Temporal differences in abundance, size-distribution and recruitment in the inner Oslofjord Green sea urchin *(Strongylocentrotus droebachiensis)* population and its possible implications on the population's future health.

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II

IV

Acknowledgements

Abstract

The Green sea urchin (*Strongylocentrotus droebachiensis*) is an echinoderm found along coasts and in fjords in the Northern Hemisphere, and is an important algal grazer in kelp forests. The inner Oslofjord population has been studied by scientists over the past decades and has been regarded as healthy. However, in the 1990s an urchin front along the Norwegian coast was pushed back because, of what scientists discovered later, was an increase in water temperature along the Norwegian coast which was unfavourable for the urchin. In more the recent times there have been reports from local divers and fishermen of diminishing urchin numbers. In response to these reports, this study investigated the current condition of the Green sea urchin population in the inner Oslofjord through comparing present data with previous studies, in order to determine if a collapse in the urchin population is indeed occurring in the fjord. Furthermore, because the Green sea urchin is a cold water species, it is sensitive to temperature as well as salinity, the study wanted to determine to what extent temperature and salinity has affected the sea urchin population in the fjord. This study found that the Green sea urchin population is still healthy and abundant. However, the study revealed some indicators which may become a concern for urchin growth in the future. Abundance was found to vary significantly with depth, with the majority of urchins occurring at 15 m and 20 m below sea level. Average urchin diameter was significantly different from previous studies, where the average diameter was 2.5 cm smaller now than in 1992. Levels of recruitment in the fjord are still healthy, but significantly lower than in 1992. This study, when comparing to a previous study from the fjord, found that recruitment occurs regularly. Gonad Index did not vary significantly between 1992 and 2013. Average water temperature at 4 m and 5 m depths increased with 2 °C at Drøbak and 4 °C at Svartskog since 1999, while average salinity increased by 0.2 ppt. Correlations between temperature and urchin depth ($R^2 = 0.0871$), and salinity and urchin depth ($R^2=0.245$) were found to be quite weak. Further research on temperature and salinity limits for *S. droebachiensis* will be an important tool to assess the vulnerability of the species' populations in fjords to climate change.

Glossary

NIVA – Norwegian Institute for Water Research.

Sill – A submerged threshold separating the basins of two water bodies.

Drop camera registration – The use of a submerged camera to film or take photos of the benthic community.

Sample sites – Locations utilized by the present study to collect sea urchin data. The sample sites used in this study are Drøbak, Flaskebekk and Svartskog.

Environmental monitoring station – Location at which environmental parameters are measured. The environmental monitoring stations used in this study are Drøbaksterskelen, Oksval and Svartskog.

Urchin monitoring station – Location at which changes in urchin population was measured and monitored. The urchin monitoring stations used in this study are Oksval and Svartskog.

Gonad Index (GI) – Calculation of gonad mass as a proportion of total mass.

Shallowest urchin depth – The shallowest depth at which a sea urchin has been registered during a sampling session.

Tolerance limit – The level of an environmental variable where, if exceeded, the sea urchin will move away to find a more suitable location, but the sea urchin will not necessarily perish if exposed to it.

Lethal limit – The level of an environmental variable where, if exceeded, the sea urchin will most likely perish if exposed to it.

Upper temperature threshold – The tolerance limit of the Green sea urchin with regards to water temperature.

Lower salinity threshold – The tolerance limit of the Green sea urchin with regards to water salinity.

Contents

List of Figures

List of Tables

1.0 Introduction

1.1 Problem statement

The Green sea urchin is the Oslofjord's most prominent grazer of macroalgae, and plays a vital role in ecosystem maintenance. Until recently, it has been believed to maintain a healthy population in the fjord. However, repeated reports by fishermen and observations by scientists conducting research in the area have drawn attention to the possibility that the urchin population may be declining in the southernmost and isolated fjords in Scandinavia, specifically Gullmars-, Oslo- and Lysefjord (observations by H Christie, KM Norderhaug and E Svensen). A decline in the Green sea urchin population would be an issue of concern as abundance of the Green sea urchin in an ecosystem is a key determinant of health and stability in many marine ecosystems (Leinaas & Christie, 1996). However, while the benthic community in the Oslofjord - of which the Green sea urchin is a part - has been monitored in recent years by NIVA, a study which focuses specifically on the health of the Green sea urchin population has not been conducted since 1992.

1.2 Background

1.2.1 State of the Green sea urchin in the Oslofjord

The Green sea urchin *(Strongylocentrotus droebachiensis)* was initially found and described by Otto Friedrich Müller at Drøbak in 1776. The Green sea urchin (Figure 1) is an echinoderm found from the low tide mark down to 1200 meters in the Artic and Northern Atlantic waters, stretching south to New Jersey, USA in the Northwest Atlantic and to Northern Denmark in the Northeast Atlantic (Hayward & Ryland 1990; Mortensen 1924). It is also found in the Northern Pacific. In the past decades the Norwegian Institute for Water Research (NIVA), and the University of Oslo (UiO) have been monitoring the general species assemblage, including the sea urchin population in the Oslofjord through both monitoring programmes (Källqvist et al., 1982; Magnusson et al., 1984a, 1984b, 1992) and independent studies (Green, 1983; Frid & Thomassen, 1995; Fredriksen, 1999).

The condition of the Green sea urchin population, along with the rest of the benthic community in the inner Oslofjord was surveyed once by Green (1983) from 1977 to 1979 at Flaskebekk, Nesodden. Green (1983) found a healthy population of the Green sea urchin was discovered. Between 1992 and 1994 two studies assessed the condition of the Green sea urchin population at Drøbak (Frid & Thomassen 1995; Fredriksen 1999). Fredriksen (1999) investigated the size-distribution, Gonad Index and vertical-distribution while Frid & Thomassen (1995) assessed the recruitment. These studies also confirmed the high abundance of the Green sea urchin at Drøbak. At the time Fredriksen (1999) and Frid & Thomassen (1995) found urchins at depths

Figure 1: Photos of the Green sea urchin: from the side (right) and from underneath (left).

ranging from 5m to 20m below the surface. The population displayed a healthy growth rate, strong gonad production and recruitment.

More recent scientific research on the Green sea urchin's abundance in the inner Oslofjord is lacking, as the latest study was conducted in the early 1990s. Over the last couple of years a decline in the sea urchin abundance has been reported, especially in the shallower depths. This would be an issue due to the important role that the Green sea urchin plays in ecosystem health and maintenance.

1.2.2 Role of Green sea urchin in ecosystem maintenance

Destructive kelp grazing is a phenomenon that occurs mostly in the mid-latitudes (40-60° N and S) (Steneck et al., 2002). Destructive kelp forest grazing is the consumption of kelp at a rate which exceeds the rate at which the kelp is able to replenish itself (Steneck et al., 2002). Urchin species within the *Strongylocentrotus* genus are usually the culprit of these types of extreme grazing events (Paine & Vadas, 1969; Harrold & Pierce, 1987; Steneck et al., 2002).

The Green sea urchin is known as an important grazer of seaweeds. However, the sea urchins are not detrimental to kelp beds in low abundances, as they are unable to consume the kelp quickly. For grazing to become destructive, the urchin front must reach a biomass of at least 2 kg urchins/m^2 according to Gagnon et al. (2004). The sea urchins will climb and weigh down of the kelp fronds so more individuals get access (Breen & Mann, 1976a, 1976b). To reach this biomass, the front tends to consist of large adults (Gagnon et al., 2004). Reported rates at which a front can remove a forest are up to 4 m/month (Scheibling et al., 1999).

Destructive grazing changes the ecosystem dramatically and high biodiversity kelp and seaweed bed systems are transformed into unproductive barrens dominated by sea urchins, where only little more than calcareous algae are able to survive and grow (Lawrence, 1975; Mann, 1977; Chapman, 1981; Steneck, 1983, 1986; Scheibling & Hatcher, 2001). The urchin barrens are ecologically stable and may persist for decades (Himmelman et al., 1983) as sea urchins have a high phenotypic plasticity which enables them to survive with very little food (Russell, 1998).

The largest grazing event occurring in the Northeast Atlantic was along the Norwegian coast, from 63-71° N (Norderhaug & Christie, 2009). The reason for the more northern expansion of destructive grazing along the East Atlantic is due to the distribution of kelp is further North along the coastlines (Steneck et al., 2002). This extreme grazing event along the Norwegian coast started in the 1970's, and the dominant grazer was found to be the Green sea urchin (Sivertsen, 1982). Approximately 2000 km of *Laminaria hyperborea* and *Saccharina latissima* beds on the outer and inner coast were transformed into urchin barrens over the next two decades (Sivertsen, 1997). When the grazing event was at its greatest, the barren

grounds extended from Nordmøre (63°N) in to Russia (Figure 2) (Sivertsen, 1997; 2006; Norderhaug & Christie, 2009). However, destructive grazing has happened in fjords as well. The kelp in Vestfjord, Northern Norway was grazed in the early 1980s and experienced minor grazing events of immature kelp in to the 1990s (Hagen, 1995).

Macroalgal beds and urchin barrens are two alternative stable systems which are very difficult to switch between due to feedback mechanisms which help to stabilize the system and make it resilient against disturbance. Examples of such feedback mechanisms increased predation of adults in kelp forests, and high settling mortality for kelp on barren grounds (Gagnon et al., 2004). The persistence of urchin barrens is mainly due to the continuous grazing of adult and juvenile kelp, the loss of urchin predators in the system, bulldozing of kelp recruits (Green, 1983) and the ability to survive without food for over four weeks without harm (Garnick, 1978).
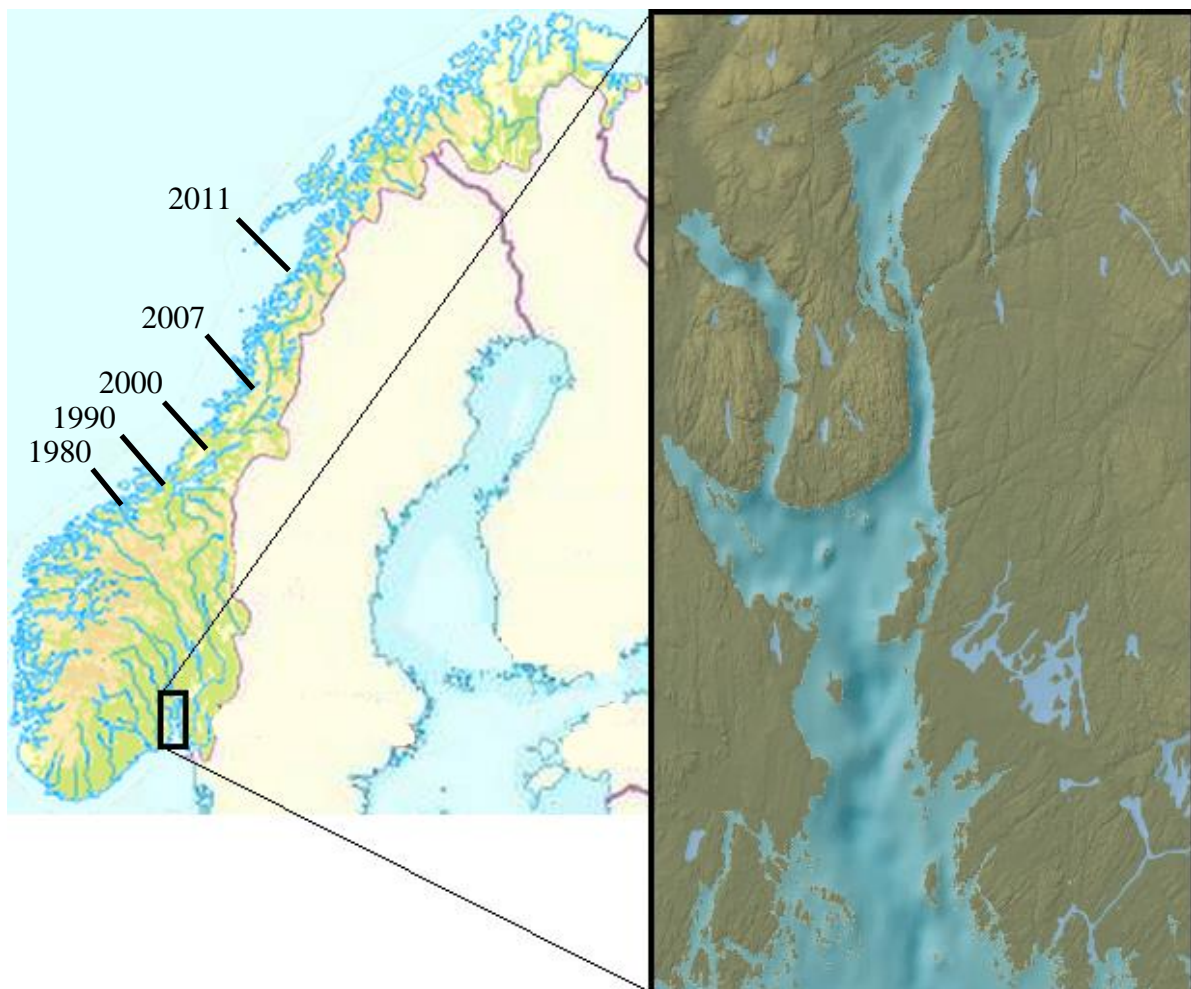
Figure 2: Map over Norway. The enlarged image is of the Oslofjord. The black lines represent the southern border of the urchin barrens at different times. The 1980 border is when the sea urchin grazing was at its greatest, while the 2011 border is the most recent sighting of the southern border.

These mechanisms inhibit the regrowth of kelp forests, even when the sea urchins are at low abundances (< 10 ind./m^2) (Leinaas & Christie, 1996). The kelp forest would be able to start recovering, in the timespan following the reduction of urchin abundance. However, this is a time-consuming process. Collapses in urchin abundance might be induced by parasites (Skadsheim et al., 1995; Sivertsen, 1996), disease (Scheibling et al., 1999), predation (Fagerli et al., 2014) or changing environmental parameters, like salinity or temperature (Rinde et al., 2014). Even when the conditions for regrowth of macroalgae are ideal, the average time it takes for a kelp forest of *Laminaria hyperborea* to grow and become dominant is approximately 3-4 years (Foreman, 1977; Christie et al., 1998). The biodiversity associated with kelp forests needs more than five years in order to fully recover after kelp regrowth has started (Christie et al., 1998). The movement of sea urchins in and around kelp forests can unsettle newly settled kelp or other organisms (Strain et al., 2013) while the older individuals are grazed down before they can withstand the grazing pressure. The kelp forest's role as a spawning ground, nursery, food source and refuge for organisms (Christie et al., 2003) will be disrupted, and further removal of kelp will push the system into an urchin barren state again (Sivertsen, 1997).

These kelp-urchin interactions are important for the health of the system. Through grazing activity the sea urchins are able to maintain a level of disturbance which increases the species diversity in the system in accordance to the intermediate disturbance hypothesis as suggested by Connell (1978). However, if the sea urchin population gets too dense it becomes unfavourable for the system, as the sea urchins will directly reduce the biodiversity of the system by keeping the benthic substrate free from vegetation and other sessile organisms. Some of the more severe consequences from this is energy loss in the system, reduced shelter from predators, reduced spawning ground and transportation corridors, reduced production and diversity (Estes et al., 2004; Graham, 2004; Byrnes et al., 2006). Due to the critical role that the Green sea urchin plays in maintaining ecosystem health and biodiversity in the Oslofjord, it is important to investigate the claims that the Green sea urchin has disappeared from the Oslofjord, and then assess the current condition of the sea urchin population.

1.2.3 Morphology of the Oslofjord and the possible impacts on the Green sea urchin's future

The Green sea urchin is believed to have grazed in many of Norway's southern fjords, including the Oslofjord, regularly since the last ice age (Vasseur, 1952; Anon, 2002). Being a cold-water species, Green sea urchin populations on Norway's coast have continued to retreat further north as water temperatures have risen. This gave the kelp a possibility to recover on the coast (Skadsheim et al., 1995). The sea urchins retreated up to Vega in 2007 (64.5°N) (Norderhaug & Christie, 2009) and in more recent times further regrowth of kelp has been reported almost as far north as Lofoten (Figure 2) (Rinde et al., 2014). Temperature increases unfavourable for the sea urchins' larval development was indicated as a plausible cause for the retreat (Fagerli et al., 2013), and that it is temperature that is limiting southern expansion along the coast.

Despite the Green sea urchin migrating North on the coast, Oslofjord has not experienced any change in its population. This is a main point of interest as the Oslofjord is located further south than current coastal populations of the Green sea urchin (Figure 2). Furthermore, while the coastal urchin population has been affected by ocean warming since around 1990 (Norderhaug & Christie, 2009), the Oslofjord populations appear unaffected. It is possible that the isolating effect of the fjord's morphology is what has allowed the populations there to persist so far south (Fredriksen, 1999; Norderhaug & Christie, 2009).

An important characteristic of the Oslofjord is that Norway's two largest rivers – the Glomma and Drammenselva – flows out at mouth of the fjord. As a result of this, a hyposaline water body may sometimes be present in the outer parts of the fjord during the Spring and Summer (Walday et al., 2014). This water body may be preventing the sea urchin population from colonising the fjord's outer coast (Figure 2) as juvenile sea urchins and recruits are most vulnerable to low salinities (Lange, 1964). The inner Oslofjord is further isolated from the outer Oslofjord by the Drøbak sill, which separates the deep-water of the inner basins from the outer basins for long periods, especially during the stratification in the Spring (Staalstrøm et al., 2012). There is another sill at Oksval, separating the Vestfjord basin and Bunnefjord basin (Staalstrøm et al., 2012). The combination of a sill, seasonal stratification and water

6

circulation at Drøbak may create a low connectivity between the outer and inner fjord urchin populations. This low connectivity can be caused by hyposalinity in the surface water flowing out. However, as ocean water temperatures continue to rise as climate change progresses, the morphology of the Oslofjord will also restrict migration of the urchins into suitably cooler waters. High resolution analyses of sea surface temperatures have shown that coastal regions are heating up quicker than offshore regions (Lima & Wethey, 2012). Because the Green sea urchin is a cold-water species it will be strongly affected long-term by the temperature and salinity changes predicted by IPCC (2013).

1.2.4 Environmental changes and possible impacts on the sea urchin

Predictions for climate change in the North Atlantic suggest that the already affected shallow- and surface water temperatures will increase between 1 and 3 degrees Celsius by the year 2100 (IPCC, 2013). Salinity is predicted to decrease by approximately 1 ppt (parts per thousand) within the same timeframe (IPCC, 2013). Sea urchins have been found to be affected by various low salinities (Russell, 2013), and high temperatures have been found to affect urchins' distribution (Fagerli et al., 2013). Echinoderms live in osmotic equilibrium with the surrounding water (Krogh, 1939), thus salinity plays a major role in limiting the urchin's depth distribution, even more so than temperature does (Drouin et al., 1985; Roller & Stickle, 1985; 1994). If either salinity or temperature exceeds the tolerance limits of the Green sea urchin, it may force the urchins to migrate into colder, more saline waters or kill them (Lange, 1964; Stephens, 1972). With the observations of fewer sea urchins in the Oslofjord, one of Norway's southernmost fjords, and predicted increases in temperature in the fjord, it is timely to question if the urchin collapse occurring along the Norwegian coast also is occurring in the Oslofjord?

There remains some dispute in the literature as to the exact environmental thresholds which the Green sea urchin can tolerate. The Green sea urchin's upper temperature and lower salinity thresholds have been suggested to lie at 10 °C (Stephens, 1972; Foreman, 1977) and 21.5 ‰ (Lange, 1964), respectively. However, these suggested thresholds have later been disputed by Pearce et al. (2005) and

Siikavuopio et al. (2008; 2012) with respect to the temperature; both of whom concluded that the 10 °C threshold is actually where the sea urchins experience optimal somatic growth. A study by Pearce et al. (2005) found that Green sea urchins were able to tolerate water temperatures up to 19 °C. However, this study used Green sea urchins from the Pacific. With regards to salinity, Stickle et al. (1990) claimed that the limit is at 13.0‰ while Himmelman et al. (1984) concluded that it is at 14.0‰. These tolerance differences might be due to local. The various studies have been conducted at Drøbak, southeast Alaska and Nova Scotia, respectively. Another possibility as that some sort of synergetic relationship between temperature and salinity is affecting the urchins. With this in mind, it is timely to question how the Green sea urchin population is being affected by the impending salinity and temperature changes.

1.2.5 Biology of the Green sea urchin

Green sea urchins grow at a rate of approximately one centimetre per year when conditions are good, but the growth rate decreases when food is scarce or of poor quality, or when the urchin reaches approximately four cm in diameter (Larson et al., 1980; Fagerli et al., unpubl. manuscript). Although the Green sea urchin's growth rate generally flattens out at six cm, some individuals may reach a diameter of up to eight
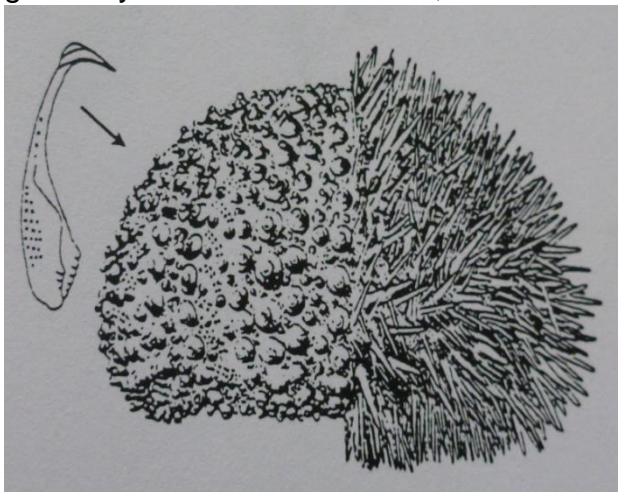
Figure 3: The globiferal pedicels of the Green sea urchin.

cm. The Green sea urchin has a flattened disc with 10 plates. Each plate has five or six pore pairs, while the globiferal pedicels are large and robust (Figure 3) (Mortensen, 1924). The Green sea urchin can vary in colouration from a green hue to a deep purple. The spikes on adults usually have white tips. The primary and secondary spikes are the same length.

The reproduction of the Green sea urchin is external and occurs around March-April (Emlet et al., 1987; Sivertsen, 2006; Fagerli et al., 2013). The species is dioecious and both eggs and sperm are released into the water column. After fertilization the egg floats in the upper water layer while it develops and hatches into a pelagic feeding larvae. This larval stage lasts between 5 to 21 weeks, depending on water temperature and egg size (Strathmann, 1978). Within a reproductive cohort the longest timespan between the first and the last larvae settling is normally five weeks. With such a long pelagic stage the dispersal potential is high (Underwood & Fairweather, 1989; Miller & Emlet, 1997). Settlement occurs mainly in the Summer months (Falk-Petersen & Lønning, 1983).

The Green sea urchin is omnivorous (Russell, 1998; Scheibling & Hatcher, 2001). Though the main sources of food vary between life stages; detritus is more important for small juveniles, while adults primarily eat macroalgae when close to kelp beds (Himmelman & Steele, 1971; Scheibling & Anthony, 2001), or drift algae and newly-settled organisms when on barren grounds (Chapman, 1981).

For the first two years of its life, the urchin exhibits cryptic behaviour, hiding in cracks, crevices and between spines of adult sea urchins (Himmelman, 1986; Raymond & Scheibling, 1987; Scheibling & Hamm, 1991; Frid & Thomassen, 1995; Fredriksen, 1999; Dumont et al., 2004). As juveniles the urchins are more vulnerable than adults to changes in temperature and salinity. After reaching two centimetres, the juvenile urchins abandon their cryptic lifestyle and move into open terrain (Himmelman, 1986; Raymond & Scheibling, 1987). These first years in the open are crucial as this is the life stage during which the urchins are most vulnerable to predation (Shears & Babcock, 2002; Fagerli et al., 2013). As the urchin grows, its rate of movement also increases, needing to spend less time in hiding (Dumont et al., 2006). When reaching four cm the individuals are too big for most predators (Clemente et al., 2013) and their growth rate decreases (Fagerli et al., unpubl. manuscript). In order to accurately assess the Green sea urchin population's condition in the inner Oslofjord, this study aimed at investigating members at different life stages and roles, namely recruits and juvenile individuals, and adult, old individuals.

1.3　Objectives and hypotheses:

Populations of the Green sea urchin (*Strongylocentrotus droebachiensis)* are declining along the Norwegian coast and it has been suggested that the some fjord populations also are in decline. This study's first objective was to investigate the health of the Green sea urchin population in the inner Oslofjord, and secondly, to assess if the possible changes in the population can be attributed to temperature increases or salinity decreases. This study tested two hypotheses:

Hypothesis 1: There is a difference in the Green sea urchin population in the inner Oslofjord with regards to density, size-distribution, recruitment and/or gonad production from previous studies.

Hypothesis 2: Changes in temperature and salinity correlate to the distribution of the Green sea urchin population in the inner Oslofjord.

1.4　Thesis plan

Chapter 2 describes the study site, the methods used to collect data, the benefits and shortcomings of the methods, and how the data was analysed. The results of the research are presented in Chapter 3 before they are discussed in a broader sense in Chapter 4. Finally, the most important findings from this study are summarized in Chapter 5 and recommendations for further research on this topic are presented.

12

2.0   Material and methods

2.1   Introduction

The study was conducted in the inner Oslofjord (59.66 – 59.91°N, 10.46 – 10.78°E). Previous studies provide a historical record of the condition of the Green sea urchin population in the fjord. An introduction to the different sampling sites used is provided in Section 2.2, while an explanation of the design of the study and a brief discussion of methods used to collect data are found in Section 2.3. The method of sampling adult individuals, density counts and recruits are discussed in Sections 2.3.1, 2.3.2 and 2.3.3, respectively. The environmental and monitoring data are explained in Section 2.3.4. Finally, Section 2.4 will go through the various statistical analyses and correlation tests utilised in this study.

Figure 4: Overview map of the inner Oslofjord. Study sites used in this thesis are pinpointed.

2.2   Sample sites

The study used three different urchin sampling stations in the inner Oslofjord where data from previous studies were available (Figure 4). One urchin sampling station was located outside the University of Oslo's research station in Drøbak (59.66°N, 10.63°E), while the two other stations were at the respective ferry wharfs at Flaskebekk, Nesoddtangen (59.86°N, 10.65°E) and Svartskog, Oppegård (59.78°N, 10.73°E). The availability of previous data on the Green sea urchin varied between stations. Drøbak had quantitative historical data. Flaskebekk had some historical quantitative data and semi-quantitative monitoring data. Svartskog only had semi-

13

quantitative monitoring data. Despite the lack of complete historical records, it was still possible to compare the data from previous studies to the present study's results for the analysis of recruitment, densities and distributions of the sea urchin populations.

2.2.1 Drøbak

The sampling station in Drøbak was located furthest out the fjord. It is located just outside a sill where the depth is only 19.5 m deep. During the year the freshwater input may cause a strong stratification in the upper ten metres, where the freshwater flows out of the fjord while saline deep-water go over the sill to replenish the surface-water. The bottom is a mosaic of hard substrate, with patches of soft bottom in between. The tidal currents are strong in the area. Data collection took place in the same area as previous studies in order to compare pre-existing and newly collected data on recruitment (Frid & Thomassen, 1995), size-distribution, abundance and Gonad Index (Fredriksen, 1999) from 1992 to 1994.

2.2.2 Flaskebekk

This was the northernmost urchin sampling station in the study, with an ocean floor consisting of a mosaic of hard substrate with patchy sediment. The wharf was located at the western side of Nesoddtangen, towards Vestfjord. As the wharf still operational at the time of the data collection, there was some disturbance from boat traffic in the area. Vestfjord has a maximum depth of 100 m. Similar to Svartskog station, Flaskebekk station also lies on the inside of the Drøbak sill, resulting in a poor replenishment of the deeper bottom water. However, the water quality at the depths surveyed in this study was most likely not affected by this phenomenon. The floor in this location slopes gently downwards for the first eight metres and then becomes steeper and more irregular in profile. Here, Green (1983) assessed the benthic community structure and its development from 1977 to 1979. This study compared its data on size-distribution to Green's findings (1983).

2.2.3 Svartskog

This sampling station was located in the Bunnefjord, close to the wharf at Uranienborg, Roald Amundsen's estate. The floor of this location is composed mainly of soft sediment in shallower depths and becomes progressively rockier and steeper once deeper than seven metres. Ice and ice melting may reduce access to the station during Winter and early Spring. Semi-quantitative transect data from Svartskog station spanning June 2005 to May 2011 was provided by NIVA.

2.3   Study design

This study sampled data in order to compare results between stations and also with data from previous studies. A crossed design was used where three urchin sampling stations and four depths per station (5, 10, 15 and 20 m) were investigated. At each depth the density of sea urchins was examined before specimens were collected for further measurements of size and Gonad Index. All individuals were classified; any individuals of *P. miliaris* collected were discarded. Standardized collection units were also placed at different depths at each site to compare the number of recruits settled (see Section 2.3.3). The first data collection at Drøbak and Flaskebekk took place on March 26th 2013, while the collection at Svartskog was conducted on May 23rd 2013 due to ice cover in March. The second round of data collection at all stations occurred on June 28th 2013 and the final round of data collection was done on October 3rd 2013. Sampling at these times throughout the year was necessary to investigate whether the Oslofjord population reproduces at the same time as previous studies as well as other populations. Settlement season has been shown in previous studies to be in the Summer months in the Oslofjord (Frid & Thomassen, 1995) and other populations (Fagerli et al., 2013; Falk-Petersen & Lønning, 1983), but since the pelagic stage may last between 5 and 21 weeks (Strathmann, 1978) another set of scours were set out to register recruits settling later. Sampling at these dates also provided the opportunity to register changes in the depth distribution of the urchins.

2.3.1 Sampling of adult urchin specimens

Adult urchin specimens were collected by a SCUBA diver. This is the only practical way to collect sea urchins representatively with regard to densities and size-distribution. Each dive lasted approximately 60 minutes per station and approximately 15 minutes per depth. As far as possible were 100 urchins per depth at each sampling station collected in order to analyse the demographics between depths and stations. The individuals were collected in the same plots used for the density counts to get a representative sample of the populations. All sea urchins inside each frame including the last frame were collected. If the density at a certain depth was low, the diver would collect all individuals found at that depth within the available time. The individuals were placed into gathering nets labelled with the station and depth and kept separate. In some cases fewer than 100 urchins were collected at each depth due to limited specimen availability and time constraints.

Once the collected specimens were brought onto land, extra care was taken to distinguish the Green sea urchin from a very similar looking echinoderm species, *Psammechinus miliaris*. The best way of distinguishing the two species from each other is by examining the ambulacral plates. However, due to the time-consuming nature of this approach, the decision was made to use the colouration of the body and tips of the spikes, and the shape of the sea urchin to distinguish between the species instead. This latter method is less time consuming but still yields fairly accurate results. *P.miliaris* has a green hue, often with purple tips on the spikes, while the Green sea urchin is green or purple with white tips on the spikes. *P. miliaris* is usually found higher in the water column than the Green sea urchin.

2.3.2 Density count

During every session of data collection density counts were conducted at each urchin sampling station. These were conducted at four different depths (5; 10; 15 and 20 m). A 0.25 m^2 square frame was placed at random ten times at each depth. The number of sea urchins within the square frame was registered by the SCUBA diver and reported to the crew on land using a communication cable. The crew on land wrote down the number of urchins in each frame and calculated a mean density for the

16

respective depth. By using square frame plots instead of a drop-camera the study was able to acquire accurate density measurements instead of semi-quantitative registrations as one would get from using a drop-camera. However, due to the time constraint posed by SCUBA diving, the study was only able to assess one transect per sampling session. Although a drop-camera would have allowed for several transects and deeper depths to be sampled, the use of a diver allowed for both physical specimens and information on density to be collected simultaneously. As such, the use of diving with scuba gear was selected as the most appropriate method for achieving the objectives of this study.

2.3.3 Sampling of newly settled juveniles

Standardized collection units were placed at three depths (5; 10 and 15 m) at each station, in order to study the recruitment success. These units consisted of four pan scours attached to a two metre long chain (see Fagerli et al., 2013). The standardization of the units facilitated quantitative comparisons of settlement of recruits between stations and previous studies. The scours were attached to the chain with cable ties, approximately 0.5 m between them. During the following round of data collection the scours were carefully enclosed in ziplock-bags by the diver and released from the chain before they were sealed. New scours were then attached to the chain to measure settlement of recruits during the next season. Once on land the contents of the ziplock-bags were emptied into containers labelled with station, depth and scour number. The ziplock-bags themselves were also rinsed thoroughly to make sure that all recruits were transported to the container. All samples were fixated with 70% alcohol on location. Some chains were lost and it was believed that this was caused by fishing activity. Fishing gear was found at all sampling stations.

2.3.4 Environmental and monitoring data

Salinity and temperature data was provided by the *Academic Council of Water and Sewage Technical Cooperation in the Inner Oslofjord*, while monitoring data with sea urchin registrations has been collected by NIVA. In order to test for the presence of relationships between environmental factors and sea urchin distributions, changes in temperature and salinity were compared to data on sea urchins over the last decade. Temperature and salinity data came from Drøbaksterskelen, Oksval and Svartskog environmental monitoring stations (Figure 5). The environmental measurements were taken throughout the year. The study made use of records spanning from 1999 up until 2009 at at Drøbaksterskelen, 2010 at Oksval and 2011 at Svartskog. While some environmental records earlier than 1999 did exist, they were not included in this study due to a number of inconsistencies which made accurate comparison impossible. These data varied in number of samplings per year, dates the sampling occurred and contained lapses of several years between each sampling.

Figure 5: Geographical location of the environmental monitoring stations. environmental monitoring stations are coloured red while the stations which this study collected urchins from are coloured blue. Sea urchin monitoring data from Svartskog and Oksval were taken from the same location as the environmental data.

The monitoring data on urchins had been collected by NIVA during 2005-2008 and 2011-2012. The transects were conducted close to the environmental monitoring stations Oksval and Svartskog. In some instances there was a temporal gap between the collection of environmental data and the urchin data. NIVA used a 2-tier system to record the presence of sea urchins; level 1 was that the urchin was present while level 2 meant that several urchins were found. Data from 2011 and 2012 was only registered as Echinoderm, not as species, which creates some uncertainty as to which species were found

18

during those years. While Frid & Thomassen (1995) and Fredriksen (1999) worked with absolute numbers of urchins, NIVA's reports worked with a semi-quantitative system. Therefore, this study compiled all data into a 4 tier system: level 1 is a single individual (present), level 2 is 1 to 20 individuals (scattered), level 3 is 20 to 40 individuals (common), and level 4 is over 40 individuals (dominating). Figure 5 shows the proximity of the environmental monitoring stations and the urchin sampling stations used by this study.

2.4 Data analysis

All collected physical specimens were analysed in the laboratory within two weeks after collection. Adult individuals were measured immediately or stored in a freezer, while the scours were stored in a dark and well-ventilated area.

2.4.1 Analysis of adult sea urchins

The diameter of the sea urchins was measured down to the nearest millimetre using a calliper. Size was used as a proxy for the age of the individual, as the 1 cm/year grow rate is a reliable indicator of urchin age (Robinson & MacIntyre, 1997; Vadas et al., 2002) until the individual reaches approximately four cm (Fagerli et al., unpubl. manuscript; Russell & Meredith, 2000). All individuals collected during the second and third sampling sessions and larger than 3.5 cm in diameter were weighed using an electronic scale and then opened to weigh the gonads. This was done to calculate Gonad Index for the individuals, in accordance with James & Siikavuopio's (2012) guide to reproduction assessment. By also measuring the Gonad Index in the Autumn, one is able to assess at what stage in the reproductive cycle the population is (James & Siikavuopio, 2012). Gonad Index can be used as a proxy for fitness and the general condition and reproductive potential of the sea urchins. Higher index numbers indicate better conditions for the sea urchins. The formula to determine Gonad Index is:

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

2.4.2 Analysis of settlement

The containers with scours were rinsed thoroughly and emptied into a 250 μm sieve. Sea urchin recruits have a size of 500 μm and up, so the mesh is small enough to avoid loss of recruits. The scours were cut into pieces and rinsed under water to retrieve all organisms in the scour. The content was then transferred to a petri dish, and placed under a microscope to be counted.

2.4.3 Statistical analyses

Microsoft Excel 2010 and R x64 3.0.0 were used to statistically analyse the data. Because this study used a crossed design the data compiled had a nested structure. The study had three sampling sessions, each with three sampling stations where four depths were assessed. In order to be able to test the different variables (for example depth) the study needed a test which took into account any variation in the other variables (in this example sampling session and station) to avoid untrue significances. Thus, this study used nested ANOVA to take into account these possible variations. The present study tested for significant differences between sampling season, year, depth and stations, and for interactions between the respective variables (a more accurate description of the different ANOVA tests conducted can be found in the Appendix).

This study wanted to determine if any size classes were particularly important for reproduction. To find this out, two correlation tests were conducted: one on the relationship between urchin diameter and Gonad Index, and one on the relationship between urchin diameter and gonad production. Trend line and R^2 were calculated to assess the strength and direction of these relationships. Similar tests were performed on temperature and salinity. The shallowest depth where sea urchins were registered was plotted against the temperature and salinity at that depth to look into whether either variable had any effect. The trend lines and their corresponding R^2 numbers indicated the strength and direction of these relationships.

3.0   Results

3.1   Chapter introduction

In this chapter, the raw data collected during this study is presented and compared with pre-existing data from external sources. Section 3.2 begins with a description of sea urchins collected, and their distribution between sampling sessions and sampling locations. Section 3.3 provides a comparison of this study's findings on sea urchin characteristics with that found in previous studies. Section 3.4 then examines the possible effect of environmental factors on the health of the sea urchin population in the inner Oslofjord.

3.2   Description of samples collected

The number of urchins collected at different locations and during different seasons varied in accordance with natural occurrence. A total of 2,509 urchins were collected and measured in this study. Of this total, 637 individuals were collected in the Spring (27%), 665 in the Summer (25%) and 1,207 in the Autumn (48%). In regards to sampling locations, 846 individuals were collected at Drøbak (34%), 1,118 individuals at Flaskebekk (44%) and 545 individuals at Svartskog (22%). Table 1 shows the sample distribution of urchins collected across the different seasons and stations included in this study.

Table 1: Summary of the number of sea urchins registered during the different sampling sessions at each station. Spring sampling occurred at March 26th and May 23rd, Summer sampling was done June 28th and Autumn sampling was done October 3rd.

	Drøbak	Flaskebekk	Svartskog	Total
Spring sampling	261	246	130	637
Summer sampling	291	153	221	665
Autumn sampling	294	719	194	1,207
Total	846	1,118	545	2,509

3.3 Indicators of the condition of the Green sea urchin population

3.3.1 Urchin abundance & depth distribution

Urchin abundance and depth distribution were measured to assess the health and possible migration of the Green sea urchin populations in the Oslofjord. The abundance and depth distribution of the Green sea urchin was measured at three locations within the Oslofjord: Drøbak, Flaskebekk and Svartskog. For all of the locations, urchin abundance and depth distribution was found to vary between seasons. Urchin abundance and depth distribution also differed noticeably between this year and previous studies (Fredriksen, 1999).

Drøbak

At Drøbak in 2013 (Figure 6, top right), the majority of individuals were found to occur at 20 m during all three sampling dates. However, the range of their depth distribution did vary throughout the year. In March (Spring) and June (Summer) no urchins were registered shallower than 20 metres. In October (Autumn), the range expanded and urchins were registered at all four depths investigated by this study. This depth distribution of urchins also varies compared to the findings of Fredriksen (1999) who conducted a similar study in 1992 (see Figure 6, top left) and found the majority of urchins in Drøbak occurred at 15 m. Fredriksen (1999) also found more individuals occurring at the depths of 5 and 10 m than this study did in 2013.

Flaskebekk

At Flaskebekk (Figure 6, bottom left) as well, the greatest densities were found at 20 m, except in March (Spring) where it was greater at 15 m (34.4 urchins/ m^2). However, urchins occurred as shallow as 5 m in Autumn, 10 m in the Summer and 15 m in the Spring. In June (Summer) urchins were found at 10, 15 and 20 m, but the density was lower than 10 urchins/ m^2. During the final sampling, in October (Autumn), the distribution gradually increased with depth, starting at 4.4 urchins/ m^2 at 5 m and ending up at 21.6 urchins/ m^2 at 20 m.
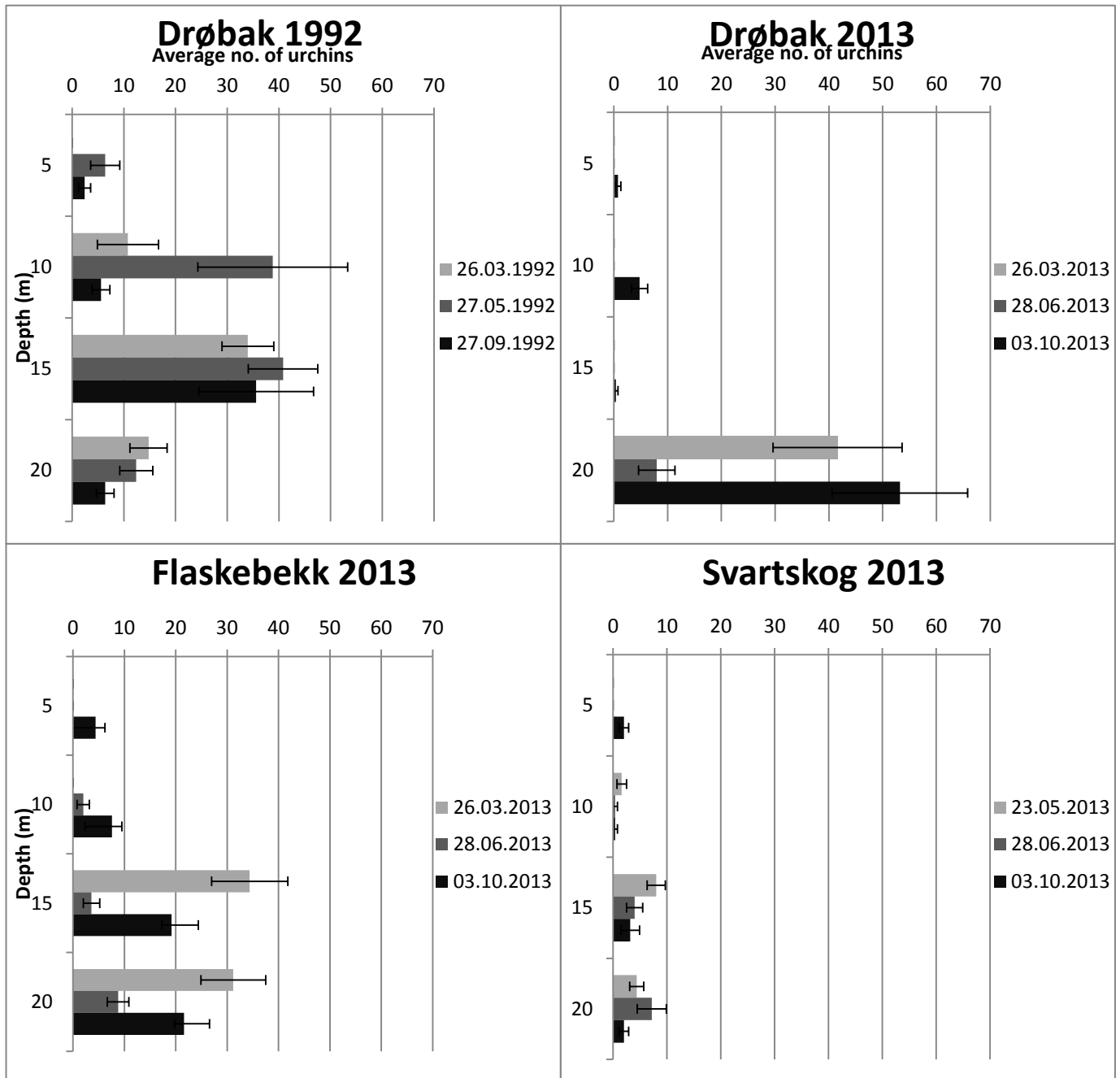
Figure 6: Average density distribution with depth. Data from Drøbak in 1992 (top left), Drøbak in 2013 (top right), Flaskebekk 2013 (bottom left) and Svartskog 2013 (bottom right). The y-axis is the different depths where sea urchins were registered. X-axis shows the average number of sea urchins per square meter (urchin/m^2) at the different depths.

Svartskog

Svartskog (Figure 6, bottom right) had a low density, less than 10 urchins/ m^2, at all depths and all periods. The highest abundance was found at 15 m in May (Spring), 8 urchins/m^2, and June (Summer), 7.2 urchins/m^2. Urchin abundance in October (Autumn) was the lowest at Svartskog of all the different.

25

Comparison between stations

The variation in abundance between stations in 2013 was found to be significant (P=0.0321) (Table 2). Urchin abundance (or density) did vary significantly with changing depth (P<<0.001) – sea urchins were more frequently registered at 15 and 20 m rather than in shallower waters.

When comparing the raw data collected in this study with the available records from Fredriksen (1999), this study found significant interactions between year and depth (P = 0.0002), and year and season (P=0.0012). Furthermore, interactions between station and depth, and season and depth were also significant (P=0.0181 and P=0.0129, respectively). This demonstrates that (1) the seasonal density was different between the years, and (2) density at certain depths differed with season as well. This supports hypothesis 1, that there is a difference in abundance in the Green sea urchin population between 1992 and 2013.

Table 2: ANOVA test on the average urchin density. The independent variables are year, station, season, depth and the interactions between these variables, on the number of sea urchins registered. Response variable was average density. P-values < 0.05 are deemed significant.

	DF	Sum Sq	Mean Sq	F value	Pr(>F)	
Year	1	470.40	470.40	8.2286	0.0081	**
Station	2	450.32	225.16	3.9386	0.0321	*
Season	2	56.45	28.23	0.4938	0.6159	
Depth	3	2066.75	688.92	12.0510	$3.93*10^{-5}$	***
Year:Depth	3	1687.26	562.42	9.8382	0.0002	***
Year:Season	2	1012.67	506.33	8,8571	0.0012	**
Station:Depth	6	1086.43	181.07	3.1674	0.0181	*
Season:Depth	6	1168.58	194.76	3.4069	0.0129	*
Residuals	26	1486.34	57.17			

3.3.2 Average size-distribution by depth

The size of individual urchins was measured to assess the age distribution and reproductive potential of current urchin populations within the Oslofjord. The average diameter of the sea urchins seemed quite uniform between seasons in 2013. Depth-wise, the largest, and hence most likely the oldest, sea urchins were most commonly found at deeper depths (20 m) than in shallower waters, except at Svartskog. This differs from Fredriksen (1999), who found the majority of large sea urchins at 10 and 15 m in 1992.

Drøbak

At Drøbak in 2013 (Figure 7, top right), the largest urchins were found on average at 20 m at all sampling dates. The average diameter at 20 m was 3.0 to 3.6 cm. Ten metres and 20 metres were the only depths where urchins were found at all sampling dates in 2013. The averages at ten metres were substantially lower than at 20 m, being 2.3 cm (Spring), 1.5 cm (Summer) and 1.8 cm (Autumn). While at Drøbak in 1992 (Figure 7, top left) the average diameter at 10 and 20 m was over 4.0 cm at all depths.

Flaskebekk

The average diameter measured at Flaskebekk in 2013 (Figure 7, centre right) was smaller than Drøbak. Here, the highest average urchin diameters each season were found at 20 m in March (Spring) (2.4 cm), and at 15 m in June (Summer) (2.6 cm) and October (Autumn) (2.6 cm). In 1979 (Figure 7, centre left), the measurements showed a higher average diameter than in 2013, being between 3 and 4 cm at 10 and 15 m (Green, 1983).

Svartskog

Svartskog (Figure 7, bottom) had similar variation in average diameter to Flaskebekk, being around 2.5 cm throughout all depths and sampling dates. The highest mean size was found in March (Spring) at 10 m (3.0 cm), while the lowest mean size was from October (Autumn) at 5 m (1.7 cm). Urchins were only found at 5 m in October (Autumn).

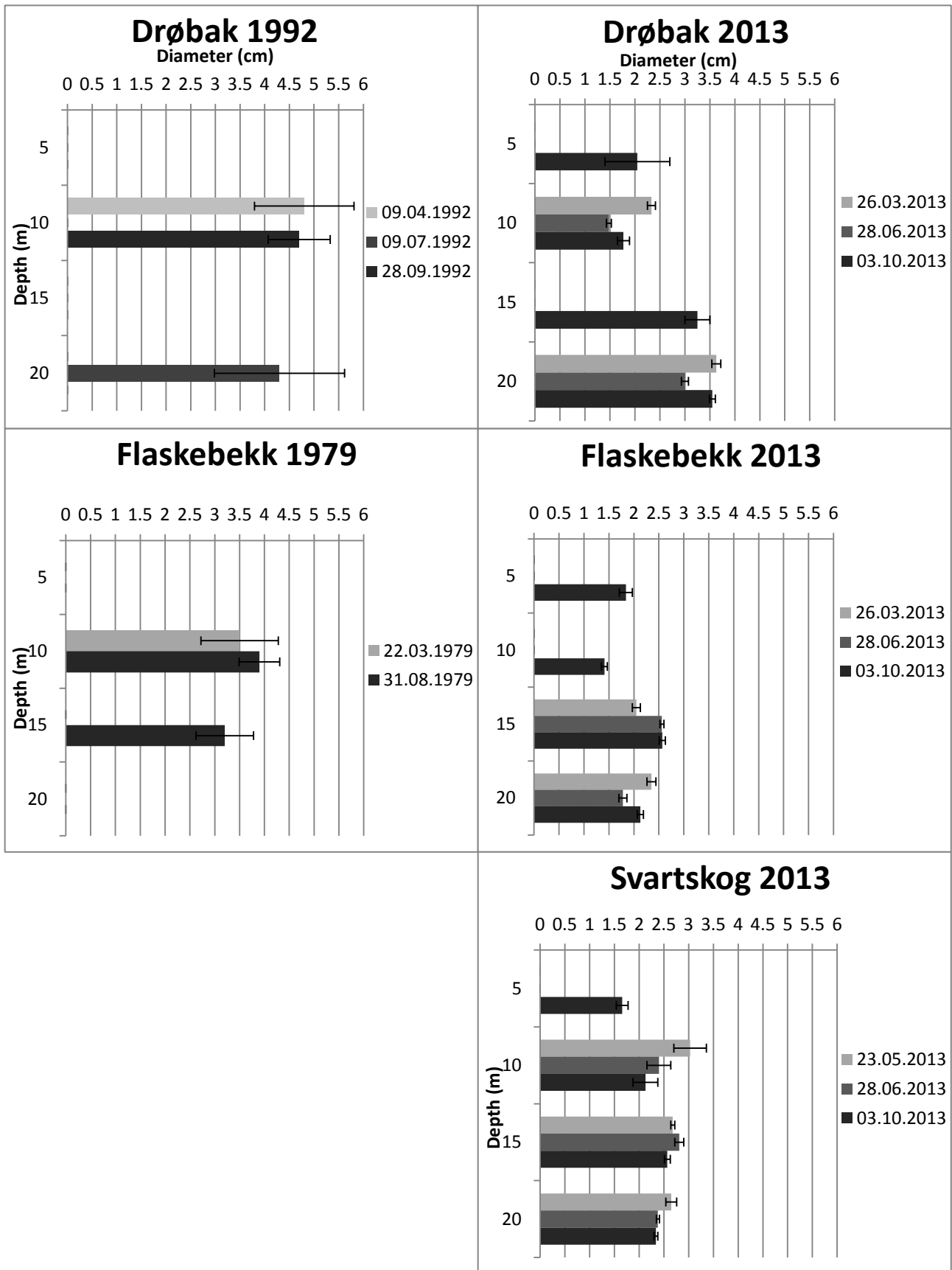Figure 7: Average urchin diameter. Drøbak in 1992 (top left) and 2013 (top right), Flaskebekk in 1979 (centre left) and 2013 (centre right) and Svartskog in 2013 (bottom). Light grey colours signify spring season, medium-grey summer and dark grey autumn. The y-axis is the different depths where the diver stopped and collected sea urchins for measurements. X-axis shows the average diameter at the different depths.

28

Factors affecting size-distribution

The ANOVA test conducted to analyse for any significant difference in average diameter (Table 3) found that there was a significant difference in average urchin diameter between stations (P= 0.003) and depth (P= 0.0042), affirming that larger individuals generally stood deeper than smaller individuals. Year had a very significant effect (P<<0.001).This means that over the years the average diameter has seemingly gotten smaller compared to previous studies. Season was shown to have a statistical significant effect (P=0.0178). There were also significant effects of the interactions between year and depth, and station and depth (P= 0.0037 and = 0.006), confirming the difference in average diameter found at Drøbak and Flaskebekk. This supports hypothesis 1, specifically that there is a difference in urchin diameter compared to previous studies.

Table 3: ANOVA test on the average urchin diameter. The independent variables are year, station, depth, and season on the average diameter of the sea urchins. The interactions between year and depth, station and depth, station and season, and depth and season. Response variable was average urchin diameter. P-values < 0.05 are deemed significant.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)	
Year	2	33.662	16.8308	398.2236	$6.17*10^{-8}$	***
Station	2	1.266	0.6328	14.9716	0.003	**
Depth	6	2.457	0.4095	9.6889	0.0042	**
Season	2	0.64	0.32	7.5719	0.0178	*
Year:Depth	2	1.174	0.5870	13.8889	0.0037	**
Station:Depth	5	2.176	0.3627	8.581	0.006	**
Station:Season	4	0.565	0.1412	3.342	0.0785	.
Depth:Season	6	0.575	0.1149	2.7196	0.1123	
Residuals	7	0.296	0.0423			

3.3.3 Size-distribution by season

Spring

During the Spring sampling (Figure 8, top row) no urchins were found at 5 m at any stations. The majority of individuals were found at 20 and 15 m and tended to be larger than 2 cm. All stations had the highest frequency of individuals registered in the 2.1-2.5 and 2.6-3.0 cm size classes. At 20 m depth at Drøbak (Figure 8, top left) a fairly even distribution of large adult individuals was found (diameter > 4 cm). Two large adults were registered at Flaskebekk, while none were registered at Svartskog. Flaskebekk (Figure 8, top middle) presented a bimodal curve for the sea urchins found at 15 and 20 m, both with their peaks at 1.1-1.5 cm and 2.6-3.0 cm size classes. Svartskog had the fewest individuals registered in the whole study (Figure 8, top right). Only a total of 130 individuals were found.

Summer

The Summer sampling (Figure 8, centre row) presented a size-segregation by depth where the larger individuals were found deeper than the smaller ones. As in Spring, no sea urchins were registered at 5 m. The curve at 10 m peaked at the 1.1-1.5 cm size class with a single individual in the class 5.1-5.5. At 20 m the size frequency peaked around 3.1-3.5 cm. No sea urchins were found at 15 m at Drøbak. Flaskebekk exhibited a bimodal curve at 20 m, with peaks at 1.1-1.5 cm and 2.6-3.0 cm, where the 1.1-1.5 cm class dominated while the other size classes were rather low in abundance (Figure 8, centre). However, at 15 m there was only one peak, at 2.1-2.5 cm. Svartskog (Figure 8, centre right) had one dominant size class at 20 m, 2.1-2.5 cm, where 61 out of the 153 individuals registered at this sampling date fell in to. Only a few individuals were registered at 10 m, while 15 m presented a bimodal distribution with peaks at 1.6-2.0 cm and 3.1-3.5 cm.
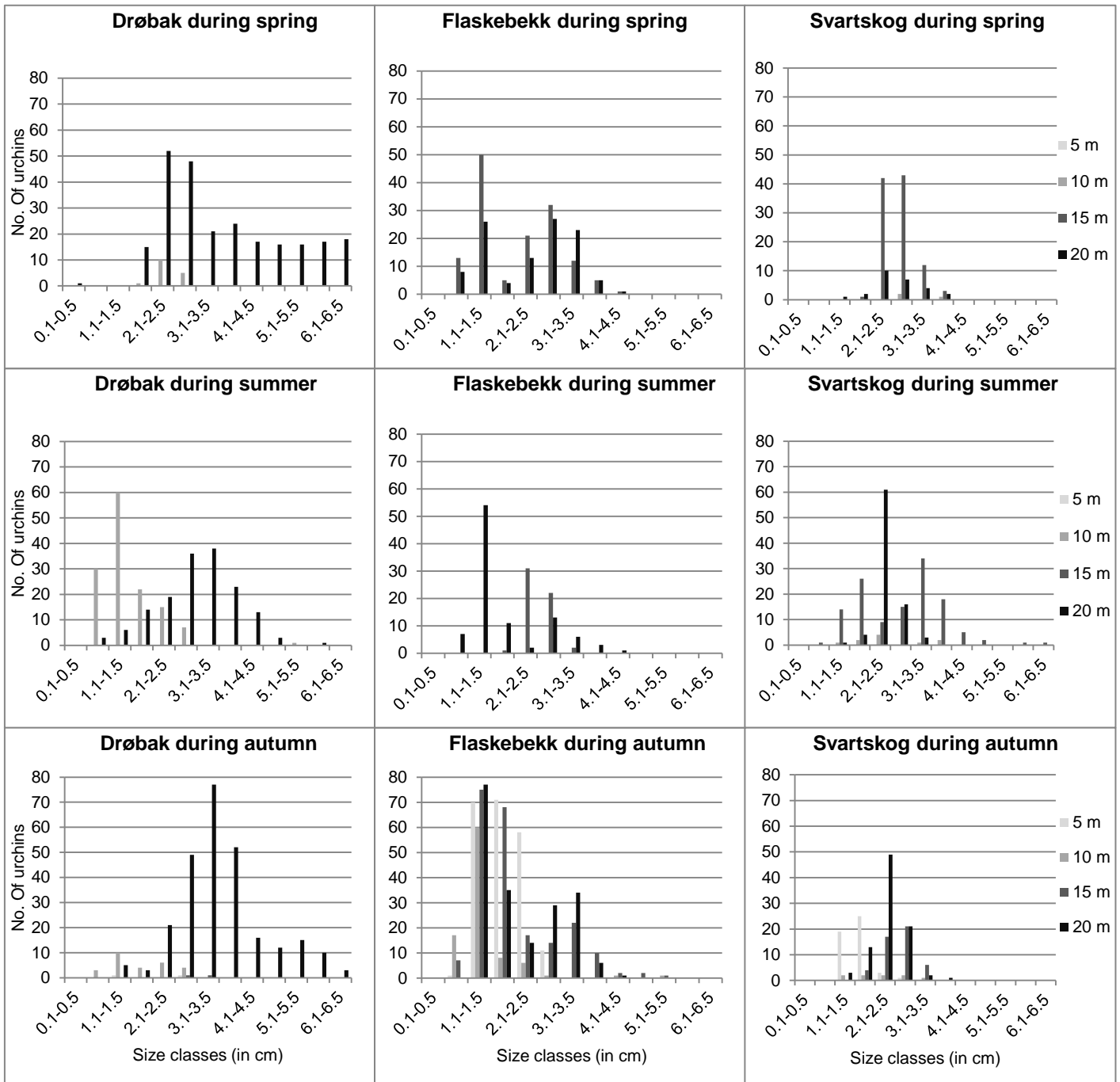
Figure 8: Size-distribution at Drøbak (left), Flaskebekk (middle) and Svartskog (right) for the different seasons sampled. The x-axis represents 0.5 cm size classes from 0.1 to 6.5 cm. Y-axis is the number of urchin individuals in each size class.

Autumn

The Autumn sampling (Figure 8, bottom) was the only time where sea urchins were found at all depths. At Drøbak (Figure 8, bottom left) almost all individuals were located at 20 m depth (263 individuals out of 294), with only two individuals found at 5 m and 15 m, and 27 individuals at 10 m. The most abundant at 20 m was 3.1-3.5

31

cm, just as in the Summer sampling. Flaskebekk (Figure 8, bottom middle) had the most individuals registered with a total of 719 individuals distributed between all depths and almost all size classes (0.1-0.5 cm and over 5.1-5.5 cm). At 15 and 20 m depth it was found bimodal distributions with peaks in the 1.1-1.5 cm and 3.1-3.5 cm size classes. Only 194 individuals were registered at Svartskog (Figure 8, bottom right). The peak at 5 m was in the 1.6-2.0 cm size class, with no individuals smaller or bigger than 1.1 cm and 3.0 cm. 15 m and 20 m peaked at 2.6-3.0 cm and 2.1-2.5 cm, respectively.

3.3.4 Recruitment

Recruitment was measured in order to investigate if reproduction was still occurring and to what extent. Recruitment is occurring at the same time and rate as previous studies (Table 4). This study also found that the short time the scours were placed at Svartskog, from May 23rd to June 28th (Summer) did not affect the number of settlers per day found in the scours. The majority of settlement happened in late Spring, early Summer with still some late recruits in the Autumn. The study found that season and year were significant variables for recruitment success, which could validate hypothesis 1.

Table 4: Calculations of number of settlers per day in the scours. The data from 1992 was collected by Frid & Thomassen (1995) and the data from 2013 was collected by the present study.

Station	Time period	Season	No. Settlers	No. Days	Settlers/day
Drøbak	23.04.92 – 27.09.92	All year	306	157	1.95
Drøbak	23.04.92 – 25.05.92	Spring	7	32	0.22
Drøbak	25.05.92 – 09.07.92	Summer	2	45	0.04
Drøbak	09.07.92 – 27.09.92	Autumn	275	80	3.44
Drøbak	27.09.92 – 05.12.92	Autumn	4	67	0.06
Drøbak	26.03.13 – 28.06.13	Summer	113	94	1.20
Drøbak	28.06.13 – 03.10.13	Autumn	53	97	0.55
Flaskebekk	26.03.13 – 28.06.13	Summer	264	94	2.81
Flaskebekk	28.06.13 – 03.10.13	Autumn	54	97	0.56
Svartskog	23.05.13 – 28.06.13	Summer	81	36	2.25
Svartskog	28.06.13 – 03.10.13	Autumn	3	97	0.03

Drøbak

The majority of settlement at Drøbak in 2013 occurred at 10 m both during Summer and Autumn (Figure 9, top right). There were no recruits registered at any other depth during the Summer sampling, and only very few recruits at 15 and 20 m during Autumn. Some of the chains with scours were lost. Scours missing is marked with asterisks in Figure 9.

The scours in 1992 (Figure 9, top left) had a high number of settled recruits (over 15 settlers/ scour) at all depths during Autumn. At the most it was an average of 26.8 settlers/ scour (15 m), while the highest number of settlers over the whole duration of the study was 6.8, also at 15 m. The highest average number during Summer settlement occurred at 10 m, and was 1.3 settlers/ scour. It was found urchins at the other depths, though these were less than 1 per scour.

Flaskebekk

The scours that were collected at Flaskebekk (Figure 9, bottom left) had a higher number of recruits settling at 10 m, with an average of 50 recruits per scour during Summer, than at 5 m. Flaskebekk and Drøbak seemed to have approximately the same amount of recruits in the Autumn, though at Flaskebekk they were found only at 10 m.

Svartskog

Recruitment at Svartskog (Figure 9, bottom right) yielded an average of 20.3 recruits per scours at 15 m during Summer (no scours were found at any other depth during this season). In the Autumn sampling, some recruits were found at 5 m (but again, no scours were found at any depth besides this).

Figure 9: Average number of recruits per scour. Drøbak in 1992 (top left), Drøbak in 2013 (top right), Flaskebekk in 2013 (bottom left) and Svartskog in 2013 (bottom right). The x-axis represents the different depths where the scours were collected from. Y-axis is average number of settled recruits per scour. The scours in the summer sampling were collected between March and July, and the autumn sampling was collected between July and December. One asterics (*) signifies where scours were not found in the summer while two asterics (**) signifies scours lost during autumn. If both sampling sessions are lacking, could not the number for the full study be established.

Significant changes in recruitment

ANOVA testing for any significant effect by year, season, depth or an interaction between year and depth or season on number of settlers per day (Table 5) showed that the season was significant (P= 0.0021) for number of settlers registered. Year was also found to be significant (P= 0.0007), meaning that there was a difference in the amount of recruits between 1992 and 2013, where there were more recruits in 1992 (see Table 4). Lastly, the interaction between year and season was found to be significant (P=0.0006), which means that the different seasons matter differently between 1992 and 2013. In 1992, Autumn was when most of the settling occurred while this happened in the Summer in 2013. This significance may support the change in recruitment suggested by hypothesis 1.

Table 5: ANOVA test on the average number of settlers per day. The independent variables are year, season and depth. Interactions between year and depth, year and season, and season and depth were also analysed.

Response variable was average number of settlers per day. P values < 0.05 are deemed significant.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)	
Year	1	420.77	420.77	89.0882	0.0007	***
Season	1	234.26	234.26	49.5985	0.0021	**
Depth	3	39.80	14.27	2.8087	0.172	
Year:Season	2	451.43	451.43	95.5794	0.0006	***
Year:Depth	1	8.68	4.34	0.919	0.4695	
Season:Depth	2	10.31	5.16	1.0917	0.4195	
Residuals	4	18.89	4.72			

3.3.5 Gonad Index (GI)

This study assessed the Gonad Index (GI) in sea urchins at the three sampling locations to determine the investment of resources in reproduction material, and to acquire an indicator for the general condition of the urchin population. GI in sea urchins sampled at Drøbak, Flaskebekk and Svartskog in 2013 (Figure 10, top right, bottom left and right) exhibited the same variation of GI, between 5 and 15 %, as the sampling done in 1992 (Figure 10, top left). This study found that GI was highest in June in 2013 at all three sampling locations. Drøbak's urchins possessed the highest GI during both Summer and Autumn, while Flaskebekk's and Svartskog's displayed a lower, more similar GI in Summer and Autumn. There were some depths and

sampling locations that there were not found any sea urchins with a diameter larger than 3.5 cm. These are stated in the Appendix (Table 10). The lack of significant difference in GI disproves hypothesis 1.
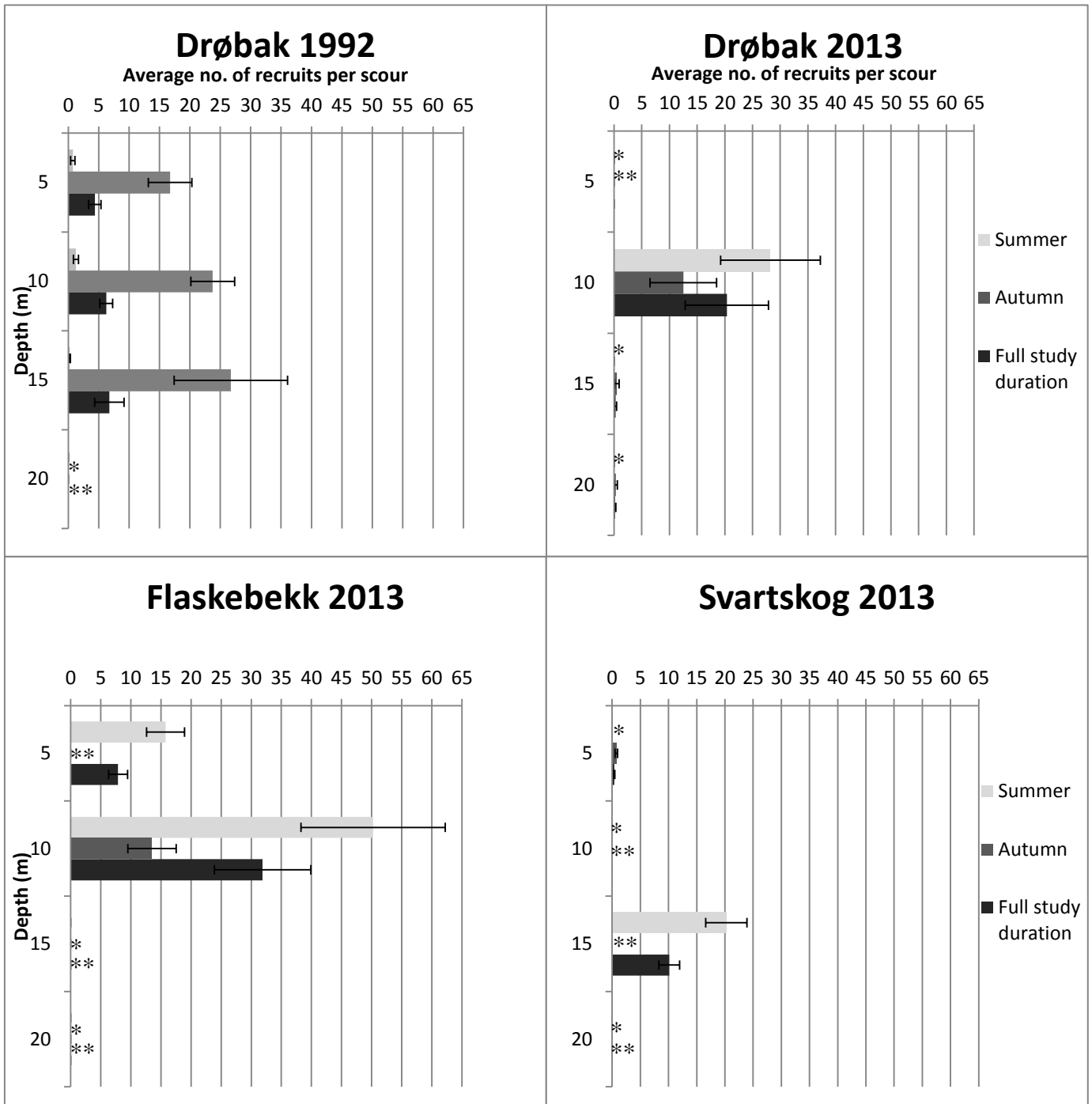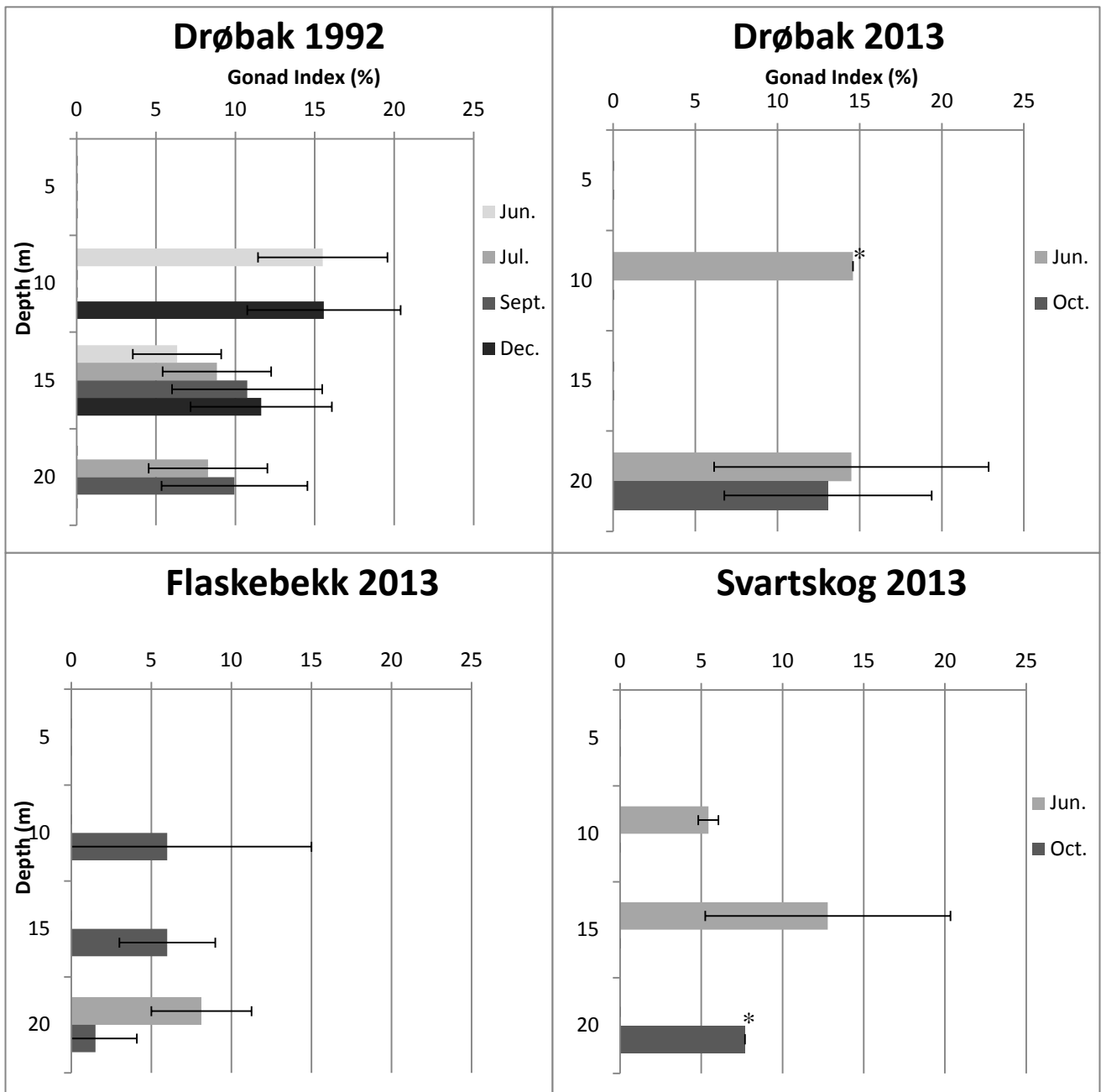

Figure 10: Average Gonad index. Drøbak in 1992 (top left), Drøbak in 2013 (top right), Flaskebekk in 2013 (bottom left) and Svartskog in 2013 (bottom right). The x-axis represents the depth where the sea urchins were collected from, and the y-axis is the gonad index (% of total urchin weight that is gonads) . Summer sampling occurred June 28th, and autumn sampling was October 3rd.

*= Only one registered individual. Standard deviation = 0.

No significant effects on Gonad Index

The ANOVA test assessed if month, year, station, depth, or an interaction between month and depth, year and depth or station and depth played any role in the GI found. All variables and interactions had no significant effect (Table 6). This means that, the Green sea urchins were able to allocate approximately as much resources to gonad production now as in Fredriken's (1999) study; therefore the general condition of the urchin population seems to be good. The lack of significance of any variable on GI rejects hypothesis 1.

Table 6: ANOVA test on Gonad Index (GI). The independent varaibles are year, season, station and depth. Interactions between year and season, year and depth, station and season, station and depth and season and depth were examined.

Response variable was GI. P values < 0.05 are deemed significant.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
Year	1	15.746	15.746	4.9987	0.2678
Station	2	128.745	64.373	20.4354	0.1545
Season	2	24.464	12.232	3.8831	0.3377
Depth	2	6.987	3.494	1.1091	0.5574
Year:Season	1	10.754	1.0754	3.414	0.3158
Year:Depth	2	51.424	25.712	8.1624	0.2403
Station:Season	1	6.76	6.76	2.146	0.3813
Station:Depth	2	15.852	7.926	2.5162	0.4072
Season:Depth	3	10.24	3.414	1.0836	0.5924
Residuals	1	3.15	3.15		

The correlation analysis conducted by the study on the relationship between urchin size (diameter) and GI (see Appendix, Figure 13) showed a weak positive correlation (R^2=0.0563). There was a high variation in GI within all sizes, ranging from 0 to 35 %. All sea urchins with 0 % were between 3.5 and 5.0 cm in diameter, while the lowest GI for larger individuals than 5.0 cm was 3.4 %. The R^2 for this correlation was merely 0.0563, where 0 is no correlation and 1 is perfect correlation. The equation for the trend line explains that for every centimetre the individual increased the GI would increase by 2.4 percentage points, meaning that the larger, older individuals generally contribute more to the reproduction than young adults or old juveniles. On the other hand, the relationship between urchin diameter and gonad production (see Appendix, Figure 14) showed a strong correlation (R^2=0.5213). This means that larger individuals produce larger gonads.

3.4 The effect of environmental change on the depth distribution of the Green sea urchin population

Data on the Green sea urchin's depth distribution collected by this study, and from other sources (Fredriksen (1999) and NIVA) was compared with records on temperature and salinity in the Oslofjord in order to assess if the changes in depth distribution was correlated with changes in the environmental variables. The Green sea urchin was found continuously throughout the water column from 5 m and down to 78 m. The shallowest recorded depths for the Green sea urchin at the different sampling locations were 5 m at Drøbak, 2 m at Oksval and sea-level (0 m) at Svartskog. Table 7 gives a short overview of the findings in the urchin depth distribution. The complete Figure on urchin depth distribution can be found in the Appendix (Figure 15).

Table 7: Brief description of the depth distribution of the Green sea urchin.

* = There are no data on depth distribution below 20 m, as this study and Fredriksen (1999) used SCUBA equipment to determine urchin abundance and depth distribution.

	Drøbak	Oksval	Svartskog
Study period	1992 – 1994; 2013	2006 – 2013	2006 – 2013
Shallowest depth	5 m (1992; 2013)	2 m (2008; 2012)	0 m (2006; 2007)
Greatest depth	20 m (1992; 2013) *	48 m (2008)	78 m (2007)
Highest level	Level 4 (1994; 2013)	level 3 (2013)	Level 2 (2008; 2013)
Commonest level	Level 2	Level 1	Level 1

3.4.1 Variation in water temperature and its impact on the Green sea urchin

This study collected and compared the urchin data with the corresponding temperature measurements in order to assess the effect of temperature on the shallowest depth urchins were registered. A weak negative correlation between shallowest urchin depth and temperature was found, but still might validate hypothesis 2.

The temperature data provided by *the Academic Council of Water and Sewage Technical Cooperation in the Inner Oslofjord* showed an overall trend in the study period of increasing water temperature, though at varying rates (see Appendix,

38

Figure 16). The seasonal variation was greatest at surface level and was gradually reduced with depth. The Summer peak in water temperature at 4 or 5 m depth was over 15 °C at all environmental monitoring stations. Table 8 presents the average temperature at 4 or 5 m at the beginning of the study period (1999), the end of the study period (2009 – 2012) and the difference in average temperature.

Table 8: Comparison of the changes in average water temperature at 5 m over the study period (1999 to 1009/2011).

	Study period	Start of study period	End of study period	Change over study period
Drøbaksterskelen	1999 – 2009	8.2 °C	10.2 °C	+ 2 °C
Oksval	1999 – 2010	9 °C	13.1 °C	+ 4.1 °C
Svartskog	1999 – 2012	9 °C	9 °C	0 °C

A weak negative correlation (R^2=0.0871) was found between water temperature and the shallowest urchin depth (Figure 11). The regression line of temperature showed that for every meter down in the water column urchins are found, temperature decreased with 0.21 °C. Though there is a fairly weak correlation between temperature and urchin depth, this correlation supports hypothesis 2, specifically that temperature exerts an effect on the depth distribution of sea urchins.
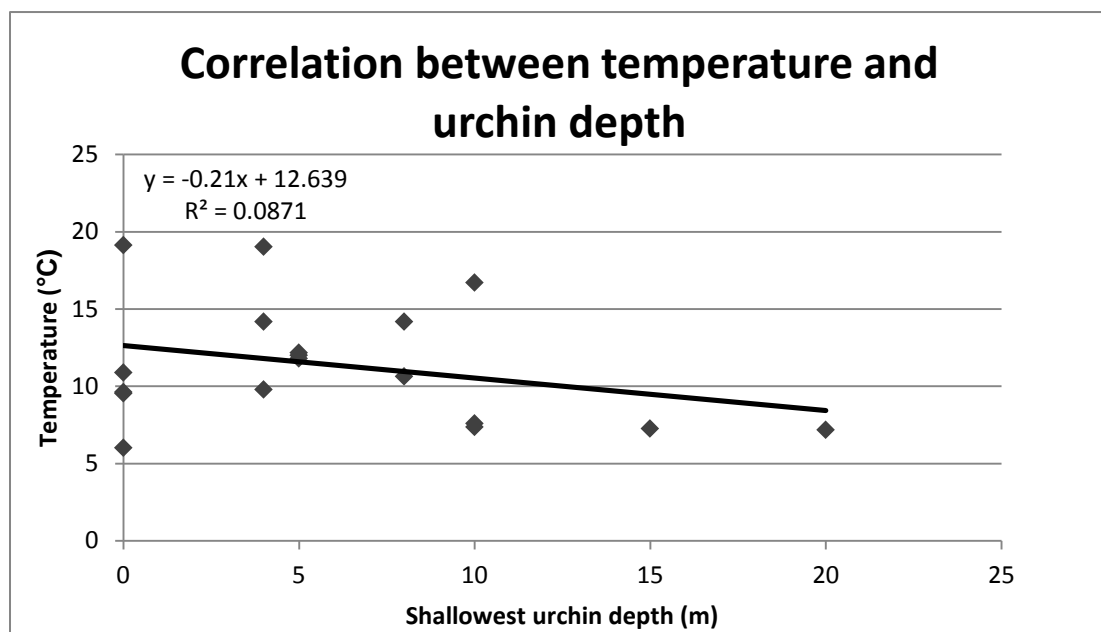

Figure 11: Correlation test of the effect of temperature on the shallowest urchin depth. The x-axis presents the shallowest depths sea urchins were registered at, while the y-axis represents the temperature at the urchin depth. R^2 represents the strength of the correlation where 1 = perfect correlation and 0 = no correlation.

3.4.2 The effect of changes in salinity on the Green sea urchin

This study investigated the relationship between salinity and the shallowest depth the Green sea urchin was registered at in order to determine the effect salinity exerts on the sea urchin's depth distribution. The study found a positive correlation between salinity and the shallowest urchin depth, which makes hypothesis 2 likely to be true.

The salinity data presented an overall trend that salinity has been slightly decreasing since 1999 (see Appendix, Figure 17). Salinity showed layering of the water column, with a layer of fresher water at water level and then increasing average salinity deeper in the water column. The surface waters exhibited more fluctuations in salinity than on deeper water. The salinity average in the inner Oslofjord still remained around 25 parts per thousand (ppt) over the duration of the study, though some changes were found over the study period (Table 9).

Table 9: Comparison of the changes in average salinity at 5 m depth over the study period (1999 to 2009/2011).

	Study period	Start of study period	End of study period	Change over study period
Drøbaksterskelen	1999 – 2009	25.0 ppt	24.8 ppt	- 0.2 ppt
Oksval	1999 – 2010	25.0 ppt	24.8 ppt	- 0.2 ppt
Svartskog	1999 – 2012	24.9 ppt	25.1 ppt	+ 0.2 ppt

Salinity had a positive correlation with the depth sea urchins were first registered at (Figure 12). The R^2 was higher for salinity (R^2=0.245) than temperature though this is still a weak correlation. This correlation supports hypothesis 2, that salinity may affect the depth distribution of the Green sea urchin.
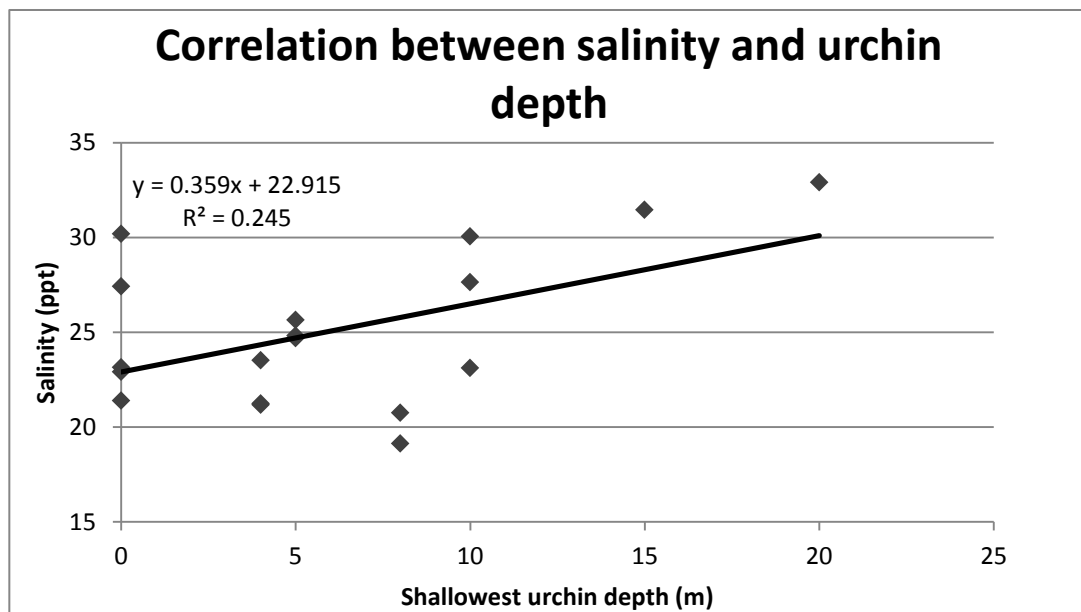
Figure 12: Correlation test of the effect of salinity on the shallowest urchin depth. The x-axis presents the shallowest depths sea urchins were registered at, while the y-axis represents the salinity at the urchin depth. R^2 represents the strength of the correlation. 1 = perfect correlation and 0 = no correlation.

3.5 Chapter summary

By comparing the raw data collected by this study in 2013 and pre-existing data on sea urchins, a number of significant differences in urchin abundance, urchin diameter and recruitment over time have been identified. The urchins were found to occur deeper in the water column throughout Spring and Summer now than in Fredriksen's study (1999). A significant reduction in size has also been found at Drøbak and Flaskebekk. Drøbak exhibited an average urchin diameter reduction of 2.5 cm since 1992, while at Flaskebekk the average diameter decreased by approximately 1.0 cm since 1979. Recruitment is still occurring in the inner Oslofjord, but at a slightly reduced level than before. This study also identified a significant difference in the season in which the majority of recruits settled, settling in the Summer in 2013 rather than Autumn as in 1992. Regarding GI, this study did not find any significant difference between 2013 and 1992. While temperature generally increased in the fjord, and salinity decreased, the study found that neither environmental factor exhibited a strong effect on the shallowest urchin depth. Salinity had a slightly stronger effect on urchin depth (R^2 = 0.245), and temperature had a relatively weak relationship (R^2 = 0.0871). Both of the correlations validates hypothesis 2's claim that temperature and salinity correlates with the depth distribution of the Green sea urchin.

41

4.0   Discussion

4.1   The condition of the inner Oslofjord population

This study found that the sea urchin population in the Inner Oslofjord appears to remain in a healthy state, as indicated by the abundance, size-distribution, recruitment and Gonad Index measured in this study. However, there have been some significant changes within these parameters which may indicate a more uncertain future for the Green sea urchin population in the inner Oslofjord. These observed differences were: (1) Abundance was different in regards to depth and seasonal distribution (see Table 2), (2) the average size of sea urchins was smaller (see Table 3 and Figure 7) and (3) recruitment was lower compared to a previous study (Figure 9).

The number of years in which this study could compare the present condition of the sea urchins to was quite low, making it more difficult to make any substantial claims about trends in the urchin demographic and density over the past 20 years, especially when the data from 1979, 1992 and 2013 sometimes focus on different aspects of the urchin population.

4.1.1 Observed changes in depth distribution

The survey conducted for this study found few urchins occurring in shallower depths (5m and 10m below surface level) compared to available data from previous studies from the same location (Green 1983; Fredriksen, 1999). Fredriksen (1999) frequently found sea urchins at 5 and 10 m in 1992, whereas the present study only found a few, less than ten urchins per square metre if any were found at all.

The depth distribution at Drøbak between 1990s and 2013 (see Appendix, Figure 15, top left and right) showed that the urchins are occurred deeper in the Spring and Summer of 2013 than the Spring and Summer in 1992. This might be connected to the gradual sea temperature warming that is shown in Figure 16 (Appendix), but as historical environmental data is limited and there was only data points from two years available for comparison, a correlation between water temperature increase and depth distribution could not be conducted.

During the Spring of 2013, the Oslofjord experienced a severe flood. An event like this could reduce the salinity in the upper water layers, forcing them below the halocline. *The Norwegian Directorate for Civil Protection* (2014) estimate that moderately large floods, such as the flood in 2013, occur on approximately every 20 years. However, the pattern found in the depth distribution did not seem to vary too much from the depth distribution from NIVA's monitoring data, leading this study to believe the Spring flood in 2013 did not affect the data collected more than what previous floods may have affected prior samplings.

Compared to Fredriksen (1999), there were differences in depth distribution (Table 2). This suggests that there might be a non-lethal factor pushing the population in to deeper water. Neither this study or Fredriksen (1999) registered sea urchins below 20 meters, but as shown by the depth distribution at Oksval and Svartskog, Green sea urchins are most definitely found deeper than 20 m (see Appendix, Figure 15, bottom left and right). NIVA utilized a drop-camera at Oksval and Svartskog when monitoring these environmental stations, enabling them to survey deeper in the water column. This study and Fredriksen's study (1999) used SCUBA equipment, restricting the survey to the upper 20 m.

It was believed that only *Psammechinus miliaris* inhabited and dominated the upper 5 metres, while the Green sea urchin dominated from 5 to 10 metres and down, but the drop-camera registrations done by NIVA showed that the Green sea urchin can be found at shallow depths as well. The depth distribution (see Appendix, Figure 15) suggests that the sea urchins migrate upwards during Autumn and Winter while going deeper during Spring and Summer. On the other hand, though the data provided by NIVA had identified the sea urchins as the Green sea urchin in the majority of the data set utilized, drop-camera cannot be used to accurately identify species; hence this study cannot be entirely sure if the species registered is correct. However, studies from the 1980s (Källqvist et al., 1982; Magnusson et al., 1984a; 1984b) had a depth distribution which is similar with NIVA reported in the 2000s, which indicates that the Green sea urchin is able to migrate up in the water column and graze at a larger area, thus possibly reducing the negative effects of being standing earlier in the year.

4.1.2 Observed changes in seasonal abundance

The seasonal abundance has also changed over the years to fewer individuals in the Spring and Summer seasons compared to Fredriksen (1999) (Figure 6). Ojeda & Dearborn (1989) did present some seasonal vertical distribution, with urchins standing deeper in Spring and Summer before moving up to 5 m in late Autumn or Winter. This fits quite well with what this study found.

If the change in seasonal abundance is correct, where sea urchins occur deeper and migrate to shallower depths during Autumn, it means that the sea urchin population to a lesser extent can utilize a large and important food source during Spring and Summer. The macroalgae in the inner Oslofjord inhabit the upper ten metres (Berge et al., 2012; 2013), which may be out of reach for the sea urchins during periods in Spring and Summer. In addition, macroalgae tend to have have the preferred ratio of carbon and nitrogen during Spring and Summer (Sjøtun et al., 1996) while during Autumn and Winter, when sea urchins occur higher up in the water column, the C:N ratio is too high or too low (Norderhaug et al., 2003; 2006) thus making it less suitable for consumption. On the other hand, even if macroalgae is one of the most important food sources for the Green sea urchin (Himmelman & Steele, 1971; Scheibling & Anthony, 2001), this species is omnivorous (Russell, 1998; Scheibling & Hatcher, 2001) and hence not entirely dependent on macroalgae to survive. The high Gonad Index found in this study (Figure 10) suggests that at the current date the Green sea urchins are not particularly harmed by the lack of access to macroalgae.

4.1.3 Observed changes in size-distribution

The study revealed that the average size of the Green sea urchin in the Oslofjord has decreased in comparison to data from previous studies (Figure 7). The average size found by this study was almost 2.5 cm smaller now at Drøbak and 1.0 cm smaller at Flaskebekk compared to Fredriksen in 1992 (1999) and Green in 1979 (1983). Possible explanations for these observed changes are: (1) High predation pressure on urchins which have not yet reached the size refuge, (2) Shifting of population into water deeper than what was explored by this study, (3) the mature urchin size has shrunk.

Possible impact of predation pressure on change in size-distribution

Higher predation pressure is a possible explanation for the observed change in size-distribution, particularly the distributions found at Svartskog and Flaskebekk, leading to fewer individuals reaching the size refuge of approximately four centimetres. If the predation is a major concern one would expect to find a bimodal distribution of urchin sizes, with one peak for juvenile individuals (< 2 cm), which live cryptically and sheltered, and one for adult and old sea urchins (> 4 cm), which have achieved a size refuge (Clemente et al., 2013; Fagerli et al., 2013). The size-distribution found in the Oslofjord (Figure 8) showed that the majority of sea urchins were within the size range which they are most vulnerable to predation. A pattern like this suggests that the predation pressure cannot be that great, as these sea urchins should have been heavily preyed upon and thus been few.

Possibility that the urchin population has shifted to deeper depths

The combined findings that average size was lower in 2013 compared to Fredriksen (1999) and Green (1983) (Figure 7), and that there was still a strong presence of old juveniles (Figure 8), support the possibility that older individuals are now standing deeper than 20 meters. This explanation appears particularly appropriate for Drøbak station, of the 84 sea urchins larger than 5.0 cm, 80 were found at Drøbak. This pattern supports the common assumption that the Drøbak population generally has larger individuals than the rest of the Oslofjord. Size-distribution between depths was found to be quite indiscriminate, but larger urchins tended to stand at 20 m while juveniles were found at all depths (Figure 8). This indiscrimination fits well with what was found in the transition zone between kelp bed and urchin barren (Gagnon et al., 2004), and at urchin barrens (Himmelman, 1986). However, this study did not find any sea urchins at five metres depth until Autumn. This may be caused by the 10 °C critical temperature limit for larval and juvenile development suggested by Stephens (1972). During spring and summer, the water temperature regularly exceeds 10 °C before dropping below this temperature during late Summer. On the other hand, this temperature limit has been questioned by several studies (Strathmann, 1978; McEdwards, 1985; Hart & Scheibling, 1988; Siikavuopio et al., 2008; 2012), claiming that the tolerance limit is closer to 14°C. Water temperatures regularly exceed 14 °C in the Summer as well, perhaps this limit will fit better with the urchin depth

distribution. Furthermore, it is possible that higher temperatures bring other stressors on the Green sea urchin, like more pathogens or increased predation by for instance *Carcinus maenas* or *Cancer pagurus* (Fagerli et al., 2014), both of which are inhabit the Oslofjord.

The reason why sea urchins from Flaskebekk or Svartskog are smaller than Drøbak is uncertain. Nonetheless, a reduction in larger individuals might weaken the older individuals' ability to function as a reproductive buffer. Figure 13 (see Appendix) showed how larger individuals had a generally higher Gonad Index, and after reaching a size larger than five centimetres always produced gonads. Without this group of old individuals guaranteeing some reproduction, the regularity of reproduction might decrease, reducing the overall abundance over time. However, as this research is only comparing the demographics of two years, one must be cautious of drawing a causational relationship. Even though statistical differences in urchin size were found, the comparison between 1992 and 2013 may only function as an indicator of possible change over time, not definitive proof.

Gonad Index

Gonad Index (GI) from 2013 was not significantly different from GI from 1992 (Table 6), and seem to maintain at a high level throughout the water column. This is not in line with Keats et al. (1984), who found that the reproductive output varied by depth, or the food availability at the different depths. This indicates that the Green sea urchins in the inner Oslofjord have similar food availability throughout the upper 20 metres of the water column. GI can be used as a proxy to determine whether a population is in good condition and, hence, have the opportunity to allocate energy to producing gonads. A low GI can indicate poor food quality or food supply in the area (Vadas, 1977; Himmelman, 1984; Raymond & Scheibling, 1987), or physiological stress, like for instance problems with the osmotic equilibrium (Krogh, 1939). The 10 % threshold is commonly utilized to determine if the population is healthy and it uses the GI from Autumn, when it is at its highest. The present study found that GI was around 10%, and did not differ significantly between 1992 and 2013, thus this study deem it safe to conclude that the gonad production is as healthy in 2013 as it was in 1992.

The standard deviations for GI were also quite high, showing a large variation in gonad production between the individuals at the different depths. Considering the Green sea urchin' external fertilisation, one or a few individuals with a large amount of reproductive material may compensate for the lack of gonads in others. When looking at the effect of urchin diameter on GI (see Appendix, Figure 13) this study found a weak correlation (R^2=0.0563). Ebert (1985) showed that a sea urchin's fitness increases exponentially with test diameter, making larger individuals provide more reproductive material to the pool than smaller ones. This study did find a strong correlation between test diameters and gonad production (R^2=0.5127) (see Appendix, Figure 14), supporting Ebert's finding (1985). However, this is to be expected, as large individuals will have a greater capacity to allocate resources to reproduction than small individuals. The large variation in gonad production between individuals of the same size probably reflects the patchiness of the food at the station, giving a reproductive advantage to some few in the right area over the others (Russell, 1998).

Recruitment

Recruitment was found to still be at a healthy level in 2013, though compared to 1992, the present study experienced a slightly lower rate of recruitment (Figure 9 and Table 5). Settlement was also found to occur in the Summer, which concurs with the literature on settling and duration as pelagic larvae (Strathmann, 1978; Falk-Petersen & Lønning, 1983; Underwood & Fairweather, 1989; Miller & Emlet, 1997; Fagerli et al., 2013). The number of settlers in the scours at Svartskog supports this (Table 4). Furthermore, recruitment was nearly the double in the Summer than compared to Autumn (Figure 9, top right, bottom left and right). Interestingly, both the Gonad Index and recruitment found by the present study were sometimes higher than found by Fagerli et al. (2013) along the coast from 2008 to 2010. In fact, the Gonad Index found by this study corresponds well with what Meidel & scheibling (1998) found in kelp beds and grazing fronts in Nova Scotia, Canada, where the Green sea urchin was thriving.

This study concludes that the inner Oslofjord population is able to sustain the population as there is still a strong recruitment in the fjord (Figure 9). In addition, the recruitment data (Figure 9) and size-distribution (by using size as a proxy for age)

(Figure 8) suggest that recruitment occurs regularly rather than sporadically by a few strong year classes. This finding aligns with Leinaas & Christie (1996), who argue that an urchin barren population would need regular recruitment to sustain itself, while Sivertsen (2006), argues that a few strong year classes at uneven intervals is enough to sustain the population. It is important to note that using size as proxy for age, though often accurate, may create a bias if there are any factors inhibiting the normal growth rate of 1 cm/year (Larson et al., 1980; Fagerli et al., unpubl. manuscript). If the recruitment is not regular, a population can be sustained by receiving recruits from other populations, as the pelagic larvae stage has a high dispersal potential. However, this relates more to coastal populations, where the recruits are more likely to be able to travel far and colonize. There are no known coastal populations just outside of the Oslofjord, but there are local sea urchin populations along the Swedish and Danish coast which theoretically could replenish the Oslofjord population with recruits using the currents to the fjord. At the present date, there are no available data to support or reject this hypothesis. However, because of the isolating effect a fjord can exhibit (Fredriksen, 1999), there are two challenges that has to be cleared in order for the inner Oslofjord population to receive recruits from other populations. The first is the morphology of the fjord. The Oslofjord has a major freshwater outlet at the opening (Walday et al., 2013), which may also work as barrier for the Oslofjord populations to flow out and colonize the coast (Figure 1). Second challenge is the Drøbak sill, where the water flow is controlled mainly by the tides (Staalstrøm et al., 2012). If the current flow is unfavourable for urchin recruits it may restrict recruitment of the inner Oslofjord from other populations when the tide is not right. Because this study only compares two years it is not possible to make any definitive conclusions. Himmelman (1986) discovered that recruitment changes constantly between depths, locations and even years. Thus, what seemed like a decline in the recruitment success might only be a result of stochastic temporal variation.

4.2   Implications of temperature and salinity changes in the fjord

This study showed that the average salinity has decreased at Drøbaksterskelen and Svartskog from 1999 to 2009/2011 (see Appendix, Figure 17). A reduction in salinity, particularly if reduced below the tolerance limit of the Green sea urchin would render a portion of the water column uninhabitable for the species. This may have positive effect for various types of algae, particularly brown algae, as they would be released from the grazing pressure exerted by the sea urchin (Himmelman & Steele, 1971; Scheibling & Anthony, 2001) as well as the bulldozing effect of the urchin movement on the substrate, dislodging newly settled kelp (Green, 1983). This could in turn lead to increased growth of seaweeds in the upper water layers in the fjord, increasing the fjord's biodiversity.

Contrary to the reduction at Drøbak and Svartskog, the salinity at Oksval increased by 0.2 ppt from 1999 to 2010 (see Appendix, Figure 17). It is very unlikely that this pattern was caused climate change. Even though Oksval is the northernmost environmental station, the latitudal difference between the two stations is so small. Oksval is less than 10 km further north than Svartskog, making the likelihood that a large scale phenomenon like ocean warming, and the subsequent reduction of salinity in the upper water masses, affecting Svartskog, but not Oksval very unlikely. A possible explanation for this pattern is upwelling caused by the sill at Oksval (Staalstrøm et al., 2012). However, this explanation does not take into account that the upwelling has to occur regularly and with the same strength, as the effect is visible and consistent throughout the year. The most plausible explanation is that the standard deviation is so much greater than the increase by the trend line, thus creating a false trend (Staalstrøm, pers. corr.). This means that local variation may create short term patterns which may mask the large scale trends of climate change.

While the salinity decreased at Drøbaksterskelen and Svartskog, the temperature increased, as somewhat predicted by the IPCC's assessment on climate change for the northern Atlantic (2013), but more precisely by Lima & Wethey (2012). Oksval did not present any temperature change since 1999. The same Summer-Winter cycles were found with temperature as with salinity. Therefore is this discrepancy likely attributed the standard deviation being too great.

50

Some of the possible confounding factors to this study that has been discussed have been pollution and wave action. Pollution has previously been an issue in Oslofjord. However, the condition in the inner Oslofjord in 2012 was deemed to be good (Berge et al., 2013). There are some parts of the fjord that are still struggling with pollutants like mercury, TBT, PCB and PAH (Thaulow & Faafeng, 2014), but the locations this study used had low levels of these pollutants. Thus the present study does not believe that pollution have been a confounding factor. Wave action has been found to have an effect on sea urchin distribution (Ojeda & Dearborn, 1989; Rinde et al., 2014), but because the wave action in the inner Oslofjord is controlled by tidal movement (Staalstrøm et al., 2012), this study does not deem it as a major factor in the urchin distribution in the fjord.

Assessing the correlation between urchin depth and the two environmental variables, the present study found that salinity was more of a determining factor than temperature (see Figures 11 & 12). These findings support previous studies which have stated that salinity is a more determining factor than temperature (Drouin et al., 1985; Roller & Stickle, 1985; 1994), but is opposite of what is found with Norwegian coastal populations (Fagerli et al., 2013). This is most likely because salinity is normally higher and varies less along the coast However, the correlation found in this study was a weak correlation ($R^2 = 0.245$). It might be possible that the correlation would be stronger if the salinity was closer to the tolerance limit of the species than the salinity measurements were in this study, or if the urchin registration and environmental measuring were done at the same location. As a result, this research suggests investigating the salinity threshold of the Green sea urchin in order to find a more definitive lethal or reproductive limit, as the present literature is conflicted on this matter. If achieved, this will improve the ability to accurately predict environmental effects on the Green sea urchin in the future.

5.0   Conclusions

This study found that the Green sea urchin is still present in the inner Oslofjord, contrary to received reports stating that it has declined dramatically. The population seems healthy, with several year classes present and an ongoing recruitment occurring. But there were some indicators that might be worrisome in the future:

The Green sea urchin's overall abundance differed from the abundance in the previous study (from 1992 to 1994). The seasonal abundance differed as well, where there are fewer individuals found in the upper 15 metres of the water column in Spring and Summer. Average urchin diameter was also different, with an average diameter 2.5 cm smaller at Drøbak since the last study conducted there (1992-1994), and a 1.0 cm reduction in average diameter at Flaskebekk since a study in 1979. Recruitment, though still occurring at a high level, seemed to have dropped slightly since 1992. Gonad Index, however, remained similar.

Between 1999 and 2011, the average water temperature at 4 to 5 metres depth appeared to have increased by 2-4 °C at Drøbak and Svartskog, but remained constant at Oksval. In the same timespan salinity dropped 0.2 ppt, from 25.0 to 24.8 ppt, at Drøbak and Svartskog, but increased by 0.2 ppt, from 24.9 to 25.1 ppt, at Oksval. However, due to the natural variation which occurs in the upper water layers these changes might not have been significant in this study.

Weak correlations between salinity and the shallowest urchin depth, and temperature and the shallowest urchin depth were found. Salinity had a stronger impact on urchin depth distribution than temperature (R^2=0.245 compared to R^2=0.871).

Further monitoring of the Green sea urchin population in the inner Oslofjord is suggested to maintain the population, and to earlier detect any further reductions in the sea urchin population in order to avoid a collapse. Further research on the effect of temperature and salinity, both independently and synergistically, on juvenile and adult individuals is suggested as a way to efficiently monitor the condition of the urchins.

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56

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7.0   Appendix

Nested ANOVA tests were conducted in order to look at changes in size and abundance. For the abundance test, the average number of urchins classified as dependent variable and year, season, depth and station as independent variables. All interactions between the independent variables were also analysed. For the ANOVA done to investigate changes in urchin size used average diameter as dependent variable and year, station and depth as independent variables. Interactions between year:station and station:depth were analysed as well.

Nested ANOVA tests were also used to investigate the recruitment and Gonad Index. For recruitment, number of settlers per day was set as dependent variable while year, season, depth and number of days were the independent variables. The interactions between year:depth and year:season were analysed. For Gonad Index, Gonad Index was dependent variable and year, month, season and depth were independent variables. Interactions between year:depth, month:depth and season:depth were investigated as well.

Table 10: Depths and locations where Gonad Index was not assessed. No sea urchins larger than 3.5 cm were found at these depths and seasons.

	Drøbak	Flaskebekk	Svartskog
Summer sampling	15 m	5m; 10 m; 15 m	20 m
Autumn sampling	10 m; 15 m	5 m	10 m; 15 m
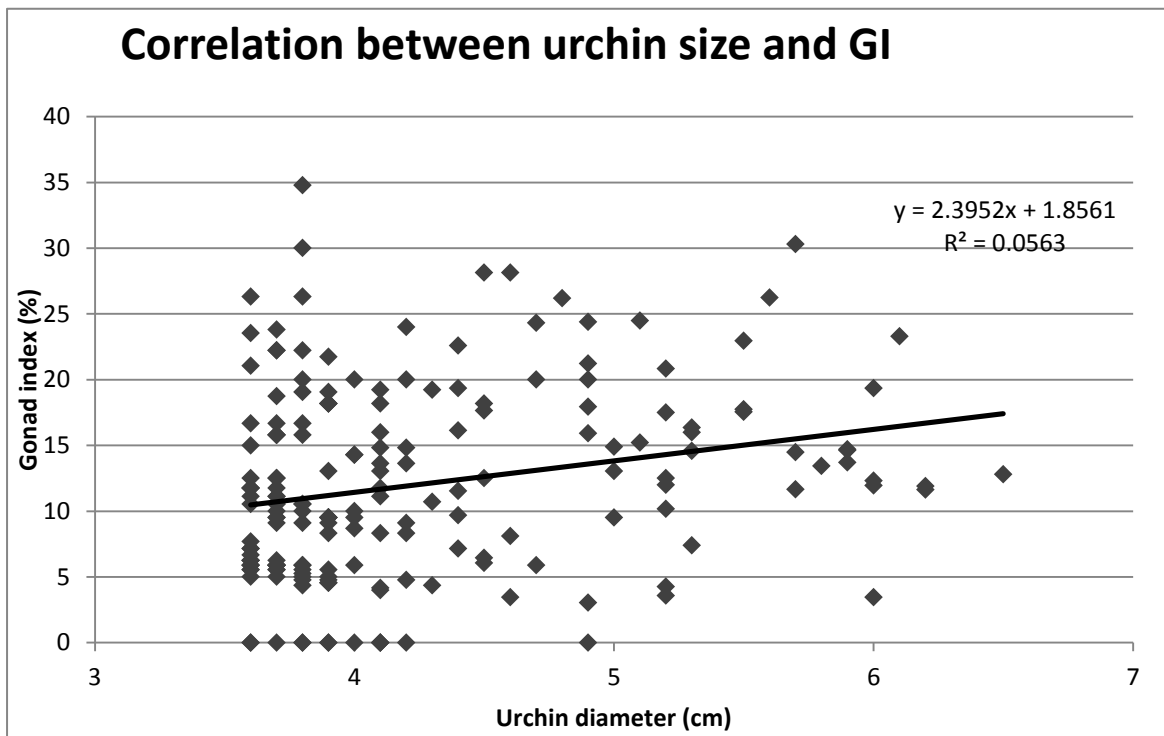
Figure 13: Correlation test of the effect of urchin diameter on GI. The x-axis represents the test diameter of registered sea urchins, while the y-axis represents the GI to the sea urchin.
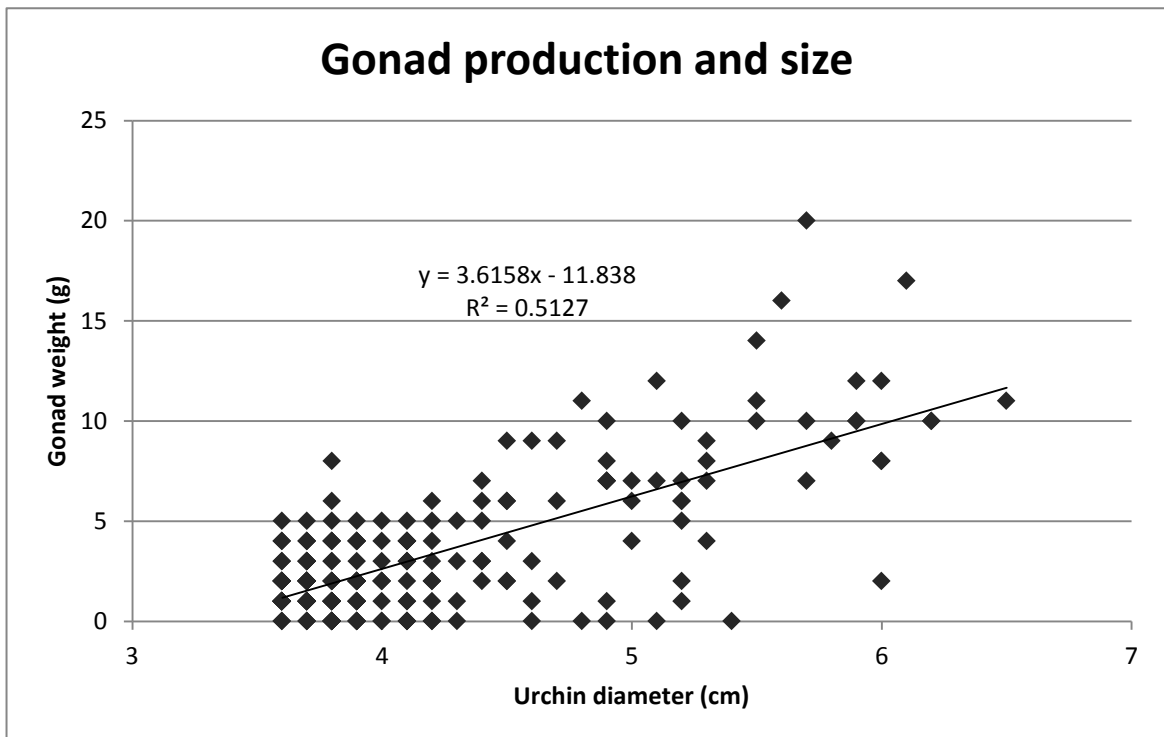

Figure 14: Correlation test of the effect of urchin diameter on gonad production. The x-axis represents the test diameter of registered sea urchins, while the y-axis represents the gonad production to the sea urchin.
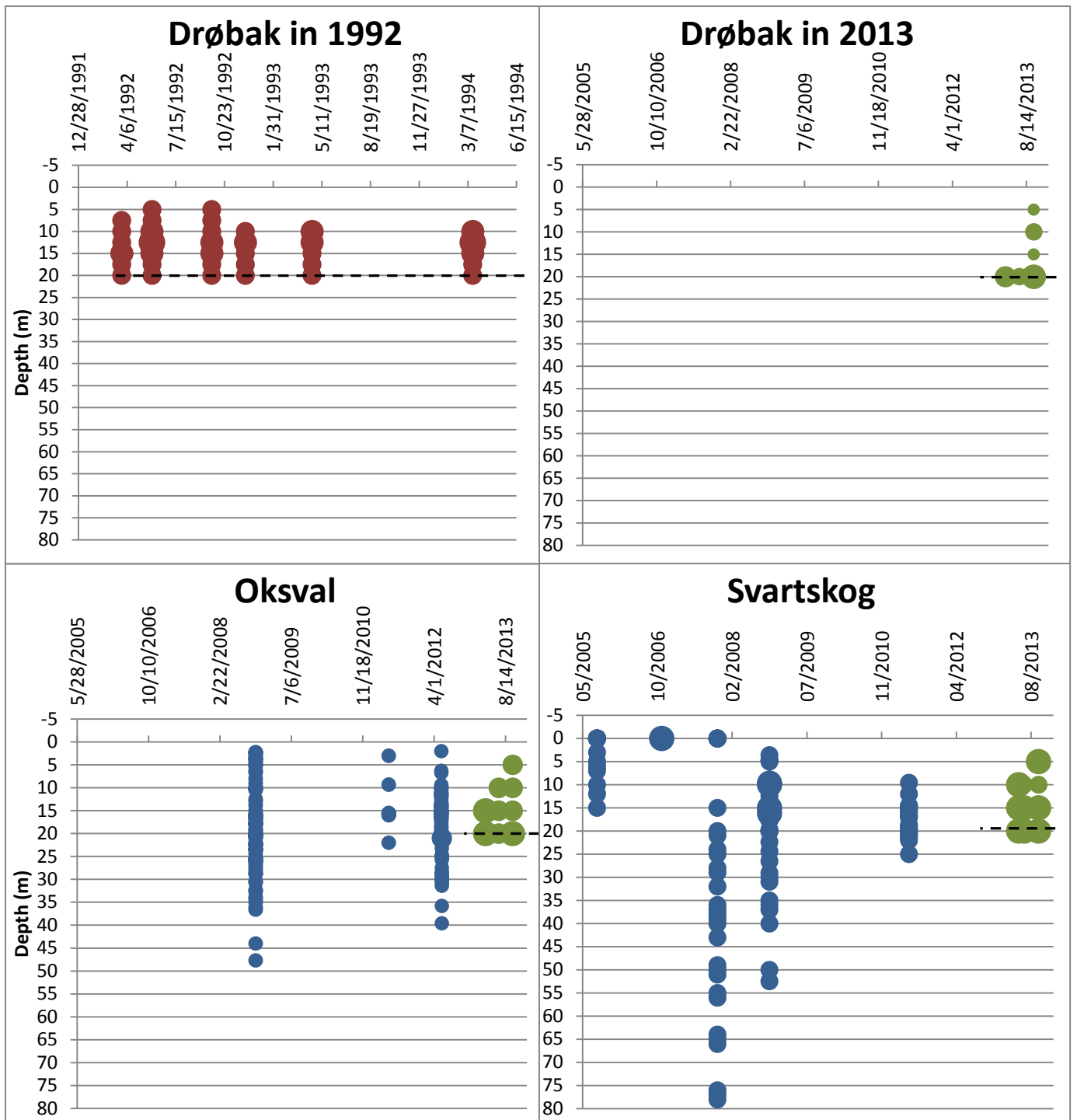
Figure 15: Urchin depth distribution. Drøbak in 1992 (top left), Drøbak in 2013 (top right), Oksval (bottom left) and Svartskog (bottom right) . Y-axis describes all the depths examined by the different studies. The black line shows the deepest divers in this study went down. X-axis is the different dates when data was collected. No data certain years mean that monitoring was not conducted, not that urchins were absent. Data from 2011 and 2012 are only registered as echinoderms, not species, Blue circles represent data from NIVA's monitoring program, red circles represent data from Fredriksen's thesis and the green circles represent the data collected by this study. Data acquired by this study from Oksval is collected at Flaskebekk.
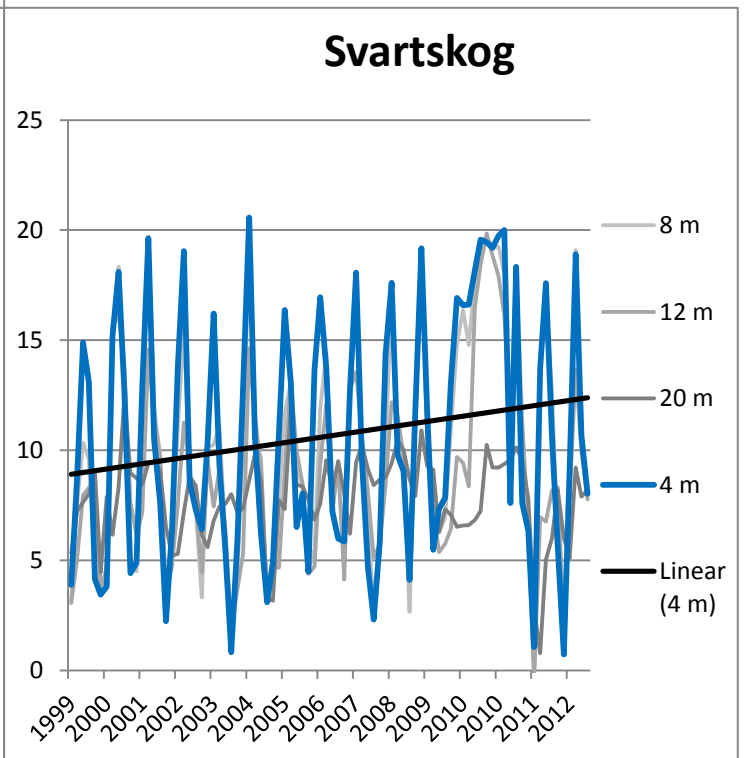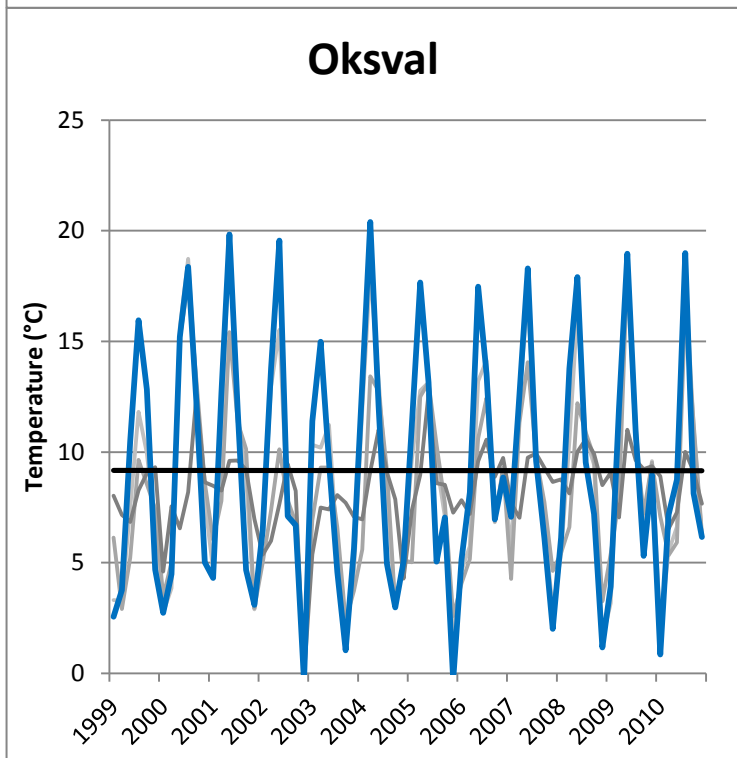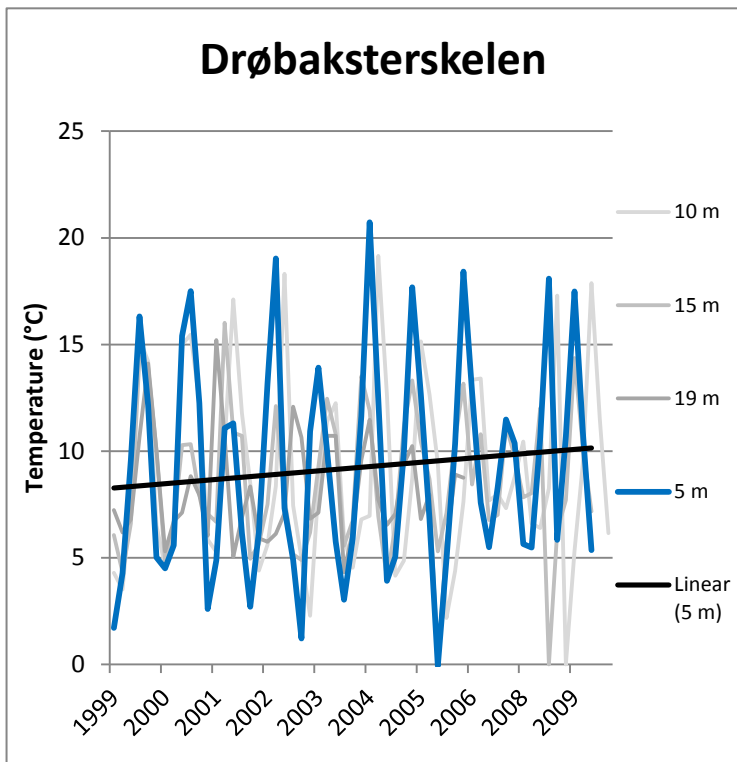
Figure 16: Temporal changes in average water temperature in the Oslofjord. Drøbaksterskelen (above), Oksval (bottom left) and Svartskog (bottom right). Data highlighted is collected at five m depth at Drøbaksterskelen and four m at Oksval and Svartskog. Data are collected by NIVA's surveillance program of the Inner Oslofjord. The x-axis is the years where temperature was measured. Y-axis is the temperature, measured in Celsius, in the different years of the study period. The black line is the overall trend at four or five m depth in the surveillance period.
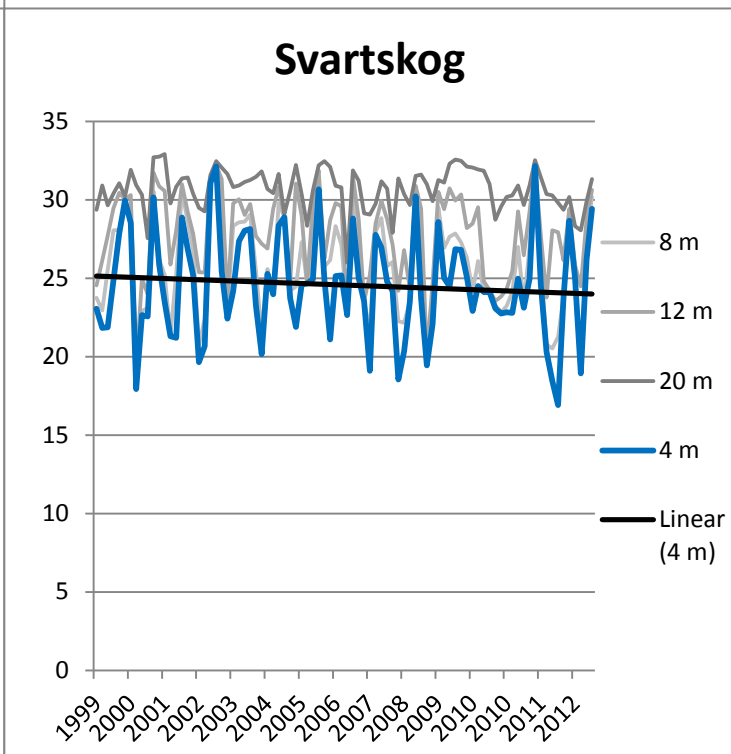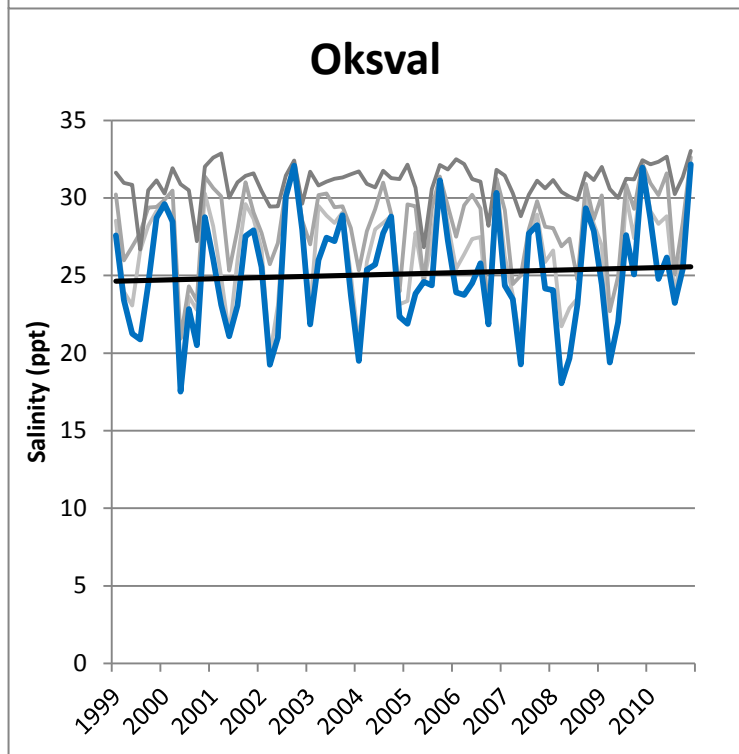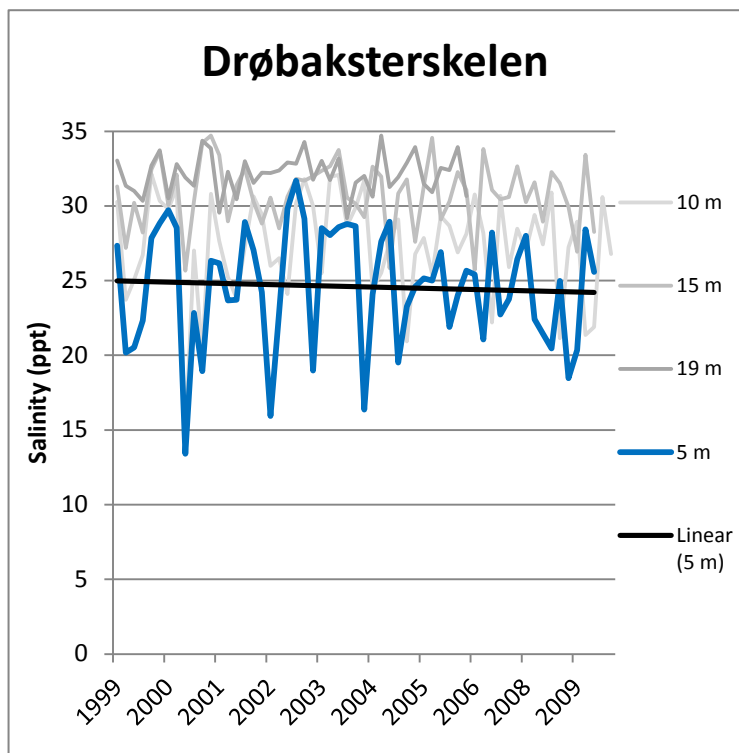
Figure 17: Temporal changes in average salinity in the Oslofjord. Drøbaksterskelen (above), Oksval (bottom left) and Svartskog (bottom right). Data highlighted is collected at five m depth at Drøbaksterskelen and four m at Oksval and Svartskog. Data are collected by NIVA's surveillance program of the Inner Oslofjord. The x-axis is the years where salinity were measured. Y-axis is the salinity, measured in parts per thousand, in the different years up of the study period. The black line is the overall trend at four or five meters depth in the surveillance period.