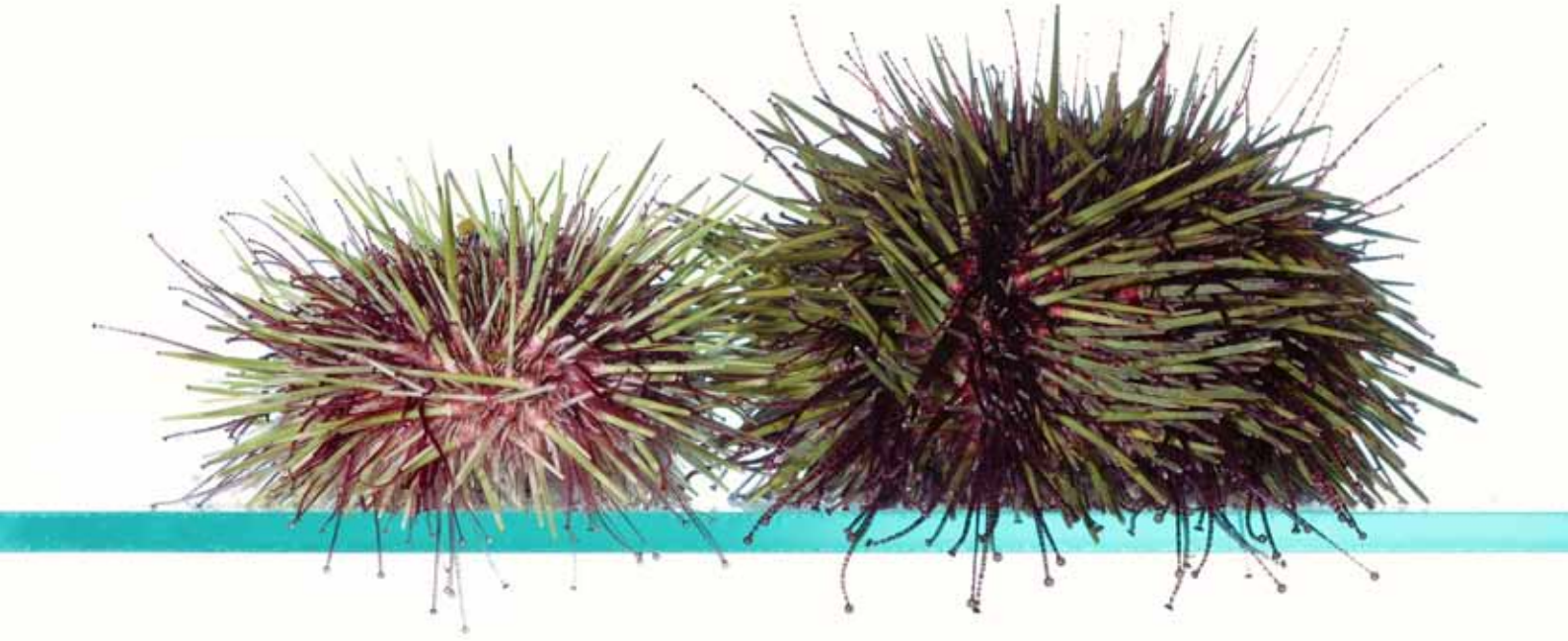
A Guide to the Sea Urchin Reproductive Cycle and Staging Sea Urchin Gonad Samples



By Philip James and Sten Siikavuopio

Nofima

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Use of this publication

This publication is intended to be used as a guide for anybody with a need to understand, or an interest, in the reproductive cycle of sea urchins. It includes the following: a description of the reproductive cycle of sea urchins; the factors that cause variations in the size and quality of the gonad; methods for sampling sea urchin gonads; and a guide to reading the histology slides of sea urchin gonads in order to be able to classify them into the different stages of the reproductive cycle. In order to further assist with this process Appendix One shows examples of histology slides taken from the two populations described in Figure 2. These clearly show the general pattern associated with the reproductive cycle of sea urchins but also the enormous variation that can occur between populations. The authors hope that this guide will enable those working with sea urchins to follow the reproductive cycle of selected populations and that it will contribute significantly to the knowledge of the reproductive cycle of sea urchins that occur in various populations around the coast of Norway.

The authors are willing to assist with any queries that may arise from the information contained in the guide and their contact details are as follows:

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Introduction to sea urchins

Sea urchins are ancient and primitive organisms that lack many of the organs found in higher animals. They have no specialized respiratory or circulatory system (i.e. no heart, no blood vessels and no oxygen binding molecules in their body fluids) and they have no specialized excretory organs. Basically, sea urchins consist of a mouth, a gut tube (digestive system), the gonads (also known as the roe) and a primitive nervous system which are all surrounded by a hard shell (also known as the test). On the outside of the test are the spines and tube feet (podia).

Despite this simple design sea urchins are capable of surviving for long periods (sometimes years) with little or no food as they have the ability to lower their body metabolism and biological functions (such as reproduction) according to environmental conditions and feed availability.

Measuring sea urchin gonads

The size of a sea urchin gonad is measured as Gonad Index (or GI). This is simply the percentage of the total body weight of the urchin that is made up by the gonad. To accurately assess GI of an individual urchin the whole urchin needs to be weighed (total wet weight of sea urchin), the gonads then need to be removed and cleaned and then also weighed (wet weight of gonad) (see following section for details of how to take sea urchin samples). The GI can then be calculated using the following formulae:

GI (%) = Wet weight of gonad (g) /
Total wet weight of sea urchin (g) x 100

Factors that affect sea urchin gonads

The GI of urchins in the wild can vary hugely and can be less than 1%, or, as high as 20% (see Figure 2) whilst for cultured sea urchins GI values can be as high as 35%. Factors that affect GI are feed availability, environmental conditions (e.g. daylight period, water temperature and presence/absence of water currents) and the reproductive cycle of the urchin. The latter also has a significant impact on the quality of the sea urchin gonad and this is discussed in more detail in the 'Reproductive cycle' section. Because of the natural variation in urchin GI, large differences can occur both between individuals within a population and between urchin populations that are very close to one another. Therefore it is extremely difficult to accurately predict the size and quality of sea urchins gonads from any given population before the sea urchin is opened and the GI is calculated.

Figure 1: *The anatomy of the sea urchin Strongylocentrotus droebachiensis.*

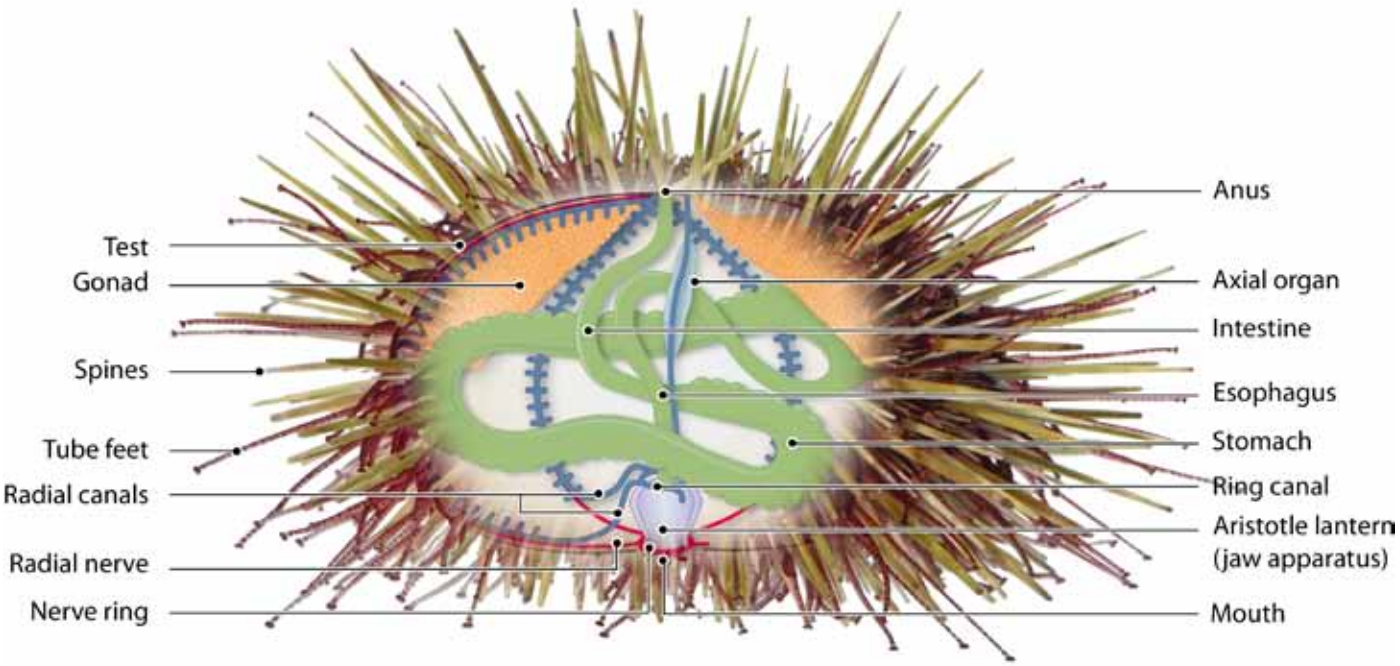


PHOTO: VIDAR MORTENSEN / ART: BY ODDVAR DAHL

Sampling sea urchins

It is not possible to accurately calculate the GI of a sea urchin without destructively removing and weighing the gonads from the urchin. Similarly, it is not possible to estimate the reproductive stage of a sea urchin without removing the gonad and taking a sample. The process for taking and examining a gonad sample is described in Figure 3 and 4. It consists of removing a thin (2-3mm) slice of the roe from the middle section of a single roe (each sea urchin has 5 roe) with a sharp clean scalpel after the urchin has been measured and opened. Once the sample has been taken it needs to be fixed and stained in order to be able to determine the sex, and the reproductive stage. The method for doing this is to place the slice into a histology labelled cassette which should then be placed in 5-10 % buffered formalin. Ideally the sample(s) should then be kept refrigerated until they can be sent to an institute with the facilities to fix and stain the samples (e.g. the Veterinærinstituttet in Harstad). The fixing and staining process involves the sample being fixed into a wax block which is thinly sliced, mounted on a glass slide and then stained (to make the various cells show up as different colours). The result is a histology slide which can then be examined under a microscope.

(NOTE: when female sea urchins are full of developed eggs it is also possible to see the eggs when a smear is taken from the gonad and examined under a microscope. However, it is not possible to accurately assess the reproductive stage of male or female urchins using this technique.)

Figure 2: *Examples of sea urchins with very high GI (left) and very low GI (right)*

Figure 3: *The process for sampling the roe of a sea urchin and preparing a histology sample: (A and B) the equipment required includes a sea urchin opener, a spoon, a scalpel; a histology cassette and a plastic container with 5-10% formalin solution; (C) weighing the whole live sea urchin; (D) measuring the test diameter of the urchin; (E) opening the urchin; (F) removing all 5 gonads; (G) weighing the 5 gonads; (H) removing a small section from the middle of the gonad; (I) placing sample into histology cassette; (J) labelling the plastic container and histology cassette; and (K) placing the sample in formalin solution.*

A

B

C

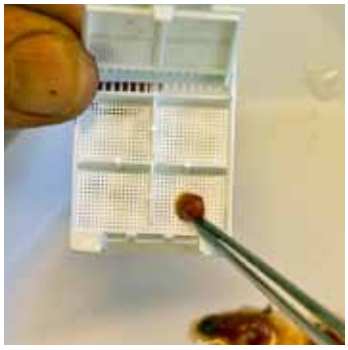
D

E

F

G

H



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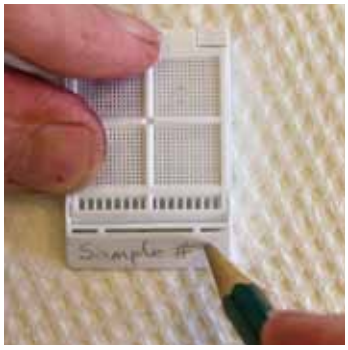


J

K

Figure 4: *Examination of a histology slide using a microscope*

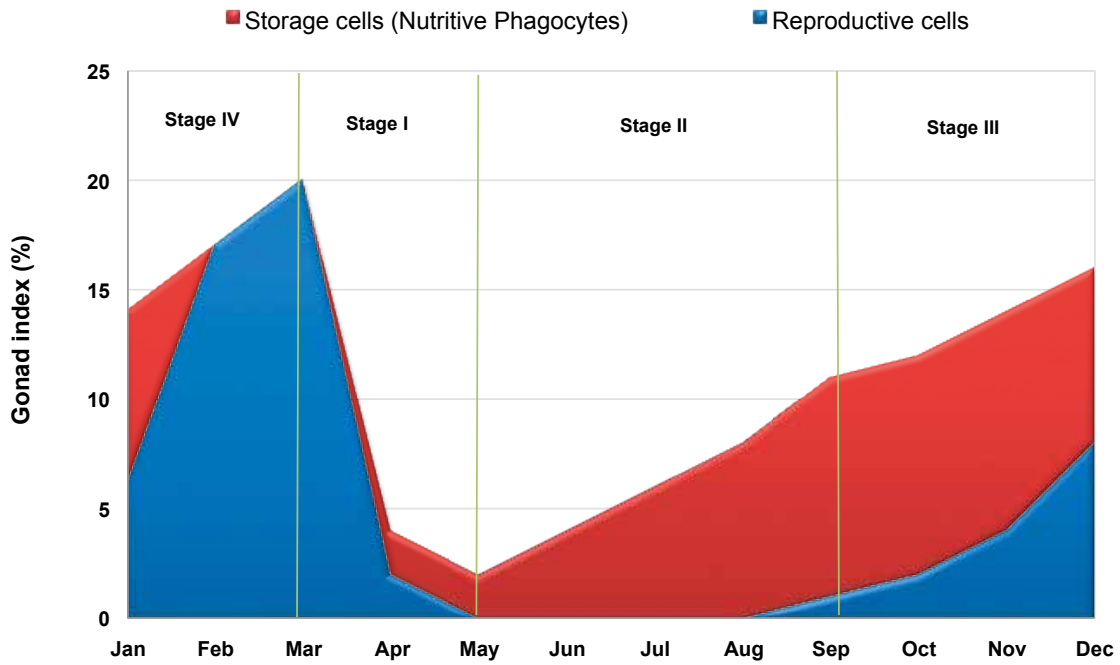
The reproductive cycle of sea urchins

Understanding the reproductive cycle of sea urchins is important to any potential sea urchin fishery in Norway as the gonad is the only part of the urchin that has any commercial value and the reproductive cycle affects both the size and the quality of the gonad and subsequently its value. Anybody involved in the aquaculture of sea urchins also need to understand both the reproductive stage of individuals and the reproductive cycles of populations in any given area where they are operating.

Description of sea urchin reproductive cycle

Adult sea urchins are either male or female, with a normal sex ratio of 1:1, they both normally spawn once per year and release their gametes (eggs or sperm) into the water column (this is called broadcast spawning) where mixing and fertilisation of the eggs occurs. Normally, in Norway, spawning occurs around April when a sharp drop in the size of the gonads occurs. Following spawning in spring/early summer the urchins go through a dormant stage when the gonad is generally small and in poor condition. In late summer the gonads slowly increase in size as it produces storage cells which increase in both size and number. In early to mid winter gametogenesis (the formation of reproductive cells) occurs and the number of storage cells in the gonad reduces and these are replaced with reproductive cells. The cues that stimulate gametogenesis are not fully understood but the primary cue is believed to be changing photoperiod. The number of reproductive cells within the gonad builds up over winter until the urchin is once again in spawning condition in late winter/Spring. The cues that trigger spawning are also unclear but the primary cues are believed to be temperature and environmental factors such as algal blooms and storms. Figure 5 shows a typical reproductive cycle in a sea urchin, with large changes in the size of the gonad (the GI) and also in the cellular composition of the gonad throughout the year. However, the reproductive cycle can vary widely between geographic locations and even within relatively limited areas and this is discussed further in the following section.

Figure 5: *A typical sea urchin (Strongylocentrotus droebachiensis) reproductive cycle showing the pre-spawning peak in the GI (February/March) followed by a rapid post-spawning decline and then a gradual rebuilding of the GI. The changes in the ratio of the storage cells (NP) and the reproductive cells are also shown. Note this cycle can vary significantly between and within sea urchin populations.*



The gonad is the only organ in sea urchins that is capable of storing nutrients and so it is both the primary organ for reproduction as well as for nutrient storage (the gut has a very limited storage capacity). This means that sea urchin reproduction and nutrient storage are very closely linked. The gonad of the sea urchin are made up of two types of cells: reproductive cells and storage cells (known as nutritive phagocytes or NP cells) (Figure 6). The reproductive cells are the eggs (oogonium, oocyte and ovum) in females and the sperm (spermatogonium, spermatocyte, spermatid and spermatozoon) in males. Nutritive phagocytes are cells that store the nutrients, such as proteins, carbohydrates and lipids that are used for gamete development (known as gametogenesis) or, for basic metabolic activity when feed availability is very low. Nutritive phagocytes also have the ability to absorb (phagocytose) unused reproductive cells after spawning has occurred. The percentage of the two types of cells present in the gonad varies throughout the reproductive cycle and has a significant effect on both the size and quality of the gonad (See Figure 5).

Variation in reproductive cycle between individual and populations of sea urchins

Apart from the natural reproductive cycle of a sea urchin, both food availability and urchin density have also been shown to influence the reproductive cycles of wild urchins. When food is limited, the size of the gonad decreases and when food is abundant the gonads are generally larger. Limited feed availability has a negative effect on the reproductive cycle and when the food supply is very low (e.g. as can occur in extensive sea urchin barrens) the roe of the urchin may be too small to produce any reproductive cells (Figure 2). Environmental conditions such as seasonal variation in seawater temperature can also impact on the reproductive cycle of sea urchins resulting in enormous variations between populations and even individuals within a population. A recent study of the GI of two populations, both situated in kvalsund, close to Tromsø that are only 0.5 km distance apart clearly shows both seasonal variation and variations between the two populations, despite their close proximity (Figure 7).

Figure 6: *The two types of cells present in the gonads of female (A) and male (B) sea urchins: Reproductive cells and storage cells (nutritive phagocytes)*

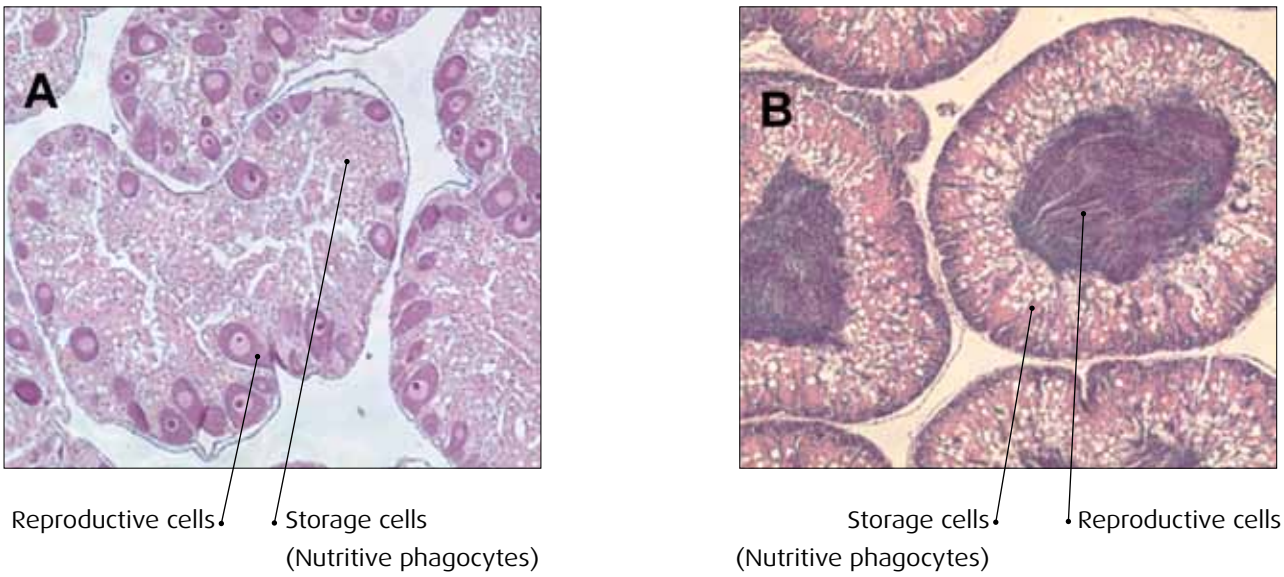
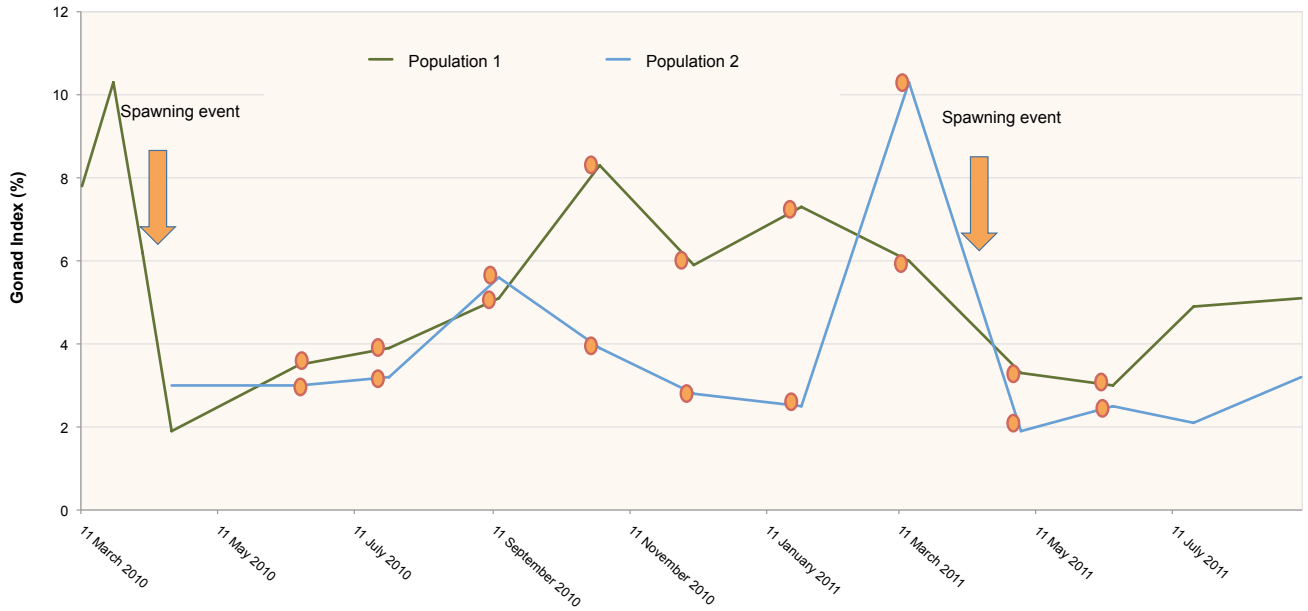


Figure 7: *The changes in GI of two populations situated 0.5 km from one another in Kvalsund near Tromsø. Note how different the GI values are between the populations despite their close proximity (the red dots indicate when the samples were taken from these two populations that are shown in Appendix 1).*



The four stages in the reproductive cycle of *Strongylocentrotus droebachiensis*

The development of the reproductive cycle in sea urchins has been has been divided into four stages by Walker *et al.* (2007). This classification is now widely around the world when describing the reproductive stage of sea urchins from a wide variety of species and has been used in this guide. The four stages are as follows:

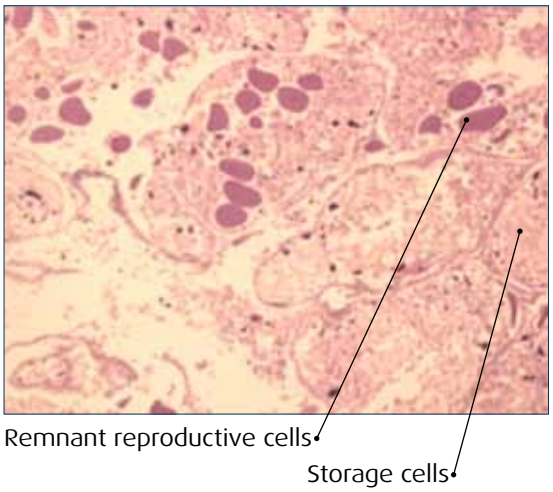
Stage I: Post spawning (Inter-gametogenesis and NP phagocytosis)

This stage occurs for approximately 3 months in spring _after spawning has occurred. Residual reproductive cells are present in the female gonads but otherwise the gonads look empty and have a 'messy' appearance with little structure. Towards the end of this stage the number of NP cells increases and reproductive cells begin to appear around the periphery of the gonads.

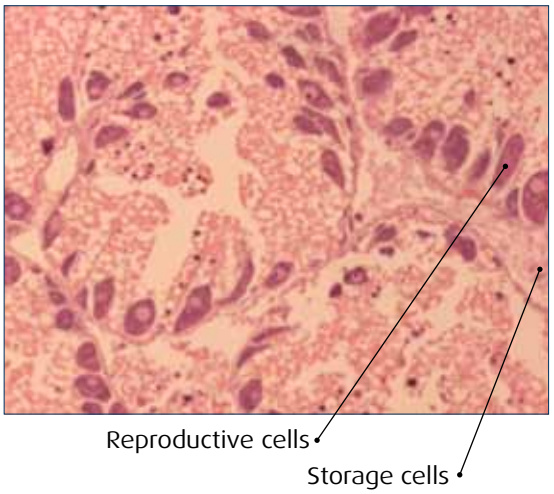
Stage II: NP growth (Pre-gametogenesis and NP renewal)

This stage occurs for approximately 3-4 months during summer. Reproductive cells continue to appear around the periphery of the gonads and start to increase in size as well as in number. Towards the end of this stage there is a substantial increase in the number and size of NP cells.

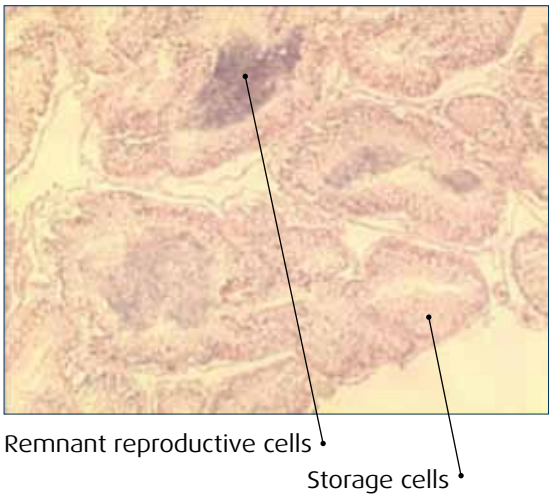
Stage I: Female



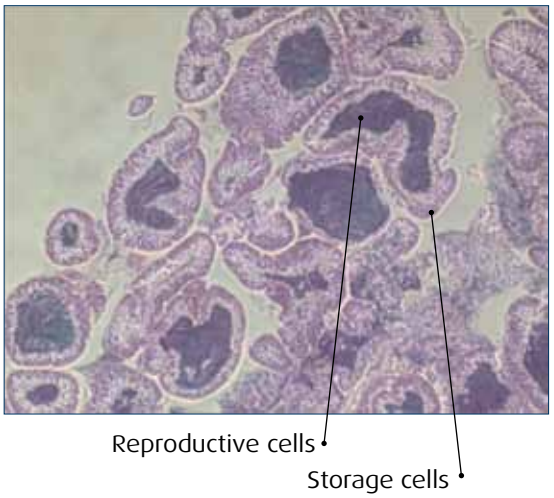
Stage II: Female



Stage I: Male



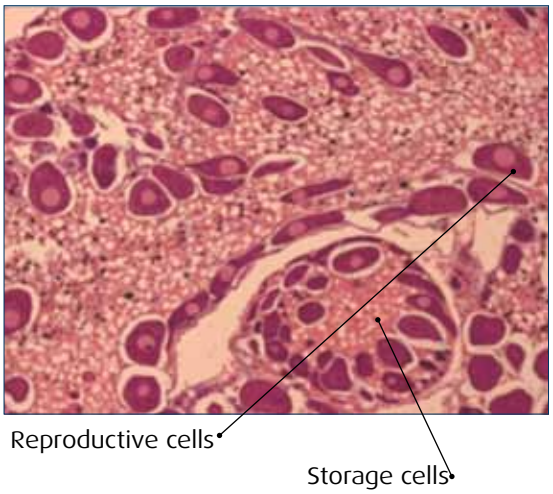
Stage II: Male



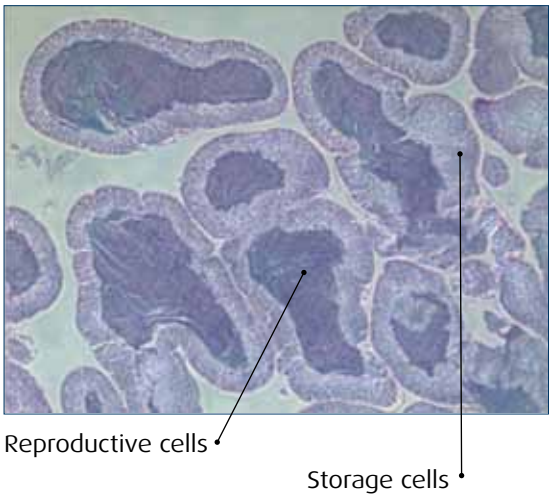
Stage III: Development of reproductive cells (Gametogenesis and NP utlilisation)

This stage occurs in early winter and lasts for approximately 5 months and overlaps with the previous stage for some time. The reproductive cells continue to develop and begin to migrate into the centre of the gonad. As the number and size of reproductive cells increases the number and size of NP cells decreases simultaneously.

Stage III: Female



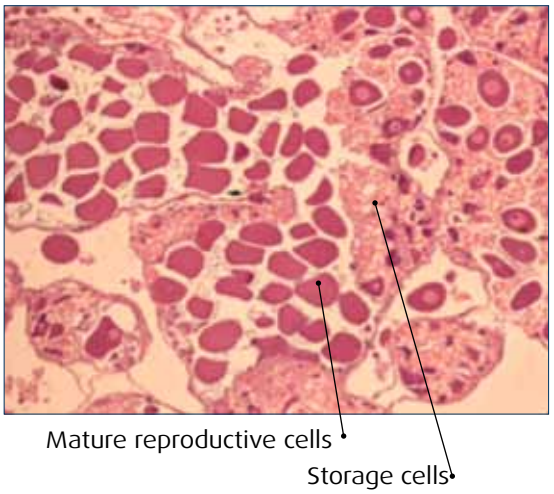
Stage III: Male



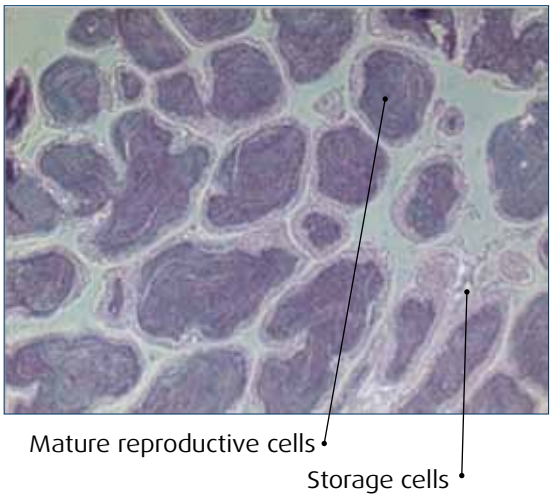
Stage IV: Spawning and pre-spawning (End of gametogenesis, NP exhaustion and spawning)

This stage lasts for approximately 2-3 months and occurs in late winter. The middle of the gonads (the lumen) is packed with fully developed (differentiated) reproductive cells (gametes) stored and ready for spawning. The NP cells are exhausted and are substantially reduced in number and size and may be completely absent. Towards the end of this stage spawning will occur when all or some of the reproductive cells will be released from the gonad.

Stage IV: Female



Stage IV: Male

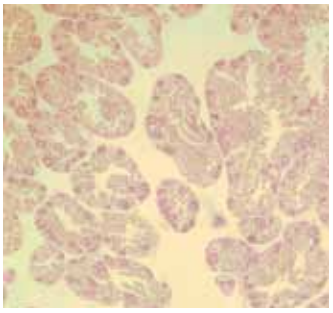


Appendix 1

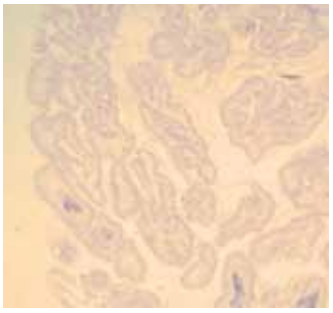
Examples of *Strongylocentrotus droebachiensis* histology from two populations in Kvalsund, near Tromsø (the magnification of the image is in brackets).

Population One

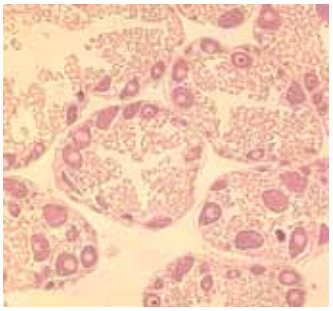
Sample 1: 16 June 2010



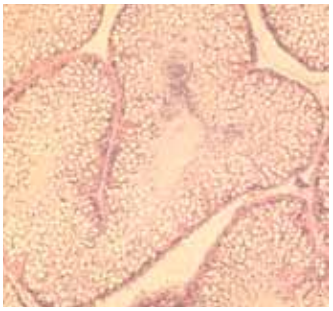
Female (x 4) Stage II



Male (x 4) Stage I

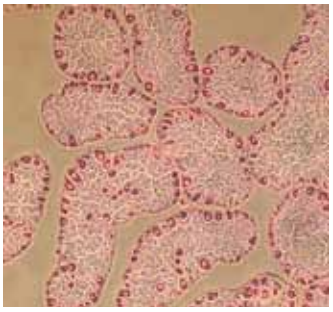


Female (x 10) Stage II

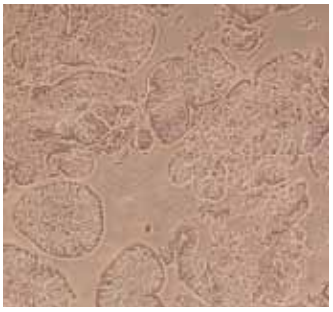


Male (x 10) Stage I

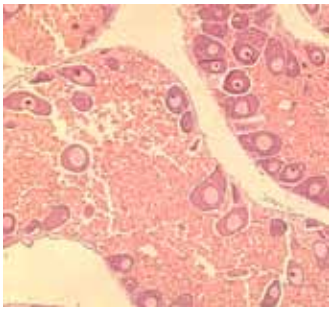
Sample 2: 26 July 2010



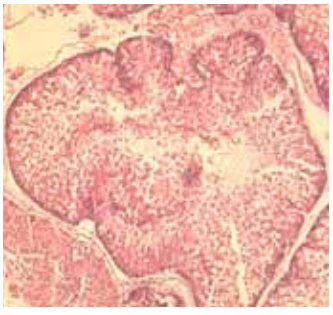
Female (x 4) Stage II



Male (x 4) Stage II



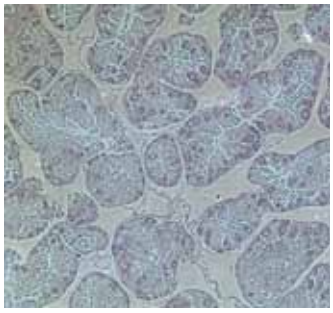
Female (x 10) Stage II



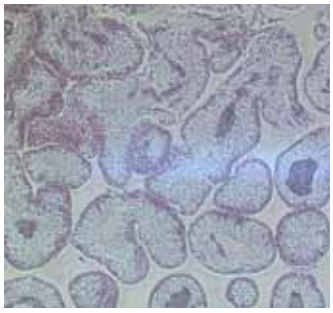
Male (x 10) Stage II

Population Two

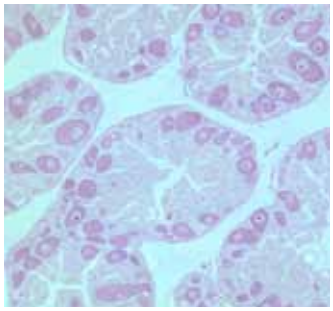
Sample 1: 16 June 2010



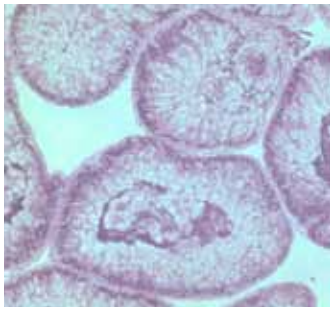
Female (x 4) Stage II



Male (x 4) Stage II

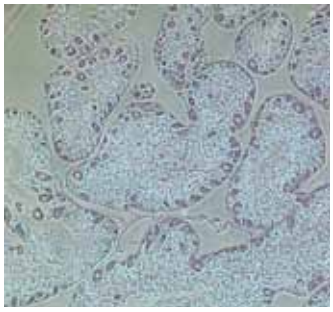


Female (x 10) Stage II

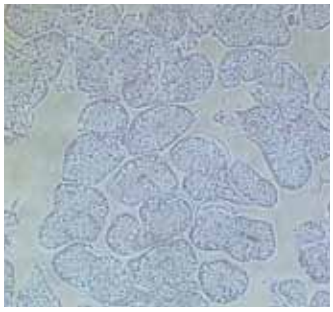


Male (x 10) Stage II

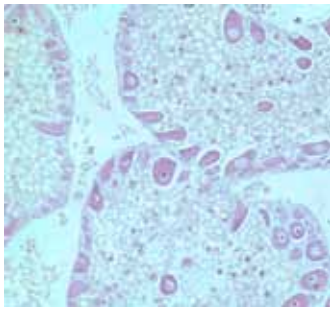
Sample 2: 26 July 2010



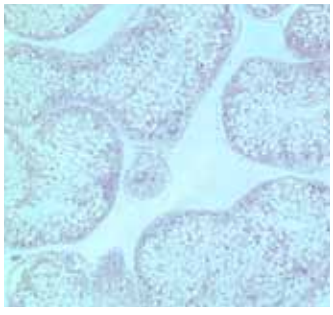
Female (x 4) Stage II



Male (x 4) Stage II



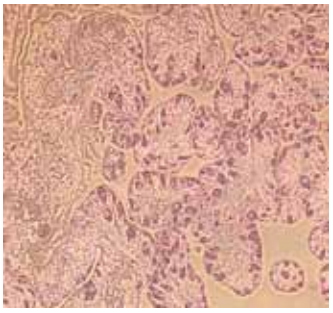
Female (x 10) Stage II



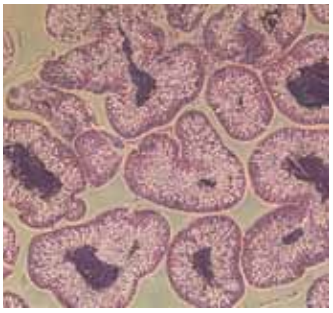
Male (x 10) Stage II

Population One

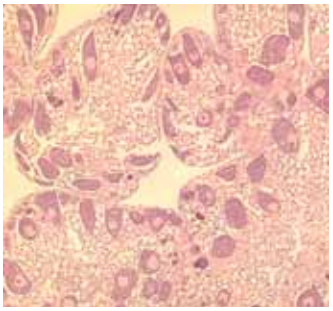
Sample 3: 13 September 2010



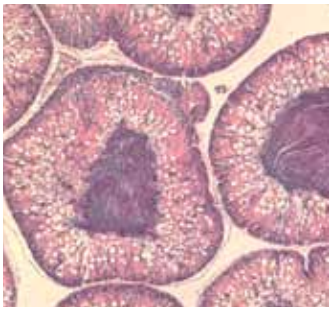
Female (x 4) Stage III



Male (x 4) Stage III

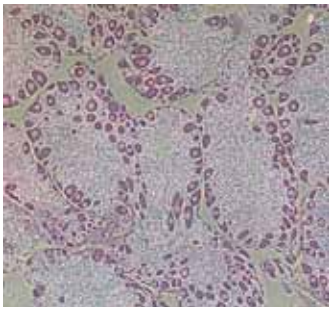


Female (x 10) Stage III

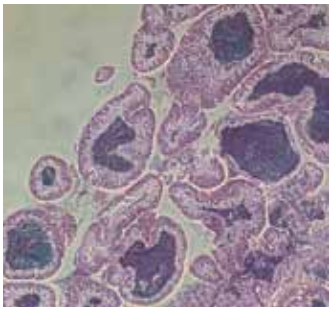


Male (x 10) Stage III

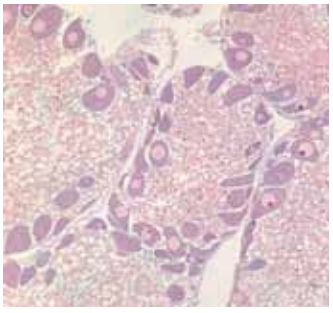
Sample 4: 28 October 2010



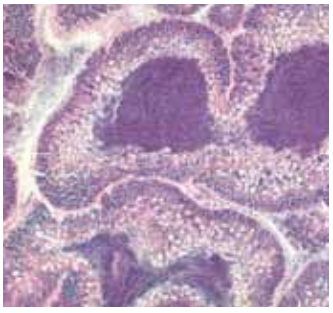
Female (x 4) Stage III



Male (x 4) Stage III



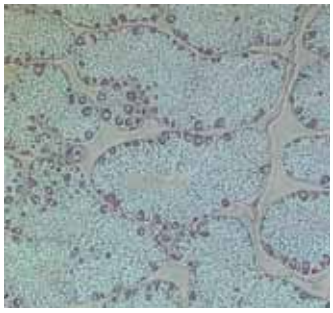
Female (x 10) Stage III



Male (x 10) Stage III

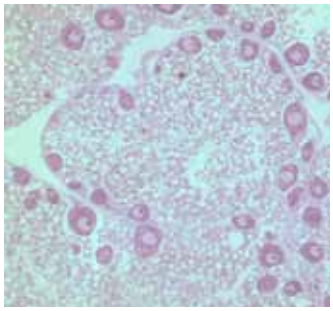
Population Two

Sample 3: 13 September 2010

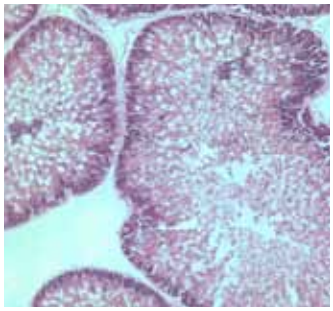


Female (x 4) Stage II

Male (x 4) Stage II

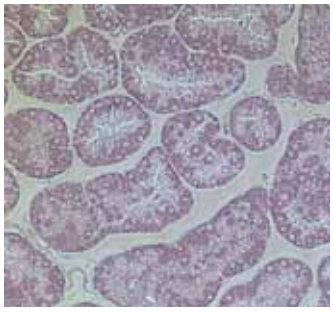


Female (x 10) Stage II

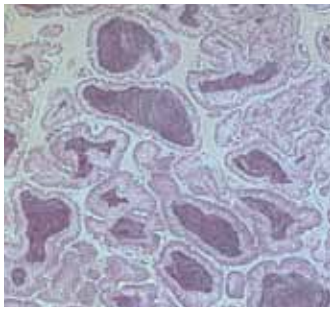


Male (x 10) Stage II

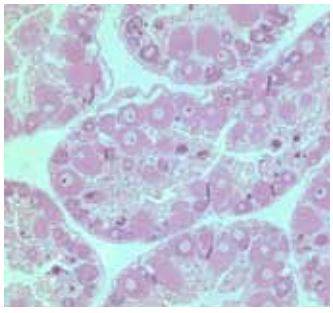
Sample 4: 28 October 2010



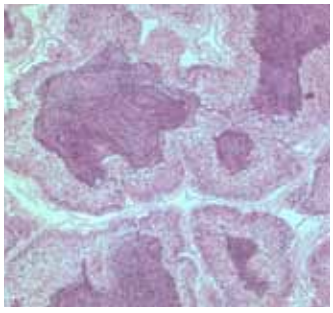
Female (x 4) Stage III



Male (x 4) Stage III



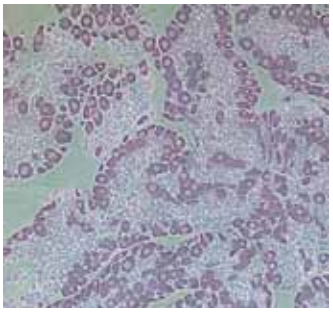
Female (x 10) Stage III



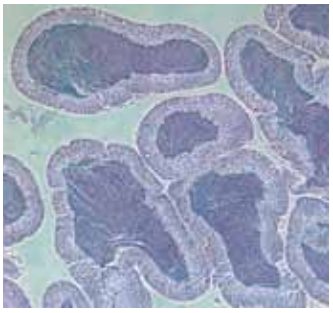
Male (x 10) Stage III

Population One

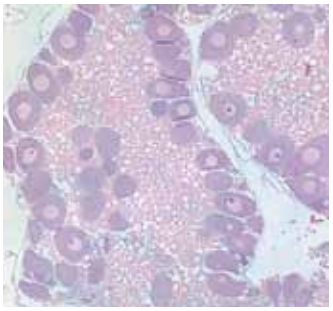
Sample 5: 9 December 2010



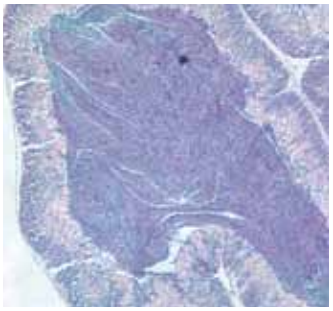
Female (x 4) Stage III



Male (x 4) Stage IV

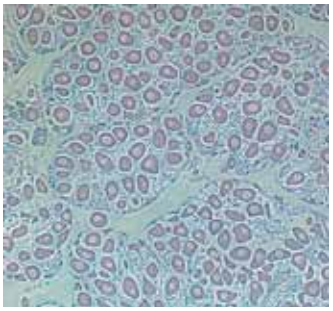


Female (x 10) Stage III

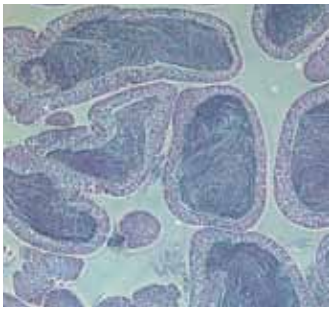


Male (x 10) Stage IV

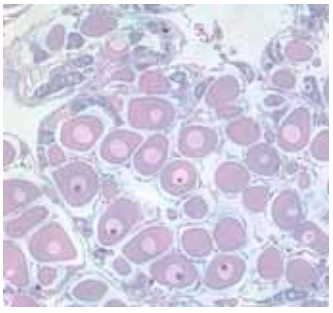
Sample 6: 26 January 2011



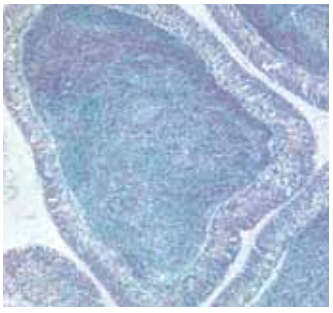
Female (x 4) Stage IV



Male (x 4) Stage IV



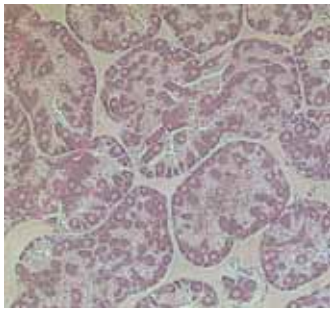
Female (x 10) Stage IV



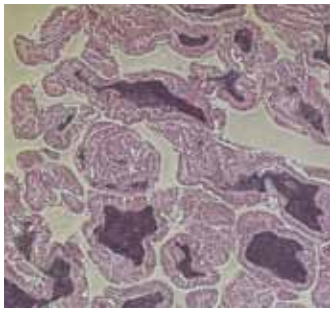
Male (x 10) Stage IV

Population Two

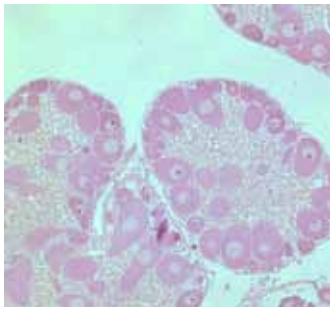
Sample 5: 9 December 2010



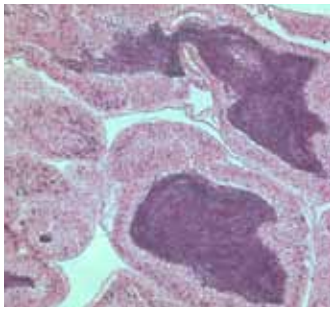
Female (x 4) Stage III



Male (x 4) Stage III

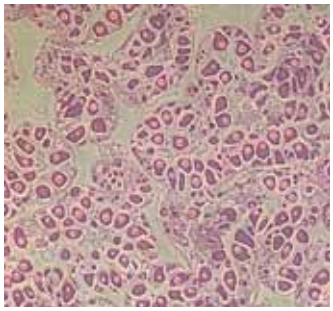


Female (x 10) Stage III

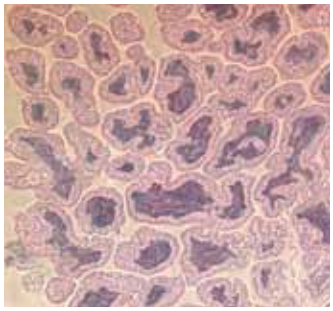


Male (x 10) Stage III

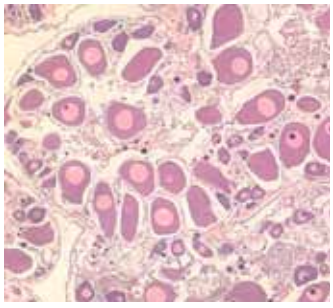
Sample 6: 26 January 2011



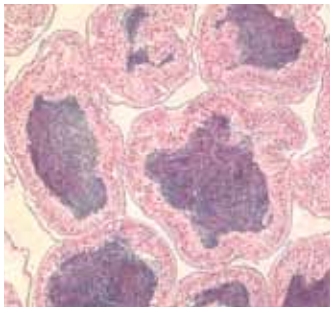
Female (x 4) Stage III/IV



Male (x 4) Stage III/IV



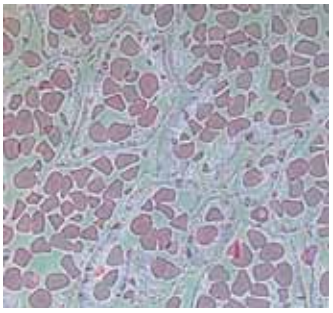
Female (x 10) Stage III/IV



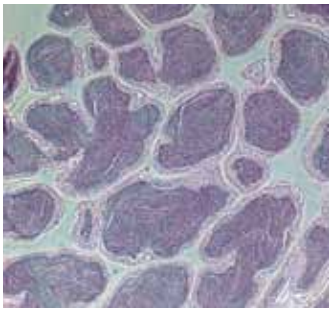
Male (x 10) Stage III/IV

Population One

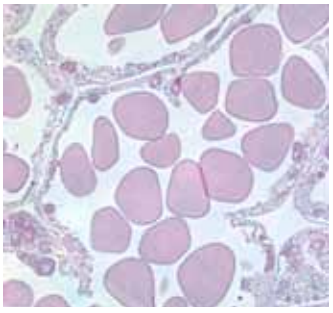
Sample 7: 15 March 2011



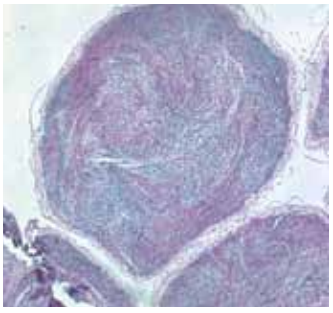
Female (x 4) Stage IV



Male (x 4) Stage IV

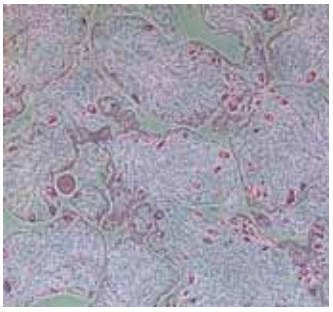


Female (x 10) Stage IV

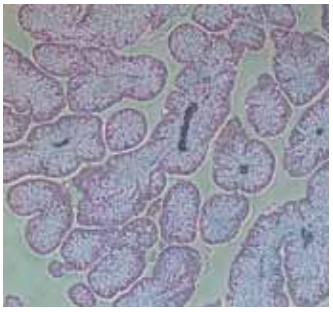


Male (x 10) Stage IV

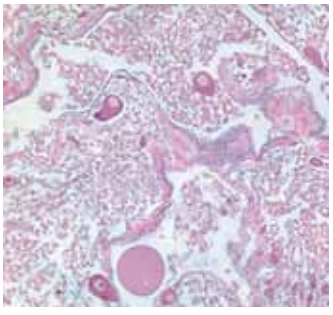
Sample 8: 4 May 2011



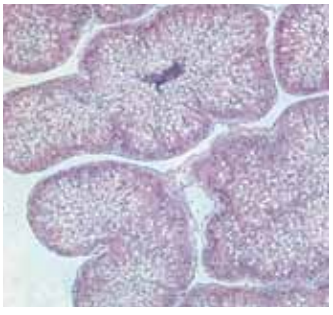
Female (x 4) Stage I



Male (x 4) Stage I



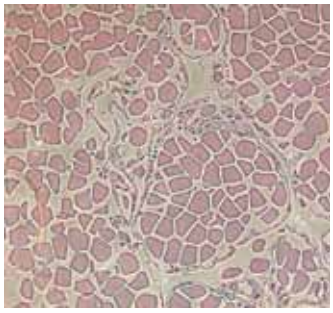
Female (x 10) Stage I



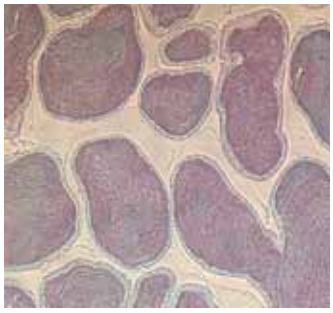
Male (x 10) Stage I

Population Two

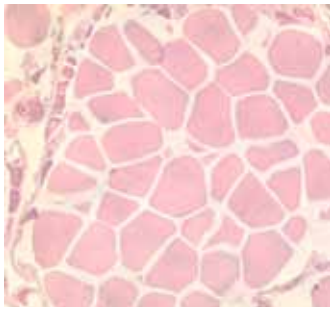
Sample 7: 15 March 2011



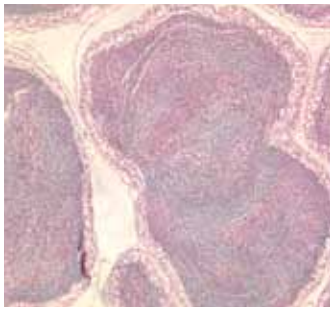
Female (x 4) Stage IV



Male (x 4) Stage IV

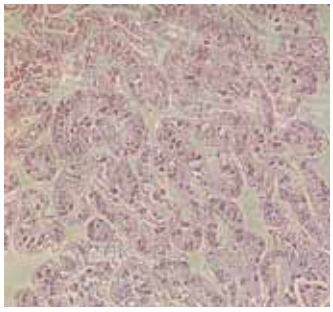


Female (x 10) Stage IV

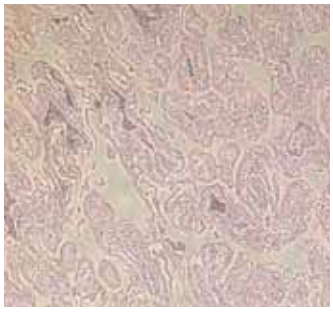


Male (x 10) Stage IV

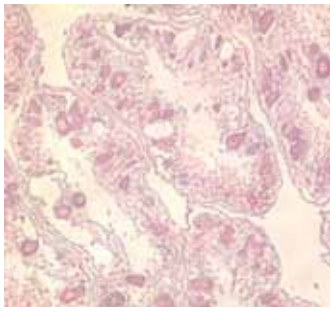
Sample 8: 4 May 2011



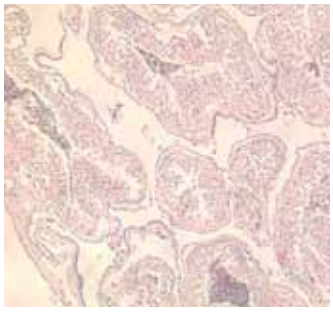
Female (x 4) Stage I



Male (x 4) Stage I



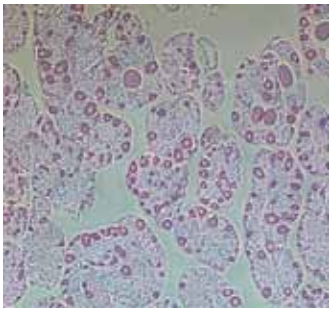
Female (x 10) Stage I



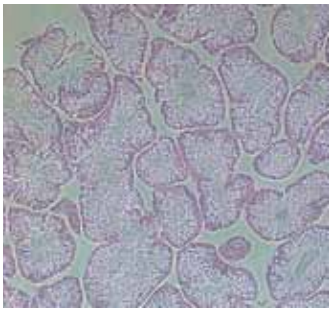
Male (x 10) Stage I

Population One

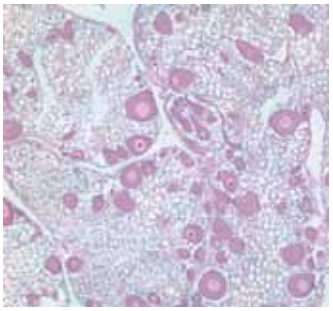
Sample 9: 14 June 2011



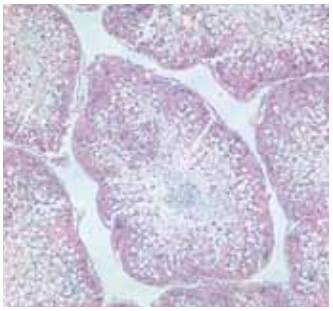
Female (x 4) Stage I/II



Male (x 4) Stage I



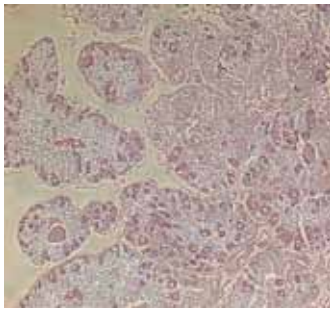
Female (x 10) Stage I/II



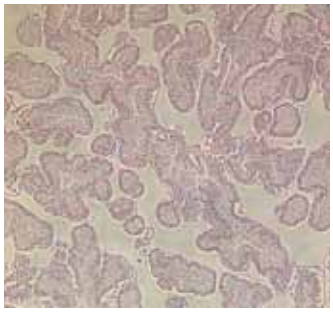
Male (x 10) Stage I

Population Two

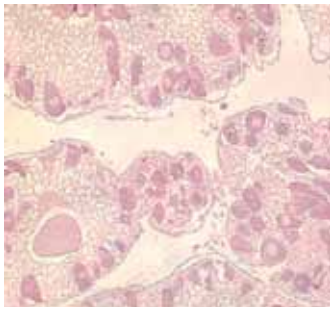
Sample 9: 14 June 2011



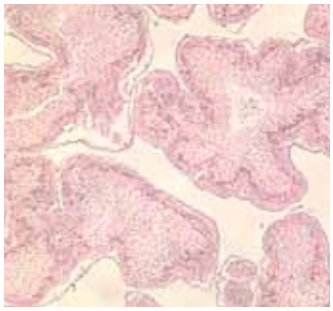
Female (x 4) Stage II



Male (x 4) Stage II



Female (x 10) Stage II



Male (x 10) Stage II

For your own notes:

Nofima is Europe's largest institute for applied research within the fields of fisheries, aquaculture and food. The institute has around 420 employees and has an annual turnover of about NOK 500 million. Nofima carries out research and develop solutions that provide a competitive edge throughout the value chain. The main office is located in Tromsø, and the research divisions are located in Averøy, Bergen, Sunndalsøra, Stavanger, Tromsø and Ås.

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The Norwegian Seafood Research Fund (FHF) is a funding scheme for industrial research and development work within fisheries and aquaculture, and is based on a levy of 0,3 percent on all exported fish and fish products. The funds shall be used for industrial R&D work for the benefit of all or part of the industry, and are distributed in the form of grants for research programmes and major projects.

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