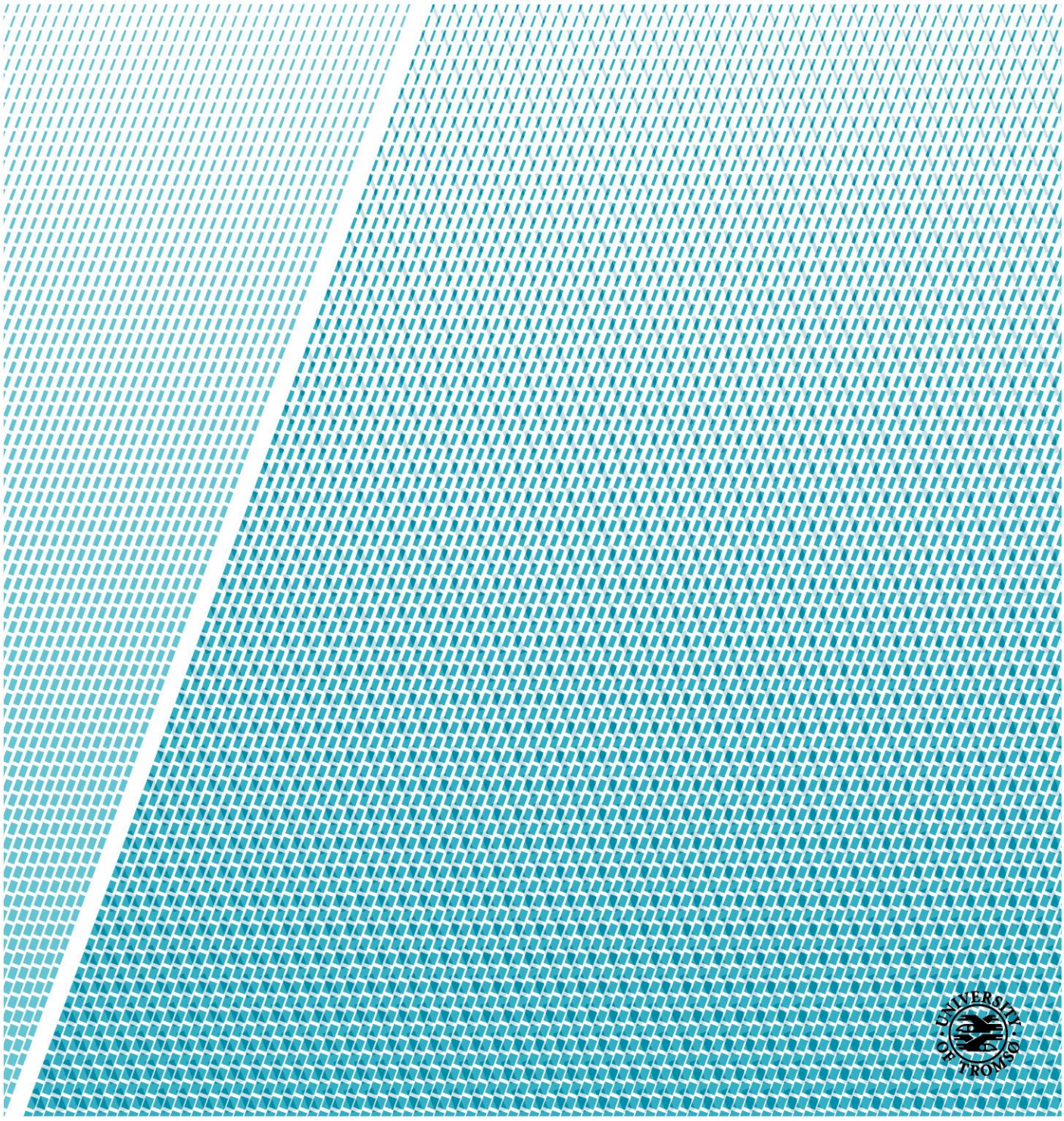
UiT
THE ARCTIC UNIVERSITY OF NORWAY

Norwegian College of Fisheries Science
Faculty of Biosciences, Fisheries and Economics

Quality aspects of wild caught and enhanced sea urchins (*Strongylocentrotus droebachiensis)*

Ingeborg Mathisen Sætra

Master thesis in Fisheries and Aquaculture Science
Aquaculture biology (60 credits)
May 2019



Acknowledgement

This master thesis was written at the Norwegian College of Fisheries Science at UiT – The Norwegian Arctic University of Norway, in cooperation with Nofima The Norwegian Institute of Food, Fisheries and Aquaculture Research. This thesis is part of The Urchin Project[1] financed by the Northern Periphery and Arctic Program. The work presented was conducted at Kårvika research station, the Norwegian College of Fisheries Science and Nofima Tromsø.

First, I would like to thank Philip James for the opportunity to work with these strange creatures, for guiding me through this project and for his time and expertise. I would also like to thank Bjørn-Steinar Sæther for his guidance and statistical expertise.

Thank you to the staff at Nofima for the including atmosphere, unlimited coffee and expertise, especially Gunhild for help in the laboratory, advising and illustrations.

Finally, I would like to thank my study group, Audun, Eskil and Malene, through my years at the College of Fisheries Science for great conversations, motivation and of course long study days.

Ingeborg Mathisen Sætra

Tromsø, May 2019.

[1] https://urchinproject.com/

Abstract

Sea urchin roe is a culinary delicacy worldwide, but with global catches are down due to overfishing and diminishing stocks, interest in aquaculture has increased. Sea urchin aquaculture lacks consistency in the quality of the product, good quality sea urchin roe should be large with an orange or yellow colour, with a distinct sweet-salty taste and have the right firm texture. The main objective of this study has been to compare quality aspects of roe from wild and enhanced sea urchins (*Strongylocentrotus droebachiensis)* using the following quality parameters: gonad index (GI), water content, reproductive stages, histological examination of relative cellular area and morphology, amino and fatty acid analysis and scanning electron microscopy (SEM). Green sea urchins were collected (*n*=250) from a wild population. The sea urchins were then held in raceways where they were fed the Nofima manufactured diet for 14 weeks (mid-August – mid-November). The GI increased significantly in the enhanced urchins during the trial, from 6.1% to 29.65% at week 12. No significant increase was recorded in wild urchins. The amino acids glycine, leucine and lysine was different between the treatments. Wild sea urchins had 6% more unidentified fatty acids, most likely a NMID fatty acid. Histological examination showed significant progress in reproductive stages in enhanced sea urchins, none in the wild sea urchins, suggesting gametogenesis is inhibited in captivity. Enhanced sea urchins had less relative percentage reproductive cells and an overall messier and less uniform appearance mid-trial (week 8-10). SEM showed less structured and greater number of surface cells in enhanced sea urchins. The results of this study suggest that a rapid increase in GI may be too fast to preserve structural integrity. Further research suggested is long-term enhancing and gonadal wall structure analysis during enhancing.

Sammendrag

Kråkebollerogn er en delikatesse over hele verden, men de globale fangstene er på tur ned på grunn av overfiske og nedgang i kråkebollebestander. Den begrensende høstesesongen, ulik kvalitet på rogna på ville kråkeboller og små kråkeboller med lite rogn i nedbeita områder, er alle faktorer som har auka interessen for havbruk av kråkeboller. Oppfôring av voksne kråkeboller løser problemet med liten rogn og en begrenset høstesesong, men det er problemer med å få tilfredstillende og jevn kvalitet. Kvalitetsrogn bør være stor med gul eller oransje farge, smake søttsalt og ha fast tekstur.

Målet med studiet har vært å sammenligne kvalitetsforskjeller hos rogn fra villfanga kråkeboller og oppfôra kråkeboller (*Strongylocentrotus droebachiensis*) ved å bruke følgende kvalitetsparametere: gonadeindeks (GI), vanninnhold, reproduksjonsstadier, histologiske undersøkelser av relativt celleområde og morfologi, amino- og fettsyreanalyser og skanningelektronmikroskopering (SEM). Kråkeboller blei plukka (n=250) fra en vill bestand og en innledende undersøkelse blei foretatt. Kråkebollene blei plassert i renner under 24-timers lys og i naturlig temperatur (gjennomsnitt=9,6°C). Der blei de fôra med Nofimas kråkebollefôr i 14 uker (fra midten av august – til midten av november). Den første prøvetakinga (n=20) blei gjort etter fire uker og deretter hver andre uke til uke 12. Ville kråkeboller blei plukka på samme tidspunkt for sammenligning. Den biokjemiske analysen blei gjort i uke 13, og SEM i uke 14.

GI økte signifikant i de oppfôra kråkebollene under forsøket, fra 6,1 ± standardavvik 3.1% i den innledende undersøkelsen til 29,65± 2.1% i uke 12. Ingen signifikant økning blei registrert hos de ville kråkebollene. Det var forskjeller i den biokjemiske sammensetninga mellom de ville og oppfôra kråkebollene. Aminosyrene med størst forskjell var glysin, leusin og lysin med henholdsvis 34,0%, 55,8% og 35,2% forskjell mellom ville og oppfôra kråkeboller. Flerumetta fettsyrer var den største gruppa fettsyrer hos begge gruppene. Det var fire ganger mer linolsyre (18:2 n-6) i oppfôra kråkeboller enn i ville kråkeboller. Mengden uidentifiserte fettsyrer var 6% større i ville kråkeboller og blei identifisert som mest sannsynlig en NMID fettsyre. Histologiske undersøkelser viste en signifikant utvikling i reproduksjonsstadier hos de ville kråkebollene, men ingen hos oppfôra kråkeboller. Dette antyder at gametogenese er hemma i fangenskap. Det er tydelige histologiske forskjeller mellom gruppene, der oppfôra kråkebollers rogn har mindre relativ prosent reproduksjonsceller og en generell mer rotete framtoning i midten av forsøket (uke 8-10). SEM viste at gonader fra oppfostra kråkeboller virker å være mindre strukturert og med flere overflateceller. Teksturen av rogna fra oppfôra kråkeboller er kjent for å være mykere og mindre innbydende for markedet. Resultatene fra dette forsøket antyder at den hurtige aukinga i GI kan være for rask til å opprettholde strukturell integritet. Anbefalt videre forskning er langvarig oppfôring og å analysere strukturen av gonadeveggen under oppfôring.

Table of Contents

1 Introduction

Sea urchins have been eaten by humans since prehistoric times, with sea urchin remains having been found in excavation sites dating back 16,000 years on the Atlantic coast of Spain, in North America, especially on the Pacific coast, and in Pacific cultures in Hawaii and New Zealand. They were eaten in the Mediterranean by the Minoans and the Romans (Campbell, 2008) and even an ancient roman cookbook from the 1st century includes recipes for sea urchin (Apicius, 2009). Sea urchins are a culinary delicacy all over the world, in the markets of Europe, Chile, North America and especially Japan which consumes 90% of the current world catch. Sea urchins are harvested for their gonads, also called *uni* or roe. The taste of high quality sea urchin roe is supposed to be sweet and salty (Sun & Chiang, 2015). Uni is a traditional food in Japan and is eaten in a variety of ways, as sushi, in soups or even as a paste spread on grilled fish or shellfish. In Mediterranean countries like France and Italy it is eaten raw on bread, as a *blaff* or *tarte*, or a roe mayonnaise (Lawrence, 2007a). Modern cuisine in high end restaurants is a new lucrative market for sea urchin roe (Stefánsson *et al.*, 2017). The global landing of sea urchins in 2016 was 69 032 tonnes, with Chile being the biggest harvester of sea urchins globally (Figure 1). The main species of sea urchin landings are the Chilean sea urchins (*Loxechinus albus)*, the red sea urchin (*Strongylocentrotus franciscanus)* and the green sea urchin (*Strongylocentrotus droebachiensis).* The green sea urchin supposedly tastes sweeter than the others species (Sun & Chiang, 2015). Sea urchins are overfished in many of the conventional harvesting countries and the average harvesting size has decreased over time (Sun & Chiang, 2015). Heavy exploitation of sea urchins can lead to population collapse, due to fertilization inefficiency and lack of protection for juveniles under the spine canopy of the adults, which all relates to density effects (Quinn *et al.*, 1993). Harvesting regulations such as a restricted harvesting season or marine protection areas are incentives to develop sea urchin aquaculture with artificial feed (Lawrence, 2007b). Aquaculture of sea urchins can be sea-based or land-based with either larval rearing or gonad enhancement. Larval rearing is of interest in overfished areas where there are few individuals and low biomass remaining. Roe enhancement refers to harvesting of wild sea urchins from areas with high abundance and low gonad indices and feeding the animals for a limited timeframe to make a valuable product. The former can take up to 3 years whilst the later can be achieved in 2-3 months (Walker *et al.*, 2015). This is more economically sustainable because of the operational costs of larval rearing, but also contributes to reforestation of kelp and other macroalgae (Dale *et al.*, 2005).

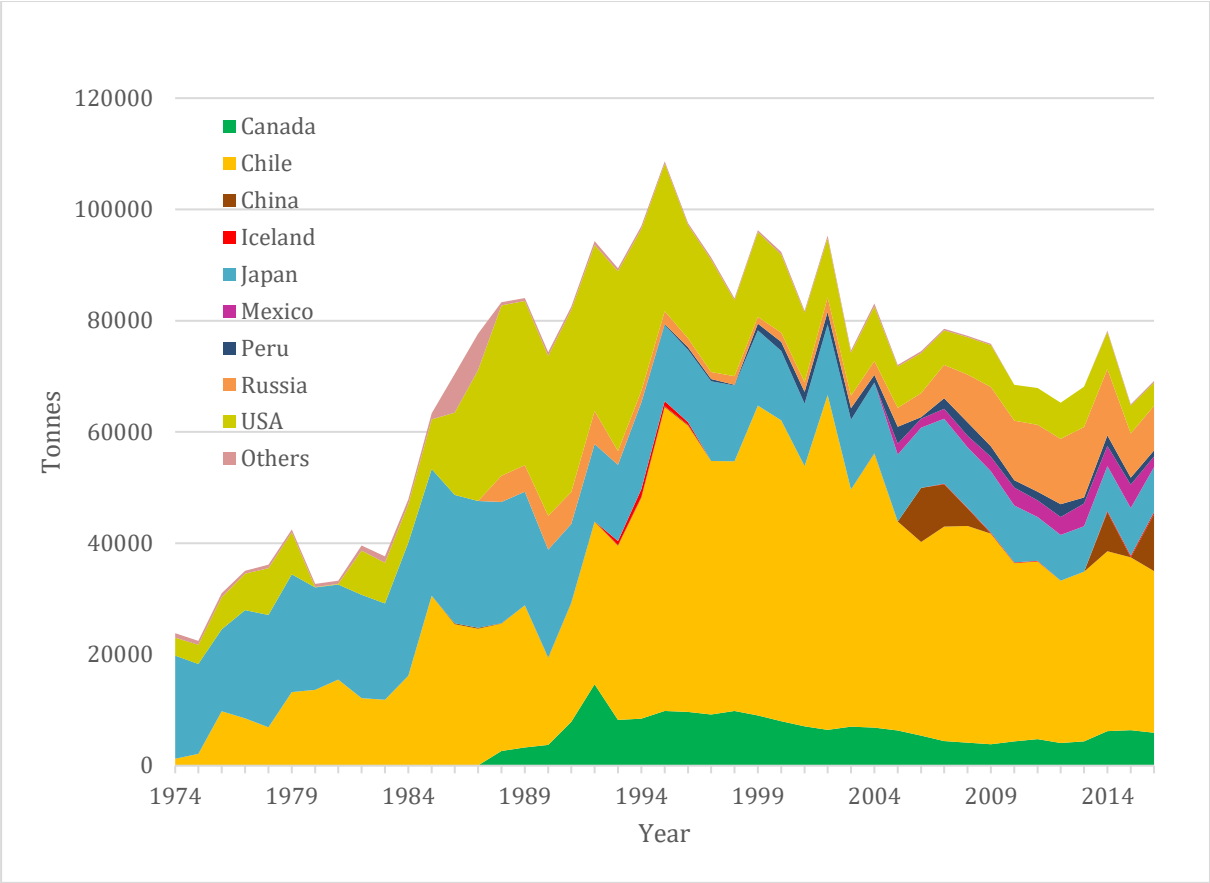


Figure 1: Global landings of sea urchins, capture and aquaculture (FAO, 2018)

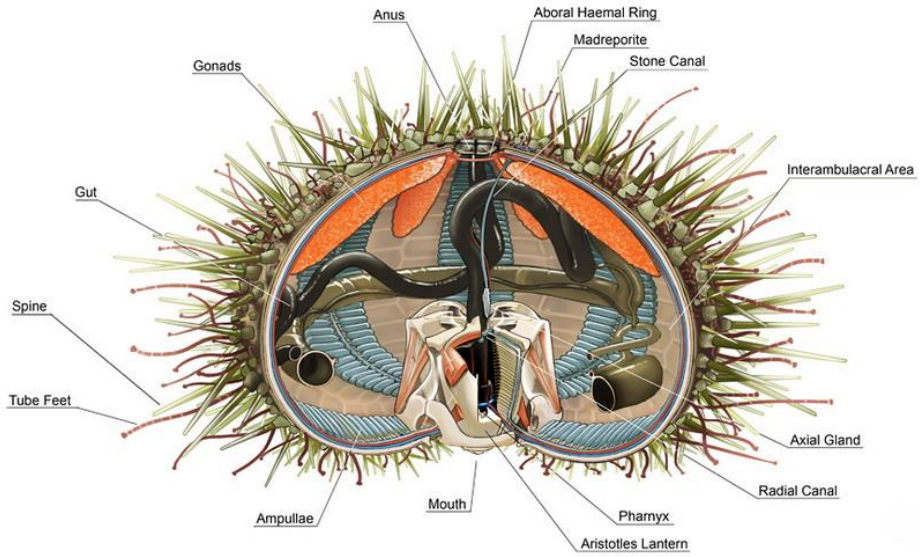


Figure 2: Longitudinal cross section of the sea urchin (*Abiogenisis, 2013*).

1.1 The sea urchin

Sea urchins are members of the marine phylum Echinodermata, which includes brittle stars, sea cucumbers, sea stars, sea lilies and sea urchins. Echinoderm means “skin with spines” as many of the species have spines protruding from the skin. They exist solely in marine environments; no freshwater species has been found yet. The body shape of echinoderms varies, but they possess a pentameric radial symmetry. There are two groups of sea urchins, irregular and regular, where all edible sea urchins are in the latter group (Yokota, 2002). The shell, or test, of sea urchins is globular and consists of separate calcareous plates equipped with spines used for defence and for locomotion. The spines are connected to the test by a joint and circular muscles enable movement. Tube feet, podia, are adhesive tubular structures protruding from the test. They are connected to the intricate water vascular system which constitute a hydraulic system used for gas exchange, sensory input and locomotion. Pedicellariae are located on the test as well and is a stalk-like structure with a claw at the end. They are used for removing debris from the body surface, defence or breaking up particles (Anderson, 2001).

The mouth is located on the flattened underside, while the anus is on the rounded top (Figure 2). Aristotle’s lantern is the mouth apparatus of the sea urchin and consists of five pointed calcareous teeth connected to muscles. The digestive tract is long because of their sedimentary diet, and empties at the top (Anderson, 2001). The five gonads of a sea urchin are the most dominant structure inside the test. These gonads serve as nutrient storage and well-fed sea urchins with large gonads are a valuable food product (Harris & Eddy, 2015).

1.1.1 Gonads as a commercial product

Many sea urchin species are considered edible worldwide, depending on cultural preferences. Taste, size, colour and texture are the main characteristics to assess quality. Pale, brown or light-coloured gonads are the least desirable colours to the market (Robinson *et al.*, 2002). The gonadal index (GI) is the ratio of the weight of the gonad to the wet weight of the whole sea urchin, where a high index means bigger gonads. The GI is at its lowest right after spawning and will increase during summer and fall, depending on species and reproductive cycle (Siikavuopio *et al.*, 2007b). Harvesting season is dependent on gonad yield, and the gonad indices of *S. droebachiensis* in Norwegian waters are highest from October to January (Falk-Pettersen & Lønning, 1983). Sea urchins are an abundant resource, it’s estimated that 80 million green sea urchins are grazing along the northern Norwegian coastline. Some areas of

the coastline are subject to destructive sea urchin grazing of the kelp forest. This creates sea urchin “barrens”, large areas where only sea urchins occur and all macroalgae species have been destructively grazed. These barrens will often result in smaller sea urchins with small gonads (Gundersen *et al.*, 2010). Low gonad yield is a worldwide problem, and commercial fishing is not always economically sustainable. Roe enhancement is a method of capturing wild sea urchins with low gonad yield and feeding them whilst in a holding system, either in sea cages or in land-based facilities. During captivity the gonad will increase to a satisfactory market size (James *et al.*, 2017a). Roe enhancement can make sea urchins commercially viable in Norway under the right conditions (James *et al.*, 2015). The global sea urchin market is accustomed to wild sea urchins. Sea urchin enhancement and aquaculture has so far lacked consistency in regards to quality aspects that the market demands (Sun & Chiang, 2015). The sea urchin market is not saturated, especially in the off-season, and the demand is higher than supply (Stefánsson *et al.*, 2017). The modern Japanese market is expecting year-round supply of seafood, due to growth and popularity of sushi restaurants. The demand of live animals is higher than frozen or processed products, and this reflects in the price of the product. Successful roe enhancing creates opportunities for supplying existing or new markets beyond the traditional harvesting season (Sun & Chiang, 2015)

1.2 Background on roe enhancement

Several studies have been conducted on roe enhancement, both on manufactured and algal diet. A review by Lourenço *et al.* (2018) compared roe enhancement studies from 2000 – 2017. Studies on *S. droebachiensis* regarding nutritional experiments are listed in Table 1. Lourenço *et al.* (2018) concludes that all sea urchins fed pellets easily develop large gonads, but further research must be done on amino acid composition of the feed and carotenoid content in relation to taste and colour.

The texture of enhanced gonads is known to be softer and more granular, but few studies have been conducted on texture of enhanced sea urchins and with different methods for comparing texture and quality. Pearce *et al.* (2002c); Pearce *et al.* (2002b, 2004), Siikavuopio *et al.* (2007a) and S. Takagi *et al.* (2017) used a subjective scale by eye, while McBride *et al.* (2004) used a texture analyser and S. Takagi *et al.* (2018) used a creep meter. There is no consensus on the cause of textural differences between wild and enhanced sea urchins.

A number of studies (Liyana-Pathirana *et al.*, 2002c; Dale *et al.*, 2006; Hammer *et al.*, 2006; Kennedy *et al.*, 2007a; González-Durán *et al.*, 2008; Prato *et al.*, 2018) have looked at biochemical composition of enhanced sea urchins, in conclusion all studies suggests that dietary lipid and protein content will affect gonad lipid and protein content.

The feed used in this study has been developed by Nofima during the last two decades and has been successful in several feeding trials (Table *1*), and is now licensed to Urchinomics who have sublicensed it to Mitsubishi in Japan for larger-scale production (Moren, 2016).

Table 1: Roe enhancement studies of *S. droebachiensis* conducted between 2000-2017 (Lourenço *et al.*, 2018).

Authors	Feed variable	Para-meters	Trial (Weeks)
Eddy *et al.* (2012)	Df protein concentration	G	24
James and Siikavuopio (2012)	Nofima feed	G	9 & 12 months
James and Siikavuopio (2013)	Nofima feed	G	12 & 34
James *et al.* (2017b)	Nofima feed	G	30-32
Kennedy *et al.* (2005)	Df protein sources and concentration	G	280 days
Kennedy *et al.* (2007b)	Df concentration of mineral and pigments	G	154 days
Kennedy *et al.* (2007a)	Df lipid sources and concentration	G, C	40
Liyana-Pathirana *et al.* (2002b)	Grain-based feed	G, C	9
Pearce *et al.* (2002b)	Df binder and concentration	G, C, T	12
Pearce *et al.* (2002a)	Prepared feed, different feeding regime	G, C, T	12
Pearce *et al.* (2002c)	Df protein source and concentration	G, C, T	12
Pearce *et al.* (2003)	Df starch type, macroalgae and β-carotene	G, C, T	12
Pearce *et al.* (2004)	Prepared feed	G, C, T	6
Siikavuopio *et al.* (2007a)	Df protein and carbohydrate	G, C, T	60 days

Df=different, G=growth, C=colour, T=taste

1.3 The green sea urchin *Strongylocentrotus droebachiensis*

The green sea urchin, *Strongylocentrotus droebachiensis*, has a northern boreal-arctic geographic distribution. It occurs from Svalbard, the Barents Sea, sometimes the White Sea all the way east to Taimyr. In the Atlantic it stretches from Scotland, Kattegat, Sweden, Norway up to Iceland, Greenland, Canada and down the east coast of America to Chesapeake Bay in the US. In the pacific it goes from Vancouver Island, Bering Strait, East Siberian Sea to the Sea of Japan. The green sea urchin occurs from 0 to ~300 m in depth, but is usually found between 0 and 50 m (Jensen, 1974; Scheibling & Hatcher, 2007). They are mainly found on hard substrates like boulders and on cobble. They also occur on soft substrate, but in exposed areas they are prone to be crushed by stones thrown around by turbulent water, especially the smaller sea urchins (Himmelman, 1969). Sea urchins are often associated with *Laminaria* kelp and can often destroy the kelp bed when the sea urchin biomass reaches a critical point, and thus create a barren (Figure 3). A wave-front is often seen in the edge of the sea urchin community at the end of the kelp bed (Lang & Mann, 1976). This grazing front is often formed by adult sea urchins, while the barren is populated by juveniles (Meidel & Scheibling, 2001). Other feeding strategies include aggregation in refuges and relying on drift algae, or browsing individuals in low density communities in the rear of the barren surviving on remnants left after the grazing front (Scheibling & Hatcher, 2007).

Figure 3: A typical barren scene of *Strongylocentrotus Droebachiensis* Photo: Hartvig Christie, NIVA

The green sea urchin is an omnivore and feeds on algae, invertebrates, mussels and other food sources it can scavenge (Mann *et al.*, 1984; Sebens, 1985; Briscoe & Sebens, 1988), but if food is abundant they will select food with high nutritional value within the given habitat and often prefers brown macroalgae such as Alaria, Chordaria, Laminaria and Petalonia. Food preference is a complex response to abiotic and biotic factors, such as environmental causes and nutritional status. Antinutrient, ash and acid content can affect the feeding behaviour of sea urchins (Scheibling & Hatcher, 2007).

1.3.1 Gonadal wall

The gonad is covered by the ciliated perivisceral peritoneum (Figure 4), this layer is found on all dermis of the sea urchin. These cells have microvilli which protrude into the coelom. These microvilli increase the surface area of the collar cells and most likely contribute to nutrient uptake (Walker, 1979). Under the peritoneum there are fibroblasts and collagen fibres, which is the collagenous layer. The next layer is smooth muscle which contracts during spawning, and the genital sinus can be voluminous at that time. The hemal space is constituted of collagenous cells and hemal fluid. The innermost layer is the germinal epithelium and consists of either gametes or nutritive phagocytes (Pearse & Cameron, 1991).

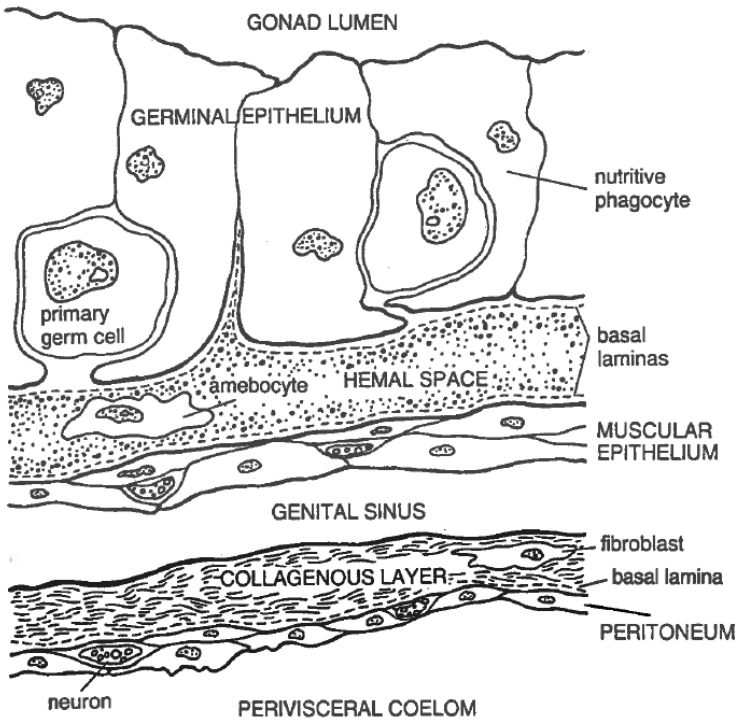


Figure 4: Gonadal wall of sea urchins (*Pearse & Cameron, 1991*)

1.3.2 Reproductive cycle and gametogenesis

Gametogenesis and nutrient storage are linked processes in the reproductive cycle. The gonad is made up by nutritive phagocytes (NP) and reproductive cells. During the annual cycle this ratio changes (Figure 5), both in number and size of the cells. Reproductive cells in the ovary are oogonia, oocytes and ova, whereas in the testes, they are spermatogonia, spermatid and spermatozoa (Walker *et al.*, 2007). The NPs serve as a nutritive source and structural support for reproductive cells during the reproductive cycle, and they will provide the nutrient requirements of reproductive cells at different gametogenic stages (Walker *et al.*, 2005).

According to Walker et al. (2007) gonadal development happens in four different stages. Stage 1 – *Inter-gametogenesis and NP phagocytosis* – occurs in the spring, after spawning, for 2-3 months and the acini are filled with NPs. Ovaries contain residual oocytes and have a messy appearance. Testes may or may not contain some residual spermatozoa but appear otherwise empty. The NPs may recycle the residual reproductive cells for nutrients. Stage 2 – *Pre-gametogenesis and NP renewal* – occurs during summer for 3-4 months. A new generation reproductive cells appear in the periphery of the acini and will increase in size. NPs start to accumulate lipids, carbohydrates and proteins and increase in size. Stage 3 – *Gametogenesis and NP utilization* – occurs in late autumn for around 5 months. Utilization of nutrients from NPs has begun and gametogenesis begins. The reproductive cells migrate to the middle of the acini. For a while NPs can still take up nutrients while at the same time deliver nutrients to the growing reproductive cells. Stage 4 – *End of gametogenesis, NP exhaustion and spawning* – occurs in late winter and lasts for 2-3 months. At this stage the NPs are at their smallest and the middle of the acini is filled with mature gametes, ova and spermatozoa. The next step is spawning.

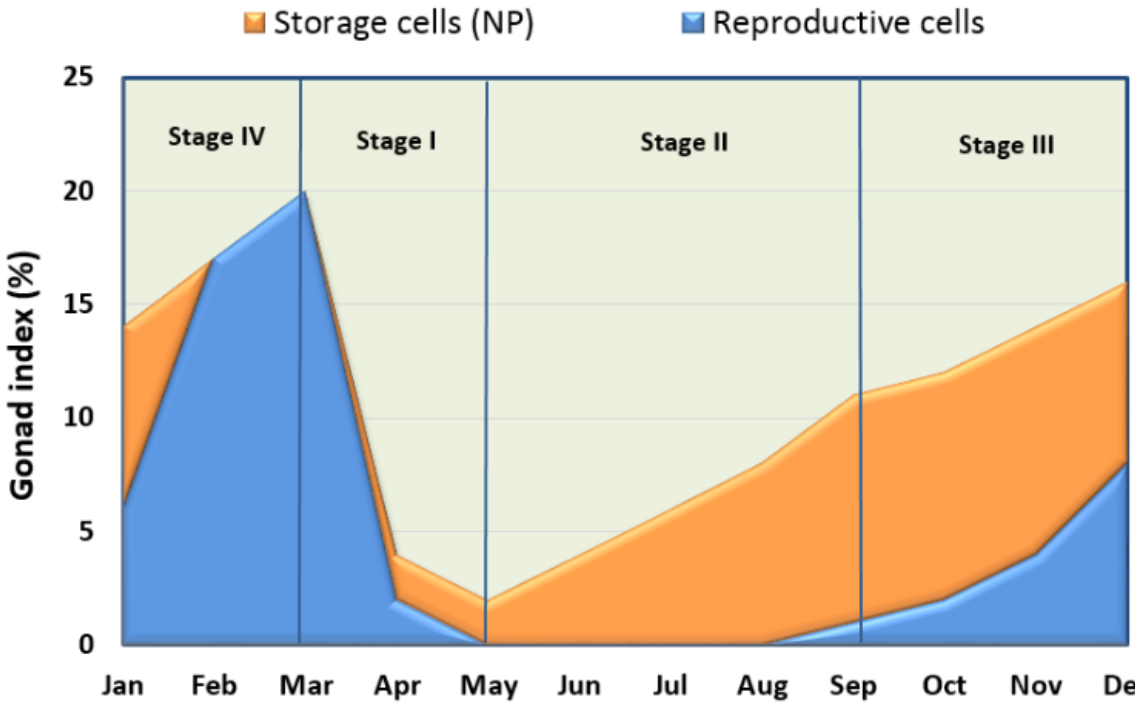


Figure 5*:* Typical reproductive cycle of the green sea urchin *(Strongylocentrotus droebachiensis).* Peak GI before spawning in spring. Gradual restoration of GI during summer, with an increase of nutritive phagocytes. (James *et al.*, 2018).

1.4 Composition

1.4.1 Amino acids

Proteins are formed by a total of 20 different amino acids in long chains. There are non-essential and essential amino acids, where the latter must be supplied in the diet of the organism because they cannot be synthesized in the body. For sea urchins the essential amino acids are threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, lysin, histidine and arginine (Fong & Mann, 1979). Deficiency of essential amino acids can lead to limited growth and protein synthesis, it is therefore important to include the right qualitative and quantitative amount of amino acids in the diet to promote health and growth. Amino acids are important for the taste of the gonad and the content will vary throughout the year, depending on season (Liyana-Pathirana *et al.*, 2002b).

1.4.2 Fatty acids

Fatty acids are a crucial item in an organism because they are an essential part of the cell membrane, energy reserve and for transport of fat-soluble substances necessary in the metabolism and physiological processes. (Blanco & Blanco, 2017). Some fatty acids are

essential and must be supplied in the diet, such as linoleic acid (C18:2 n-6) and DHA (C22:6 n-3). Sea urchins use lipids as an energy reserve during long periods with poor feed availability and season and dietary lipid will affect gonadal lipid content (Liyana-Pathirana *et al.*, 2002c). Lipids are known to influence the taste properties of the gonad by aromatic effects and flavour masking, but also oxidation of long chain polyunsaturated fatty acids which results in a putrid taste (Liyana-Pathirana & Shahidi, 2003).

1.5 Aim of study

The objective of this study is to compare quality aspects between wild caught and enhanced sea urchins and to establish how manufactured feed impacts the roe. The parameters used are: gonad index (GI), water content, reproductive development, histological examination of relative cellular area and morphology, amino and fatty acid analysis and scanning electron microscopy (SEM) over an experimental period of 14 weeks.

2 Materials and methods

2.1 Collection and holding systems

Sea urchins were collected by a SCUBA diver at Kvalsund (N69.82086, E19.01914), Tromsø in mid-August 2018 (week 0) (Figure 6). A total of 250 animals were collected into catch bags from the subtidal region and were placed in large plastic tubs and covered to avoid exposure to air during transport. They were transported to the nearby Kårvika research facility (10 minutes by car), where they were distributed to three raceways (Figure 9) and held for up to 14 weeks. Wild sea urchins were also collected at two-week intervals, respectively at week 4, 6, 8, 10 and 12 for comparison between the wild and enhanced population, the final sample was collected in mid-November. Sea urchins with a diameter close to 50 mm and above were selected to minimize any size differences and subsequent size dependant roe enhancement effects (min 41.60 mm; max 72.58 mm; mean 50.76 mm). After 13 weeks gonad samples from both wild and enhanced sea urchins were taken for scanning electron microscopy and biochemical analysis.

The raceways had perforated bottoms and internal walls forming compartments (12 x 10 x 15 cm) allowing individual storage for each sea urchin in flow through seawater. The reared sea urchins were held at ambient water temperature (average of 9.6°C) and 24-hour photoperiod under standard fluorescent lamps. Temperature was measured daily (TFX 410 laboratory

Figure 6: Collection site of sea urchins in Kvalsund outside Tromsø (Google, n.d.)

thermometer, Ebro) and oxygen (Handy Polaris, Oxyguard) levels weekly. Twice a week they were fed Nofima sea urchin roe enhancement formulated feed pellet. Only one sea urchin died during the experiment.

2.2 Water temperatures

The sea water temperature was measured at 0.2- and 1-meter depth at the collection site in Kvalsund for the last three censuses (week 8, 10 and 12). The temperature in the holding tanks at Kårvika research station was measured every day in all three tanks. The water temperature at the collection site (Figure 7) started at 9°C in mid-October and ended at 7.5°C in late November, with a mean of 8.2°C for the experimental period. The water temperature at Kårvika research station (Figure 8) started at 10°C in mid-August and increasing till mid-September and ended at 8°C in late November, with a mean of 9.6°C for the experimental period.

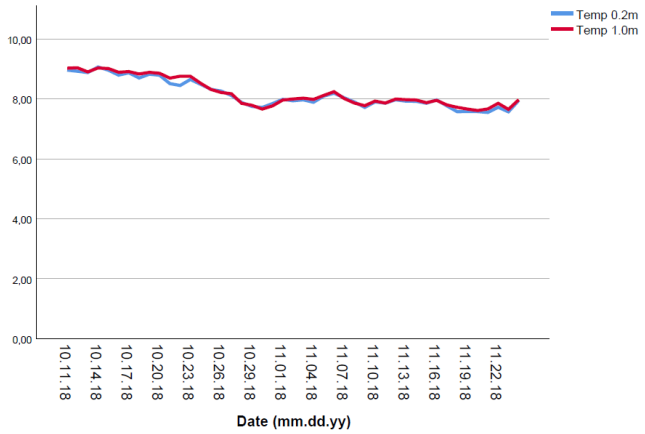


Figure 7: Water temperature (°C) at 0.02 and 1 m at the collection site in Kvalsund.

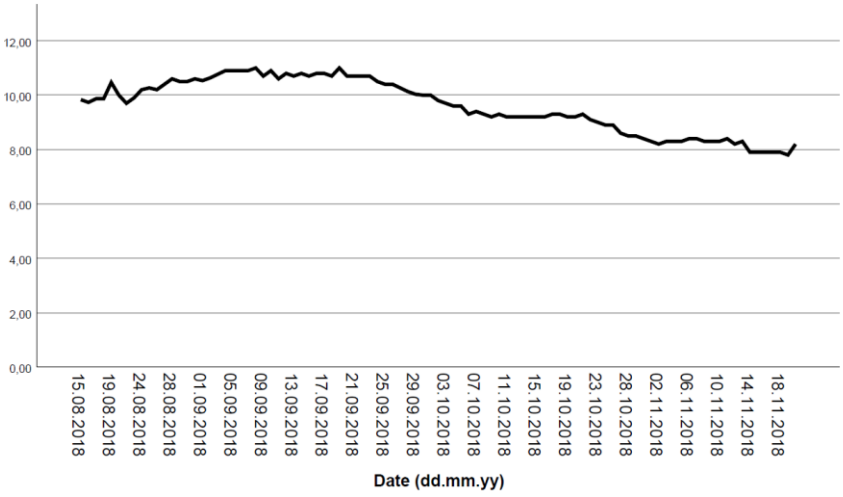


Figure 8: Water temperature (°C) at Kårvika research station.

2.3 Protocols

The experiment ran for 14 weeks. 30 animals were sampled from the initial 250 animals to establish a baseline (initial sample). The test diameter (TD) and gonad index (GI) was measured on all 30 animals. A transverse section of the gonad was removed for histology and fixed in 10% buffered formaldehyde. After 4 weeks the first sample was conducted. Enhanced sea urchins (n = 20) were taken from the experimental holding system, and wild animals (n = 20) were collected at the same time and from the same collection site in Kvalsund as the initial sample. The TD and GI were measured for and histology samples were collected from each sea urchin. After the first four weeks (census 1), sampling was conducted every second week in the same manner, except from the final sample (census 5), when a sample of 30 animals from each group (enhanced and wild) was collected.

The wet weight of whole animals was measured on a digital scale (0.01 g accuracy) to get the wet weight, then size was measured at the widest point using electronic verniera calipers to get the TD. A specialized tool (Figure 10b) was used to crack the sea urchins open and the gonads were removed, weighed and cut for histology. This specalized tool has an arrowhead shaped end, which is inserted in the mouth of the sea urchins and a spring mechanism will crack the test open when force is applied on the handle.

Figure 9: Raceway at Kårvika research station

Figure 10: Sampling of sea urchin gonads: (a) measuring the test diameter of the sea urchin; (b) opening the sea urchin using a specialized tool; (c) extracting the gonads and weighing them; (d) draining the gonads with a sieve; (e) the tip of the gonad in a histology cassette; (f) gonads in pre-weighted aluminium cups for drying.

The gonads were drained in a sieve to remove excess water and placed in pre-weighted aluminium cups and dried to constant weight in an oven at 70°C for 48-64 hours (Figure 10). The samples were weighed after drying to calculate the water content.

Gonad index and water content was calculated using these fomulae

$$GI\% = \frac{gonad\ wet\ weight}{whole\ urchin\ wet\ weight}\ X\ 100$$

$$Gonad\ water\ (\%) = \frac{(gonad\ wet\ weight - gonad\ dry\ weight)}{gonad\ wet\ weight}\ X\ 100$$

At week 13 (census 6), 20 animals from each group (wild and enhanced) were opened and the gonads were put into pottles containing 10 individuals each (pooled sample), in total four pottles, and sent off for biochemical analysis to Nofima Biolab (Bergen). At week 14 (census 7) four animals from each group were opened and the tip of one gonad was sampled for scanning electron microscopy (SEM).

2.4 Histology

All the samples were fixed in 10 % buffered formaldehyde and then stored until the end of the experiment in a 4°C refrigerator. They were transferred into 70% ethanol and stored for at least 24 hours until further processing (Table 2) in a tissue processor (Citadel 2000, Thermo Scientific). The samples were blocked (EG1150G, Leica biosystems) in wax (Sigma-Aldrich, USA) and chilled on a cooling plate (CP-4, Axel Johnsen Lab System) until fully hardened. The hardened blocks were stored in room temperature until further processing.

The blocks were sectioned at 5 µm thickness (RM2255, Leica biosystems) from ~1/3 – 2/3 into the embedded tissue, then transferred to microscope slides via water bath at 43°C. One slide, containing 2-3 sections, was prepared for each individual sample. Brittle blocks were treated pursuant to the Leica biosystem guide to no success (Rolls, 2016). The slides were placed overnight in a 63°C oven.

To remove excess paraffin wax, the slides were submerged in Histoclear (National Diagnostics Inc, UK) for at least four minutes before staining with the haematoxylin and eosin method using a 16-step Leica ST4020 machine (Table 3). Haematoxylin is a basic dye and colours acidic structures purple, while eosin is an acidic dye and colours basic structures pink (Young *et al.*, 2013). After staining, the cover glass was mounted on the slides with a non-water-soluble mounting medium (Eukitt, Sigma-Aldrich, USA). The slides were dried at room temperature overnight before further analysis.

Table 2: Dehydration steps Citadel 2000

Tray nr.	Content	Time
1	Empty	
2	Empty	
3	96 % EtOH	2 hours
4	96 % EtOH	2 hours
5	100 % EtOH	2 hours
6	100 % EtOH	2 hours
7	1:1 100% EtOH and Histoclear	1 hour
8	Histoclear	1 hour
9	Histoclear	1 hour
10	Histoclear	1 hour
11	1:1 Histoclear and paraffin wax	1 hour
12	Paraffin wax	min. 2 hours

Table 3: Dewaxing and staining in a Leica ST4020 (90 sec in each container)

Container	Content	Container	Content
1	Histoclear	**8**	Water
2	100% EtOH	**9**	Water
3	96% EtOH	**10**	Eosin
4	96% EtOH	**11**	96% EtOH
5	Water	**12**	96% EtOH
6	Haematoxylin	**13**	100% EtOH
7	Haematoxylin	**14**	100% EtOH
8	Water	**15**	Histoclear

The slides were photographed under a Nikon Eclipse Ci at 4X and some slides at 10X magnification. During photography the sex and reproductive stage was determined for the individuals using the four reproductive stages by Walker *et al.* (2007). The total area of the acini on the cross sectioned gonads was measured using thresholding of coloured areas (different saturation). Unwanted areas (noise) and white space were removed manually if necessary. Reproductive cells were selected by thresholding at a different saturation to remove the lighter nutritive phagocyte and non-tissue areas. Corrections were made manually if necessary. All measurements of each histology slide were exported to an Excel-sheet and the mean cell and reproductive area was calculated from all observations. The nutritive phagocyte area was calculated by subtracting reproductive cell area from total tissue area (Figure 11).

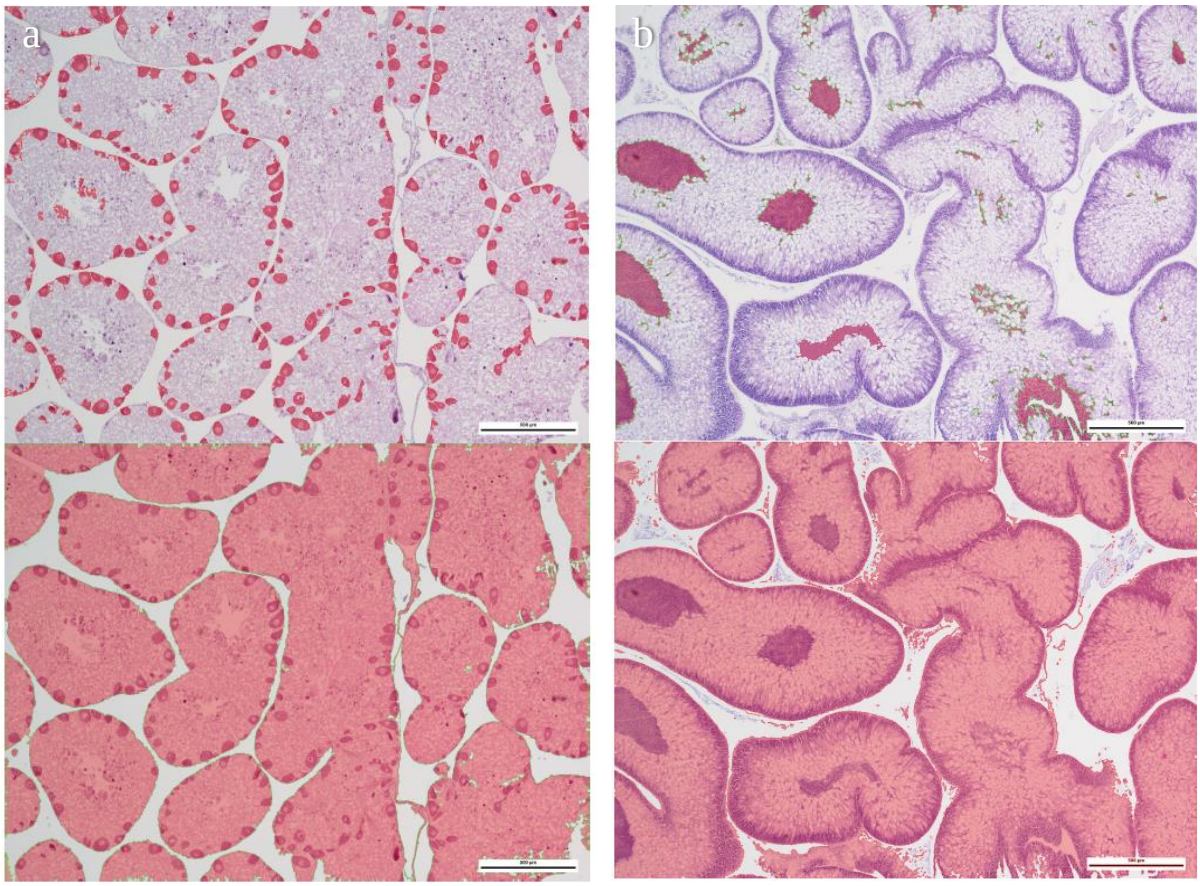


Figure 11: Cross section of gonad, top picture with area (red) of reproductive cells and bottom picture with total cell area: (a) female; (b) male. *Bar: 500 µm.*

2.5 Messiness index of gonad structure

No staging system has been developed before to grade the messiness of gonads of sea urchins. To measure the messiness of the gonad, a system was created on a scale from 1-3 (Figure 12) to quantify the messiness. The overall shape, clean edges and how hard it was to distinguish individual acinus were the key parameters. All histology slides were scored according to this system.

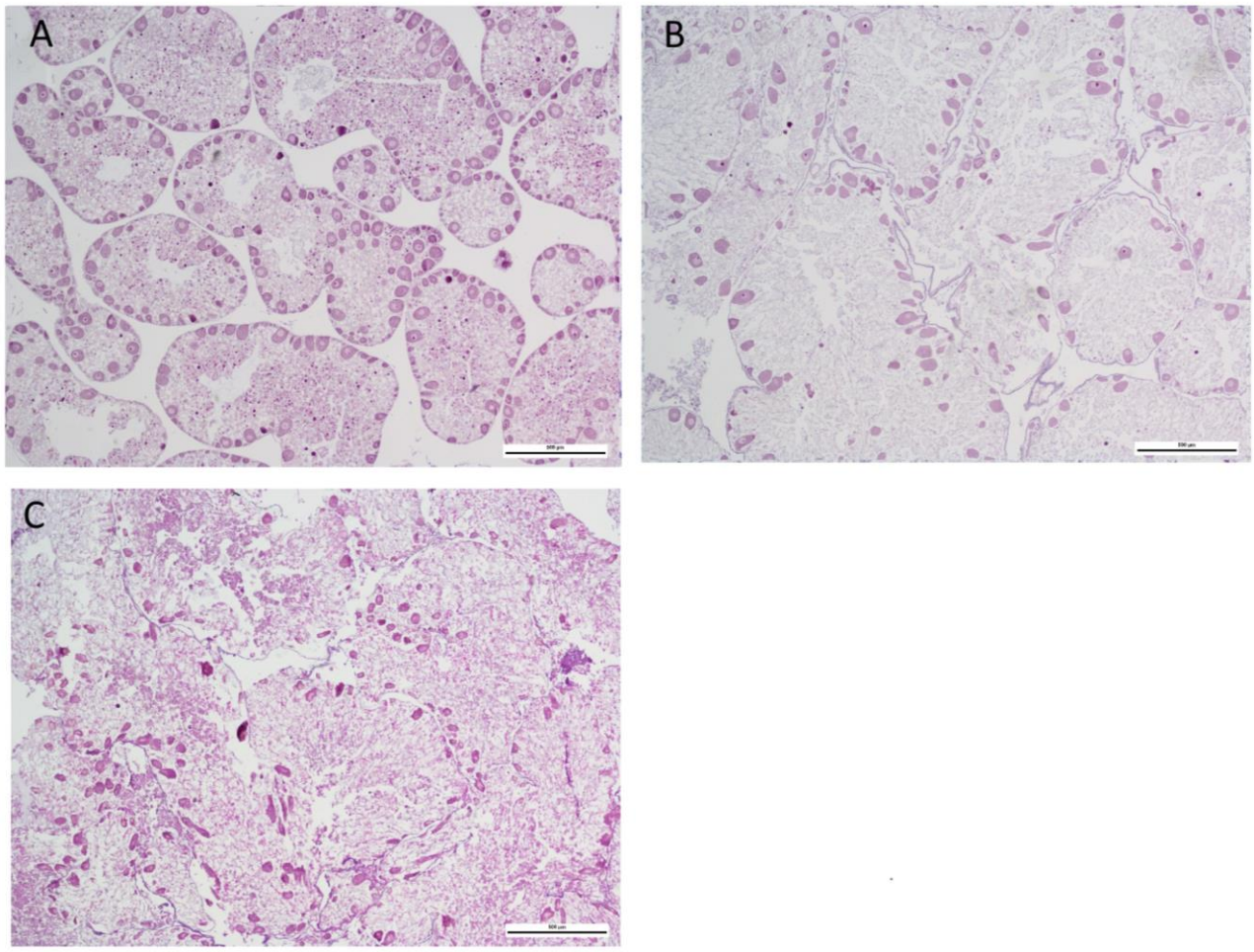


Figure 12: Cross section of female gonad. (a) score 1; distinctive acini, clean edges, (b) score 2; more messy appearance, harder to distinguish acini, edges still definable, (c) score 3, no order, acini not distinguishable, no clean edges. Bar: 500 µm.

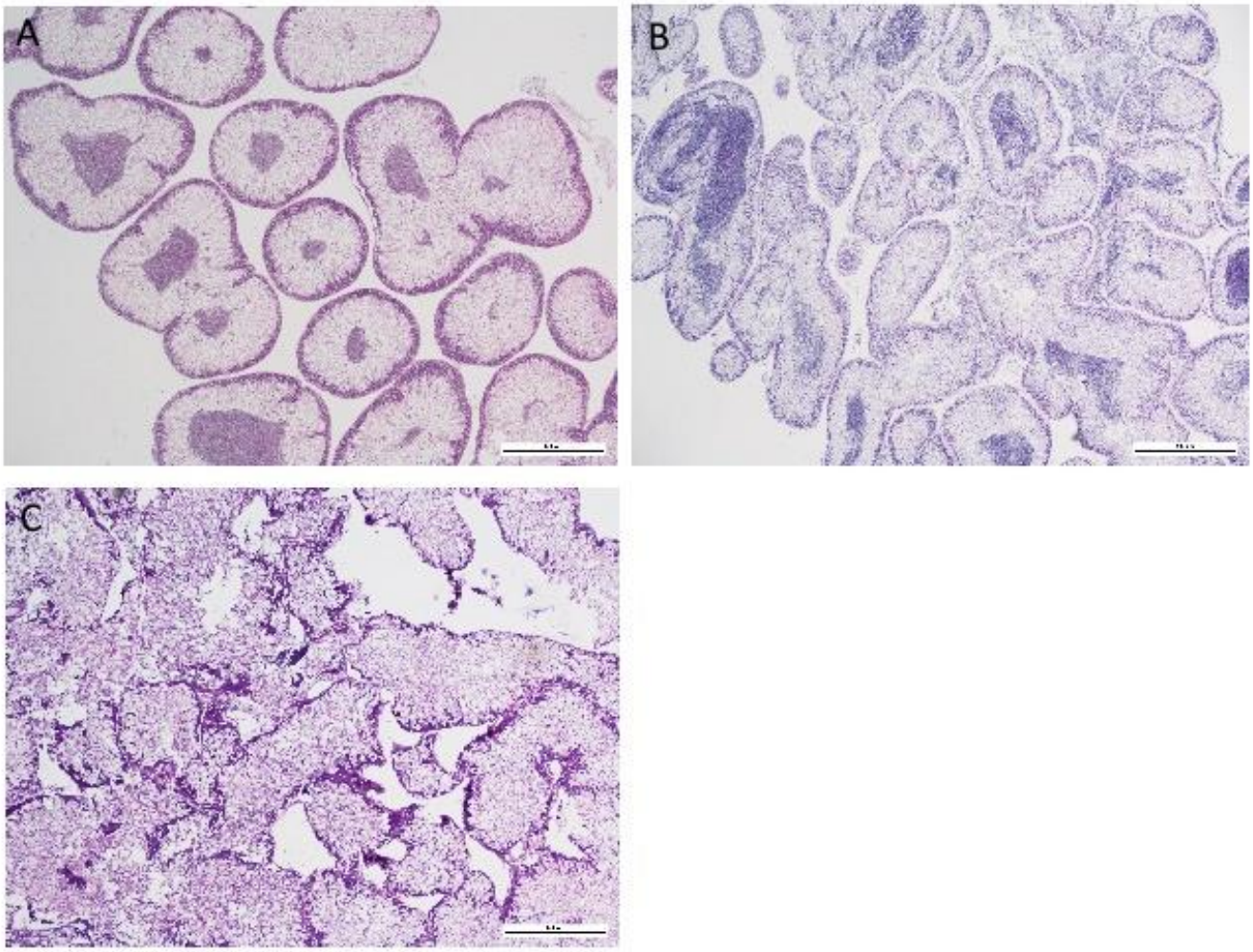


Figure 13: Cross section of male gonad. Same scoring as in Figure 12. Bar: 500 µm

2.6 Software and statistical analysis

The histology slides were captured and analysed with NIS-elements Basic Research v. 4.50.00 from Nikon.

Fishstat J was used to determine the global aquaculture and landings of sea urchins from the FAO database v.2018 1.0. The filtering used for species was “Chilean sea urchin”, “European edible sea urchin”, “Sea urchins nei”, “Sea urchins, etc. nei” and “Stony sea urchin” and the top 10 countries from 2016 were selected and everything else grouped as “Other”.

All the data was processed in RStudio version 1.1.456 (R Core Team, 2018) using the ggplot2 package (Wickham, 2016) for graphing. A Shapiro-Wilk test was used to evaluate normal distribution, and probability plots were used to visually inspect the dependent variables normality and homogeneity of variance (Appendix B). The difference between groups was significant if $p<0.05$. The effect of the diet was analysed using two-way analysis of variance (ANOVA) with type, week and their interaction type X week as factors for gonad index, and a three-way analysis of variance with week, type, sex, with interactions (week)(sex), (week)(type), (type)(sex) and (sex)(week)(type) as factors for reproductive cell area. Backward iteration was applied to the ANOVA to remove insignificant factors and increase the fit to the model, using the “olsrr” package in R (Hebbali, 2018).

If an ANVOA with significant interactions occurred, a post hoc test was applied, Tukey HSD for samples with the same number of individuals and Scheffé test if the sample sizes were unequal. A Fisher-Freeman-Halton test was used to test for differences in reproductive stages between enhanced and wild sea urchins from each two-week sample, and the following sample (e.g. week 6-8). Stages 3 and 4 were pooled due to small sample sizes. Fisher-Freeman-Halton was also utilized to analyse for differences in messiness of the gonad structure.

3 Results

3.1 Initial sea urchin sampling

The baseline variables of the initial wild sampling are listed in Table 4.

Table 4: Mean (± 1 SD) initial variables of the wild sea urchin sample

	Initial sea urchin
Test diameter (mm)	46.47 ± 3.61
Wet weight (g)	43.19 ± 13.85
Gonad weight (g)	2.63 ± 1.80
Gonad index (%)	6.11 ± 3.07
Sex	53% male / 47% female
Reproductive stage	21% stage 1 / 52% stage 2 / 26% stage 3
Relative area of reproductive cells	7.42 ± 6.61
Relative area of nutritive cells	92.58 ± 6.61

3.2 Sea urchin gonad index (GI)

Diet significantly affected GI of the enhanced sea urchins (Figure 14). Mean GI was significantly different from the initial sampling to all subsequent sampling of the enhanced sea urchins (ANOVA: $F_{5, 134}$=201; p<0,001). Mean GI of the initial sampling was not significantly different from the wild population in all subsequent sampling ($F_{5,134}$=1.25; p=0.29). After four weeks the enhanced sea urchins had a significantly higher GI than the wild. At week 6 and 8 the enhanced sea urchins increased their GI significantly. Between week 8 and 10 the GI did not increase significantly, neither between week 10 and 12. But there is a significant difference between week 8 and 12.

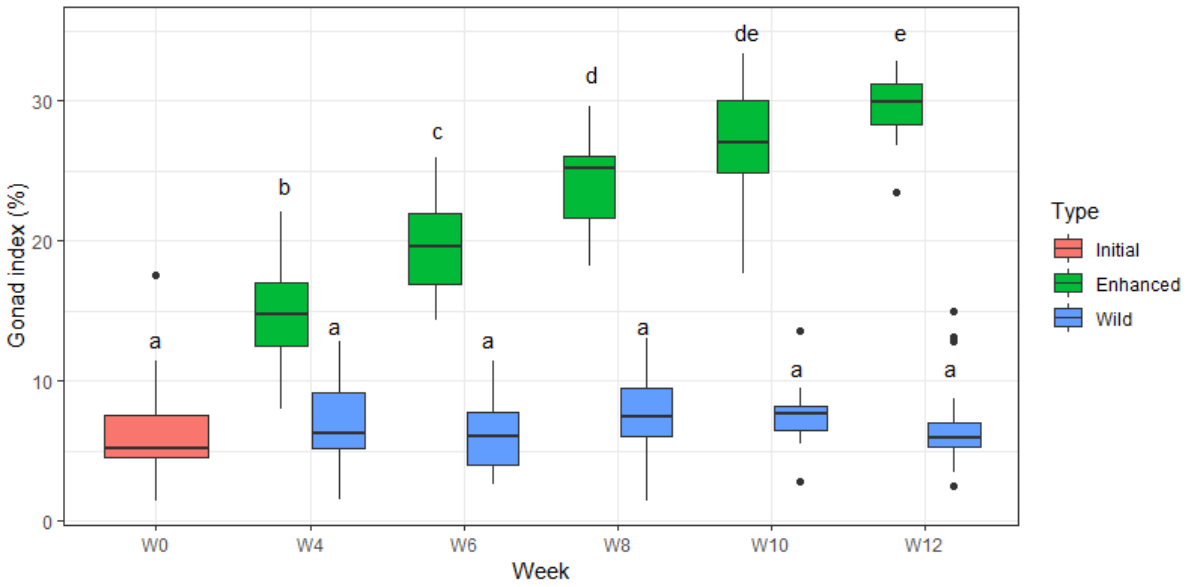


Figure 14: Mean GI of the initial sample, the enhanced sea urchins and the wild sea urchins. Letters above bars indicate a Tukey HSD test, different letters show significant differences between the 11 samples (P<0.05).

3.3 Gonad water content

Mean percent gonad water is shown in Figure 15. There is a significant difference between the sampling time, i.e. weeks, (ANOVA, F=23.14, p<0.001), but not between wild and enhanced within the same week.

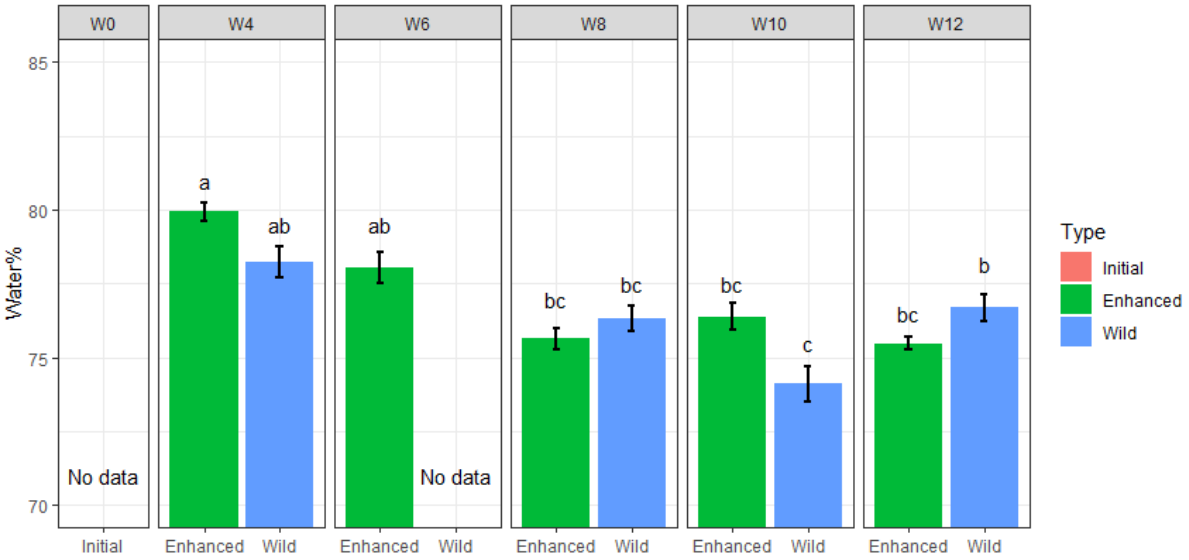


Figure 15: Mean water percentage of gonads. Letters above bars indicate a Tukey HSD test, different letters show significant differences between samples (P>0.05). Bars indicate standard error.

3.4 Reproductive stage

Histological examination shows differences in the reproductive stages between wild and enhanced sea urchins. The examination of the initial sea urchin gonads showed that the gonads were mostly inter-gametogenic (stage 1) or pre-gametogenic (stage 2). The testes contained residual spermatozoa or a new generation of reproductive cells in the periphery, and the ovaries had residual oocytes and some new reproductive cells in the periphery. During the experimental period the wild sea urchins developed to gametogenesis and NP utilization (stage 3). The primary oocytes continue to enlarge and migrate to the lumen while the NPs decrease in size. The testicular epithelium develops a distinct dark layer of spermatogonia and spermatozoa fills the testicular lumen. Ovaries and testes of enhanced sea urchins was less developed at the end of the experimental period, with a majority of individuals either at stage 1 or stage 2, showing the same maturation stage as the initial sea urchins.

Over the sampling period the wild sea urchins advanced through the reproductive stages, and at week 12 none of the wild sea urchins were at stage 1 (Figure 16). The Fisher-Freeman-Halton test showed significant differences of the reproductive stage between the wild and enhanced starting at week 8 ($p<0.01$), no significant difference at week 10, but again at week 12 ($p<0.001$).

The differences of the wild and enhanced sea urchins are tested as frequencies of different stages at different censuses (Figure 16) and are listed in Table 5. Week 12 of the wild sea urchins was significantly different from all the other samples. Week 10 of the enhanced sea urchins was significantly different from all weeks, except week 12.

Table 5: Statistical differences (p-values) between reproductive stages of (a) wild and (b) enhanced sea urchins from week 4 to week 12

a	W4	W6	W8	W10
W4				
W6	0.61			
W8	0.02	0.03		
W10	0.13	0.26	0.70	
W12	<0.001	<0.001	0.03	<0.01

b	W4	W6	W8	W10
W4				
W6	0.59			
W8	0.20	0.53		
W10	0.01	0.05	0.01	
W12	0.39	1	0.48	0.08

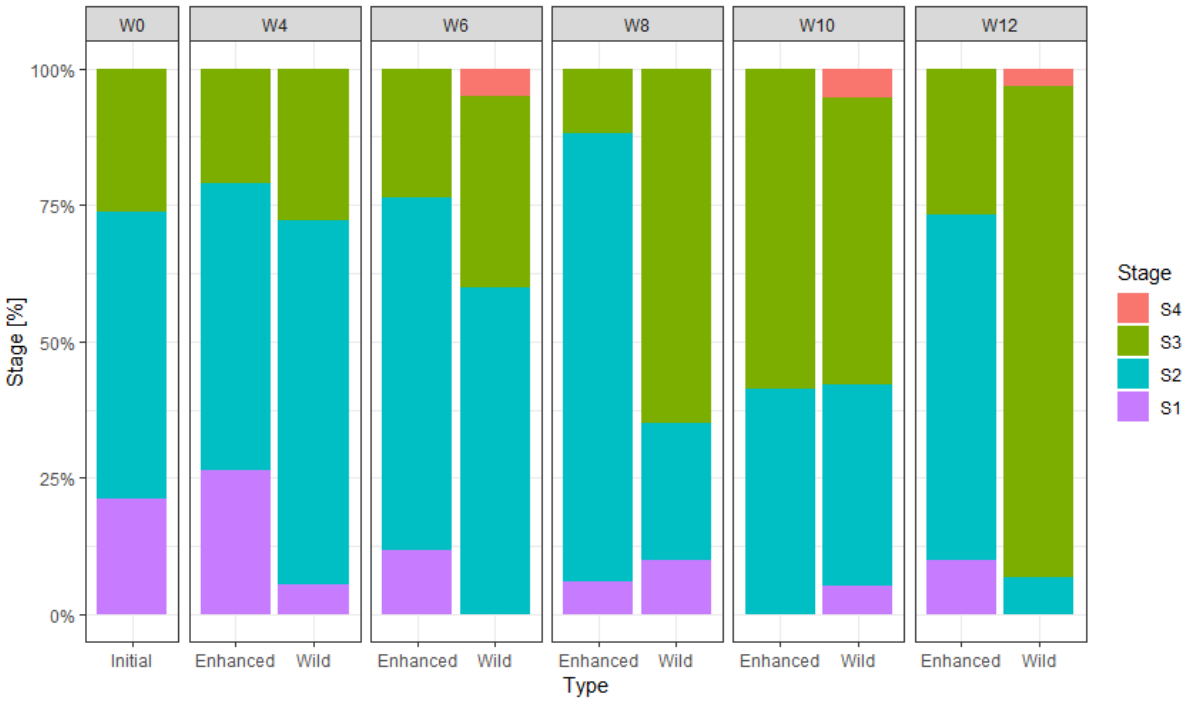


Figure 16: Proportion of reproductive stages (1-4) from the initial sampling (week 0), week 4 and every two weeks for 12 weeks for enhanced and wild sea urchins.

The wild sea urchins at week 12 were significantly different from the initial sea urchins ($p<0.001$), meaning that there was a significant progress in reproductive stages during the period. There was no difference between the initial sampling and week 12 of the enhanced sea urchins.

The male sea urchins appear to be more disparate in the distribution of reproductive stages (Figure 17). Week 4, 6, 8 and 12 of the male enhanced sea urchins have three different stages within the same census. The male wild sea urchins have three different stages at all weeks and week 10 shows four different stages. At week 12 over 90% of male wild sea urchins have developed beyond stage 2. The female sea urchins have only two different stages at all weeks for both wild and enhanced sea urchins. Stage 1 of females only appears for the initial sample and week 4 for enhanced sea urchins. The female enhanced sea urchins are 100% at stage 2 after 12 weeks, while over 90% of wild sea urchins have developed to stage 3. No female sea urchins were staged at 4 of either wild or enhanced sea urchins.

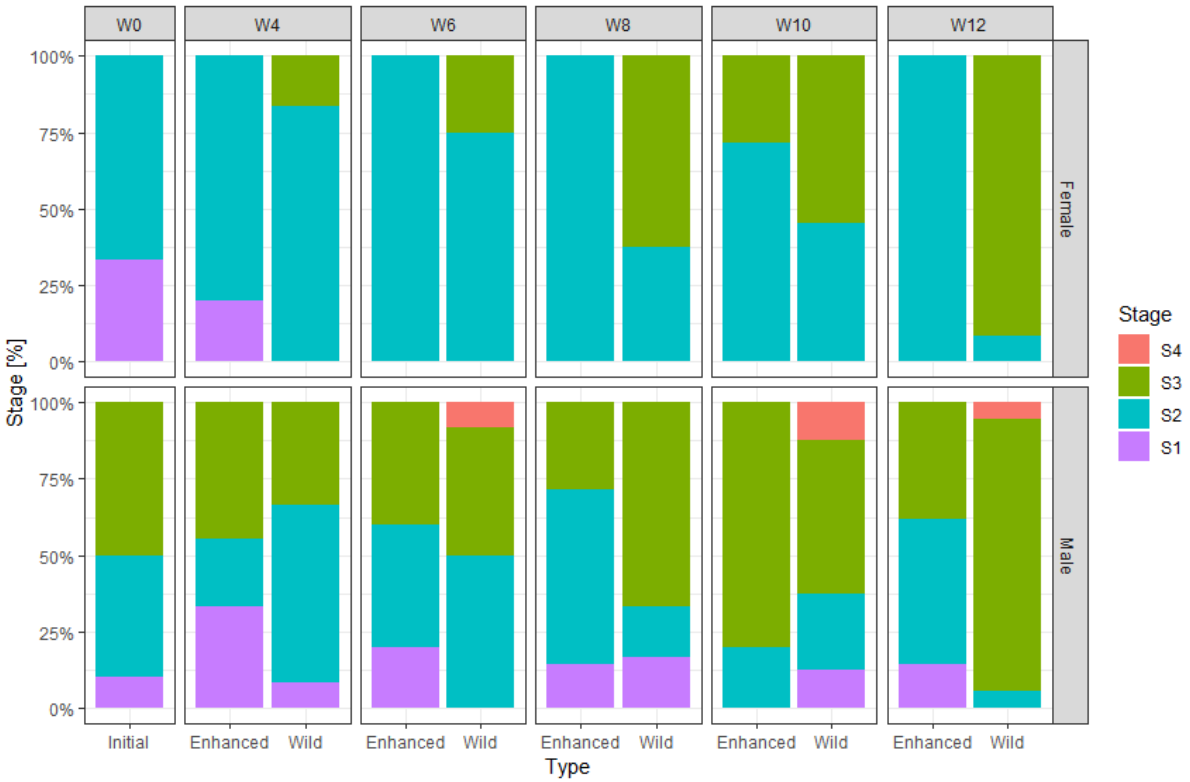


Figure 17: Proportion of reproductive stages (1-4) from the initial sampling (week 0), week 4 and every two weeks for 12 weeks for male and female sea urchins.

3.5 Gonad relative area

Histological examinations show differences in the cellular composition of the gonads from wild and enhanced sea urchins. The mean relative area of reproductive cells was 4.81% for enhanced sea urchins and 21.9% for wild sea urchins at week 12 (Figure 18). There was significant difference between treatments ($F_{1,213}$=59.50; p<0.001) and between sex ($F_{1,213}$=13.53; p<0.001). There was also a significant effect of week and treatment on gonad relative area ($F_{4,213}$=4.33; p<0.01). The enhanced sea urchins are not different from the initial sample, but the wild sea urchins are different from the initial sea urchins starting at week 10. The enhanced sea urchins are significantly different from their wild counterparts at week 8 (p<0.05) and week 12 (p<0.001).

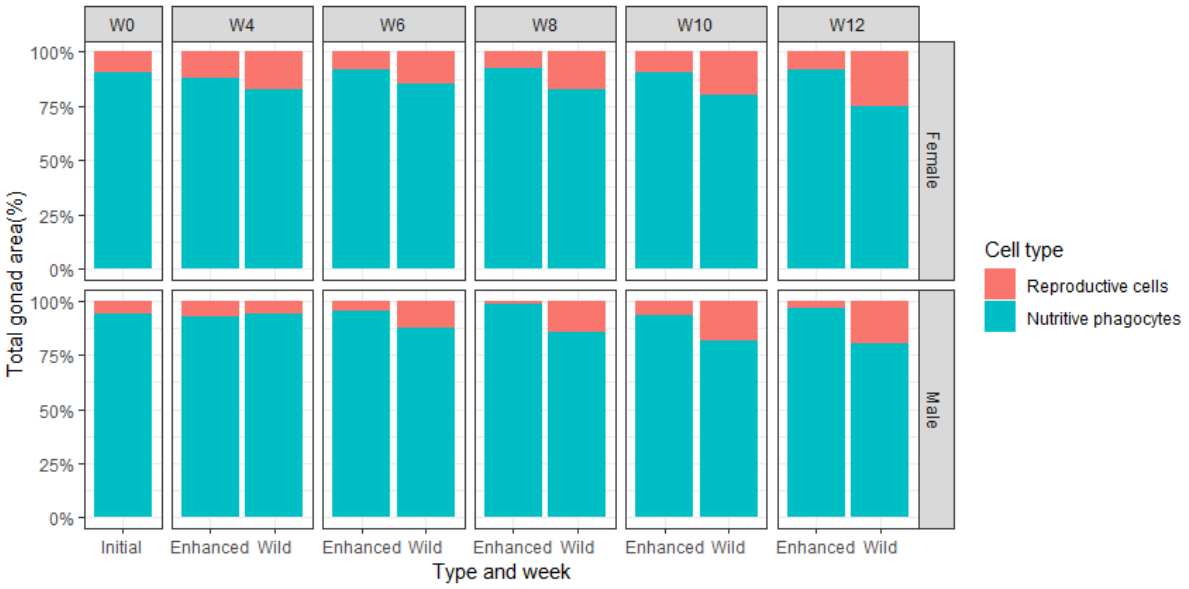


Figure 18: Mean relative gonad area of female and male sea urchins that were either enhanced or wild captured during a period of 12 weeks.

3.6 Biochemical analysis

The amino acid composition of the wild and enhanced sea urchin gonads is shown in Figure 19. Lysine, leucine and glycine were the amino acids with biggest differences between enhanced and wild sea urchins, with respectively 35.2%, 55.8% and 34.0% difference. The essential amino acids, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine were present in both groups, with 3.44 g/100g sample in total for the wild and 4.12 g/100g sample for the enhanced, representing 38.6% and 44.5% of total amino acid content, respectively. Total amino acid content for enhanced sea urchins was 9.26 g/100g sample, and 9.18 g/100g sample for the wild sea urchins

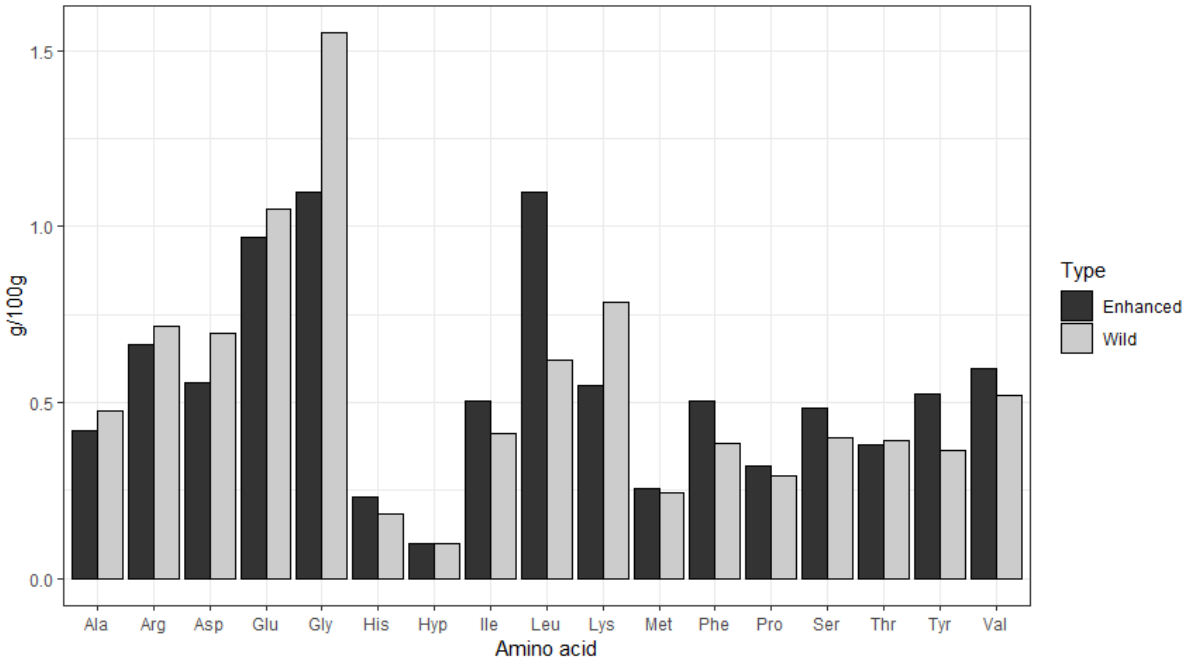


Figure 19: Amino acid composition of sea enhanced and wild sea urchins at week 13 Ala; alanine, Arg: arginine, asp; Aspartic acid, glu; glutamic acid, gly; glycine, His; histidine, Hyp; hydroxyproline, Ile; isoleucine, Leu; leucine, Lys; lysine, Met; methionine, Phe; phenylalanine, Pro; proline, Ser; serine, Thr; threonine, Tyr; tyrosine, val; valine.

Crude protein, moisture and ash content in the sea urchins are listed in Table *6*.

Table 6: Protein, moisture and ash content of enhanced and wild urchins sampled at week 13

	Enhanced	Wild
Crude protein (%)	10.7	11.3
Moisture (%)	76.4	77.6
Ash (%)	1.4	2.0

The total fat content was 5.5% in wild sea urchins and 4.4% in enhanced sea urchins. The fatty acid composition is shown in Table 7. Qualitatively the fatty acids were the same in both groups. The n-3/n-6 ratio was 1.12 in the enhanced sea urchins and 1.99 in the wild sea urchins. Polyunsaturated fatty acids (PUFA) were the most dominant fatty acids in both groups (enhanced; 32.55%, wild; 31.25%), followed by monounsaturated fatty acids (MUFA) (E; 24.45%, W; 21.95%) and saturated fatty acids (SFA) (E; 22.70%, W; 20.15%). Among SFAs, palmitic acid (C16:0) was dominant in both groups and was also the highest proportion of any fatty acid (E; 11.70%, W; 10.45%). In both wild and enhanced eicosenoic acids (C20:1 n-7 & n-9) (E; 9.35%, W; 8.95%) were the dominant MUFAs, followed by oleic acid (C18:1) (E;6.86%, W;6.00%). EPA was the dominant PUFA in both groups (E; 7.65%, W; 9.90%). Other notable fatty acids include linoleic acid (C18:2 n-6), where the enhanced sea urchins' content was four times higher than wild sea urchins (E; 6.75%, W; 1.65%), and DHA (C22:6 n-3) where the content of the enhanced sea urchins was twice of the wild (E; 4.20%, W; 1.75%). The amount of unidentified fatty acids was bigger in the wild sea urchins (E; 20.20%, W; 26.30%).

Table 7: Fatty acid composition of enhanced and wild sea urchin after 13 weeks.

Fatty acid	Lipid number	Enhanced (%)	Wild (%)
Myristic acid	C14:0	9.10	7.45
Palmitic acid	C16:0	11.70	10.45
Stearic acid	C18:0	1.65	1.80
Arachidic acid	C20:0	0.20	0.40
Palmitoleic acid	C16:1 n-7	2.70	2.95
Oleic acid	C18:1	6.85	6.00
Eicosenoic acid	C20:1 n-7 + n-9	9.35	8.95
Erucic acid	C22:1	5.50	3.95
Nervonic acid	C24:1 n-9	0.10	0.10
9,12-Hexadecadienoic acid	C16:2 n-4	0.15	0.20
6, 9,12-Hexadecadienoic acid	C16:3 n-4	0.10	0.20
Linoleic acid	C18:2 n-6	6.75	1.65
γ-Linolenic acid	C18:3 n-6	0.10	0.30
Eicosadienoic acid	C20:2 n-6	3.05	1.55
Dihomo-gamma-linolenic acid	C20:3 n-6	1.05	0.50
Eicosatetraenoic acid	C20:4 n-3	0.70	0.95
Adrenic acid	C22:4 n-6	0.10	0.20
α-Linolenic acid	C18:3 n-3	1.25	1.45
Stearidonic acid	C18:4 n-3	2.35	4.90
Eicosatrienoic acid	C20:3 n-3	0.60	1.25
Arachidonic acid	C20:4 n-6	4.25	6.20
Eicosapentaenoic acid (EPA)	C20:5 n-3	7.65	9.90
Heneicosapentaenoic acid	C21:5 n-3	0.15	0.15
Docosapentaenoic acid (DPA)	C22:5 n-3	0.35	0.30
Docosahexaenoic acid (DHA)	C22:6 n-3	4.20	1.75
Sum saturated fatty acids		22.70	20.15
Sum monounsaturated fatty acid		24.45	21.95
Sum PUFA (n-6)		15.30	10.45
Sum PUFA (n-3)		17.25	20.80
Sum EPA + DHA		11.85	11.70
Sum unidentified fatty acids		20.20	26.30

3.7 Observations of the scanning electron microscopy (SEM)

The sectioning was done as is illustrated in Figure 20. The gonad consists of several acini with an overall bobbly look. The outer acini epithelium is covered by flagella, approximately 15-25 µm in length, connected to collar cells (Figure 21). It appears the enhanced sea urchins (Figure 22A2, B2, C2, D2) have greater numbers of flagella and a more messy structure than wild sea urchins (Figure 23A2, B2, C2, D2). The acini of the enhanced sea urchins (Figure 22A1, B1, C1, D1) appears to be less structured and less uniform in shape than the wild sea urchins (Figure 23A1, B1, C1, D1).

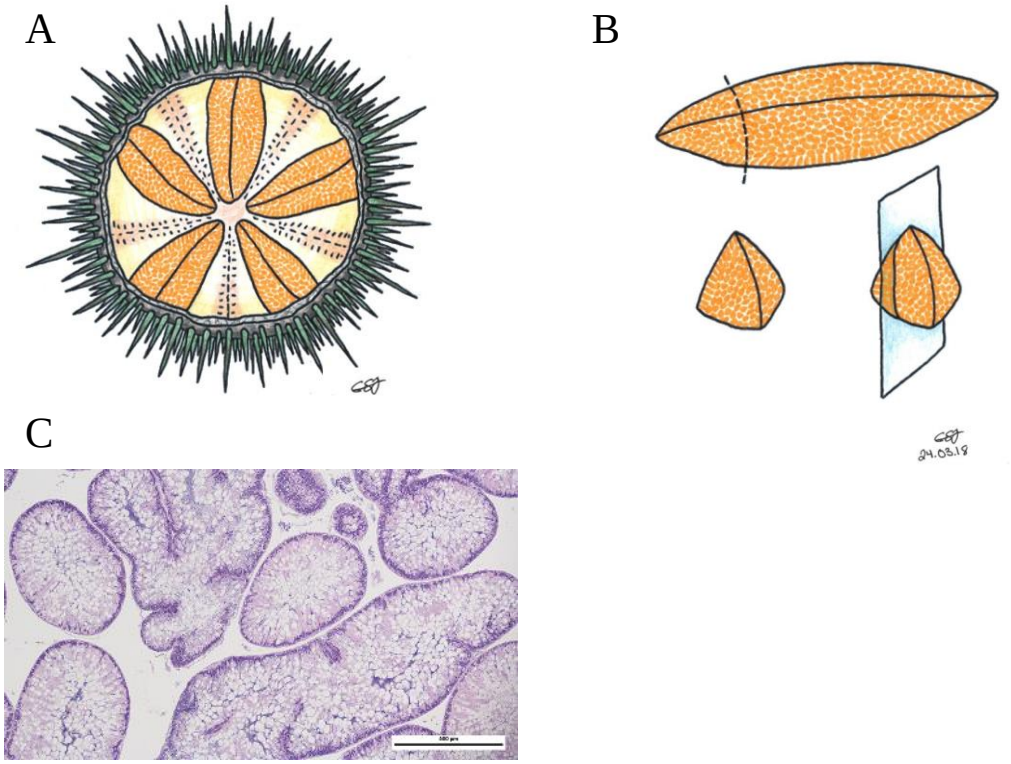


Figure 20: (A) Ventral view of sea urchin with bottom removed, (B) removal of gonad and cutting the tip off, slide showing cross section for scanning electron microscopy, (C) cross section of acini visible under light microscopy bar=500 µm. Illustrations by: Gunhild S. Johansson

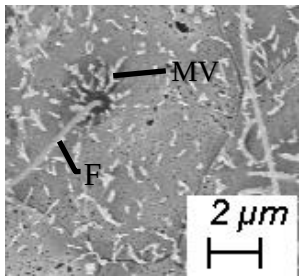


***Figure 21**: Flagellated collar cell on the gonad surface. F; flagellum, MV; microvilli*

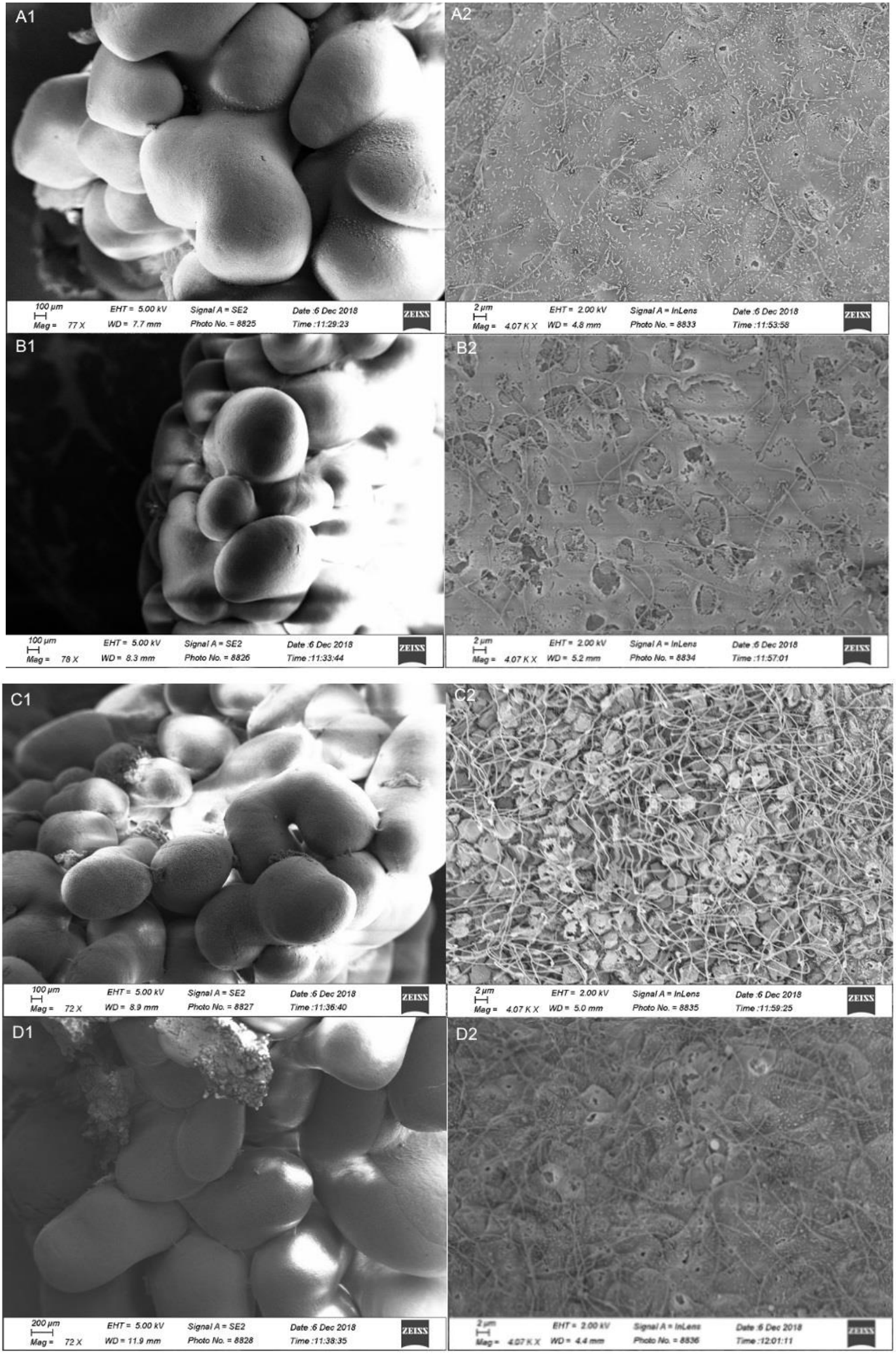


Figure 22: Face on view of enhanced urchins. Same individual (A1, A2) on left and right side with different magnification. Acini on left side, close-up of surface of acini on right side with flagella and microvilli. Notice individual C with a particularly messy appearance of the flagella.

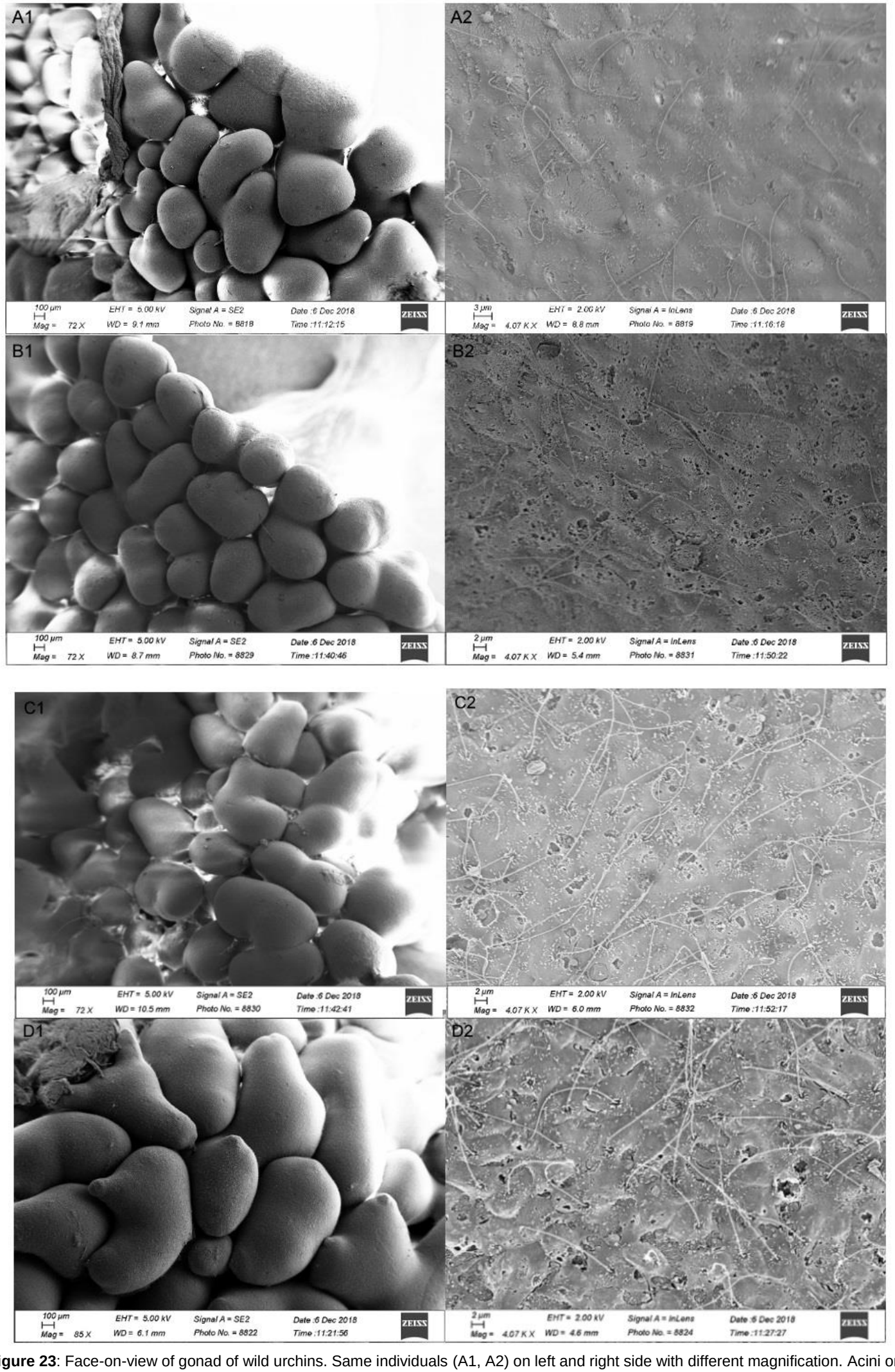


Figure 23: Face-on-view of gonad of wild urchins. Same individuals (A1, A2) on left and right side with different magnification. Acini on left side, close-up of surface of acini on right side with flagella and microvilli

3.8 Messiness index of gonad structure

In the initial sampling all slides were scored at 1 (Figure 24). All the significant differences between the enhanced sea urchins are listed in Table 8. None of wild sea urchins had significant differences between them.

Table 8: P-values from Fisher-Freeman-Halton test for enhanced W=Week

A	W4	W6	W8	W10
W4				
W6	NS			
W8	0.01	0.03		
W10	0.08	NS	NS	
W12	NS	NS	0.03	NS

The significant differences between the enhanced and wild sea urchins are listed in Table 9.

Table 9: P-values from Fisher-Freeman-Halton test between all weeks of enhanced and wild sea urchin. W=Week, NS=not significant.

	W4	W6	W8	W10	W12	Enhanced
W4	0.02					
W6		0.02				
W8			<0.001			
W10				0.003		
W12					NS	

Wild

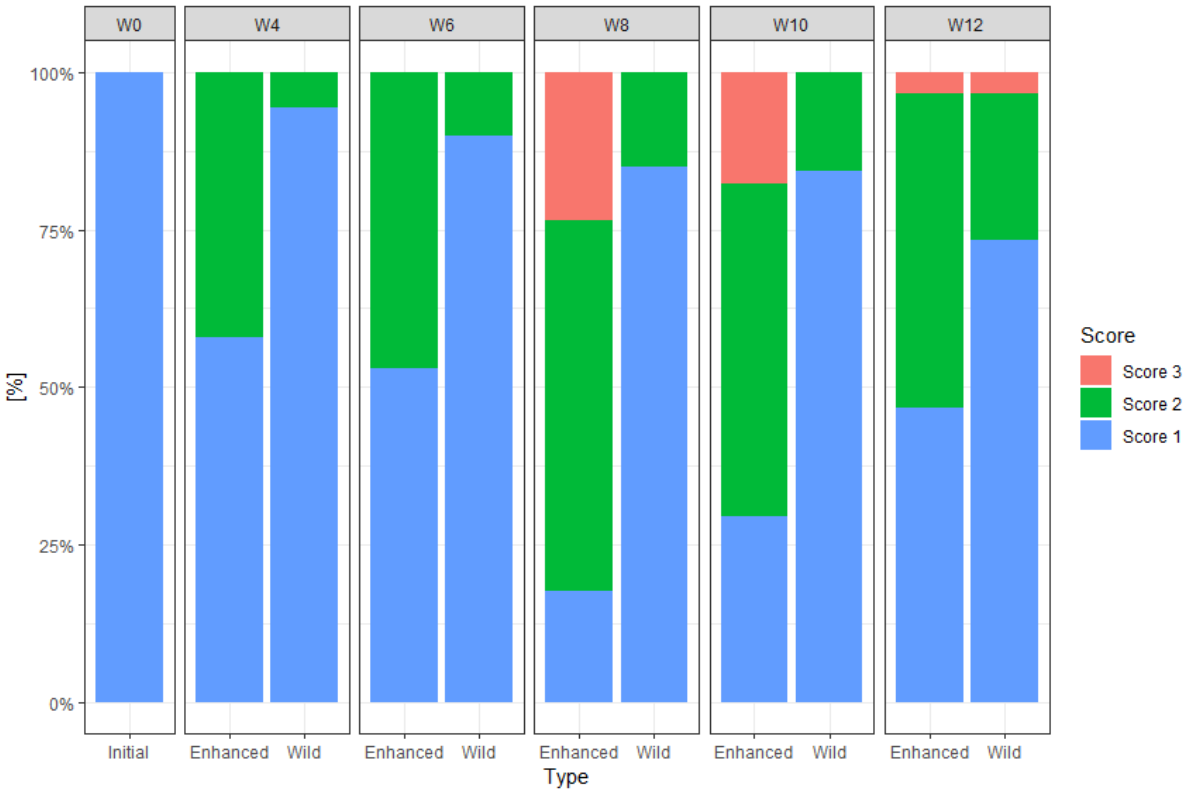


Figure 24: Proportion of messiness scores (1-3) from the initial sampling (week 0), week 4 and every two weeks for 12 weeks in total for enhanced and wild sea urchin.

4 Discussion

4.1 Gonad index

Gonad index increased from 6% in the initial sampling to 30% in the last sampling of the enhanced sea urchins, with an increase of GI of approximately 2% per week. This is above the saturation point (~0.17% GI per day) suggested by Marsh and Watts (2007) due to the metabolic absorption limit of protein. The wild sea urchins did not increase their gonad index significantly during the experimental period. This is in accordance with previous studies on increased gonad yield using formulated feed compared to natural feeds (Pearce *et al.*, 2002c; Chang *et al.*, 2005; Dale *et al.*, 2005; Shpigel *et al.*, 2005; Siikavuopio *et al.*, 2007a; Eddy *et al.*, 2015). This increase in GI suggests that the formulated feed has substantially higher nutritional value than the natural kelp. The convenience of formulated feed compared to collecting and feeding natural feeds is an advantage for commercial aquaculture, compared to harvesting natural algae which includes logistical challenges, seasonal changes and fluctuating nutritional value (Shpigel *et al.*, 2005). Both groups were subjected to natural seawater temperatures ranging from about 8°C to 10°C. The optimal temperature for the green sea urchin is in the range between 8-13°C (Pearce *et al.*, 2005; Siikavuopio *et al.*, 2006). Growth rate is correlated with ambient temperature, which is also correlated with the reproductive cycle because of the seasonal changes (Siikavuopio *et al.*, 2007b).

The wild sea urchins were exposed to natural light, while the enhanced sea urchins were exposed to 24h daylight. Fuji (1967) observed no decrease in feeding activity level under laboratory lighting conditions, but less feeding activity during high light intensity in the field. During the experimental period of 86 days (mid-August till November), 14 (16%) days were sunny with little clouds, the remaining days were either partial overcast (42%) or total overcast (42%). This means that the wild sea urchins were subjected to slightly higher intensity lighting, and that the differences in lighting condition are most likely negligible in terms of feeding activity. The feed availability for the wild sea urchins at the collection site is limited, and they most likely depend on algae drifting into the sheltered cove. This results in sporadic and clustered feeding with high competition between individuals. This could lead to some individuals getting more and better feed than others, and greater variation between GI of individuals.

4.2 Gonad water content

Gonad water content fluctuate during the annual reproductive cycle, with the lowest water content during early winter for *P. lividus* (Montero-Torreiro & Garcia-Martinez, 2003; Rocha *et al.*, 2019), winter for *Evechinus chloroticus* (Verachia *et al.*, 2012) and from September to December for *S. droebachiensis* (Hagen *et al.*, 2008). This is during the pre-gametogenesis and gametogenesis stages in the reproductive cycle. Pearce *et al.* (2004) and McBride *et al.* (2004) reported that higher gonad firmness is linked with lower gonad water content, and observed sea urchins fed a prepared diet had softer gonads than wild sea urchins. Azad (2011); S. Takagi *et al.* (2017); (2018) found no correlation between gonad texture and water content, but Azad (2011) found differences in water content for sea urchins fed prepared diet. In the current study the water content was at its highest for the first six weeks of the experimental period, but there are no significant differences between enhanced and wild sea urchins.

4.3 Biochemical analyses

These tests were run on a single replicate from week 13, so no statistical tests could be run. Gonads from wild sea urchin had more crude protein, ash and fatty acids than enhanced sea urchins. Ash content in this study is lower than reported in Montero-Torreiro and Garcia-Martinez (2003), with an ash content ranging from 7.3-12.4, with the lowest in November. Woods et al. (2008) found an ash content ranging from 0.9-1.3 in enhanced sea urchins.

4.3.1 Amino acids

Amino acids are important for the taste. Glycine and alanine, proline and serine contribute to sweetness, aspartic acid and glutamic acid taste of umami, while arginine, histidine, lysine, methionine, phenylalanine, valine, leucine and isoleucine are bitter amino acids (Woods *et al.*, 2008). Lee and Haard (1982) noted that glycine, alanine, serine, glutamic acid, methionine and arginine were important for taste in *S. droebachiensis.* Pearce *et al.* (2002c) reported a maximum gonad yield with a dietary protein content between 10% and 19%, with higher levels of protein only adding to cost of the feed. The ratio of sweet/umami/bitter amino acids were: 0.33/0.19/0.46 and 0.29/0.16/0.53 in wild and enhanced, respectively. Phillips *et al.* (2010) reported a correlation between sweetness and glycine. The glycine content of the wild sea urchins was 34% higher than enhanced which most likely implies that the wild sea urchins would have a sweeter taste. Phillips et al. (2010) also reported a correlation between bitter taste, leucine and lysine. Enhanced sea urchins had 35.2% and 55.8% higher content of lysine and

leucine than wild sea urchins. The combination of less glycine and more lysine and leucine in the enhanced sea urchins would most presumably give a bitter taste of the gonads.

Deficiency of essential amino acids is known to limit growth, reproductive maturation and protein synthesis (Murai, 1992; Lanes *et al.*, 2012; Kabir *et al.*, 2015). The current study did not find any studies conducted on effects of amino acid deficiency on sea urchin growth. Because the GI increase was significant in the enhanced sea urchins in the current study, deficiency of essential amino acids most likely did not occur.

Glycine content was the dominant amino acid in the wild sea urchins, and the leucine content was higher in sea urchins fed artificial feed than their wild counterparts, which is also consistent with other studies (Liyana-Pathirana *et al.*, 2002b; Dale *et al.*, 2006; Phillips *et al.*, 2010). Gonadal leucine content is shown to fluctuate during the year with the lowest concentrations during winter (Lee & Haard, 1982; Liyana-Pathirana *et al.*, 2002b). Varaksina and Varaksin (2002) reported that leucine is incorporated in oocytes during gametogenesis. The difference in leucine between enhanced and wild sea urchins could be due to reaching different stages of the reproductive cycle. Lysine content was lower in the enhanced sea urchins, which is not in accordance with the findings in Dale et al. (2006) or Phillips *et al.* (2010), but Liyana-Pathirana et al. 2002b found significant differences between seasons, with the highest content during fall for wild sea urchins. Feed has shown to have a significant effect on the amino acid composition in the gonads (Liyana-Pathirana *et al.*, 2002a).

4.3.2 Fatty acid composition

The fatty acid composition in sea urchin gonads is highly correlated with dietary fatty acid composition (González-Durán *et al.*, 2008), but there is also seasonal variation (Liyana-Pathirana *et al.*, 2002c). The fatty acids EPA (20:5 n-3) and DHA (22:6 n-3) are marine fatty acids, found in fish and other seafood. Because of the long carbon chain, these fatty acids will maintain the membrane structure of animals in cold temperatures. These fatty acids are predominant in both the wild and enhanced sea urchins, which is consistent with other findings (Liyana-Pathirana *et al.*, 2002c; González-Durán *et al.*, 2008). Palmitic acid (C16:0) and myristic acid (C14:0) are the major fatty acids found in both groups, which is in accordance with findings in other studies (T. Takagi *et al.*, 1980; Kaneniwa & Takagi, 1986; Liyana-Pathirana *et al.*, 2002c). Palmitic acid is synthesized in bacteria and in the chloroplast of plants and algae and is further elongated and desaturated to linoleic acid (C18:2 n-6). The latter fatty

acid cannot be synthesized by animals (Gurr *et al.*, 2002). Dietary linoleic acid can be desaturated and elongated to arachidonic acid (C20:4 n-6) by the sea urchin. In the wild samples the precursor linoleic acid was not enhanced compared to arachidonic acid, which suggests that the wild sea urchins fed on an arachidonic acid rich feed, and it was not endogenous synthesis. González-Durán *et al.* (2008) suggests that the levels of arachidonic acid (C20:4 n-6) will affect the osmoregulation, and Castell *et al.* (2004) speculates whether sea urchins produce eicosanoids from arachidonic acid, which are important for ion transport over membranes. The wild sea urchins have more arachidonic acid than the enhanced sea urchins. Zuo *et al.* (2018) reported that a higher dietary arachidonic acid increased growth, gonad development and antioxidant enzymes. These results indicate that further research should be undertaken on osmoregulation in regards of arachidonic acid content. EPA (C20:5 n-3) cannot be elongated to DHA (C22:6 n-3) in sea urchins, which implies all gonadal DHA must come from the diet (González-Durán *et al.*, 2008). The enhanced sea urchins had twice the amount of DHA compared to the wild ones. Some of the unidentified fatty acids could be an unusual type of polyunsaturated fatty called NMID (non-methylene-interrupted dienoic fatty acid). These fatty acids constituted above 10% of *gonads* in González-Durán et al. 2008, from 10-21% in *gonads* in Takagi et al. 1980, and 20% in the *body wall* of the sea urchin in Shikov *et al.* (2017). Another major fatty acid in Liyana-Pathirana et al. 2002 is C20:1 n-15, with 9.7%, which González-Durán et al. 2008 later identified as a NMID with a different methodology in their study.

Much of fresh seafood flavour is derived from polyunsaturated fatty acids, such as EPA, DHA and arachidonic acid via enzymatic activity which results in aromatic compounds such as aldehydes, alcohols and ketones. The total lipid is therefore of importance regarding taste (Josephson & Lindsay, 1986). Little research has been conducted on volatile compounds in sea urchin gonads, but Rodríguez-Bernaldo De Quirós *et al.* (2001) suggests that important compounds derived from fatty acids in sea urchins are: nonanal; gives an orange-like taste, benzaldehyde; has a nutty aroma, 2-propanone; sweet floral fruity aroma. Another factor important for taste is autooxidation of fatty acids that occurs soon after death and leads to a putrid taste due to aldehydes derived from PUFA n-3 (Lindsay, 1994). This can be prevented by limiting oxygen, light, metals or by adding antioxidants (Choe & Min, 2009).

Humans cannot synthesize the n-3 fatty acids EPA and DHA in an effective way and needs a dietary source of these fatty acids. The daily recommended intake varies from 0.5 g to 1.8 g

and seafood is the predominant source of EPA and DHA (Kris-Etherton *et al.*, 2009). Dietary lipid will incorporate into cell membranes as phospholipids. The n-3/n-6 ratio of the diet is important to human health because of the inflammatory properties of metabolic products derived from membrane phospholipid called eicosanoids. EPA/DHA and arachidonic acid (n-6) are substrates for the same eicosanoids producing enzymes and a higher n-3 content will reduce the production of more bioactive eicosanoids derived from n-6 (Calder, 2015). In this study the wild sea urchins had the best n-3/n-6 ratio with 1.99 and the enhanced had 1.12. According to Simopoulos (2002) the optimal ratio of n-3/n-6 ranges from 0.25 to 1. Both enhanced and wild sea urchins are well above the optimal limit, thus both are beneficial to human health. In addition, a study by Pozharitskaya *et al.* (2015) suggests that the 20 carbon NMIDs in sea urchin gonads will compete against arachidonic acid as substrate for the eicosanoid producing enzyme. This strengthens the potential health benefits of sea urchin gonads.

4.4 Histological examination

Gonads containing fewer reproductive cells relative to somatic cells are preferred as food in most markets, especially Japan, this is due to oozing of milt and eggs which effects the food quality, both in texture and in some cases taste (Unuma, 2002). Other cultures may have other preferences (Walker *et al.*, 2007). Photoperiod may influence gametogenesis. Böttger *et al.* (2006) reported cellular differences between sea urchins under continuous July photoperiod and ambient photoperiod. They observed an increase in volume of NPs with little or no increase of reproductive cells. Pearce *et al.* (2002b) suggests that photoperiod can delay gametogenesis, but other authors (Spirlet *et al.*, 2000; James & Heath, 2008) find no correlation between photoperiod and gametogenesis. In this study the relative gamete area of the enhanced sea urchins did not increase. The wild sea urchins' relative area of reproductive cells increased from 9.5% to 22% during the 12 weeks. In the initial sampling most of the sea urchins were at early stages of gametogenesis, either recovering or with a new generation of gametes in the periphery of the acinus. The increase in size of the enhanced sea urchins is due to growth of NPs because of accumulation of nutrients from the formulated feed (Garrido & Barber, 2001). The present study suggests that gametogenesis is inhibited in captivity. Reproductive impairment is recorded in several fish species (De Silva *et al.*, 2008; Zupa *et al.*, 2017).

Pearce et al. (2004) remarks that gonad firmness must be due to intrinsic variation of e.g. intercellular spacing, proportion of gametogenic and nutritive cells and fluidity of cell membranes. There are evident histological structural differences between wild and enhanced sea urchins (see Appendix A). Many of the enhanced sea urchins have undistinguishable acini and an overall messy appearance of the gonads. The interacinal space is limited and the gonad is cramped. This is also apparent on the SEM-imaging. It also appears that enhanced sea urchins have more flagella on the perivisceral peritoneum (Figure 22 C2). These flagellated cells peritoneal cells are attached to a basal body (Longo & Anderson, 1969), and are current-producing cells found in Echinoderms which move fluids and particles about in the animal (Walker, 1979). The peritoneal cells will increase in numbers to accommodate gonadal growth (Pearse & Cameron, 1991). Walker (1979) states that the collagenous and elastic layer of the gonad wall is stretched out during gametogenesis, therefore the appearance of the gonad is related to the fibres of this layer. These fibres are numerous in freshly spent gonads and may serve to maintain the structural stability of the gonad. Regeneration of muscle is observed in echinoderms following injury or amputation in various tissues (García-Arrarás & Dolmatov, 2010). VandenSpiegel *et al.* (2000) reported full regeneration, including the collagenous layer, of damaged defensive tubules in sea cucumber in five weeks. There should be more research done on the structure of the gonadal wall at different times during enhancing. The rapid increase in GI may be too fast for the sea urchin to synthesize essential components to preserve the gonadal structural integrity. In this study the messiness of the enhanced sea urchins was at its highest in the middle of the experimental period (week 8) and declining for the last two samples, which corresponds to less increase of GI for the last four weeks. Further research should be conducted on a slower increase in GI over several months too see whether it will improve texture of enhanced sea urchins. Few long-term enhancing trials on formulated feed have been conducted (Table *1*).

Conclusion

The results of this study show that enhanced sea urchins are different from wild captured sea urchins. Enhancing sea urchins show multiple effects on the roe. Feeding wild captured sea urchins artificial feed is an effective way to increase gonad indices. Biochemical composition was different between the treatments, most likely due to dietary input. The wild sea urchins were more advanced in terms of reproductive stage towards the end of the experiment. Enhancing sea urchins under these experimental conditions resulted in suspension of reproductive development, suggesting that captivity inhibits gametogenesis. There are clear histological differences between wild and enhanced sea urchins. The relative reproductive cell area is significantly lower in the enhanced sea urchins than in the wild sea urchins at the end of the experimental period. There is an increased messiness of the enhanced sea urchin gonads mid-trial, but it appears to decrease at the end. Further research should focus on whether enhancing has an effect on the morphology of the gonadal wall and its structural integrity, and what the effects are on the quality, especially firmness and texture, at different times and whether a rapid increase in GI will influence the quality. There should also be focus on essential fatty and amino acids on how they affect growth, osmoregulation and other physiological processes that may affect the quality of the sea urchin gonad.

5 Reference list

Abiogenisis. (2013) *S. droebachiensis* - The Green Sea Urchin. Retrived from https://www.deviantart.com/abiogenisis/art/S-droebachiensis-The-Green-Sea-Urchin-408186466 21.03.2019

Anderson, D. T. (2001). *Invertebrate zoology* (2nd ed.). Melbourne, Vic: Oxford University Press.

Apicius. (2009). *Cooking and Dining in Imperial Rome*. In Book IX, Chapter 8, F. Starr (Ed.), *De Re Coquinaria* Retrieved from https://www.gutenberg.org/files/29728/29728-h/29728-h.htm

Azad, M. A. K. (2011). *Factors influencing adult gonad production and larval growth and survival of the purple sea urchin (Strongylocentrotus purpuratus)* (Doctoral dissertation), University of British Columbia, Vancouver.

Blanco, A., & Blanco, G. (2017). *Medical Biochemistry* London: Academic Press.

Briscoe, C. S., & Sebens, K. P. (1988). Omnivory in *Strongylocentrotus droebachiensis* (Müller) (Echinodermata: Echinoidea): predation on subtidal mussels. *Journal of Experimental Marine Biology and Ecology*, *115*(1), 1-24.

Böttger, S. A., Devin, M. G., & Walker, C. W. (2006). Suspension of gametogenesis in green sea urchins experiencing invariant photoperiod. *Aquaculture*, *261*, 1422.

Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1851*(4), 469-484.

Campbell, G. J. A. (2008). Sorry, Wrong Phylum: A Neophyte Archaeomalacologists Experiences in Analyzing a European Atlantic Sea Urchin Assemblage. *Archeofauna, 17*, 77-90.

Castell, J. D., Kennedy, E. J., Robinson, S. M. C., Parsons, G. J., Blair, T. J., & Gonzalez-Duran, E. (2004). Effect of dietary lipids on fatty acid composition and metabolism in juvenile green sea urchins (*Strongylocentrotus droebachiensis*). *Aquaculture*, *242*(1), 417-435.

Chang, Y.-Q., Lawrence, J. M., Cao, X.-B., & Lawrence, A. (2005). Food Consumption, Absorption, Assimilation and Growth of the Sea Urchin *Strongylocentrotus intermedius* Fed a Prepared Feed and the Alga *Laminaria japonica*. *Journal of the World Aquaculture Society*, *36*(1), 68-75.

Choe, E., & Min, D. B. (2009). Mechanisms of Antioxidants in the Oxidation of Foods. *Comprehensive Reviews in Food Science and Food Safety, 8*(4), 345-358.

Dale, T., Siikavuopio, S. I., & Aas, K. (2005). Roe enhancement in sea urchin: effects of handling during harvest and transport on mortality and gonad growth in *Strongylocentrotus droebachiensis*. *Journal of Shellfish Research, 24*(4), 1235-1240.

Dale, T., Siikavuopio, S., Aksnes, A., Hope, B., Gebauer, R., & Carlehög, M. (2006). *Smak og tekstur på kråkebollegonader: Forholdet mellom biokjemisk sammensetning og produktkvalitet*. Retrieved from Fiskeriforskning, Rapport 4/2006

De Silva, S. S., Nguyen, T. T., & Ingram, B. (2008). Fish reproduction in relation to aquaculture. In M. J. Rocha, A. Arukwe, & B. G. Kapoor (Eds.), *Fish Reproduction* (pp. 535-575). Enfield Science Publishers.

Eddy, S. D., Brown, N. P., & Kling, A. L. (2012). Growth of Juvenile Green Sea Urchins, *Strongylocentrotus droebachiensis,* Fed Formulated Feeds with Varying Protein Levels Compared with a Macroalgal Diet and a Commercial Abalone Feed. *Journal of World Aquaculture Society, 43,* 159-173.

Eddy, S. D., Camire, M., & Kenn, U. (2015). Gonad yields and sensory attributes of green sea urchins following enhancement feeding with either dried kelp, fresh kelp, or formulated dried feeds. In: Abstracts, 107th Annual Meeting National Shellfisheries Association p. 627.

Falk-Pettersen, I. B., & Lønning, S. (1983). Reproductive cycles of two closely related sea urchin species, *Strongylocentrotus droebachiensis* and *Strongylocentrotus pallidus. Sarsia, 68*, 158-164.

Fong, W., & Mann, K. H. (1979). Role of Gut Flora in the Transfer of Amino Acids Through a Marine Food Chain. *Canadian Journal of Fisheries and Aquatic Sciences, 37,* 88-96.

Fuji, A. (1967). Ecological studies on the growth and food consumption of Japanese common littoral sea urchin, *Strongylocentrotus intermedius* (A. Agassiz). *Memoirs of the Faculty of Fisheries, Hokkaido university, 15*, 83-160.

García-Arrarás, J. E., & Dolmatov, I. Y. (2010). Echinoderms: potential model systems for studies on muscle regeneration. *Current pharmaceutical design, 16*(8), 942-955.

Garrido, C. L., & Barber, B. J. (2001). Effects of temperature and food ration on gonad growth and oogenesis of the green sea urchin, *Strongylocentrotus droebachiensis*. *Marine Biology, 138*, 447-456.

González-Durán, E., Castell, J. D., Robinson, S., & Blair, T. J. (2008). Effects of dietary lipids on the fatty acid composition and lipid metabolism of the green sea urchin *Strongylocentrotus droebachiensis*. *Aquaculture, 276*, 120-129.

Google. (n.d.) [Map of Tromsø municipality]. Snazzy Maps. Retrived from https://snazzymaps.com/style/24088/map-without-labels 21.03.2019

Gundersen, H., Hartvig, C., & Rinde, E. (2010). *Perspektivstudie av kråkeboller - fra problem til ressurs. Analyse av ressursgrunnlaget for høsting av kråkeboller og vurdering av økologiske perspektiver knyttet til høsting (Sea urchins - from problem to commercial resource. Estimates of sea urchins as a resource and an evaluation of ecological gain by sea urchin exploitation).* Retrieved from NIVA (Norwegian Institute for Water Research), Report L. NR. 6001-2010

Gurr, M., Harwood, J., & Frayn, K. (2002). The biosynthesis of fatty acids. In M. Gurr, J. Harwood, & K. Frayn (Eds.), *Lipid biochemistry* (pp. 21-58): Oxford: Blackwell Science.

Hagen, N. T., Jorgensen, I., & Egeland, E. S. (2008). Sex-specific seasonal variation in the carotenoid content of sea urchin gonads. *Aquatic Biology, 3*, 227-235.

Hammer, H., Hammer, B., Watts, S. A., Lawrence, A. L., & Lawrence, J. M. (2006). The effect of dietary protein and carbohydrate concentration on the biochemical composition and gametogenic condition of the sea urchin *Lytechinus variegatus*. *Journal of Experimental Marine Biology and Ecology, 334*, 109-121.

Harris, L. G., & Eddy, S. D. (2015). Sea Urchin Ecology and Biology. In N. P. Brown & S. D. Eddy (Eds.), *Echinoderm Aquaculture* (pp. 3-24). Hoboken, New Jersey: John Wiley & Sons, Inc. .

Hebbali, A. (2018). olsrr: Tools for Building OLS Regression Models. R package version 0.5.2. https://CRAN.R-project.org/package=olsrr.

Himmelman, J. (1969). *Some aspects of the ecology of Strongylocentrotus droebachiensis in eastern Newfoundland.* (M. Sc. thesis), Memorial university. Newfoundland, Canada.

James, P., & Heath, P. (2008). The effects of season, temperature and photoperiod on the gonad development of *Evechinus chloroticus*. *Aquaculture, 285*, 67-77.

James, P., & Siikavuopio, S. (2012). The effect of continuous and intermittent feeding regimes on survival and somatic and gonadal growths of the sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture, 364-365*, 173-179.

James, P., & Siikavuopio, S. (2013). The effects of tank system, water velocity and water movement on survival, somatic and gonad growth of juvenile and adult green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture Research, 46*(6).

James, P., Siikavuopio, S., & Mortensen, A. (2015). Sea Urchin Aquaculture in Norway. In N. P. Brown & S. D. Eddy (Eds.), *Echinoderm Aquaculture* (pp. 147-173). Hoboken, New Jersey: John Wiley & Sons, Inc.

James, P., Evensen, T., & Samuelsen, A. (2017a). *Commercial scale sea urchin roe enhancement in Norway: Enhancement, transport and market assessment*. Retrieved from Nofima, Report 7/2017

James, P., Siikavuopio, S., & Reguera, T. W. (2017b). Measuring density effects on growth and survival of two size classes of juvenile sea urchins (*Strongylocentrotus droebachiensis*) held in sea-based holding systems and impacts on holding system design and management. *Aquaculture Research, 48*(11), 5541-5549.

James, P., Siikavuopio, S., & Johansson, G. S. (2018). *A guide to the Sea Urchin Reproductive Cycle and Staging Sea Urchin Gonad Samples: (second edition)* Nofima. Retrieved from: https://urchinproject.com/2018/04/27/a-guide-to-the-sea-urchin-reproductive-cycle-and-staging-sea-urchin-gonad-samples-second-edition/ Accessed: 07.05.2019

Jensen, M. (1974). The Strongylocentrotidae (Echinoidea), a morphologic and systematic study. *Sarsia, 57*(1), 113-148.

Josephson, D. B., & Lindsay, R. C. (1986). Enzymic Generation of Volatile Aroma Compounds from Fresh Fish. In T. Parliment & R. Croteau (Eds.), *Biogeneration of Aromas* (Vol. 317, pp. 201-219): American Chemical Society.

Kabir, M. A., Ghaedi, A., Talpur, A. D., & Hashim, R. (2015). Effect of dietary protein levels on reproductive development and distribution of amino acids in the body tissues of female *Pangasianodon hypophthalmus* (Sauvage, 1878) broodstock in captivity. *Aquaculture Research, 46*(7), 1736-1747.

Kaneniwa, M., & Takagi, T. (1986). Fatty Acids in the Lipid of Food Products from Sea Urchin. *Bulletin of the Japanese Society of Scientific Fisheries, 52*, 1681-1685.

Kennedy, E. J., Robinson, S., Parsons, G. J., & Castell, J. D. (2005). Effect of protein source and concentration on somatic growth of Juvenile green sea urchins *Strongylocentrotus droebachiensis*. *Journal of the World Aquaculture Society, 36*, 320-336.

Kennedy, E. J., Robinson, S. M. C., Parsons, G. J., & Castell, J. D. (2007a). Effect of Lipid Source and Concentration on Somatic Growth of Juvenile Green Sea Urchins, *Strongylocentrotus droebachiensis*. *Journal of the World Aquaculture Society, 38*(3), 335-352.

Kennedy, E. J., Robinson, S. M. C., Parsons, G. J., & Castell, J. D. (2007b). Effect of Dietary Minerals and Pigment on Somatic Growth of Juvenile Green Sea Urchins, *Strongylocentrotus droebachiensis*. *Journal of the World Aquaculture Society, 38*(1), 36-48.

Kris-Etherton, P. M., Grieger, J. A., & Etherton, T. D. (2009). Dietary reference intakes for DHA and EPA. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 81*(2), 99-104.

Lanes, C. F. C., Bizuayehu, T. T., Bolla, S., Martins, C., de Oliveira Fernandes, J. M., Bianchini, A., . . . Babiak, I. (2012). Biochemical composition and performance of Atlantic cod (*Gadus morhua L.)* eggs and larvae obtained from farmed and wild broodstocks. *Aquaculture, 324-325*, 267-275.

Lang, C., & Mann, K. (1976). Changes in sea urchin populations after the destruction of kelp beds. *Marine Biology, 36*(4), 321-326.

Lawrence, J. M. (2007a). Sea Urchin Roe Cuisine. In J. M. Lawrence (Ed.), *Edible sea urchins: biology and ecology* (Vol. 37, pp. 521-523): Elsevier.

Lawrence, J. M. (2007b). Edible sea urchins: use and life-history strategies. In J. M. Lawrence (Ed.), *Edible sea urchins: biology and ecology* (Vol. 37, pp. 1-9): Elsevier.

Lee, Y. Z., & Haard, N. F. (1982). Evaluation of the Green Sea Urchin Gonads as a Food Source. *Canadian Institute of Food Science and Technology Journal, 15*, 233-235.

Lindsay, R. C. (1994). Flavour of fish. In F. Shahidi & J. R. Botta (Eds.), *Seafoods. Chemistry, processing technology and quality* (1 ed., pp. 75-84). Surrey: Springer Science + Business Media, B.V.

Liyana-Pathirana, C., & Shahidi, F. (2003). Effect of an Artificial Diet on Lipid, Free Amino Acid, and Carotenoid Composition of Green Sea Urchin Gonads. In *ACS National Meeting Book of Abstracts* (Vol. 223, pp. 83-93).

Liyana-Pathirana, C., Shahidi, F., & Whittick, A. (2002a). Comparison of nutrient composition of gonads and coelomic fluid of green sea urchin *Strongylocentrotus droebachiensis*. *Journal of Shellfish Research, 21*, 861-870.

Liyana-Pathirana, C., Shahidi, F., Whittick, A., & Hooper, R. (2002b). Effect of season and artificial diet on amino acids and nucleic acids in gonads of green sea urchin *Strongylocentrotus droebachiensis*. *Comparative Biochemistry and Physiology Part A, 133*, 389-398.

Liyana-Pathirana, C., Shahidi, F., Whittick, A., & Hooper, R. (2002c). Lipid and lipid soluble components of gonads of green sea urchin (*Strongylocentrotus droebachiensis*). *Journal of Food Lipids, 9*, 105-126.

Longo, F. J., & Anderson, E. (1969). Sperm differentiation in the sea urchins *Arbacia punctulata* and *Strongylocentrotus purpuratus*. *Journal of Ultrastructure Research, 27*(5), 486-509.

Lourenço, S., Valente, L. M. P., & Andrade, C. (2018). Meta-analysis on nutrition studies modulating sea urchin roe growth, colour and taste. *Reviews in Aquaculture*, 1-16.

Mann, K. H., Wright, J. L. C., Welsford, B. E., & Hatfield, E. (1984). Responses of the sea urchin *Strongylocentrotus droebachiensis* (O.F. Müller) to water-borne stimuli from potential predators and potential food algae. *Journal of Experimental Marine Biology and Ecology, 79*, 233-244.

Marsh, A. G., & Watts, S. A. (2007). Biochemical and Energy Requirements of Gonad Development. In J. M. Lawrence (Ed.), *Edible Sea Urchins: Biology and Ecology* (Vol. 37, pp. 35-53): Elsevier.

McBride, S. C., Price, R. J., Tom, P. D., Lawrence, J. M., & Lawrence, A. L. (2004). Comparison of gonad quality factors: color, hardness and resilience, of *Strongylocentrotus franciscanus* between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery. *Aquaculture, 233*(1), 405-422.

Meidel, S. K., & Scheibling, R. E. (2001). Variation in egg spawning among subpopulations of sea urchins *Strongylocentrotus droebachiensis* a theoretical approach. *Marine Ecology Progress Series, 213*, 97-110.

Montero-Torreiro, M. F., & Garcia-Martinez, P. (2003). Seasonal changes in the biochemical composition of body components of the sea urchin, *Paracentrotus lividus*, in Lorbé (Galicia, north-western Spain). *Journal of the Marine Biological Association of the United Kingdom, 83*, 575-581.

Moren, M. (2016). Teaching secret feed production. Accessed: 26.04.2019 Retrieved from https://nofima.no/en/nyhet/2016/06/teaching-secret-feed-production/

Murai, T. (1992). Protein nutrition of rainbow trout. *Aquaculture, 100*, 191-207.

Pearce, C., Daggett, T. L., & Robinson, S. (2002a). Effect of binder type and concentration on prepared feed stability and gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture, 205*(3), 301-323.

Pearce, C., Daggett, T. L., & Robinson, S. (2002b). Optimizing prepared feed ration for gonad production of the green sea urchin *Strongylocentrotus droebachiensis*. *Journal of the World Aquaculture Society, 33*, 268-277.

Pearce, C., Daggett, T. L., & Robinson, S. (2002c). Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture, 214*(1), 307-332.

Pearce, C., Daggett, T. L., & Robinson, S. (2003). Effects of starch type, macroalgal meal source, and beta-carotene on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis* (Müller), fed prepared diets. *Journal of Shellfish Research, 22*(2), 505-519.

Pearce, C., Daggett, T. L., & Robinson, S. (2004). Effect of urchin size and diet on gonad yield and quality in the green sea urchin (*Strongylocentrotus droebachiensis)*. *Aquaculture, 233*, 337-367.

Pearce, C., Williams, S. W., Yuan, F., Castell, J. D., & Robinson, S. M. C. (2005). Effect of temperature on somatic growth and survivorship of early post-settled green sea urchins, *Strongylocentrotus droebachiensis* (Müller). *Aquaculture Research, 36*(6), 600-609.

Pearse, J. S., & Cameron, R. A. (1991). Echinodermata: Echinoidea. In A. C. Giese, J. S. Pearse, & V. B. Pearse (Eds.), *Reproduction of Marine Invertebrates, Vol. VI, Echinoderms and Lophophorates*. Pacific Grove, CA: The Boxwood Press.

Phillips, K., Hamid, N., Silcock, P., Sewell, M. A., Barker, M., Weaver, A., . . . Bremer, P. (2010). Effect of manufactured diets on the yield, biochemical composition and sensory quality of *Evechinus chloroticus* sea urchin goniads. *Aquaculture, 308*(1), 49-59.

Pozharitskaya, O. N., Shikov, A. N., Laakso, I., Seppänen-Laakso, T., Makarenko, I. E., Faustova, N. M., . . . Makarov, V. G. (2015). Bioactivity and chemical characterization of gonads of green sea urchin *Strongylocentrotus droebachiensis* from Barents Sea. *Journal of Functional Foods, 17*, 224-234.

Prato, E., Chiantore, M., Kelly, M. S., Hughes, A. D., James, P., Ferranti, M. P., . . . Fanelli, G. (2018). Effect of formulated diets on the proximate composition and fatty acid profiles of sea urchin *Paracentrotus lividus* gonad. *Aquaculture Int, 26*, 185-202.

Quinn, J. F., Wing, S. R., & Botsford, L. W. (1993). Harvest refugia in marine invertebrate fisheries: models and applications to the red sea urchin, *Strongylocentrotus franciscanus*. *American Zoologist, 33*(6), 537-550.

R Core Team. (2018). R: A Language and Environment for Statistical Computing. https://www.R-project.org/: R Foundation for Statistical Computing.

Robinson, S. M. C., Castell, J. D., & Kennedy, E. J. (2002). Developing suitable colour in the gonads of cultured green sea urchins (*Strongylocentrotus droebachiensis*). *Aquaculture, 206*(3), 289-303.

Rocha, F., Baião, L. F., Moutinho, S., Reis, B., Oliveira, A., Arenas, F., . . . Valente, L. M. P. (2019). The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of *Paracentrotus lividus* along the North Atlantic coast of Portugal. *Scientific Reports, 9*(1), 2994.

Rodríguez-Bernaldo De Quirós, A., López-Hernández, J., González-Castro, M., de la Cruz-García, C., & Simal-Lozano, J. (2001). Comparison of volatile components in fresh and canned sea urchin (*Paracentrotus lividus*, Lamarck) gonads by GC–MS using dynamic headspace sampling and microwave desorption. *European Food Research and Technology, 212*(6), 643-647.

Rolls, G. O. (2016). *Difficult Blocks and Reprocessing.* Retrieved from: https://www.leicabiosystems.com/pathologyleaders/difficult-blocks-and-reprocessing/ Accessed:

Scheibling, R., & Hatcher, B. (2007). Ecology of *Strongylocentrotus droebachiensis*. In J. M. Lawrence (Ed.), *Edible sea urchins: biology and ecology* (Vol. 37, pp. 1-9): Elsevier.

Sebens, K. (1985). *Community ecology of vertical rock walls in the Gulf of Maine, USA: small-scale processes and alternative community states*. Kent, UK: Hodder & Stoughton Educational Press.

Shikov, A. N., Laakso, I., Pozharitskaya, O. N., Seppänen-Laakso, T., Krishtopina, A. S., Makarova, M. N., . . . Makarov, V. (2017). Chemical Profiling and Bioactivity of Body Wall Lipids from *Strongylocentrotus droebachiensis*. *Marine drugs, 15*(12), 365.

Shpigel, M., McBride, S. C., Marciano, S., Ron, S., & Ben-Amotz, A. (2005). Improving gonad colour and somatic index in the European sea urchin *Paracentrotus lividus*. *Aquaculture, 245*(1), 101-109.

Siikavuopio, S., Christiansen, J., & Dale, T. (2006). Effects of temperature and season on gonad growth and feed intake in the green sea urchin (*Strongylocentrotus droebachiensis)*. *Aquaculture, 255*(1-4), 389-394.

Siikavuopio, S., Dale, T., & Carlehög, M. (2007a). Sensory quality of gonads from the green sea urchin *Strongylocentrotous droebachiensis,* fed different diets. *Journal of Shellfish Research, 26*(2), 637-643.

Siikavuopio, S., Christiansen, J., Sæther, B.-S., & Dale, T. (2007b). Seasonal variation in feed intake under constant temperature and natural photoperiod in the green sea urchin (*Strongylocentrotus droebachiensis*). *Aquaculture, 272*, 328-334.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*(8), 365-379.

Spirlet, C., Grosjean, P., & Jangoux, M. (2000). Optimization of gonad growth by manipulation of temperature and photoperiod in cultivated sea urchins, *Paracentrotus lividus* (Lamarck) (Echinodermata). *Aquaculture, 185*(1-2), 85-99.

Stefánsson, G., Kristinsson, H., Ziemer, N., Hannon, C., & James, P. (2017). *Markets for Sea Urchins: A Review of Global Supply and Markets*. Retrieved from Icelandic Food and Biotech R&D

Sun, J., & Chiang, F. S. (2015). Use and Exploitation of Sea Urchins. In N. P. Brown & S. D. Eddy (Eds.), *Echinoderm Aquaculture* (pp. 25-45). Hoboken, New Jersey: John Wiley & Sons, Inc.

Takagi, S., Murata, Y., Inomata, E., Endo, H., & Aoki, M. N. (2017). Improvement of gonad quality of the sea urchin *Mesocentrotus nudus* fed the kelp *Saccharina japonica* during offshore cage culture. *Aquaculture, 477*, 50-61.

Takagi, S., Murata, Y., Inomata, E., Endo, H., Aoki, M. N., & Agutsama, Y. (2018). Dietary effect of kelp (*Saccharina japonica)* on gonad quality and quality in sea urchins (*Mesocentrotus nudus)* collected from a barren before the fishing season. *Journal of Shellfish Research, 37*, 659-669.

Takagi, T., Eaton, C. A., & Ackman, R. G. (1980). Distributin of Fatty Acids in Lipids of the Common Atlantic Sea Urchin *Strongylocentrotus droebachiensis*. *Canadian Journal of Fisheries and Aquatic Sciences, 37*, 195-202.

Unuma, T. (2002). Gonadal growth and its relationship to aquaculture in sea urchins. In Y. Yokota, V. Matranga, & Z. Smolenicka (Eds.), *The Sea Urchin: From Basic Biology to Aquaculture*. Lisse: Balkema.

VandenSpiegel, D., Jangoux, M., & Flammang, P. (2000). Maintaining the line of defense: regeneration of Cuvierian tubules in the sea cucumber *Holothuria forskali* (Echinodermata, Holothuroidea). *The Biological Bulletin, 198*(1), 34-49.

Varaksina, G. S., & Varaksin, A. A. (2002). Effect of Estradiol Dipropionate on Protein Synthesis in Oocytes and Ovary of the Sea Urchin *Strongylocentrotus intermedius* at Different Stages of the Reproductive Cycle. *Biology Bulletin of the Russian Academy of Sciences, 29*(5), 496-500.

Verachia, W., Sewell, M. A., Niven, B., Leus, M., Barker, M. F., & Bremer, P. J. (2012). Seasonal changes in the biochemical composition of *Evechinus chloroticus* gonads (Echinodermata: Echinoidea). *New Zealand Journal of Marine and Freshwater Research, 46:3*, 399-410.

Walker, C. W. (1979). Ultrastructure of the Somatic Portion of the Gonads in Asteroids, with Emphasis on Flagellated-collar Cells and nutrient transport. *Journal of Morphology, 162*, 127-162.

Walker, C. W., Unuma, T., & Lesser, M. P. (2007). Gametogenesis and Reproduction of Sea Urchins. In J. M. Lawrence (Ed.), *Edible Sea Urchins: Biology and Ecology* (Vol. 37, pp. 11-33): Elsevier.

Walker, C. W., Harrington, L. M., Lesser, M. P., & Fagerberg, W. R. (2005). Nutritive phagocyte incubation chambers provide a structural and nutritive microenvironment for germ cells of *Strongylocentrotus droebachiensis,* the green sea urchin. *The Biological Bulletin, 209*(1), 31-48.

Walker, C. W., Boettger, S. A., Unuma, T., Watts, S. A., Harris, L. G., Lawrence, A. L., & Eddy, S. D. (2015). Enhancing the Commercial Quality of Edible Sea Urchin Gonads - Technologies Emphasizing Nutritive Phagocytes. In N. P. Brown & S. D. Eddy (Eds.), *Echinoderm Aquaculture* (pp. 263-286). Hoboken, New Jersey: John Wiley & Sons, Inc. .

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Woods, C., James, P., Moss, G., Wright, J., & Siikavuopio, S. (2008). A comparison of the effect of urchin size and diet on gonad yield and quality in the sea urchin *Evechinus chloroticus* Valenciennes. *Aquaculture Int, 16*, 49-68.

Yokota, Y. (2002). Introduction to the sea urchin biology. In Y. Yokota, V. Matranga, & Z. Smolenicka (Eds.), *The Sea Urchin: From Basic Biology to Aquaculture*. Lisse: Balkema.

Young, B., Woodford, P., & O'Dowd, G. (2013). *Wheater's functional histology: a text and colour atlas*: Elsevier Health Sciences.

Zuo, R., Li, M., Ding, J., & Chang, Y. (2018). Higher Dietary Arachidonic Acid Levels Improved the Growth Performance, Gonad Development, Nutritional Value, and Antioxidant Enzyme Activities of Adult Sea Urchin (*Strongylocentrotus intermedius*). *Journal of Ocean University of China, 17*(4), 932-940.

Zupa, R., Rodríguez, C., Mylonas, C. C., Rosenfeld, H., Fakriadis, I., Papadaki, M., . . . Corriero, A. (2017). Comparative Study of Reproductive Development in Wild and Captive-Reared Greater Amberjack *Seriola dumerili* (Risso, 1810). *PloS one, 12*(1).

Appendix

A Plates

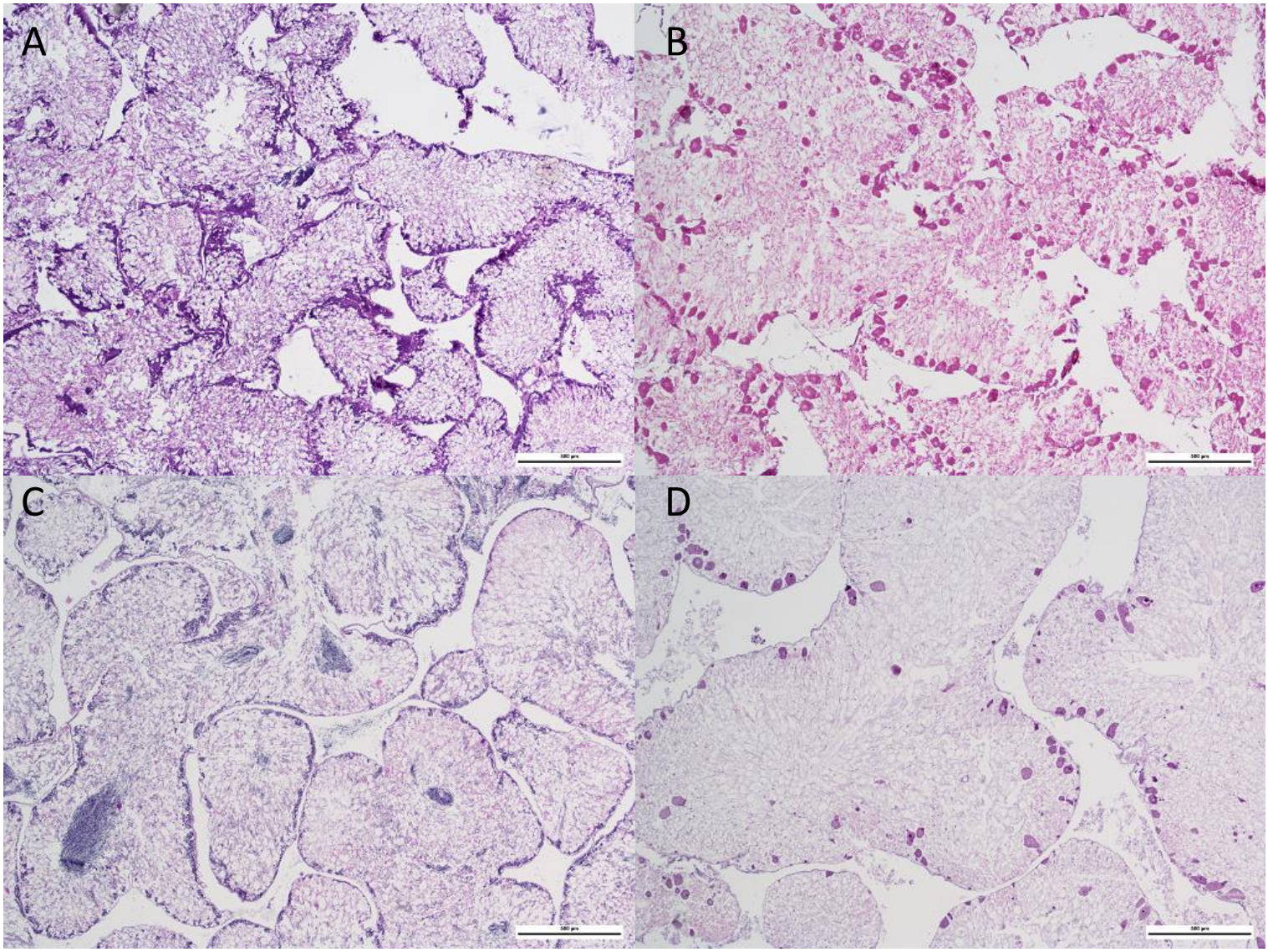


Figure 25: Light microscopy of gonads of enhanced sea urchins. (A) male week 8; (B) female week 8; (C) male week 12; (D) female week 12. Bar=500µm

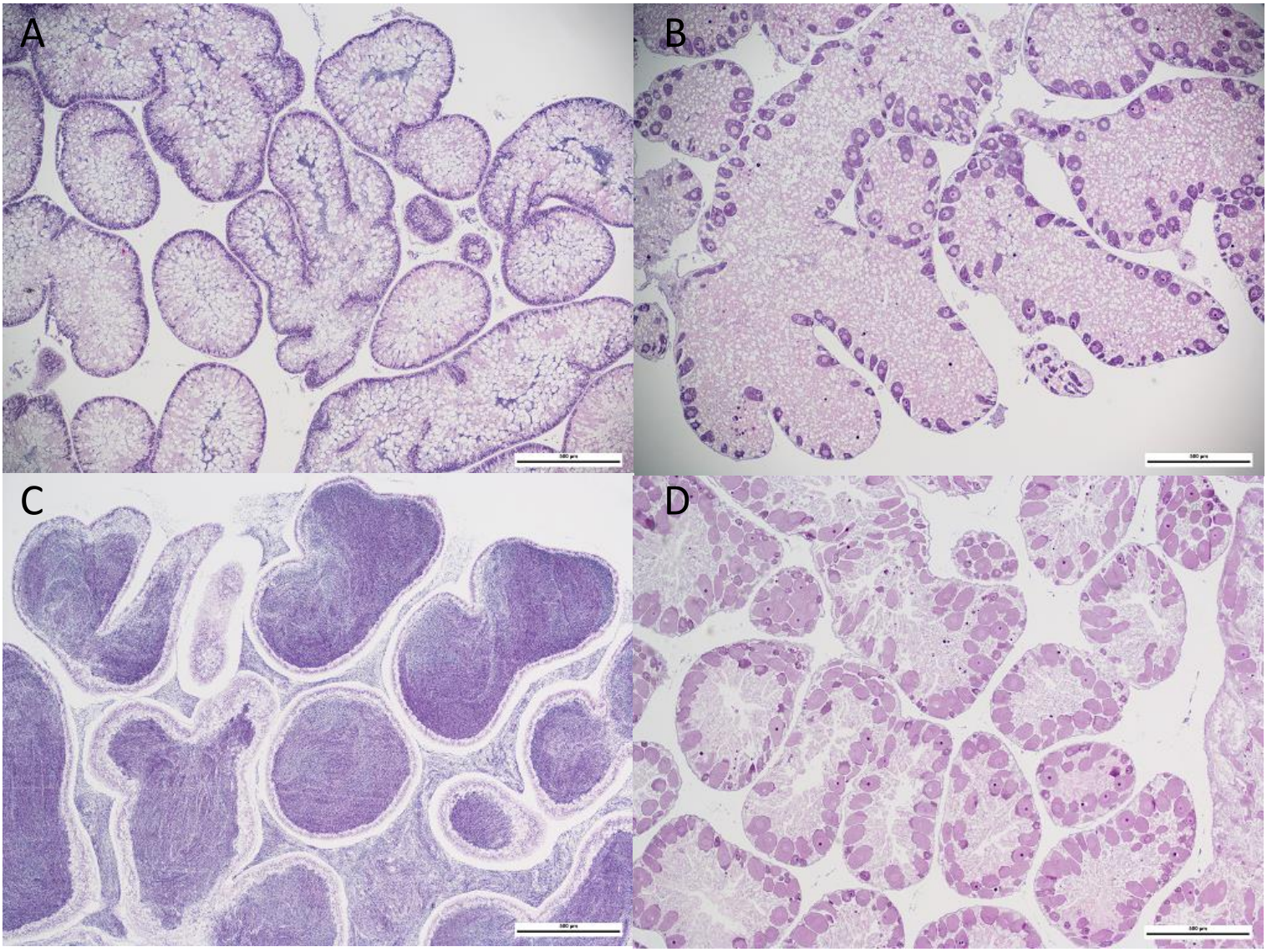


Figure 26: Light microscopy of gonads of wild sea urchins. (A) male week 6; (B) female week 6; (C) male week 10; (D) female week 12. Bar=500µm

B Statistical evaluation

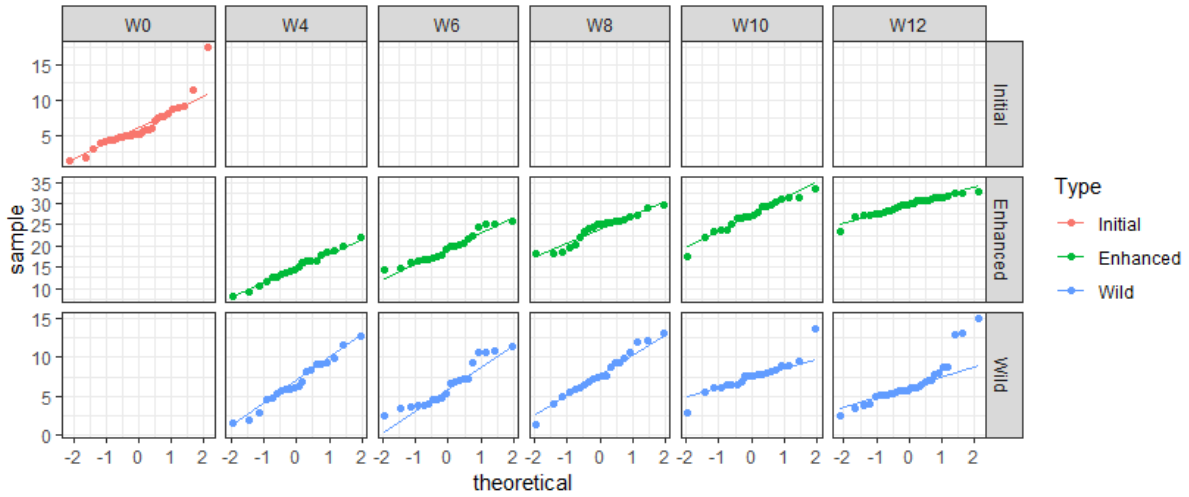


Figure 27: Probability plots of GI measurements from each type and week. Samples following the line is assumed to be following normal distribution. Theoretical values on x-axis. The Shapiro-Wilk test for wild urchins at week 12 was rejected ($p<0.05$), but the visual inspection shows only three outliers of n=30. The initial sample is also rejected by Shapiro-Wilk but has only one outlier. All the outliers have been determined by residual plotting of the ANOVA to not affect the model significantly.