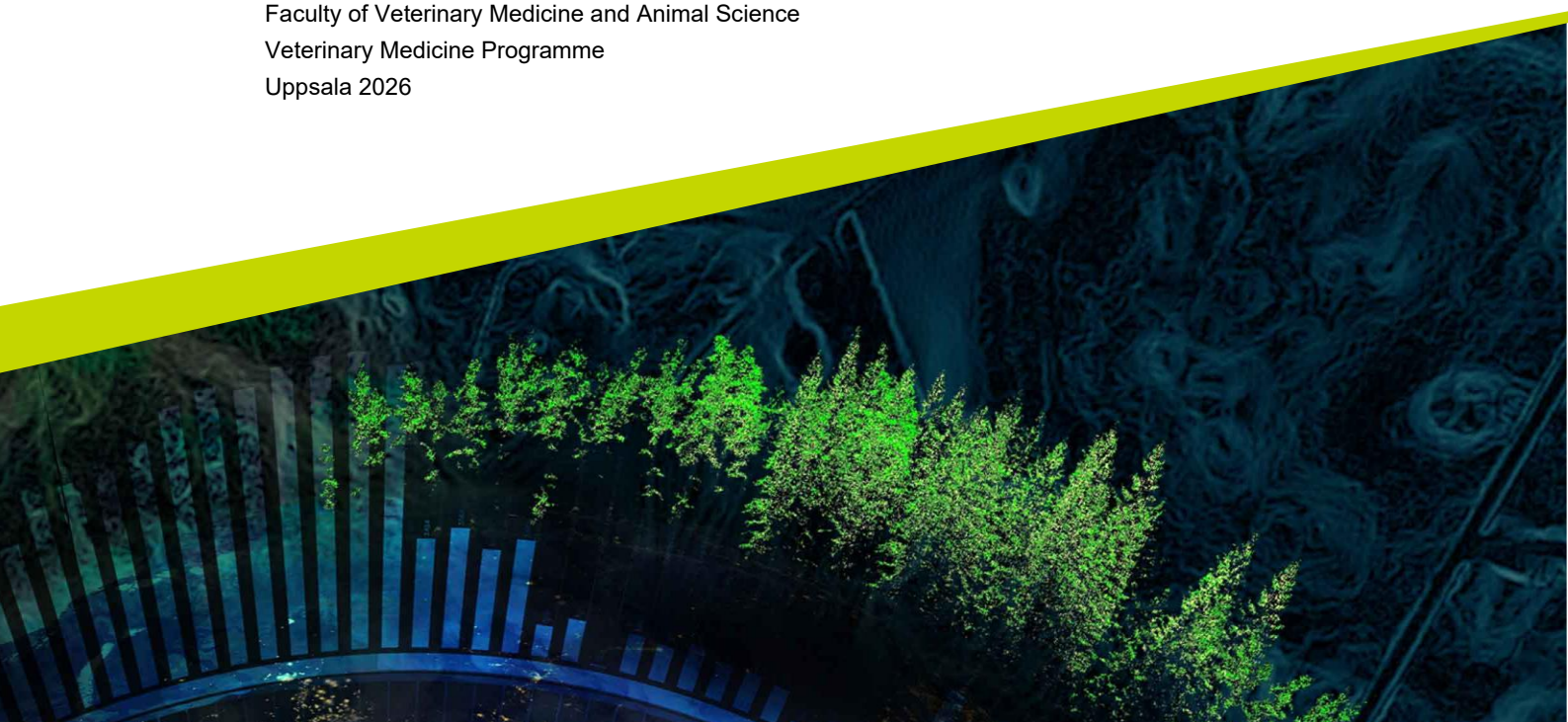
Reproductive maturity of the male Baltic grey seal

Alva Bergfors

Independent Project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme
Uppsala 2026



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Keywords:	grey seal, Baltic sea, seasonality, reproduction, spermatogenesis

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Abstract

Grey seals (*Halichoerus grypus*) are top predators in the Baltic Sea and important indicators of ecosystem health. In Sweden, their health status is investigated by long-term monitoring conducted by the Swedish Museum of Natural History (SMNH). Although there is some knowledge about female grey seal reproduction, male sexual maturity and seasonal activity are less studied. This is partly due to the reproductive seasonality, which reduces the availability of comparable samples throughout the year. Understanding these aspects is essential to adequately monitor reproductive health in male seals and detecting deviations from normal patterns over time. The aim of this master's thesis was therefore to investigate whether histological criteria in testis sections, together with testis weight, could be used to indicate sexual maturity in male Baltic grey seals, including periods when spermatogenetic activity is absent.

From paraffin embedded tissue blocks, histological sections of testicle and epididymis were prepared at SMNH and studied semi-quantitatively under a light microscope. The tissue originated from three presumed juvenile seals and seven adult seals. In addition, sperm smears from nine grey seals were examined for presence of spermatids. Testis weights from 1,042 seals were obtained from the SMNH and analysed for their relation to age and seasonal variation.

The findings in this study indicate that both age and season strongly influences male reproductive physiology. Testis weight increased sharply from 4 to approximately 6 years of age, reflecting the onset of puberty, followed by a gradual plateau. In males older than 8 years sampled outside of the breeding season, the testis weight was consistently above 50g, while peak testis weight was during January to April. Histological observations of juvenile seal testes included a high proportion of Sertoli cells, and a large amount of interstitial tissue. Adult testes showed thicker seminiferous epithelium, a higher number and variety of spermatogenic cells, and the presence of spermatozoa in the epididymal duct before and after the breeding season. During autumn, outside the breeding season, the seminiferous epithelium was thinner and spermatozoa and spermatids were absent, suggesting arrest at spermatocyte stage.

In order to assess sexual maturity, histological evaluation could be used as a complement to testis weight and macroscopic findings. During the seasonally active period, the finding of spermatids in the seminiferous epithelium, as well as spermatozoa on smears from the epididymis, is indicative of sexual maturity. Outside of the reproductive season, a small amount of interstitial tissue, accompanied by a low proportion of Sertoli cells may serve as an indicator of sexual maturity. Further studies are needed to confirm these findings, as the small sample size, variability in tissue preservation, and lack of tissue samples from certain months and ages limit the current conclusions.

Keywords: grey seal, Baltic sea, seasonality, reproduction, spermatogenesis

Table of contents

List of tables **9**

List of figures **10**

1. Introduction **11**

2. Literature Review **13**

2.1 The grey seal 13

2.1.1 Anatomy and histology of the testicle and epididymis 13

2.1.2 Spermatogenesis 14

2.1.3 Sexual maturity 14

2.2 Seasonality of the Baltic grey seal 15

2.3 Sexual maturity and seasonality from a histological perspective 16

3. Material And Methods **18**

3.1 The monitoring programme 18

3.2 Statistical analysis of testis weights 18

3.3 Selection of samples for histology 18

3.4 Histological preparation and study 19

3.5 Sperm smears 21

3.6 Methodology of text processing 22

4. Results **23**

4.1 Testicular weight 23

4.2 Sperm smear observations 25

4.3 Histological findings 25

4.3.1 Juveniles 25

4.3.2 Adults 27

4.3.3 Collective table of all seals 32

5. Discussion **33**

5.1 Testis weight statistics 33

5.2 Microscopical findings 33

5.2.1 Differences between juvenile and adult descriptive histology 33

5.2.2 Differences in descriptive histology between the adults 34

5.2.3 Seasonal differences in descriptive histology of the adults 34

5.2.4 Sperm smears 34

5.3 Criteria of sexual maturity 35

5.4 Study design; limitations and future directions 36

5.4.1 Artefacts 36

5.4.2 Prospects for future studies 37

6. Conclusion **39**

Acknowledgments **40**

References **41**

List of tables

Table 1. Compilation of selected seals for histological examination 19

Table 2. Collective table of descriptive results from cytological study of grey seal sperm smears. 25

Table 3. Descriptive findings of the histological examination 32

List of figures

Figure 1. Left picture: Premade paraffin embedded epididymis and testicle tissue from a grey seal. Right picture: Microtome including a water bath. 20

Figure 2. Mean testis weight in grams (y) per age (x) of seals caught between July and November. Error bars show confidence interval (95%). N=624 seals. 23

Figure 3. Age related monthly changes in testicular mass, ages 0-8 years old. N: 46 seals. 24

Figure 4. Age related monthly changes in testicular mass, adults 8-34 years old. Error bars show confidence interval (95%). N=272 seals. 24

Figure 5: Left picture; histological section of left testis, Seal A. Right picture; histological section of left testis, Seal B. H&E. 26

Figure 6. Histological section of left testis, Seal C. The black arrows highlight examples of spermatocytes in the section. H&E. 27

Figure 7. Histological section of left testis, Seal D. H&E. 27

Figure 8. Histological section of left testis, Seal E. H&E. 28

Figure 9.Histological section of left testis, Seal F. H&E. 29

Figure 10. Histological section of left testis, Seal G. H&E. 29

Figure 11. Picture 1 & 2: Histological section of left epididymis seal G, both showing spermatozoa in epididymal duct. H&E. 30

Figure 12. Histological section of left testicle, seal H. H&E. 30

Figure 13. Histological section of left testicle, seal I. H&E. 31

Figure 14.Histological section of left testicle, seal J. H&E. 31

1. Introduction

The grey seal*, Halichoerus grypus (*subspecies *grypus)*, is the largest and most common seal species inhabiting the Baltic Sea (MarineFinland.fi n.d.). The Swedish Museum of National History (SMNH) has been commissioned by the Swedish Agency for Marine and Water management, to perform monitoring of the health of the Baltic seal populations). Since the 1970s, stranded or bycaught seals have been investigated. In recent years, bycaught seals as well as samples from hunted seals are necropsied at the SMNH.

The Baltic grey seal population faced a marked decline from about 100,000 seals in the beginning of the 20th century to 3000- 4000 seals by the 1970s, mainly due to excessive hunting and contamination of the environment with reproductive toxicants affecting seal reproduction negatively (SLU Swedish Species Information Centre n.d.). Since then, the grey seal population has been recovering and since the early 2000´s, seal hunting is permitted on a limited scale.

Regarding grey seal reproduction, research has included studies on, for instance, uterine occlusions due to high levels of polychlorinated biphenyls, PCB, and dichlorodiphenyltrichloroethane, DDT (Helle *et al.* 1976; Bergman 1999b). In Atlantic grey seals (*Halichoerus grypus atlantica*), research on subjects such as age at sexual maturity and the impact of environmental factors on seasonal reproduction, has been conducted (Coulson 1981; Hammill & Gosselin 1995). However, the reproduction of the Atlantic grey seals differs from that of the Baltic, and there are still details, including male sexual maturity, which remain undiscovered.

A deeper understanding of seal reproductive biology could ultimately lead to more reliable research results. In addition, knowing what is within normal range enables the determination of deviations from it. However, studying seal reproduction is challenging due to strong seasonal variation in reproductive activity. Seasonal spermatogenetic activity can lead to situations where a male grey seal has no active spermatogenesis at the time of death, for example when it becomes caught in fishing nets and later sent for necropsy at SMNH. In this situation, it may be difficult for the veterinary pathologists to safely tell if the seal was sexually mature by the time of death.

The aim of this master´s thesis was to investigate whether histological criteria for testicular tissue, together with testis weight, can be used to indicate sexual maturity in male Baltic grey seals and to ensure that the criteria are reliable even

during non-active reproductive periods. This was achieved by comparing samples from juveniles and adults across different seasons.

2. Literature Review

2.1 The grey seal

The Baltic Sea is inhabited by three seal species; the ringed seal (*Pusa hispida*), harbour seal (*Phoca vitulina*) and the grey seal (*Halichoerus grypus*) (MarineFinland.fi n.d.). Among these, the grey seal is the largest and most common one. All of these species are considered to be apex predators (Almkvist 1980). Because the Baltic grey seal is an apex predator, it is a sensitive indicator of pressures in the ecosystem. Because of this, Baltic grey seal health has been monitored in Sweden since the late 1970´s (Bergman 1999). As part of the national monitoring programme, a subset of this population is investigated each year. Their reproductive health is examined as a core indicator for the status of the Baltic Sea marine environment within the Helsinki Commission, with the main purpose to study long term effects of human impact on the environment (HELCOM 2023). The pupping season of the Baltic grey seals occurs in February and March (Galatius *et al.* 2024). Mating then occurs at the end of the short, on average 18 day long, lactation period (Hall & Russell 2018).

2.1.1 Anatomy and histology of the testicle and epididymis

The male gonads consist of paired testes where spermatogenesis occurs (Knobil & Neill 1998). In all phocid seals, including the grey seal, the testicles are ascrotal and the anatomical location of the gonads is positioned along the para-abdominal wall, and imbedded by a thick layer of subcutaneous blubber (Blix *et al.* 1983; Atkinson 2009).

The testes of male mammals consist of both endocrine and exocrine tissue within a shared capsule (Bacha & Bacha 2012; Ross 2016). The testicular parenchyma is divided into lobules by connective tissue, originating from the tunica albuginea and consists of seminiferous tubules and interstitial tissue. Sertoli cells and the spermatogenic cells form the stratified epithelium lining the seminiferous tubules. Sertoli cells are recognised by their light nucleus, varying in shape from triangular to oval, and a distinct nucleolus. During spermatogenesis, the immature spermatogonia at the basal lamina gradually develop into mature spermatids closer to the seminiferous lumen. The spermatogenic cells are located in between the tall Sertoli cells, and their different developing stages form the stratified epithelium. The interstitial tissue, located between the seminiferous tubules, consists of irregular connective tissue and Leydig cells with a small, rounded, nucleus and acidophilic cytoplasm.

The epididymis is anatomically divided into head, body and tail (Bacha & Bacha 2012; König & Liebich 2020). As the efferent ducts from the testicle enter the head of the epididymis, they join into a single epididymal duct that runs all the way to the tail. The spermatozoa mature as they pass through the epididymis and they are stored in the epididymal tail. Histologically, the epididymis is outlined by a columnar epithelium arranged in a pseudostratified formation. In the epididymal head, the epithelium is surrounded by myocytes, which decrease in numbers further down the epididymal duct.

2.1.2 Spermatogenesis

Spermatogenesis, the process where spermatozoa develop from germinal stem cells, occurs in the seminiferous tubules (Boyd 1991). The progression of germ cell development is not well researched in pinnipeds, particularly not the Baltic Grey seals, but available studies indicate that spermatogenesis seems to follow the same patterns as those of other mammals. Generally, in mammals, sperm development starts with the spermatogonia (Reece & Rowe 2017). In the first stages of differentiation, multiple steps of mitotic division cause the spermatogonia to differentiate into primary spermatocytes. The spermatocytes then enter two meiotic divisions, resulting in the formation of spermatids. After further maturation, spermatids differentiate to spermatozoa and enter the seminiferous tubules lumen and the efferent ducts. The spermatozoa then continue their maturing process as they pass through the epididymal duct.

In another marine mammal inhabiting the Baltic Sea, the harbour porpoise, researchers have been able to histologically describe eight stages of spermatogenesis (Kesselring *et al.* 2019). All stages occurred simultaneously in the same cross section of the stratified epithelium. This finding was described as a multistage arrangement, where the first of the eight stages included presence of rounded and elongated spermatids, young spermatocytes and spermatogonia. This first defined stage was then followed by seven more, with the last one also including spermatogonia, as well as the two stages of meiosis, secondary spermatocytes and spermatids still attached to the epithelium.

2.1.3 Sexual maturity

Sexual maturity is defined as the time when the animal is capable of successfully reproducing, and puberty is defined as the maturation process leading up to it (Sjaastad *et al*. 2016). Younger individuals, who have not yet reached sexual maturation can be defined as juvenile (Atkinson 1997; Pistorius *et al.* 2011).

When studying testis weight of Northwest Atlantic grey seals, a rapid increase in testis mass was used as an indication of puberty onset (Hammill & Gosselin

1995). The results showed a significant increase in testis weight in males above the age of three. In the study, testes were collected during August to September. The cut off mark for the seals to be classified as sexually mature was a testis weight of a minimum of 60g, based on a prior study by Hewer (1964). Using this definition, the average age to reach sexual maturity, in 89 individuals, was 5,6 years. At seven years all seals had reached that mark (Hammill & Gosselin 1995).

In another study, with mainly testis weight as an indicator, the age of sexual maturity in Icelandic grey seals was investigated (Hauksson 2007). The males collected varied in age, from the youngest being foetal to the oldest being 23 years old. Age determination was performed by counting the growth layer groups of the canine teeth of the seals. The researchers then weighed and/or measured testes from 325 individuals and defined age of sexual maturity based on when seals reached a testis mass >75g. The average age of sexual maturity was 4.9 years and at 8 years of age all the seals were considered to be mature using this definition.

2.2 Seasonality of the Baltic grey seal

In seasonally breeding mammals, sexually mature males exhibit a seasonally fluctuating sensitivity to steroid feedback inhibition of Gonadotropin Releasing Hormone, GnRH (Knobil & Neill 1998). During the breeding season, this sensitivity decreases, leading to increased spermatogenesis. During the rest of the year the sensitivity increases and ultimately results in decreased GnRH secretion, resulting in a reversible suppression of spermatogenesis.

Pinnipeds, such as seals, sea lions and walruses, exhibit seasonally varying spermatogenic activity. Spermatogenesis has been shown to gradually increase before and reach its highest level during the breeding season (Knobil 1998). When comparing this to the reproductive season of female pinnipeds, the time of spermatogenic activity extend beyond this time (Würsig *et al.* 2018). However, there are still not enough studies on the spermatogenic window of Baltic grey seals, or other seals, to reliably determine its exact duration.

When studying seasonal changes in seal reproduction, many species show signs of varying testicular activity throughout the year. In a study done on male harbour seals, a seasonal variation in luteinizing hormone responsiveness to GnRH administration was present in both juvenile and adult male seals (Gardiner *et al.* 1999). In ringed seals, significant increases in relative testis weight were measured during the annual breeding season (Ryg *et al.* 1991; Griffiths 1984). A similar pattern, where testis weight increased with season, was also seen in juvenile males. Regarding the juveniles however, the seasonal weight difference was not as major as in the adults.

The main factors controlling the timing and characteristics of the reproductive period in grey seals are not fully understood. In a study on grey seals in the Northeast Atlantic, correlations between environmental factors such as mean sea-surface temperature, social structures and the annual median date of pup births were investigated (Coulson 1981). The median pupping dates in grey seal populations at different latitudes were found to follow an irregular geographical distribution, a finding that made the researchers exclude the photoperiod as the main factor controlling the time of pupping in grey seals. Instead, sea-surface temperature and social factors, where a lower temperature and smaller colonies were shown to delay the reproductive processes, were mentioned as aspects of importance.

2.3 Sexual maturity and seasonality from a histological perspective

In a study on male Southern elephant seals (*Mirounga leonina*), another phocid, seasonally varying mass of the seminiferous tubules was found to be the main reason for significantly increased testis weight during the breeding season (Griffiths 1984). Histological changes in the seminiferous tubules during that time were an increase in both tubular diameter and number of rows with spermatogenetic cells in the tubular epithelium. Meanwhile, the interstitial tissue did not show the same seasonal fluctuation.

Research done on male Cape fur seals (*Arctocephalus pusillus*), showed a clear seasonal variation in the morphology of the testes (Stewardson *et al.* 1998). In this study, testicles originating from 59 males, four years of age and older, were examined with a light microscope. The researchers observed four yearly phases of spermatogenesis, where a seasonal change in testis weight and seminiferous tubule diameter was significant. In the germinal epithelium, signs of early spermatogenesis, defined as presence of spermatogonia and spermatocytes but the lack of sperm cells in the epididymis, started 3-4 months before the breeding season. The peak in spermatogenic activity correlated with the highest testis weight. During the breeding season spermatogenic cells, such as primary spermatocytes and maturing spermatids, were present histologically. During the following months, the tissue regressed, characterized by a thinner, inactive seminiferous epithelium and a reduced seminiferous tubule diameter. Also, the epididymal duct was empty during this inactive phase.

In the harbour porpoise, testicular morphology was studied during different life stages (Kesselring *et al.* 2019). The study included both histological changes linked to tissue maturation in neonatal and juvenile porpoises and annual changes

in adult testes. When comparing individuals of different ages histologically, the seminiferous tubules of the juveniles were of a much smaller diameter than the adults, and the seminiferous epithelium consisted only of Sertoli cells and spermatogonia. Mature porpoises, on the other hand, presented a far more developed seminiferous system. Their tubules possessed a wider diameter, and the epithelial cells included differentiated germ cells, with the possibility to identify most stages of spermatogenic activity during the breeding season.

In a histological study investigating the reproductive physiology of male Pacific spotted dolphins (*Stenella attenuata*), criteria for differentiating testicles into three groups- immature, pubertal and mature were described (Hohn *et al.* 1985). These criteria have later been used by other marine mammal researchers to help categorise histological findings, for example in a study done on sea lions (Grandi *et al.* 2010). The dolphin testicles that were classified as immature were characterrised by having small seminiferous tubules and by lacking a seminiferous lumen (Hohn *et al.* 1985). The cell types identified in the seminiferous epithelium were found to only consist of undifferentiated spermatogonia and Sertoli cells. In the animals with an ongoing development of sexual maturity, classified as pubertal, the most determining part of this developmental step was the presence of spermatogenetic cells such as spermatogonia, and spermatocytes in the stratified epithelium. In the seminiferous epithelium of individuals classified as mature, spermatids were present, and spermatozoa were found in the seminiferous lumen.

The same study of Pacific spotted dolphins also investigated the seasonality of the adult males and found a similar pattern as in other marine mammals, i.e. testis weight was highest in the breeding season and there was a distinct monthly pattern in histological findings (Hohn *et al.* 1985). During the month of the highest reproductive activity (April for the spotted dolphins studied), the seminiferous tubules increased in size and the number of individuals having spermatids was the highest.

3. Material And Methods

3.1 The monitoring programme

As part of the national monitoring programme, Baltic grey seal necropsies are performed at SMNH. Bycaught seals are collected from fishers throughout the year, and hunters send in samples from harvested grey seals during autumn and winter. Age determination is routinely performed on each seal by counting the number of growth layer groups in the cementum of the canine tooth. Tissue samples from multiple organs are routinely archived at the museum. For this study, premade paraffin blocks with fixated tissue from testicle and epididymis were provided by SMNH. Additionally, the museum provided testis weights and premade smears of epididymal fluid.

3.2 Statistical analysis of testis weights

In this study, data collected at SMNH from 1042 male grey seals were analysed. The year of sampling ranged from 1982 to 2024, and variables included in this study were age, testis weights, and month of death. Excel was used for statistical analysis. Mean testes weight was calculated as the average of the two testicles for each individual seal.

3.3 Selection of samples for histology

The primary selection criterion was the inclusion of testicular and epididymal tissue from individuals that had not been frozen prior to fixation. Available individuals that met the inclusion criteria were a total of 14 Baltic male grey seals. From this group, three individuals presumed to be juvenile because of their young age were selected, all in the age range of 2-3 years. When selecting these individuals, a low testis weight also contributed to the assumption of them being juvenile. Seven adults were also chosen based on age and month of death, with an adult age span varying from 6 to 25 years, aiming to reflect differences in reproductive maturity and seasonal differences, see

Table 1. A higher number of adults were selected, as greater histological variation was expected in this age group.

Table 1. Compilation of selected seals for histological examination.

Seal	SMNH ID	Age (years)	Sampling month	Presumed maturity
A	A2013/05482	2	September	Juvenile
B	A2013/05597	2	November	Juvenile
C	A2013/05379	3	August	Juvenile
D	A2015/05628	6	October	Adult
E	A2016/05606	6	October	Adult
F	A2021/08004	7	September	Adult
G	A2021/05118	11	January	Adult
H	A2013/05611	14	November	Adult
I	A2017/0558770	15	September	Adult
J	A2010/05282	25	June	Adult

3.4 Histological preparation and study

Preparation of the sections

The histological sections were prepared from archived paraffin-embedded tissue blocks using a manual microtome, see Figure 1. In the first step, excess paraffin surrounding the tissue was removed with a scalpel. The block was then placed on the microtome and trimmed using thick sections until the whole surface of tissue was exposed. After that, the microtome settings were adjusted for thin sectioning at 5 µm and the sections of highest quality were transferred to a water bath for flattening and then mounted on a glass slide to dry. From each tissue block, one slide was prepared including two adjacent sections. The slides were then placed on a slide warmer until completely dried.

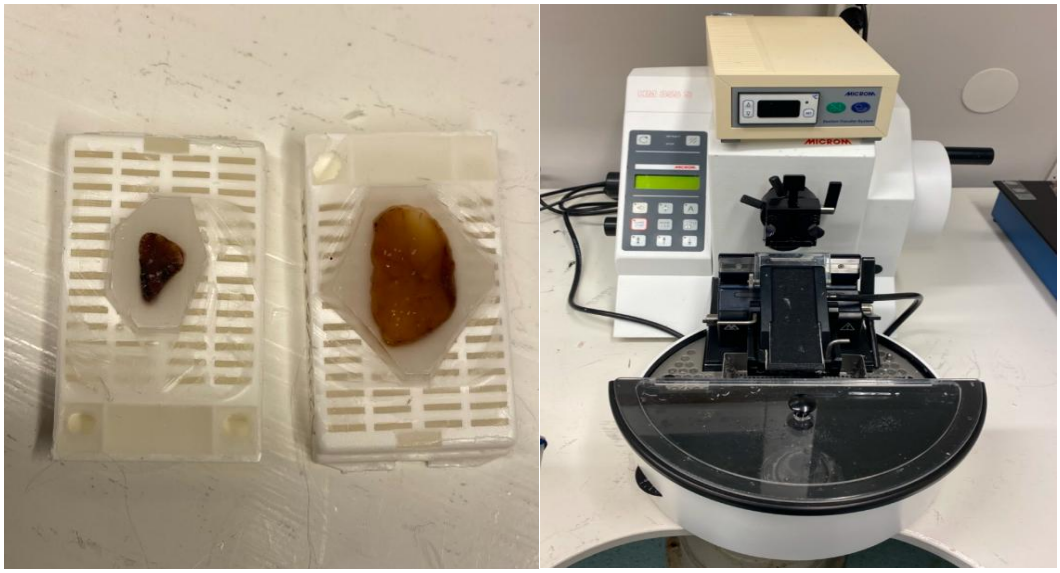


Figure 1. Left picture: Premade paraffin embedded epididymis and testicle tissue from a grey seal. Right picture: Microtome including a water bath.

Consequently, the sections were stained with haematoxylin and eosin, according to standard histological protocols. This step was followed by immediate mounting of coverslips using Permount mounting medium.

Microscopical study

After the sections had dried for two weeks, they were studied under a light microscope, and a representative area of each section was photographed. When examining all the sections, the testes and epididymides tissues were described using a consistent set of measures developed for the purpose of this study. These were for example level of artifacts, size of lumen, amount of Sertoli cells and were used throughout for all sections. This set of characteristics, which formed the basis for the descriptive results, was noted in a collective table including all ten study subjects. In this table, each parameter was semi-quantitatively classified into one of four categories; absent/none (-), minimal (+), noticeable/moderate (++) or extensive/marked (+++).

When grading artifacts into these four categories, overall tissue damage was estimated based on loss of organisation in the tissue. If minimal or no loss was present, artifacts were graded (-). Sections graded (+) indicated mostly retained structure with only minimal detachment, vacuolisation and loss of structure in both the seminiferous epithelium and the interstitial tissue, see Figure 5. More pronounced artifacts (++) included damage such as detachment in seminiferous epithelium and over all loss of tissue structure, but cell types remained distinguishable, see Figure 7. Extensive artifacts (+++) involved severe damage, including marked loss in tissue structure, detachment of seminiferous epithelium and substantial cell damage, making cell differentiation difficult. See

Figure 13.

The amount of interstitial tissue was estimated for each section. An absence, or minimal presence, of interstitial tissue was classified as (-). Sections graded (+) only displayed a small amount of interstitial tissue, occupying a minor proportion of the total tissue, see Figure 8. A moderate amount (++) of interstitial tissue was characterised by a larger proportion and a noticeable separation of the tubules, see

Figure 9. Sections graded (+++) showed prominent interstitial areas between the tubules, see Figure 5.

Thickness of the seminiferous epithelium was described using the same grading categories. Thin epithelium, with only 1-2 rows of germ cells, was graded as (+), see Figure 5. Sections with approximately 3-4 rows were graded as (++), see Figure 7. Testes where a majority of the tubules had a width of five or more rows of germ cells were described with (+++), see
Figure 14. If no cells were present, the thickness was categorised as (-).

When determining the approximate amount of each cell type, as done for spermatocytes and spermatids, the cell amount was described as (-) when the specified cell type was not identified in the section. In sections graded (+) the cell type was identified in one or more seminiferous tubules but only made up a small fraction of the total epithelium, for example amount of Sertoli cells in

Figure 10. A notice-able/moderate amount was (++) selected when the cell type was observed in several tubules and to a greater extant, but without dominating the total epithelial cell proportion, for example, amount of spermatids in

Figure 10. In sections where the specified cell type was found in the majority of the tubules and made up a substantial part of the total cell proportion, the marked/extensive grading (+++) was used. For example, amount of spermatocytes in Figure 8.

3.5 Sperm smears

Eight already premade sperm smears from the SMNH were also studied. The sperm smears were made by cutting the epididymis and dipping the end of a glass slide on the surface of the cut. A rolled smear was prepared by repeatedly lifting and reapplying the slide while spreading the epididymal fluid. The slides were then studied under a light microscope and evaluated by two categories, (-) if no spermatozoa were seen and (+) if spermatozoa were found in the sample (See Table 2*)*. The number of spermatozoa in the smear was graded as follows: (-) not detected; (+) a few spermatozoa present;(++) a noticeable/moderate amount present and (+++) extensive when spermatozoa were found in abundance.

3.6 Methodology of text processing

In this master thesis, artificial intelligence has, on occasional instances, been used as a tool for translation and grammar correction. The purpose of this use has been to enhance the readability and clarity of the writing. Importantly, artificial intelligence has only been used for refinement of already existing sentences and has not been used for generating text.

4. Results

4.1 Testicular weight

In Figure 2, the age-related changes in testis weight are shown, including seals sampled between July and November, out of breeding season. It seems that there is a gradual increase in weight during juvenile years, starting slowly during year 2 and 3 and then more pronounced from 4 years of age up until approximately 6 years of age, where the increase seems to level off at approximately 60 grams on average, with exception for the eight-year-olds (N=23) being closer to 50g.

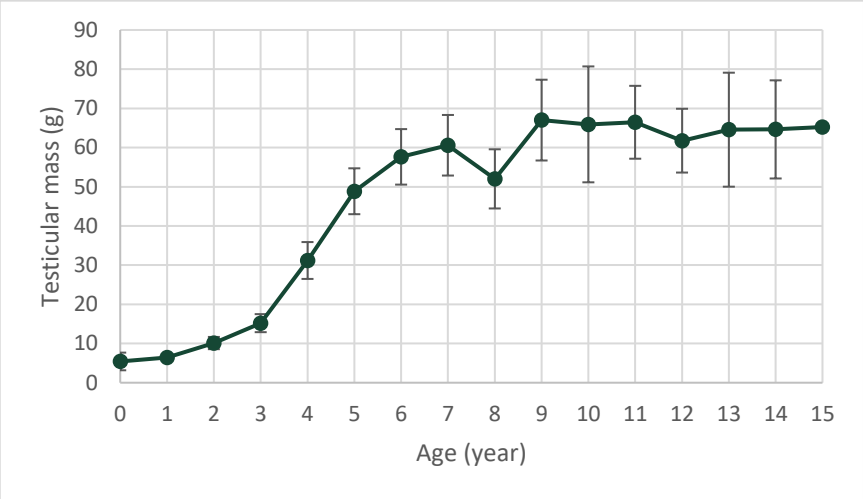


Figure 2. Mean testis weight in grams (y) per age (x) of seals caught between July and November. Error bars show confidence interval (95%). N=624 seals.

In Figure 3, the age-related monthly changes in mean testis weight are shown individually for each age group between 1-8 years of age. The highest testis weight appears to be during April and May. During July-October, the age groups show the lowest testis weight. The 0-3 years old do not show as clear seasonal trends as the 4 to 8-years old. Also, a rapid increase in testis weight is shown between the age of 4 and 5 years old.

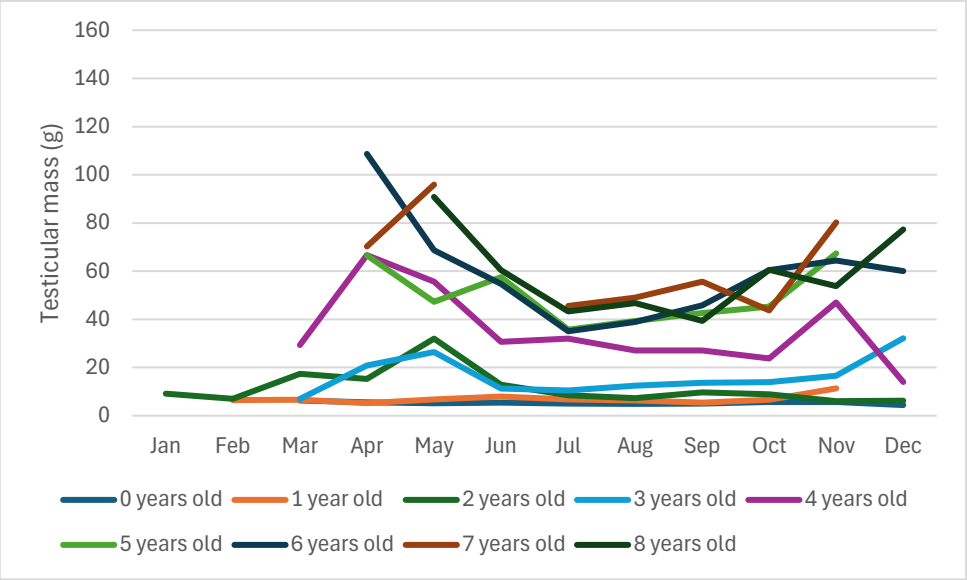


Figure 3. Age related monthly changes in testicular mass, ages 0-8 years old. N: 46 seals.

In Figure 4, age related monthly changes in mean testis weight are shown for seals aged 8-34 years old. During July-October, the age group shows the lowest mean testis weight. The larger variation in testicular mass during November to April, and especially the large confidence interval in March, is probably due to a small sample size during these months (N= 2 in March).

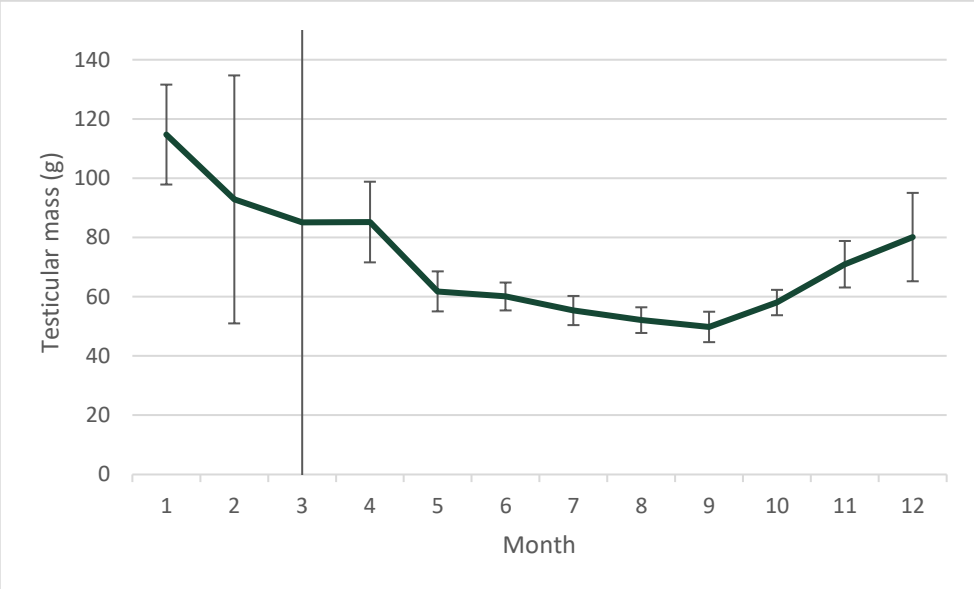


Figure 4. Age related monthly changes in testicular mass, adults 8-34 years old. Error bars show confidence interval (95%) Confidence intervals are truncated at zero and 150g for visual clarity, but also because testicular mass cannot be negative. Wide intervals in March reflect small sample size (N=2). N=272 seals.

4.2 Sperm smear observations

In Table 2, descriptive results from the cytological study of the collected sperm smears are presented together with age and month of death. The largest amount of spermatozoa (+++) was seen in the seals sampled in January. The other seals showed either a minimal amount or no spermatozoa present.

Table 2. Collective table of descriptive results from cytological study of grey seal sperm smears.

SMNH individual code	Age (years)	Month of death	Estimated amount of sperm cells
A2019/05148	15	October	-
A2021/05119	4	January	+++
A2019/05635	8	August	-
A2020/05860	13	August	+
A2021/05118	11	January	+++
A2019/05211	14	August	-
B2022/00093	14	November	+
B2023/05038	13	May	-

4.3 Histological findings

4.3.1 Juveniles

Seal A

Seal A (see left picture in Figure 5), was two years old at the time of death. It was bycaught in September and transported to SMNH. The testis section had artifacts such as vacuolised seminiferous epithelium and detached cells, although most of the spermatogonia were still adjacent to the basal membrane. The seminiferous lumen was very small and sometimes unidentifiable. As a result, the size of seminiferous lumen could not be assessed with confidence. Regarding the interstitial tissue, there was much less damage to this than to the seminiferous epithelium and the amount of interstitial tissue was noted as substantial.

Although a lot of cells were detached from the basal membrane, different cell types were still possible to categorize in this sample. The seminiferous epithelium appeared as very thin, and the only types of cells present were spermatogonia and Sertoli cells. In summary, there were no signs of spermatogenic activity, which was in line with the small testicle weight (9.12 grams, Table 3)

Seal B

Seal B was two years old and bycaught in November (see right picture in Figure 5). The testis tissue had a lot of vacuolisations which were more prominent in the seminiferous tubules than in the interstitial tissue. The interstitial tissue and basal membrane were not detached and most of the spermatogonia were still adjacent to basal membrane. In contrast, Sertoli cells were often detached and located in the vacuolised centre of the tubules. Due to this artifact, there were no clearly defined lumina, but the seminiferous epithelium appeared very thin as there were no identifiable spermatocytes or spermatids. This section had a lot of interstitial tissue and Leydig cells were often located in clusters. The quality of the epididymis section was described as good and the epididymal duct lacked spermatozoa.

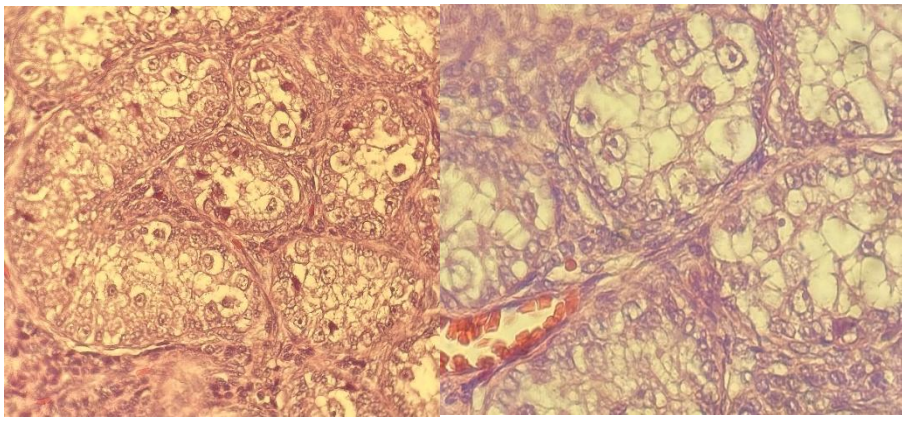


Figure 5: Left picture; histological section of left testis, Seal A. Right picture; histological section of left testis, Seal B. H&E.

Seal C

Seal C, a bycaught seal that died in August 2013, was determined to be 3 years. When selected, this seal was presumed to be juvenile. However, it was possible to identify more cells and there was a larger variety of cell types compared to the previous two-year-olds (A and B) (See

Figure 6). The seminiferous epithelium consisted of a lot of Sertoli cells and spermatogonia, but also some spermatocytes. There were, however, no spermatids and the epididymal duct lacked spermatozoa. In summary, these findings are indicative of pubertal onset (but not necessarily sexual maturity). The weight of the left testis (17.8 g) was superior to those of seal A and B (9.12 and 7.98 g, respectively). In addition, the amount of interstitial tissue was considered to be substantial (+++), but slightly less than in the younger seals.

Regarding the section quality, the structure of both the interstitial tissue and the seminiferous tubules was damaged. A lot of vacuolisation and loose epithelial cells made it hard to determine the size of the seminiferous lumen.

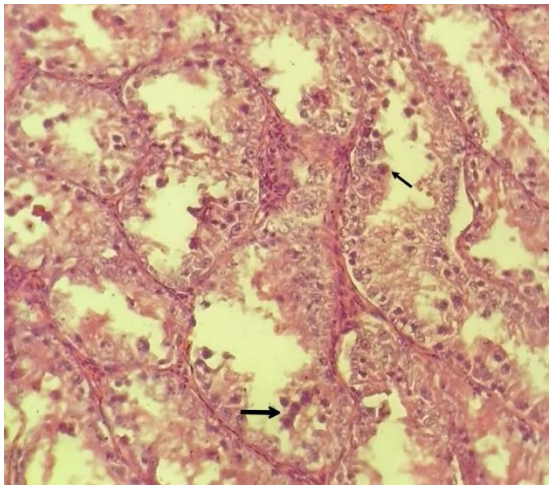


Figure 6. Histological section of left testis, Seal C. The black arrows highlight examples of spermatocytes in the section. H&E.

4.3.2 Adults

Seal D

Seal D was found stranded in October 2015 and was determined to be six years old at the time of death. The necropsy revealed a gunshot as the most likely cause of death. The left testis weight was 59.64 g, but the epididymis weights were not recorded. This section had artifacts including vacuolisation and partly detached seminiferous epithelium, leading to difficulties in determining the seminiferous lumen width. The interstitial tissue was generally of better quality but still vacuolised.

Compared to the younger individuals (A-C), the seminiferous epithelium was thicker and had generally more cells, and a different proportion of cell types (see Figure 7). Sertoli cells and spermatogonia were fewer and instead, the epithelium consisted of a lot of spermatocytes. No spermatids were found in the section and the epididymal duct lacked spermatozoa.

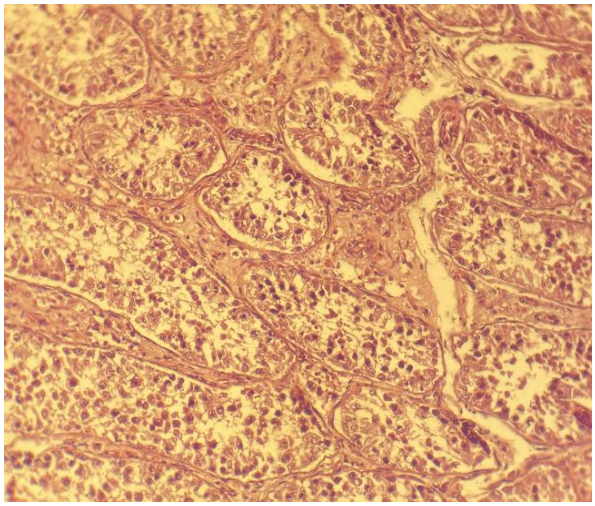


Figure 7. Histological section of left testis, Seal D. H&E.

Seal E

Seal E was bycaught in October and was determined to have died at the age of 6. The left testis weight was 46.46 g and the epididymis weight was not noted. The level of artifacts was categorised as the same as the other 6-year-old (seal D, see Table 3). Vacuolisation and an almost completely detached epithelium were the major damages to the tissue, leading to difficulty determining the seminiferous lumen size (Figure 8). Despite these artifacts, it was still possible to find spermatogonia, Sertoli cells and a lot of spermatocytes in the seminiferous epithelium. No spermatids could be found in this section. The section of the left epididymis showed a lot of cells and debris in the epididymal duct. However, these cells most likely originated from the damaged epithelium. No spermatozoa could be detected in the duct.

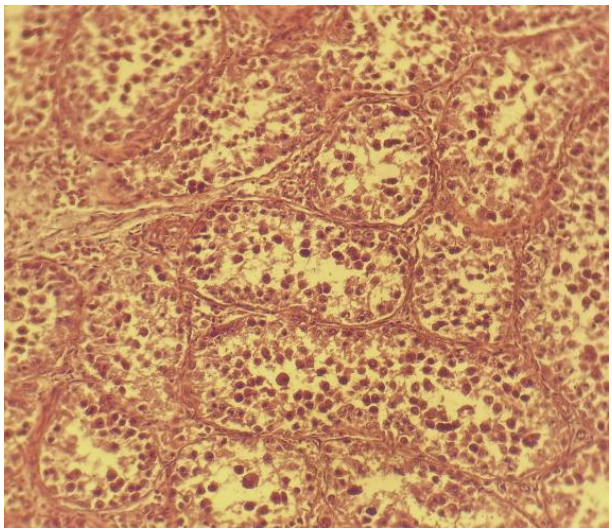


Figure 8. Histological section of left testis, Seal E. H&E.

Seal F

Seal F was a 7-year-old seal that was shot during the hunt in September 2021. The left testis weight was 52.74 g and the left epididymis weight was 15.26 g. The seminiferous lumen was prominent but due to the widespread vacuolisation and cell detachment, its size could not be reliably measured (

Figure 9). The relatively thin epithelium consisted mostly of spermatogonia, Sertoli cells and spermato-cytes. No spermatids were definately found in the section, but it was not possible to fully exclude their presence either due to artifacts or autolysis. Regarding the interstitial tissue, this individual had a larger amount than the other adults but less than the presumed juveniles. The epididymal duct lacked spermatozoa.

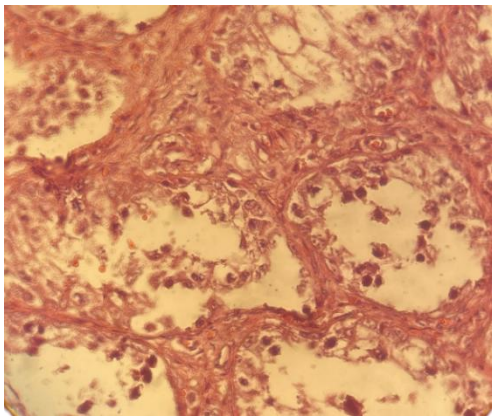


Figure 9.Histological section of left testis, Seal F. H&E.

Seal G

Seal G was 11 years old when shot by a hunter in January 2021. The cut surface of the testes was described as prominent and white fluid exuded when the epididymis was incised. A sperm smear was performed from this seal and when examining these smears microscopically as a part of this master thesis, an abundance of sperm cells was seen from both the left and right epididymis.

The artifacts in this tissue were determined to be major, which made it impossible to locate the original structure of the tissue and lumen. However, a lot of the detached cells were still possible to categorise. The seminiferous epithelium appeared to be thick with a lot of cells. The proportion of Sertoli cells and spermatogonia were small in this section, whereas there was a large quantity of spermatocytes and spermatids. Due to the disorganised structure, seminiferous

spermatozoa were not possible to distinguish. There was only a small amount of interstitial tissue in between the seminiferous tubules, but these two tissues were detached in most places of the section (

Figure 10).

When studying the histological section of the epididymis a lot of spermatozoa were present in the epididymal duct (Figure 11). Considering the characteristics of the testes and epididymis, seal G was classified as having active spermatogenesis at the time of death.

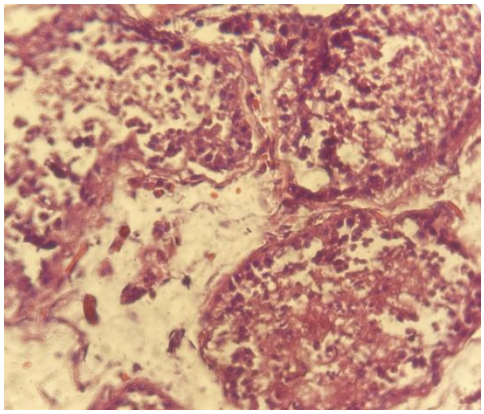


Figure 10. Histological section of left testis, Seal G. H&E.

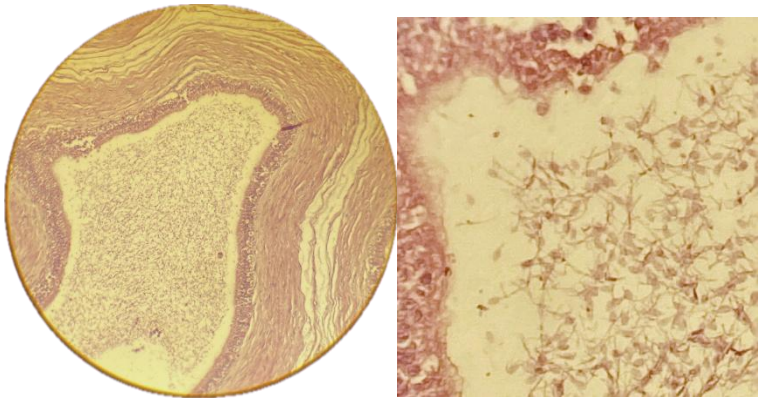


Figure 11. Picture 1 & 2: Histological section of left epididymis seal G, both showing spermatozoa in epididymal duct. H&E.

Seal H

Seal H, a 14-year-old that was hunted in November 2013, had a left testis weight of 45.3 g and a left epididymis weight of 22.1 g. The artifacts of the left testicle section included vacuolisation and a detached epithelium. When looking at the seminiferous epithelium, some of the loosened cells were autolysed and it was not possible to distinguish between the different cell types. The cells possible to categorise were mostly Sertoli cells and spermatogonia, but also some spermatocytes. No spermatids were found in this section, and the epithelial thickness was thinner than in the other adults studied (Figure 12). Due to the artifacts, the size of lumen varied troughout the tissue and like the other seals studied, is not thought to con-

tribute to reliable results. When looking at the epididymis section of seal H, the epididymal duct was empty.

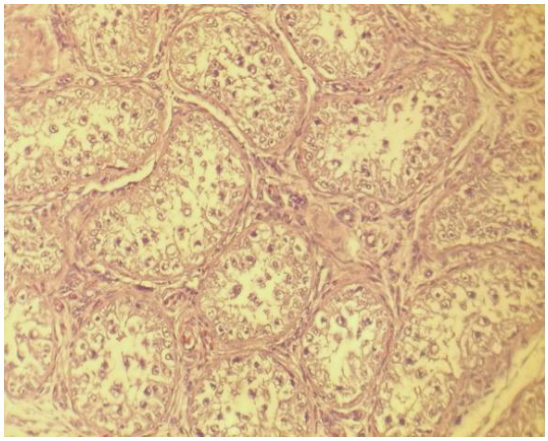


Figure 12. Histological section of left testicle, seal H. H&E.

Seal I

Seal I was 15 years old when it was bycaught in September 2017. When looking at this section (

Figure 13), the artifact level was categorised as (+++), all the cells had detached from the epithelium and were so autolysed and disorganised in the tissue that the possibility of quantifying cell types in an adequate way was limited. Cell types that were possible to identify, but not quantify, were Sertoli cells, sper-matogonia and spermatocytes. The interstitial tissue was described as scant. Regarding the epididymis, the paraffin-embedded tissue block labelled as epidi-dymis turned out to be of another tissue origin and could not be used.

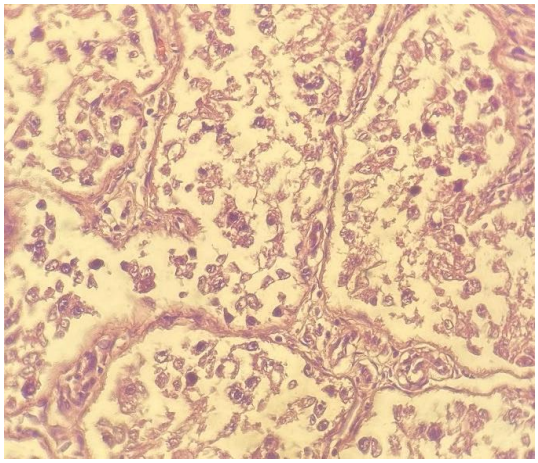


Figure 13. Histological section of left testicle, seal I. H&E.

Seal J

Seal J was the oldest seal included in this study at 25 years old. It was shot in a seal trap in June 2010. The left testis weighed 71.08 g and the left epididymis weighed 18.6 g. Artifacts in the testis section included mostly vacuolisation and some detachment of the cells from the seminiferous membrane. At some places, the basal membrane and interstitial tissue were also detached from each other, resulting in a loss of structure of the seminiferous tubules.

This testis had a thick epithelium with a lot of spermatogenetic cells, sometimes hard to distinguish from each other due to the mentioned artifacts (
Figure 14). A lot of spermatocytes were present in the seminiferous epithelium, but also some Sertoli cells, spermatogonia and spermatids. When looking at the left epididymis, some spermatozoa were seen in the epididymal duct. The number of spermatozoa was a lot less than in seal G but still observed in multiple parts of the section.

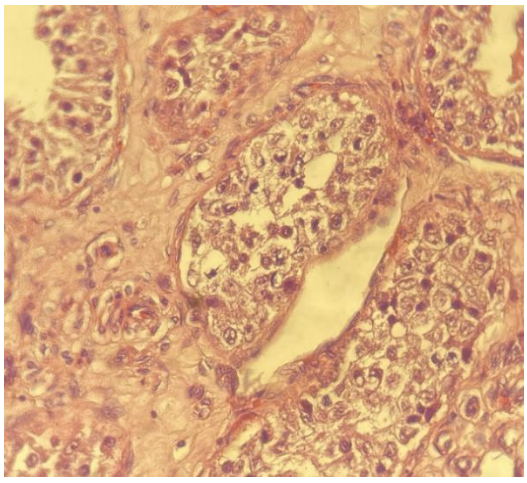


Figure 14.Histological section of left testicle, seal J. H&E.

4.3.3 Collective table of all seals

In Table 3, a collective table of results from seals A-J, observations were graded semi quantitatively and information such as month of death and testis weight were compiled.

Table 3. Descriptive findings of the histological examination.

Seal	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
ID number	A2013/05482	A2013/05597	A2013/05379	A2015/05628	A2016/05606	A2021/08004	A2021/05118	A2013/05611	A2017/05587	A2010/05282
Age (years)	2	2	3	6	6	7	11	14	15	25
Presumed maturity (Juvenile/ Adult)	J	J	J	A	A	A	A	A	A	A
Time of death (month)	Sep	Nov	Aug	Oct	Oct	Sep	Jan	Nov	Sep	Jun
Left testicle weight (g)	9.12	7.98	17.28	59.46	46.46	52.74	128.06	45.3	46.26	71.08
Mean testis weight (g)	9.12	7.77	17.19	60.1	45.01	52.11	124.63	44.49	46.18	69.57
Left epididymis weight (g)	3.72	3.62	5.76			15.26	38.04	22.1		11.16
Level of artifacts	+	+	++	++	++	++	+++	++	+++	++
Amount of interstitial tissue	+++	+++	+++	+	+	++	+	++	+	+
Epithelial thickness	+	+	+/++	++	++	+/++	++	+/++	n.a*	+++
Size of lumen	n.a*	-	+	++	++	++	n.a*	++	n.a*	++
Sertoli cells; Estimated proportion	+++	+++	+++	+	++	++	+	++	n.a*	++
Amount of spermatocytes	-	-	+	+++	+++	++	+++	+	n.a*	+++
Amount of spermatids	-	-	-	-	-	-	++	-	n.a*	++
Amount of spermatozoa in epididymal duct:	-	-	-	-	-	-	+++	-	n.a*	+

*The descriptive results were semi-quantitatively classified into four categories: Absent/none (-), Minimal (+), Noticeable/moderate (++) and Extensive/marked (+++). *= not applicable due to extensive level of artifacts.*

5. Discussion

5.1 Testis weight statistics

In the first graph, Figure 2, testis weight starts to plateau at 6 years old to then consistently stay just above 50 g for seals older than 8 years old. These findings are interpreted as being about 10 g lower compared to current literature of testis weight correlation to age in grey seals (Hammill & Gosselin 1995; Hauksson 2007). However, the testis weight generally being lower in Baltic grey seals compared to Atlantic grey seals could be explained by their size difference, with the Atlantic grey seal being larger than the Baltic grey seal.

When analysing the age related monthly changes in testicular mass (Figure 2 and Figure 3), a marked increase in testis weight from four to six years is seen. The seasonal fluctuations are also more marked in the 4-8 years old than in the youngest individuals. In Figure 3*,* a significant peak in testis weight is seen in about April-May, likely due to the reproductive peak expected in March. Studying Figure 4*,* a seasonal pattern is seen, with the lowest testis weight during the autumn. However, it is not a clear peak in April. Importantly, a source of error in both graphs is the low sample size during winter and early spring due to hunting being prohibited during this season. Therefore, most likely due to the limited sample size, the expected testis weight peak in March is not shown in the graphs.

5.2 Microscopical findings

5.2.1 Differences between juvenile and adult descriptive histology

When comparing the descriptive results (Table 3), some histological observations were consistently similar within one age group and differed between the juveniles and adults. The amount of interstitial tissue was more pronounced in the younger seals. This parameter showed more variation among the adult seals, but all sections showed a clear reduction compared with the juvenile tissue composition. In contrast, due to an increased amount and variety of the spermatogenic cells, the seminiferous epithelium was categorised as the thickest in the adult testes. In the juvenile two-year-olds, the only cell types present in the very thin seminiferous epithelium were spermatogonia and Sertoli cells. The three-year-old was the youngest seal to exhibit spermatocytes, while still having a thin epithelium and pronounced interstitial tissue. When compared to the adults, all of them had a greater number of spermatocytes than the three-year-old. The observation of the seminiferous epithelium consisting of fewer and undifferentiated, germ cells in

the juvenile individuals is consistent with the results of other marine mammal studies (Hohn *et al.* 1985; Kesselring *et al.* 2019)

5.2.2 Differences in descriptive histology between the adults

As expected, the sections from adult seals showed a greater variety in testicular morphology. The thickness of the seminiferous epithelium was highest in seals G and J. Additionally, these two were the only studied seals also presenting spermatozoa in the epididymal duct, where the duct of seal G contained by far the most of the two. Comparing the testis and epididymal weights, the adults showed considerable variation in testicular mass. However, there was no clear observation of differences in amount of interstitial tissue, or number of spermatocytes, between adults during the breeding season and seals sampled outside the breeding season.

5.2.3 Seasonal differences in descriptive histology of the adults

Seal G was the only one showing signs of active spermatogenesis. This observation is in alignment with what would be expected during this season. As January is close to the pupping season, then followed by mating shortly after, an increase in spermatogenetic activity during this time would be necessary to ensure reproductive success during the early spring. Seal J was caught in June, two months after presumed spermatogenetic peak, which could explain the histological findings showing signs of spermatogenesis such as a thicker epithelium and higher testis weight but a lower number of spermatozoa in the epididymal duct than seal G. The testis weight being seemingly higher in the two seals closer to the breeding season corresponds well with existing literature comparing testicular weight to annual spermatogenetic activity in male pinnipeds (Griffiths 1984; Stewardson *et al.* 1998).

The rest of the adults were sampled between August and November and had a decreased spermatogenetic activity. When comparing to seal G and J, common characteristics of these sections were a thinner epithelium, no spermatozoa in the epididymal duct and no spermatids in the seminiferous epithelium, suggesting arrest at spermatocyte stage. These seasonal differences between seals closer to the breeding season and ones not expected to show signs of spermatogenetic activity are similar to the observations of other studies on seasonal changes to testicular histology in marine mammals (Hohn *et al.* 1985; Stewardson *et al.* 1998).

5.2.4 Sperm smears

Out of the sperm smears studied, the most extensive number of spermatozoa were seen in the individuals sampled in January. Interestingly, no spermatozoa could be detected in the smear from the seal in May, but a few sperm cells were found in

smears from August and November. Likely, this finding is not due to ongoing spermatogenetic activity during autumn, but remnants from the previous breeding season. Findings of spermatozoa could be a great complement to histological evaluation of the testicular and epididymal tissue, where presence of spermatozoa in the smear supports the assessment of an individual being sexually mature. Importantly, sexual maturity is still possible even when no spermatozoa are detected on the smear. This is primarily due to seasonal variation, as reflected in the studied seals.

5.3 Criteria of sexual maturity

Characteristics that consistently were described as absent in the presumed juvenile sections, but found in all the adults, could be used as the base for a set of criteria as an indicator of sexual maturity. The amount of interstitial tissue differed significantly between the juvenile and the adult individuals. Scant interstitial tissue could therefore be a possible histological criterion for sexual maturity. Also, a thicker seminiferous epithelium, with a larger total amount of germ cells, could indicate sexual maturity. All presumed juveniles had a very thin epithelium, 1-2 rows in height with a high Sertoli cell proportion, meanwhile all the adults presented a thicker epithelium with a larger cell type variety and therefore a lower Sertoli cell proportion, even during the autumn.

Spermatocytes were identified in all individuals except the two youngest seals. Considering the finding of spermatocytes in the three-year-old, an individual still lacking other signs of sexual maturity, could suggest that spermatocyte presence is not a reliable indicator of sexual maturity, but more of an indicator of ongoing puberty. The number of spermatocytes seemed to be higher in the adults than in the three-year-old, except for seal H which makes the observation inconclusive. Given the small sample size and variability in tissue preservation, further studies are needed to investigate whether the amount of spermatocytes could be used as a supporting parameter for assessing sexual maturity during the non-active reproducetive season.

In this study, spermatids were only found in individuals showing clear signs of spermatogenetic activity such as spermatozoa in the epididymal duct, suggesting that presence of this cell type could be used as a criterion for sexual maturity. In summary, this study suggests that sexual maturity could be identified histologically by the presence of spermatids in the seminiferous epithelium, however it seems that this can only be done from approximately January to June. Furthermore, a small amount of interstitial tissue in combination with a low proportion of Sertoli cells may also indicate sexual maturity, including in individuals out of breeding season.

5.4 Study design; limitations and future directions

5.4.1 Artefacts

One main challenge when researching wild animals is to obtain the specimens in a fresh condition. The seals used in this study first had to be found in the nets or be hunted, and then sent to SMHN for necropsy and fixation. When preparing and examining the testicular tissue, structural damage such as germ cell detachment and vacuolisation was observed, strongly indicating that degradation processes had taken place prior to tissue fixation.

In a study on American mink (*Neovison vison,* now *Neogale Vison*), researchers investigated how freezing and delayed time before fixation affected the characteristics of the testicular tissue (Spörndly-Nees *et al.* 2015). The process of freezing the testis 6 hours postmortem resulted in tissue alterations including major damages to germinal cell morphology and decreased width of seminiferous epithelium and lumen. Changes got more severe with increased delay times. After 42 hours of prefixation delay, marked signs of biological degeneration, such as germ cells and morphological changes, were present, making cell differentiation challenging.

It is likely that the pre-fixation delay of the seals in this study was a lot longer than 42 hours. All sections showed similar degenerative artifacts as described in the minks and were thought to be a result of the significantly delayed pre-fixation time. If the seals were frozen earlier postmortem, the damage caused by degeneration processes could have decreased, but then damage due to freezing artifacts would be present instead. As it is rarely possible to study wild animal tissue instantly postmortem, researchers must take mentioned factors into consideration when interpreting the results. For example, due to degenerative artifacts in the tissue, exact measuring of luminal diameter and epithelial thickness was not possible and were therefore only estimated descriptively.

As access to fresh seal tissue for fixation is often very limited, wildlife researchers typically must choose between a longer delay time or freezing the tissue before fixation, with both methods causing damage to the tissue. This means that, in most cases, researchers must evaluate histological slides of comparable quality to these sections. Even though details and some structures were lost in the sections in this study, there is still a lot of information to obtain. The criterion suggested for sexual maturity in this thesis does not require an absence of artefacts, which is also why it could be of practical use to researchers evaluating tissue of similar preservation status. Thus, the reduced quality is not a reason not to study more seals of similar quality, rather, it highlights the need for further studies of it. In addition to further studies on non-frozen tissue, it would also be interesting to

compare these findings to sections frozen prior to fixation. Possibly, most the knowledge regarding grey seal sexual maturity would be obtained from studying both techniques in parallel.

5.4.2 Prospects for future studies

The sample size and quality of sections in this study contributed to an inability to rely on these results for definitive conclusions. Therefore, a suggestion of improvement would be to include more seals in the studied group, to increase the relevance of the conclusions on a population level. Of those for which quality of the histological sections allows it, measures of lumen diameter could potentially be an interesting criterion to investigate. For example, in the histological study done on spotted dolphin testes a large seminiferous lumen was suggested as a potential criterion for sexual maturity even outside of their reproductive season (Hohn *et al.* 1985). To investigate if lumen size indicates the same pattern in Baltic grey seals, a consistent sampling and measuring technique would be essential. When sampling, prioritizing seals with a known time of death, such as hunted seals or seals caught in nets, and enabling a quick postmortem fixation process would be important for limiting artifacts. A possible study design for comparing lumen sizes across age groups could involve selecting a set number of randomized cross-sectioned seminiferous tubules. In these, morphometry programmes such as ImageJ could be used to measure the lumen diameter, over for example, 4 axes within one tubule, to obtain a representative mean diameter. Even with a changed lumen due to, for example, freezing or vacuolisation, it might still be possible to see patterns of lumen sizes. The total number of cells in cross-sectioned tubules is another potential parameter that could be quantified with the help of software, although artifacts may limit the accuracy of the counts.

An established way of investigating and assessing sexual maturity in grey seals populations is by analysing testis weights, and from this define a cut of value as a criterion of sexual maturity. An older study, which has influenced more recent research on the determination of sexual maturity of the grey seal, was done by H. R. Hewer in 1964. In this study, teeth cement growth layer groups were used for age determination. Knowing the chronological age of the seals, the average age of sexual maturity was estimated using testis weight, body size, macroscopical changes to gonads and behavioural observations during breeding season (Hewer 1964). The criteria set in this study to be considered sexually mature was a testis weight at a minimum of 60g. In more recent studies, the correlation between age and testis weight remains a key factor in determining sexual maturity in grey seals (Hammill & Gosselin 1995; Hauksson 2007).

To further our knowledge of sexual maturity in Baltic grey seals in general, and particularly how to recognize this on an individual level outside of the breeding season, histological evaluation of the testes can be a valuable complement to traditional study designs. The mass of the testes does not provide direct information on their functionality. The occurrence of seasonal changes, also observed in this study, emphasises the importance of histological reference points to support the evaluation of non-spermatogenically active testes.

6. Conclusion

The results of this thesis indicate that Baltic grey seals exhibit seasonal changes in spermatogenesis related to the annual breeding season. Signs of active spermatogenesis were seen approximately from January to May, although this finding requires further confirmation with a larger sample size.

This thesis also highlights a possibility of using histological evaluation as a complement to testis weight and macroscopic findings when evaluating sexual maturity, both during and outside of the reproductive season. Criteria suggested based on this study include the presence of spermatids in the seminiferous epithelium, alternatively spermatozoa seen in the epididymal duct or in sperm smears. In addition, a small amount of interstitial tissue, accompanied by a low proportion of Sertoli cells, seems to be a promising method to determine sexual maturity in adults outside the reproductive season.

Using the proposed testicular weight cut-off value of 50 grams during autumn, the majority of male seals are likely to have reached sexual maturity by the age of 6 on a population level, although some individuals may attain this threshold, and potentially sexual maturity, at 5 years.

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Populärvetenskaplig sammanfattning

Gråsälen, *Halichoerus grypus*, är en toppredator i Östersjön och en viktig del av det marina ekosystemet. Naturhistoriska Riksmuseet (NRM) i Stockholm har, fått i uppdrag att övervaka gråsälpopulationernas hälsoläge i Östersjön. Eftersom gråsälen är högst upp i näringskedjan är den särskilt känslig för förändringar i ekosystemet. På grund av detta kan förändringar i gråsälens hälsoläge fungera som en tidig varningssignal för mänsklig påverkan i Östersjön.

I dagens forskning på gråsälar saknas detaljer om de fysiologiska processer i testiklarna som slutligen leder till att en säl kan fortplanta sig och bli könsmogen. Det är viktigt att öka förståelsen kring vad som är normalt för att göra det möjligt att upptäcka när något avviker från det normala. En tidig indikation på ökad belastning på ekosystemet, exempelvis orsakad av mänsklig påverkan, kan i sin tur leda till att åtgärder för att förhindra ytterligare skada kan sättas in i tid.

Syftet med denna studie var att beskriva testikelvävnadens utseende i ett mikroskop, för att se om det var möjligt att föreslå kriterier för hur testiklarna ser ut hos könsmogna individer. Detta gjordes även när sälarna var fångade utanför parningssäsongen och testiklarna därför inte hade någon aktiv spermieproduktion. Totalt valdes tio sälar ut till denna studie: tre av dessa var unga och tänkta att inte vara könsmogna ännu och sju vuxna sälar som antogs vara könsmogna. Testikel- och bitestikelvävnad från samtliga individer skivades mycket tunt och placerades på ett objektglas för att sedan undersökas under mikroskop.

Vid mikroskopering beskrevs sedan testikelvävnaden enligt samma kategorier för alla sälar. I detta ingick bland annat att uppskatta mängden av de olika celltyperna och proportion av olika typer av vävnad. Observationerna var sedan grund för att jämföra likheter och skillnader både inom och mellan vuxna och unga sälar. Utöver detta tillhandahöll NRM testikelvikter från 1042 sälar, som användes för att studera hur storleken på testiklarna ändras med ålder och över året.

Vid mikroskopisk undersökning av de unga sälarnas testikelvävnad fanns mycket vävnad som omgav de spermieproducerande områdena. Den omgivande vävnaden kallas för interstitiell vävnad. I de unga sågs även mindre utvecklade stadier av spermieceller i den spermiebildande vävnaden jämfört med hos de vuxna. Förutom att ha mindre utvecklade stadier av cellerna sågs även en mindre mängd celler hos de unga, men en högre andel av stödjeceller (sertoliceller). Hos de vuxna sågs generellt en större variation i celltyper och mängd av dessa. De vuxna sälar som samlats in närmast parningssäsongen visade mest tecken på spermie-

produktion, såsom mer mogna stadier av blivande spermieceller i testikeln och spermier i bitestikeln.

Vid analys av testikelvikterna sågs en signifikant ökning i testikelvikt från fyra till sex års ålder för att sedan plana ut på en jämn nivå. För sälar som dött utanför parningssäsongen och också var över åtta år, sågs medeltestikelvikten konsekvent ligga över 50g. När testikelvikt analyserades i förhållande till månad på året sågs den högsta testikelvikten i april och maj. Den lägsta testikelvikten sågs utanför parningssäsongen, och särskilt under sensommaren och höstmånaderna.

Efter analys av resultaten från mikroskoperingen i denna studie, med förbehåll för att begränsningar så som vävnadsartefakter och ett litet urval av sälar behövde tas i beaktning, föreslogs ett antal kriterier som skulle kunna bidra till att utröna om individen är könsmogen. Det första förslaget på sådant observationskriterium var en liten mängd interstitiell vävnad kombinerat med en låg proportion av stödjeceller (sertoliceller). Ett ytterligare kriterium som föreslogs var fyndet av långt utvecklade förstadier av spermieceller i testikelvävnaden, så kallade spermatider, alternativt att spermieceller sågs i bitestikelgången.

En slutsats som kan dras ur denna studie var att Östersjögråsälen visar tecken på säsongsmässig reproduktion, där aktiv spermieproduktion ses under månaderna innan och under parningssäsongen. I den här studien kunde det ses från ungefär januari till maj, men det är viktigt att understryka att större studier behövs för att kunna säga säkert. Från viktstatistiken föreslogs en testikelvikt på 50 g som ett gränsvärde som indikerar könsmognad för hanar utanför parningssäsong. De allra flesta sälar verkar ha nått den gränsen vid 6 års ålder, fast vissa kan ha blivit könsmogna tidigare. Det är även möjligt att mikroskopbedömning av testikelvävnaden i framtiden skulle kunna användas som ett komplement till dessa testikelvikter för att öka möjligheten att göra en säkrare bedömning av könsmognaden, särskilt betydelsefullt hade detta i sådant fall varit för sälar som skickats in utanför parningssäsongen.

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