drift can also occur just by the nature of becoming a newly (or not so newly) distinct population with little or no gene flow from the source population. Since our animals are recently derived from a wild, natural population, they have not been subjected to the same degree of laboratory artificial selection and inbreeding as typical laboratory rodents (Heideman, 2004). Presumably, these mice have not

undergone as many of the changes associated with long-term laboratory selection, which could confound the investigation of natural variation in neuroendocrine systems, making *Peromyscus leucopus* is a good model for studying the differences in seasonal reproduction.

b- Peromyscus life history traits

During winter, subsets of natural populations of *Peromyscus* may still remain reproductively active (Desjordans et al., 1986: Heideman and Bronson 1991); they maintain the reproductive pathway through which GnRH is released, maintaining the release of FSH and LH, and preventing the gonads from regressing (Blank and Desjardins, 1986: Mintz et al., 2007). However, other individuals in the winter may respond to longer durations of melatonin release by causing repression of GnRH (Ansel et al., 2011: Tsutsui et al., 2012: Servili et al., 2013). One hypothesis is that because GnRH may be suppressed in amount or in pulse frequency, a low amount of LH and FSH may be released, causing the gonads to regress (Heideman and Pittman, 2009). Individuals that respond to the photoperiod (longer durations of melatonin) and undergo this gonadal regression, becomes reproductively inactive, are referred to as photoperiod responsive individuals. Conversely, individuals that do not respond to the photoperiod and maintain their gonads, remaining reproductively active, are referred to as non-responsive individuals.

In the wild, a reasonable explanation for the maintenance of these two very distinct subsets of the population is that selection pressure is variable upon individuals within a population (Prendergast et al., 2001). During some winters

that are mild, there might be increased pressure to maintain reproduction, because the animals that are reproducing more have the selective advantage (Prendergast et al., 2001). Conversely, during harsh winters, animals that are allocating energy toward reproduction might perish, so the selective pressure would favor individuals who allocate their energy towards survival (Prendergast et al., 2001).

c- Background into previous related experiments

Heideman et al. (1999) developed two selection lines of Peromyscus continued through approximately 18 generations (Heideman and Pittman 2009). One of these lines is reproductively responsive (R) to photoperiod, while the other line is non-responsive (NR) to photoperiod. The responsiveness to photoperiod, measured by gonad size, was shown to be heritable, responding to selection by the second laboratory generation (Heideman et al., 1999). In later studies, the selection lines were shown to differ in the number of immunoreactive-GnRH (hereafter IR-GnRh) neurons, with the number of IR-GnRH neurons heritable in an unselected control line (Avigdor et al., 2005: Heideman et al., 2007).

In order to determine the relationship between GnRH neuron number and fertility in a wild population of mice, animals were collected from the wild in the winter season to determine differences in phenotype, with regards to reproductive activity. Mice were also collected in summer season to determine the overall variation in GnRH neurons that exists within natural populations and

to attempt to further understanding for reproductive inactivity under summer conditions.

d- The laboratory versus the field

This study will examine how accurately these lab models apply to a natural system. This is important because the goal of most scientific studies is to model an aspect of a natural system. In order to extrapolate our lab findings to a natural environment, we have asked if the same relationship between the number of IR-GnRH neurons and reproductive status is present in a wild, natural population of mice.

This experiment is valuable because, complex systems are often tested in a controlled laboratory environment and the findings used to make inferences about the natural environment. However, many of these findings need to be tested in the field before such conclusions can be made. In the field of endocrinology, this is especially true because the laboratory and natural environment(s) are different. Hormone receptors react to the environment acutely and chronically, inducing a change in minutes to days (Winter and Flataker, 1951). These hormonal changes can induce transcriptional and translational changes (Jensen et al., 1968). Because endocrine systems are dynamic and affected quickly by environmental factors, the endocrine system of a laboratory animal and a wild animal can be in very different states simply because of the difference in environment. For example, Meijer and Schwabl (1989) concluded that androgen levels during the breeding season of wild kestrels (*Falco spraverius*) were three-fold higher than levels in kestrels raised in captivity. They

also found that GnRH and LH were higher in the laboratory population than in the wild (Meijer and Schwabl, 1989). The study performed with kestrels indicates that an animal taken from the wild can have a different endocrine status, because they are in a different environment. Currently, it is acceptable in endocrinology to take a study in the lab that accounts for single factors or a group of factors to learn the mechanistic basis of a system. However, before the study is extrapolated to the natural environment, it must be tested in a natural setting. In addition, the mice in our laboratory have been isolated from the natural environment for approximately 18 generations, potentially allowing genetic divergence from the natural population. Artificial selection of the mice for responsiveness to photoperiod could cause an array of unanticipated changes in the endocrine system. In addition, there could be other changes in the endocrine system which arise from the differences in environmental conditions between the laboratory and the natural habitat of the mice.

e- Potential benefits for humans

The kind of variation exists among each class of neuron within the mammalian brain is poorly known. However, humans have a wide range of reactions to various drug treatments. Much of this diversity, which potentially causes varying reactions to medications such as anti-depressants, is likely due to phenotypic variation within the brain (Bittner and Friedman, 2000). More research needs to examine the variation that exists within neuronal systems and how this variation affects physiological systems in mammals. As we gain more of an understanding about how neuronal variation affects physiological systems,

researchers can likely develop drug treatments to create fewer side effects for patients.

Specific Aims:

Overarching questions:

a.) How similar is the variation in our laboratory selection lines of mice to variation in the wild population?

b.) How good are physiological measures of fertility at representing reproductive status?

2- Core thesis:

METHODS

f- Animal collection

Animal collection occurred during the summer of 2013 and the winter seasons of 2012-2013 and 2013-2014. This collection was conducted in the woods around the College of William and Mary, which is located in Virginia's Coastal Plain region (37° 16' N 76o 42'W). Mice were collected using Sherman live traps, which were baited with oats. Traps also contained water resistant polyfiberfill, for nest building material to help insulate the mice from the cold. On days when the temperature fell below -12°C, trapping was avoided because of the potential for increased mortality rates in mice and other small mammals. This cold temperature mortality is especially associated with animals which do not use

nesting materials within the trap (Churchfield, 1990). The winter field season began in December and the summer field season began in July.

There was a need for special equipment to resolve trap disturbance issues (Fig. 1, Fig. 2). Due to the high level of disturbances from wildlife, such as raccoons, a special apparatus was constructed to decrease the number of trap disturbances. Trap disturbances included the following: relocation of the Sherman trap (often relocated meters away from the original placement), trap disassembly (the pin holding the trap together was removed). To avoid these problems traps were placed within a plastic corrugated pipe, to minimize access to the actual Sherman trap. The apparatus was constructed using a metal stake attached to plastic corrugated tubing (tubing was ~0.5m long). This corrugated pipe had a metal stake (approximately 30cm in length) attached to it, using a malleable metal wire to attach the stake to the corrugated pipe. This was the base for the apparatus. Then, two designs were applied to this basic structure. I decided to use two different designs to try and stay ahead of the raccoon learning curve, as well as to try and determine if one style was more feasible to use than another.

One design was a "pin" style (fig.1), the basic apparatus structure had 8 holes drilled into the corrugated pipe. Pins which were designed from metal wire to have a metal loop on one end were placed into the pre-drilled holes in the corrugated pipe. After each of these four pins were placed into the eight pre-drilled holes, the metal loops were tied together with a heavy nylon string. These pins within the corrugated pipe created a barrier so that the mice could get into

the Sherman trap, but other animals could not remove the Sherman trap from the corrugated pipe.

The other design was called the "wire mesh" design. This design implemented chicken wire with 2.5cm mesh, on the front and the back holes of the corrugated pipe (fig. 2). The wire mesh allowed mice to enter the tubing which contained the Sherman trap, but did not allow larger animals to pass through, as long as the mesh was intact. The mesh was placed over the end of the tube, then molded down around the sides of the tube. After the mesh was fitted, a malleable but sturdy wire was used to weave the mesh around the sides of the tube. One side was made into a removable mesh cover, by using the wire to circle around the tube, and making a loop on each end with the wire. Then, the cover could be removed to replace traps. In the loops, a metal ring was fitted to put enough tension on the removable cover to hold it in place on the corrugated tubing. The other side of the corrugated tubing was permanently fitted with mesh.

b- Animal Housing

After capture, animals were classified as adult or juvenile. To classify animals as juvenile or adult, they were weighed and pelage color was examined. On average, *Peromyscus leucopus* females mature between 46-51 days and males mature ~10 days later (Clark, 1938). Juveniles are grey in color (Gottschang, 1956). Molt of the grey juvenile pelage begins at 40-50 days of age; on average, it takes 24 days after the molt begins to fully transform into a brown or reddish-brown color (Gottschang, 1956). Females appear to undergo their

first estrous cycle before they reach their full body size (Clark, 1938). Yet, one study suggests no correlation between weight and pelage color (Gottschang, 1956), implying weight cannot be used to act as an approximation of age. However, we decided to assign a minimum weight value in conjunction with pelage color to determine if mice were adults, because color alone to determine age could be subjective. The average mass of an adult wild *Peromyscus* appears to be approximately 19g, at 30 days old, mice are approximately 2/3 the weight of an adult (approximately 13g) (Gottschang, 1956). Therefore mice were collected only if they weighed over 15 grams and had the same pelage color as a typical adult mouse.

Animals that were determined to be juvenile or sub-adult were released at the site of capture. Animals which were classified as adult were housed in polypropylene cages with pine bedding and nesting material, and fed ad *lib*. Body mass was collected three times: on the day of capture, the third day in lab, and the day of perfusion. Specimens were euthanized within 14 days of their arrival. While a shorter period between capture and tissue collection would have reduced effects of acclimation, for logistical reasons mice were housed for this longer period. The photoperiod was matched within 30 minutes of natural conditions in order to minimize effects on the melatonin cycle of the animals. Statistical analyses (mostly ANOVAS) were conducted to test whether amount of time the animals spent within the laboratory was a factor in this study.

Tissue Collection

Animals were weighed, and then euthanized with an overdose of isoflurane (Abbot Laboratories, North Chicago, IL); animals completed respiratory arrest before perfusion. After euthanasia, animals were perfused using intra-cardiac puncture to the left ventricle, followed by an infusion of 0.1M Phosphate-Buffered Saline (PBS: pH 7.4), then a 4% paraformaldehyde solution was infused to preserve tissues following a technique previously used by other researchers (Avigdor et al., 2005; Heideman et al., 2007). After the perfusion, the brain, testes, and seminal vesicles were removed for evaluation. Brains were cryoprotected in 30% sucrose with a Phosphate Buffered Saline (PBS) solution (Heideman et al., 2007). After cryoprotection, brains were sliced in coronal sections (30 micron) using a freezing sliding microtome These coronal sections were placed into wells, each well contained every fourth section (Avigdor et al., 2005). One out of every four brain sections were analyzed with immunocytochemistry (ICC) to quantify the number of IR-GnRH neurons (Heideman et al., 2007).

The masses of the reproductive organs were used as a measure of fertility. Testes size is highly correlated with sperm count in this species (Heideman and Bronson, 1991, Broussard, et a 2009). Therefore, in males, the reproductive organs were removed: testes and seminal vesicles masses were recorded. In females, the uterine horns and ovaries were removed, and their respective masses were recorded. If there were any botfly parasites, then these botfly parasites were removed and the total number of botflies per animal was counted.

Immunocytochemistry

Mature IR-GnRH in the brain was labeled using a single-label avidin-botin-peroxidase-complex method. Brain slices were washed four times, in ten minute increments, in cold 0.02M TBS (4°C) (Avigdor et al., 2005). Brain slices underwent a 48 hour incubation with SMI-41 monoclonal antibody at 4°C (Sternberger Monoclonals, Lutherville, MA) at a dilution of 1:20,000 in 0.02M TBS (Avigdor et al., 2005). SMI-41 was designed for 5 amino acids on the C-terminus adjacent to the GnRH peptide amidation site: therefore, SMI-41 is proposed to detect only mature GnRH peptide (Tai et al., 1997). The antibody carrier solution for SMI-41 was made with 0.50% lambda-carrageenan, 1% bovine serum albumin, and 0.3% Triton X-100 in 0.02M TBS at a pH of 7.8 (Avigdor et al., 2005). All subsequent treatments were conducted at room temperature with gentle agitation unless stated differently (Avigdor et al., 2005).

After the 48-hour incubation, sections were washed 4 times in 0.02M TBS, and then slices were incubated with a biotinylated horse anti-mouse IgG at a dilution of 1:500 in 0.02M Tris Buffered Saline (TBS) for 1 hour (Sternberger Monoconals, Lutherville, MA) (Avigdor et al., 2005). After brain sections were washed three times with 0.02M TBS, sections were incubated in avidin-biotin-peroxidase (Vector Laboratories Elite ABC-Peroxidase kit) in 0.02M TBS for 60 min (Avigdor et al., 2005). Sections were then rinsed three times in 0.02M TBS and then placed in a solution of diaminobenzidine Enhanced Liquid Substrate System (2.5ml of the buffer and 30 µl of the chromagen) (Sigma). The color reaction was allowed to proceed for 10 minutes before three 10-minute washes

in 0.02M TBS to stop the reaction. A total of 10 independent runs were conducted within this experiment.

Neuron Assessment

IR-GnRH neurons were counted on a compound light microscope by Melissa Proffitt (MRP), and separate independent counts were done by an undergraduate (Gabrielle Smith, GKS). Two different counts for IR-GnRH neurons were implemented. One count was the total number of observed IR-GnRH neurons. The other count was a separate attempt, to distinguish the number of "light" IR-GnRH neurons. The qualification for the light neuron count is as follows: neurons were considered "light" if they had a prominent outline of a cell body and cell processes above the background color, with the area between the outline of the cell body being around the color of the background tissue. However, if the cell body was a consistent color throughout, then the neuron was not considered to be a "light neuron." This attempt to measure numbers of light neurons was subjective, but consistent across different individuals. The objective was to quantify the numbers of these neurons to investigate potential trends for further investigation in future studies. The results of these independent counts (for the number of total observed IR-GnRH and number of "light" IR-GnRH) between MRP and GKS were very similar.

Landmark brain areas were identified using the rat brain atlas (Paxinos and Watson, 1989), because Avigdor et al., (2005) used this rat brain atlas to identify the areas where IR-GnRH neurons were most prevalent in *Peromyscus*

leucopus (plates 12-25 in this atlas) (Avigdor et al, 2005). Therefore, we identified mature IR-GnRH neurons to count between plates 12-25 in the stereotaxic coordinate atlas for the rat brain (Paxinos and Watson, 1989). Statistical tests were carried out using R version 2.15.3 (R core development team 201, 2013).

B- RESULTS

a- Perfusion quality, ICC run, and slice number

Because the mean number of GnRH neurons could vary across ICC run, a linear model was created for the number of IR-GnRH neurons and run of ICC. This was tested with a two-way ANOVA. Also, the quality of perfusion could have created potential inconsistencies in our counts, which could potentially result in numbers of different IR-GnRH neurons. Another linear model was created for the number of IR-GnRH neurons and the perfusion quality rank, and this model was analyzed using a two-way ANOVA. Neither the run of ICC nor the perfusion quality had statistical effects on the numbers of IR-GnRH neurons (ICC run $p=0.98$; perfusion quality $p=0.99$). An interaction between the run of ICC and perfusion quality on the number of GnRH neurons was also tested using the same linear model and two-way ANOVA method; this test also did not approach significance ($p=0.64$). Finally, another liner model was analyzed using an ANOVA to determine if there was a relationship between the number of slices counted and number of counted IR-GnRH neurons (plates 12-25 Paxinos and Watson, 1989), no significant effect of slice number on the number of IR-GnRH neurons

was detected. Therefore, perfusion quality, ICC run, and slice number were not considered in subsequent statistical analysis.

b- Percentage of reproductively suppressed mice collected in winter 1995 and 2013.

The percentage of reproductively suppressed mice collected in the winter of 1995 appeared similar to the percentage of reproductively suppressed mice collected from the winters of 2013 and 2014 (Fig. 3). These percentages were also similar to the findings from a previous study of this population (Terman, 1993).

Data for Male Mice:

c- Body mass and number of days in lab

Animals varied in the number of days they spent in lab (0-17 days). A linear model was run on males collected in winter, to test for changes in body mass over the number of days in the laboratory. There was no significant effect of days in the laboratory on body mass of mice caught in winter ($p=0.55$). When mice captured in winter and summer were analyzed together using a linear model and ANOVA, again there was no statistical relationship between the change in body mass during the number of days an animal remained in the laboratory (Fig. 4, $p= 0.97$). Also, there was no statistically significant relationship between final body mass (on day of perfusion) and number of days in lab (Fig. 5, $p=0.45$)

d- Male body mass and reproductive organ mass correlations

A Pearson's correlation analysis indicated a positive statistically significant correlation between initial body mass (mass on the date of capture) and testes mass (Fig. 6, df= 33, R=0.512, p=0.0017*). Another Pearson's correlation analysis indicated a positive statistically significant correlation between testes mass and final body mass (body mass on day of euthanasia) (Fig. 7, df=43, R= 0.618, p= 6.14e-06*). Seminal vesicles mass was positively correlated with initial body mass (Fig. 8, df=33 R= 0.382, p =. 0.0236*). There was also a positive correlation between seminal vesicles mass and final body mass (Fig. 9, df=43, R= 0.614, p =. 7.33e-06*).

e- Number of days in lab and testes mass, seminal vesicles mass, and IR-GnRH neuron number

An ANOVA on a linear model that included the number of days the animals spent in lab did not indicate a relationship with testes mass (Fig. 10, p=0.39), seminal vesicles mass (Fig. 12, p=0.43), or IR-GnRH neuron number (Fig. 14, p=0.97). There was a suspicion as to whether the data points for seminal vesicles mass and testes mass had a log relationship with the number of days in lab, due to the appearance of the graph. Therefore, this was also tested after transformation. An ANOVA on a linear model did not indicate a relationship between days the animals spent in lab and the log transformation of testes mass (Fig. 11, p=0.60). An additional ANOVA did not indicate a relationship between days the animals spent in lab and the log transformation of seminal vesicles mass (Fig. 13, p=0.42)

f- Testes mass, seminal vesicles mass, and IR-GnRH neuron distribution by season and capture date in male mice

A Welch's two tailed t-test indicated that there was no statistically significant relationship between season and testes mass (Fig. 15, t=0.39 p= 0.70), season and seminal vesicles mass (Fig. 16, t=0.64 p=0.53), or season and numbers of IR-GnRH neurons (Fig. 17, t=0.46 p=0.65). An ANOVA indicated that there was no statistically significant effect of capture date on testes mass (Fig. 18, p=0.77), seminal vesicles mass (Fig. 19, p= 0.65), or IR-GnRH neuron number (Figure 20, p=0.90).

g- GnRH neurons and male reproductive organ mass correlations

Testes Mass and Seminal vesicles mass:

There was a statistically significant positive correlation between testes mass and seminal vesicles mass was demonstrated (Fig. 21, df=43, R= 0.881, p=1.33e-15*).

GnRH neuron number and Testes Mass:

There was a statistically significant negative correlation between male testes mass and number of GnRH neurons (Fig. 22, df=43, R=-0.40, p=0.007*). Because there was a relationship between body mass and testes mass, we analyzed the data using an ANOVA, ANCOVA, and then a multiple linear regression. To show these relationships, we present figures as the residuals of testes mass regressed on body mass. A Pearson's correlation analysis on the

residuals for testes mass regressed on body mass graphed against the number of IR-GnRH neurons indicated a negative correlation, (Fig. 23, df=43, R=-0.50, p= 0.0005*) (ANOVA results, p=0.0005*). Since there are statistical concerns about using ANOVA to analyze residuals in a dataset (García-Berthou, 2001), we decided to analyze these data using ANCOVA. An ANCOVA indicated a statistically significant relationship between the total number of counted IR-GnRH neurons and testes mass (p= 0.0001*) and with body mass (Fig. 23, p= 0.011*). A multiple linear regression, which took into account the interaction between testes mass and body mass to compare to the number of IR-GnRH neurons, indicated a statistically significant relationship (Fig. 23, p= 0.010*).

In the graphs where IR-GnRH neurons were plotted against the residuals for testes mass regressed on body mass, there were different patterns for individuals captured in winter or in summer. Therefore, summer and winter individuals were separated for subsequent statistical tests.

Winter males: relationship between interaction of testes/body mass and IR-GnRH neurons

A Pearson's correlation analysis of the residuals for testes mass regressed on body mass and the number of IR-GnRH neurons indicated a statistically significant negative correlation, (Fig. 24, df=17, R=- 0.806, p= 0.00003*) and an ANOVA using the residuals indicated a statistically significant relationship (Fig. 24, p= 0.00003*). Again, because there are statistical concerns about using an ANOVA on residuals or correlations from a residuals plot to

analyze data (García-Berthou, 2001), we further analyzed these data using ANCOVA. An ANCOVA indicated a statistically significant relationship between the total number of counted IR-GnRH neurons and testes mass (p= 0.0002*) and with body mass (p= 0.014*) (Fig. 24). A multiple linear regression, which took into account the interaction between testes mass and body mass to compare to the number of IR-GnRH neurons, indicated a statistically significant relationship (Fig. 24, p= 0.001*).

Summer males: lack of relationship between interaction of testes/body mass and IR-GnRH neurons

A Pearson's correlation analysis of residuals for testes mass regressed on body mass and the number of IR-GnRH neurons did not indicate a statistically significant correlation, (Fig. 25, df=24, R= -0.099, p= 0.63). An ANOVA of the residuals from testes mass regressed on body mass and IR-GnRH neurons did not indicate a relationship (p=0.63). Again, because there are statistical problems with using a correlation analysis of a residuals plot to analyze these data (García-Berthou, 2001), we decided to analyze these data with ANCOVA. An ANCOVA did not indicate a statistically significant relationship between the total number of counted IR-GnRH neurons and testes mass (p= 0.96) or with body mass (p= 0.46) (Fig. 25). A multiple linear regression, which took into account the interaction between testes mass and body mass to compare to the number of IR-GnRH neurons, did not indicate a statistically significant relationship in summer males (Fig. 25, p= 0.97).

GnRH neuron number and Seminal vesicles mass:

For summer and winter males analyzed together, there was a statically significant negative correlation between seminal vesicles mass and number of GnRH neurons (Fig. 26, df=43, R=-0.370, p = 0.012*). For winter males, a Pearson's correlation analysis of seminal vesicles mass and number of IR-GnRH neurons indicated a statistically significant negative correlation (Fig. 27, df=17, R=-0.616, p=0.005*). For summer males, a Pearson's correlation analysis between seminal vesicles mass and number of IR-GnRH neurons did not indicate a statistically significant correlation (Fig. 28, df=24, R=-0.026, p=0.898)

h- Neuron counts for wild mice

The number of light neurons was an exploratory measure; presumably these light neurons do not contain as much IR-GnRH as more darkly stained neurons. If individuals have many darkly stained neurons, and a few lightly stained neurons, this may be telling us something different than individuals who have a few darkly stained neurons, with a few lightly stained neurons. If we only looked at the number of lightly stained neurons, we could not distinguish this difference. Therefore, we decided to investigate the number of lightly stained neurons and make it a proportion to the total number of observed IR-GnRH neurons. When the proportion of light neurons to the total number of GnRH neurons was graphed against testes mass, a positive correlation was indicated in winter males (R=0.686, p=0.001*).

A Pearson's correlation analysis of the residuals of testes mass regressed on body mass compared to the proportion of "light" IR-GnRH neurons to the total number of neurons, indicated a statistically significant positive correlation in winter males (df=17, Fig. 29, R=0.821, p= 1e-05*). Further analysis with an ANCOVA indicated a statistically significant relationship between the proportion of "light" IR-GnRH neurons and testis mass (p=0.0001*) and body mass (p=0.005*) (Fig. 29) in winter males. A multiple linear regression, which took into account the interaction between testes mass and body mass to compare to the number of IR-GnRH neurons, indicated a statistically significant relationship in winter males (Fig. 29, p=0.002*).

When the number of darkly stained IR-GnRH neurons (all of the neurons that were not considered to be "light IR-GnRH neurons") was graphed against testes mass, a negative correlation was indicated in winter males (df=17, R=-0.660, p= 0.002*).

A Pearson's correlation analysis of the residuals of testes mass regressed on body mass was graphed against the number of "darkly stained" IR-GnRH neurons, indicated a statistically significant negative correlation in winter males (Fig. 30, df= 17, R= -0.806, p= 3.15e-05*). Further analysis with an ANCOVA indicated a statistically significant relationship between the number of "darkly stained" IR-GnRH neurons and testes mass (p= 0.0006*) and body mass (p= 0.007*) (Fig. 30) in winter males. A multiple linear regression, which took into account the interaction between testes mass and body mass to compare to the

number of IR-GnRH neurons, indicated a statistically significant relationship in winter males (Fig. 30, p= 0.004*).

There was no statistically significant correlation demonstrated by a Pearson's correlation analysis between testes mass and proportion of light IR-GnRH neurons in summer males (df= 24, R=0.33, p=0.10). A Pearson's correlation analysis of the residuals of testes mass regressed on body mass compared to the proportion of "light" IR-GnRH neurons to the total number of neurons, indicated no statistically significant correlation in summer males (Fig. 31, df=24, R=0.22, p=0.27). An ANCOVA on data from summer males did not indicate a statistically significant relationship between the proportion of "light" IR-GnRH neurons and testes mass (p= 0.09) or body mass (p= 0.69) (Fig. 31). A multiple linear regression, which took into account the interaction between testes mass and body mass in relation to the number of IR-GnRH neurons, did not indicate a statistical relationship in summer males (Fig. 31, p= 0.054).

When the number of darkly stained IR-GnRH neurons were graphed against testes mass, no statistically significant correlation was indicated in summer males (df=24, R=-0.12, p=0.56).

A Pearson's correlation analysis of the residuals of testes mass regressed on body mass graphed against the number of "darkly stained" IR-GnRH neurons, indicated no statically significant correlation in summer males (Fig. 32, df= 24, R= -0.14, p= 0.48). Further analysis with an ANCOVA did not indicate a statistical relationship between the number of "darkly stained" IR-GnRH neurons and testes mass (p= 0.57) or body mass (p=0.699) (Fig. 32) in summer males. A multiple

linear regression, which took into account the interaction between testes mass and body mass to compare to the number of IR-GnRH neurons, did not indicate a statistical relationship in summer males (Fig. 32, p= 0.50).

i- In Summer males, parasite load was related to reproductive phenotype

The presence of botflies on male mice was related to reproductive phenotype in summer (Fig. 33 shows testes mass, Fig. 34 shows seminal vesicles mass). Animals which had a botfly parasite had a testes size (Fig. 36) which implies impaired sexual behavior based on unpublished data (Sharp et al., in manuscript). A Welch's two-sample t-test on animals that had parasites vs. animals that did not have parasites indicated that mice with one or more botflies had significantly smaller mean testes mass (Fig. 33, t=3.39, p=0.009) and mean seminal vesicles mass (Fig. 34, t=2.86 p=0.003) than animals which did not have botfly parasites. The presence of botfly parasites was not correlated with the number of IR-GnRH neurons (Fig. 35, t= 0.050 p=0.96).

j- Body mass comparison between male laboratory and wild populations

A t-test was used to detect differences between the laboratory control line and the wild population of Peromyscus. Animals captured in winter were significantly heavier than mice in short photoperiod in the laboratory (Fig. 37, t=2.60 p=0.014*). Animals captured during summer did not differ statistically in body

mass from those housed in long-day conditions in laboratory (Fig. 38, t=1.12 p=0.27).

k- Reproductive organ mass comparison between male laboratory and wild mice

A Welch's two-sample t-test comparing males in our unselected control line in the laboratory in short-day to males captured in winter indicated smaller testes in wild males (t = 17.35, P =2.2e-16 (Fig. 39).

l- Number of GnRH neurons in wild versus laboratory populations

Neuron counts between the laboratory population (Avigdor et al., 2005) and the wild population males under short day/winter conditions did not indicate a statistical difference (Fig. 40, t=0.58, p=0.57). Also, a comparison between the laboratory population conditions (Avigdor et al., 2005) and wild population males under long day/summer did not indicate a statistical difference (Fig. 40, t=0.57, p=0.57).

Wild-caught males had an average of 140 mature IR-GnRH neurons in the sections counted. Because only 1 out of every 4 brain slices was immunostained for GnRH, the mean number of neurons can be approximated by multiplying this mean by 4. Therefore, the mean estimate for these wild mice is approximately 560 IR-GnRH neurons. The minimum estimated number of IR-GnRH neurons detected was 76, and the maximum was 996. Because some neurons would be present in more than one section, the total estimated mean, estimated maximum, and estimated minimum is a slight overestimate (West, 2012: Schmitz and Hof

2005). Stereological adjustment would likely reduce these estimates (Schmitz and Hof 2005).

Data for Female Mice:

m- Female Body mass and number of days in lab

Animals varied in the number of days they spent in lab (0-15 days). A linear model was run on females collected in winter and in summer, to test for changes in body mass over the number of days in the laboratory. There was no significant effect of days in the laboratory on body mass of mice caught in winter and summer ($p=0.07$, Fig. 41).

n- Female Body mass and reproductive organ mass correlations

A Pearson's correlation analysis did not indicate a statistically significant correlation between initial body mass (mass on the date of capture) and ovary mass (Fig. 42, df= 15, $R=0.17$, $p=0.507$). A different Pearson's correlation analysis indicated a statistically significant positive correlation between ovary mass and final body mass (body mass on day of euthanasia) (Fig. 43, df=17, $R= 0.74$, $p= 0.0003$*). Uterine horn mass was not significantly correlated with initial body mass (Fig. 44, $R= 0.11$, df=15, $p=0.66$). There did not appear to be a statistically significant correlation between uterine horn mass and final body mass (Fig. 45, df= 17, $R= 0.40$, $p= 0.09$).

o- Number of days in lab and ovary mass, uterine horn mass, and IR-GnRH neuron number

An ANOVA on a linear model that included the number of days the animals spent in lab did not indicate a relationship with ovary mass (Fig. 46, p=0.062), uterine horn mass (Fig. 47, p=0.76), or on IR-GnRH neuron number (Fig 48, p=0.48).

p- GnRH neurons and female reproductive organ mass (ovary, uterine horn, and total reproductive mass)

Number of IR-GnRH neurons and Ovary Mass:

There appeared to be no statistically significant correlation between ovary mass and number of GnRH neurons (Fig. 49, df=17, R=.26, p=0.27).

Because there was a relationship between body mass and ovary mass, we analyzed these data using an ANCOVA, and then a multiple linear regression. To show these relationships, we present figures as the residuals of ovary mass regressed on body mass. An ANCOVA indicated a no statistical relationship between the total number of counted IR-GnRH neurons and ovary mass (p=0.295) or with body mass (p=0.85) (Fig. 50). A multiple linear regression, which took into account the interaction between ovary mass and body mass to compare to the number of IR-GnRH neurons, did not indicate a statistical relationship (Fig. 50, p= 0.23).

Number of IR-GnRH neurons and and uterine horn mass:

There appeared to be no statistically significant correlation between uterine horn mass and number of GnRH neurons (Fig. 51, df=17, R=.019, p= 0.94).

Number of IR-GnRH neurons and total reproductive organ mass:

There appeared to be no statistically significant correlation between total reproductive organ mass (ovary + uterine horn mass) and number of GnRH neurons for summer and winter females (Fig. 52, df=25, R=0.17, p=0.40). When winter females were analyzed, there appeared to be no statistically significant correlation between total reproductive organ mass and number of GnRH neurons for winter females (Fig. 53, df=11, R=0.08, p=0.80). When summer females were analyzed, there appeared to be no statistically significant correlation between total reproductive organ mass (and number of GnRH neurons for summer females (Fig. 54, df=11, R=0.21, p=0.49). There did not appear to be a statistically significant correlation between total reproductive organ mass and body mass for winter females (Fig. 55, df=11,R= 0.44, p=0.14), or for summer females (Fig. 56, df=11, R=0.53, p=0.07). However, since there was a statistically significant correlation between total reproductive organ mass and body mass for all females combined (Fig. 57, df=25, R=0.46, p=0.017*), I decided to analyze this female reproductive organ mass data further, using body mass as a covariate.

Because there was a relationship between body mass and total female reproductive organ mass when summer and winter females were analyzed together, we analyzed these data using an ANCOVA, and then a multiple linear regression. To show these relationships, we present figures as the residuals of total reproductive organ mass regressed on body mass. An ANCOVA indicated no statistical relationship between the total number of counted IR-GnRH neurons

and total reproductive organ mass (p=0.42) and with body mass (p=0.43) (Fig. 58) for summer and winter females combined. A multiple linear regression for summer and winter females combined, which took into account the interaction between total reproductive organ mass and body mass to compare to the number of IR-GnRH neurons, did not indicate a statistical relationship (Fig. 58, p= 0.31).

For winter females, reproductive organ mass and body mass were analyzed as covariates in an ANCOVA, and then a multiple linear regression was used to further analyze the data. To show these relationships, we present figures as the residuals of total reproductive organ mass regressed on body mass. An ANCOVA indicated a no statistical relationship between the total number of counted IR-GnRH neurons and total reproductive organ mass (p=0.82) or with body mass (p=0.62) (Fig. 59) in winter females. A multiple linear regression on winter females, which took into account the interaction between total reproductive organ mass (ovary + uterine horn mass) and body mass to compare to the number of IR-GnRH neurons, did not indicate a statistical relationship (Fig. 59, p= 0.74).

For summer females, reproductive organ mass and body mass were analyzed as covariates in an ANCOVA, and then a multiple linear regression was used to further analyze the data. To show these relationships, we present figures as the residuals of total reproductive organ mass regressed on body mass. An ANCOVA indicated no statistical relationship between the total number of counted IR-GnRH neurons and total reproductive organ mass (p=0.52) or with

body mass (p=0.67) (Fig. 60) in summer females. A multiple linear regression on summer females, which took into account the interaction between total reproductive organ mass and body mass to compare to the number of IR-GnRH neurons, did not indicate a statistically significant relationship (Fig. 60, p=0.41).

q- Comparison between laboratory and wild mice reproductive organ mass

Females:

Females were more strongly suppressed by short days in the laboratory than by winter in nature. There was significantly greater suppression of reproductive tract mass in the lab among short day females in our unselected control line than observed in winter in the wild (Fig. 61, t=3.4, p<0.01)

3- DISCUSSION

Overall, the wild population appears to be similar to the laboratory population with respect to the distribution of seminal vesicles mass and testes mass (Fig. 39). In addition, the distribution of IR-GnRH neurons appears to be similar between the laboratory and wild populations (Fig. 40). In wild populations, there were few seasonal differences between the distributions of body mass, IR-GnRH neuron number (Fig. 17), testes mass (Fig. 15), & seminal vesicles mass (Fig. 16). In the summer, the wild population has as many reproductively suppressed males as the wild population in winter (Fig. 3), whereas few mice in the

laboratory are reproductively suppressed under long-day conditions. This is consistent with previous findings from the wild natural population in Williamsburg, Virginia (Terman, 1993).

In this study, there was no evidence that the number of days mice were held in the laboratory affected reproductive phenotype, body mass, or number of GnRH neurons (Figs. 4-14). Wild mice captured during winter gained an average of 1.3g in the laboratory, while mice collected during summer gained an average of 0.95g in the laboratory, but these increases were not statistically significant. However, in both cases, this change in weight was not significantly related to the number of days in the laboratory (Fig. 4). This is consistent with findings in similarly sized rodent species, such as the Syrian and Siberian hamster, in which seasonal changes in body mass usually occur on the timescale of weeks or months (Bartness et al., 2002). In addition, there was no significant relationship between the number of days spent in the laboratory and testes mass/seminal vesicles mass (Figs. 10 & 12). This finding is also consistent with finding by Terman (1999) within the *Peromyscus* genus, showing that access to supplemental food in the wild population did not affect reproduction (Terman, 1999). Finally, there was no statistically significant variation in the number of GnRH neurons in relation to the number of days in the laboratory (Fig. 14). Therefore, we did not include days in the laboratory in further analyses.

Interestingly, winter and summer-caught mice were similar in most measures. Capture date (Julian date) did not appear to have a significant relationship with testes mass (Fig. 18), seminal vesicles mass (Fig. 19), or IR-GnRH neuron

number (Fig. 20). Season also did not appear to have a significant relationship with testes mass, seminal vesicles mass, or IR-GnRH neuron number.

There was an unexpected negative correlation between reproductive organ mass and number of IR-GnRH neurons (Fig. 24). Mice with larger reproductive organ mass appear to have lower numbers of neurons, while mice with lower reproductive organ mass did not demonstrate a relationship between seminal vesicles mass and numbers of IR-GnRH neurons. Specifically, males that were assessed as likely to be azoospermic or likely to have reduced reproductive behavior, based on laboratory findings (Sharp et al., in manuscript) had no relationship between reproductive organ mass and number of IR-GnRH neurons (Fig. 22). Only in reproductively active animals did it appear that lower number of GnRH neurons was related to larger reproductive organs.

However, when the residuals of male reproductive organs regressed on body mass were analyzed with IR-GnRH neurons, a difference between summer and winter animals was discerned. In winter animals, there was a negative correlation between the male reproductive organ mass (when adjusted for body mass) and number of IR-GnRH neurons (Fig. 24), whereas in summer, there was no relationship (when male reproductive organ mass was adjusted for body mass) (Fig. 25). Therefore, this indicates there is likely a different mechanism controlling the reproductive cascade, at least on the level of GnRH, between summer and winter in these populations.

In winter males, it is possible that the low counts of IR-GnRH neurons in reproductively active animals was due to our inability to visualize all of the IR-GnRH neurons. It has been shown in other rodents that GnRH receptors are not down regulated in the non-breeding season, despite the decreased levels of LH and T (Oosthuizen and Bennett 2007). Therefore, it is plausible the GnRH hormone itself is being down regulated. Therefore, GnRH might be stored in the neurons of reproductively inactive animals because they are not releasing GnRH at a high rate when not in breeding condition. Conversely, in reproductively active animals in winter GnRH stores within GnRH neurons could be low due to rapid release. The result would be greater difficulty detecting GnRH neurons in the most reproductively active males. This interpretation is consistent with our finding that the mice with the largest testes mass also have the highest proportion of lightly-stained GnRH neurons (Fig. 29) and lowest number of darkly-stained GnRH neurons (Fig. 30). This is a possible explanation for observing fewer immunoreactive GnRH neurons in reproductively active male animals.

Females may be affected differently than males, as there did not appear to be a statistically significant relationship between the number of IR-GnRH neurons and reproductive organ mass (Fig. 50). It is poorly understood why animals are reproductively suppressed during the summer season, because the reproductive suppression is not due to the short photoperiodic signal. In this experiment, we did not observe a correlation between male reproductive organ mass (if we adjust reproductive organ mass for body mass) and GnRH neurons in summer

males. However, I speculate that the summer reproductive suppression in *Peromyscus leucopus* might be due at least in part to parasite load (Figs. 33 & 34). In Fig. 25, in which testes mass is adjusted for body mass, most of the animals with botfly parasites had a lower testes mass then we would expect for the individuals body mass. My data indicates that males with botfly parasites had suppressed reproduction during the summer season (Figs. 28, 31, 33, 34). Reproductive suppression in the presence of parasitic infections has been documented by other studies (Vandegrift et al., 2008).

It may be that there are a number of environmental stressors that can suppress reproduction in the wild. If the reproductive axis in these mice is sensitive to environmental inputs of such environmental stressors as parasitic infection or illness, then any stressor, including short photoperiod, illness, disease, or reduced food, might suppress reproduction. Laboratory mice are not exposed to all of these stressors on a regular basis, which may allow males in the laboratory to maintain larger testes masses than those in the wild. Wild mice might be responding to additional factors which are suppressing reproduction in the wild more than in the laboratory.

The body masses of males captured in winter were significantly larger than the mass of mice in the laboratory in short-day conditions (Fig. 37). However, summer-caught wild mice appeared to be similar in mass to those in the laboratory in short-day conditions (Fig. 38). The wild populations and laboratory population of mice appear to have similar distributions of IR-GnRH neuron number (Fig. 40). The means for the counted number of IR-GnRH neurons

between the summer animals in the wild (from this study) and long-day animals in the laboratory (Avigdor et al., 2005) did not differ significantly (Fig. 40). Also, the mean numbers of IR-GnRH neurons between the winter animals in the wild (from this study) and short-day animals in the laboratory (Avigdor et al., 2005) were not significantly different (Fig. 40).

In the laboratory, testes mass has not been found to be correlated with the number of GnRH neurons (Avigdor et al., 2005). In the wild population, in contrast, high numbers of immunoreactive GnRH neurons were correlated with low testes mass (Fig. 24) and low seminal vesicles mass.

During winter in the wild, males appeared to be more reproductively suppressed than in the laboratory in short-day. Conversely, females under short-day conditions in the laboratory were significantly more reproductively suppressed in than in the wild (Fig. 61). The distribution of the data points appears to differ between males in the wild versus males in the laboratory in the first winter season (Fig. 39). Within the distribution of "wild" males, two individuals have a testes mass, of around 0.65g, with no individuals in the range of 0.35-0.65g. Conversely, the laboratory population had an even distribution of individuals up to 0.4g. Many individuals had a testes mass in the range of 0.1g in the wild population, while the laboratory population had a more even distribution between 0.1g and 0.4g. Wild females appeared to demonstrate a similar pattern (Fig. 61). For total reproductive organ mass, there are no data points between 0.03g and 0.045g. This difference between lab and field could be the result of a difference in selective pressures: for those in the wild that suppress their

reproductive system, it may be imperative to minimize energy costs when not producing sperm or eggs. Conversely, in the laboratory with food in excess, it might be advantageous to support enough tissue to resume reproduction as quickly as possible. This may explain why the testes mass of many males is concentrated around 0.1g; this testes mass has also been determined in our laboratory to be just below the threshold necessary to fertilize a female (Sharp et al., in manuscript). However this trend seemed to disappear in the second season. Two winter seasons may not be enough to resolve whether this gap and clustering of points in the first winter season was due to random chance, or if this is a real phenomena and the second winter did not have a large enough sample size to visualize this pattern.

Interestingly, seminal vesicle and testes mass were positively correlated with body mass (Figs. 7 & 8). It has been demonstrated that under short-day laboratory conditions, food intake and body mass is associated with testes mass (Heideman et al., 2005: Kaseloo et al., 2014). However, in wild males, there was a correlation between body mass and reproductive organ mass in both summer and winter conditions. Previous results have indicated midsummer suppression of reproduction in this population (Terman, 1993: Terman, 1998: Terman, 1999), even though mice in long-day in the laboratory are not reproductively suppressed (Avigdor et al., 2005). In male mice, it also appears that more wild males became reproductively inactive than did laboratory control line males. This may be due to environmental stressors that occur under natural conditions, but not in the laboratory, such as parasite load.

Overall, mice in the wild appear to have responses to environmental factors that are not present in the laboratory. These responses included body mass, testes mass, and seminal vesicles mass. In contrast, total IR-GnRH neuron number may have low plasticity, causing these to be shared between the laboratory and wild populations. The differences between laboratory and wild mice identified in this study support the proposal that researchers should verify their findings in natural populations before data can be extrapolated from the laboratory to natural populations.

Future Directions

It would be interesting to collect animals from the same population in the spring and fall seasons, when these animals are reported to be reproductively active, to see if animals show more numbers of IR-GnRH neurons on average (that reproductively active animals have large numbers of IR-GnRH neurons), as well as no correlation with between reproductive organ mass and IR-GnRH neurons. If animals do display more IR-GnRH neuron numbers in the breeding season, it would lend support to the idea that we are not seeing all of the existing neurons in the animals which remain reproductively active in the non-breeding season. Studies examining mRNA production could be conducted on wild mice to see if the GnRH is down regulated during the non-breeding season. If GnRH is down regulated, it would be easier to deplete the GnRH, which is stored in these neurons.

It would also be interesting to expose laboratory animals to environmental stressors which mimic those found in the wild to see if they react similarly to animals in the wild (with respect to the negative correlation between IR-GnRH neuron number and reproductive organ mass, or suppression of reproduction under long day conditions). It would also be interesting to restrict food access in long day mice, and examine if the photoperiod responsive animals suppress reproduction under long days in the lab, and then examine the numbers of IR-GnRH neurons to see if the negative correlation between testes mass and IR-GnRH is similar to the wild population under these conditions.

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FIGURE LEGENDS

Figure 1. Illustration of the "Pin" style apparatus designed to hold the Sherman trap in place, so the trap was less likely to be disturbed.

Figure 2. Illustration of the "wire mesh" style apparatus designed to hold the Sherman trap in place, so the trap was less likely to be disturbed.

Figure 3. Percentage of mice reproductive suppressed (Reproductively inactive male mice defined as a paired testes mass of 0.1, and inactive female mice as a total reproductive organ mass of 0.02 or less) during winter 1995 (Heideman et al. 1999) and winter 2012-13.

Figure 4. Change in male body mass and number of days in lab (p= 0.97) (Winter animals are the filled points [p=0.55, for winter animals and days in lab]), while summer animals are the open points)

Figure 5. final male body mass (on the day of perfusion) and the number of days in lab (Winter animals are the filled points, while summer animals are the open points)

Figure 6. Comparison of initial body mass (on date of capture) and testes mass. R=0.51, p= 0.0017*

Figure 7. Comparison of final body mass (on perfusion date) and testes mass.(R= 0.618, p=6.14e-06*)

Figure 8. Comparison of initial body mass (on date of capture) and seminal vesicles mass.(R= 0.382, p= 0.024*)

Figure 9. Comparison of final body mass (on perfusion date) and seminal vesicles mass.(R= 0.614, p = 7.33e-06)

Figure 10. Number of days in lab and testes mass. p=0.39 (Winter animals are the filled points, while summer animals are the open points)

Figure 11. Number of days in lab and the log transformation of testes mass. p=0.60.

Figure 12. Number of days in lab and Seminal vesicles mass. p=0.43 (Winter animals are the filled points, while summer animals are the open points)

Figure 13. Number of days in lab and the log transformation of seminal vesicles mass. p=0.42.

Figure 14. Number of days in lab and total observed IR-GnRH neuron number. P=0.97 (Winter animals are the filled points, while summer animals are the open points)

Figure 15. Testes mass distribution by season. p=0.68

Figure 16. Seminal vesicles mass distribution by season. p= 0.49

Figure 17. Total observed number of IR-GnRH neurons by season. p=0.64

Figure 18. Testes mass and capture date (in Julian date) p=0.77.

Figure 19. Seminal vesicles mass and capture date. p= 0.65

Figure 20. IR-GnRH neuron count and capture date. p=0.90

Figure 21. Scatter plot of testes mass and seminal vesicles mass. R= 0.881, p=1.33e-15

Figure 22. The relationship between testes mass and number of Immunoreactive GnRH neurons in a defined region of the brain (plate 12-25 (Paxinos and Watson, 1989)) R=-0.397, p =0.007* (the grey line at 0.1g indicates males predicted to be azoospermic, and the grey line at 0.25g indicates animals predicted to have impaired sexual behavior (Sharp et al., in Manuscript and Broussard et al., 2009))

Figure 23. Plot of residuals from testes mass regressed on body mass, compared to the total number of IR-GnRH neurons. R=-0.498, p= 0.0005*.

Figure 24. Plot of residuals for winter males from testes mass regressed on body mass, compared to the total number of IR-GnRH neurons. R=- 0.806, p= 3.068e-05*

Figure 25. Plot of residuals for summer males from testes mass regressed on body mass, compared to the total number of IR-GnRH neurons. R= -0.099, p= 0.63.

Figure 26. The relationship between Seminal vesicles mass and number of Immunoreactive GnRH neurons in a defined region of the brain (plate 12-25 (Paxinos and Watson, 1989)) R=-0.370, p = 0.012* (Winter animals are the filled points, while summer animals are the open points)

Figure 27. Scatter plot of winter male seminal vesicles mass and number of total observed IR-GnRH neurons. R=-0.616, p=0.00496*

Figure 28. Scatter plot of summer male seminal vesicles mass and number of total observed IR-GnRH neurons. R=-0.026, p=0.898

Figure 29. Plot of residuals for winter males from testes mass regressed on body mass, compared to the proportion of "light" IR-GnRH neurons to the total number of IR-GnRH neurons. R=0.821, p= 1.62e-05.

Figure 30. Plot of residuals for winter males from testes mass regressed on body mass, compared to the number of "darkly stained" IR-GnRH neurons (dark

neurons are defined in this paper as all of the observed IR-GnRH neurons that were not considered to be "light" neurons). R= -0.806, p= 3.15e-05*.

Figure 31. Plot of residuals for summer males from testes mass regressed on body mass, compared to the proportion of "light" IR-GnRH neurons to the total number of IR-GnRH neurons. R= 0.23, p= 0.27

Figure 32. Plot of residuals for summer males from testes mass regressed on body mass, compared to the number of "darkly stained" IR-GnRH neurons (dark neurons are defined in this paper as all of the observed IR-GnRH neurons that were not considered to be "light" neurons). R= -0.14, p= 0.48

Figure 33. Summer animal testes mass with and without botflies (t= 3.388, p=0.009*)

Figure 34. Summer animals seminal vesicles mass with and without botflies (t=2.859, p=0.003*)

Figure 35. Summer animals, total counted IR-GnRH neurons with and without botflies t= 0.05 p=0.96

Figure 36. Scatterplot containing IR-GnRH neuron number and testes mass (the grey line at 0.1g indicates males that would be azoospermic, and the grey line at 0.25g indicates animals that would have impaired sexual behavior (Sharp et al., in manuscript and Broussard et al., 2009) (open circles indicate summer animals without parasites, and open circles with x's indicate animals in summer with parasites. Closed circles indicate winter animals)

Figure 37. Comparison between the body mass of the laboratory population (Kaugars et al., 2013) and the wild population. Short day/winter animals are statistically different between the wild and laboratory populations (t=2.602 p=0.014*)

Figure 38. Comparison between the body mass of the laboratory population (Kaugars et al., 2013) and the wild population. Long day/summer animals are not statistically different between the wild and laboratory populations (t=1.12 p=0.27).

Figure 39. Comparison of male reproductive organ masses between the laboratory control (minimal artificial selection) line (SeJun, Ives, and Heideman, unpublished), and the wild natural population. Light line located at 0.1 g indicates the testes mass used to determine if an animal is reproductively active or inactive (Heideman et al., 1999, Sharp et al., in manuscript, Broussard et al., 2009). The black line indicates the mean for each group. (t=17.35 p=2.2e-16)

Figure 40. Comparison of total number of observed IR-GnRH neurons between laboratory and wild populations (Winter/Short day males: t=0.5758, p=0.5687) (Summer/Long day males: t=0.57, p=0.57).

Figure 41. final female body mass (on the day of perfusion) and the number of days in lab (Winter animals are the filled points, while summer animals are the open points). p=0.07

Figure 42. Comparison of initial body mass (on date of capture) and ovary mass. R=0.1731, p=0.5065

Figure 43. Comparison of final body mass (on date of perfusion) and ovary mass. R= 0.736, p= 0.0003*

Figure 44. Comparison of initial body mass (on date of capture) and uterine horn mass. R= 0.11, p=0.66.

Figure 45. Comparison of final body mass (on date of perfusion) and uterine horn mass. R= 0.40, p=0.09.

Figure 46. Scatterplot of ovary mass and the number of days in lab. p=0.06

Figure 47. Scatterplot of uterine horn mass and the number of days in lab. p=0.76

Figure 48. Scatterplot number of observed IR-GnRH neurons and the number of days in lab. p=0.48

Figure 49. Scatterplot between ovary mass and the total number of observed IR-GnRH neurons. R=.26, p=0.27.

Figure 50. Residuals of ovary mass regressed on body mass plotted against the total number of observed IR-GnRH neurons.

Figure 51. Scatterplot between uterine horn mass and the total number of observed IR-GnRH neurons. R=0.02, p= 0.94.

Figure 52. Scatterplot showing female reproductive organ mass (ovary + uterine horn mass) and number of GnRH neurons for summer and winter females (R=0.17, p=0.40).

Figure 53. Scatterplot showing winter female reproductive organ mass (ovary + uterine horn mass) and number of GnRH neurons for winter females (R=0.08, p=0.80).

Figure 54. Scatterplot showing summer female reproductive organ mass (ovary + uterine horn mass) and number of GnRH neurons for summer females (R=0.21, p=0.49)

Figure 55. Scatterplot for total reproductive organ mass (ovary + uterine horn mass) and body mass for winter females (R= 0.44, p=0.14),

Figure 56. Scatterplot for total reproductive organ mass (ovary + uterine horn mass) and body mass for summer females (R=0.53, p=0.07),

Figure 57. Scatterplot for total reproductive organ mass (ovary + uterine horn mass) and body mass for all females combined (summer and winter females) (R=0.456, p=0.017*)

Figure 58. Residuals of total reproductive organ mass (ovary + uterine horn mass) regressed on body mass plotted against the total number of observed IR-GnRH neurons for all females (winter and summer females) (p=0.31).

Figure 59. Residuals of total reproductive organ mass (ovary + uterine horn mass) regressed on body mass plotted against the total number of observed IR-GnRH neurons for winter females (p=0.74).

Figure 60. Residuals of total reproductive organ mass (ovary + uterine horn mass) regressed on body mass plotted against the total number of observed IR-GnRH neurons for summer females (p=0.41).

Figure 61. Comparison of female reproductive organ masses between the laboratory control (minimal artificial selection) line (Mahoney and Heideman, unpublished), and the wild natural population. ($t = 3.4$, $P < 0.01$,)

Figure 1.

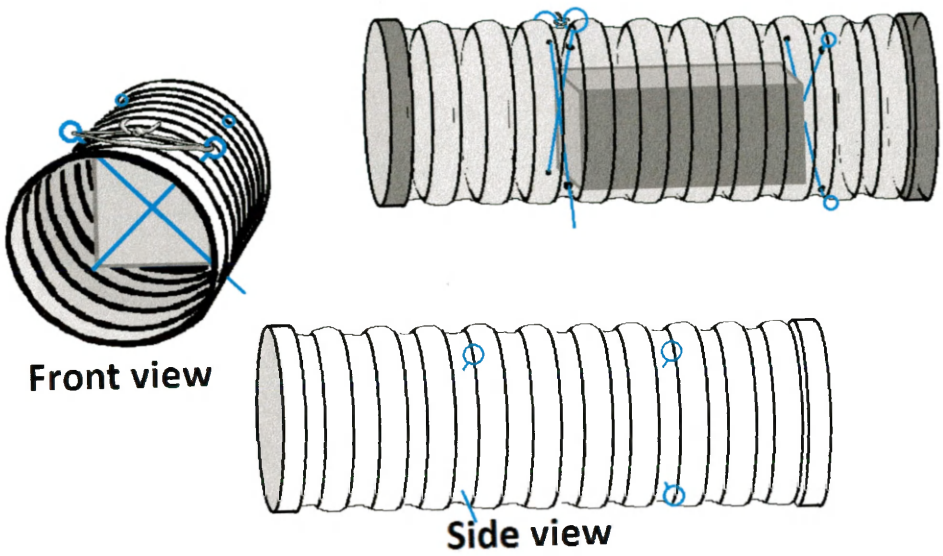


Figure 2

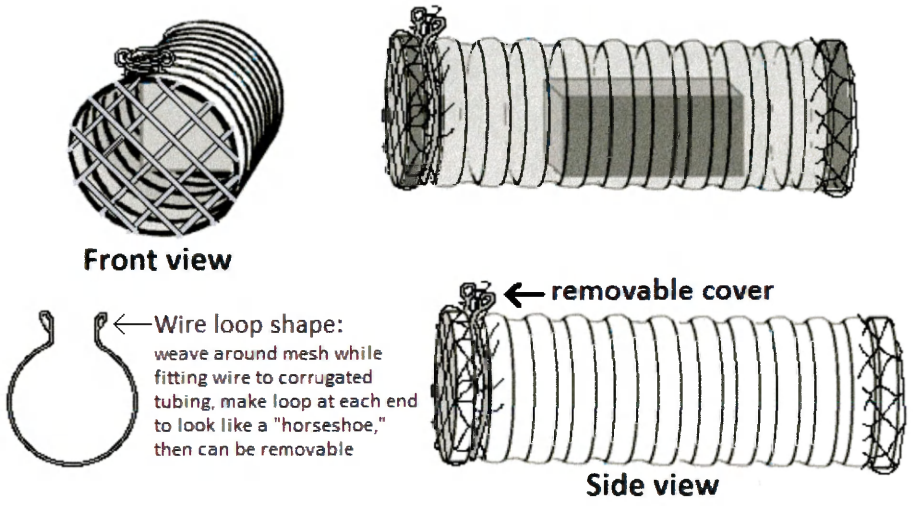

Front view
Wire loop shape:
weave around mesh while fitting wire to corrugated tubing, make loop at each end to look like a "horseshoe," then can be removable
removable cover
Side view

Figure 3.

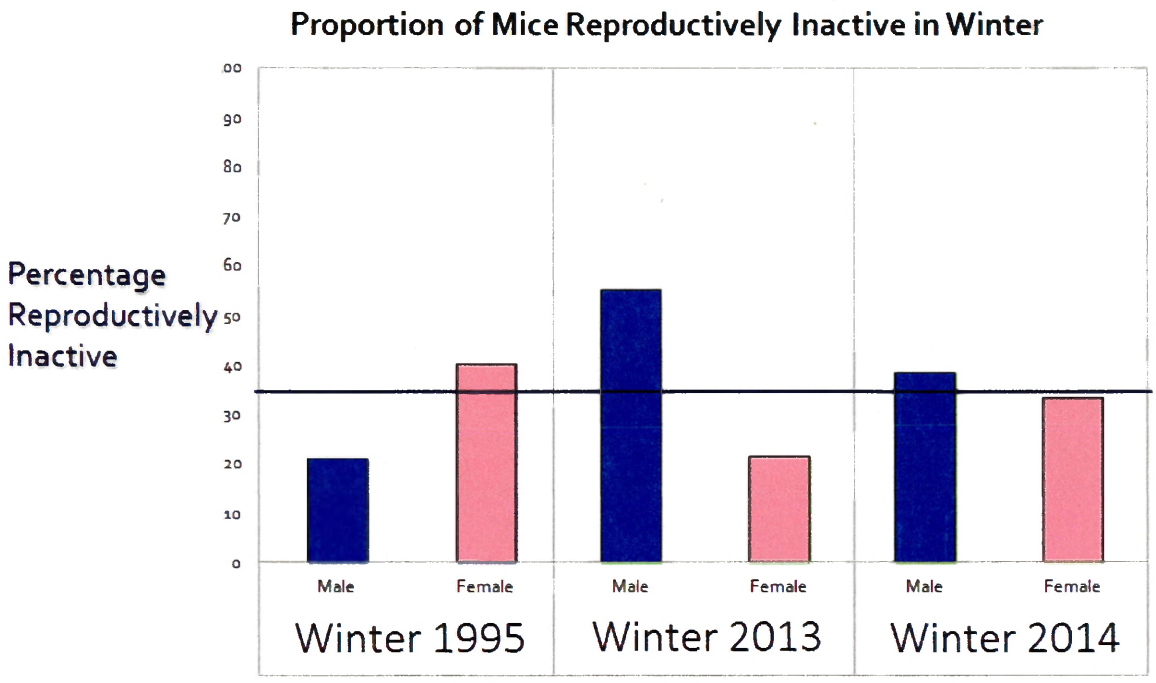

Proportion of Mice Reproductively Inactive in Winter
Percentage Reproductively Inactive
00
90
80
70
60
50
40
30
20
10
0
Male
Female
Male
Female
Male
Female
Winter 1995
Winter 2013
Winter 2014

Figure 4

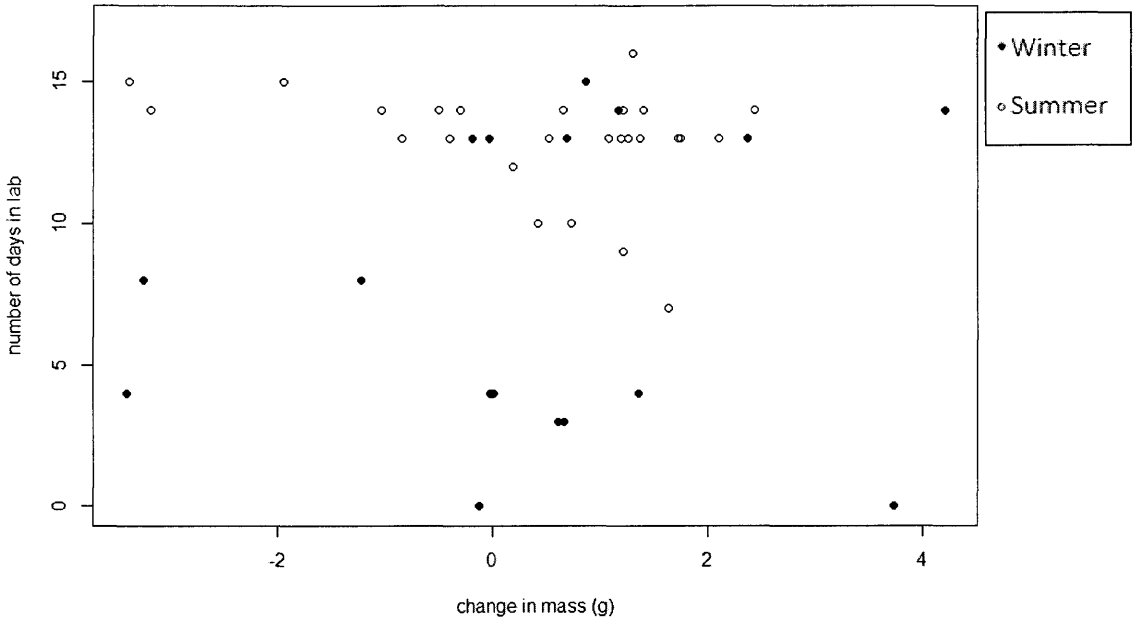

Winter
Summer
number of days in lab
0
5
10
15
-2
0
2
4
change in mass (g)

Figure 5.

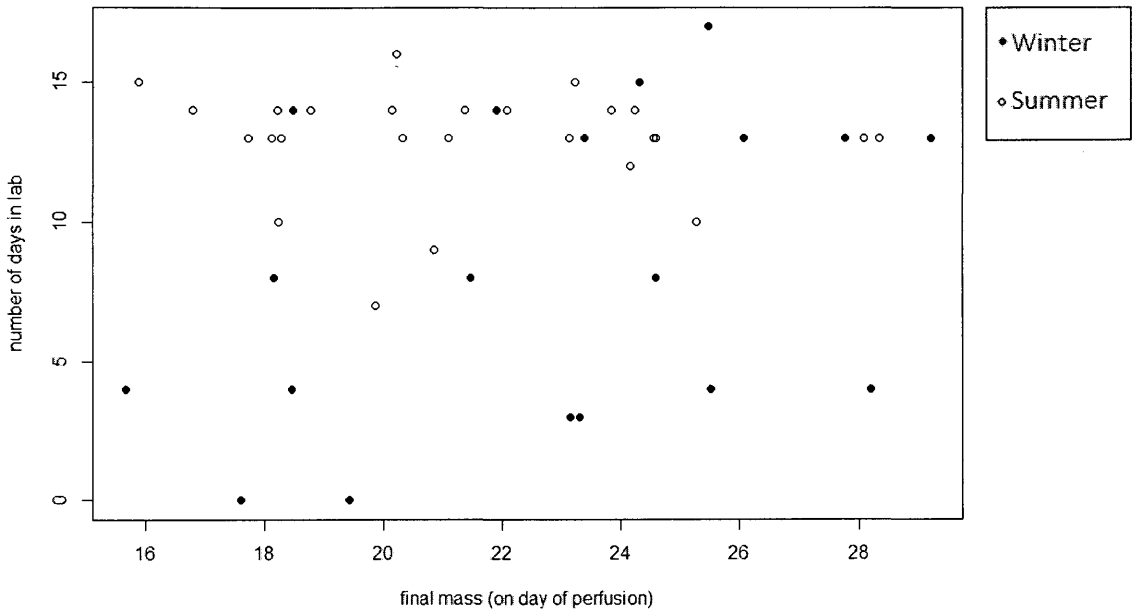

Winter
Summer
number of days in lab
0
5
10
15
16
18
20
22
24
26
28
final mass (on day of perfusion)

Figure 6.

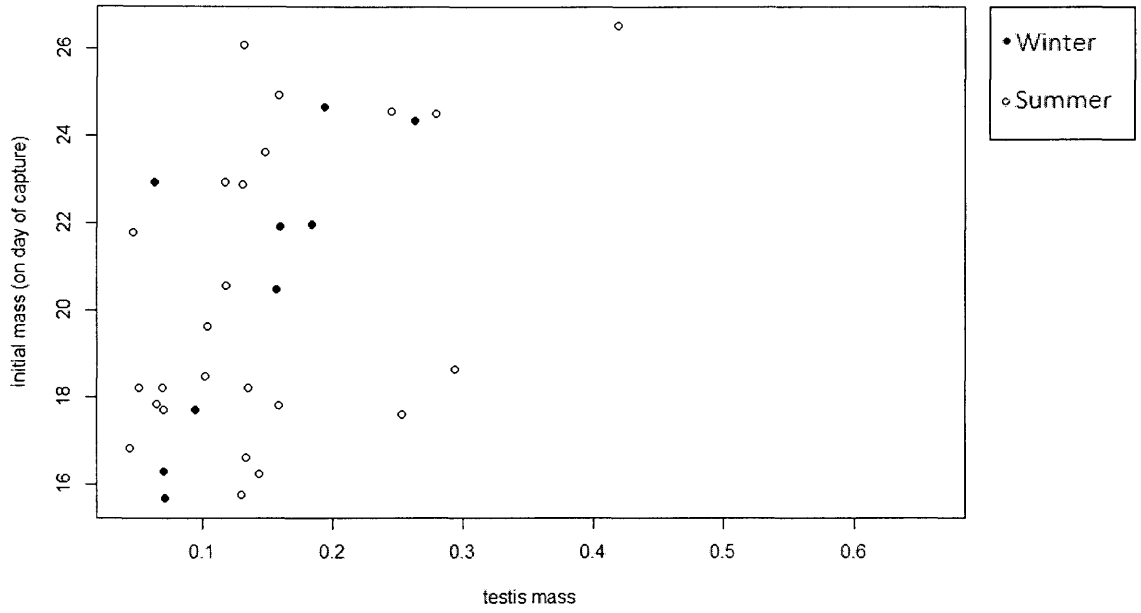
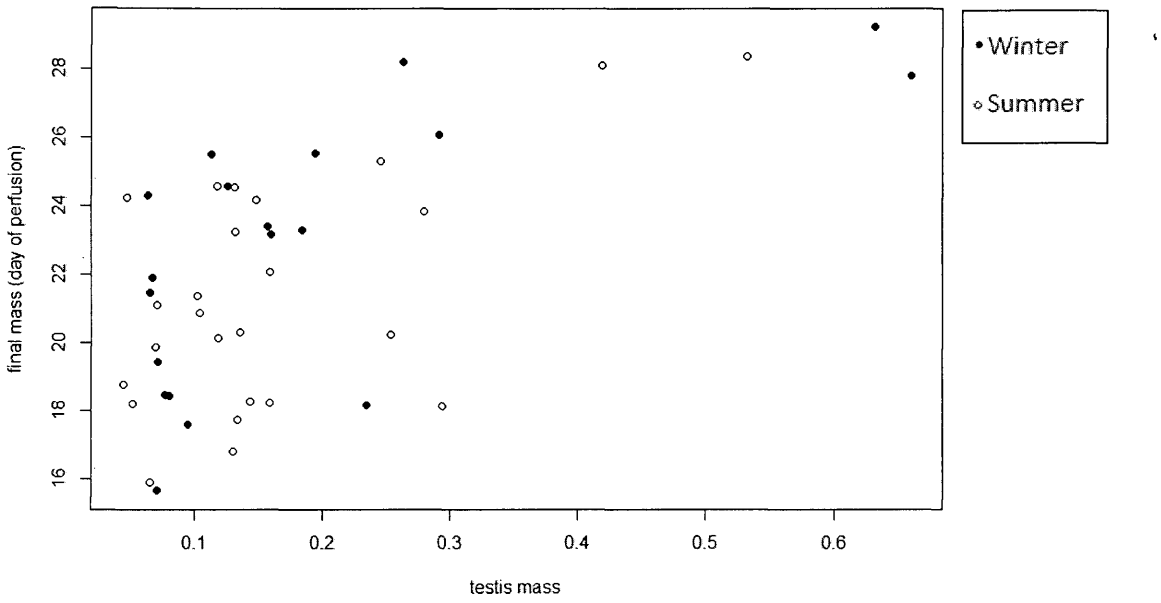

Winter
Summer
Initial mass (on day of capture)
16
18
20
22
24
26
0.1
0.2
0.3
0.4
0.5
0.6
testis mass

Figure 7.



R=0.6178328

p= 6.136e-06

Figure 8.

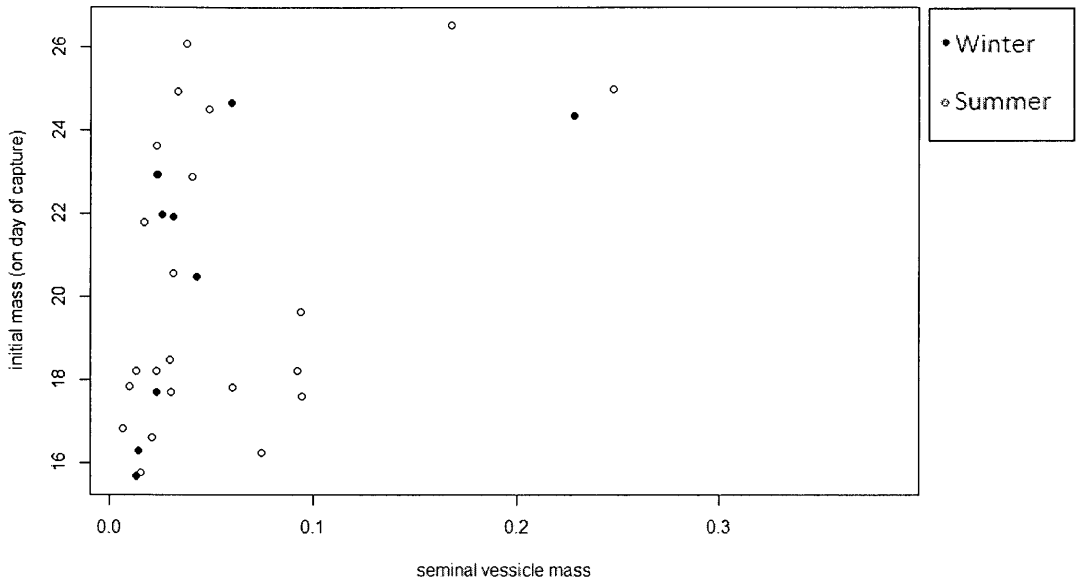

initial mass (on day of capture)
16
18
20
22
24
26
0.0
0.1
0.2
0.3
seminal vessicle mass
Winter
Summer

Figure 9.

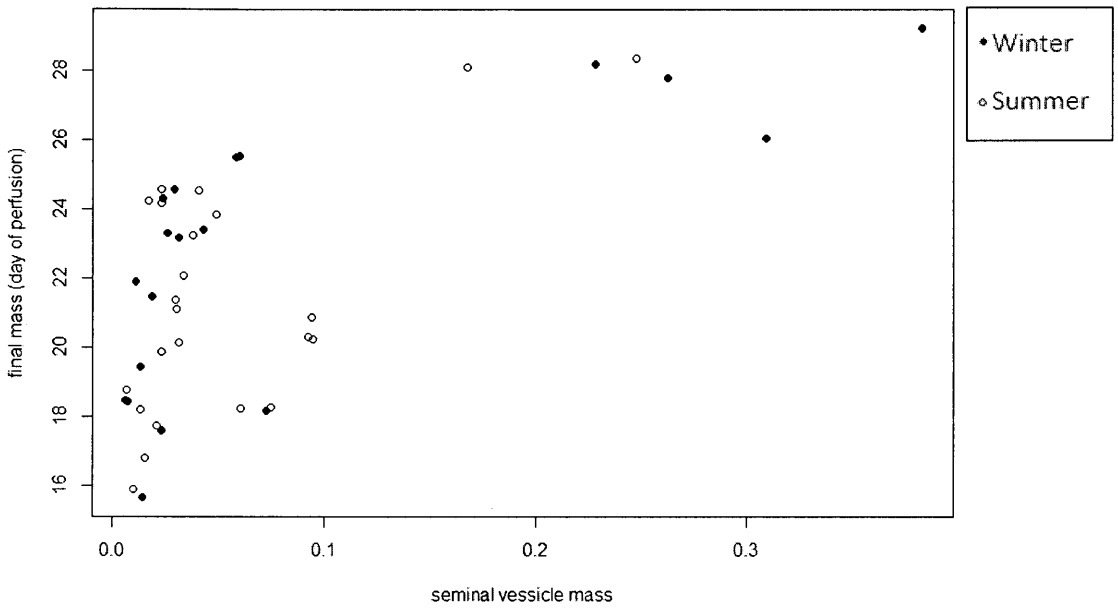
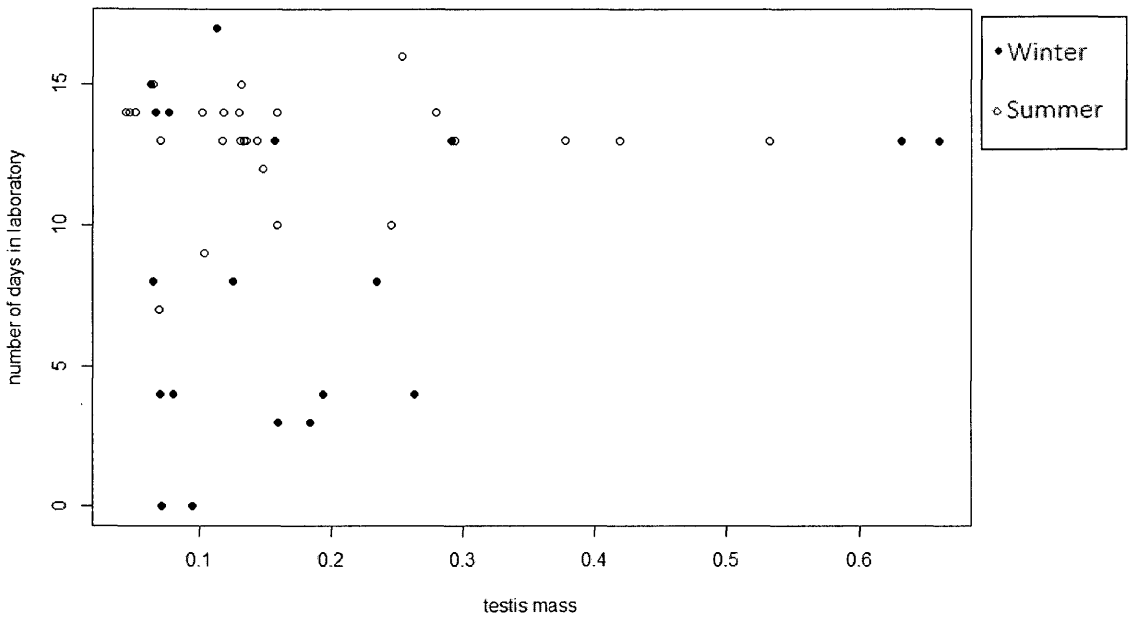
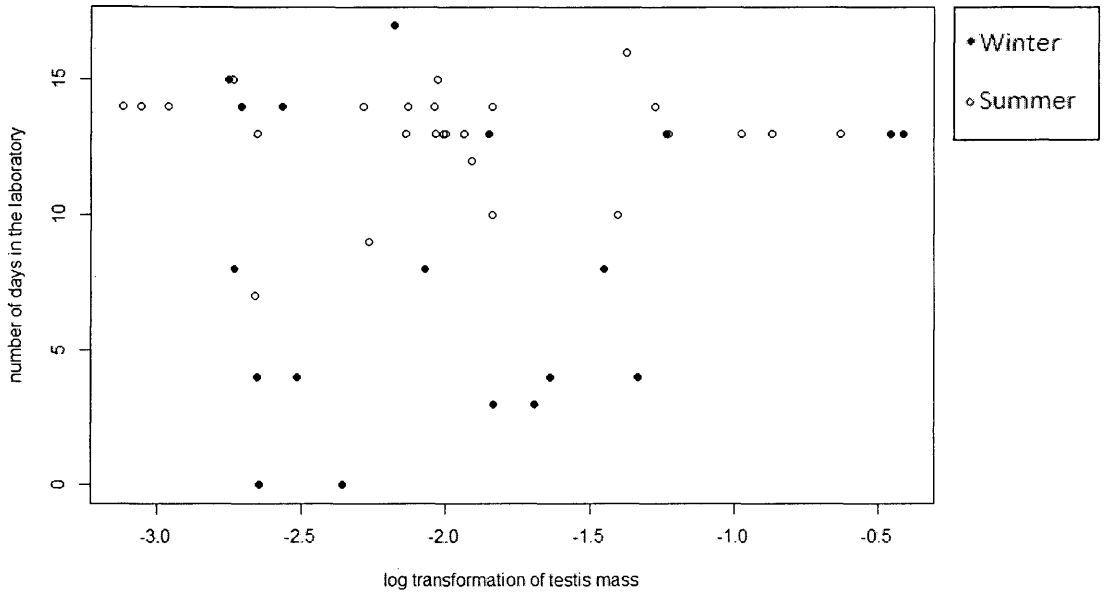

final mass (day of perfusion)
16
18
20
22
24
26
28
0.0
0.1
0.2
0.3
seminal vessicle mass
Winter
Summer

Figure 10.



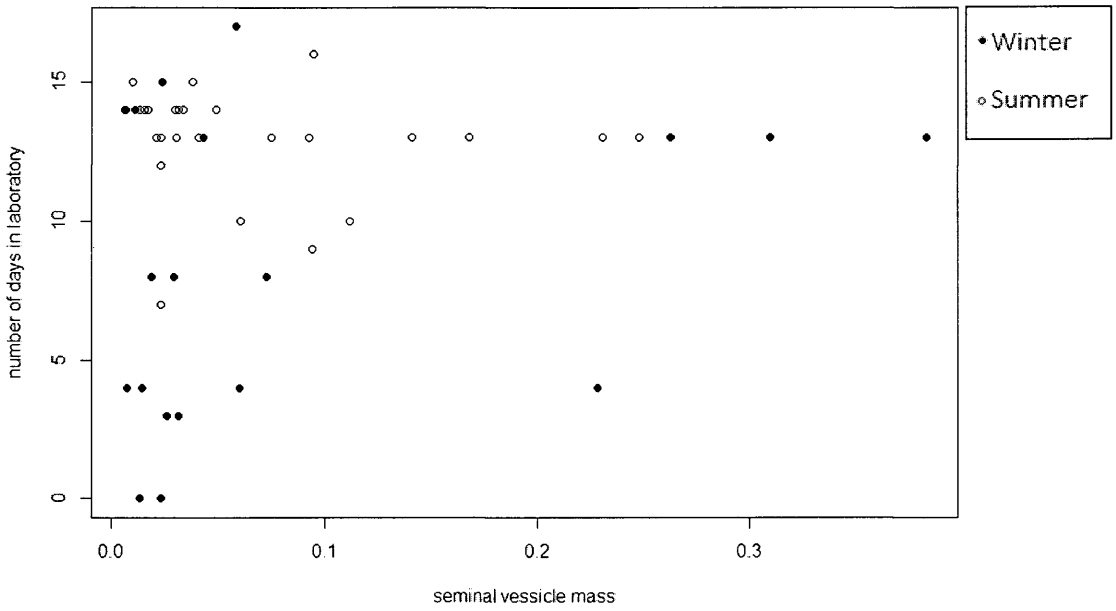
p= 0.3925

Figure 11.



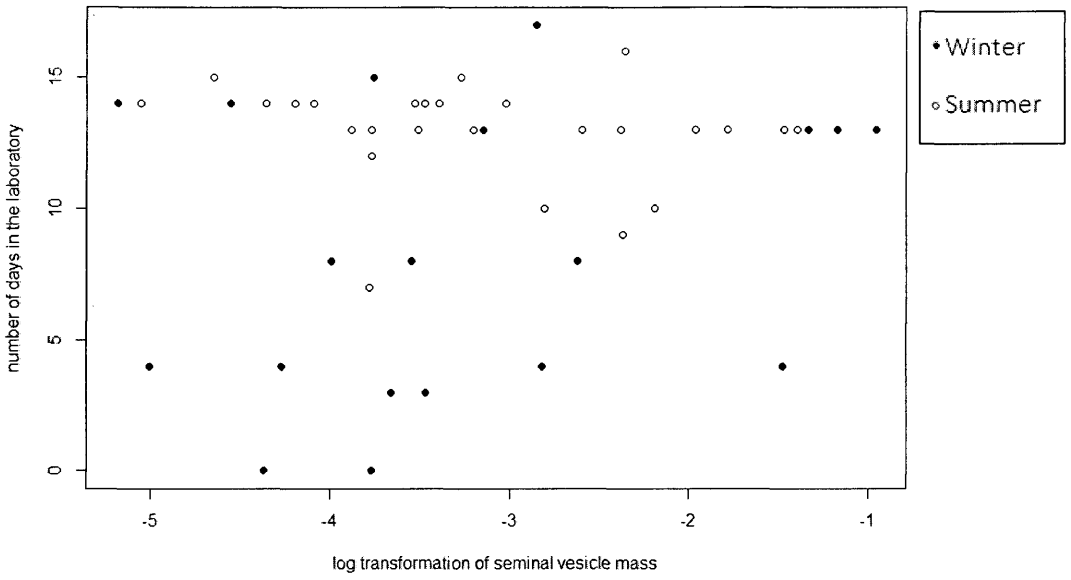
p=0.6029

Figure 12.



p= 0.4268

Figure 13.



p= 0.418

Figure 14.

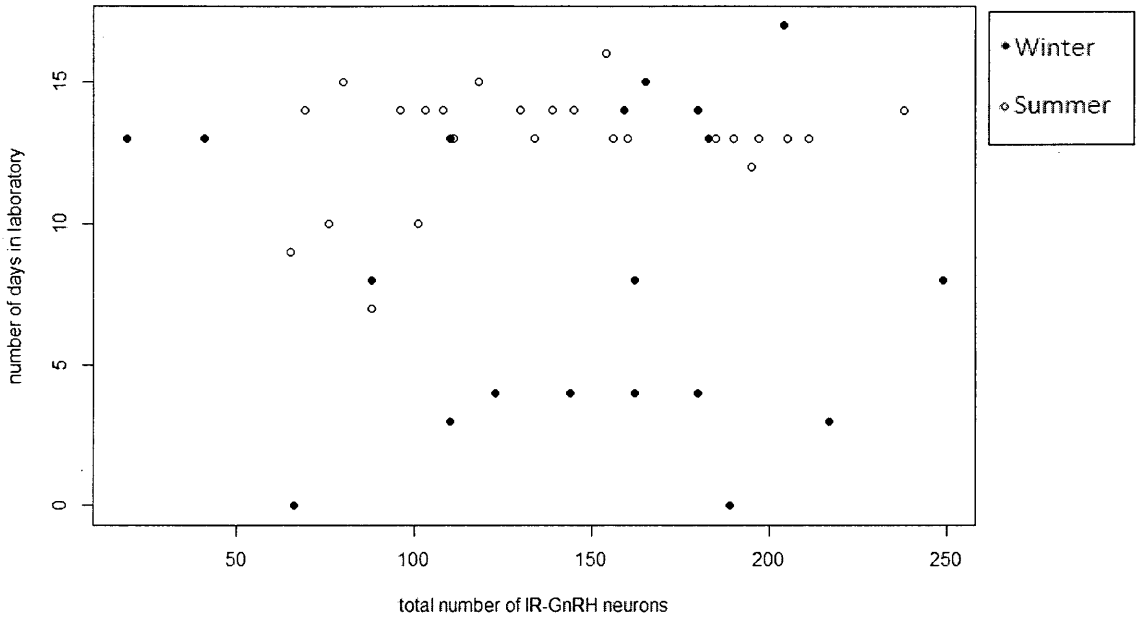

number of days in laboratory
0
5
10
15
50
100
150
200
250
total number of IR-GnRH neurons
Winter
Summer

Figure 15.

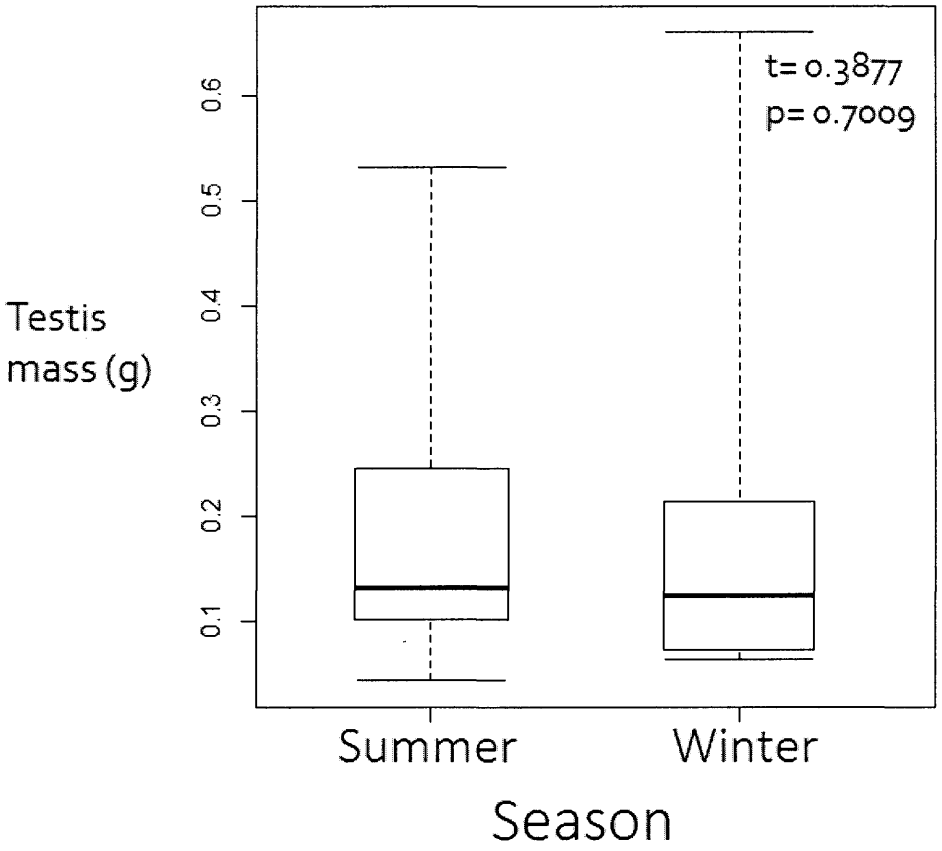

t= 0.3877
p= 0.7009
Testis mass (g)
0.1
0.2
0.3
0.4
0.5
0.6
Summer
Winter
Season

Figure 16.

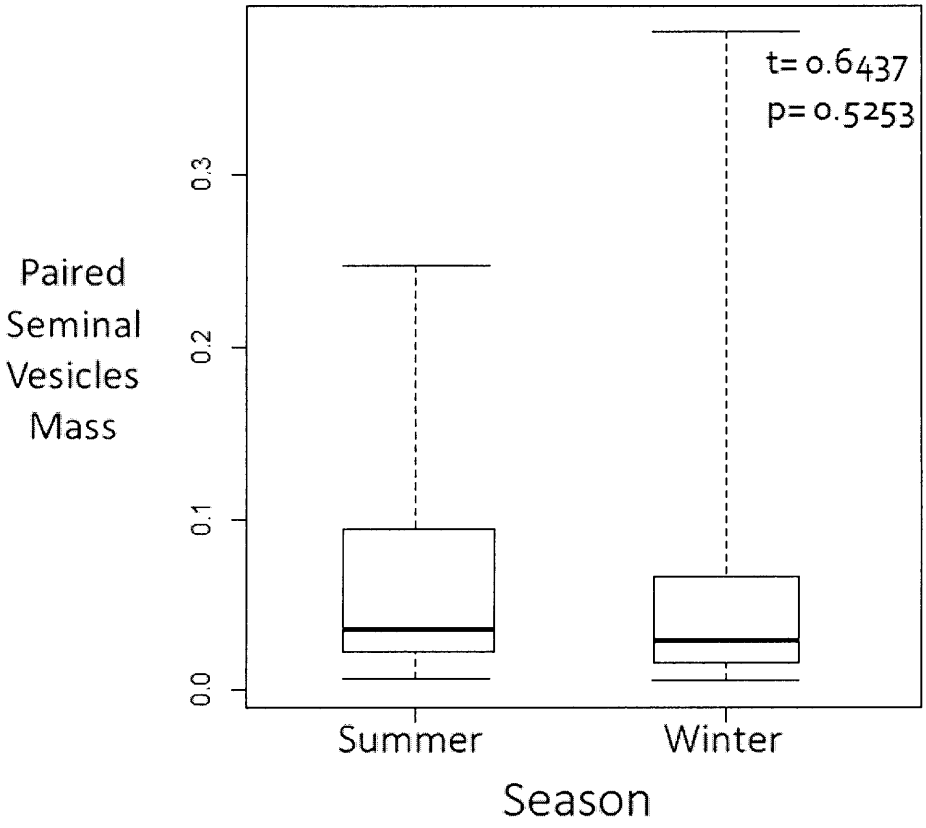

t= 0.6437
p= 0.5253
Paired Seminal Vesicles Mass
0.0
0.1
0.2
0.3
Summer
Winter
Season

Figure 17.

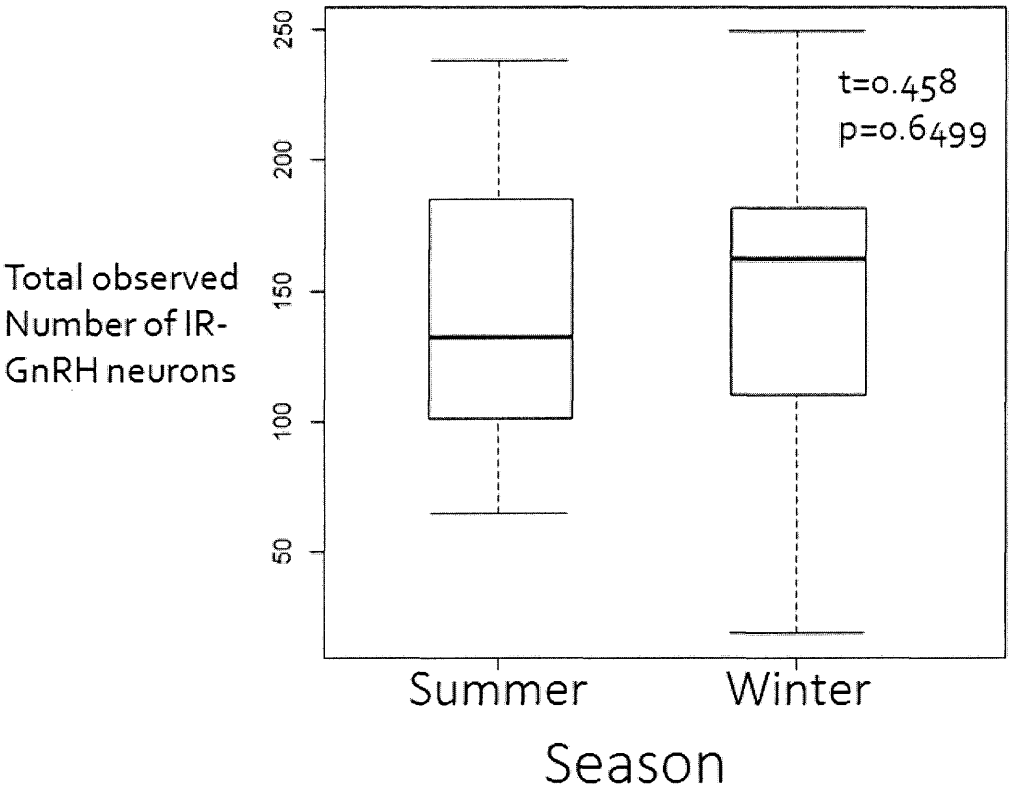

Total observed Number of IR-GnRH neurons
250
200
150
100
50
t=0.458
p=0.6499
Summer
Winter
Season

Figure 18.

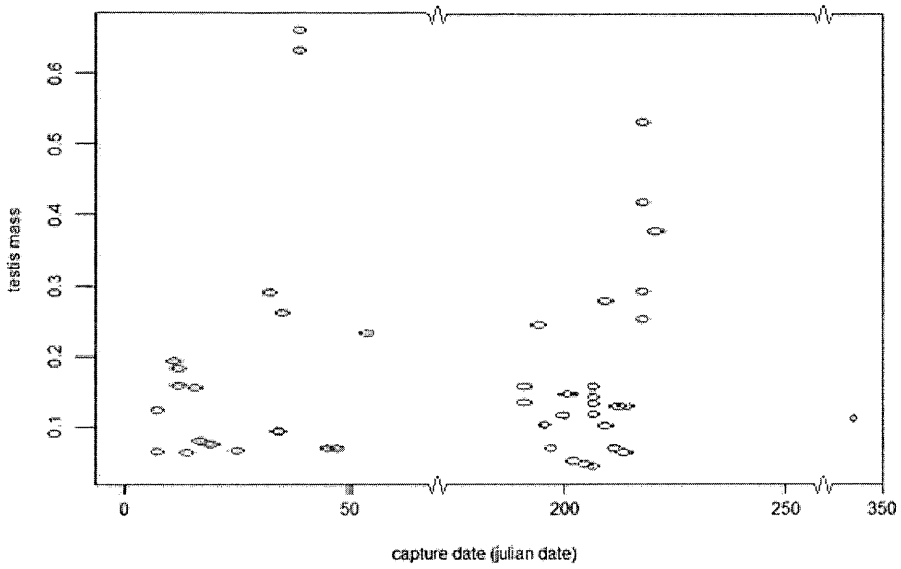

testis mass
0.1
0.2
0.3
0.4
0.5
0.6
0
50
200
250
350
capture date (julian date)

Figure 19.

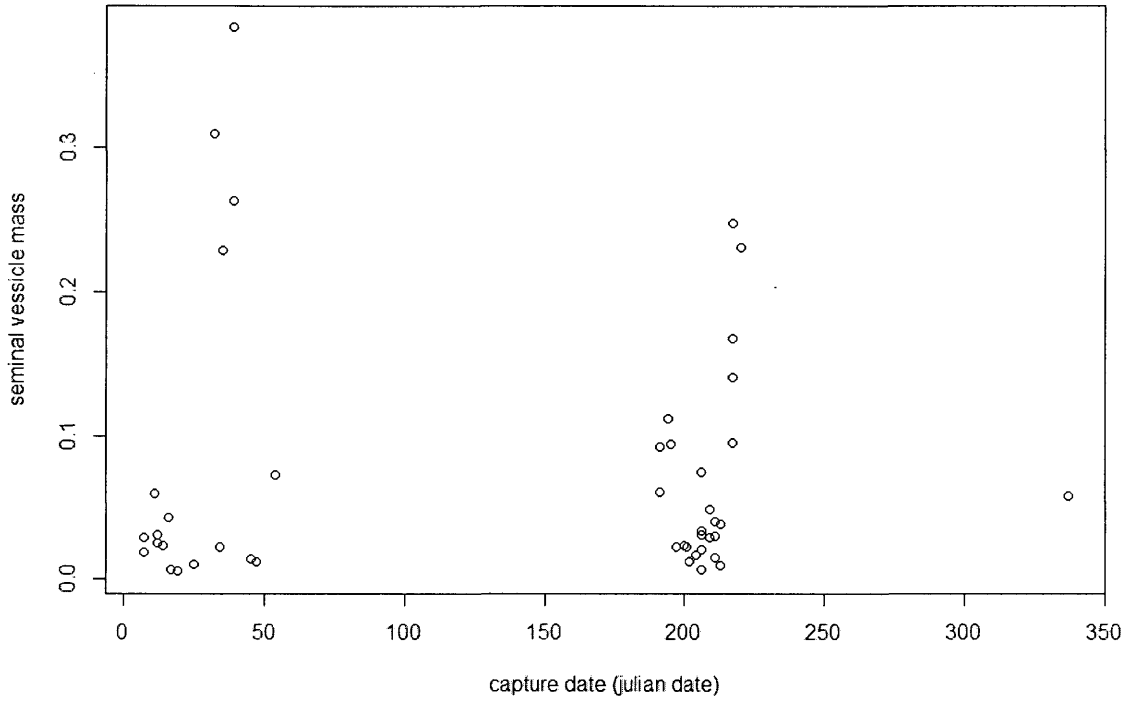

seminal vessicle mass
0.0
0.1
0.2
0.3
0
50
100
150
200
250
300
350
capture date (julian date)

Figure 20.

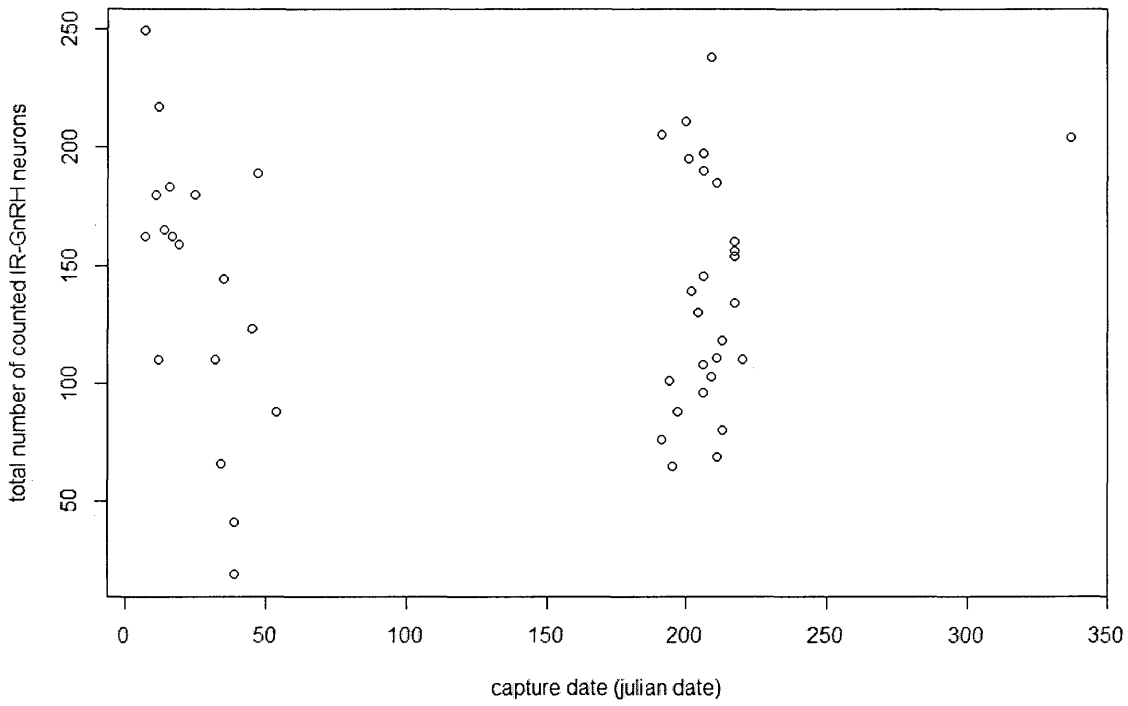

total number of counted IR-GnRH neurons
50
100
150
200
250
0
50
100
150
200
250
300
350
capture date (julian date)

Figure 21.

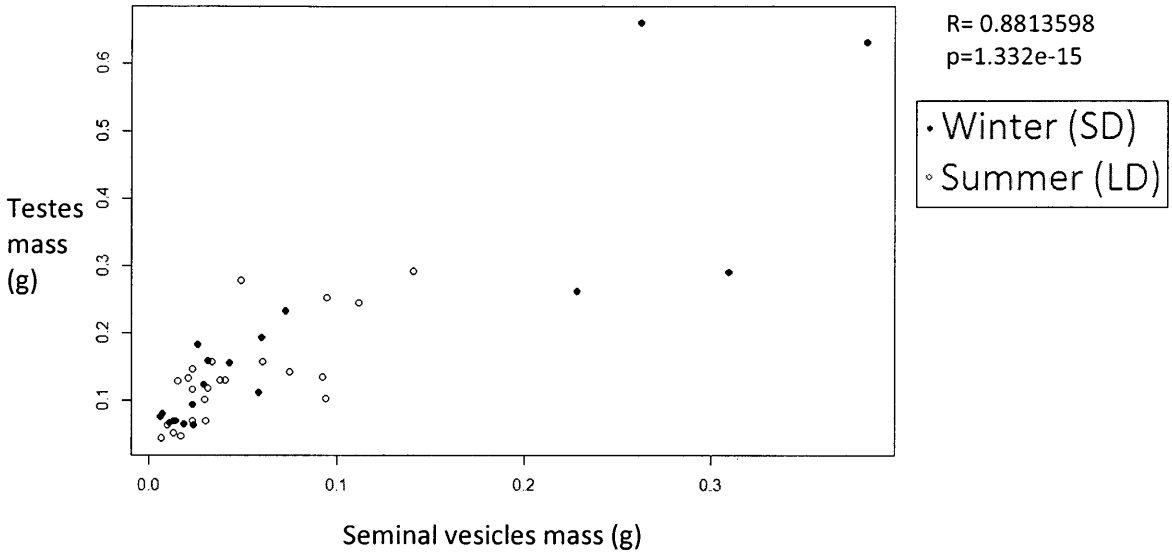

R= 0.8813598
p=1.332e-15
Winter (SD)
Summer (LD)
Testes mass (g)
0.1
0.2
0.3
0.4
0.5
0.6
0.0
0.1
0.2
0.3
Seminal vesicles mass (g)

Figure 22.

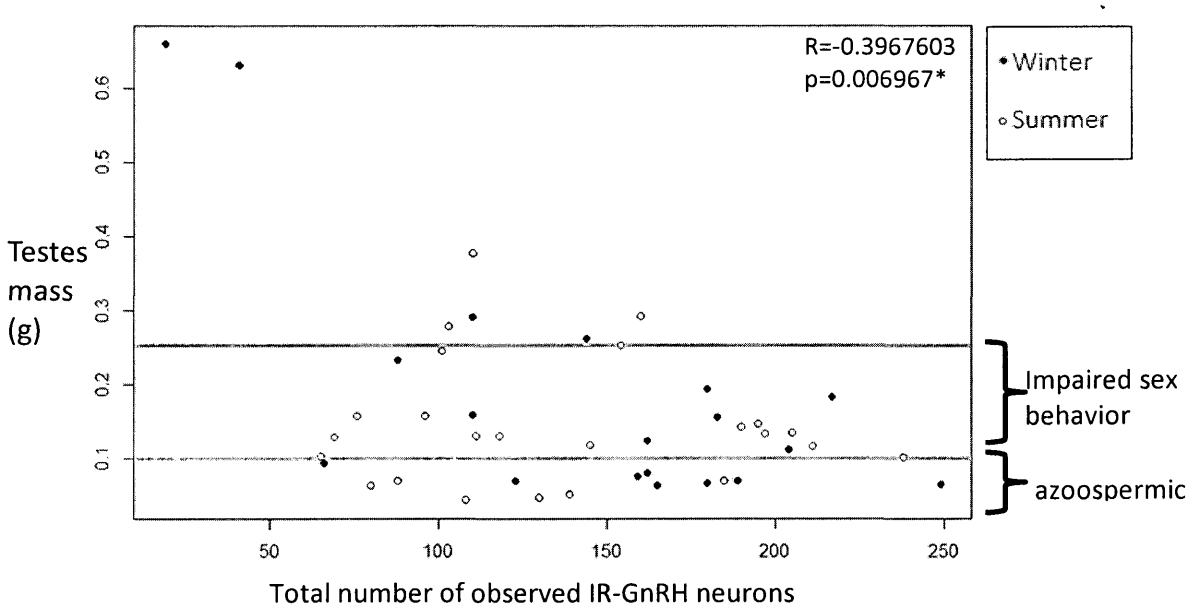

R=-0.3967603
p=0.006967*
Winter
Summer
Testes mass (g)
0.1
0.2
0.3
0.4
0.5
0.6
50
100
150
200
250
Total number of observed IR-GnRH neurons
Impaired sex behavior
azoospermic

Figure 23.

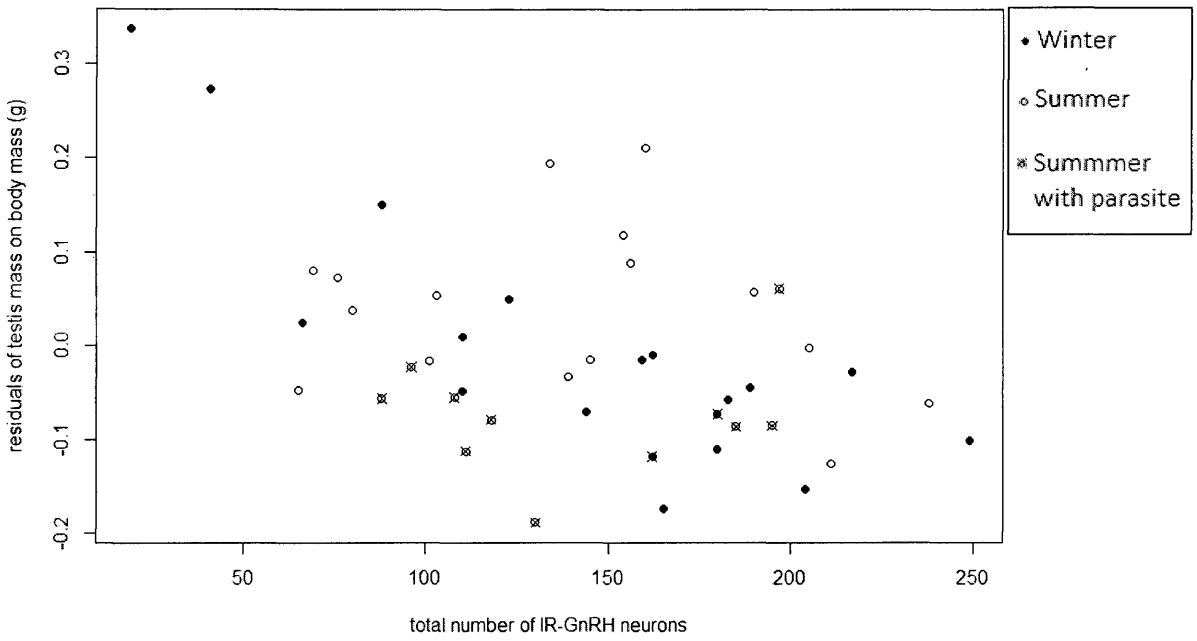

residuals of testis mass on body mass (g)
-0.2
-0.1
0.0
0.1
0.2
0.3
50
100
150
200
250
total number of IR-GnRH neurons
Winter
Summer
Summmer with parasite

Figure 24.

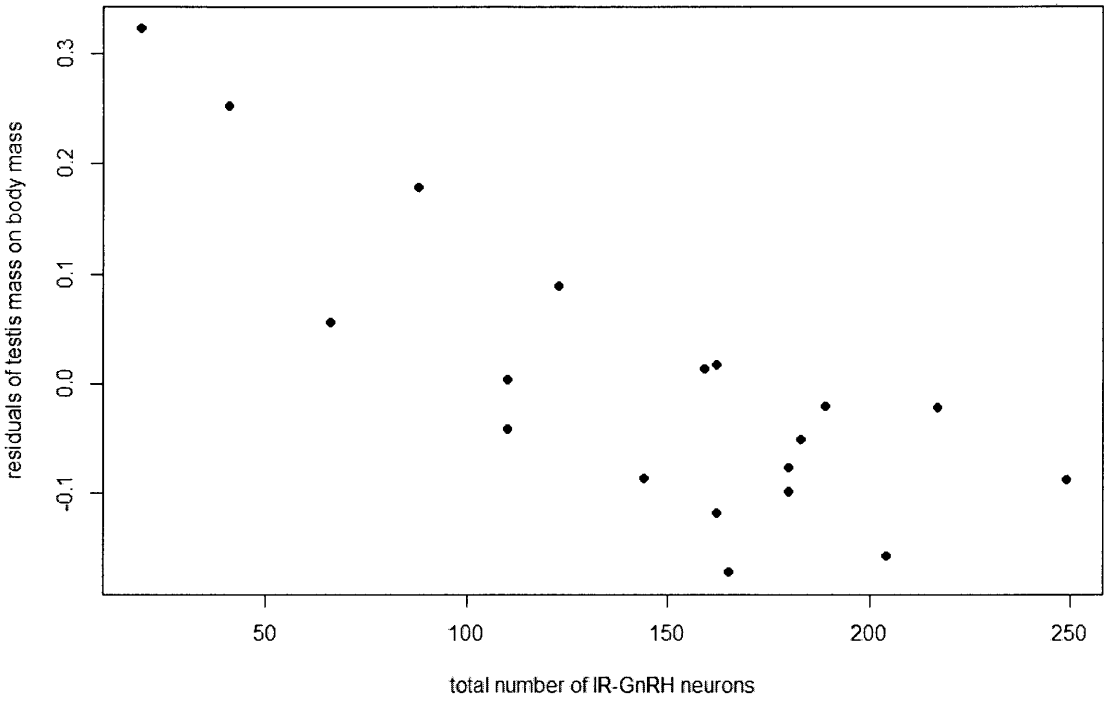

residuals of testis mass on body mass
0.3
0.2
0.1
0.0
-0.1
50
100
150
200
250
total number of IR-GnRH neurons

Figure 25.

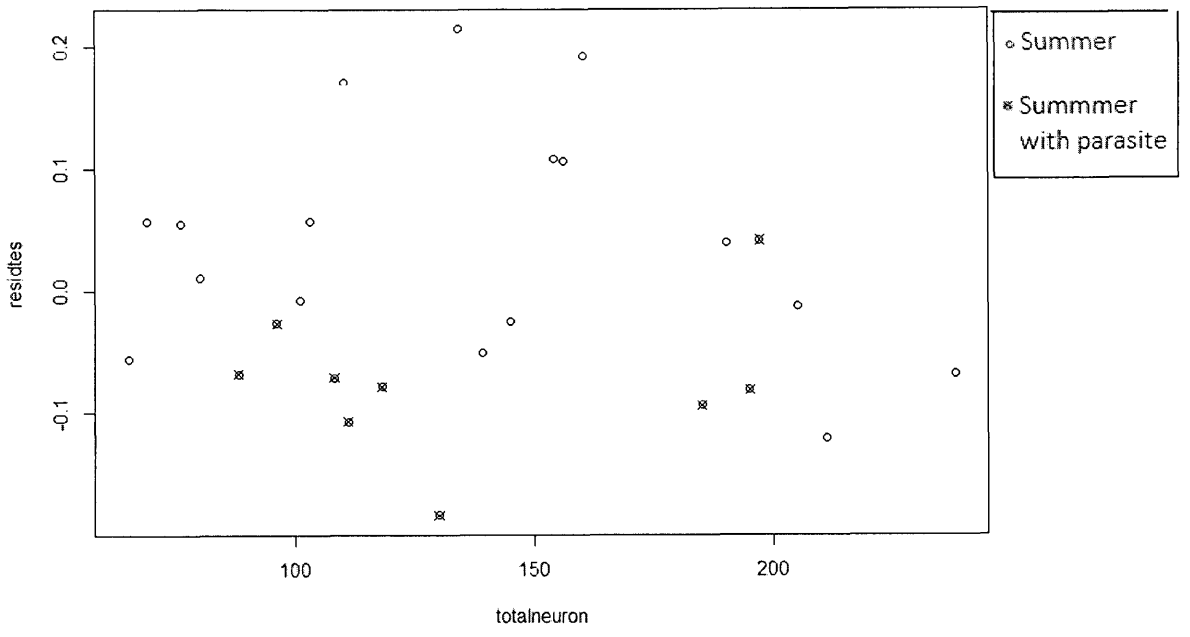

Summer
Summmer with parasite
residtes
0.2
0.1
0.0
-0.1
100
150
200
totalneuron

Figure 26.

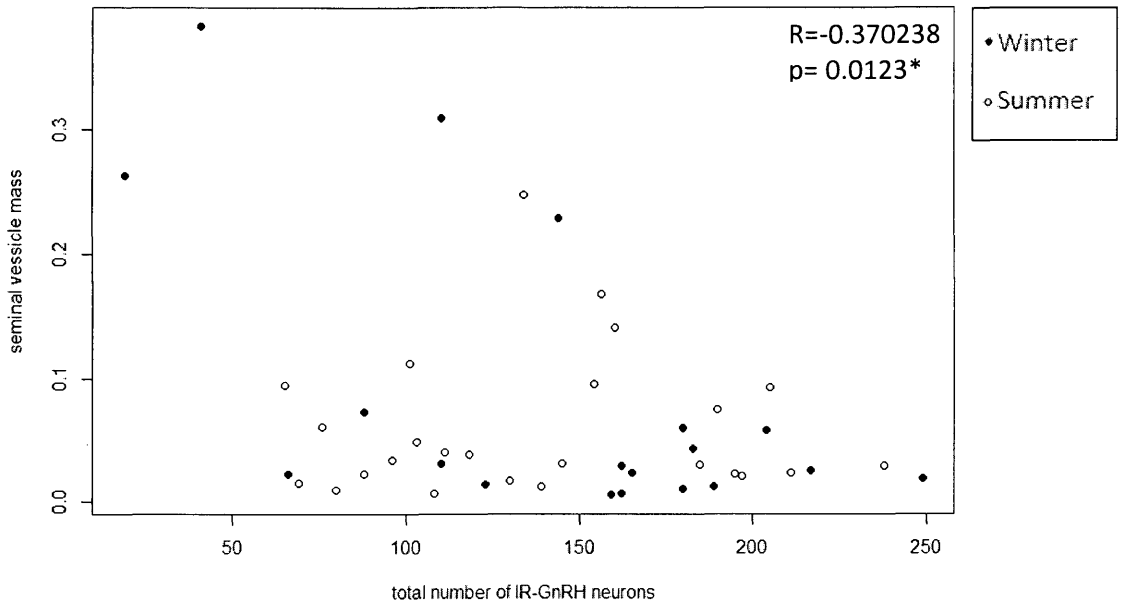

R=-0.370238
p= 0.0123*
Winter
Summer
seminal vessicle mass
0.0
0.1
0.2
0.3
50
100
150
200
250
total number of IR-GnRH neurons

Figure 27.

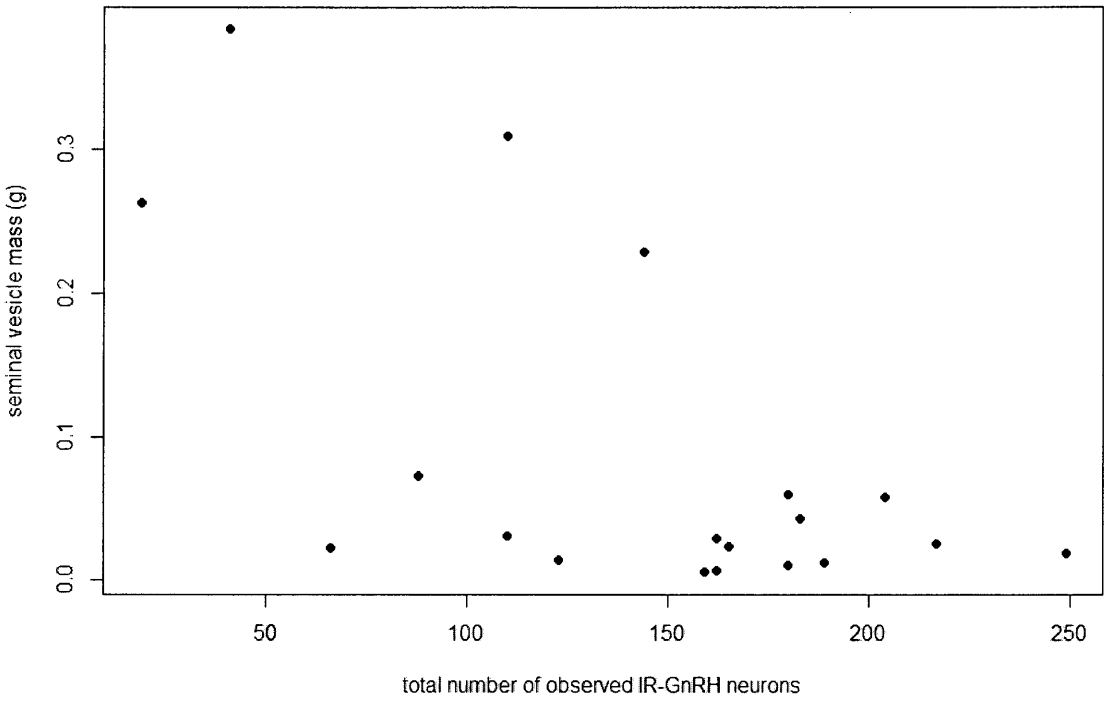

seminal vesicle mass (g)
0.0
0.1
0.2
0.3
50
100
150
200
250
total number of observed IR-GnRH neurons

Figure 28.

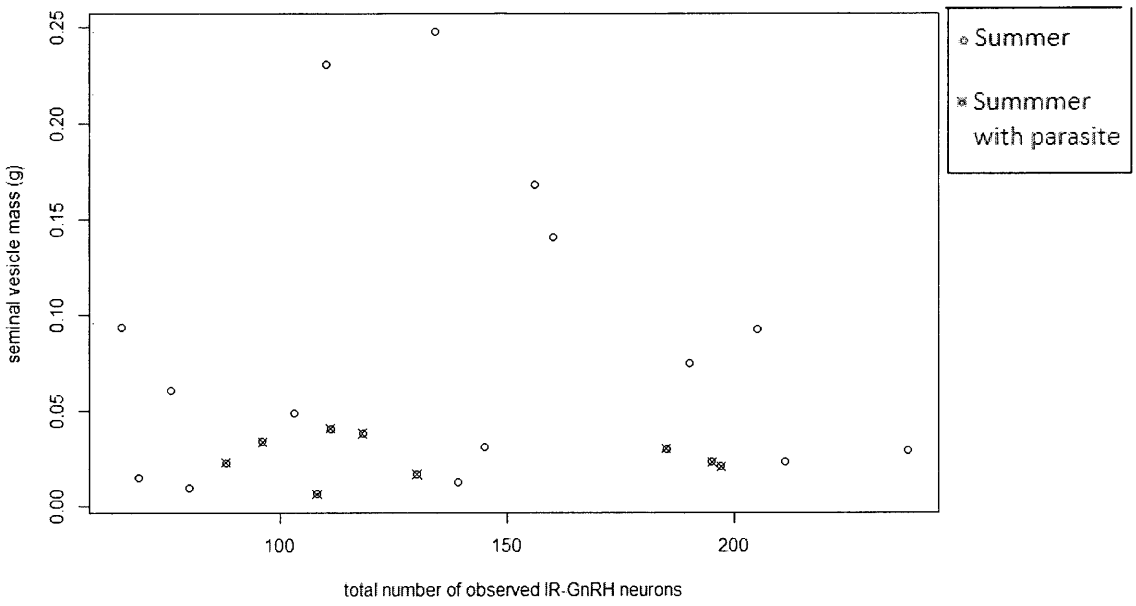

seminal vesicle mass (g)
0.00
0.05
0.10
0.15
0.20
0.25
100
150
200
total number of observed IR-GnRH neurons
Summer
Summmer with parasite

Figure 29.

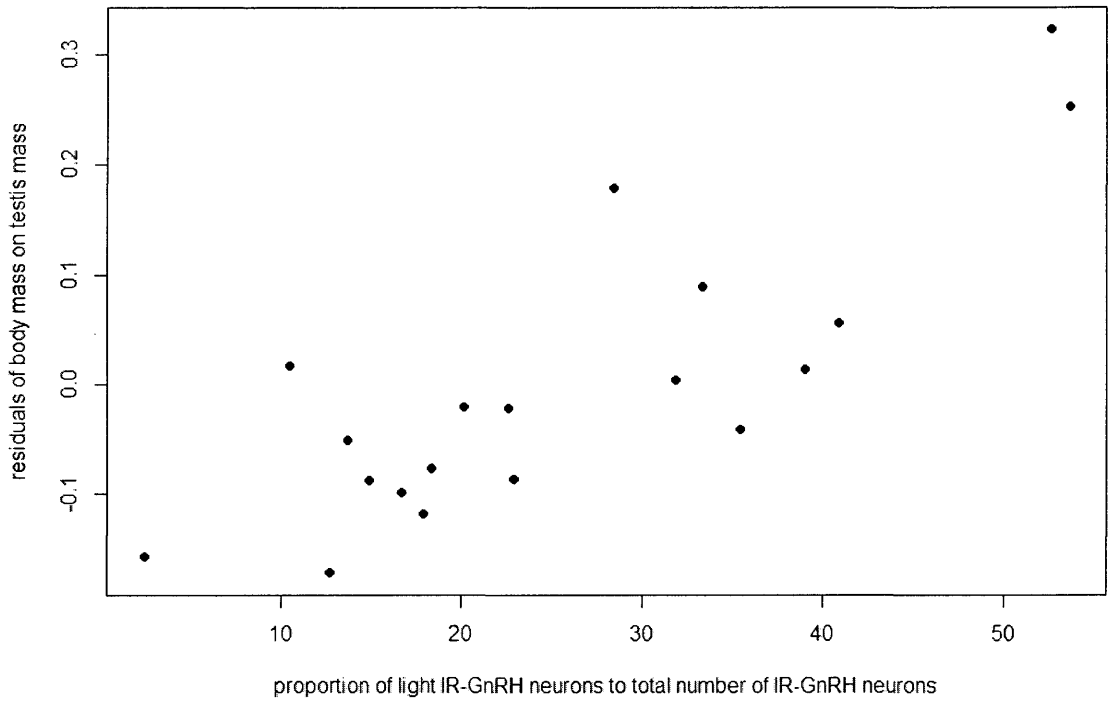

residuals of body mass on testis mass
-0.1
0.0
0.1
0.2
0.3
10
20
30
40
50
proportion of light IR-GnRH neurons to total number of IR-GnRH neurons

Figure 30.

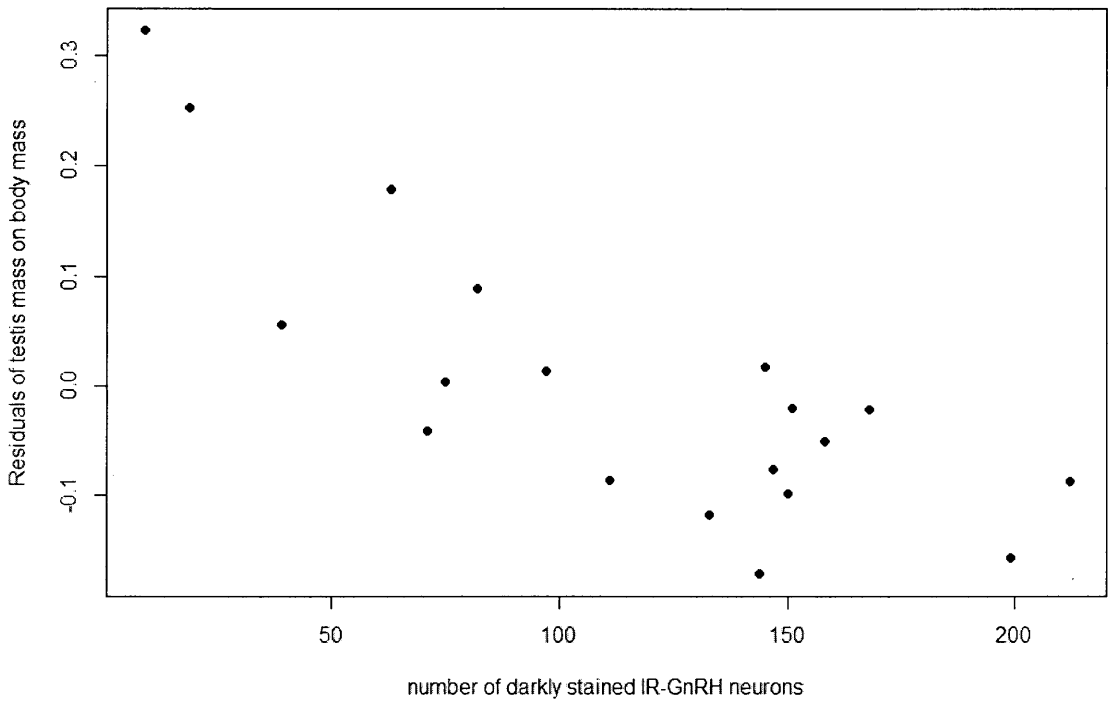

Residuals of testis mass on body mass
-0.1
0.0
0.1
0.2
0.3
50
100
150
200
number of darkly stained IR-GnRH neurons

Figure 31.

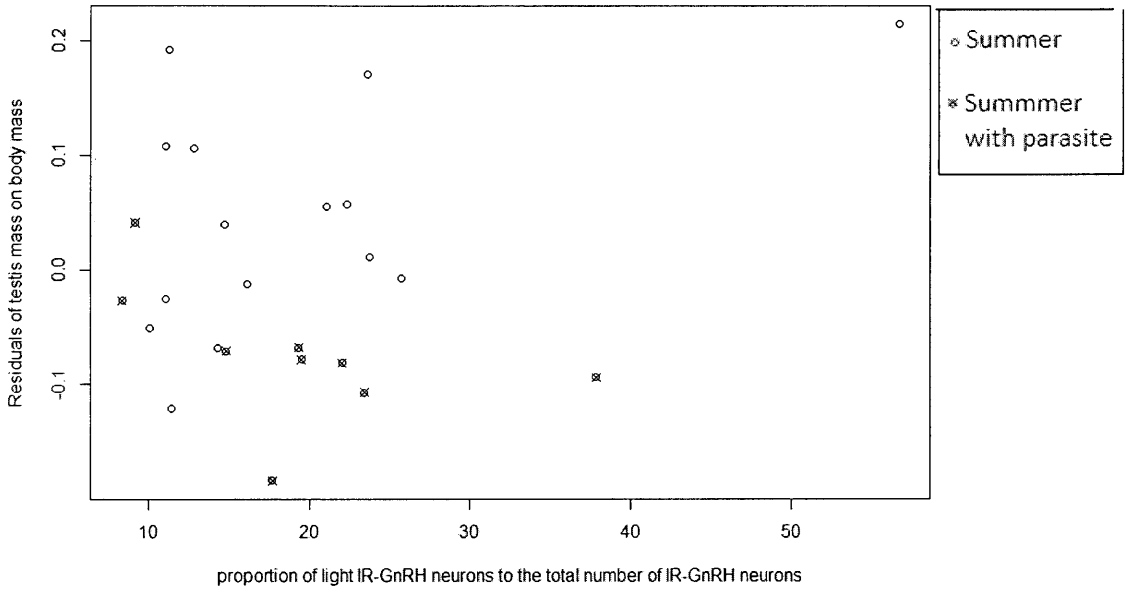

Residuals of testis mass on body mass
0.2
0.1
0.0
-0.1
10
20
30
40
50
proportion of light IR-GnRH neurons to the total number of IR-GnRH neurons
Summer
Summmer with parasite

Figure 32.

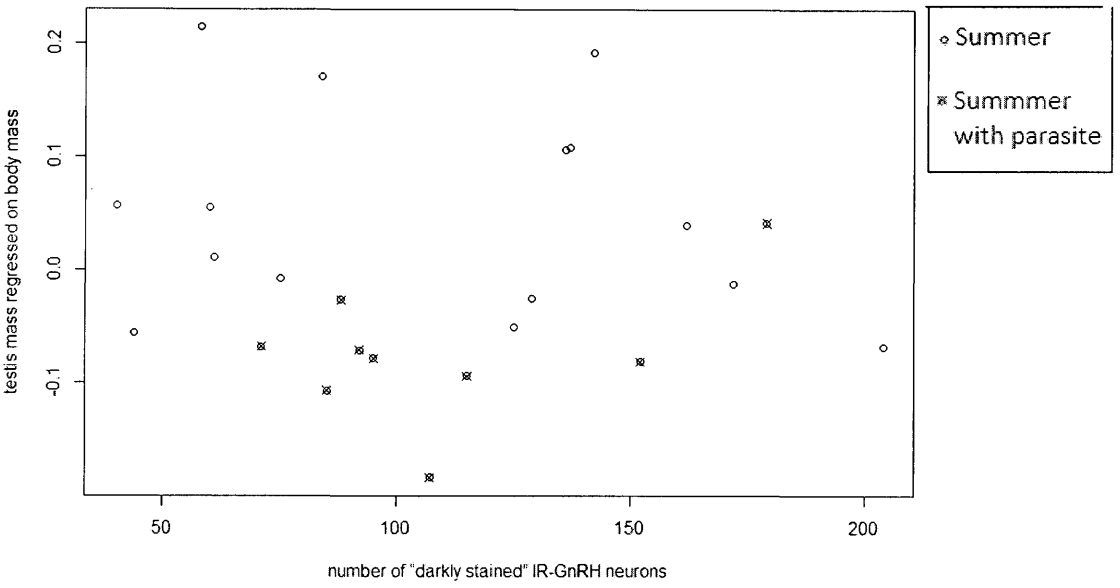

testis mass regressed on body mass
0.2
0.1
0.0
-0.1
50
100
150
200
number of "darkly stained" IR-GnRH neurons
Summer
Summmer with parasite

Figure 33.

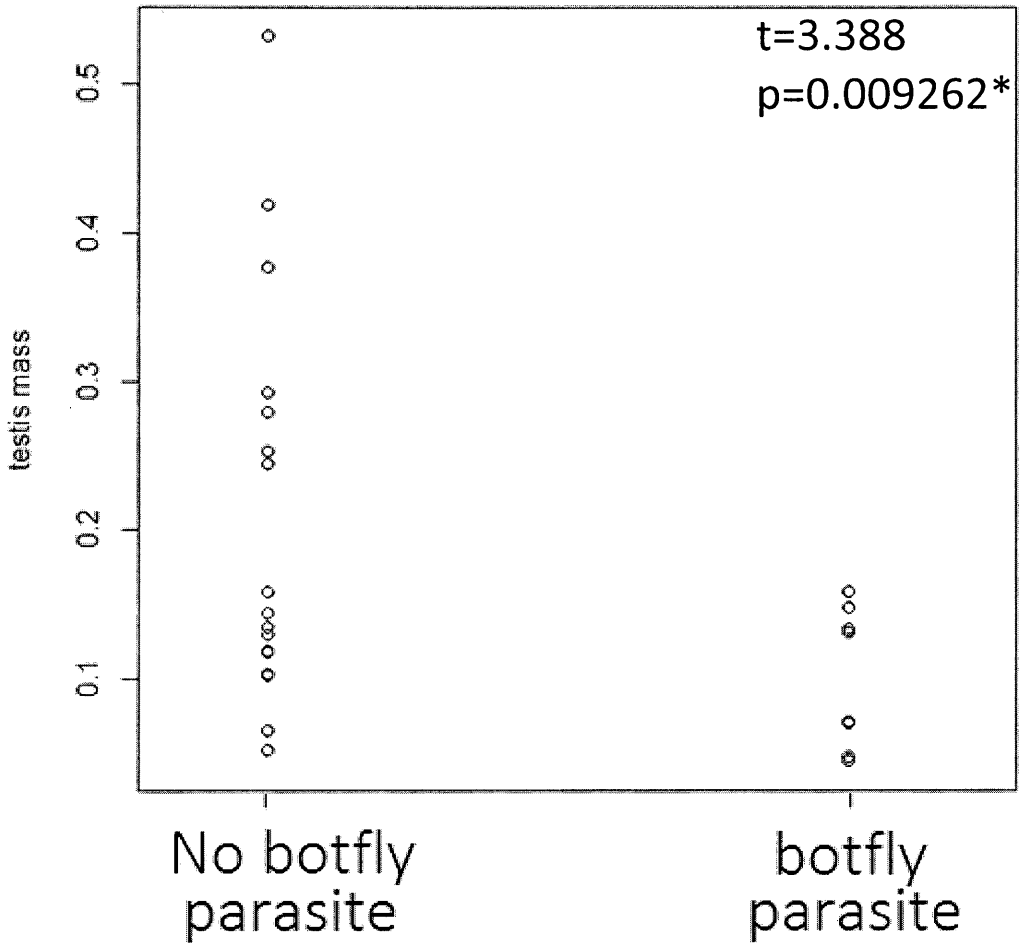

t=3.388
p=0.009262*
testis mass
0.1
0.2
0.3
0.4
0.5
No botfly parasite
botfly parasite

Figure 34.

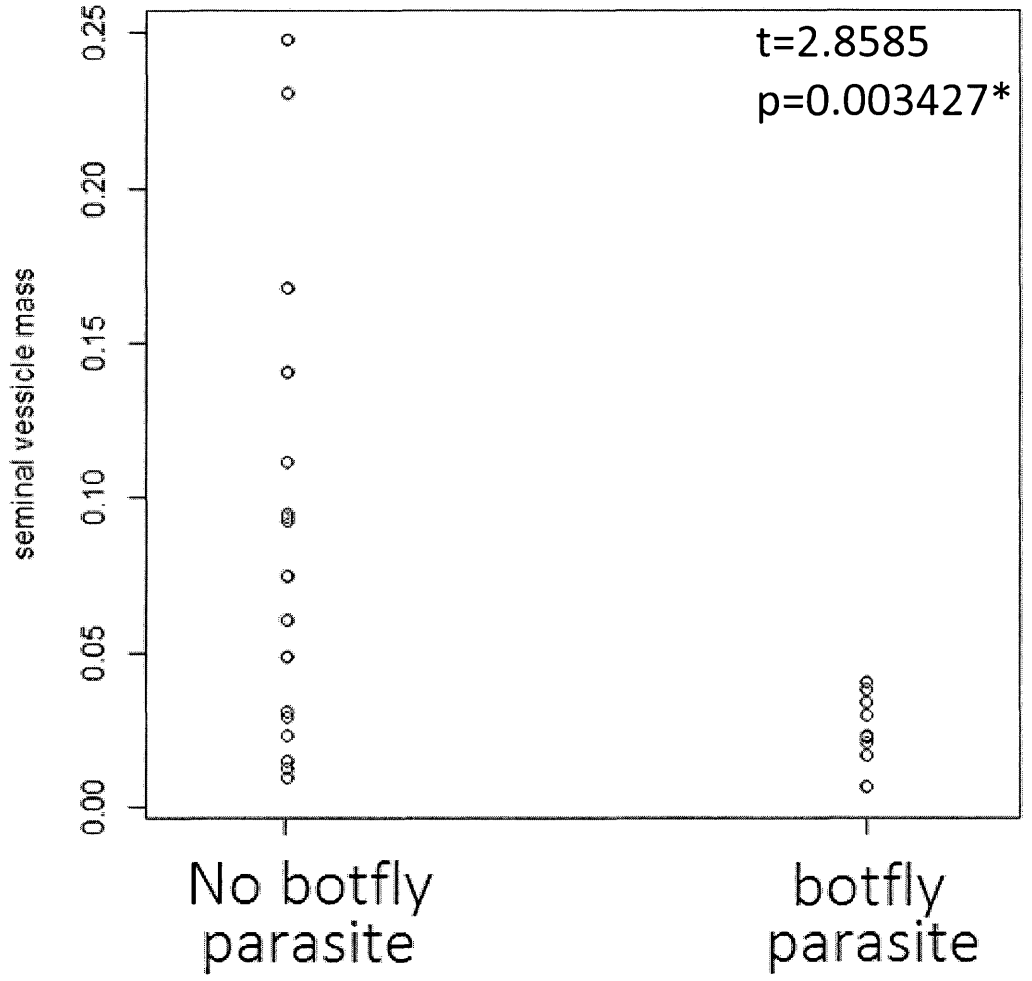

t=2.8585
p=0.003427*
seminal vessicle mass
0.00
0.05
0.10
0.15
0.20
0.25
No botfly parasite
botfly parasite

Figure 35.

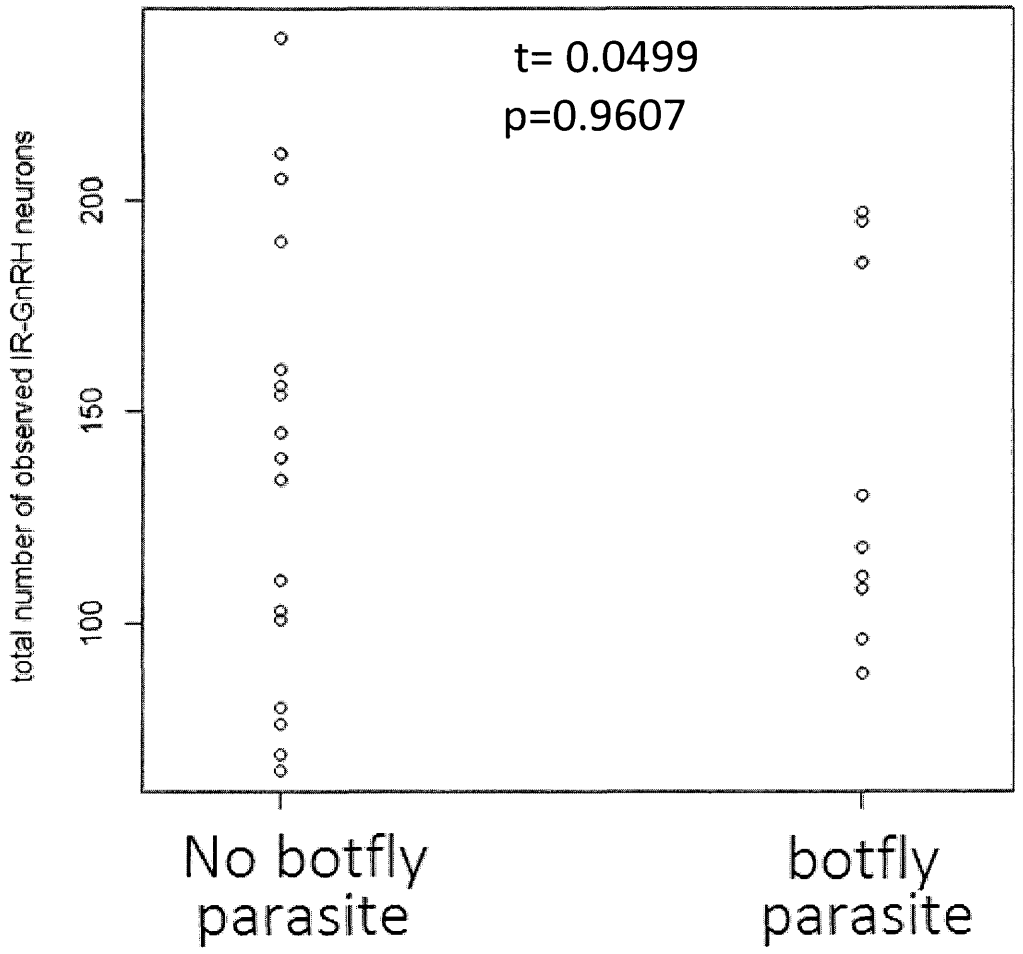

t= 0.0499
p=0.9607
total number of observed IR-GnRH neurons
100
150
200
No botfly parasite
botfly parasite

Figure 36.

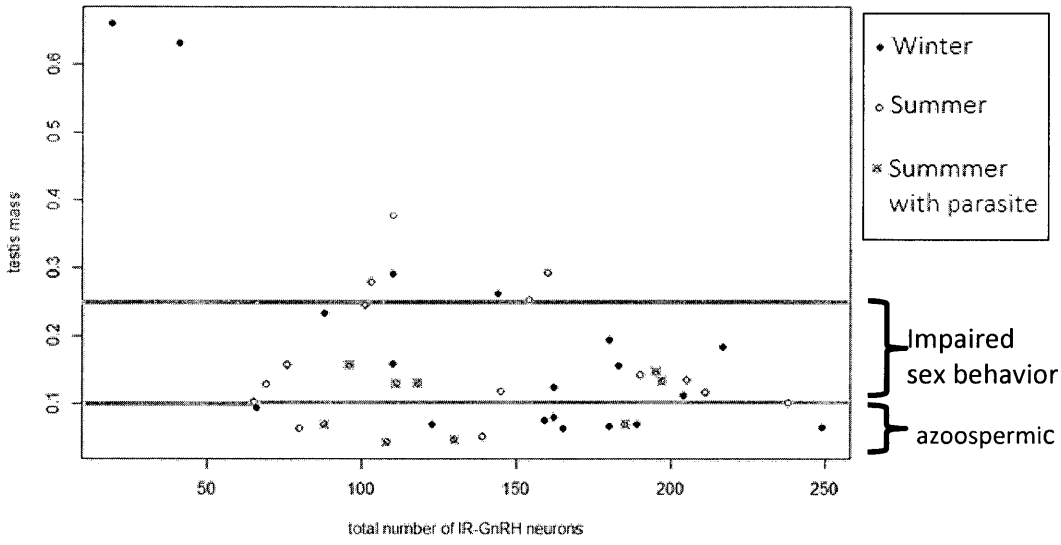

testis mass
0.1
0.2
0.3
0.4
0.5
0.6
50
100
150
200
250
total number of IR-GnRH neurons
Winter
Summer
Summmer with parasite
Impaired sex behavior
azoospermic

Figure 37.

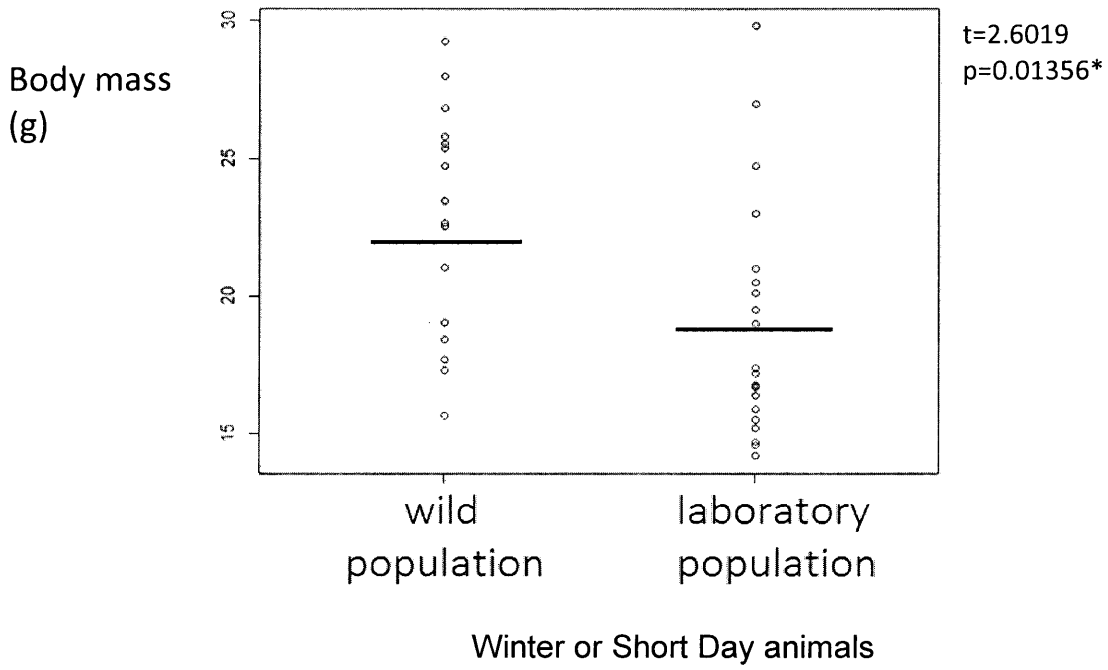

Body mass (g)
30
25
20
15
t=2.6019
p=0.01356*
wild population
laboratory population
Winter or Short Day animals

Figure 38.

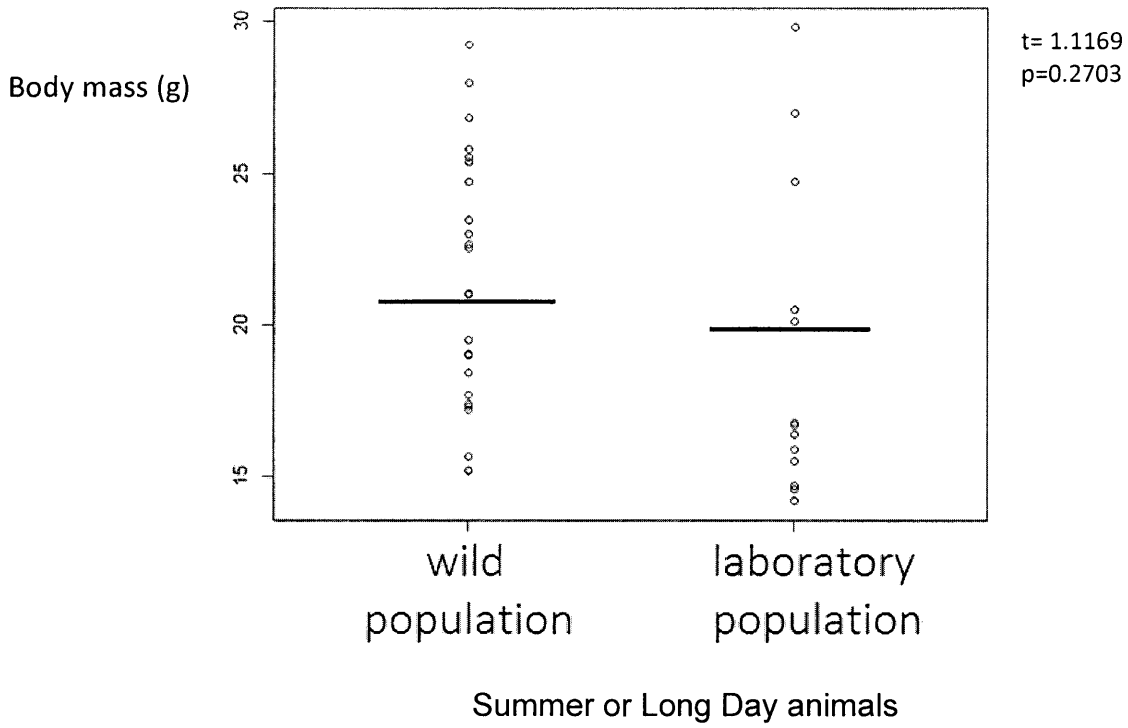

Body mass (g)
30
25
20
15
t= 1.1169
p=0.2703
wild population
laboratory population
Summer or Long Day animals

Figure 39.

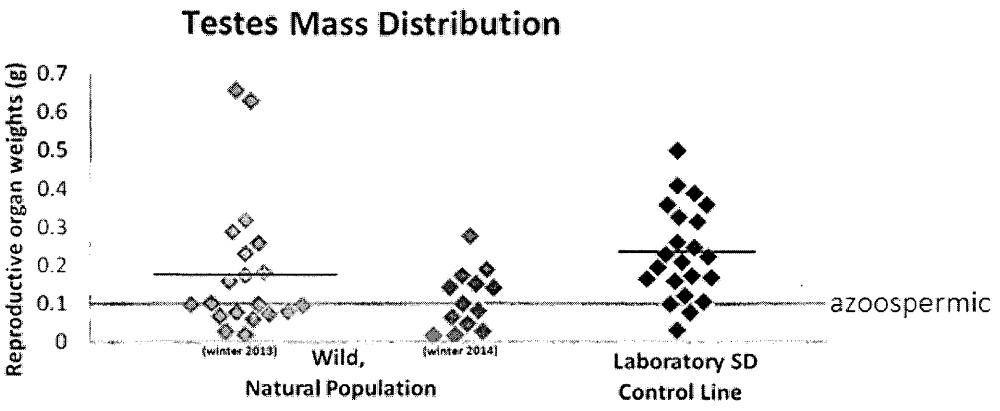
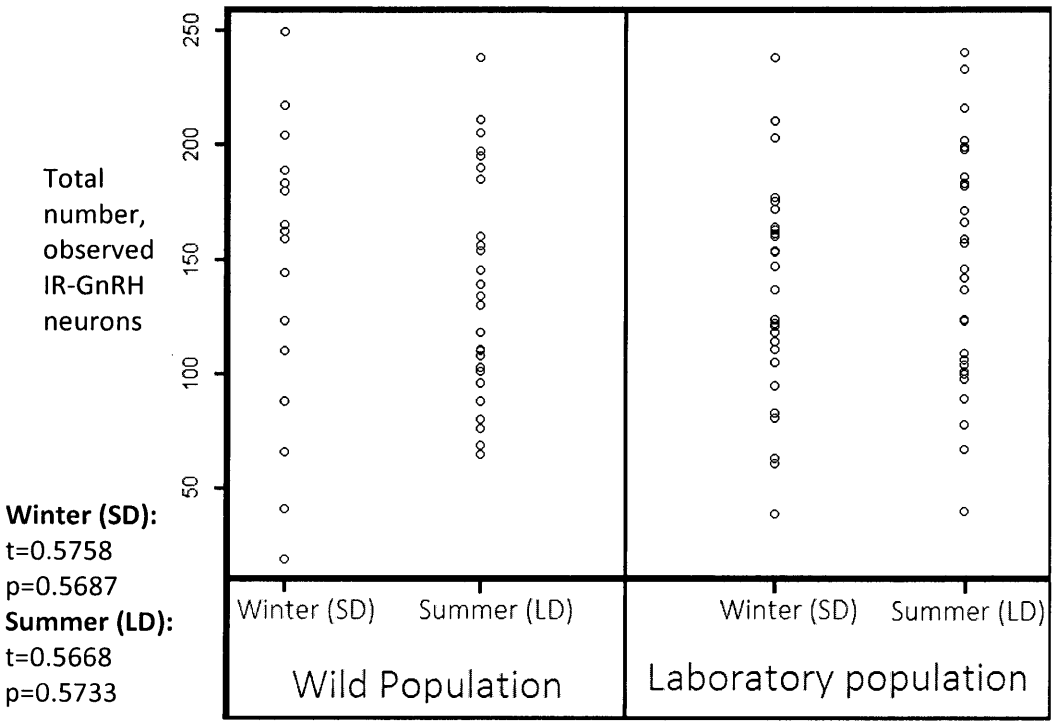

Testes Mass Distribution
Reproductive organ weights (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
azoospermic
(winter 2013)
(winter 2014)
Wild,
Natural Population
Laboratory SD
Control Line

Figure 40.


Total number, observed IR-GnRH neurons
250
200
150
100
50
Winter (SD):
t=0.5758
p=0.5687
Summer (LD):
t=0.5668
p=0.5733
Winter (SD)
Summer (LD)
Wild Population
Winter (SD)
Summer (LD)
Laboratory population

Females:

Figure 41.

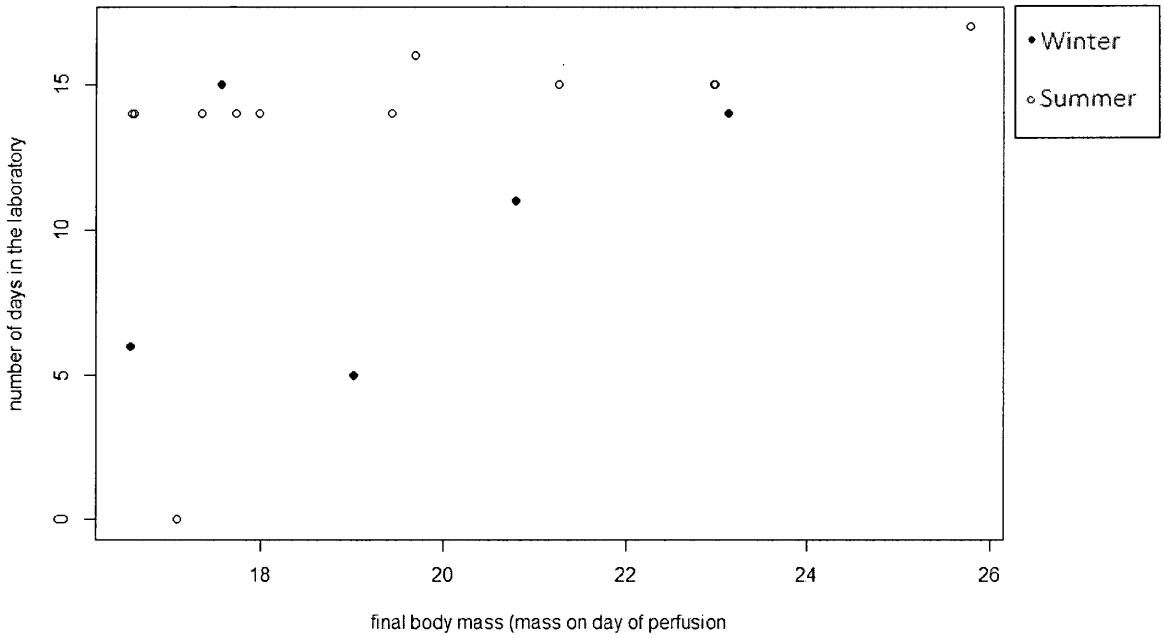


Figure 42.

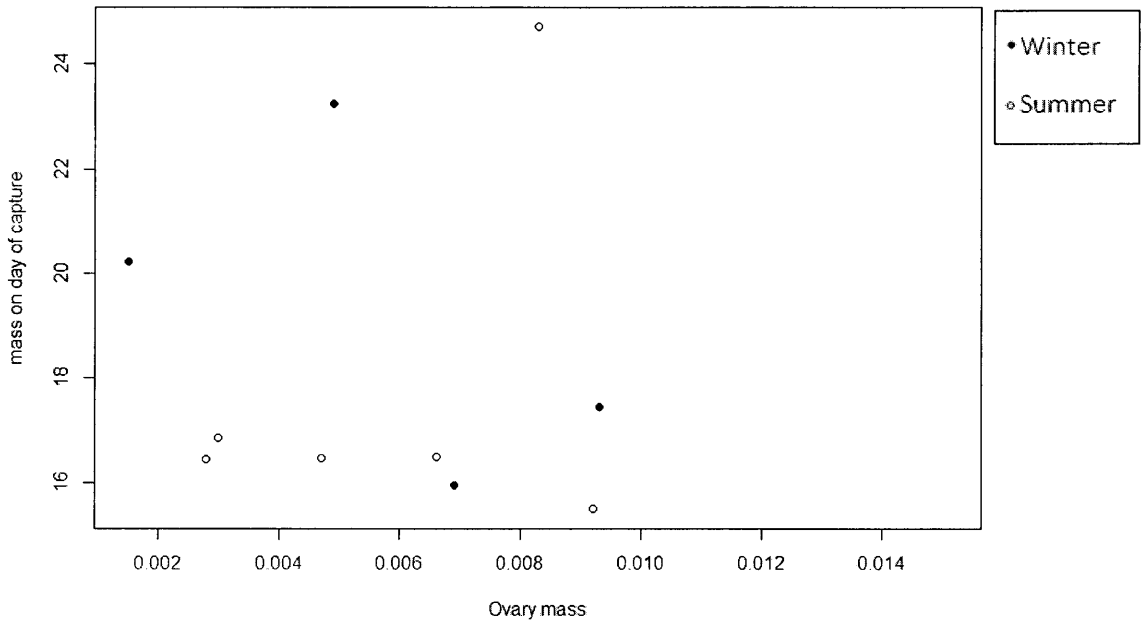

mass on day of capture
16
18
20
22
24
0.002
0.004
0.006
0.008
0.010
0.012
0.014
Ovary mass
Winter
Summer

Figure 43.

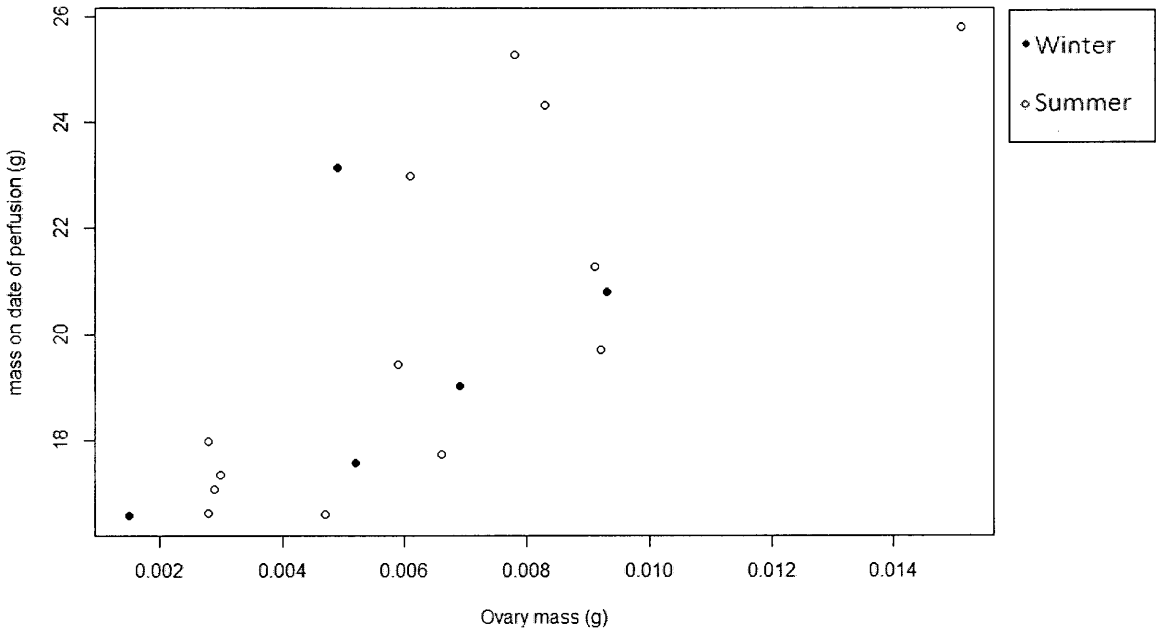

mass on date of perfusion (g)
18
20
22
24
26
0.002
0.004
0.006
0.008
0.010
0.012
0.014
Ovary mass (g)
Winter
Summer

Figure 44.

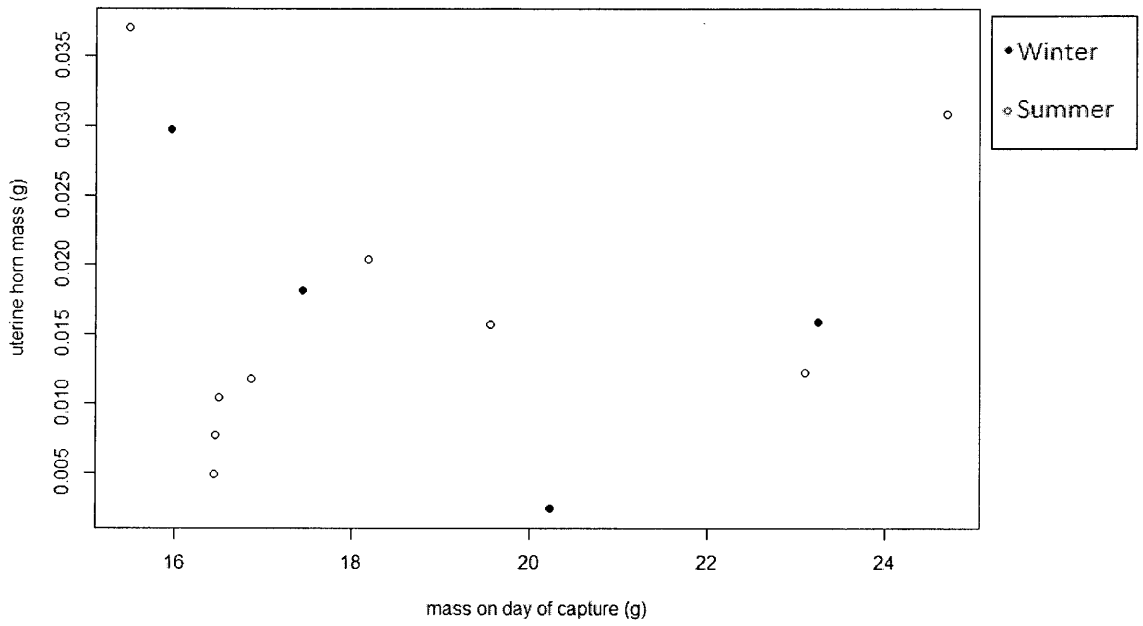

uterine horn mass (g)
0.005
0.010
0.015
0.020
0.025
0.030
0.035
16
18
20
22
24
mass on day of capture (g)
Winter
Summer

Figure 45.

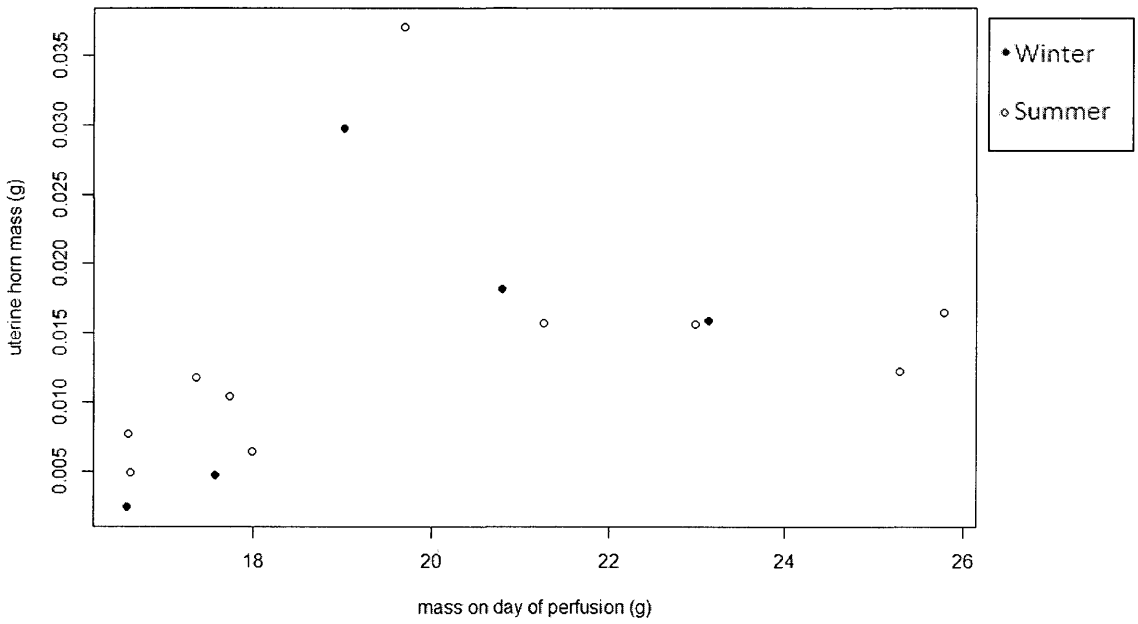

uterine horn mass (g)
0.005
0.010
0.015
0.020
0.025
0.030
0.035
18
20
22
24
26
mass on day of perfusion (g)
Winter
Summer

Figure 46.

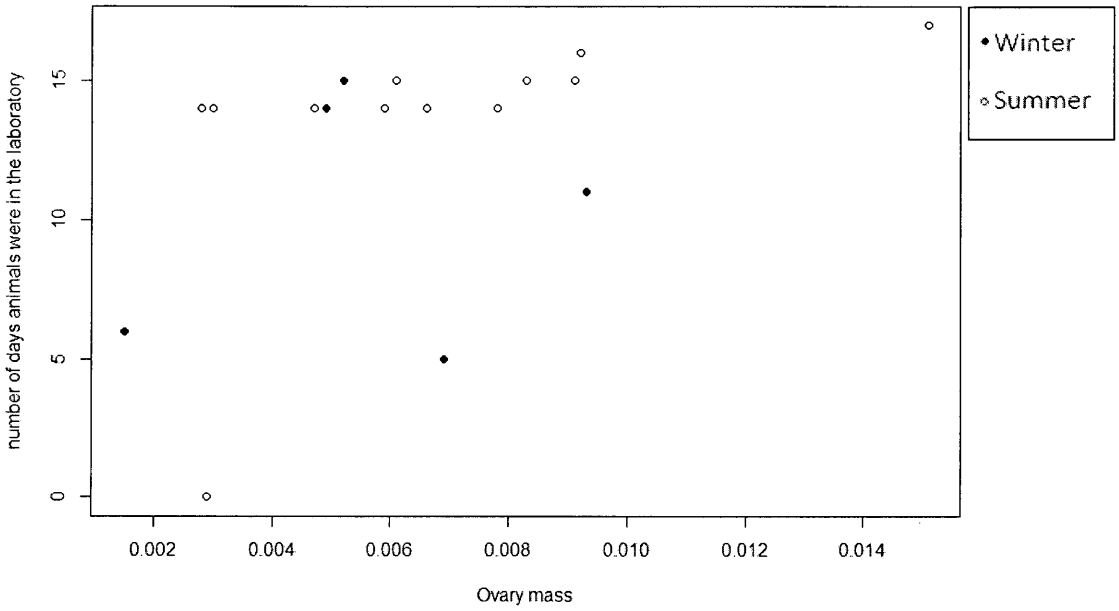

number of days animals were in the laboratory
0
5
10
15
0.002
0.004
0.006
0.008
0.010
0.012
0.014
Ovary mass
Winter
Summer

Figure 47.

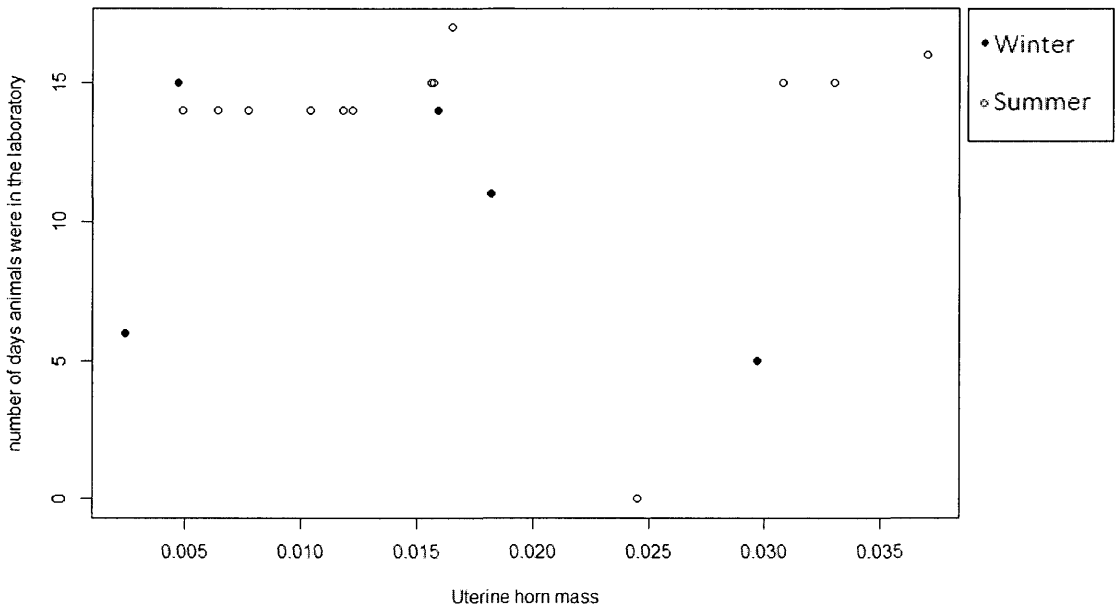

number of days animals were in the laboratory
0
5
10
15
0.005
0.010
0.015
0.020
0.025
0.030
0.035
Uterine horn mass
Winter
Summer

Figure 48.

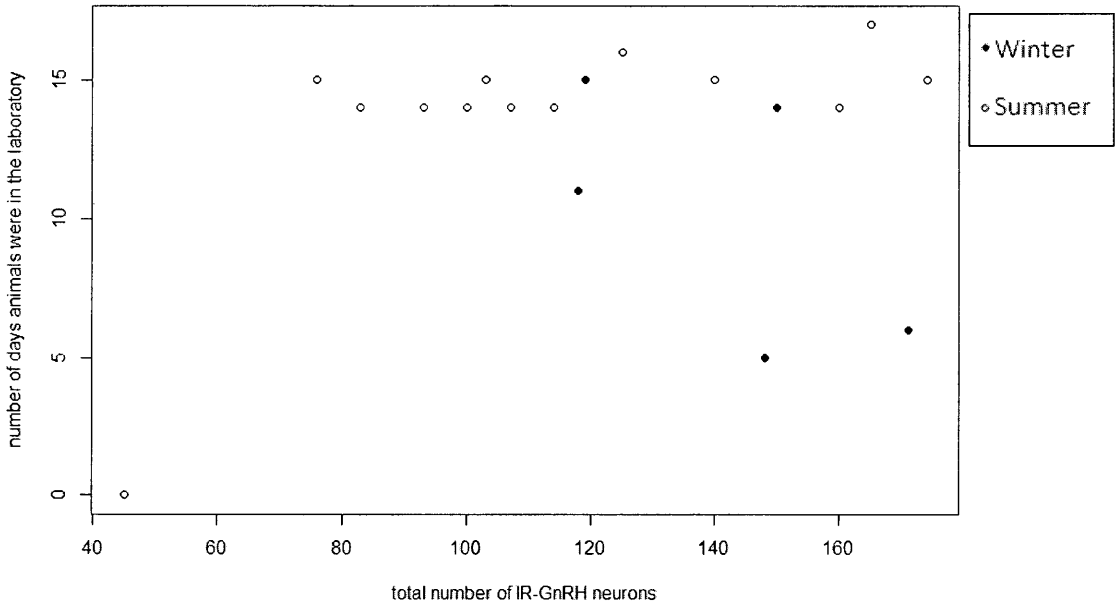

number of days animals were in the laboratory
0
5
10
15
40
60
80
100
120
140
160
total number of IR-GnRH neurons
Winter
Summer

Figure 49.

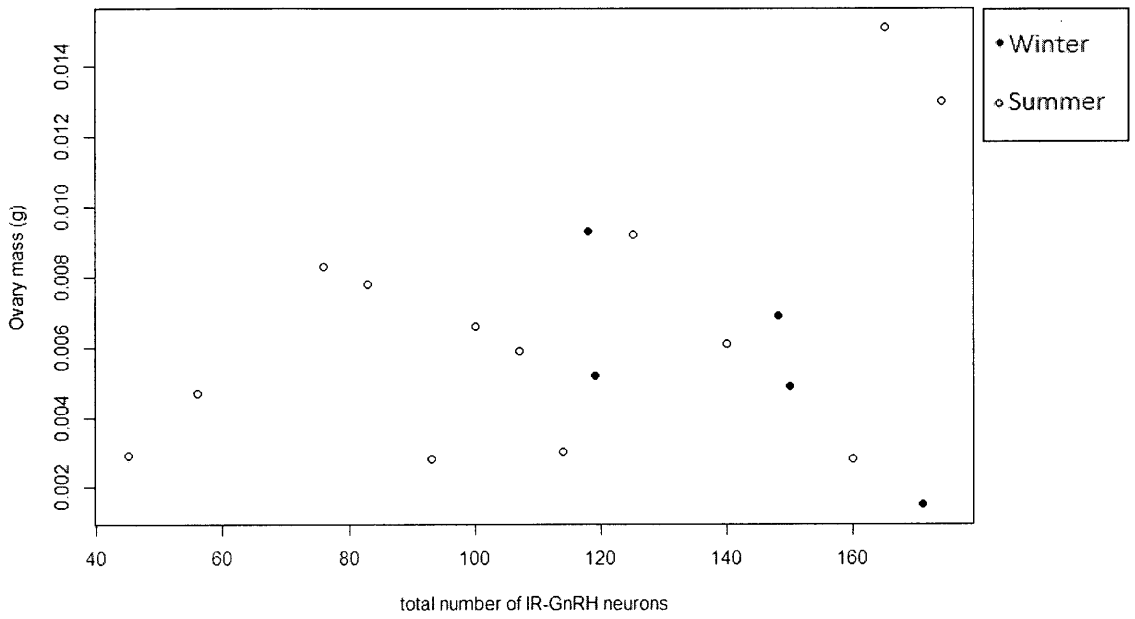

Winter
Summer
Ovary mass (g)
0.002
0.004
0.006
0.008
0.010
0.012
0.014
40
60
80
100
120
140
160
total number of IR-GnRH neurons

Figure 50.

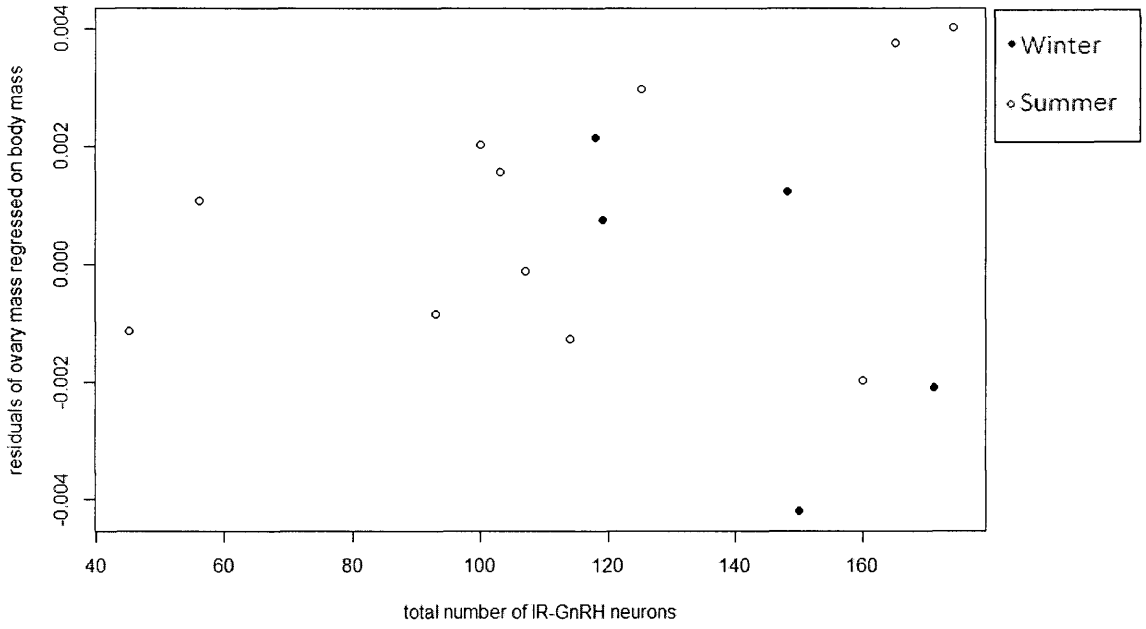

residuals of ovary mass regressed on body mass
0.004
0.002
0.000
-0.002
-0.004
40
60
80
100
120
140
160
total number of IR-GnRH neurons
Winter
Summer

Figure 51.

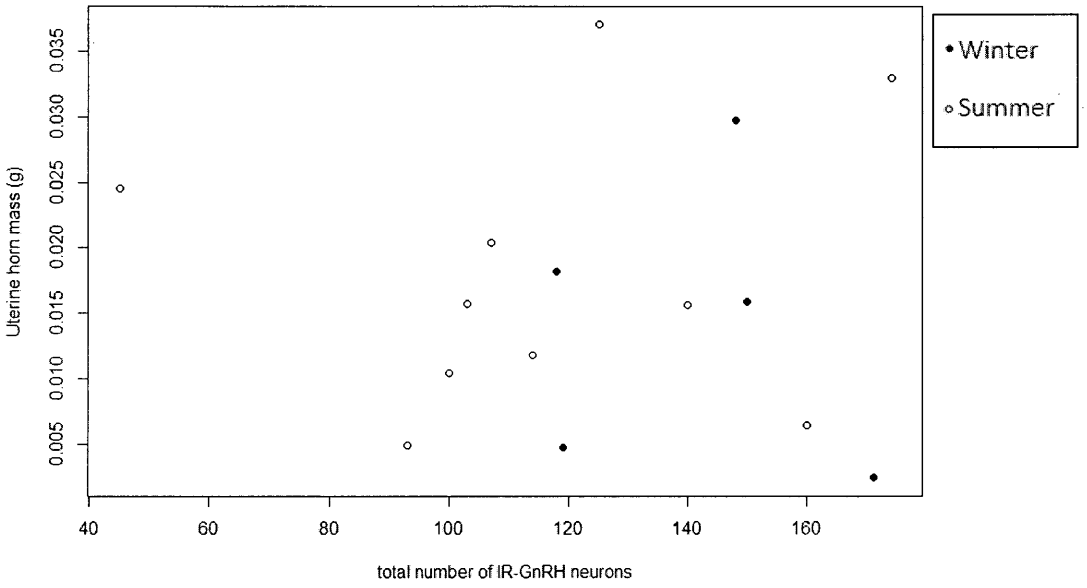

Uterine horn mass (g)
0.005
0.010
0.015
0.020
0.025
0.030
0.035
40
60
80
100
120
140
160
total number of IR-GnRH neurons
Winter
Summer

Figure 52.

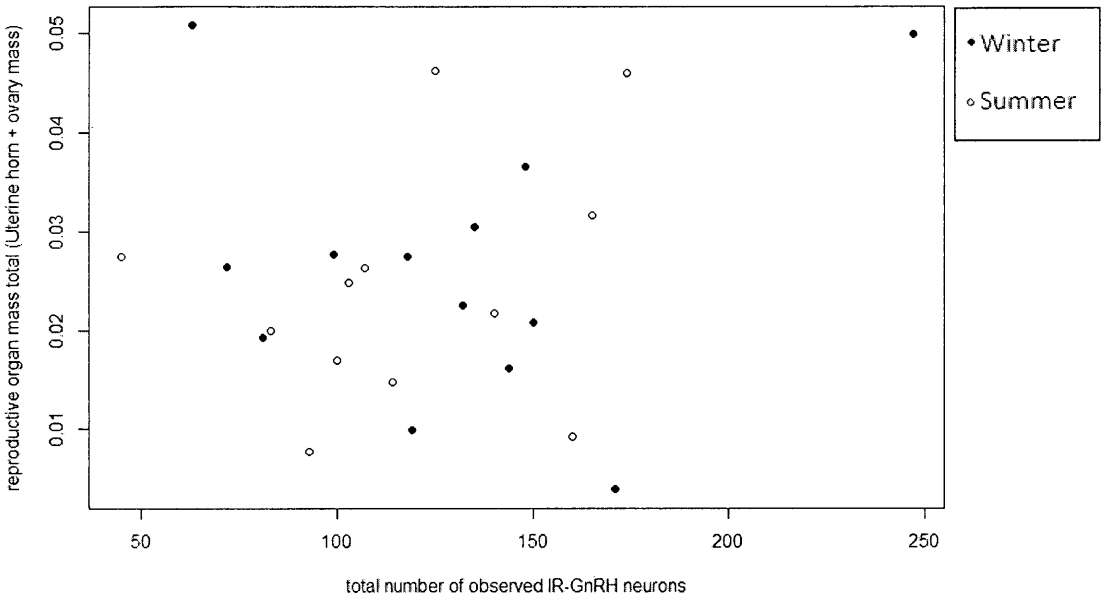

reproductive organ mass total (Uterine horn + ovary mass)
0.01
0.02
0.03
0.04
0.05
50
100
150
200
250
total number of observed IR-GnRH neurons
Winter
Summer

Figure 53.

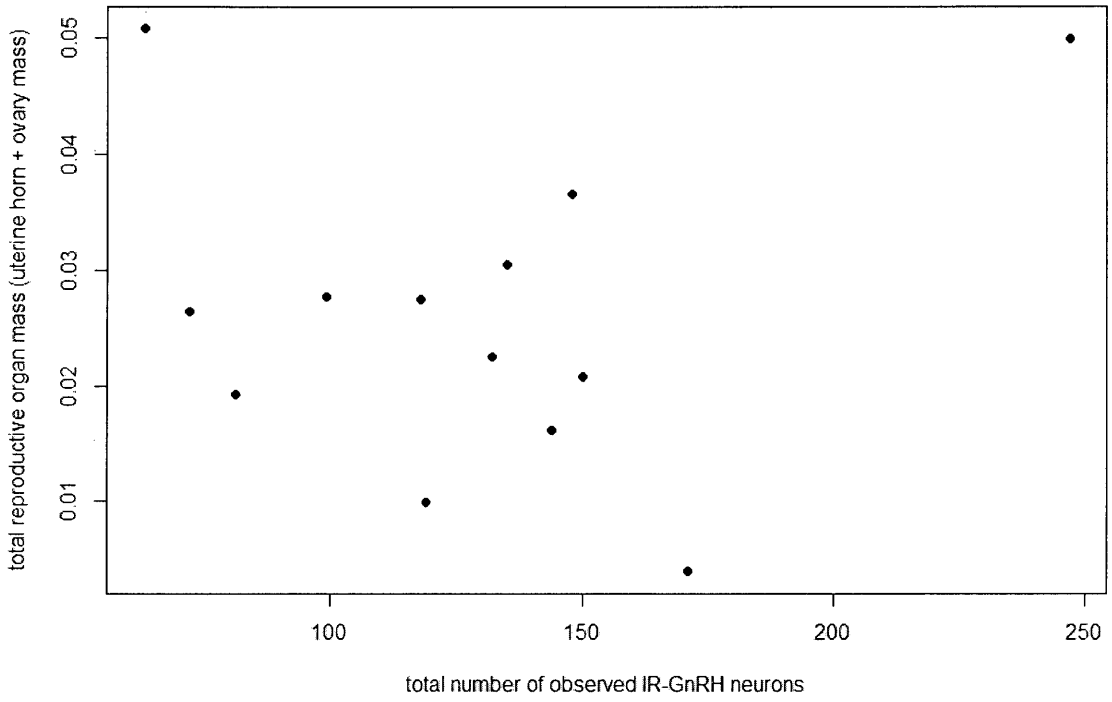

total reproductive organ mass (uterine horn + ovary mass)
0.01
0.02
0.03
0.04
0.05
100
150
200
250
total number of observed IR-GnRH neurons

Figure 54:

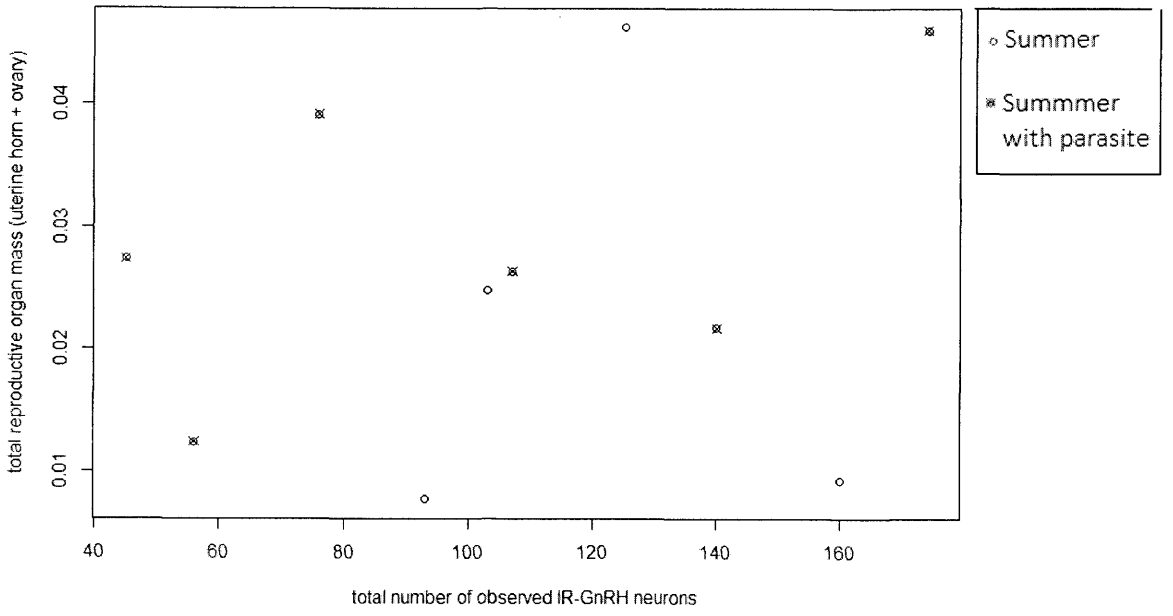

total reproductive organ mass (uterine horn + ovary)
0.01
0.02
0.03
0.04
40
60
80
100
120
140
160
total number of observed IR-GnRH neurons
Summer
Summmer with parasite

Figure 55.

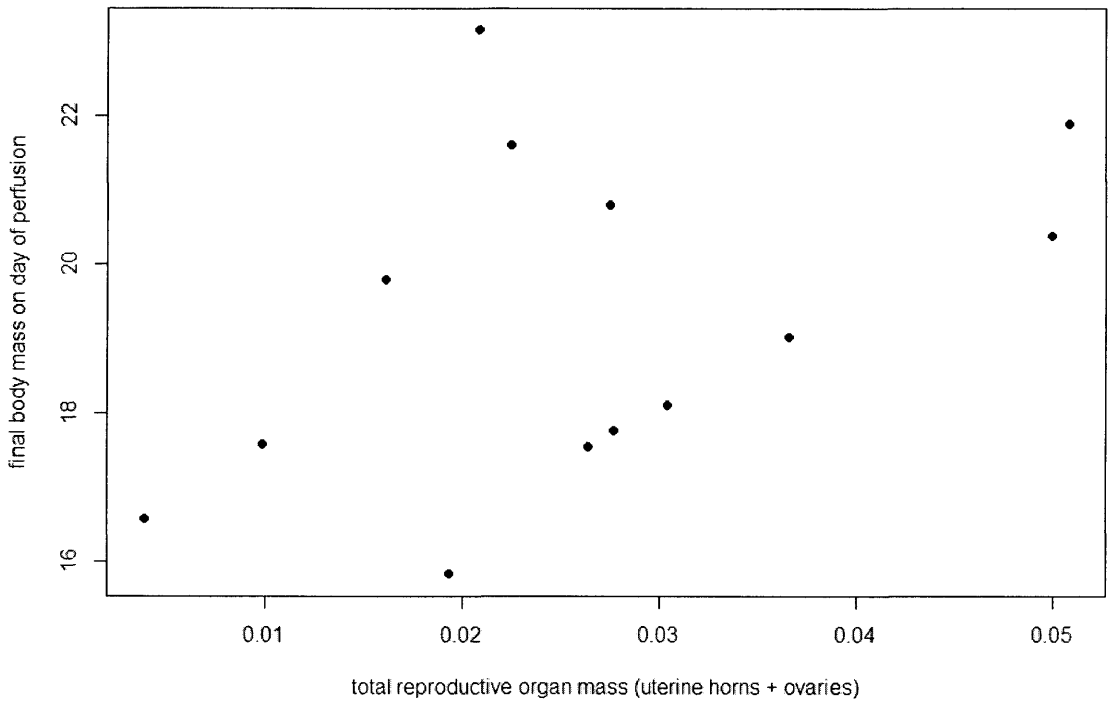

final body mass on day of perfusion
16
18
20
22
0.01
0.02
0.03
0.04
0.05
total reproductive organ mass (uterine horns + ovaries)

Figure 56.

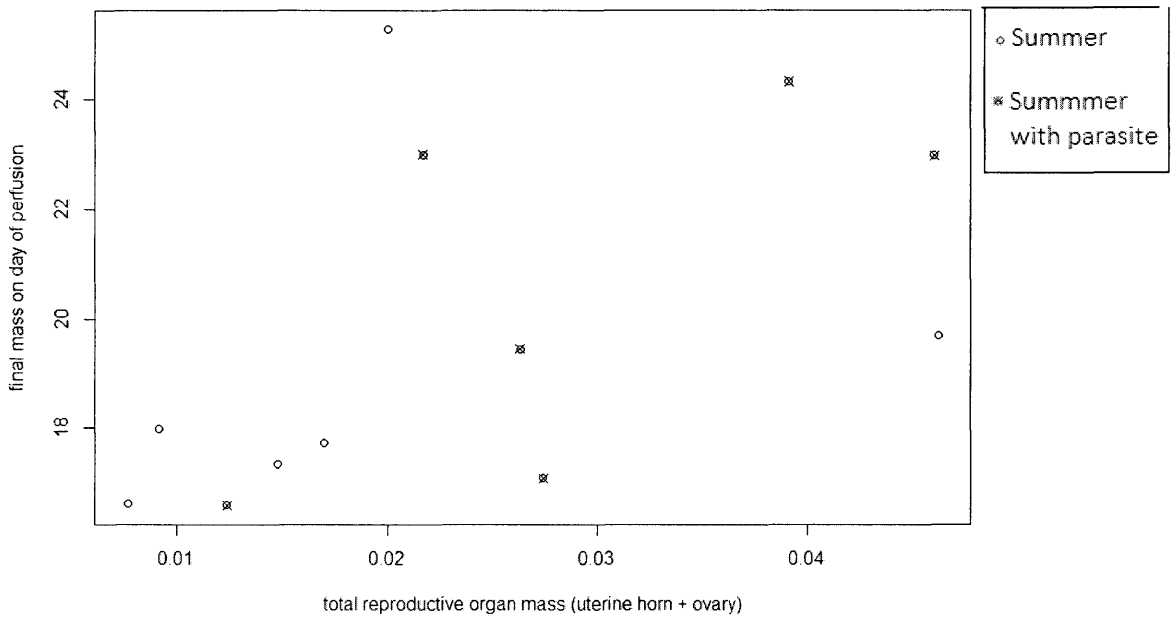

Summer
Summmer with parasite
final mass on day of perfusion
18
20
22
24
0.01
0.02
0.03
0.04
total reproductive organ mass (uterine horn + ovary)

Figure 57.

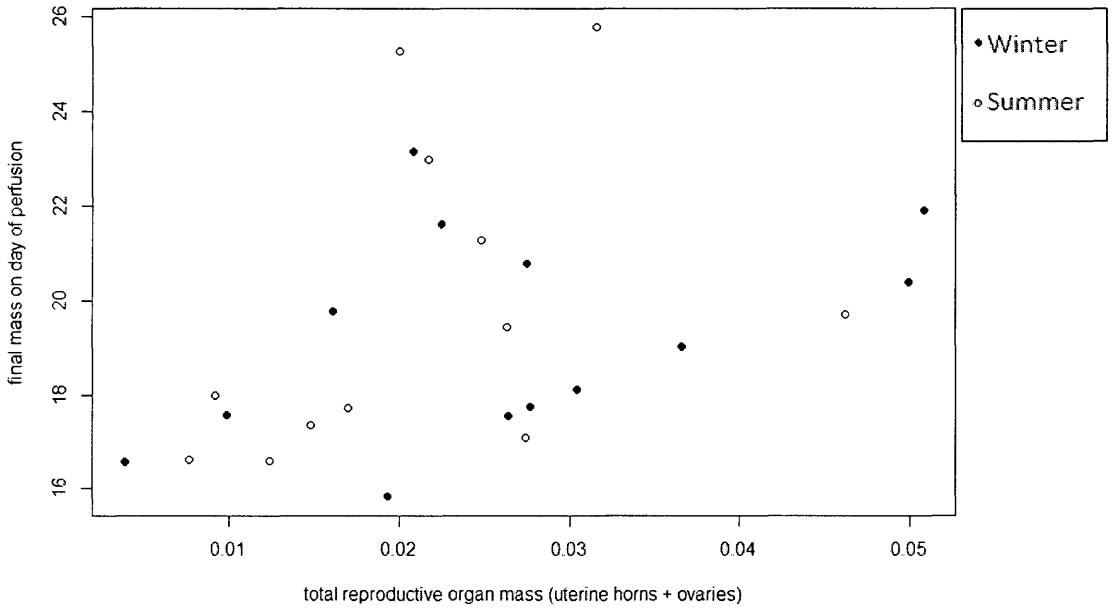

final mass on day of perfusion
16
18
20
22
24
26
0.01
0.02
0.03
0.04
0.05
total reproductive organ mass (uterine horns + ovaries)
Winter
Summer

Figure 58.

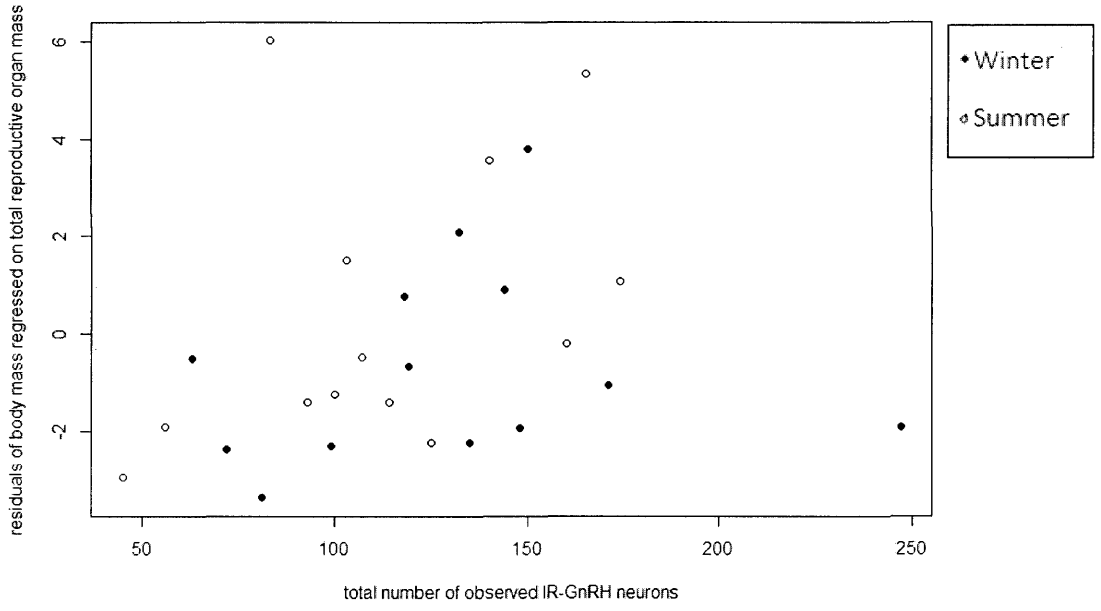

residuals of body mass regressed on total reproductive organ mass
-2
0
2
4
6
50
100
150
200
250
total number of observed IR-GnRH neurons
Winter
Summer

Figure 59.

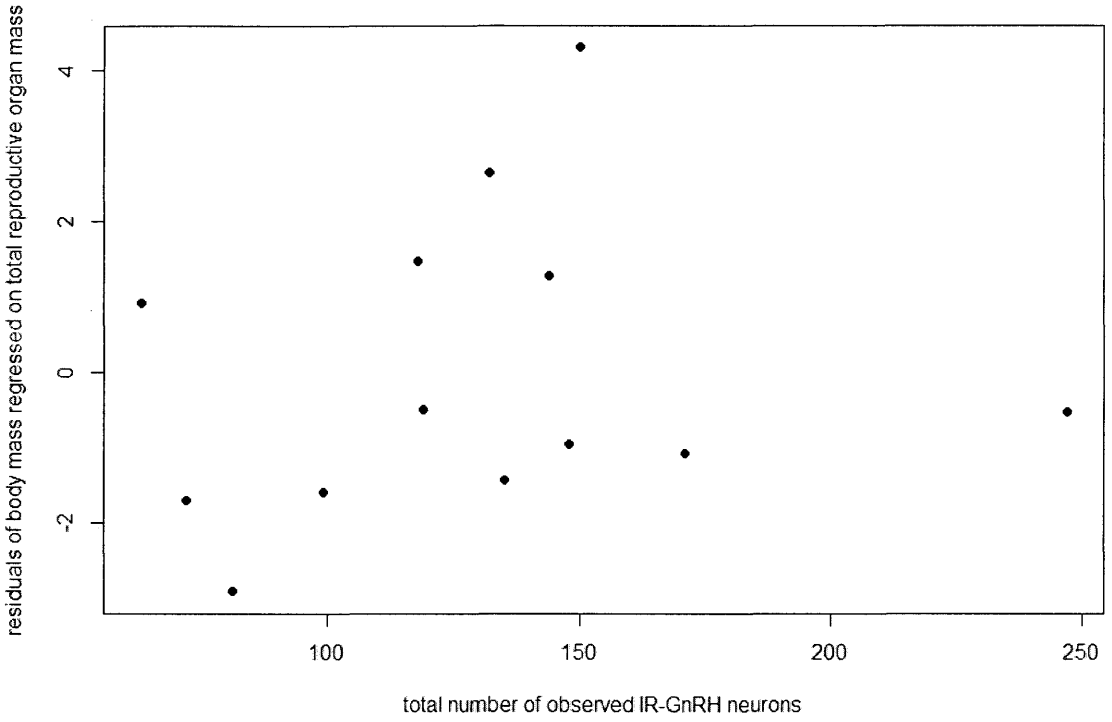

residuals of body mass regressed on total reproductive organ mass
4
2
0
-2
100
150
200
250
total number of observed IR-GnRH neurons

Figure 60.

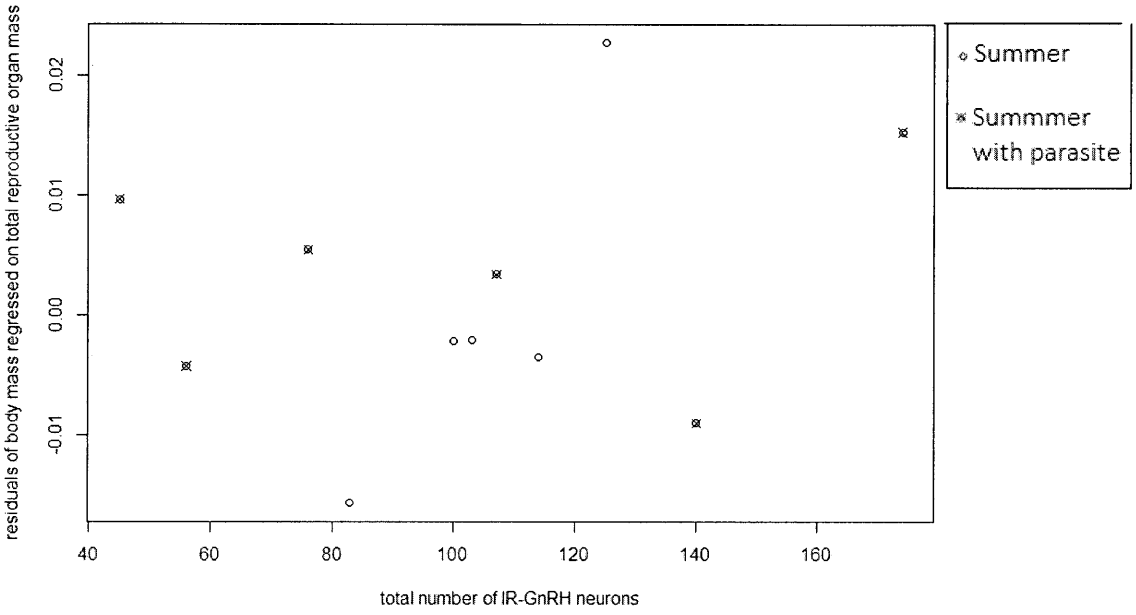
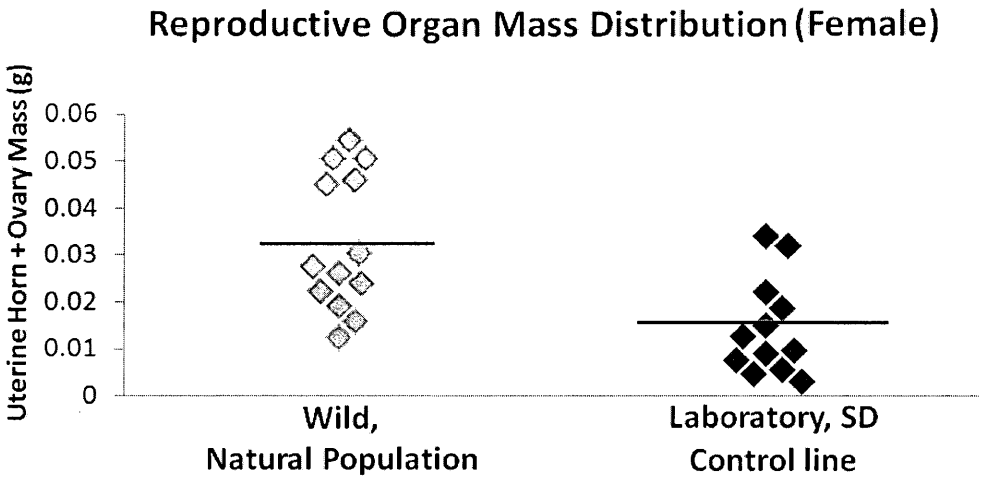

residuals of body mass regressed on total reproductive organ mass
0.02
0.01
0.00
-0.01
40
60
80
100
120
140
160
total number of IR-GnRH neurons
Summer
Summmer with parasite

Figure 61.


Reproductive Organ Mass Distribution (Female)
Uterine Horn + Ovary Mass (g)
0.06
0.05
0.04
0.03
0.02
0.01
0
Wild,
Natural Population
Laboratory, SD
Control line

Melissa Renee Proffitt

mrproffitt@email.wm.edu

The College of William and Mary
GSH Box 0091, 110 Sadler Center
Williamsburg, VA 23185
(276) 202-1432

Permanent address:
2037 Lake Witten Road
North Tazewell VA, 24630
(276) 988-6773

EDUCATION:

M.S. Biology: The College of William and Mary: Williamsburg, VA

Expected summer of 2014

Major- Biology

Cumulative GPA: 3.54

Relevant Coursework:

Animal physiology, molecular genetics, molecular genetics laboratory, wetlands ecology, thesis writing. Two semesters of colloquium and three semesters of graduate research.

B.S. Biology: Concord University: Athens, WV

Obtained May 2012

Major – Biology: Recombinant Gene Technology

Minor - Chemistry

Final GPA: 3.33

Relevant Coursework:

Scuba diving, zoology, ethology, ecology, ornithology, cell/molecular biology, microbiology, senior biology seminar, immunology, introduction to research methodology, math through calculus 1, analytical chemistry, introductory physics, intermediate physics, chemical lab safety, biochemistry, both semesters of organic chemistry, and two semesters of independent research.

CAREER OBJECTIVE:

Multi-disciplined "overachiever" who seeks to earn a Ph.D working in the following areas: endocrinology, physiology, and animal behavior.

PROFILE:

Detail oriented with an uncanny ability to see patterns, think critically, and connect seemingly unrelated concepts. Patient, but driven individual with good time management, communication, and organizational skills. A team player, with mentoring abilities: but able to work well independently.

RESEARCH EXPERIENCE:

Current Research: College of William and Mary, Williamsburg VA

Examining natural variation in winter fertility and GnRH neurons in a population of white-footed mice (*Peromyscus leucopus*)

NSF Research for Undergraduates Experience: Indiana University, Bloomington IN

I interned in the Evolution, Ecology, and Behavior program at Indiana University. I worked with the steroid regulation of seasonal behavior in Siberian hamsters (*Phodopus sungorus)*

Independent Research Projects: Concord University, Athens WV

Independent Research- Examining the roles of natural barriers on mechanisms of gene flow and genetic diversity in the Northern Dusky Salamander (*Desmognathus fuscus*). Data will be examined using maximum parsimony analysis.

Other research projects completed in classes at the undergraduate level:

"Non-Mendialian Inheritance Patterns in Drosophila Melanogaster," genetics (Fall 2011)

"Identification of Unknown Bacterial Strain" in microbiology (Fall 2011)

"Identification and characterization of egg antibacterial agents" biochemistry (Fall 2011)

"Determination of binding partner for Ezrin, a structural protein" in cell/molecular biology (spring 2011)

"Organic synthesis of a Benzoxazol from Toluene" in organic chemistry (Spring 2011)

"Chlorophyll as a chelating agent," in my analytical chemistry course (Fall 2010)

"Crayfish defensive response in relation to size," in my zoology course (Spring 2010)

WORK EXPERIENCE:

Teachers Assistant: The College of William and Mary, Williamsburg VA

August 2012-Current

Laboratory Courses Taught:

- Animal Physiology
- Integrative Biology (Zoology)
- Introduction to Cells, Molecules and Development

IT help-desk worker: Concord University, Athens WV

January 2010-August 2012

- Assist students, faculty, and staff with technological issues
- Assess minor computer problems, and dispatch technicians when necessary

Volunteer: Animal Shelter, Tazewell VA

May 2008-August 2008

- Furthered trained in animal husbandry techniques

Volunteer: Valley Animal Hospital, Cedar Bluff VA

October 2004- August 2008

- Safely restrain animals for examinations
- Assist in surgery for small domestic animals
- Furthered animal husbandry techniques

- Monitor IV's and other recovery regiments, such as recovery from anesthesia.

PRESENTATIONS:

College of William and Mary Graduate Research Symposium, 2014- Poster presentation on Natural Variation in Fertility and GnRH Neurons in a Wild, Natural Population of White-Footed Mice, *Peromyscus leucopus.*
SBN 2013- Society for Behavioral Neuroscience- Poster presentation on Natural Variation in Fertility and GnRH Neurons in a Wild, Natural Population of White-Footed Mice, *Peromyscus leucopus*

College of William and Mary Graduate Research Symposium, 2013- Poster presentation on Natural Variation in Fertility and GnRH Neurons in a Wild, Natural Population of White-Footed Mice, *Peromyscus leucopus.*

Behavior 2011- Meeting of the International Ethological Society and the Animal Behavior Society: poster presented on steroid regulation of aggression in female Siberian hamsters (*Phodopus sungorus*).

Concord University Summer Research Symposium- Presentation given on "Steroid Regulation of seasonal aggression in female Siberian hamsters (*Phodopus sungorous*)."

Concord University Undergraduate Research Symposium, Spring 2011- Posters, "Enhancement of Quantum Dot Fluorescence, Determination of Ezrin's Binding Partner Through Immunofluorescence, and Organic Synthesis of a Benzoxazol from Toluene."

SKILLS:

Lab Skills: Perfusions, castrations, dissections, retro-orbital blood collections, vaginal cytology, and microtome experience

Immunocytochemistry (ICC), various gel electrophoresis techniques (such as SDS page), DNA extraction, PCR, spot blots, and a variety of spectrophotometric assays.

GC MS, Liquid Chromotography (including some HPLC experience), Gravimetric analysis, potentiometric titrations, spectrophotometry.

Field work skills: Sherman small mammal live trapping and wild salamander collection

Computer: Adobe Photoshop, OD log, Phylip, lab scribe, Windows. Microsoft Word, Excel, Publisher, Open Office.

HONORS & AWARDS:

87th percentile on the GRE analytical writing section (2012)

87th percentile out of the nation on the ETS major field test for biology (2011)

84th percentile out of the nation in Analytical Chemistry: ACS standardized exam (2010).

Obtained "biology researcher of the year" award during my senior year of undergraduate

Highest average in high school veterinary science (2007).

CERTIFICATIONS/ACTIVITIES:

Graduate Activities: Biology department representative in Graduate Student Association (2013-2014)

Certification: open water scuba diving (obtained March 2012)

Undergraduate Activities:

Concord University American Chemical Society secretary (2010-2011)

Animal Behavior Society

National American Chemical Society

Sigma Zeta Honors Society

Cardinal Key Honors Society

Gamma Beta Phi Honors Society