HORMONE PROFILES OF CAPTIVE HARP SEALS (PAGOPHILUS GROENLANDICUS) IN RELATION TO PUBERTY, REPRODUCTION AND SENESCENCE

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A dissertation submitted to the Department of Biology in partial fulfilment of the requirements

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ABSTRACT

The main objectives of this study were to describe sex-steroid hormones in the harp seal (*Pagophilus groenlandicus*), in relation to seasonality in reproduction, puberty and senescence. Blood samples from 3 males and 2 females were collected at ~2 week intervals from 1999-2007. Plasma concentrations of estradiol, progesterone and prolactin in females and testosterone in males were measured by radioimmunoassay. Estradiol in one mature female and testosterone in 3 males during their years of reproductive maturity increased across years roughly as would be expected from the breeding season of the wild Northwest Atlantic harp seal population. Estradiol and progesterone levels suggested that 1 young female did not reach maturity at 5 yr of age. Testosterone levels in 1 young male suggested that physiological maturity was reached at 4 yr of age. Estradiol levels in a mature female after she was ~22 yr of age declined substantially, suggesting senescence. This study extends our knowledge of reproductive physiology of phocid seals in general and the harp seal in particular, and shows that hormonal activities are not suppressed in the captive environment.

ACKNOWLEDGEMENTS

I would like to give special thanks to Daryl Jones and Edward Miller for their support, patience and suggestions throughout my dissertation. I would also like to thank Dr. Miller for welcoming me into his lab and critically reviewing drafts of this paper. I would also like to thank the many people over the years that helped to obtain and analyze the samples. Without their cooperative effort over many years this project would not have been possible. I thank Connie Short for her help in retrieving data, Garry Stenson for giving helpful hints and Paul Regular for his statistical advice.

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INTRODUCTION

1. Background to study

The Ocean Sciences Centre (OSC, St. John's, NL, Canada) has housed phocid seals for decades. Since the 1970s four species of seal have been maintained there for research: harbor seal (*Phoca vitulina*); harp seal (*Pagophilus groenlandicus*); hooded seal (*Cystophora cristata*); and ringed seal (*Pusa hispida*; common and scientific names follow Committee on Taxonomy, 2011). The OSC is one of only a few facilities in the world to hold captive harp seals in a research environment. Currently there are five harp seals at the facility. Past research has explored behaviour, physiology, energetics, perception, diet, development, sensory systems and reproduction. Since December 1998 blood samples have been taken to monitor health; portions of these samples were saved and analyzed for sex hormones. I took advantage of these data because reproductive endocrinology of pinnipeds is poorly known but presumably varies importantly in relation to the diverse life history and ecology in the group.

Reproductive physiology is broadly similar across mammalian species. Most species of mammals breed once annually (Feldhamer et al., 2007), a conservative pattern that applies even to marine species like pinnipeds. The sole exception is the Australian sea lion (*Neophoca cinerea*), which has an 18-month breeding cycle (Gales et al., 1992). In addition pinnipeds are monestrous (one oestrus per breeding cycle, usually a year) except the walrus (*Odobenus rosmarus*) and Hawaiian monk seal (*Monachus schauinslandi*), in which females are polyestrous (two or more oestrus periods per year). Walruses have an initial oestrus period 4 months after giving birth when males are infertile, and a second oestrus 6 months later when males are fertile (Reidman, 1990;

Pomeroy, 2011).

The physiology of steroid hormones in pinnipeds is poorly documented and has been assumed to be similar to that of other mammals (Table 1; Robeck et al., 2001). However in some pinnipeds it is known that levels of sex hormones vary throughout life: over the annual cycle, at sexual maturity and possibly at senescence (St. Aubin, 2001; Atkinson et al., 2008).

The main goal of my study was to contribute to knowledge of lifetime and seasonal variation in sex hormones of the harp seal, and to place these findings in the context of the species' life history and annual cycle. I studied steroid hormones of five captive harp seals to determine correlations with puberty and senescence, and with the annual cycle of wild animals. Sex hormones of harp seals have not been analyzed, apart from an investigation into the seasonal relationships between testosterone levels and vocal activity (Serrano, 2000) and progesterone levels in relation to pseudopregnancy (Renouf, 1994). This study will aid in filling the gap in information available about endocrine and reproductive physiology of harp seals.

In the following sections I review the current state of knowledge about reproductive hormones of pinnipeds through the annual cycle, and at puberty and senescence.

2. Literature Review

a) Seasonality of reproduction in pinnipeds

High-latitude pinnipeds have a highly seasonal synchronized reproductive cycle; e.g. harp seals pup and mate from February-March (Sergeant, 1991). The breeding season is longer

Table 1. Published literature on sex steroids in phocid seals shows data gaps in and how generalizations have been made based on few species (8 of 19 species).

Species	**Testosterone**	**Prolactin**	**Estrogens**	**Progesterone**
Hawaiian monk seal *Monachus schauinslandi*	Atkinson and Gilmartin, 1992; Atkinson et al., 1994		Pietraszek and Atkinson, 1994	Pietraszek and Atkinson, 1994
Southern elephant seal *Mirounga leonina*	Ferreira et al., 2005		Ferreira et al., 2005	Ferreira et al., 2005
Harbor seal *Phoca vitulina*	Gardiner et al., 1999		Gardiner et al., 1999; Reijnders, 1990	Reijnders, 1990, Gardiner et al., 1996; Katsumata, 2010;
Spotted seal *Phoca largha*				Katsumata, 2010
Harp seal *Pagophilus groenlandicus*	Serrano, 2000			Renouf et al., 1994
Gray seal *Halichoerus grypus*	Seely and Keith, 1991; Sangalang and Freeman, 1976.		Mellish and Iverson, 2005	Hobson and Boyd, 1984; Mellish and Iverson, 2005.
Hooded seal *Cystophora cristata*	Noonan, 1991	Mellish et al., 1999	Mellish and Iverson, 2005; Noonan, 1989	Mellish and Iverson, 2005; Noonan, 1989
Weddell seal *Leptonychotes weddellii*	Bartsh et al., 1992			

in low-latitude environments (e.g. Hawaiian monk seals breed from March-July; Atkinson and Gilmartin, 1992; Atkinson et al., 1994).

Pinnipeds spend most of their time in the water but return to land or ice to give birth and then enter oestrus shortly thereafter, when mating occurs. In the Otariidae (fur seals and sea lions) oestrus begins approximately 1 week postpartum, whereas in phocid seals oestrus begins after lactation and weaning (Atkinson, 1997; Boyd et al., 1999). Males aggregate around the time of parturition and oestrus. In this study I predicted that the timing of breeding in captive harp seals would be similar to that in the wild population (i.e., February and March in the Northwest Atlantic).

b) Annual cycle of reproductive hormones in general

Mammal hormones have particular functions in different phases of reproduction, for example in the female oestrous cycle. Phases of this cycle are anestrus (non-breeding stage), proestrus (beginning stage), estrus, fertilization, gestation and lactation. During proestrus the animal's reproductive system is preparing for the estrus stage. The main female sex organs are the ovaries. Follicles in the ovary each enclose a single egg. The ovarian follicle produces steroid sex hormones including androgens, estrogens and progestins. Follicular development is controlled by FSH (follicle-stimulating hormone) and LH (luteinizing hormone). The oestrus stage is the brief period before and after ovulation. Oestrus is entered as the egg in the follicle matures, the follicle enlarges, and finally the egg is released (ovulation). Ovulation is triggered by the secretion of estradiol by the developing follicles, which elevates levels of LH. The follicle then fills with yellow follicular cells and is now termed the corpus luteum. The corpus luteum maintains pregnancy by producing progesterone. Progesterone promotes the growth of female sex tissues including the uterus. The majority of mammals including phocid seals have spontaneous ovulation, meaning that ovulation occurs without copulation. Once ovulation occurs the egg enters oviduct where it can be fertilized.

Testosterone is responsible for the production of sperm. Testosterone is a steroid hormone which is synthesized in the testes. Testosterone is also responsible for the development of male sex organs. If fertilization occurs the embryo develops into a blastocyst (embryo at 32-64 cells), which is now ready to implant into the uterine wall (Feldhamer et al., 2007). Gestation follows after implantation, which is the time between fertilization and parturition. During pregnancy ovarian hormones such as estradiol and progesterone are secreted causing the enlargement of the mammary glands. This prepares the glands for milk production (lactation). Following parturition, hormone levels decrease signaling the anterior pituitary gland in the brain to secrete prolactin. Prolactin is a peptide hormone which indirectly stimulates milk production. In contrast to steroid hormones, peptide hormones are proteins that have an endocrine function and indirectly act on the reproductive organs (in this case on the mammary glands to produce milk). Once lactation is over estrus begins again and the oestrus cycle continues.

c) Annual cycle of reproductive hormones in pinnipeds

In female pinnipeds the annual cycle of reproduction includes the fertile oestrous period, delayed implantation and gestation. Oestrus consists of physiological and behavioural components; in my study the focus was physiology. Oestrus is the phase of the oestrous cycle when females are sexually receptive and pregnable. In the pinniped oestrous cycle, a peak in oestrogen is followed by a peak in progesterone in the Hawaiian monk seal (Pietraszek and Atkinson, 1994), harbor seal (Reijnders, 1990) and Antarctic fur seal

(*Arctophoca gazella*; Boyd, 1991). Progesterone levels increase after ovulation and continue to increase if the seal is pregnant (Reijnders, 1990). This pattern shows pre-ovulatory follicular maturation and formation of the corpus luteum (Atkinson, 1997). Follicular formation is similar across seal species, but hormonal production by the corpus luteum differs. In the Hawaiian monk seal circulating progesterone decreases 3 weeks after ovulation, with the next ovulation occurring 2 weeks after showing luteolysis (degradation of the corpus luteum; Pietraszek and Atkinson, 1994). In the harbor seal (Reijnders, 1990) and hooded seal (Noonan, 1989) progesterone concentrations are high for 4-5 months, revealing a long luteal phase (i.e. continuing function of the corpus luteum). Following oestrus and mating, fertilized pinnipeds express delayed implantation, lasting ~4 months (Boyd et al., 1999). Delayed implantation is when the embryo is suspended until environmental conditions become favorable. This is important in harp seals in particular so they can to give birth when sea is present, as parturition and lactation take place on the ice surface. In phocids, progesterone levels increase gradually over delayed implantation and gestation (Boyd 1983; Reijnders, 1990). In otariids, progesterone concentrations remain low during delayed implantation and increase just before the blastocyst is activated for implantation (Boyd, 1991; Gales et al., 1997). In one species (northern fur seal, *Callorhinus ursinus*), a rise in oestrogen occurs before embryonic reactivation (Gales et al., 1997). Prolactin may also function in the reactivation process because it has luteotrophic effects, although levels decline during delayed implantation in the Antarctic fur seal (Boyd, 1991). Once the pup is born and lactation begins, levels of prolactin are high and of progesterone are low (Boyd, 1991). Lactation lasts for 4-36 months in different otariid species, during which females alternate periods ashore with pups to feed (when females fast) with periods at sea to feed. Lactation is briefer in most phocid species and in some species females fast throughout lactation (especially large species with more fat stores; Atkinson, 1997; Mellish et al., 1999; Lydersen and Kovacs, 1999). Unfertilized females may express pseudopregnancy, when progesterone levels remain high due to the persistence of the corpus luteum (Atkinson, 1997). This has been noted in the harbor seal (Reijnders, 1990), hooded seal (Noonan 1989) and harp seal (Renouf et al., 1994).

Testosterone levels vary systematically over the male's annual cycle. High levels occur for 1-3 months in species with brief breeding seasons such as hooded and Weddell seals (*Leptonychotes weddellii*; Noonan et al., 1991; Bartsh et al., 1992). For subtropical species such as the Hawaiian monk seal, testosterone increases in the breeding period but the onset and end are more gradual in temperate and polar species (Atkinson and Gilmartin, 1992). In this study I predicted that mature female harp seals would show high levels of estrogen during the mating season, followed by a progressive increase in progesterone over the year, as in other phocid species. I predicted an increase in testosterone in mature male harp seals before and during the mating season, as in other high-latitude phocid seals.

d) Population biology and the annual cycle of harp seals

The harp seal is distributed in subarctic and arctic regions of the North Atlantic Ocean. Three populations are distinguished based mainly on where they whelp (Sergeant, 1991). One population is in the Barents Sea; it pups in the White Sea. A second population is off the eastern coast of Greenland, with whelping grounds near Jan Mayen. The third population is in the Northwest Atlantic off Canada, with two breeding localities: the

Front off the northeastern coast of Newfoundland and southern Labrador; and the Gulf of St. Lawrence near the Magdalen Islands (Fig. 1; Sergeant, 1991).

Harp seal populations differ in whelping times: February-March in the Barents Sea; late March in the Greenland Sea (Frie et al., 2003); and late February-early March in Canada (Sjare and Stenson, 2009). Mating takes place approximately 2 weeks after pupping and is followed by moulting a month after the pup is born (Sergeant, 1991). After moulting Canadian harp seals migrate northward in late May to early June (Sergeant, 1991).

e) Puberty

Harp seals can live to 25-35 yr of age but mature early so they have a long reproductive life. Physiological sexual maturity (puberty) is defined as the first ovulation for female seals and the onset of sperm production (spermatogenesis) for males. The age of puberty in harp seals differs between males and females, across populations and over time. Females and males become mature at 4-6 yr of age; males may take an extra year or two to reach sexual maturity (Frie et al., 2003). In the Barents Sea the mean age at maturation was 5.4 yr from 1962-1972 and 6.6 yr from 1976-1993 (Frie et al., 2003). Seals in the Greenland Sea population averaged 5.6 yr at age of sexual maturity from 1959-1990 (Frie et al., 2003). In Canada sexual maturity averaged 6.2 yr in 1952 to 4.5 yr in 1979 (females; Bowen et al., 1981), 5.5-5.7 yr in the 1990s and 4.9-6.0 yr from 2000-2004 (Sjare and Stenson, 2009). Variation over time can be attributed in part to a density-dependent response in increasing or high-density populations (Bowen et al., 1981; Sjare et al., 1996).

Before puberty, sex-steroid levels are undetectable to low in females (Hawaiian

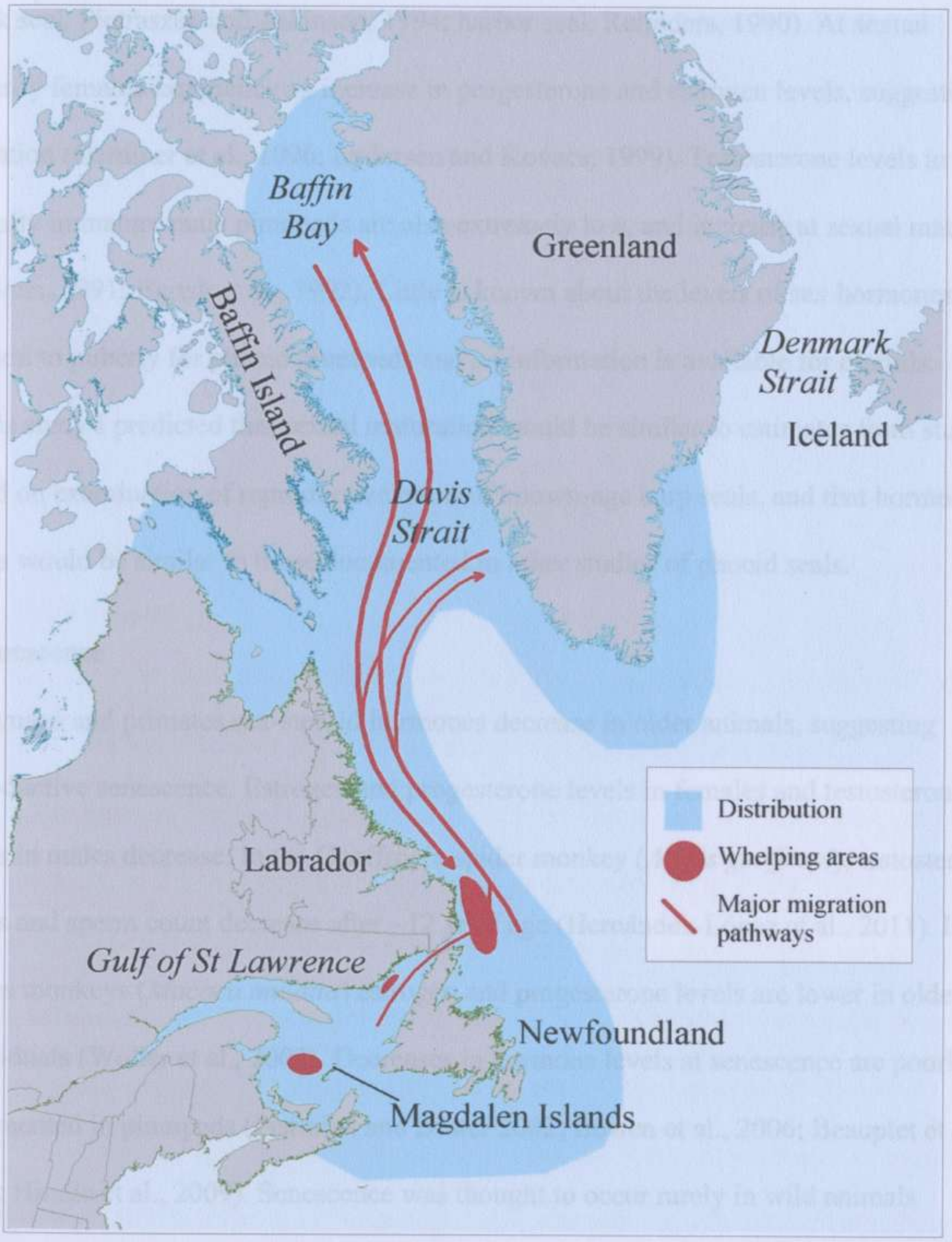

Figure 1. Distribution, migratory movements and whelping grounds of the Northwest Atlantic harp seal (*Pagophilus groenlandicus*). Map courtesy of Garry Stenson, Department of Fisheries and Oceans.

monk seal, Pietraszek and Atkinson, 1994; harbor seal, Reijnders, 1990). At sexual maturity females experience an increase in progesterone and estrogen levels, suggesting ovulation (Gardiner et al., 1996; Lydersen and Kovacs, 1999). Testosterone levels in sexually immature male pinnipeds are also extremely low, and increase at sexual maturity (Noonan, 1991; Bartsh et al., 1992). Little is known about the levels of sex hormones in relation to puberty for phocid pinnipeds and no information is available for otariids. In this study I predicted that sexual maturation would be similar to estimates from studies based on examination of reproductive tracts of known-age harp seals, and that hormone levels would be similar to those documented in other studies of phocid seals.

f) Senescence

In humans and primates sex-steroid hormones decrease in older animals, suggesting reproductive senescence. Estrogen and progesterone levels in females and testosterone levels in males decrease. In the Geoffroy's spider monkey (*Ateles geoffroyi*), testosterone levels and sperm count decrease after ~12 yr of age (Hernández-López et al., 2011). In rhesus monkeys (*Macaca mulatta*) estrogen and progesterone levels are lower in older individuals (Woller et al., 2002). Decreases in hormone levels at senescence are poorly documented in pinnipeds (Pistorius and Bester 2002; Bowen et al., 2006; Beauptet et al., 2006; Hindle et al., 2009). Senescence was thought to occur rarely in wild animals because natural mortality levels are typically high (Hindle et al., 2009). Senescence in the gray seal (*Halichoerus grypus*) is accompanied by decreased lactation time and smaller neonatal and post-weaning mass in pups (Bowen et al., 2006). In the Weddell seal, biological aging is evident in the musculature, with remodeling of myofibers and the extracellular matrix (Hindle et al., 2009). In one study of southern elephant seals

(*Mirounga leonina*) no individual survived to an age where physiological aging could be detected: 5% of females survived to 10 and 0.5% to 17 yr of age (Pistorius and Bester, 2002). In otariids senescence has been documented in one population of subAntarctic fur seals, with a decrease in survival and reproduction after 13 yr of age (Beauptet et al., 2006). In this study I predicted that long-term hormone records for one old seal would show decreased hormone levels either during the reproductive season as age increased, or throughout the year.

METHODS

1. Location of study

I studied five harp seals at the OSC in Logy Bay, Newfoundland and Labrador (47^{o}38'N 52^{o}40'W). The seals were of different sexes and ages and were born at the OSC or captured in the wild (Table 2; Appendix 1). They had free access to three tanks; the two round tanks were 2.5 m in depth, 4.9 and 7.5 m in diameter, respectively; the third tank was rectangular and measured 3.8 by 0.9 m. The tanks were surrounded by approximately 125 m^2 of deck on which they could move freely (Fig. 2). The tanks were filled with seawater that was pumped in directly from the Atlantic Ocean beside the OSC by a continuous flow-through system. Temperature of the sea water varied seasonally from ~ -1.0 °C in February to about ~ 17 °C in September and salinity was constant at ~33 ppt. Caretakers drained, cleaned and refilled the tanks weekly. The seals were fed ~2-5 kg of herring daily, depending on their body mass and time of year (e.g., at approximately 120 kg Babette was fed ~2.5-3.0 kg per day). Crushed ice was provided daily as a source of freshwater.

2. Data Collection

Caretakers took blood samples from each seal every ~2 weeks from 1999 to 2007, except 2000-2001. The interval varied due to weather and availability of caretakers. Seals were kept out of the tanks for ~5 hr before samples were taken, to allow the animals to become warm and hence to increase peripheral blood flow to facilitate blood collection. Blood was sampled ~20 hr after the last meal. To facilitate sampling, seals were trained to stay in one place when a wooden V-shaped structure with soft mesh was placed in front of them (Fig. 3). The blood sample was taken from the plantar interdigital veins of the rear flipper with a 20-gauge 1.5-inch needle attached to a 10-ml BD Vacutainer blood-serum collection tube. We sampled ~3 ml of blood each time. After the needle was withdrawn, firm pressure was applied with the thumb to the sample site to stop bleeding. Blood samples were centrifuged with a Sorvall RC-5B refrigerated super-speed centrifuge at 1°C, at 5000 rpm for 10 min. The plasma was then pipetted into 1.5-ml Eppendorf tubes and frozen at -70C for later analysis. Assays were done to estimate plasma concentrations of testosterone in males and estradiol-17β, 17α-OH-progesterone and prolactin in females. Concentrations were estimated by immunoradiometric assays using Coat-a-Count procedure kits (Siemens Medical Solutions Diagnostic Products Corporation, Los Angeles, CA) following procedures in the kits' manuals (estradiol -- PITKE2-7, 2005-06-06 and PITKE2-4, 2004-11-01; progesterone -- PITKOP-6, 2006-12-29 and PITKOP-4, 2003-11-04; prolactin -- PIIKPR-6, 2006-12-29 and PITKPR-1, 2003-01-20; and total testosterone -- PITKTT-4, 2005-03-18 and PITKTT-1, 2002-12-11).

Table 2. Summary of information on age and origins of captive harp seals (*Pagophilus groenlandicus*) at the Ocean Sciences Centre, Logy Bay, Newfoundland and Labrador, from which blood samples were taken for reproductive hormone analyses (further details are in Appendix 1).

Sex	**Name**	**Age (yr) in 2012**	**Place of birth/capture**
Female	Babette	~30	Captured as breeding adult near Magdalen Islands, 1989
Male	Tyler	22	Captured as whitecoat near Magdalen Islands, 1990
Male	Jamie	18	Born at OSC, 16 March 1994
Male	Lenny	12	Born at OSC, 8 March 2000
Female	Deane	10	Born at OSC, 18 March 2002

Figure 2. Enclosure at the Ocean Sciences Centre where harp seals (*Pagophilus groenlandicus*) are maintained and blood samples used in this study were collected.

Figure 3. Harp seals (*Pagophilus groenlandicus*) at the Ocean Sciences Centre were trained to not move and to accept blood sampling from a hind flipper when a soft net attached to a wooden frame was placed in front of them.

Animals were maintained, handled and sampled following guidelines of the Canadian Council on Animal Care and under permit from the Animal Care Committee of Memorial University.

3. Data analysis

Samples were not always made biweekly, so I averaged data over each month of each year. Using statistical software R, I tested for concordance in hormone levels (a) across years within each individual and (b) across individuals with Kendall's coefficient of concordance. The scaled values of the monthly means were used in these computations (within each year, all readings were divided by the maximal reading, which thus was set at 1.0).

RESULTS

1) Female steroid hormones

a) Estradiol-17β in Babette

Estradiol concentrations showed a seasonal pattern in the years 1999, 2002, 2004 and 2005, with a peak level in or near the breeding period of harp seals in the Northwest Atlantic population in each of those years (Fig. 4). Concentrations decreased each year beginning in 1999 at 1121 pg/ml, reaching a low of 9.5 pg/ml in 2006-2007. High concentrations lasted for 5 months (January-May) in 1999, for at least 2 months in 2002 when Babette gave birth to her daughter Deane (no samples were available before April) and for 3 months in 2004 (January-March; Fig. 4). Only a slight increase was evident in 2005 but this occurred during the breeding period (38.1 pg/ml; 22 March). Estradiol levels did not change measurably throughout the years 2003, 2005, 2006 or 2007.

Patterns in those years showed that a rise in estradiol were significantly concordant ($W = 0.503$, $p = 0.0036$).

b) Estradiol-17β in Deane

Estradiol concentrations of Deane did not vary throughout the years except in 2002, her year of birth (Fig. 5). In that year her peak estradiol level was similar to that of her mother, Babette: 145.0 +/- 29.6 pg/ml and 140.8 +/- 15.9 pg/ml, respectively (Fig. 5).

c) 17α-OH-Progesterone in Babette

Progesterone concentrations showed an overall trend of a gradual increase throughout the year after the breeding period, except for 2004 (Fig. 6). Progesterone concentrations ranged from 20.0 (in 1999) to 0.045 ng/ml and were not concordant across years (Kendall's $W = 0.169$, $p = 0.293$).

d) 17α-OH-Progesterone in Deane

Progesterone levels in Deane were low in 2006-2006 and increased slightly (to a high of 1.93 ng/ml) in 2007 (Fig. 7). Levels were not concordant across years ($W = 0.185$, $p = 0.353$).

e) Prolactin in Babette and Deane

Prolactin levels varied substantially within the years 1999, 2002 and 2003 for Babette (2002 was the year of birth of her daughter Deane) and in 2003 for Deane, when she was 1 yr old (Fig. 8). Subsequently (2004-2007) prolactin levels did not vary measurably for either seal.

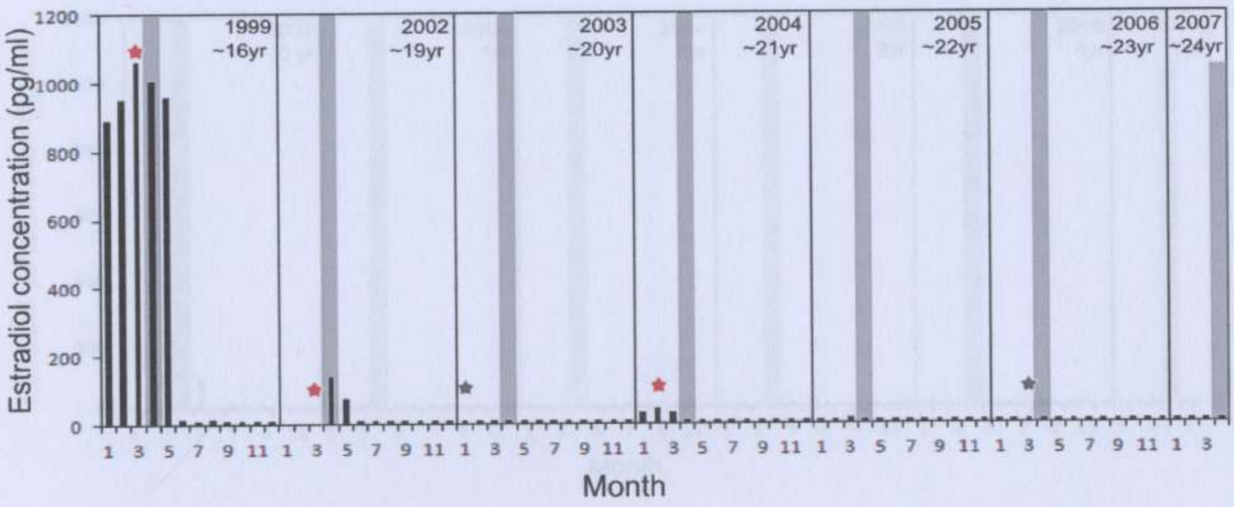

Figure 4. A pattern of seasonality with high levels of estradiol (plasma estradiol-17β) around and during the natural breeding period was evident for several years between the ages of 16-21yr of age in the captive female harp seal (*Pagophilus groenlandicus*) Babette. The shaded area in each calendar year represents the period of breeding for wild Northwest Atlantic harp seals (Sjare and Stenson, 2009). Red stars indicate live birth (1999, 2002, 2004) and grey stars indicate stillbirths (2003, 2006). Monthly mean data are plotted for years 1999-2007 (estimated ages 16-24 yr).

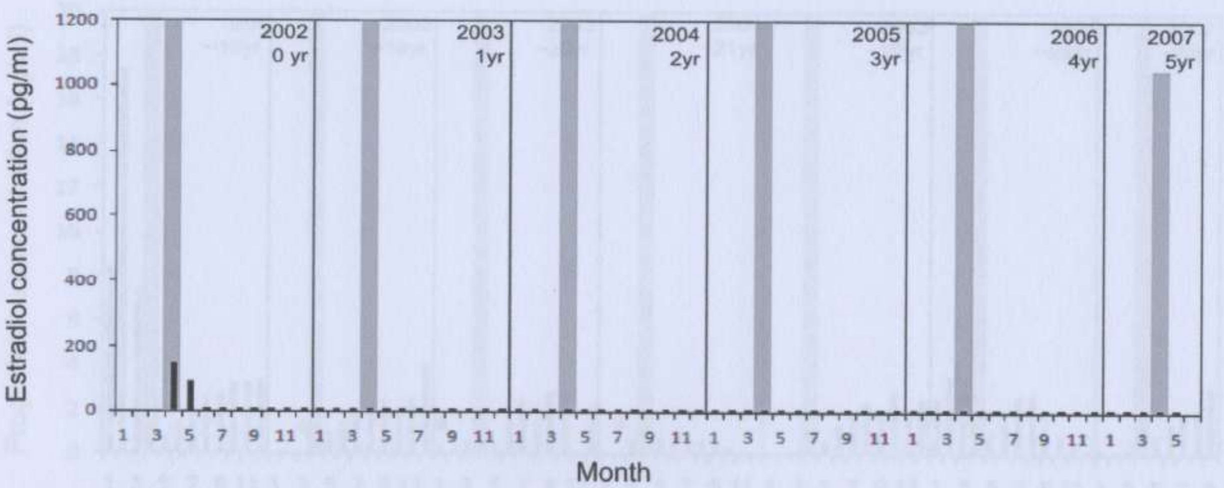

Figure 5. Levels of estradiol (plasma estradiol-17β) showed no variation within or across the 6 years of life in the captive female harp seal (*Pagophilus groenlandicus*) Deane, except in her year of birth (2002). The shaded area in each calendar year represents the period of breeding for wild Northwest Atlantic harp seals (Sjare and Stenson, 2009). Monthly mean data are plotted for years 2002-2007 (ages 0-5 yr).

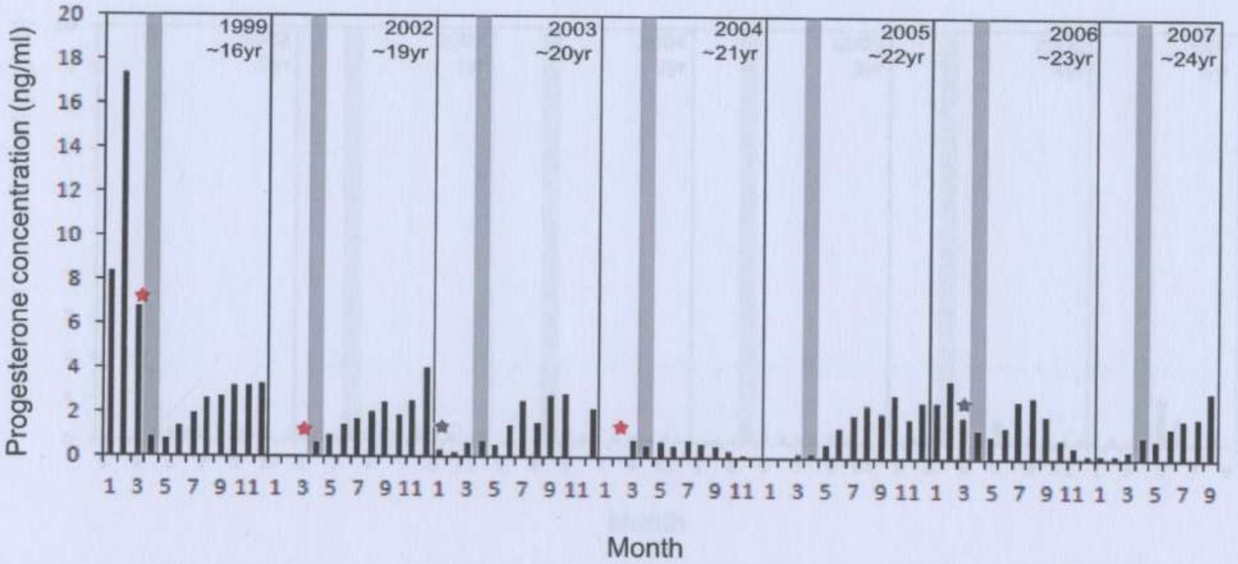

Figure 6. Levels of progesterone (17α-OH-progesterone) in the captive harp seal (*Pagophilus groenlandicus*) Babette increased gradually after mating in most years but varied unsystematically across years. The shaded area in each calendar year represents the period of breeding for the wild Northwest Atlantic harp seals (Sjare and Stenson, 2009). Red stars indicate live birth (1999, 2002, 2004) and grey stars indicate stillbirths (2003, 2006). Monthly mean data are plotted for years 1999-2007 (estimated ages 16-24 yr).

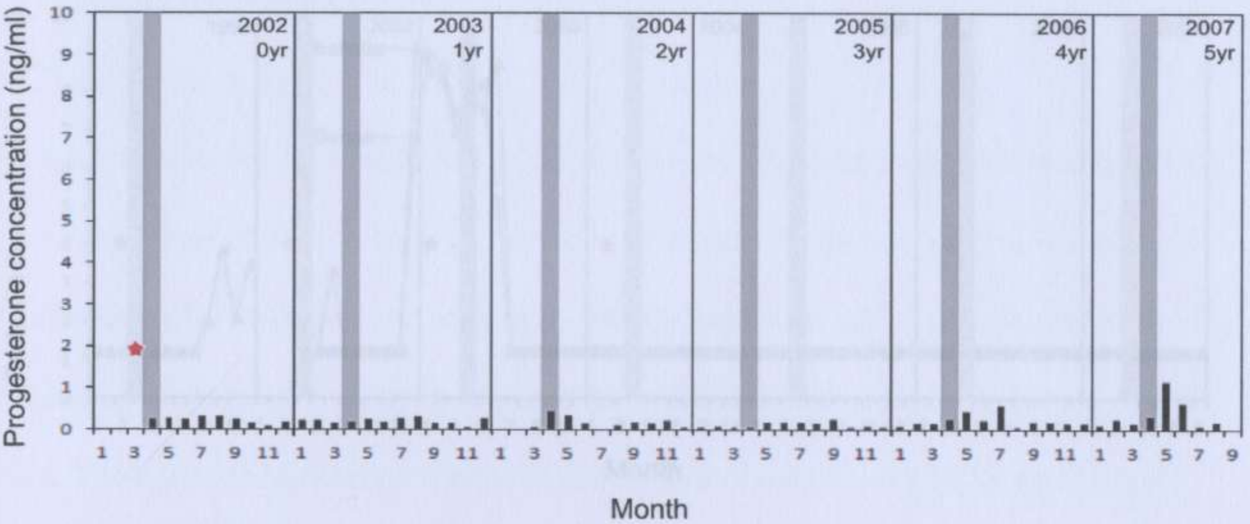

Figure 7. Progesterone (17α-OH-progesterone) levels varied unsystematically within and across years in the captive harp seal (*Pagophilus groenlandicus*) Deane. The shaded area in each calendar year represents the period of breeding for wild Northwest Atlantic harp seals (Sjare and Stenson, 2009). Red star indicates date of birth. Monthly mean data are plotted for years, 2002-2007 (ages 0-5 yr).

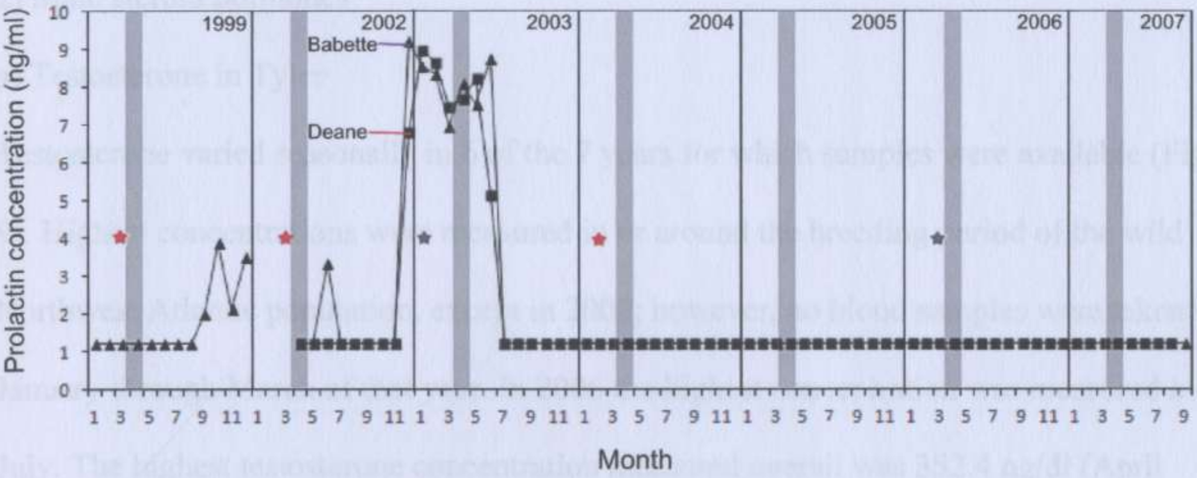

Figure 8. Prolactin levels varied within some years (1999-2003) for a captive harp seal (*Pagophilus groenlandicus*) Babette and her 1-yr-old daughter Deane, then did not vary measurably for either seal (2004-2007). The shaded area in each calendar year represents the period of breeding for the wild Northwest Atlantic harp seals (Sjare and Stenson, 2009). Red star indicates Deane's date of birth. Monthly mean data are plotted.

2) Male steroid hormones

a) Testosterone in Tyler

Testosterone varied seasonally in 6 of the 7 years for which samples were available (Fig. 9). Highest concentrations were measured in or around the breeding period of the wild Northwest Atlantic population, except in 2002; however, no blood samples were taken in January through March of that year. In 2006 the highest concentration was measured in July. The highest testosterone concentration measured overall was 352.4 ng/dl (April 2003). Patterns across years were significantly concordant ($W = 0.337$, $p = 0.0226$).

b) Testosterone in Jamie

Testosterone concentrations in Jamie also varied seasonally in all years (Fig. 10). The pattern was more erratic year-to-year than for Tyler, but nevertheless highest levels within years generally were found in or around the breeding period of the wild Northwest Atlantic population. Notable deviations were 2005, when the year's highest level was measured in October; and 2006, when the five highest concentrations were measured from June-December. Testosterone levels increased during the breeding period and increased with age. The highest concentration (394.3 ng/dl) was from the latter period (August 2006). Patterns across years were not significantly concordant ($W = 0.207$, $p = 0.25$); if 2006 is removed from the analysis statistical significance is found ($W = 0.469$, $p = 0.007$).

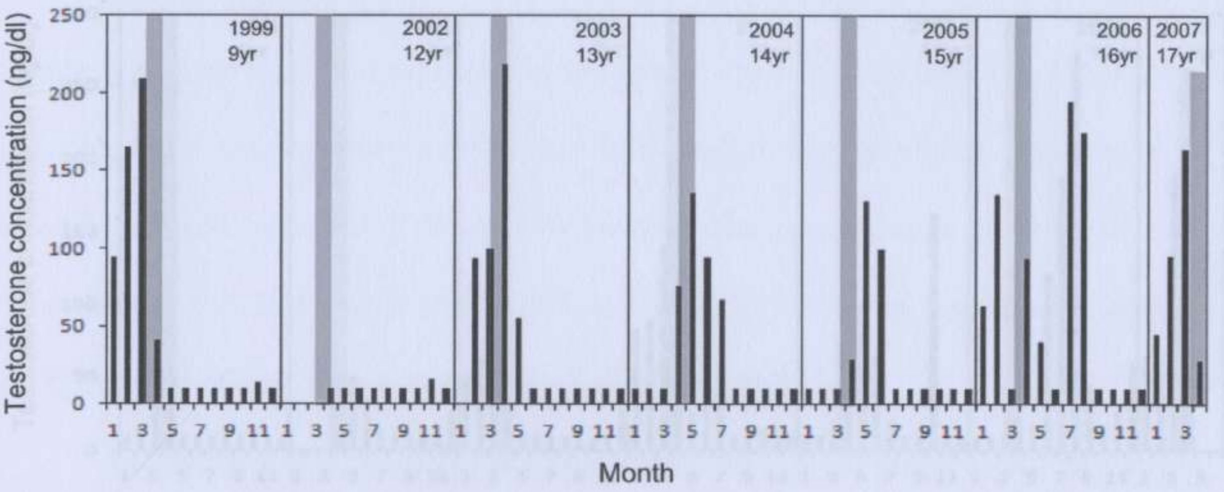

Figure 9. Plasma testosterone concentrations in a captive adult male harp seal (*Pagophilus groenlandicus*), Tyler, showed strong peaks in and around the breeding period of the wild Northwest Atlantic population (shown in grey; Sjare and Stenson, 2009) except in one year. Monthly mean data are plotted, 1999-2007 (ages 9-17 yr).

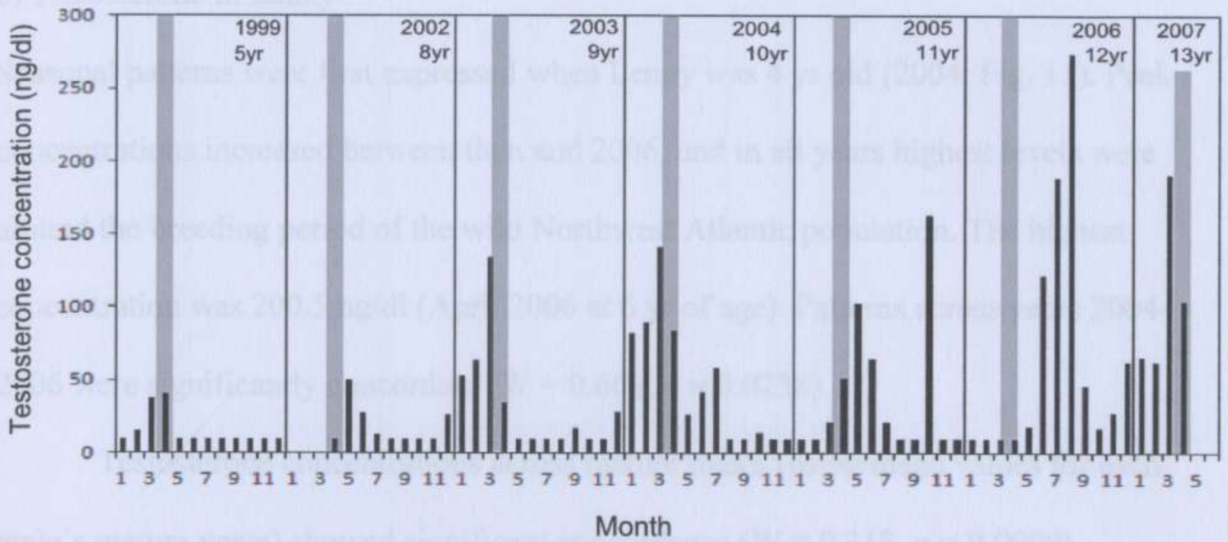

Figure 10. Plasma testosterone concentrations in a male adult harp seal (*Pagophilus groenlandicus*), Jamie, varied substantially across years but showed a similar pattern within most years: high levels in and around the breeding period of the wild Northwest Atlantic population (shown in grey; Sjare and Stenson, 2009). Monthly mean data are plotted for years, 1999-2007 (ages 5-13).

c) Testosterone in Lenny

Seasonal patterns were first expressed when Lenny was 4 yr old (2004; Fig. 11). Peak concentrations increased between then and 2006, and in all years highest levels were around the breeding period of the wild Northwest Atlantic population. The highest concentration was 200.5 ng/dl (April 2006 at 6 yr of age). Patterns across years 2004-2006 were significantly concordant ($W = 0.669$, $p = 0.0238$).

Testosterone concentrations across mature males (using mean values for each male's mature years) showed significant concordance ($W = 0.318$, $p = 0.0009$).

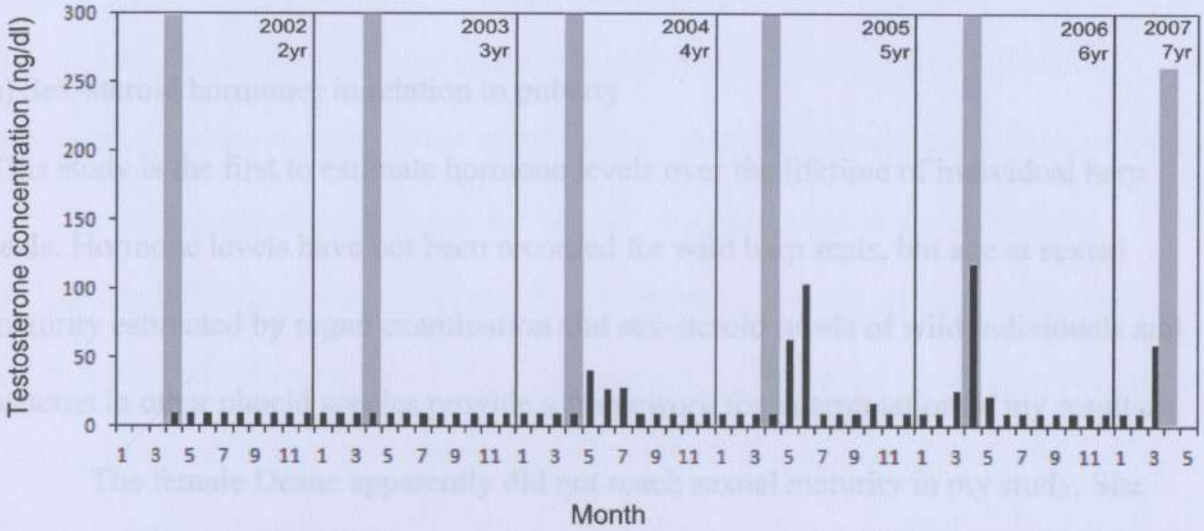

Figure 11. Plasma testosterone concentrations in a young captive male harp seal (*Pagophilus groenlandicus*), Lenny, showed the achievement of sexual maturity, a progressive increase after puberty, and highest activity in and around the period of breeding in the wild Northwest Atlantic population (shown in grey; Sjare and Stenson, 2009). Monthly mean data plotted, 2002-2007 (ages 2-7 yr).

DISCUSSION

a) Sex-steroid hormones in relation to puberty

This study is the first to estimate hormone levels over the lifetime of individual harp seals. Hormone levels have not been recorded for wild harp seals, but age at sexual maturity estimated by organ examination and sex-steroid levels of wild individuals and patterns in other phocid species provide a framework for interpretation of my results.

The female Deane apparently did not reach sexual maturity in my study. She showed undetectable levels of estradiol with the exception of 2002 (her year of birth), presumably due to transfer from her mother, Babette. Deane's progesterone levels remained below 0.5 ng/ml (except 2007), similar to pre-pubertal hooded seal (Noonan, 1989), Hawaiian monk seal (Pietraszek and Atkinson, 1994) and harbor seal (Gardiner et al., 1996). The slight increase in 2007 (at 5 yr of age) might have been an indication that physiological puberty would follow in 2008.

Based on testosterone levels, the male Lenny apparently attained sexual maturity at 4 yr of age, when his testosterone reached a measurable level. Over the following few years Lenny's testosterone levels increased progressively. Before sexual maturity his testosterone levels were <1 ng/ml, similar to the hooded seal (Noonan, 1991) and Hawaiian monk seal (Pietraszek and Atkinson, 1994). Estimates of puberty in wild harp seals in Canada were 4.9-6 yr in 2000-2004 (Sjare and Stenson, 2009). Therefore if Lenny was sexually mature at 4 yr of age it was at a younger age than in the wild population. His early maturation could be explained by the abundant food resources available in a captive environment. The captive environment did not suppress the rise in sex-hormone concentrations in this male.

b) Sex-steroid hormones in relation to reproduction in females

Babette, the mature female in my study, showed seasonal patterns in sex-steroid hormones. In years when she was pregnant her progesterone levels gradually increased after the mating period and also toward the end of gestation, as in the harbor seal (Reijnders, 1990). In years when she was not pregnant, her progesterone levels were variable but were elevated for ~4-5 months, suggesting pseudopregnancy, a phenomenon previously reported for captive harp seals (Renouf, 1994) and harbor seals (Reijnders, 1990). A gradual increase in progesterone followed a peak in estradiol in some years, as also reported for several other phocid species: harbor seal (Reijnders, 1990) gray seal (Hobson and Boyd, 1984), hooded seal (Noonan, 1989) and Hawaiian monk seal (Pietraszek and Atkinson, 1994).

Patterns in estradiol were concordant across years, with increased levels around the breeding period of wild seals (the same population from which Babette was captured). In 2004 estradiol did not increase, perhaps due to the lack of interaction with males: in that year Babette and her pup were separated from the male harp seals. In other studies on phocids that measured estrogen levels no information was given about the presence of males. The annual seasonal rise in estradiol that I observed also has been reported for hooded seal (Noonan, 1989) and harbor seal (Reijnders, 1990).

Prolactin increases during lactation in seals (Boyd 1991), but Babette's high levels did not always occur around that time. Her prolactin levels increased in November-December 1999, perhaps related to the birth of Lenny in March 2000. Other studies on pinnipeds have not reported such a pattern. Lactation occurred in Babette in 2002 but no blood samples were taken; however a slight increase was noted in June of that year. In

2003 prolactin was high in January-July in both Babette and her immature daughter Deane. Babette was pregnant that year and had a stillborn pup in January 2003 (Fig. 8). The high levels of prolactin in Babette presumably were associated with her pregnancy. This pattern was unexplainable in Deane and could be due to a laboratory contamination as the rise was similar in both females (Fig. 8).

c) Sex-steroid hormones in relation to reproduction in males

My main finding was that testosterone levels of reproductively mature male harp seals showed a seasonal pattern like that expected from the brief pupping and breeding period in the wild Northwest Atlantic population. Tyler and Lenny showed a clear seasonal pattern through all their mature years. Jamie's testosterone levels varied more, but these nevertheless increased near the time of breeding in the wild population. Testosterone levels in the three males were similar to those reported for captives of this species by Serrano (2000) and for other phocid species: hooded seals exhibit elevated testosterone 1 month before breeding and this continued through the breeding period with a gradual decrease afterwards (Noonan, 1991); the gray seal shows a 4 month rise in testosterone from November-February with the highest peak during the breeding period (Seely and Keith, 1991); and the Hawaiian monk seal exhibits a gradual increase in testosterone levels leading up to the breeding period in February-June (Atkinson and Gilmartin, 1992).

d) Sex-steroid hormones in relation to senescence

The mature female harp seal Babette appeared to show physiological senescence, since she had her last live offspring in 2004, at an estimated age of 21 yr. In female primates, physiological senescence is reflected in decreased estrogen and progesterone levels (Woller et al., 2002). Since 1999 Babette's estradiol levels around the breeding period decreased gradually. Progesterone levels decreased slightly since 1999 but still showed the annual trend of pregnancy or pseudopregnancy. Senescence has been reported for other pinnipeds but not with hormonal measurements: gray seal (Bowen et al., 2006), Weddell seal (Hindle et al., 2009) and subAntarctic fur seal (Beauptet et al., 2006).

e) Conclusion and suggestions for future research

This study elaborated on the reproductive physiology of the harp seals and revealed that: (a) hormone levels fluctuated seasonally, roughly, as would be expected from the timing of reproduction in the Northwest Atlantic harp seal population; (b) trends were similar to those of other phocid species and; (c) patterns of hormone levels and fluctuations were not suppressed in a captive environment.

Future research should be conducted on wild harp seals, in particular the Northwest Atlantic, to document levels and seasonal patterns and compare them with the results of my study. In addition sex-steroid hormones should be sampled on a finer temporal scale than was possible in this study, in both wild and captive individuals. Levels and seasonal fluctuations in pinniped sex-steroid hormones should be studied in other species, particularly those with different ecology, to assess adaptive patterns (e.g. Australian sea lion, having an 18 month reproductive cycle). Finally senescence in wild and captive individuals of different pinniped species is poorly known but may have important effects on fitness, particularly in females (Bowen, et al., 2006).

Superficially, different pinniped species appear to be similar in sex-steroid hormonal patterns in relations to the reproductive cycle, but knowledge of the endocrinology of reproduction is inadequate (Boyd, 1999). For example, sex-steroid hormones have been examined in 8 of 19 phocid species (Table 1).

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APPENDICES

Appendix 1. Biographies of harp seals used in the study

Babette

- Female
- Captured from the Magdalen Islands on 2 March 1989
- Estimated date of birth - March 1983
- Mother of Jamie, Lenny, Deane, and other seals formely housed at the OSC
- First harp seal in the world to give birth in captivity
- Mass has fluctuated from 110 kg to an allowed maximum of 200 kg (She was before pupping)
- Amount of herring consumed varied with body mass (2-6 kg/day)
- Distinctive features: a distinct harp on her back, a black mask on her face, and scar on the ventral part of her neck

Tyler

- Male
- Captured as a whitecoat from the Magdalen Islands in 1990
- Date of birth - March 1990
- Father of Lenny and Deane
- Mass has fluctuated from 110 kg to an allowed maximum of 200 kg.
- Amount of herring consumed varied with body mass (2-6 kg/day)
- Distinctive features: a genetic colour morph called 'smutty'. This colouration covers the harp and mask making the typical features of a harp seal difficult to recognize.

Jamie

- Male
- Born at the OSC on 16 March 1994
- Son of Babette (father unknown)
- Mass varied from 80 kg to 120 kg.
- Distinctive features: harp and dark mask covering face, multiple dark spots along ventral side.

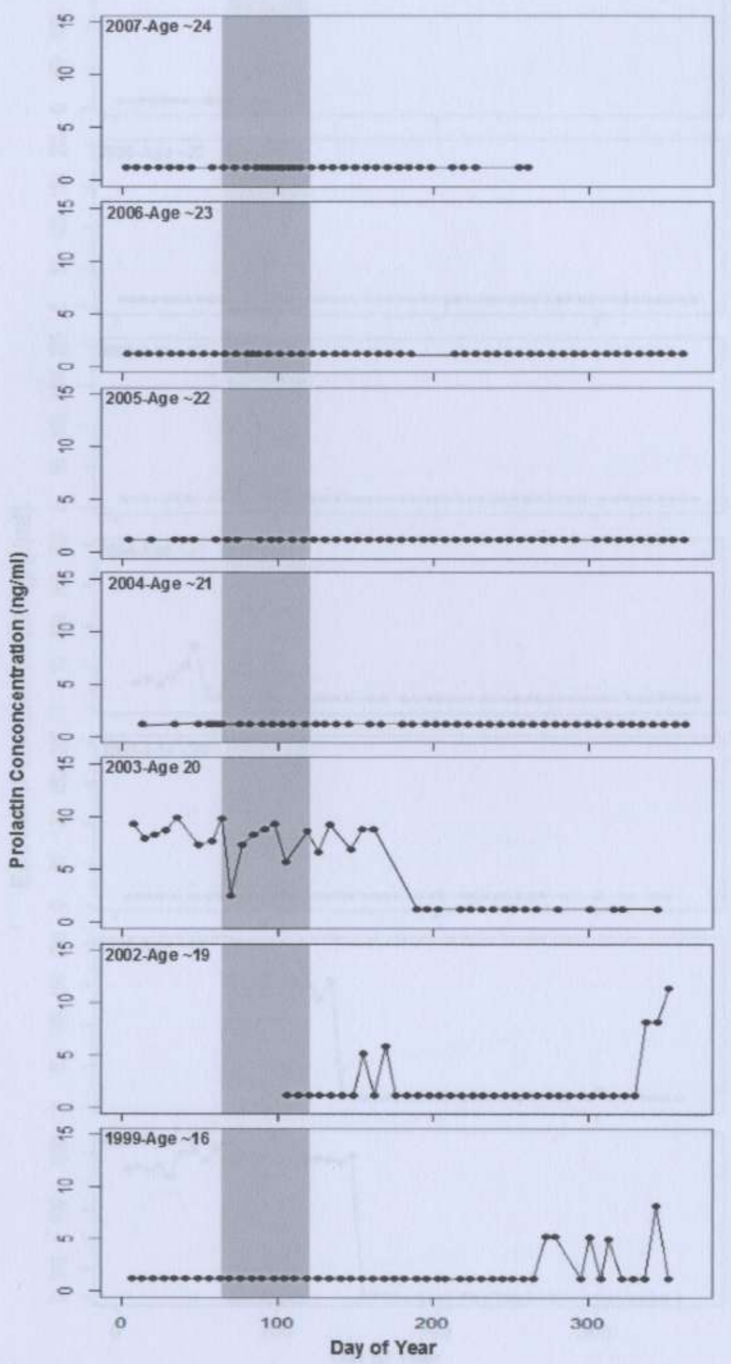
Lenny ('Millennium')

- Male
- Born at the OSC on 8 March 2000
- Son of Babette and Tyler
- Mass averaged 85 kg
- Distinctive features: smutty morph

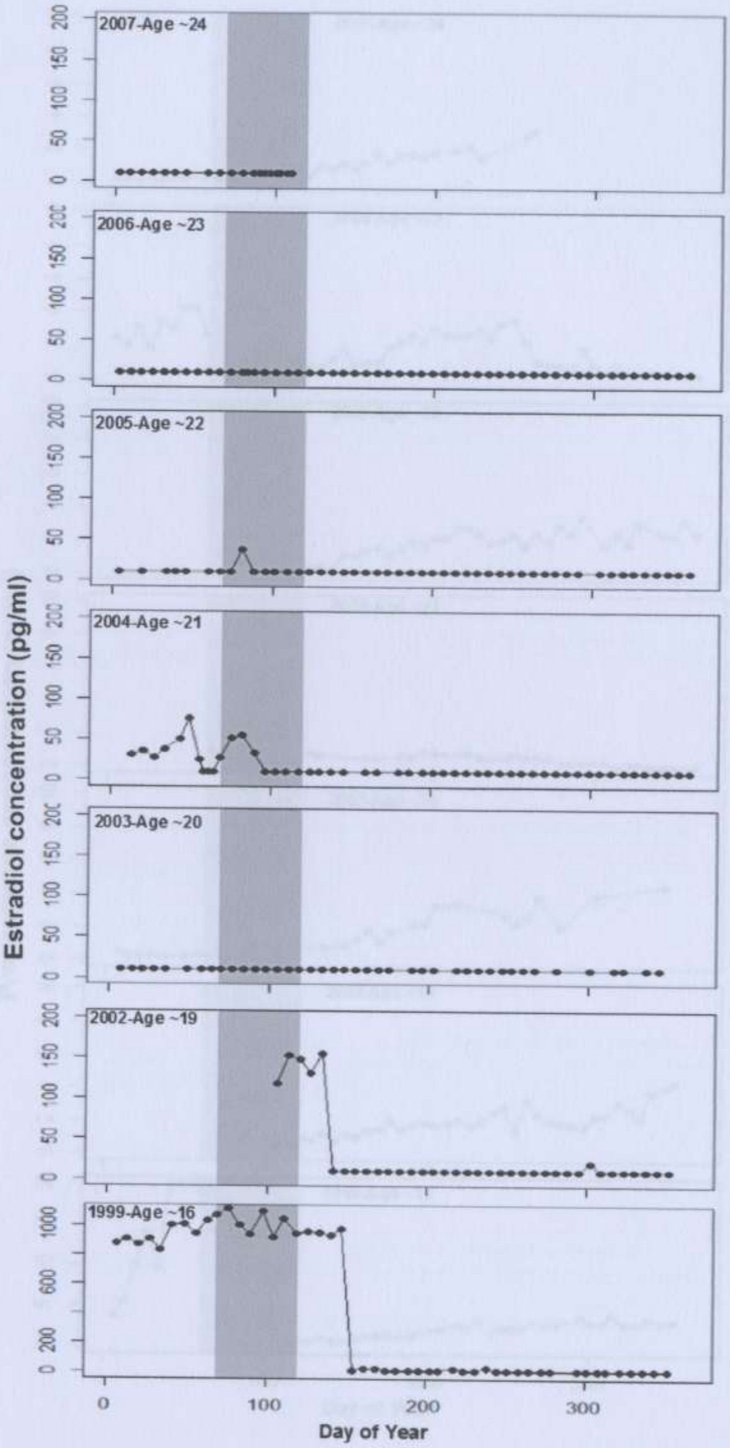
Deane (DEE-nee)

- Named after Dr. Deane Renouf
- Female
- Born at the OSC on 18 March 2002
- Daughter of Babette and Tyler
- Mass averaged 75 kg
- Distinctive features: a silvery coat with few large spots located on each side (typical colouration of a bedlamer)

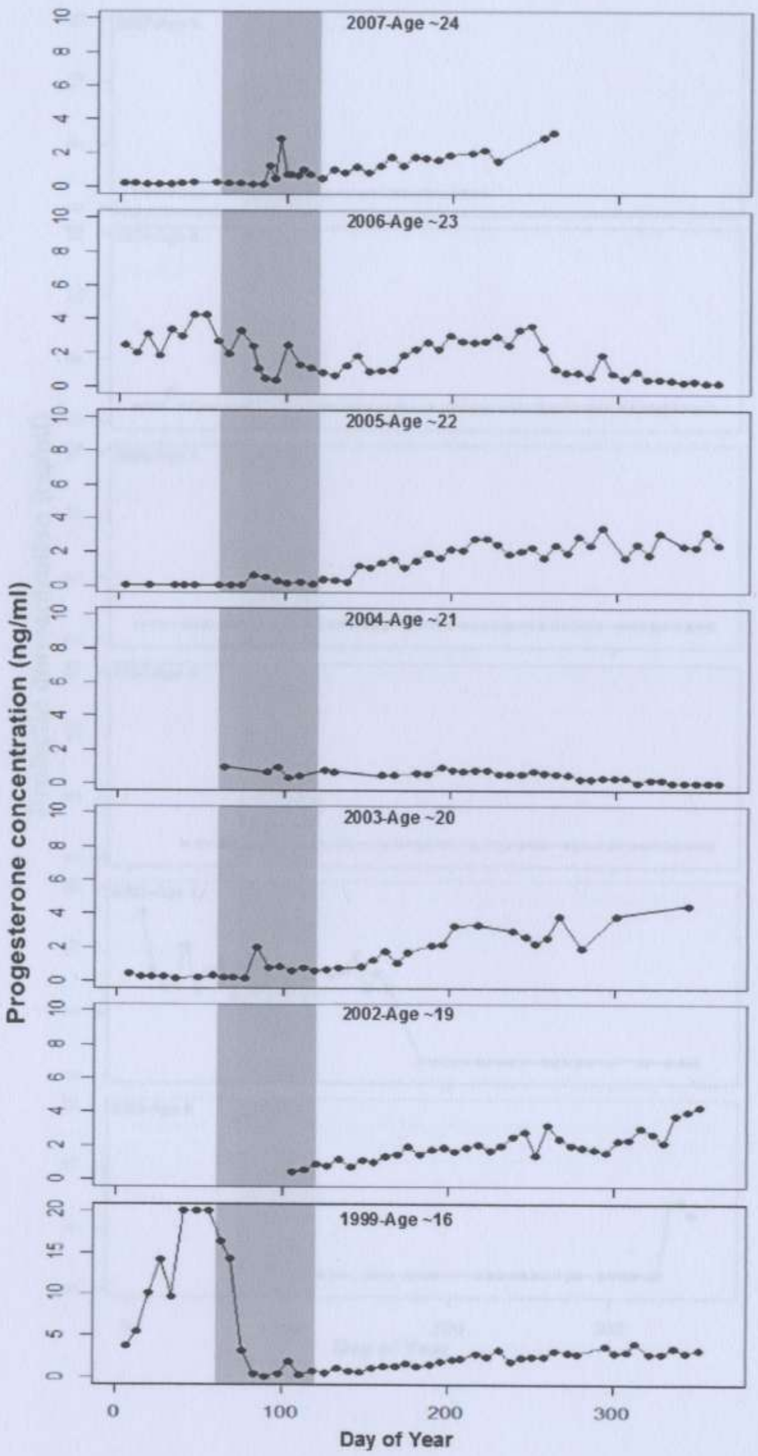
Appendix 2. Graphs of raw data collected for the study.



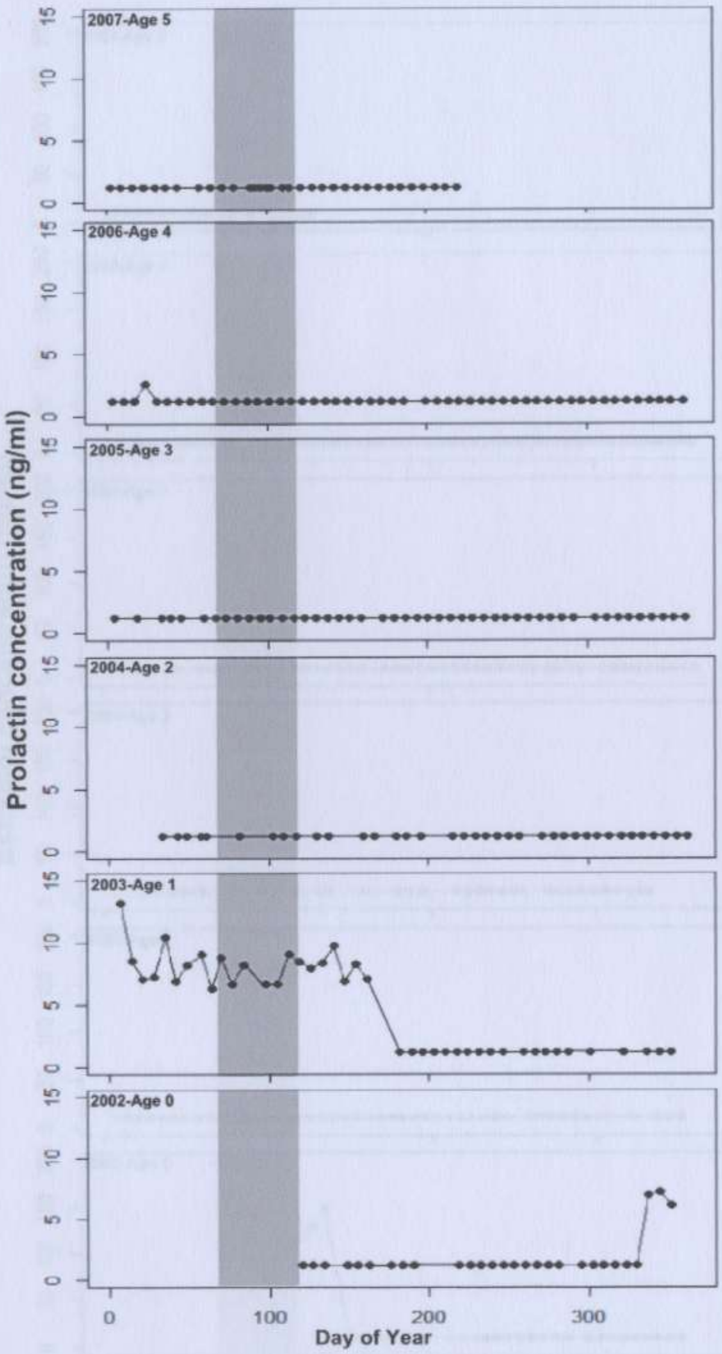
Appendix Figure 2.1. Prolactin levels for a captive harp seal, Babette. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 1999-2007 (ages ~16-24 yr).



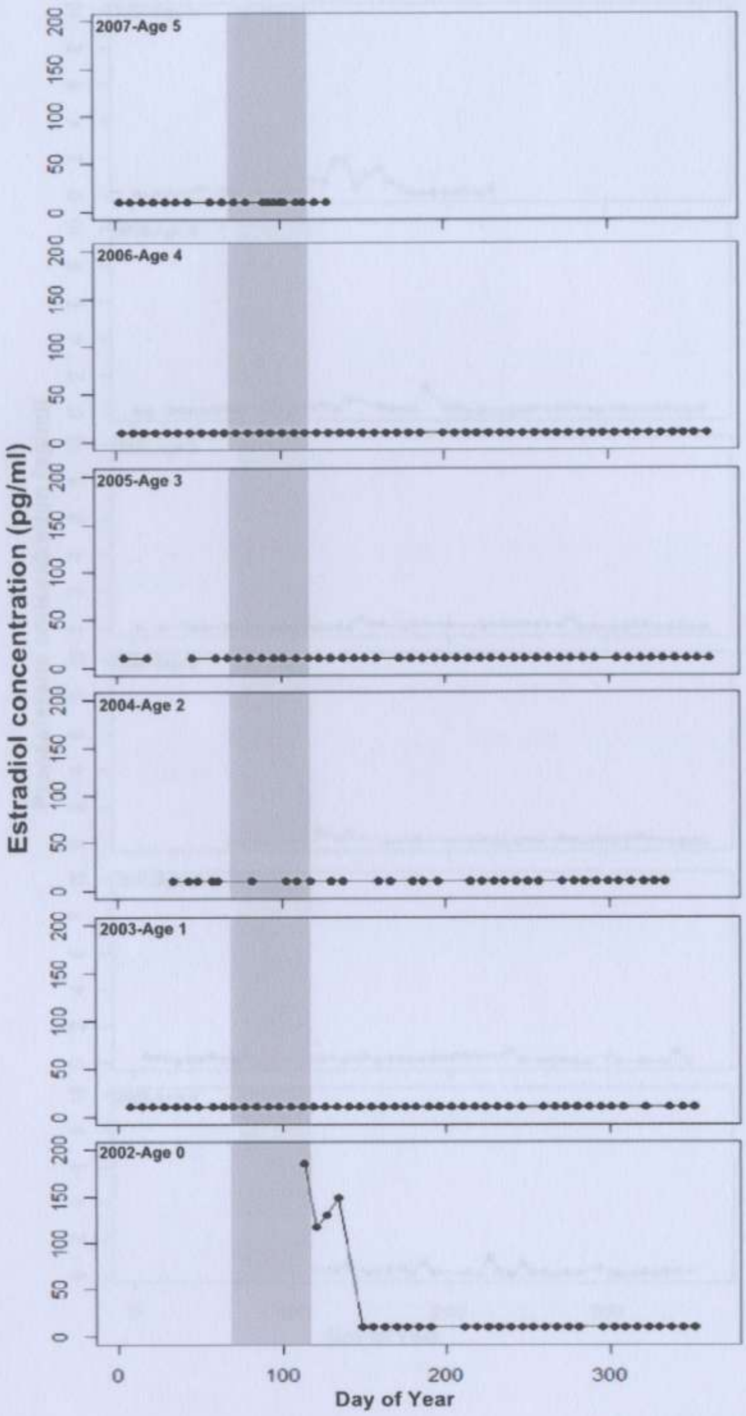
Appendix Figure 2.2. Estradiol levels for a captive harp seal, Babette. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 1999-2007 (ages ~16-24 yr).



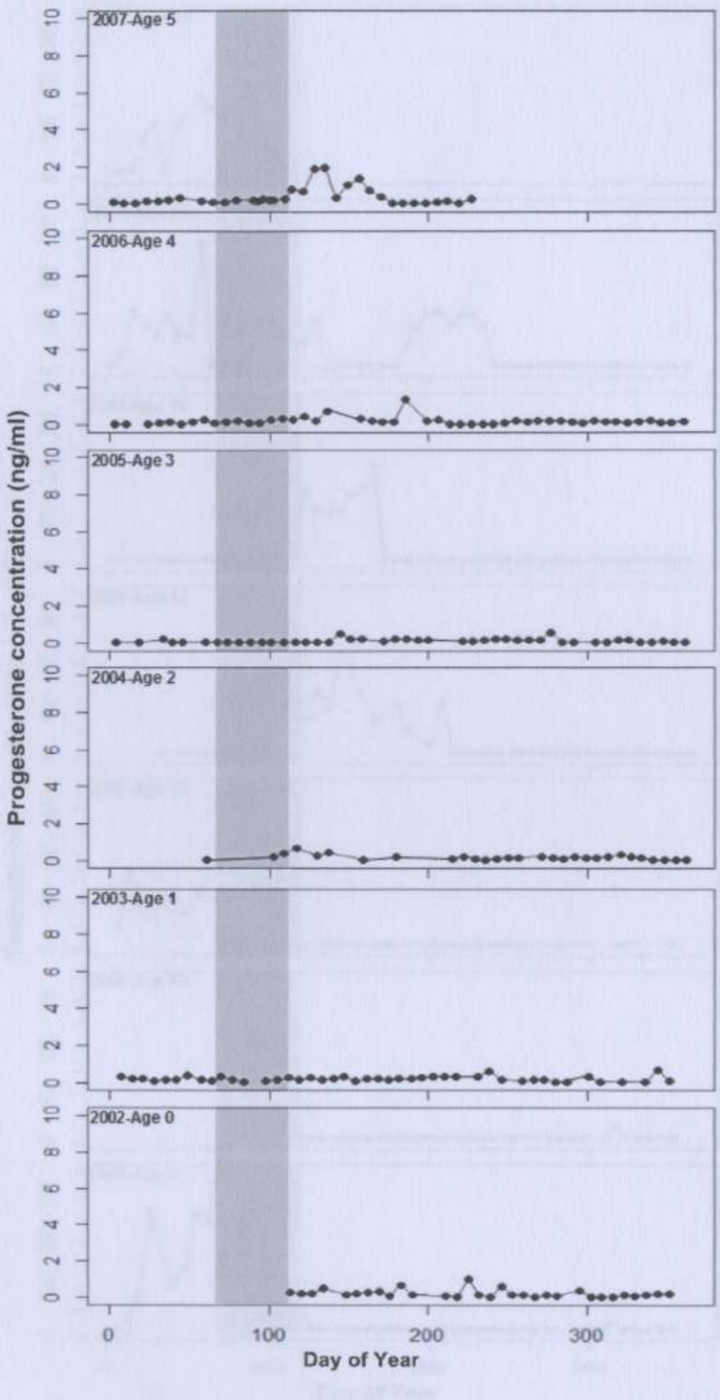
Appendix Figure 2.3. Progesterone levels for a captive harp seal, Babette. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 1999-2007 (ages ~16-24 yr).



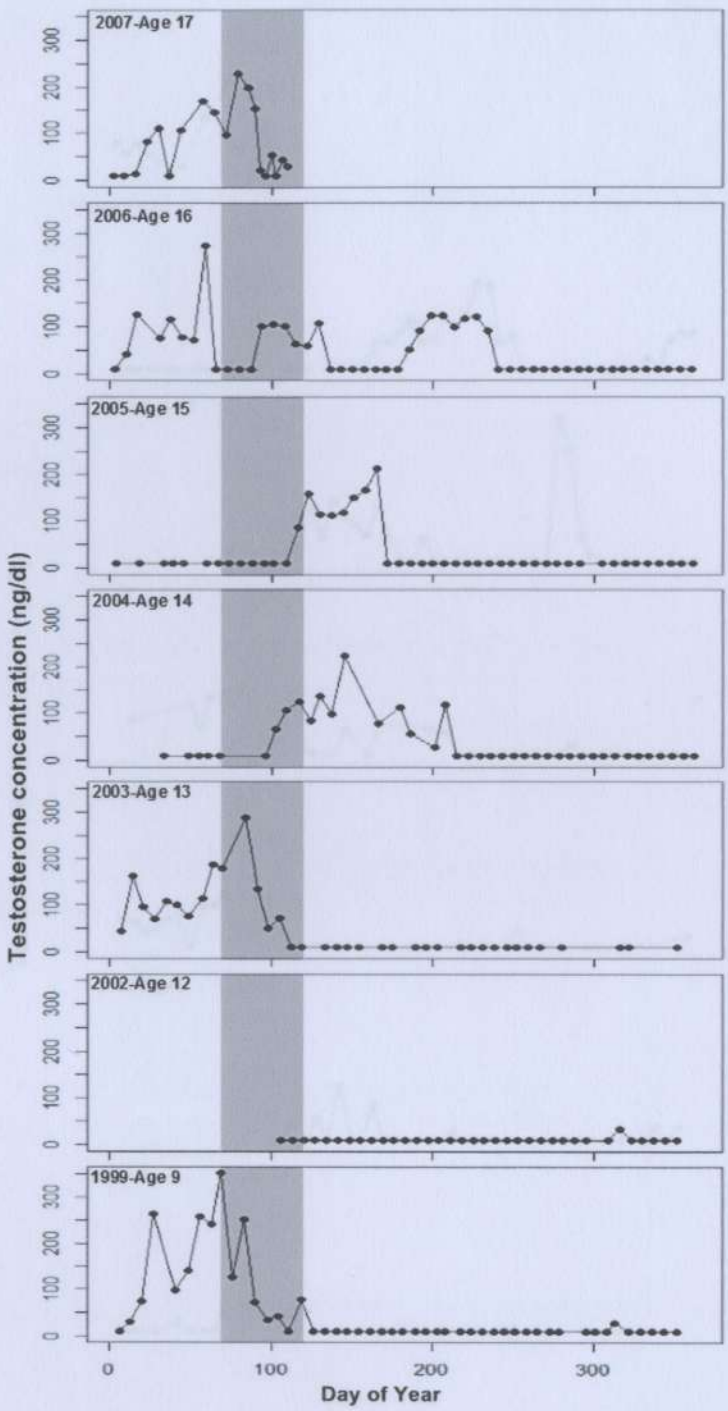
Appendix Figure 2.4. Prolactin levels for a captive harp seal, Deane. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 2002-2007 (ages 0-5 yr).



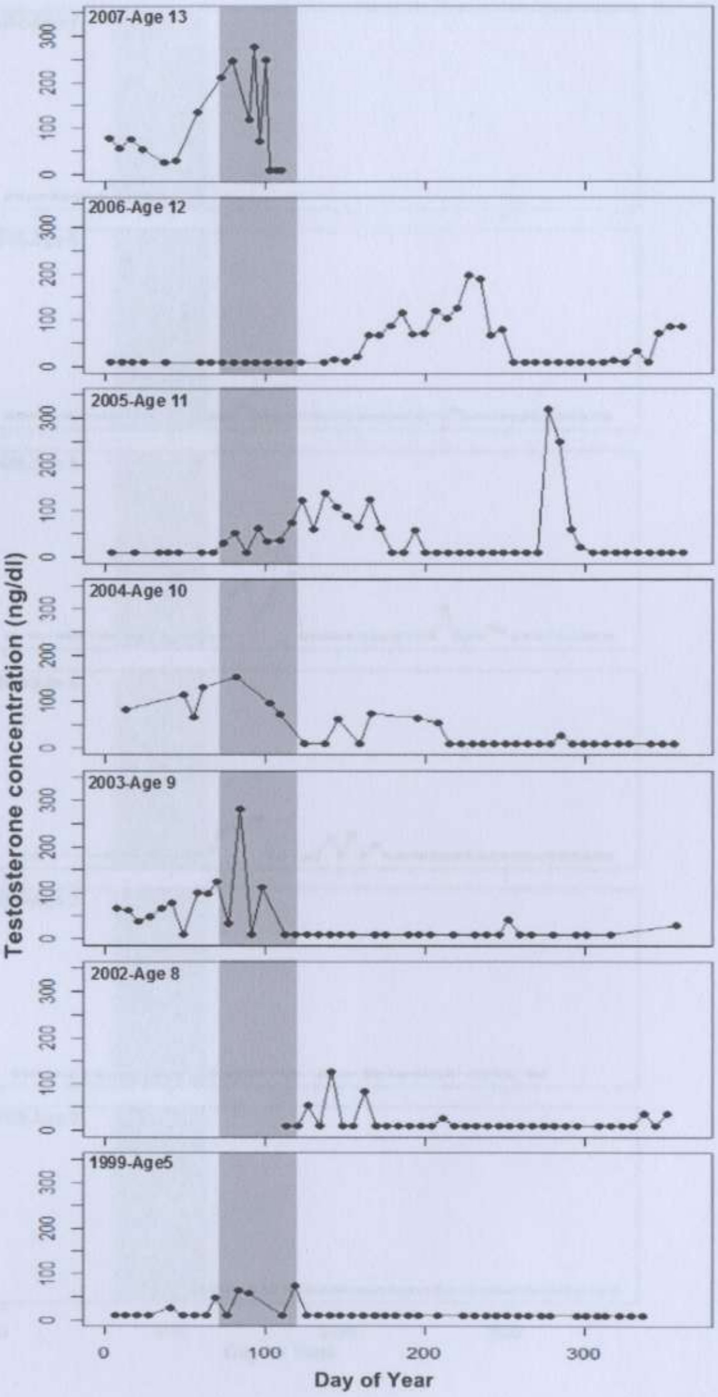
Appendix Figure 2.5. Estradiol levels for a captive harp seal, Deane. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 2002-2007 (ages 0-5 yr).



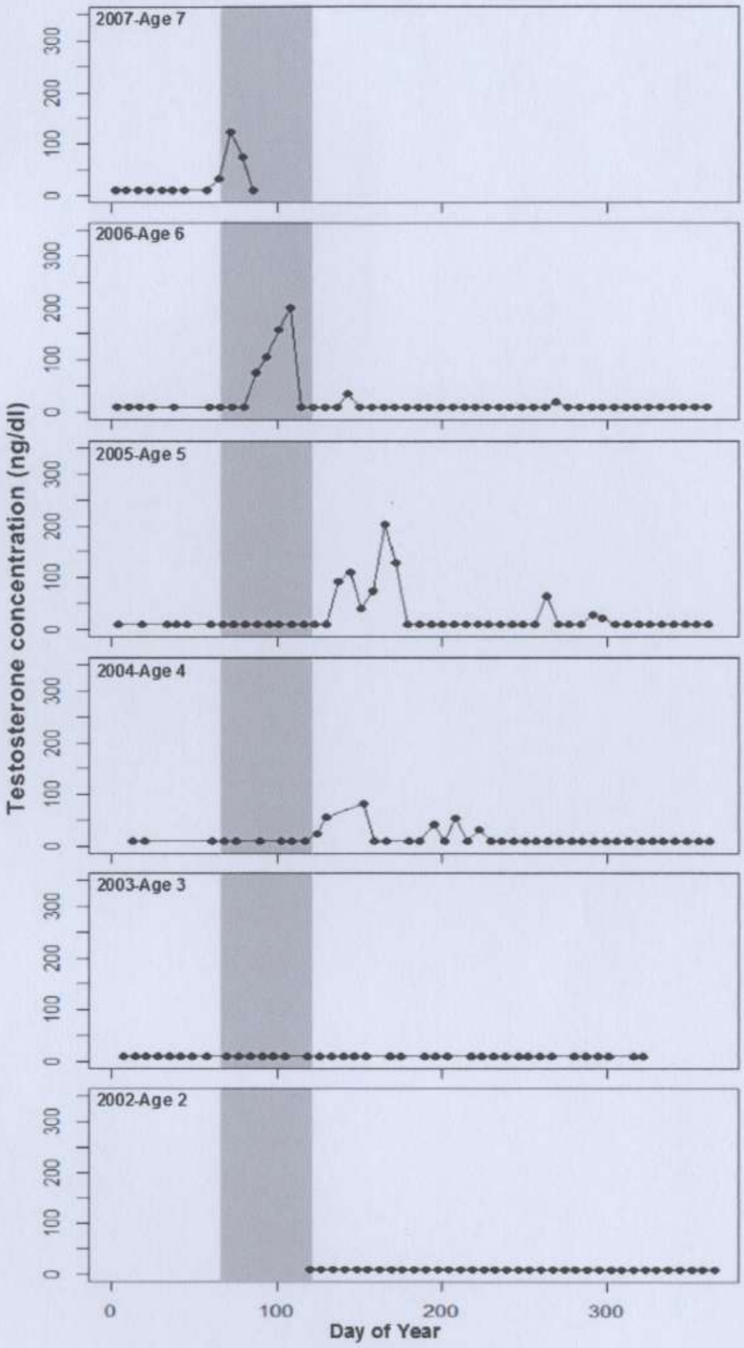
Appendix Figure 2.6. Progesterone levels for a captive harp seal, Deane. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 2002-2007 (ages 0-5 yr).



Appendix Figure 2.7. Testosterone levels for a captive harp seal, Tyler. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 1999-2007 (ages 9-17 yr).



Appendix Figure 2.8. Testosterone levels for a captive harp seal, Jamie. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 1999-2007 (ages 5-13 yr).



Appendix Figure 2.9. Testosterone levels for a captive harp seal, Lenny. Shaded areas indicate breeding period of wild Canadian harp seal population (Sjare and Stenson, 2009). Raw data are plotted for years 2002-2007 (ages 2-7 yr).