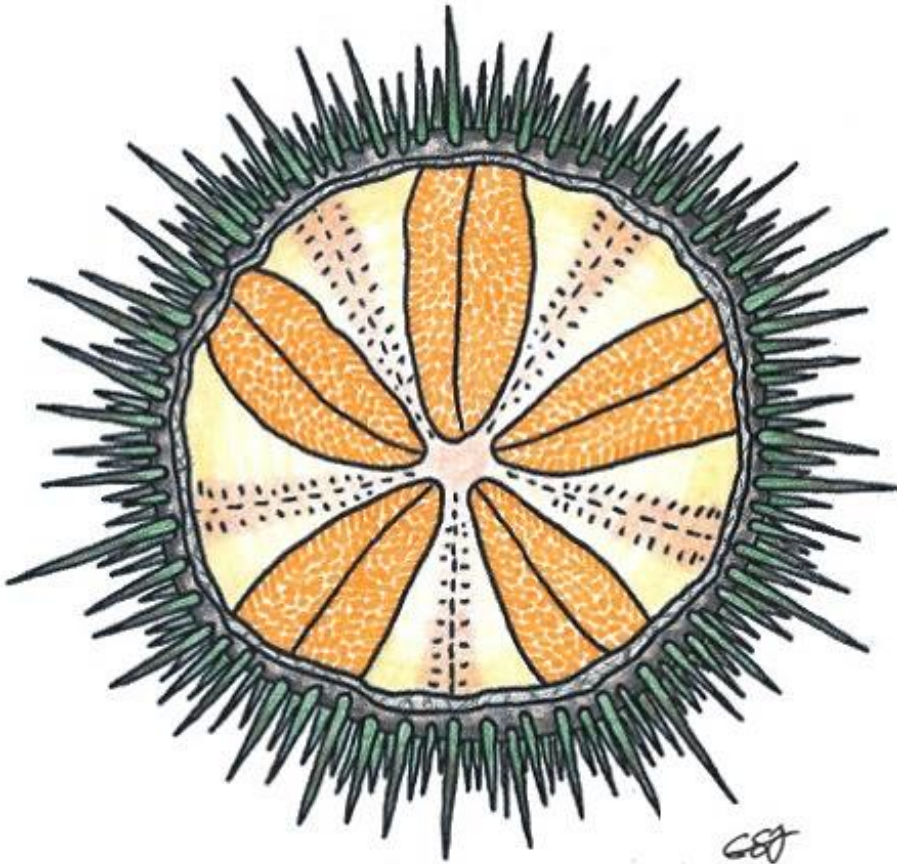


A Guide to the Sea Urchin
Reproductive Cycle and Staging
Sea Urchin Gonad Samples:
(Second Edition)



2018

By Philip James, Sten Siikavuopio and
Gunhild Seljehaug Johansson

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 I show examples of histology slides taken from the two wild populations as well as histology slides from an enhanced captive sample. These clearly show the general pattern associated with the reproductive cycle of sea urchins but also the enormous variation that can occur between populations and treatments.

This revised version of the original document has been improved and updated with scanning electron microscopy images as well as histology images of enhanced sea urchin roe. It is 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 to the knowledge of the reproductive cycle of sea urchins that occur in various populations around the coast of Norway and elsewhere around the world.

Cover illustration: Gunhild S. Johansson

Acknowledgements

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

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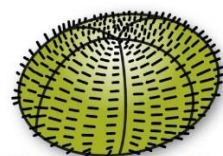
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URCHIN
Utilising the Arctic Urchin Resource

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, blood vessels or, 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) (Figure 1). 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. Because of this, both the size and the quality of the gonad and the rhythm of the reproductive cycle are highly variable between individuals and between populations of sea urchins.

Measuring sea urchin gonads

The size of a sea urchin gonad is measured as Gonad Index (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, cleaned and then weighed (Wet weight of gonad). The GI can then be calculated using the following formulae:

$$\text{GI (\%)} = \frac{\text{Wet weight of gonad (g)}}{\text{Total wet weight of sea urchin gonad (g)}} \times 100$$

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%, whilst for cultured sea urchins GI values can be as high as 35% (Figure 2). Factors that affect GI are feed availability, environmental conditions (e.g. daylight period, water temperature/quality 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.

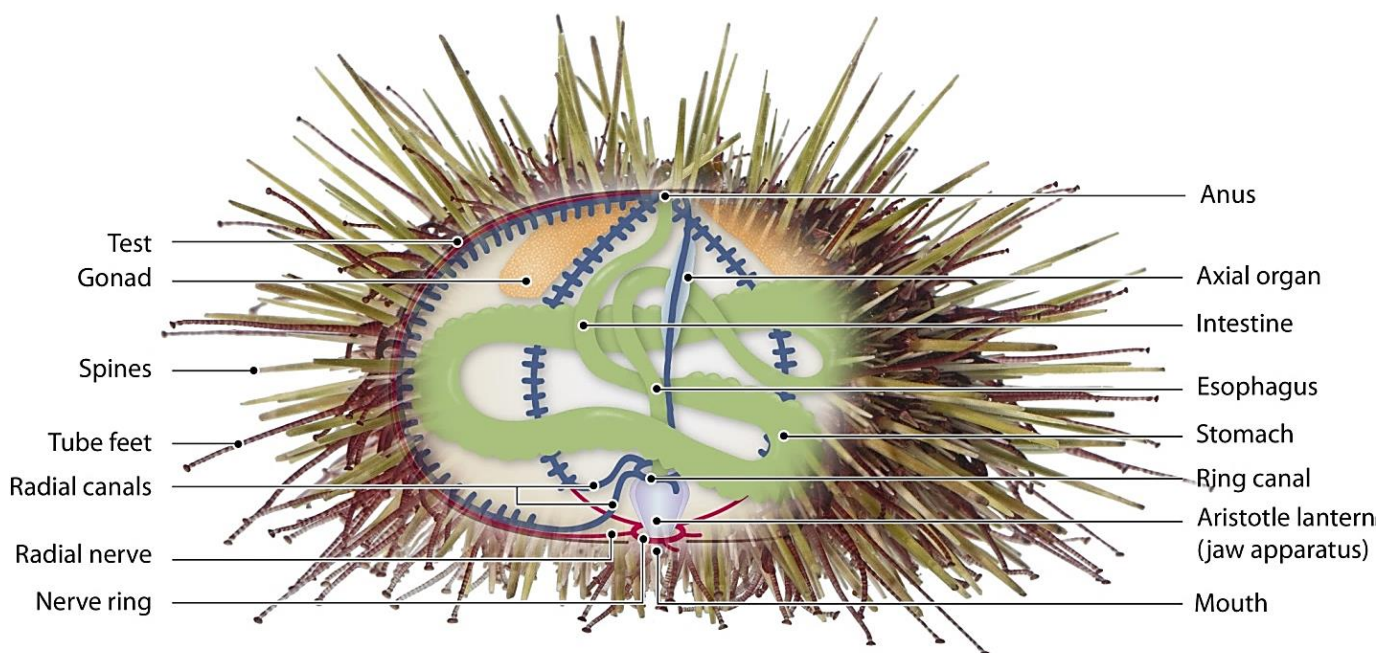


PHOTO: VIDAR MORTENSEN / ART: BY ODDVAR DAHL

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

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 making a histological examination of a portion of the gonad. The process for taking a gonad sample is described in Figure 3 and 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. 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 labelled histology cassette which should then be placed in 10% buffered formalin (Figure 3). Ideally the sample(s) should then be kept refrigerated until

they can be sent to an institute with the facilities (if not available in house) to fix and stain the samples. 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 fully 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).

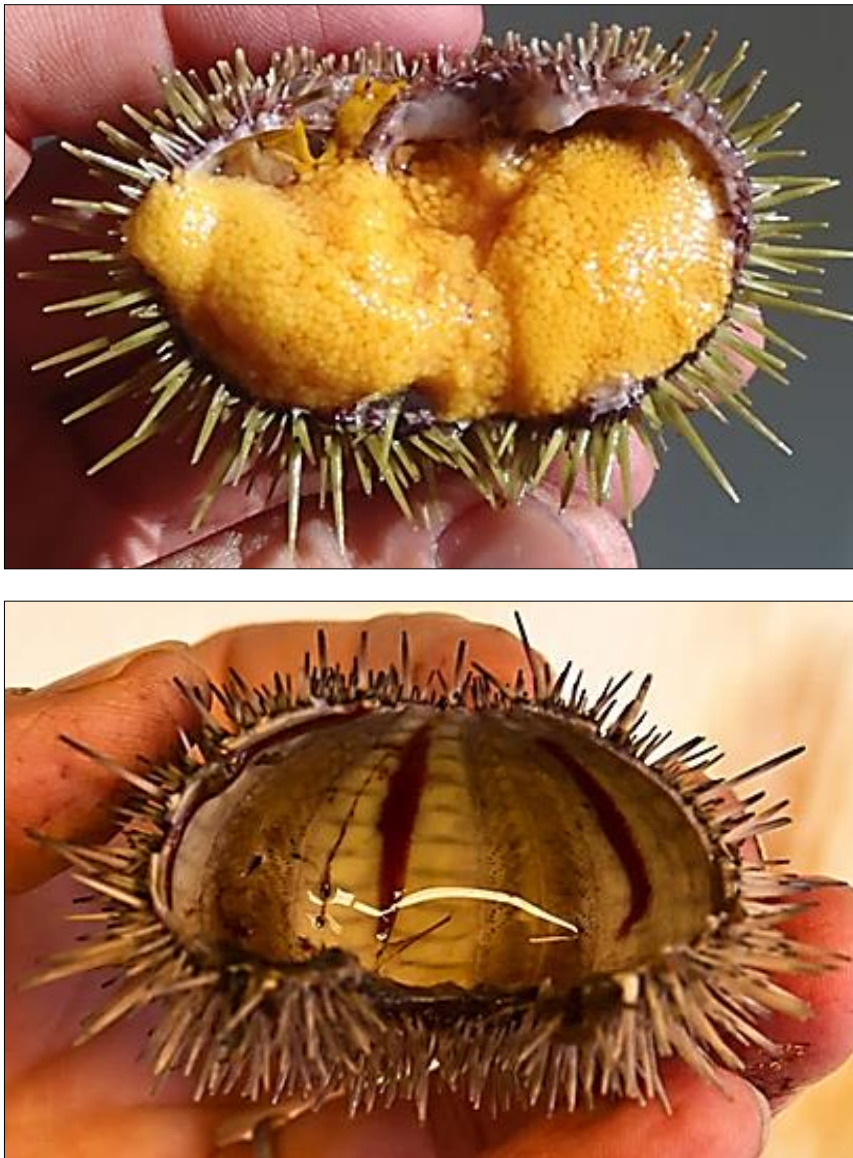


Figure 2. Example of sea urchins (*Strongylocentrotus droebachiensis*) with very high GI (top) and very low GI (bottom)

Sampling sea urchins

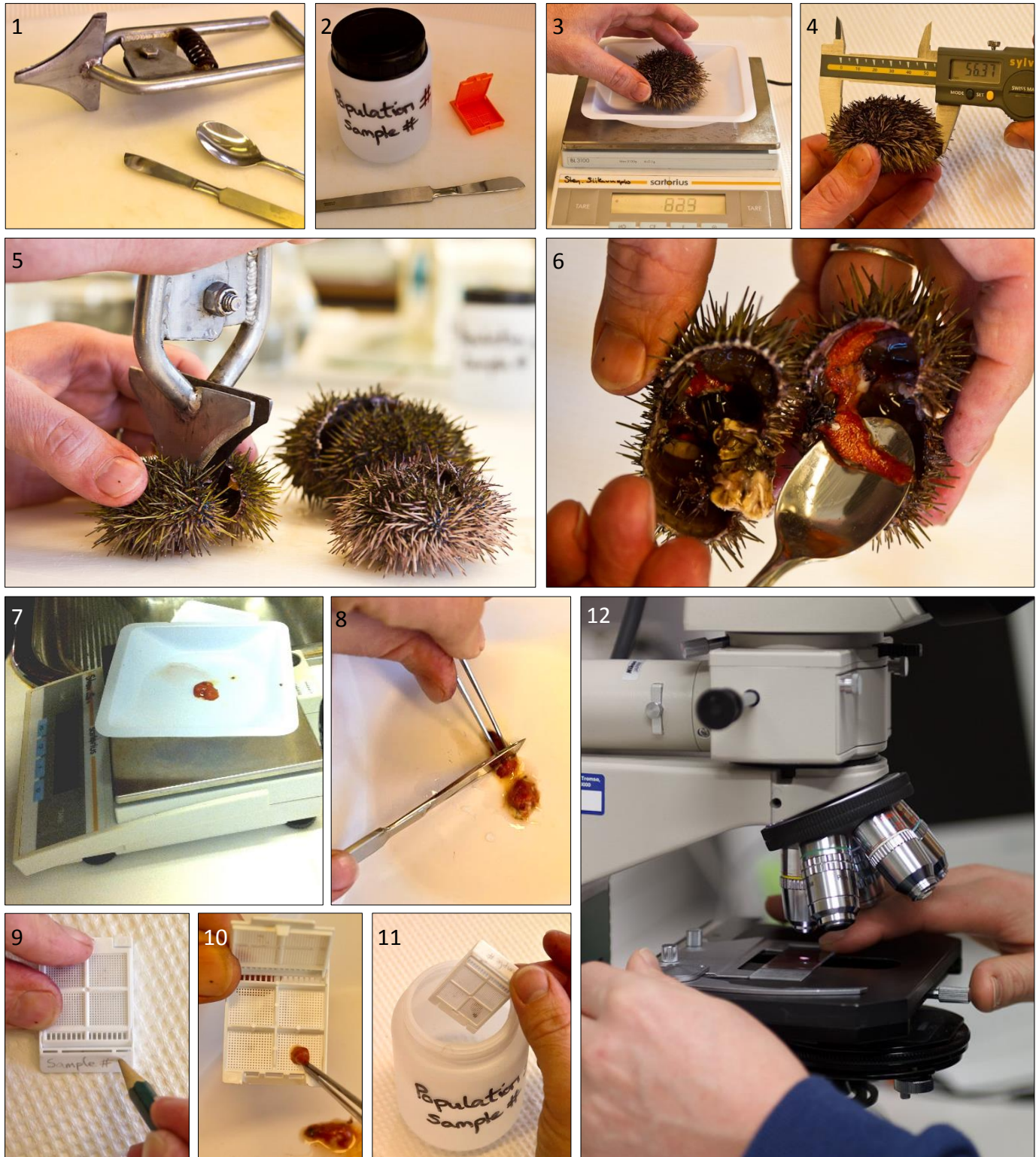


Figure 3. The process for sampling the roe of a sea urchin and preparing a histology sample: (1) the equipment required includes a sea urchin opener, a spoon, a scalpel; (2) a histology cassette and a plastic container with 10% formalin solution; (3) weighing the whole live sea urchin; (4) measuring the test diameter of the urchin; (5) opening the urchin; (6) removing all 5 gonads; (7) weighing the 5 gonads; (8) removing a small section from the middle of the gonad; (9-11) labelling the plastic container and histology cassette and placing the sample in formalin solution; (12) Examination of histology slides using a light microscope.

Understanding the morphology of sea urchin gonads

There are a number of publications, book chapters and references describing the histological morphology of sea urchin gonads. However, the authors believe that there is a paucity of information on the gross morphology (structure) of sea urchin gonads. As an example it is difficult to imagine how it is possible to get the strange shapes in histology slides made from the gonads of sea urchins (Figure 4). By taking a closer look at the shape and structure of the gonad it is easier to imagine why we get the resulting histology. Imagine a series of 'bobbles' (picture a balloon filled with water) that are all joined together to form a whole gonad structure that we see when we look at the gonad. This is a solid structure made up from a series of interconnected 'bobbles' (Figure 5B). When we take a slice through the gonad (as we do when taking a histology sample) then we do not get a solid section. Instead where we cut across the 'bobbles' we get solid histology sections surrounded by gaps.

This is illustrated in Figure 4 and 5. Figure 5 shows scanning electron microscopy (SEM) three dimensional images of how the sea urchin gonad looks when fixed and cut. There are a number of physical differences in the gonads of enhanced sea urchins compared to gonads from wild sea urchins. The images in Figure 5 are from a wild caught urchin and it would be interesting to compare the gross morphology of wild and enhanced gonads.

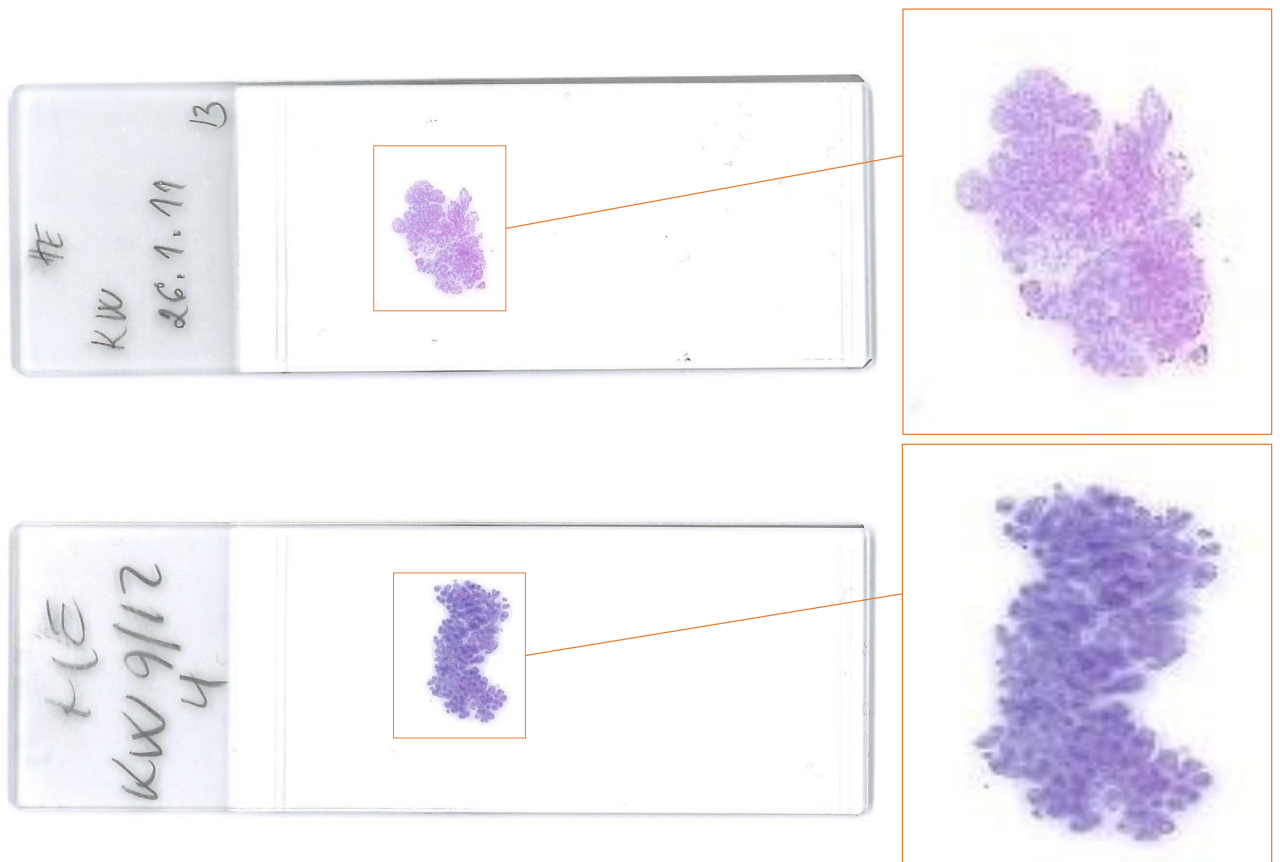


Figure 4. An example of a histology slide resulting from very thin slices taken from the fixed gonad. This slide shows the roe of a female (top) and male (bottom) sea urchins.

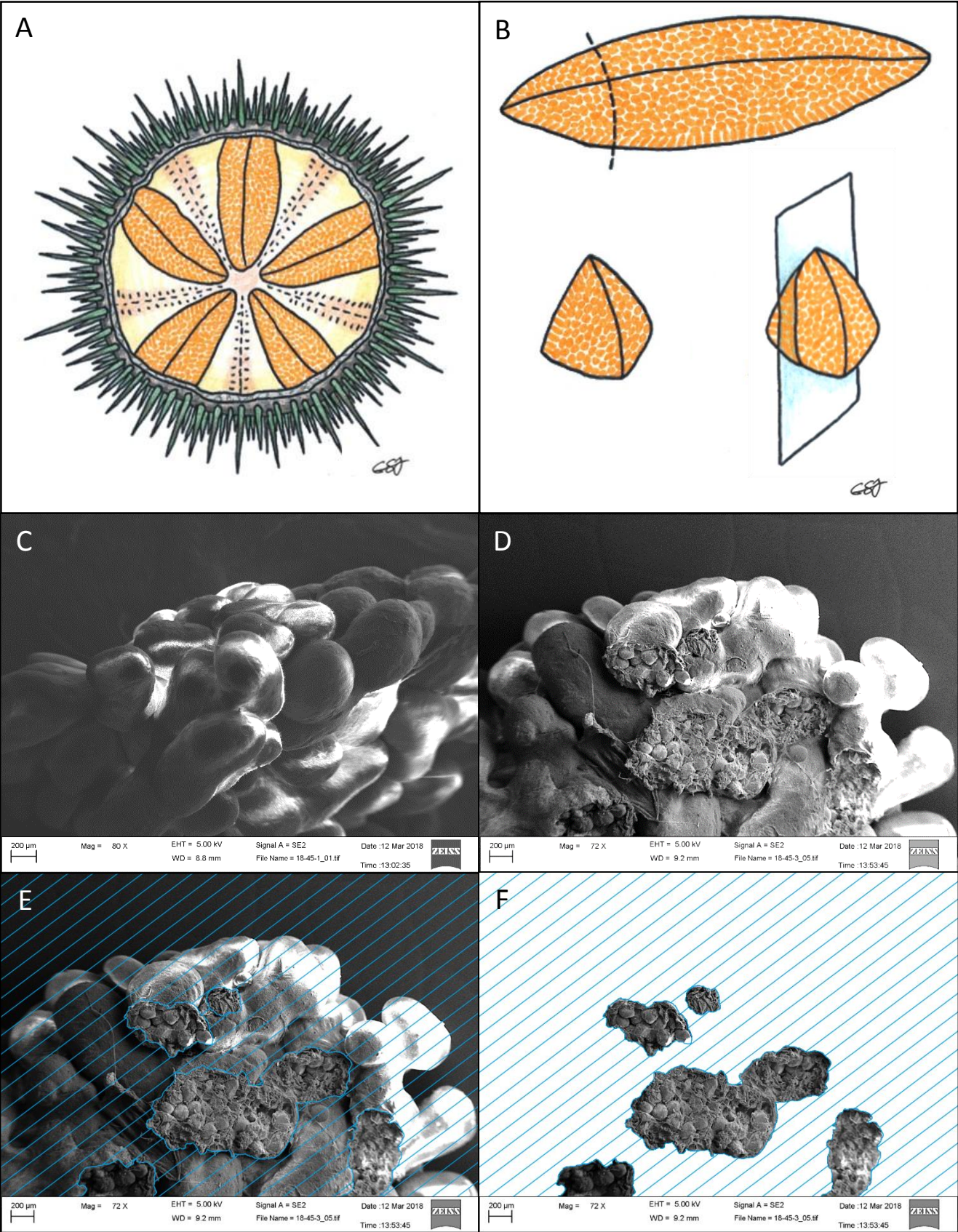


Figure 5. (A) Dorsal (top) view of sea urchin with mouth removed to reveal the 5 gonads. (B) Removal of the gonad followed by removal of the tip and subsequent slicing of the tip. (C) Scanning electron microscopy (SEM) image of the tip of the gonad. (D) SEM of the slice through the tip. (E) The slice through the tip with hatching showing where the histology slide would show details. (F) The resulting view in a histology slide from this preparation (in this case it is from a female with mature eggs).

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 (often referred to as the ‘roe’) 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 (also know as nutritive phagocytes or NP) 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 (Figure 6). 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 6 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.

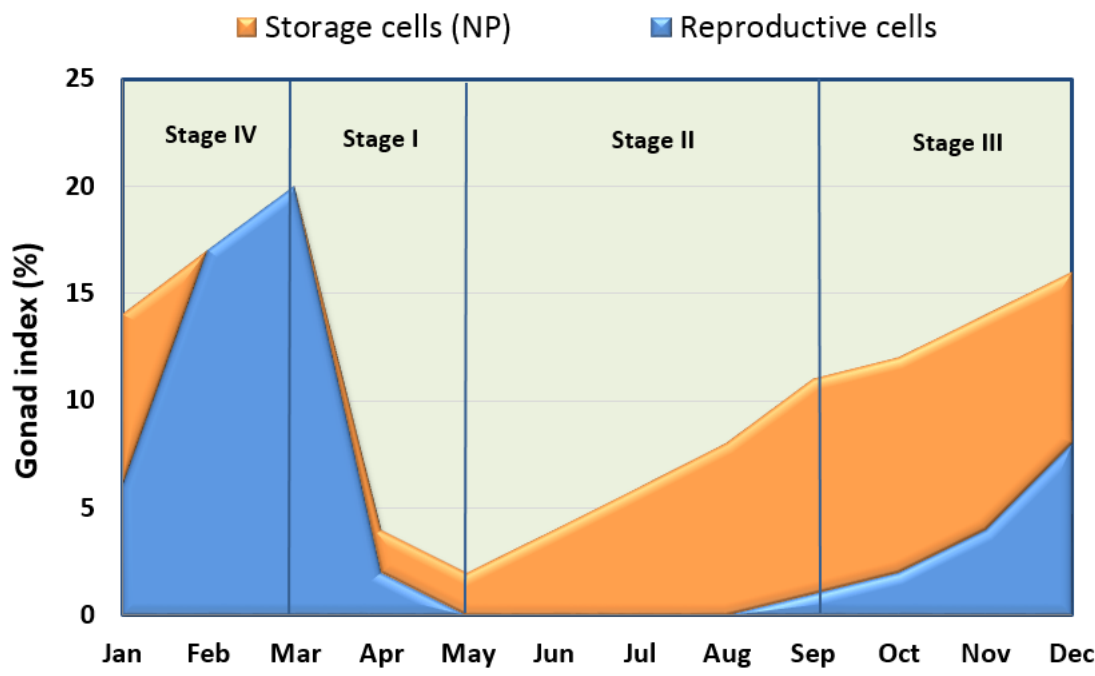


Figure 6. A typical sea urchin (*Strongylocentrotus droebachiensis*) reproductive cycle showing the pre-spawning peak in the GI (February/March) followed by a rapid post-spawning decline and then a gradual rebuilding of the GI. The changes in the ratio of the storage cells (NP) and the reproductive cells 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. 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 (Figure 7). The reproductive cells are the eggs (oogonium, oocyte and ovum) in females and the sperm (spermatogonium, spermatocyte, spermatid and spermatozoon) in males. Storage cells 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. Storage cells 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 (Figure 2 and 6).

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 gonads 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 and light (photoperiod) can also impact on the reproductive cycle of sea urchins resulting in enormous variations between populations and even individuals within a population. A 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 8).

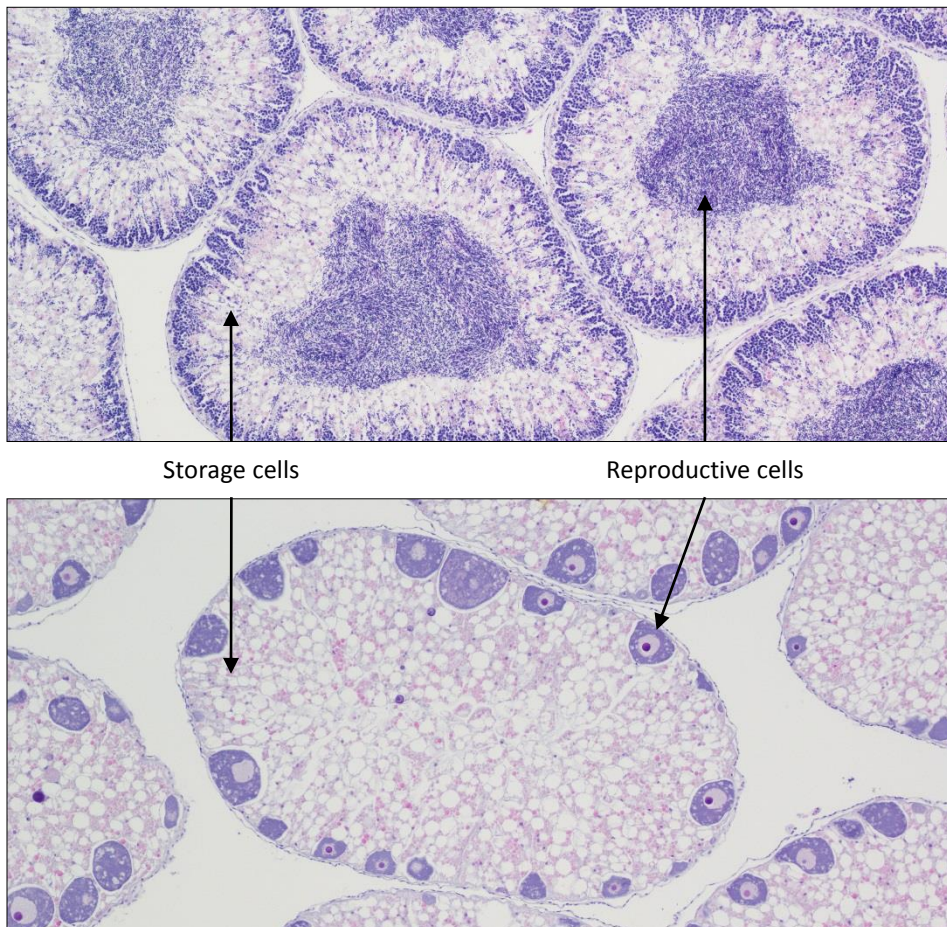


Figure 7. The two types of cells present in the gonads of male (top) and female (bottom) sea urchins: Reproductive cells and storage cells (nutritive phagocytes).

Roe enhancement of sea urchins

Roe enhancement is defined as the capture of wild, mature sea urchins that are of suboptimal quality for selling whole and live or for processing and selling the gonad (roe). The sea urchins can be collected from areas of low feed availability, poor environmental conditions or from urchin ‘barrens’ where sea urchins are present at very high densities. Once collected they can be held in either land or sea-based holding systems and fed natural or manufactured feeds to enhance the roe. Enhancement means increasing the quantity of the roe (i.e. increasing the percentage GI) as well as improving the quality and consistency of the product. This varies according to a number of factors but can normally be achieved within 2-3 months.

There are many references and publications describing roe enhancement trials on a range of sea urchin species from a range of different countries using a variety of feeds and holding systems^[1].

The results of these trials are usually expressed as the increase in GI resulting from roe enhancement but a number of studies have also investigated the resulting changes in quality (such as colour and taste).

It is clear that roe enhancement can cause a very rapid increase in the size of the gonad and resulting GI, especially compared to the GI of wild populations (Figure 8). It is not entirely clear what the impact is on the physical qualities of the gonad other than taste and colour. For example, the texture of ‘enhanced’ gonads appear to be softer than those from wild sea urchins and the reasons for this are unclear. The following four pages compare the histology slides of wild sea urchin gonads with enhanced sea urchin gonads from the four stages of the reproductive cycle. There is an obvious difference in the amount of storage cells between the two (see also Appendix I) with enhanced sea urchin gonads having significantly larger amounts. This is a result of the enhanced sea urchins being held in optimal conditions with regular feed.

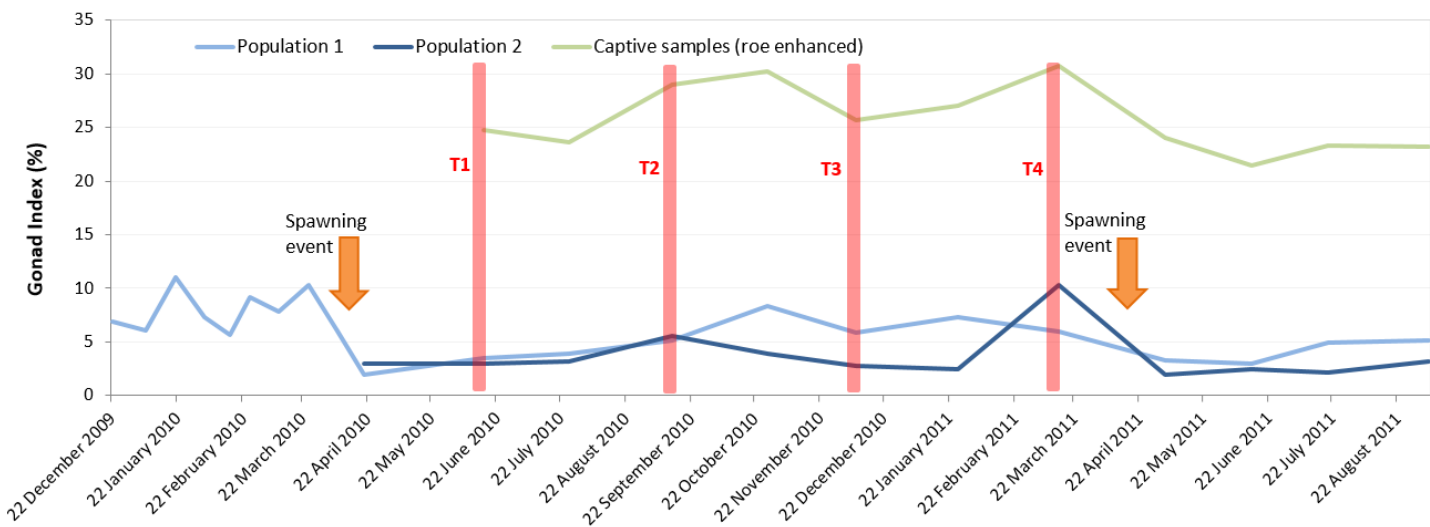


Figure 8. The changes in GI of two wild populations situated 0.5 km from one another in the Kvalsund near Tromsø. Note how different the GI values are between the populations despite their close proximity. The captive samples show the GI of roe enhanced sea urchins collected from Population 2 and held in a land based facility (the red bars T1-4 indicate when the samples that are shown in Appendix 1 were taken from these three sample groups).

NOTE: a full description of the two population sites and the conditions that the captive samples were held in is given in Appendix 1

[1] Brown, N.P. & Eddy S.D., 2015. Echinoderm Aquaculture. John Wiley & Sons, Inc., Hoboken, New Jersey, pp367.

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

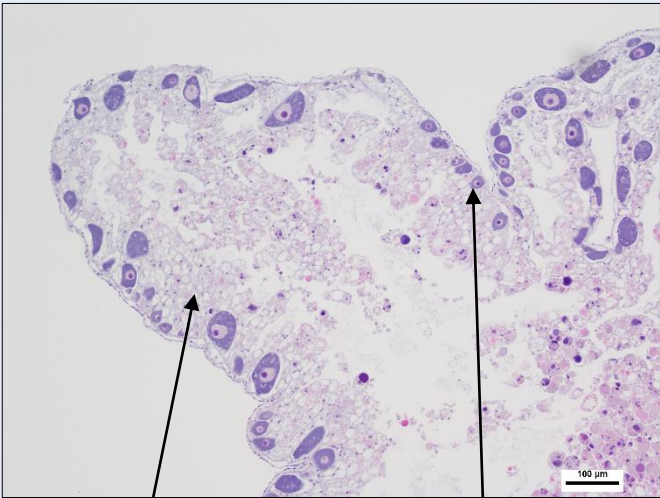
The development of reproductive cycle in sea urchins has been divided into four stages by Walker *et al.* (2007). This classification is now widely used around the world when describing the reproductive stage of sea urchins from a wide variety of species and has been used in this guide. The four stages are as follows (examples are given from male and female urchins collected from wild populations as well as captive sea urchins that have been roe enhanced):

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

This stage occurs for approximately 3 months in spring after spawning has occurred. Residual primary oocytes are present in the cells of 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 storage cells increases and reproductive cells begin to appear around the periphery of the cells.

Stage I: Female

Wild

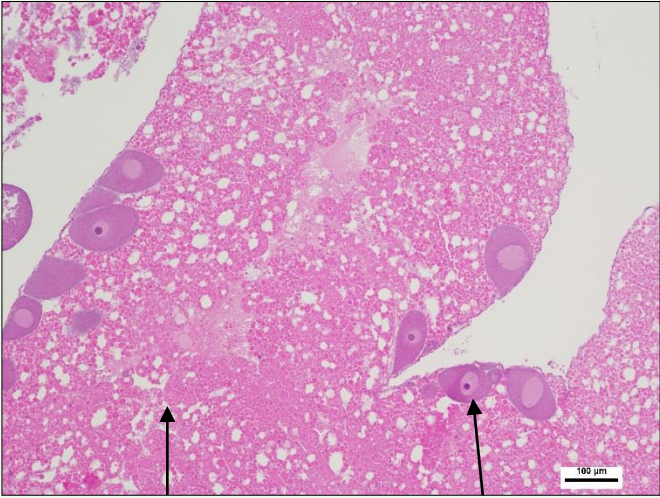


Storage cells

Reproductive cells

Stage I: Female

Captive

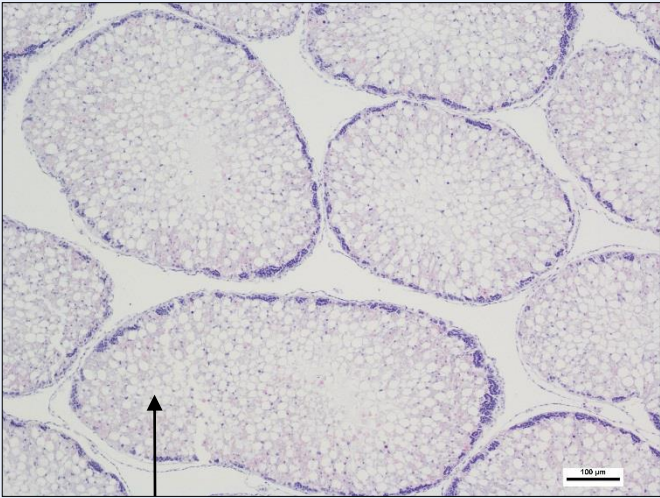


Storage cells

Reproductive cells

Stage I: Male

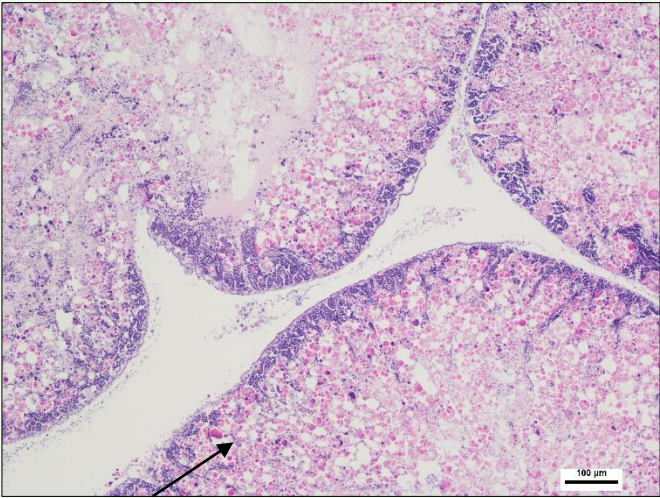
Wild



Storage cells

Stage I: Male

Captive

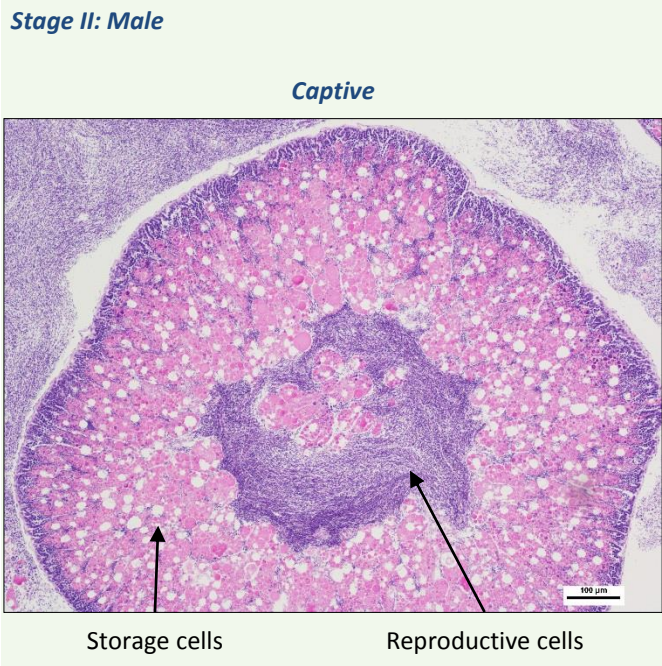
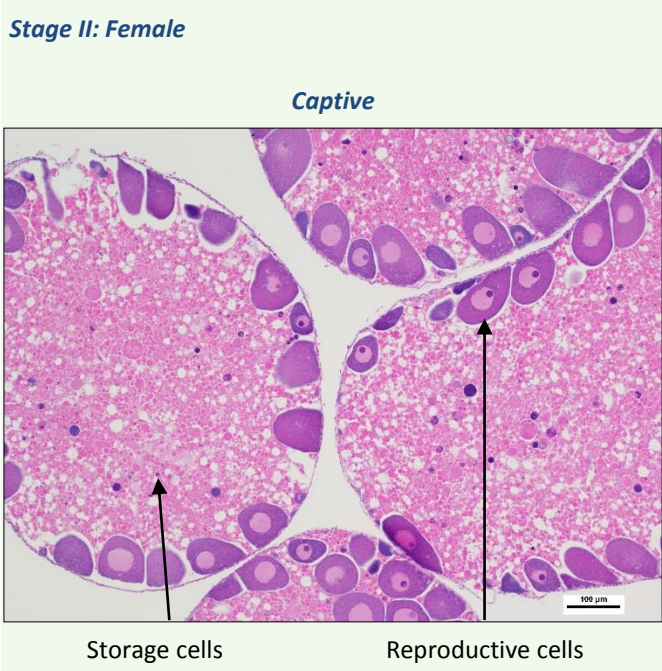
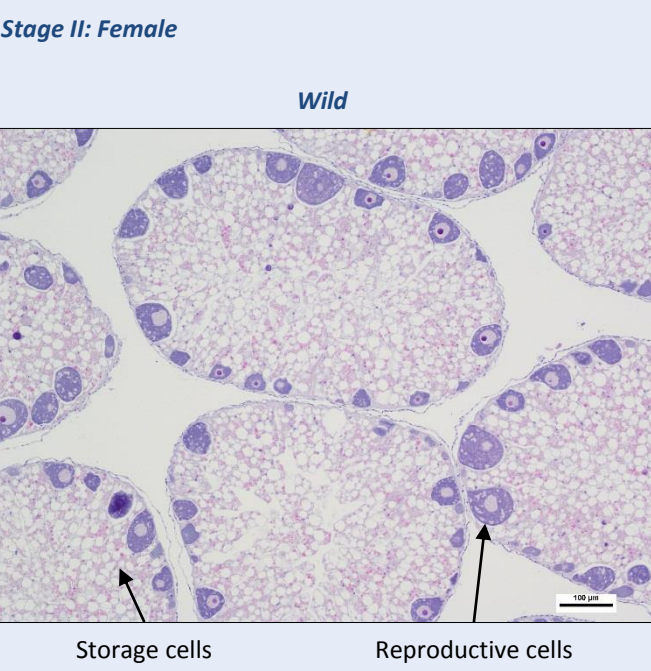


Storage cells

[2] Walker, C.W., Unuma, T., McGinn, N.A., Harrington, L.M., Lesser, M.P., 2001. Reproduction of sea urchins. In: Lawrence, J.M. (Ed.), Edible Sea Urchins: Biology and Ecology. Elsevier, Tampa, Florida, pp. 5 - 26.

Stage II: Storage cell growth (Pre-gametogenesis and storage cell renewal)

This stage occurs for approximately 3-4 months during summer. Reproductive cells continue to appear around the periphery of the cells 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 storage cell cells.

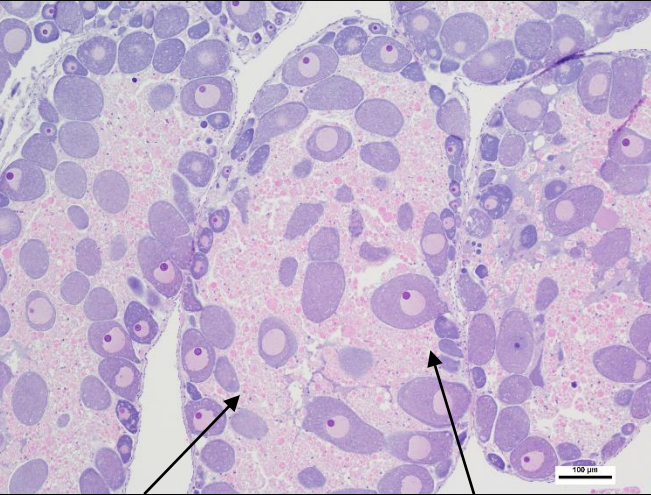


Stage III: Development of reproductive cells
(Gametogenesis and storage cell utilisation)

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 cell. As the number and size of reproductive cells increases the number and size of storage cell cells decreases simultaneously.

Stage III: Female

Wild

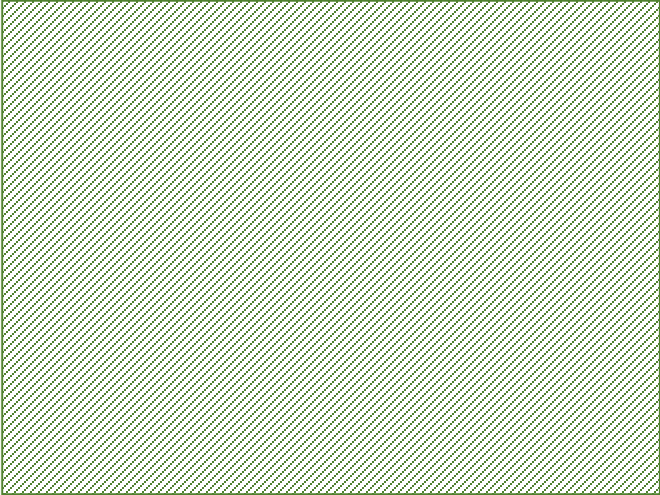


This histological section shows several large, rounded cells. Some have a clear, pale center (storage cells), while others have a darker, more granular center (reproductive cells). Arrows point to these two types of cells. A scale bar in the bottom right corner indicates 100 μm.

Storage cells Reproductive cells

Stage III: Female

Captive

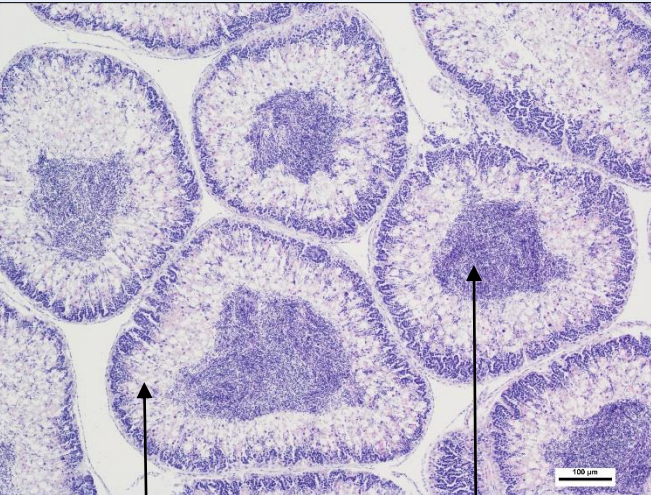


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Image is not available from samples

Stage III: Male

Wild




This histological section shows several large, rounded cells. Some have a clear, pale center (storage cells), while others have a darker, more granular center (reproductive cells). Arrows point to these two types of cells. A scale bar in the bottom right corner indicates 100 μm.

Storage cells Reproductive cells

Stage III: Male

Captive



This histological section shows several large, rounded cells. Some have a clear, pale center (storage cells), while others have a darker, more granular center (reproductive cells). Arrows point to these two types of cells. A scale bar in the bottom right corner indicates 100 μm.

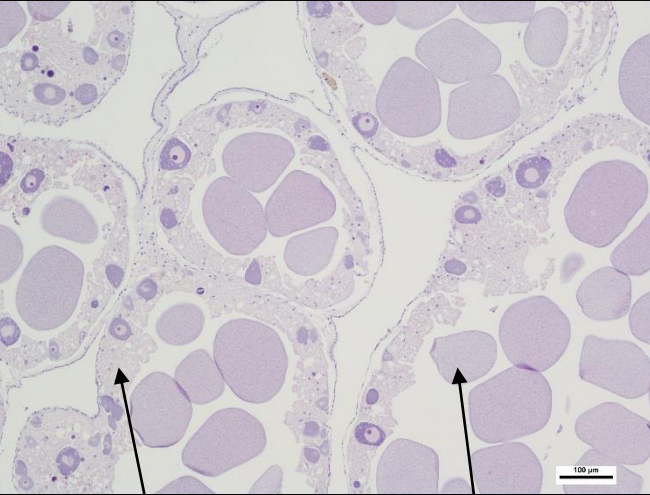
Storage cells Reproductive cells

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

This stage lasts for approximately 2-3 months and occurs in late winter. The middle of the cells (the lumen) is packed with fully developed (differentiated) reproductive cells (gametes) stored and ready for spawning. The storage cell 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

Wild




Storage cells Mature reproductive cells

This micrograph shows a cross-section of a wild female gonad in Stage IV. The lumen is filled with numerous mature reproductive cells (gametes). Storage cells are present but appear reduced in number and size compared to earlier stages. Two black arrows point to storage cells, and a scale bar in the bottom right indicates 100 µm.

Stage IV: Female

Captive

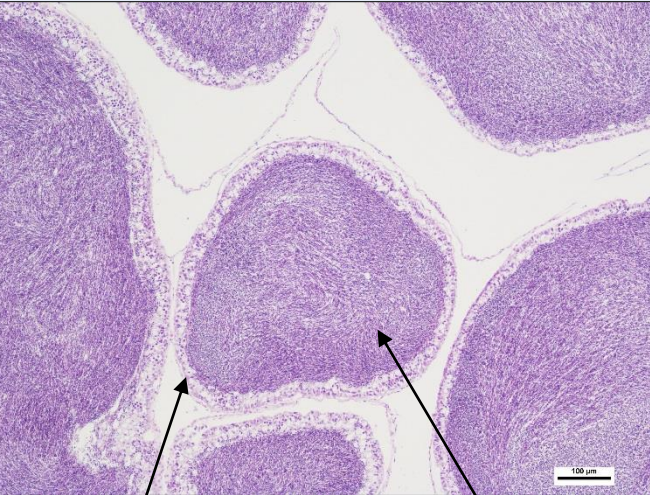


Storage cells Mature reproductive cells

This micrograph shows a cross-section of a captive female gonad in Stage IV. The lumen is densely packed with mature reproductive cells. Storage cells are visible but appear exhausted and reduced in size. Two black arrows point to storage cells, and a scale bar in the bottom right indicates 100 µm.

Stage IV: Male

Wild

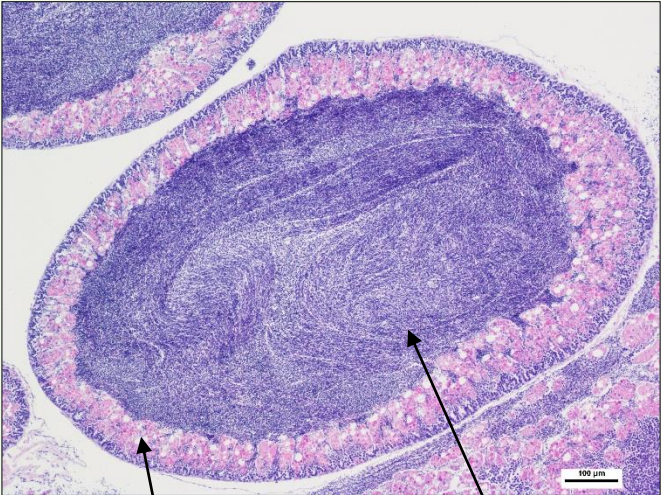


Storage cells Mature reproductive cells

This micrograph shows a cross-section of a wild male gonad in Stage IV. The lumen is filled with mature reproductive cells. Storage cells are present but appear reduced in number and size. Two black arrows point to storage cells, and a scale bar in the bottom right indicates 100 µm.

Stage IV: Male

Captive



Storage cells Mature reproductive cells

This micrograph shows a cross-section of a captive male gonad in Stage IV. The lumen is densely packed with mature reproductive cells. Storage cells are visible but appear exhausted and reduced in size. Two black arrows point to storage cells, and a scale bar in the bottom right indicates 100 µm.

APPENDIX I: Comparison of wild vs. captive histology samples from Jun 2010-Mar 2011

The following histology images are included in this publication as examples taken from two wild populations of sea urchins as well as a sample of sea urchins that were collected from one of the wild populations and held in a land-based facility and enhanced over a 12 months period. The authors believe this is the first time a visual comparison of wild versus enhanced gonad histology has been presented in a publication and we hope that it will be of use in subsequent studies.

Description of collection sites

Population 1 (Kårvika) (Figures 9 & 10)

The sea urchins were collected from an area between 2-6m deep. It had a hard rocky bottom interspersed with soft gravel. The currents at this site were relatively small for the Kvalsund area (which is known to have very strong currents) as it is very broad at this point and the site offers a natural small sheltered harbour which further reduces currents. The sea urchins were collected by free divers using catch bags. The enhanced sea urchins were collected from this population.

Population 2 (Tunnel) (Figures 9 & 10)

The sea urchins were collected from an area that was between 2-4m deep and slowly shelving. It had a hard rocky bottom interspersed with soft gravel. The currents at this site were stronger (2-3knots of current is common) as the sound is very narrow at this point. The sea urchins were collected by free divers using catch bags.

Captive population

Sea urchins collected from Population 1 were transferred to the Havbruksstasjonen (land-based research facility) at Kårvika. They were placed in shallow trays and fed once per week with the Nofima sea urchin roe enhancement feed. They were held at ambient seawater temperatures and at 12/12 hour light period for 12 months.

Regular amples were removed and histology samples were collected over this period. Examples of the resulting histology are shown in the following pages.



Figure 9: The the two collection sites for Population 1 and 2 in the Tromsø area of North Norway

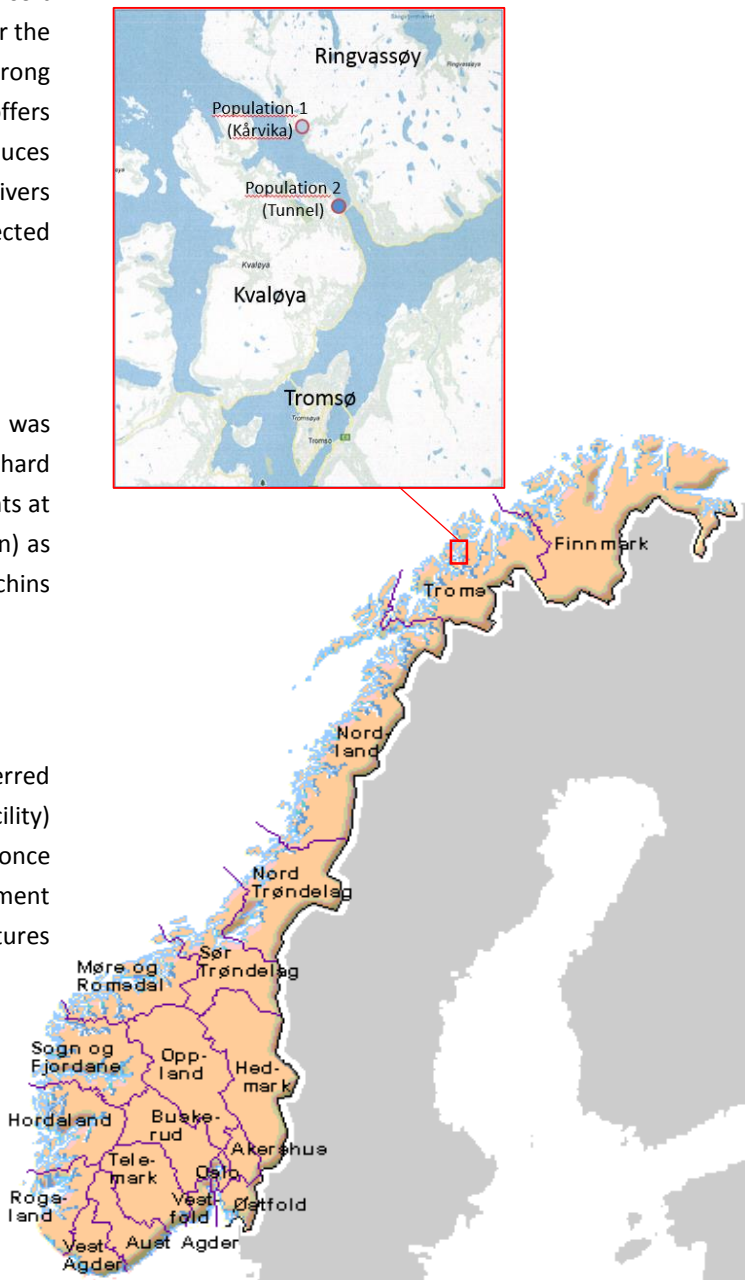


Figure 10: The position of the two collection sites in the north of Norway

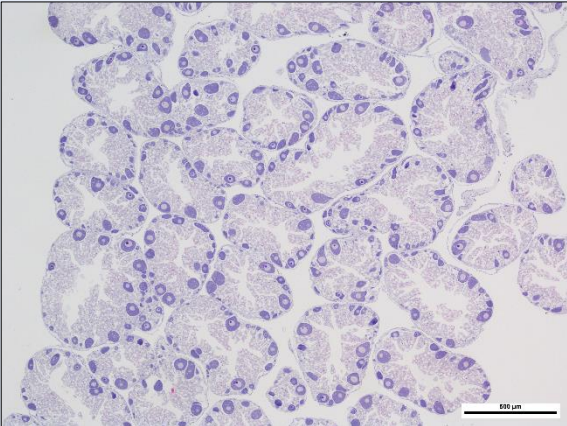
APPENDIX I

T1 collection: Female urchins, 16 June 2010

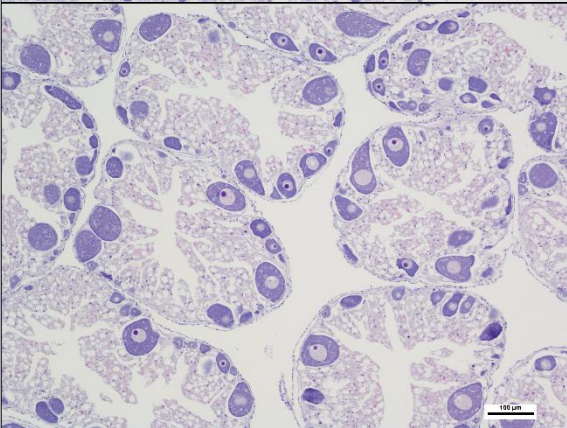
Wild

Population I

Stage II



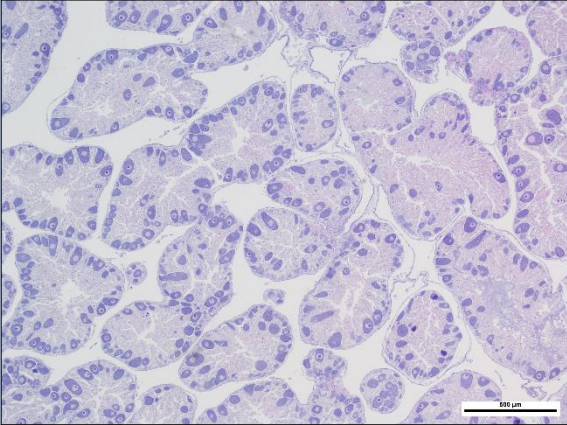
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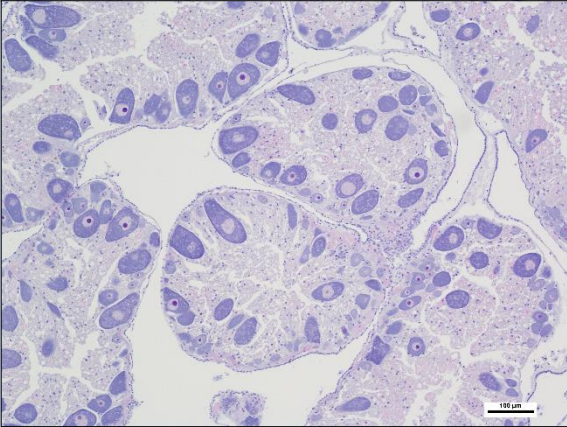
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Population II

Stage II



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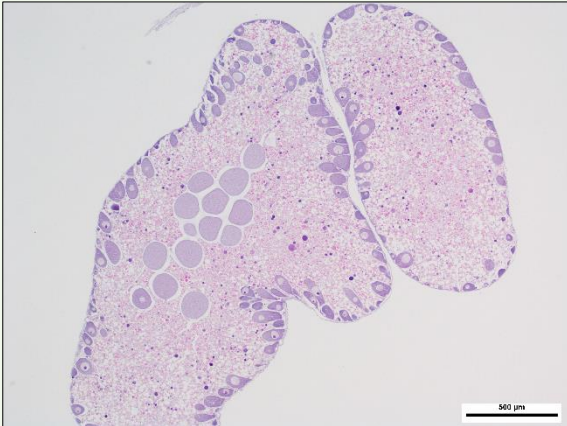


(10x)

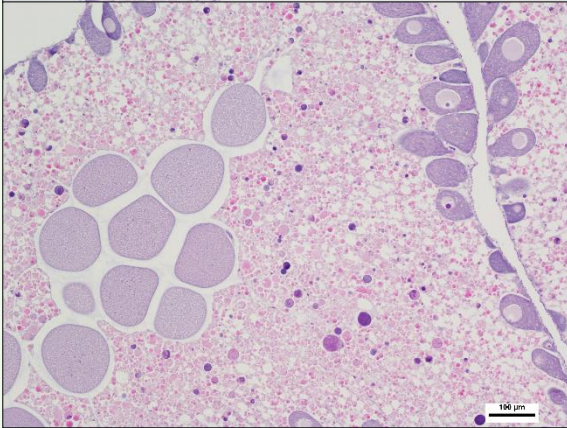
Captive

Example 1

Stage I



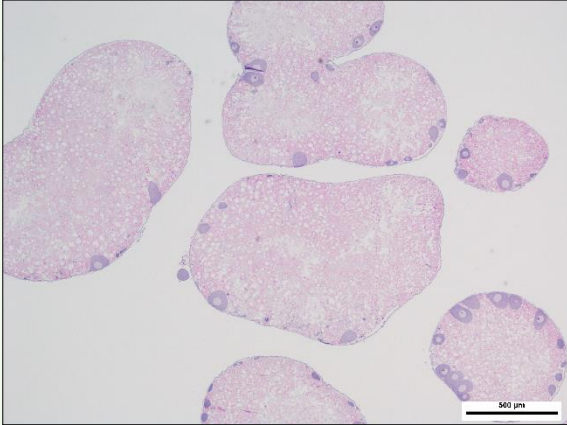
(4x)



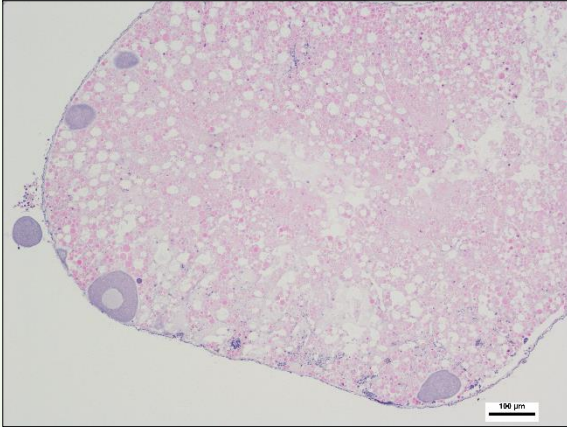
(10x)

Example 2

Stage I



(4x)



(10x)

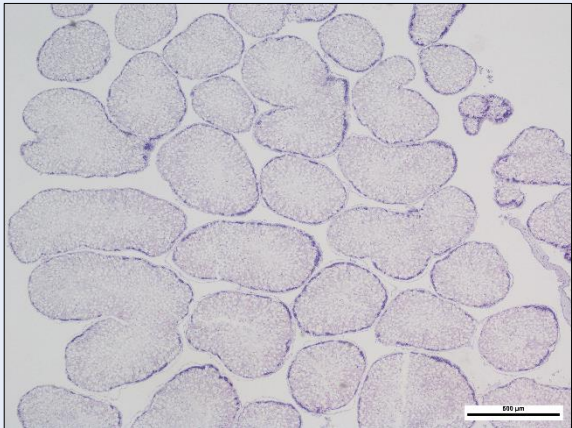
APPENDIX I

T1 collection: Male urchins, 16 June 2010

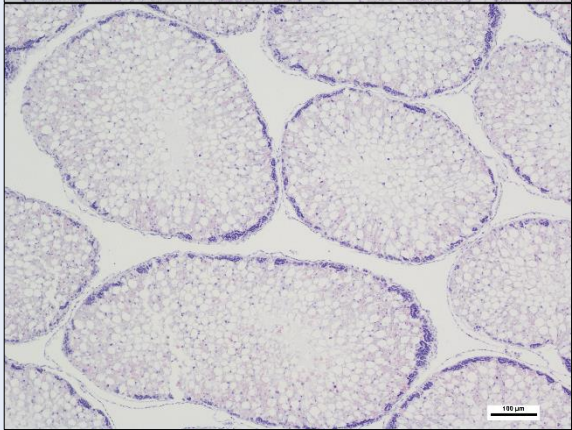
Wild

Population I

Stage I



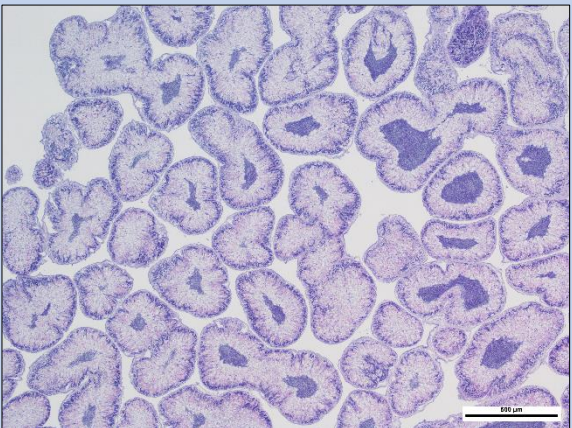
(4x)



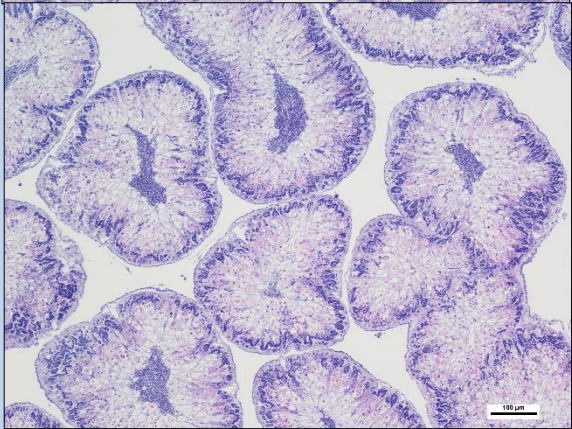
(10x)

Population II

Stage II



(4x)

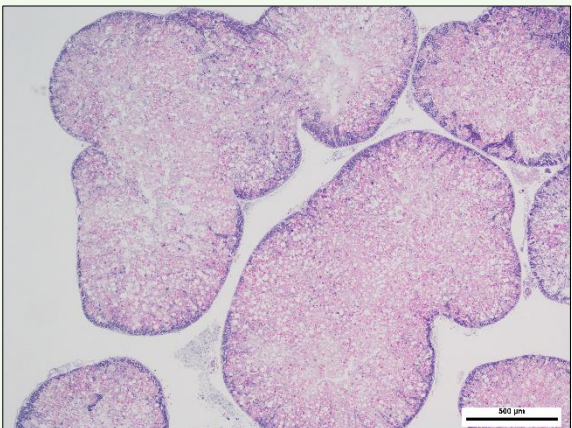


(10x)

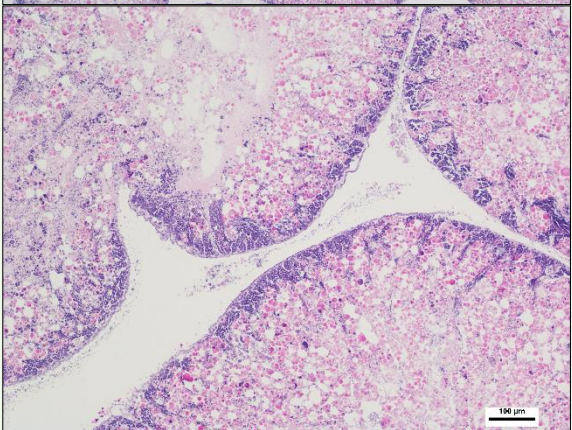
Captive

Example 1

Stage I



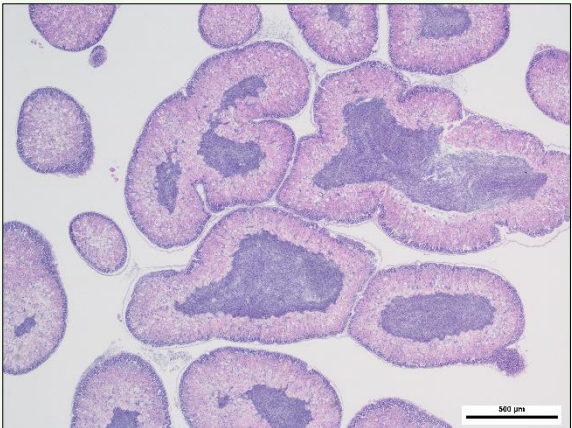
(4x)



(10x)

Example 2

Stage II/III



(4x)



(10x)

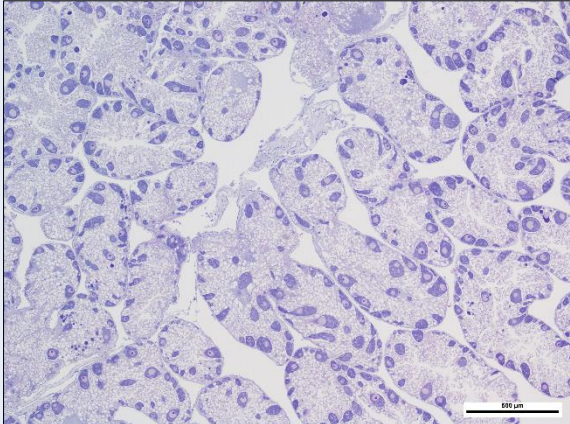
APPENDIX I

T2 collection: Female urchins, 13 September2010

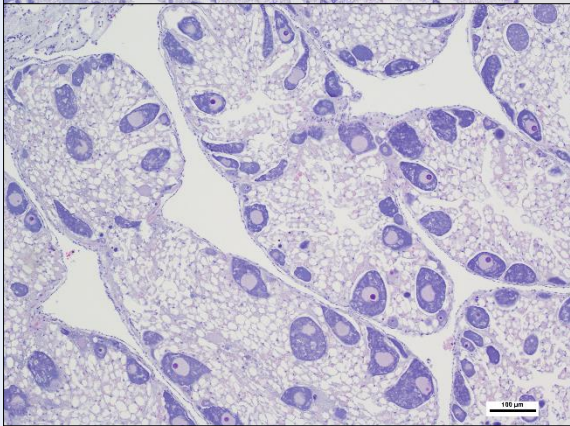
Wild

Population I

Stage II/III



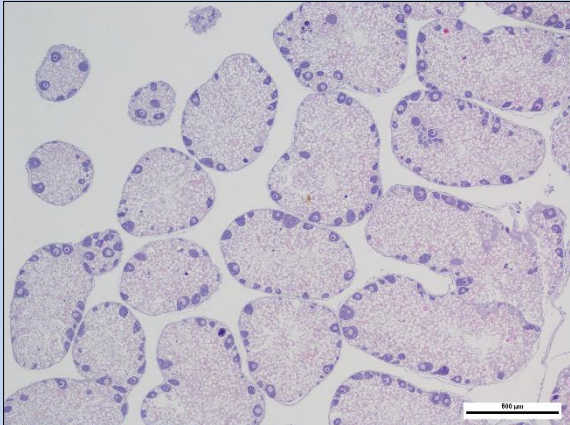
(4x)



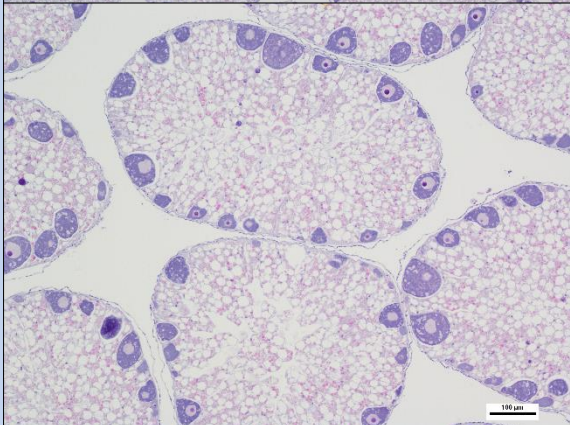
(10x)

Population II

Stage II



(4x)

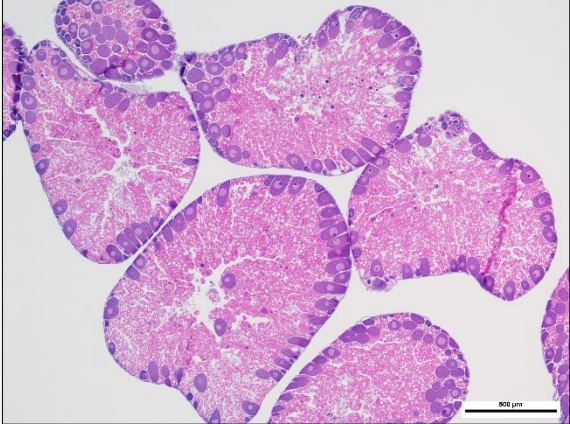


(10x)

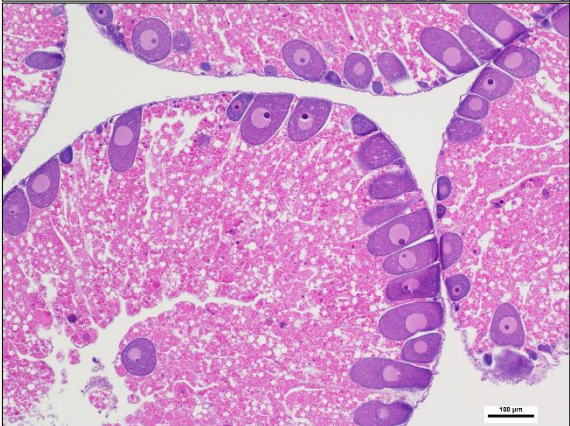
Captive

Example 1

Stage II



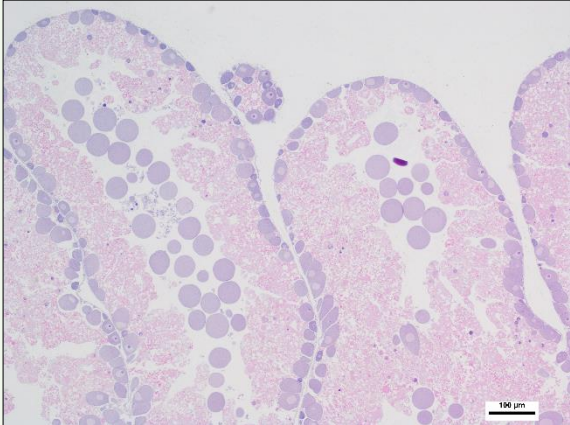
(4x)



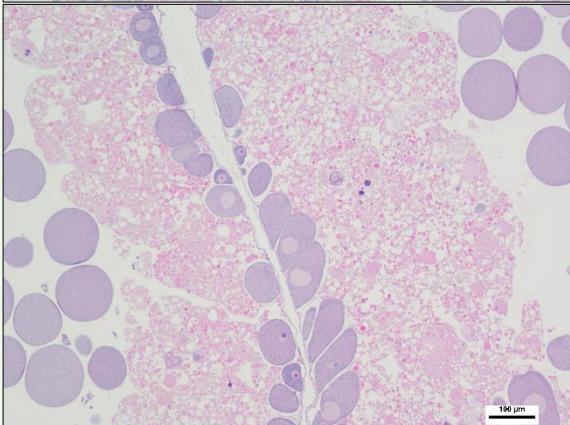
(10x)

Example 2

Stage II



(4x)



(10x)

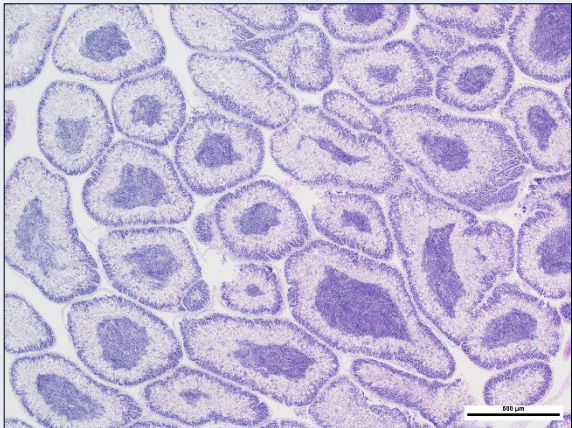
APPENDIX I

T2 collection: Male urchins, 13 September 2010

Wild

Population I

Stage III



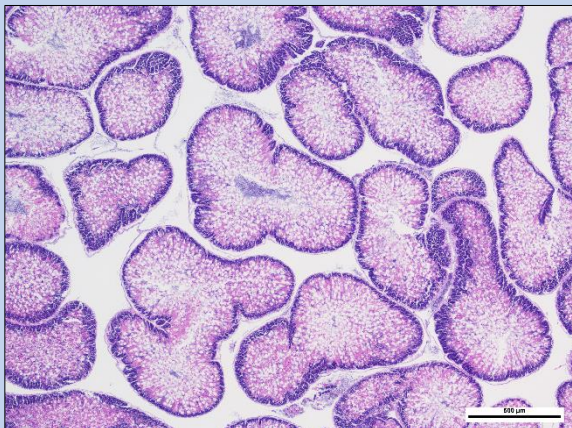
(4x)



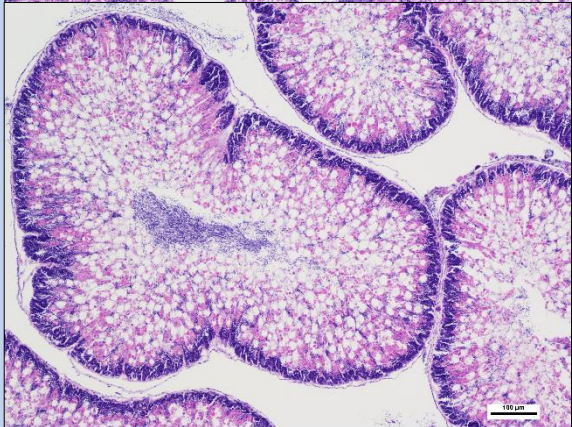
(10x)

Population II

Stage II



(4x)

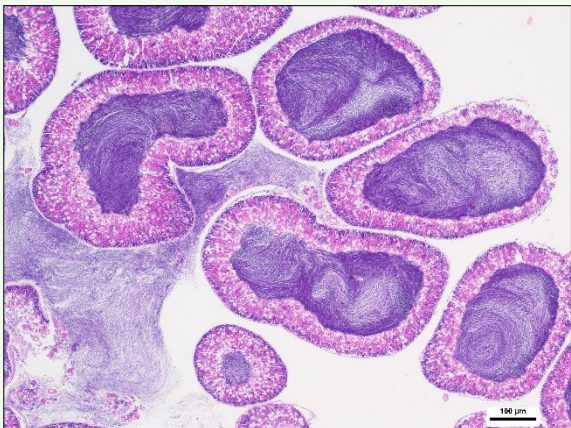


(10x)

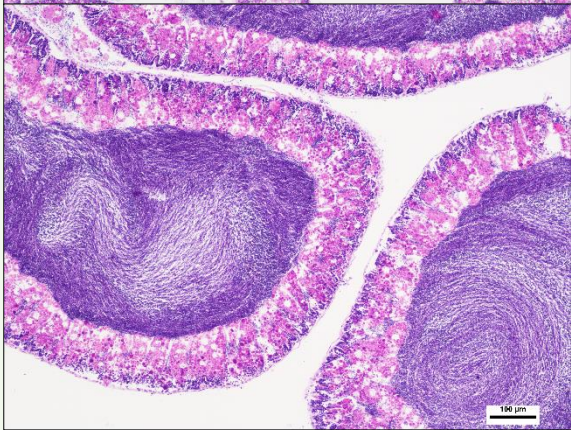
Captive

Example 1

Stage III



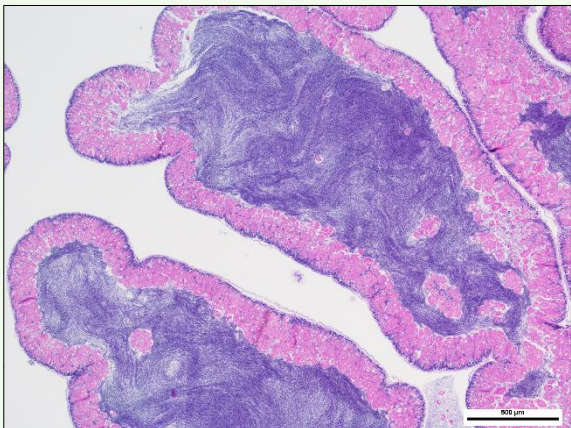
(4x)



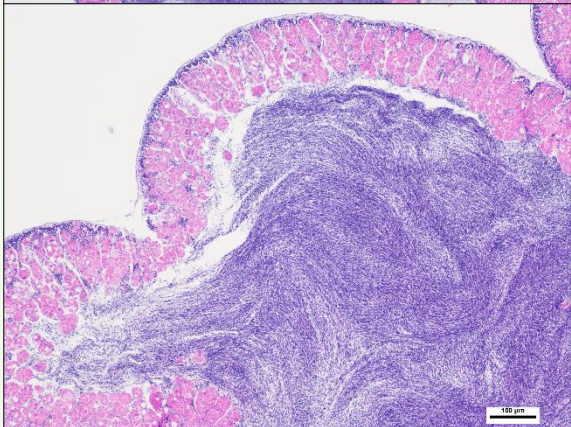
(10x)

Example 2

Stage III



(4x)



(10x)

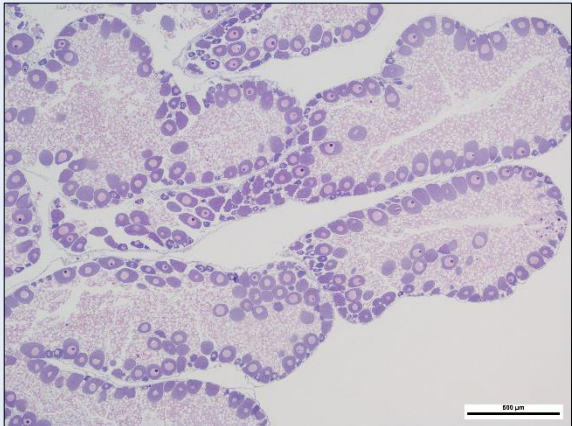
APPENDIX I

T3 collection: Female urchins, 9 December 2010

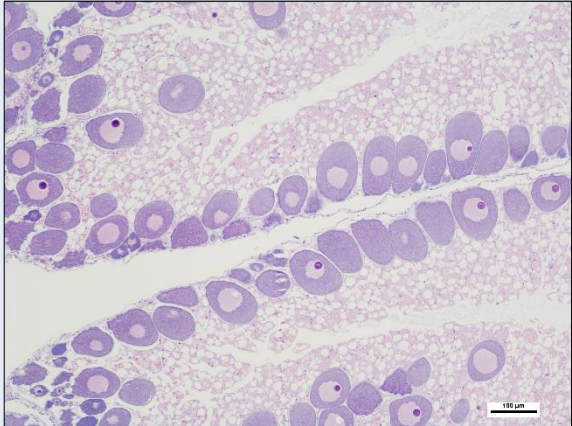
Wild

Population I

Stage II



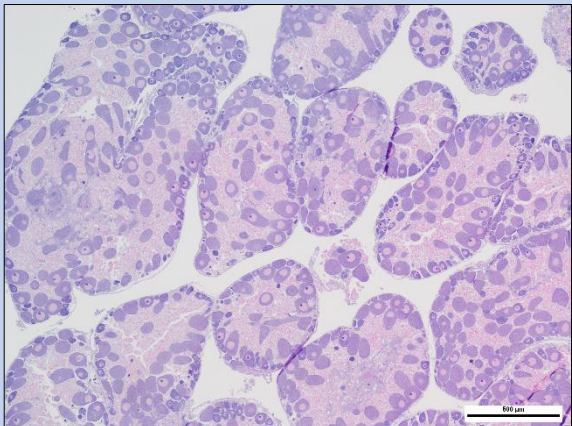
(4x)



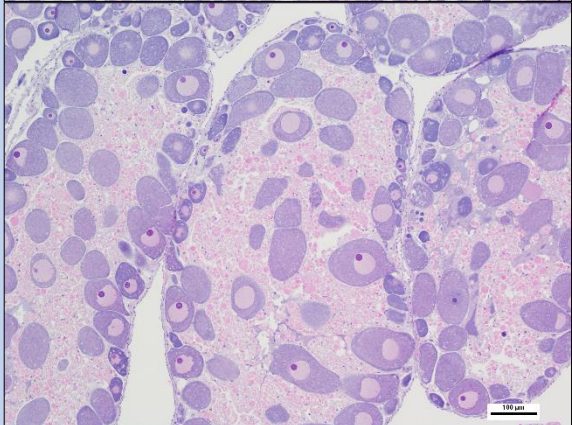
(10x)

Population II

Stage III



(4x)

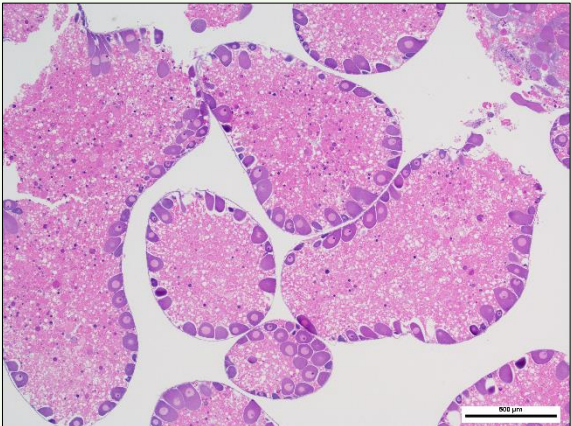


(10x)

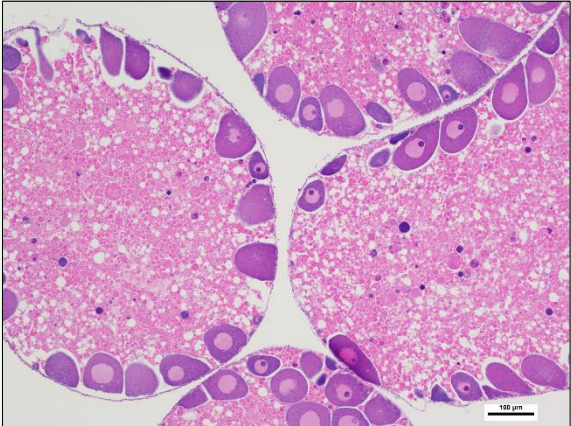
Captive

Example 1

Stage II



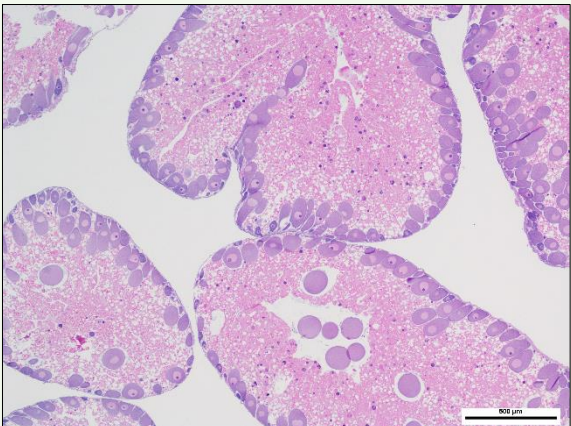
(4x)



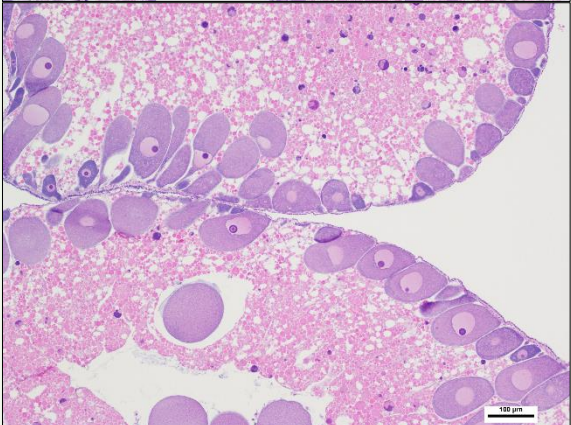
(10x)

Example 2

Stage II



(4x)



(10x)

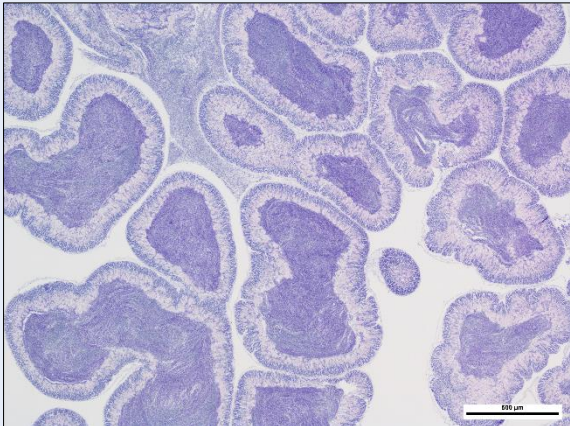
APPENDIX I

T3 collection: Male urchins, 9 December 2010

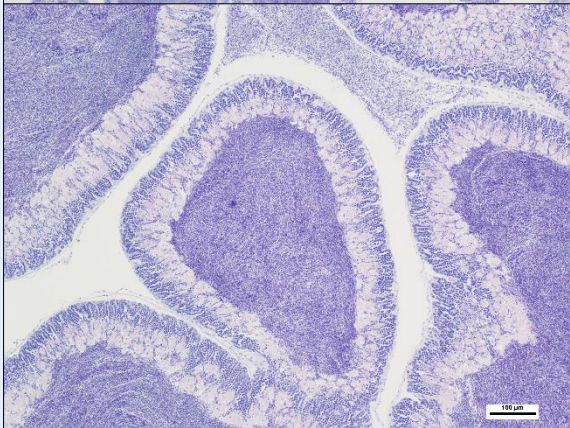
Wild

Population I

Stage III



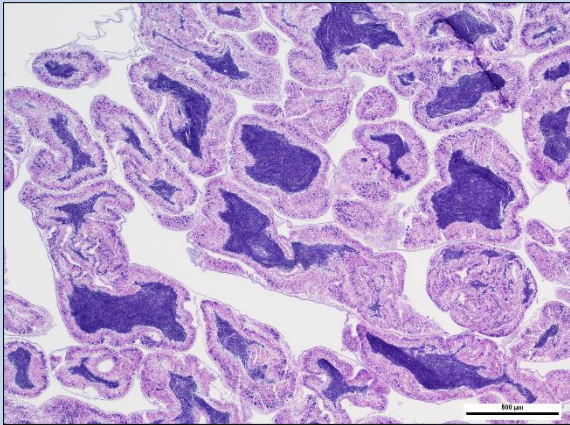
(4x)



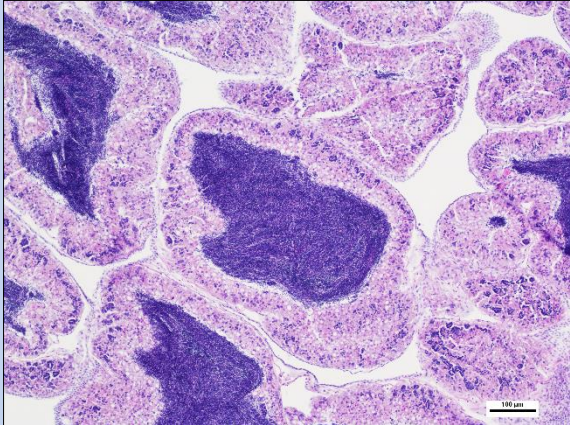
(10x)

Population II

Stage II/III



(4x)

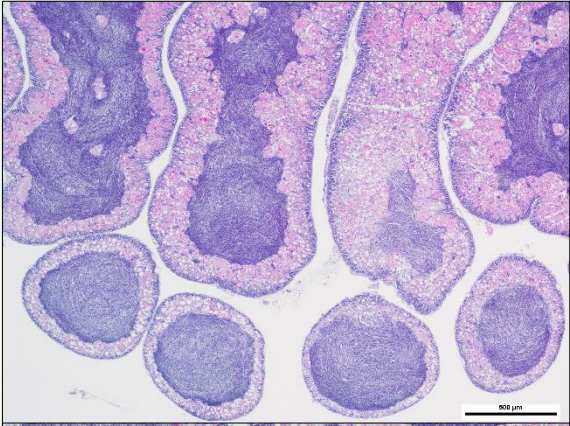


(10x)

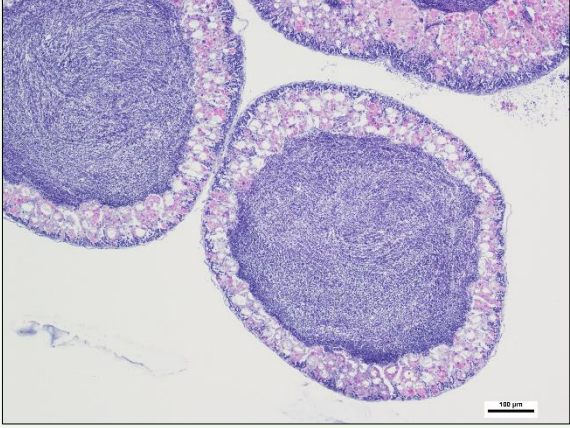
Captive

Example 1

Stage III



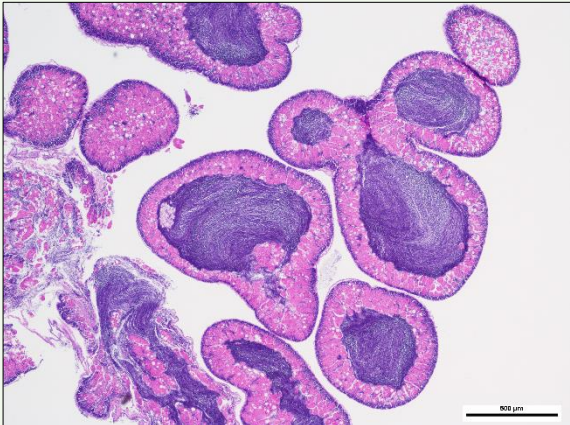
(4x)



(10x)

Example 2

Stage III



(4x)



(10x)

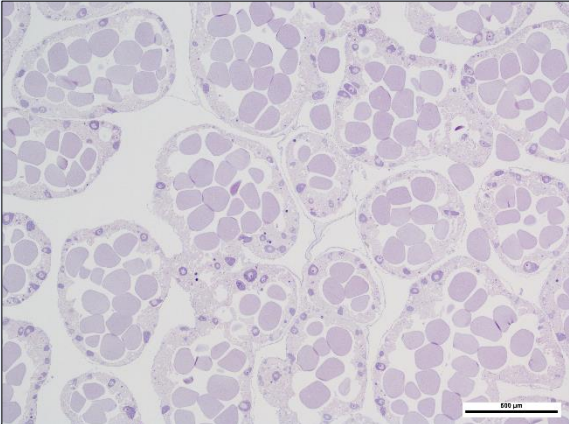
APPENDIX I

T4 collection: Female urchins, 15 March 2011

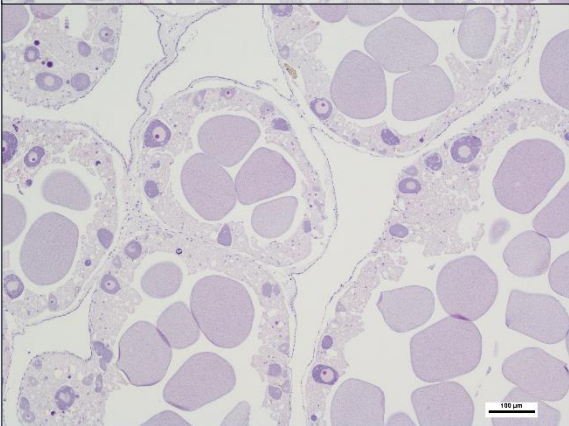
Wild

Population I

Stage IV



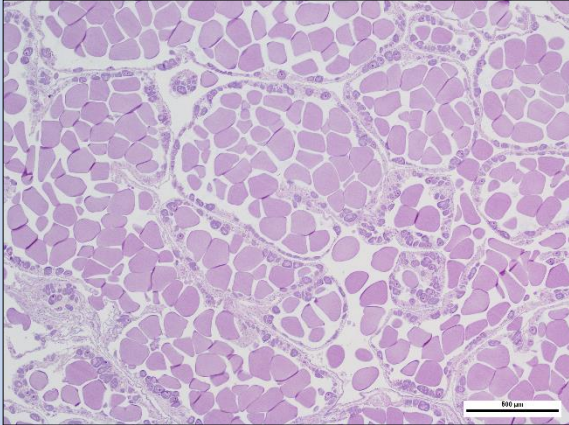
(4X)



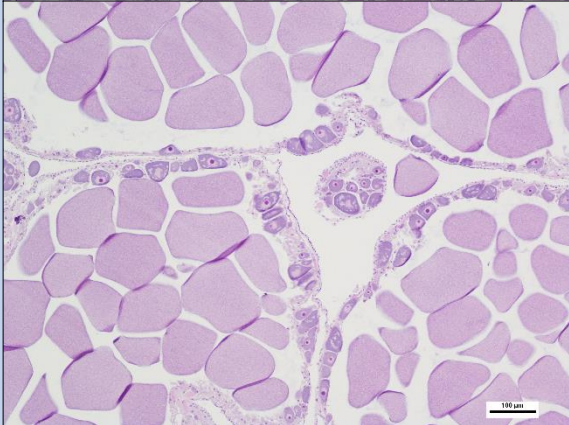
(10X)

Population II

Stage IV



(4X)

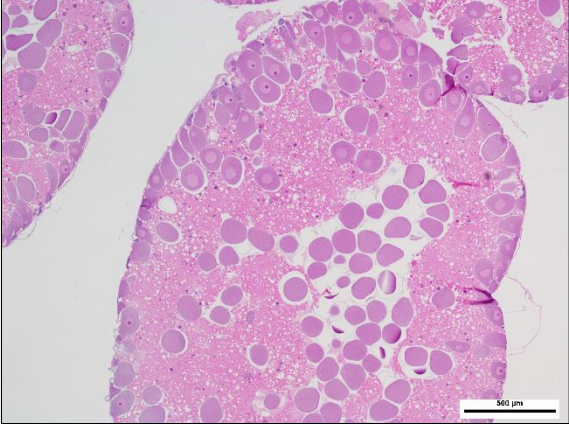


(10X)

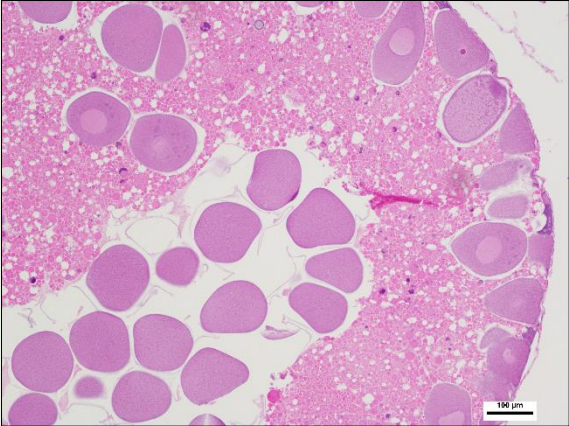
Captive

Example 1

Stage IV



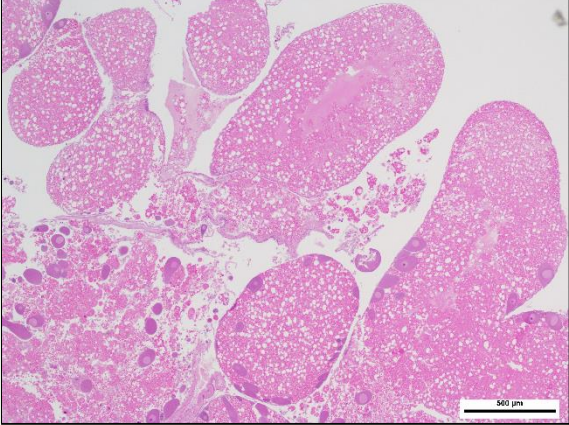
(4X)



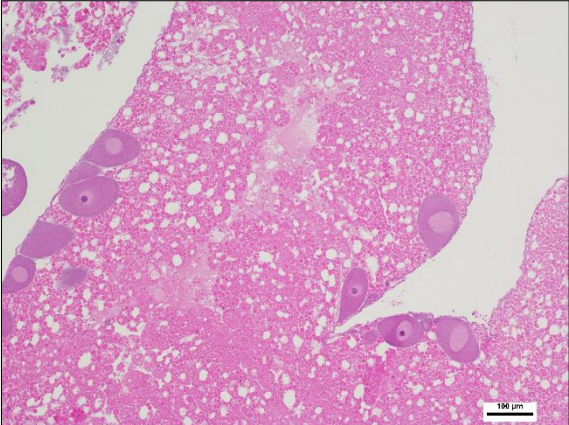
(10X)

Example 2

Stage I



(4X)



(10X)

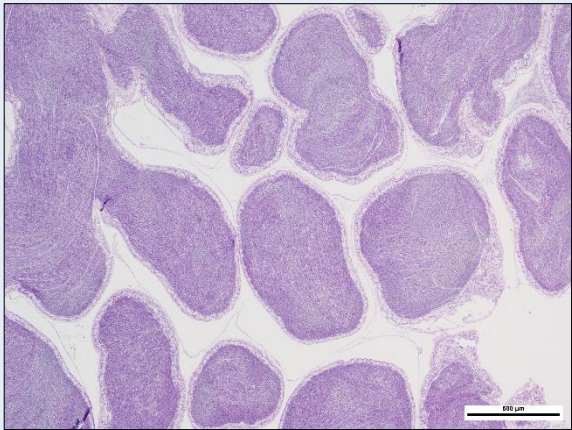
APPENDIX I

T4 collection: Male urchins, 15 March 2011

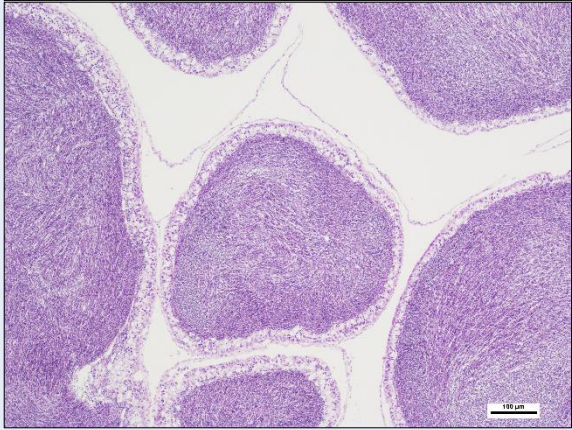
Wild

Population I

Stage IV



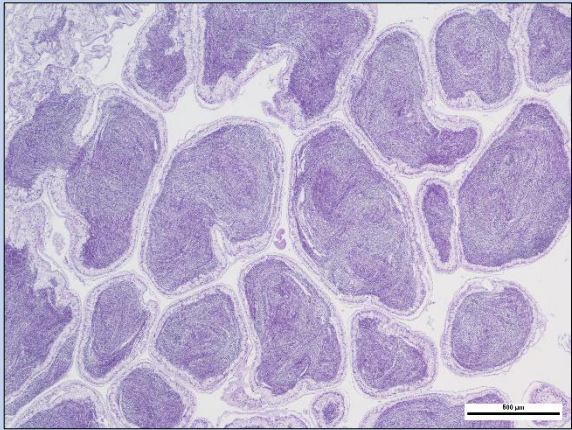
(4x)



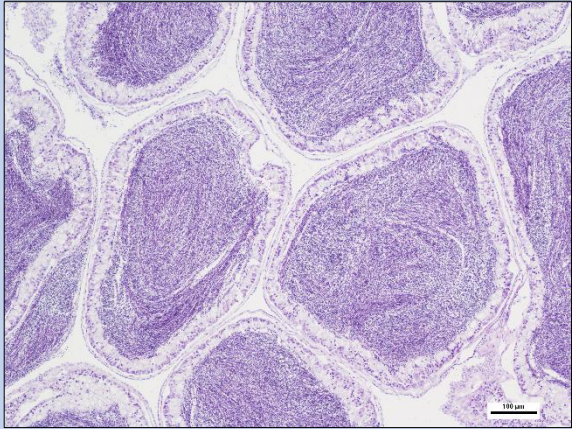
(10x)

Population II

Stage IV



(4x)



(10x)

Captive

Example 1

Stage IV



(4x)



(10x)

Example 2

Stage IV



(4x)



(10x)