Spawning, settlement and growth of *Ciona intestinalis* in Øygarden, Hardangerfjorden and Kvitsøy

Master of Science in Aquaculture Biology

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Abstract

Due to the increasing aquaculture industry, new resources for feed should be assessed. A high protein content in *Ciona intestinalis* have increased the interest to farm *C. intestinalis*, and use as a feed resource. In addition, *C. intestinalis* has a tunic of cellulose, which can be used in biofuel production. The ascidian *C. intestinalis* is abundant in temperate regions, and is characterized by rapid growth, early maturation and high reproductive output. To examine the maturation, broodstock animals were collected from 4 different locations, in three different areas; Øygarden, Hardangerfjorden and Kvitsøy. Collection occurred between March and June. Broodstock animals were examined, and dry weight and maturation index was found. Eggs in the gonoduct were collected, and examined using a stereo microscope. The growth and settlement were found using submerged PVC plates at 5 to 25 m depth at 5 locations in three different areas; Øygarden, Hardanger and Kvitsøy. Plates were deployed in March to June, and retrieved in mid to end October. 30 individuals were collected from each depth for the different deployment times from each location in order to investigate differences in dry weight with time of deployment and depth. This study showed that maturation of eggs occurred earlier than expected, and with large variations between locations. The number of eggs varied considerably between individuals. High settlement of *C. intestinalis* was found in all locations except Kvitsøy. This study showed that depth and time of deployment are important factors to obtain a high biomass. The highest recruitment and dry weight was found at 10 and 15 m depth at all locations, and on plates deployed from March to May.

Acknowledgements

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1. Introduction

Norway is well known for salmon farming, and is the dominant producer of Atlantic salmon (S*almo salar*). Due to an increase in global food requirements, it is expected that the amount of farmed fish will increase (Gillund & Myhr 2010). The fish stocks used for fish feed occasionally become fully- or overexploited (Bendiksen et al. 2011). This means that new resources should be assessed as a feed for aquaculture industry. Due to a high protein content, *Ciona intestinalis* are being assessed as a resource for fish feed, as well using their cellulose-rich tunic in biofuel production. This thesis' focus is to investigate time of spawning, settlement and growth for *C. intestinalis* in order to explore the production possibilities.

1.1 Habitat requirements

Ciona intestinalis, commonly known as the vase tunicate, is an ascidian in the subphylum Tunicata (Stolfi & Christiaen 2012), and is one of the most common species of ascidians in the world (Berrill 1947). Populations of *C. intestinalis* are usually found heterogeneously distributed, both spatially and temporally (Petersen & Svane 1995). Densities in the temperate regions show a seasonal pattern (Schmidt 1983). The distribution, spread and persistence are factors that are heavily influenced by salinity and temperature (Vercaemer et al. 2011), and populations of *C. intestinalis* are commonly found in fjords and estuaries (Petersen & Svane 1995). In Scandinavia, the density can be high, and there can be up to several thousand individuals in one square metre (Svane & Havenhand 1993). In natural populations, they are often found on hard substrates, such as bedrocks. *C. intestinalis* often dominate artificial substrates such as harbours, or equipment related to aquaculture sites (Carver et al. 2006). The introduction of man-made structures, such as floating docks and aquaculture gear, have changed the composition of the biofouling communities in the oceans (Connell & Glasby 1999; Holloway & Connell 2002). *C. intestinalis* is considered a cold water and temperate organism, but can occasionally be found in tropical harbours (Manniot & Manniot 1994).

C. intestinalis has a wide range of tolerance for temperature, and the temperature vary among geographical populations and ecotypes (Carver et al. 2006). Results from monitoring of the populations in Scandinavia showed a higher mortality in the cold water populations during the coldest periods of the year (< 1 °C) (Dybern 1965), while populations in the Mediterranean have a low survival rate when the temperature is lower than 10 °C. When temperature is lower than 10 °C, only the younger individuals survives due to a higher tolerance of cold temperature (Marin et al. 1987). The Mediterranean populations experiences temperatures of 25-28 °C on a regular basis without any apparent stress. For the Scandinavian populations however, Petersen and Riisgård (1992) found a decline in filtration rate when temperatures exceeded 21 °C, suggesting that temperatures over 21 °C are stressful for these populations. *C. intestinalis* is classified as a euryhaline organism, which means that they tolerate a wide range of salinities between 11 and 40 ‰. The requirements for salinity vary among populations. Scandinavian populations have a lower limit of salinity of 11 ‰ (Dybern 1965). Observations have shown that *C. intestinalis* has a high survival in turbid conditions. *C. intestinalis* is a sessile filter feeder, and some movement in the water is important to ensure food availability (Carver et al. 2006).

1.2 Anatomy

C. intestinalis is an invertebrate with a yellowish green colour (Carver et al. 2003). The body is cylindrical with a gelatinous tunic, which acts as a skeletal structure (Zhao & Li 2014). This tunic is chemically similar to cellulose (Millar 1970, Sato et al. 2012). Inside the tunic there is a thin sac-like membrane that encloses the organs (Van name 1945). The body is divided into two parts- a large atrial cavity that contains the branchial sac, and a smaller, visceral cavity that contains the digestive and reproductive organs (Millar 1971) (Figure 1.1). *C. intestinalis* has two siphons, an oral siphon with inflow of water, and an atrial siphon where waste and faeces are expelled. *C. intestinalis* is a filtrating organism that traps particles in a rod-like endostyle, where the wall of the branchial sac is continuously secreting a layer of mucus that trap particles with a size of 1 μm (Petersen & Riisgård 1992; Flood & Fiala-Médioni 1981; Petersen 2007). *C. intestinalis* is a potential

key organism for controlling phytoplankton in shallow fjords in late summer (Bone et al. 2003). However, *C. intestinalis* does not have the ability to sort particles (Petersen et al. 1997).

1.3 Reproduction

C. intestinalis is characterised by rapid growth, early maturation and high reproductive output (Carver et al. 2006). They are hermaphroditic, which means that they have both sperm and eggs (Howes et al. 2007). In the early stages of maturity, *C. intestinalis* showed a tendency towards being a protandric hermaphrodite, which means the production of sperm precedes the production of eggs (Carver et al. 2006). The sperm was shown to be viable for at least 12 hours in tanks, and seemed to be activated by the presence of egg water from *C. intestinalis* (Bolton & Havenhand 1996). Eggs are set free into the water column, either separate or in clusters, where the actual fertilization happens (Berrill 1947; Svane & Havenhand 1993). The fertilized eggs then develop into a tadpole larvae (Nakayma-Ishimura et al. 2009), which are non-feeding and free swimming. This larva consists of 2600 cells, and a few tissues (epidermis, endoderm, mesenchyme, notochord, central nervous system and muscles) (Matsushima et al. 2013). After one to five days the larva will metamorphose into the adult and sessile stage (Howes et al. 2007). McLaughlin et al. (2013) found that water containing high levels of suspended inorganic matter could reduce the ability of *C. intestinalis* to develop into the adult stage. The dispersal of larvae are limited, and influenced by reproductive mode, as well as the mobility of the larvae (Petersen & Svane 1995;
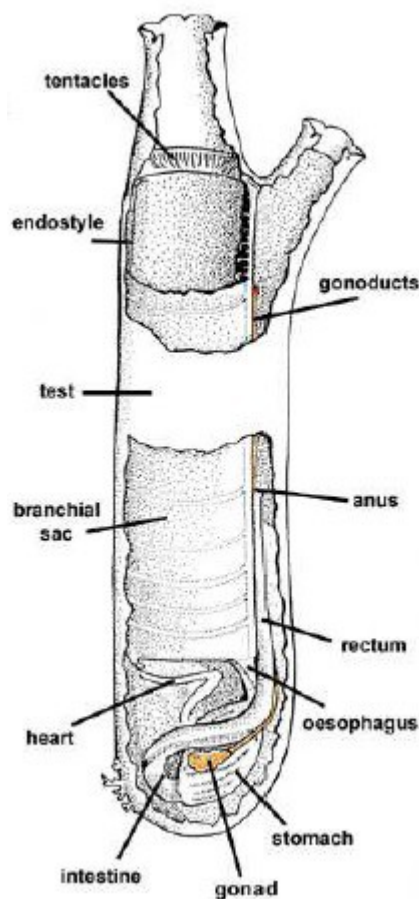
Figure 1.1: Anatomy of *Ciona intestinalis* (Carver et al. 2006)

Kanary et al. 2011).

Size are important for maturation, and the size for maturation varies among populations (Carver et al. 2006; Dybern 1965). Yamaguchi (1975) found that the gonads started to develop when the individuals were about 20 mm long. However, the populations in cold water along the coasts of Scandinavia must reach 5 to 8 cm before they can produce fertile gametes (Carver et al. 2006). Individuals that are large enough for maturity can continually produce gametes as long as the temperature is suitable (Carver et al. 2006). The eggs and sperm are stored in the gonoduct (Figure 1.1) prior to spawning (Yamaguchi 1975). Spawning consists of three steps. (1) Localisation of the egg and binding of the sperm to the coat (2) penetration of the sperm through the coat of the egg, and (3) fusing of the sperm and egg (Byrd & Lambert 2000). The eggs is released in cycles, and in each cycle 2000 to 3000 eggs are released, with spawning every second or third night (Yamaguchi 1975). The spawning of *C. intestinalis* is induced by short periods of light after dark, and *C. intestinalis* often spawns at dawn (Lambert & Brandt 1967; Whittingham 1967). At continuous light, spawning can be inhibited (Carver et al. 2006).

The coastal populations in Scandinavia have a life cycle that last for 12 to 18 months, and they produce two generations (Carver et al. 2006). The first generation is recruited when the temperature in the spring is suitable, while the second is recruited when the first generation reaches maturity and gives rise to the second generation. These two generations coexist through the winter until first spawning (Carver et al. 2006). The generation recruited during fall will grow in the fall as long as temperatures are suitable, but will not reach sexual maturity until the spring spawning (Carver et al. 2006).

The reproduction of *C. intestinalis* is thought to be seasonal (Berrill 1947). In places where the seasonal changes are big and the temperatures in winter and summer are very different, the spawning of *C. intestinalis* is more distinct (Dybern 1965). The temperature has been registered to be minimum 8 ºC for spawning in the Scandinavian populations (Dybern 1965). In 1970, Gulliksen (1972) registered that the spawning started at a temperature

between 8 ℃ and 12 ℃ in May to June, and continued in August to October. When temperatures are low, the maturation of eggs can be inhibited (Joly et al. 2007). *C. intestinalis* in central Japan reached sexual maturity after 1 month in the summer season, and after 2 months during winter (Yamaguchi 1975).

C. intestinalis can refill its gonoduct within 24 hours after spawning in the summer season (Yamaguchi 1975). Yamaguchi (1975) estimated that a total fecundity of one individual in central Japan is about 100 000 eggs. The larva of *C. intestinalis* develop during the night after fertilization, and hatch the next morning (Yamaguchi 1975). Experiments conducted along the European coast have shown that the embryonic development from fertilized eggs occurred when the temperature was between 8 ℃ and 23 ℃ (Dybern 1965). Gulliksen (1972) observed that in North- Tröndelag the main settlement of *C. intestinalis* was in June.

1.4 Settlement and Growth

C. intestinalis can appear in high densities on artificial structures, as well as in natural benthic habitats (Petersen et al. 1995). Dumont et al. (2011) found that the occurrence of adult populations on artificial structures were due to high predator pressure in benthic communities. Research by Osman & Whitlatch (2004) concluded that the predation on ascidians at the post settlement stage might be more important than the recruitment for the composition of epibenthic communities. Growth is very rapid for *C. intestinalis*, and experiments have shown that the growth can be exponential until they are 10 mm in length (Yamaguchi 1975). Marshall & Keough (2003) found that the larger individuals had a higher survival than the smaller individuals after settlement. In temperate areas with shallow waters there are two growth seasons, one in spring/early summer and one in summer/beginning of fall (Petersen et al. 1995). A linear relationship was found between growth and increased temperature (Petersen & Riisgård 1992). Sampling conducted in different locations in northern Europe showed that the growth can be as high as 10-20 mm/month (Dybern 1965). The fast growth rate of *C. intestinalis* has been recorded to be 0.26-0.76 mm in diameter in seven days (Collin & Johnsen 2014). A laboratory experiment

revealed a growth rate of 0.7% in length per day for *C. intestinalis* fed with cultured algae (Petersen et al. 1995). The upper limit for rapid growth has been set to 23 ºC for Scandinavian populations, but temperatures this high are associated with water pollution and an increased mortality rate (Cirino et al. 2002). Petersen & Riisgård (1992) found that temperatures over 21 ºC can be stressful. There are big differences between locations. Deep water populations can reach up to 150 mm, and only reproduce once a year in temperatures that exceeds 8 ºC. The growth of *C. intestinalis* has an isometric growth where the organs accounts for 36 % of the total wet weight and 45 % of the dry weight (Carver et al. 2006). The cost for maintenance is higher for large individuals, while the cost of organ growth is similar regardless of size (Carver et al. 2006). There are big variations in the populations, and the temperature seems to be an important factor for deciding life cycles. In warmer waters, like Japan (temperature > 10 ºC), the life span of *C. intestinalis* is much shorter and the total body length is much shorter than for the individuals living in cold water (Yamaguchi 1975).

The growth of *C. intestinalis* started to decrease when they became sexually matured (Yamaguchi 1975). Grant et al. (1998) found that the mean biomass at a mussel farm was 1.35 kg of *C. intestinalis* per m ropes in South Africa. Petersen et al. (1995) found a logarithmic relationship between algal concentration and growth rate, and that the growth rate increased with an increased algal concentration. The growth seems to be determined by the amount of suspended particles in the water, and an increased amount of particulate suspension has been shown to reduce the growth and increase the death rate for filter feeders (Robbins 1985).

1.5 Impacts on other species

Space limitation is often seen as the limiting factor for sessile suspension feeders, and the competition rate is high (Lesser et al. 1992). Research has shown that other species of ascidians had an impact on the abundance of microzooplankton (Bingham & Walters 1989). A study conducted by Blum et al. (2007) showed that dense aggregation of *C. intestinalis* could reduce the species richness at a local scale, as well as the community

composition. Two mechanisms seem to be important for *C. intestinalis* to dominate a community; (1) a high recruitment and the ability to successfully occupy a settlement space, and (2) reduce the settlement on or among *C. intestinalis* by other species (Blum et al. 2007).

From the beginning of salmon farming in Norway in the 1970's the biofouling of *C. intestinalis* has increased due to settlement on gear used in the aquaculture industry (Carver et al. 2006; Skogen et al. 2009). The main areas for farming salmon have been the fjords along the Norwegian coastline (Skogen et al. 2009). Waste from these farming locations can be particulate substances that contain nutrients, which can lead to eutrophication (Skogen et al. 2009). One hypothetical scenario is that *C. intestinalis* can have a positive impact on systems with a nutrient loading (Carver et al. 2006). Conley et al. (2000) suggested that benthic filter feeders, like *C. intestinalis*, could play an important role of managing the development of phytoplankton blooms, which might cause hypoxia and anoxic events. Due to its high filtration capacity, *C. intestinalis* can be used as a cleaning agent in fjords with high numbers of aquaculture sites. This means that farming of *C. intestinalis* could improve water quality for the ecosystems in the fjords.

1.6 Aim of study

1.6.1 Reproduction study

The aim of the reproduction study is to examine the maturation of *C. intestinalis* in populations in Hardanger, Øygarden and Kvitsøy, and to look at possible differences between locations. Several studies indicate that spawning starts when the temperature reaches 8 ºC (Dybern 1965; Gulliksen 1972). Several of the studies conducted on Scandinavian populations show that spawning does not occur until May, with a few observations of spawning in the end of April. Observations from Ulvesundet in 2013 (Øygarden municipality) indicates that spawning starts earlier than these studies claim.

1.6.2 Biomass study

The aim of the biomass study is to examine the growth and time of settlement of *C. intestinalis* on PVC (polyvinyl chloride) plates. Several studies conducted on growth and recruitment have shown good results with PVC plates for recruitment of *C. intestinalis* (Vercaemer et al. 2011). In addition, the tunic of *C. intestinalis* consist of cellulose (Millar 1970; Sato et al. 2012), which can be used in biofuel production. A research project is looking at the biochemical content of *C. intestinalis* and the possibility using *C. intestinalis* as a bio-resource. Since *C. intestinalis* contains a lot of proteins, it can hopefully be used in the aquaculture industry as feed for salmon and other farmed species. This will reduce the need for wild fish as a marine protein source. This Master thesis will investigate variations between location, time of deployment and depth.

2. Materials and methods

2.1 Reproduction study

2.1.1 Sampling sites

Ciona intestinalis was collected from four locations along the west coast of Norway. Sampling sites were located in Hardanger (Kvam municipality), Øygarden municipality and in Kvitsøy municipality. The sampling sites were 1a- Ulvesundet (Øygarden municipality), 3a- Steinstø (Kvam municipality), 4a- Øystese (Kvam municipality) and 5a- Kvitsøy municipality (Figure 2.1). Ulvesundet (1a) is located in a strait from Hjeltefjorden, outside of Bergen city. The sampling sites in Hardangerfjorden are located in the middle part of the fjord. Steinstø (3a) is located in a strait in Hardangerfjorden, called Fyksesundet, while Øystese (4a) is further out the fjord. Kvitsøy (5a) is an island outside Stavanger city.
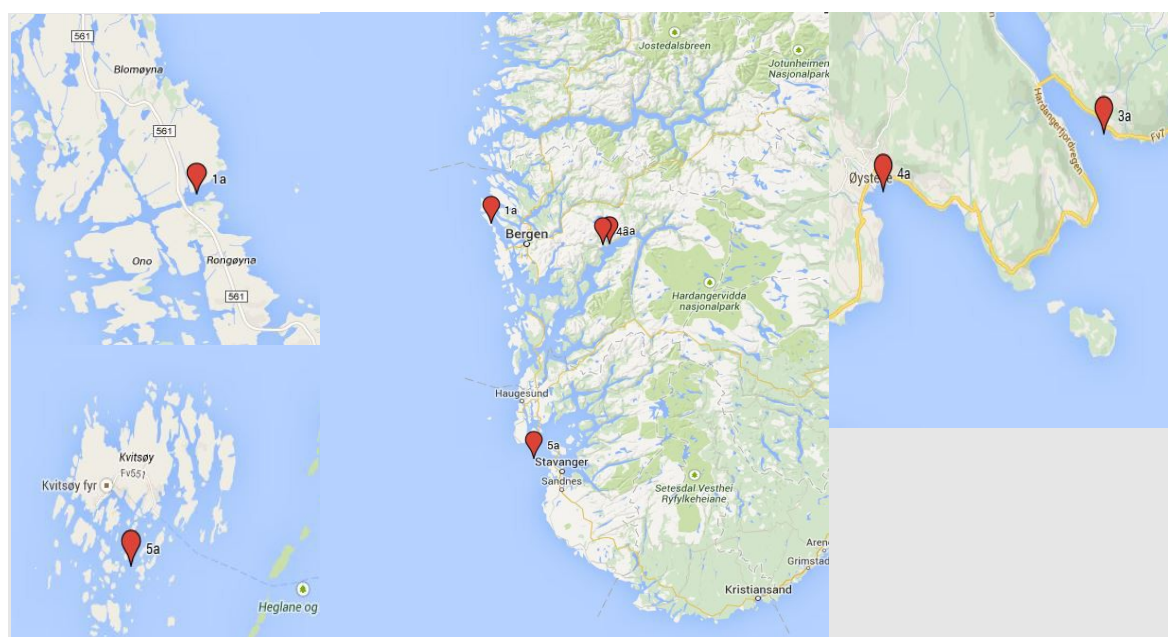
Figure 2.1: Locations (Western part of Norway) used for collecting broodstock animals at different dates (Table 2.1)

2.1.2 Sampling

Collection of broodstock animals of *C. intestinalis* was conducted between March and June 2014. 30 individuals were collected from each location, performed every second week until week 25 (Table 2.1), providing six to eight collections from each sampling sites. In Ulvesundet (1a) and Kvitsøy (5a) broodstock animals were picked from PVC (Polyvinyl chloride). In Hardanger (3a and 4a), broodstock animals were collected from natural populations on floating docks by diving (Hotate AS). The individuals were brought back to the laboratory at the Department of Biology in Bergen for further analysis.

Table 2.1: Overview over dates and locations for collection of broodstock animals

Location name	Location	Date	Location name	Location	Date
Rong	1a	10.03.2014	Øystese	4a	26.03.2014
Rong	1a	25.03.2014	Øystese	4a	09.04.2014
Rong	1a	07.04.2014	Øystese	4a	23.04.2014
Rong	1a	22.04.2014	Øystese	4a	12.05.2014
Rong	1a	05.05.2014	Øystese	4a	21.05.2014
Rong	1a	19.05.2014	Øystese	4a	11.06.2014
Rong	1a	02.06.2014	Kvitsøy	5a	01.04.2014
Rong	1a	16.06.2014	Kvitsøy	5a	10.04.2014
Steinstø	3a	26.03.2014	Kvitsøy	5a	28.04.2014
Steinstø	3a	09.04.2014	Kvitsøy	5a	07.05.2014
Steinstø	3a	23.04.2014	Kvitsøy	5a	22.05.2014
Steinstø	3a	12.05.2014	Kvitsøy	5a	12.06.2014
Steinstø	3a	21.05.2014	Kvitsøy	5a	24.06.2014
Steinstø	3a	11.06.2014			

2.1.3 Analysis

The wet weight of the collected individuals was measured, and divided into aluminium beakers. The aluminium beakers weight was measured before they were used, and this weight was subtracted from the dry weight of individuals afterwards. The wet weight was measured using a scale, Kern EW Zero tare. After the wet weights were recorded, each animal was opened to examine the gonads and the gonoduct. A table (Table 2.2) was constructed on the basis of Dybern' (1965) table of the stages of sexual maturation. Each

broodstock animal was examined and the maturation stage was registered in relations to the maturation stages described in Table 2.2.

After examination of maturity stage, the individuals were heated to obtain dry weight. The heating was conducted in a heating cabinet (Fermarks type TS 8024) at 60 ºC, and the weight was monitored until dry weight was obtained (approximately four days) (Appendix A1). Dried samples were moved from the heating cabinet to a desiccator with silica gel, until they obtained ambient temperature. The dry weight was measured using Sartorius scale type ME2355.

Table 2.2: The criteria for each maturation index, used as a guide to identify the development of maturation, inspired by Dybern (1965).

Maturation index	Gonad	Gonoduct
1	No colouring of gonad	No eggs in gonoduct, no sperm
2	Light orange/orange gonad	No eggs in gonoduct, but have sperm
3	Orange/red gonad	Eggs in gonoduct, lower part of animal, have sperm
4	Orange/red gonad	Eggs in gonoduct, middle part of the animal
5	Orange/red gonad	Eggs in gonoduct, upper part of animal, whole gonoduct filled with eggs, have sperm
6	Orange/red gonad	Eggs in gonoduct, from middle part to upper part, have sperm
7	Light orange/orange gonad	Only small amount of eggs in the gonoduct, have sperm

Eggs were collected from five individuals with maturation index between 4 and 6 (Table 2.2) from each location. The collecting of eggs was done by cutting the end of the gonoduct, and the eggs were pressed out of the gonoduct. The eggs were then collected using a pipette, and put in the refrigerator for further analysis.

2.1.4 Egg analysis

The eggs were examined in a stereo microscope (Leica M125). This stereo microscope was connected to a camera (Nikon DS-U3). Some eggs were very sticky, and laid in clusters. In order to separate the eggs, different methods were tested (ethanol, freshwater, seawater, sieve and pipette). The pipette turned out to be the most efficient method to separate the eggs from each other. By dragging/sucking the eggs in and out of the pipette a couple of times, most of the eggs were separated. Eggs from the individuals were divided into different compartments and photographed with 0.8x magnification on the stereo microscope, and 10 x magnification on the camera, a total of 8x magnification. Egg counting was done in Image J (Version 1.48), with ROI management, where the total number of eggs was estimated.

2.2 Biomass study

2.2.1 Sampling sites

In this study, *C. intestinalis* was collected in five different locations (Figure 2.2). These locations are in the same area as the reproduction study locations. Four of the locations were in Hordaland; two in Øygarden municipality and two in Hardanger (Kvam municipality). The fifth location was in Rogaland, in Kvitsøy municipality. The sites were 1b- Ulvesundet (Øygarden municipality), 2b- Hjeltefjorden (Øygarden municipality) 3b- Tveitnes (Kvam municipality), 4b- Saltkjelen (Kvam municipality) and 5b- Kvitsøy municipality. Tveitnes (3b) and Saltkjelen (4b) are salmon farming sites.
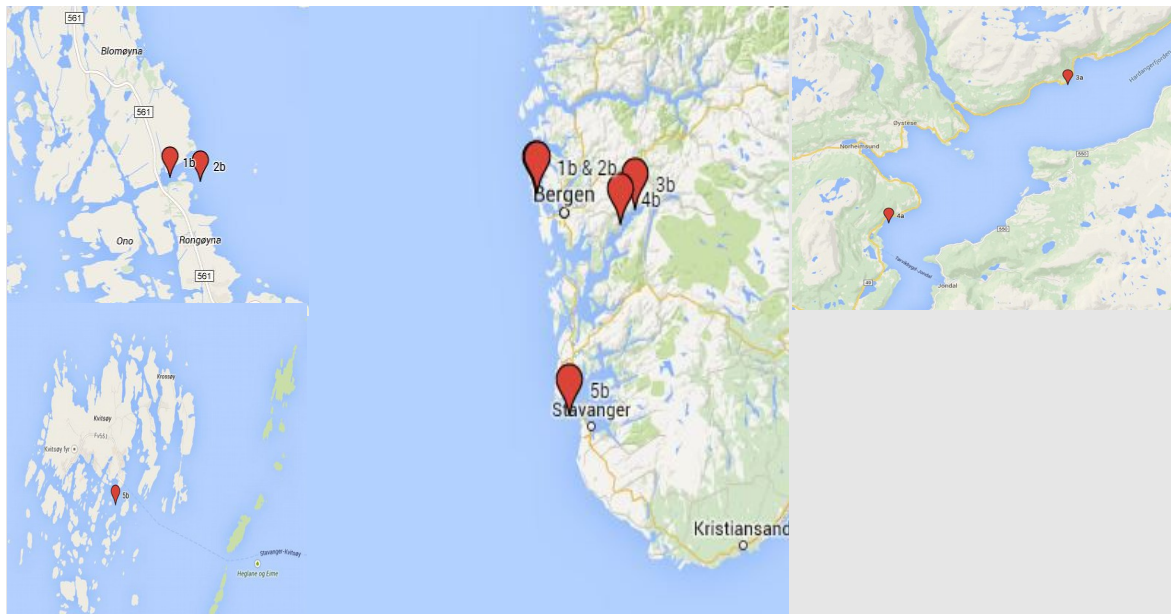

Figure 2.2: Locations (Western part of Norway) where collectors where placed out at different dates (Table 2.3)

2.2.2 Sampling

Submerged PVC (Polyvinyl chloride) plates of 20×20 cm (0.04 m^2) were placed in five different locations (Figure 2.2), during spring and early summer (Table 2.3). These PVC plates have shown good results for collecting the pelagic larvae from *C. intestinalis* in previous studies. Plates were placed between 5 and 25 metres on a rope with 5 metres between each plate (Figure 2.3). In Kvitsøy (5b) the collectors were placed on a line at 5 m below sea surface. The first plate was placed at 10 m below sea surface, the second at 15 m, and then a weight, and the third plate at 15 m, the fourth plate at 10 m and the fifth plate at 5 m. This was done due to limitations in depth, with maximum depth of 20 metres. The first collector deployed had a temperature logger (Tinytag aquatic 2) at 10 metres. The temperature was measured every 6th hour.
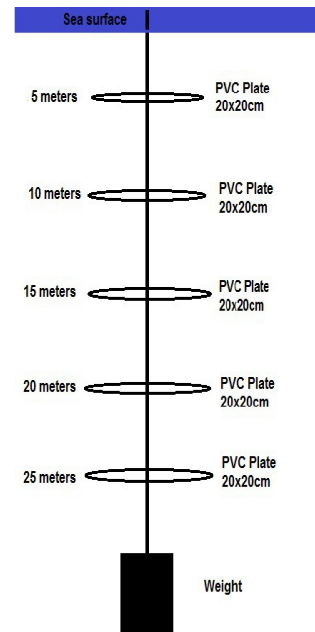
Figure 2.3: Illustration of collector with 5 PVC (polyvinyl chloride) plates placed from 5 m to 25 m.

The collectors were retrieved in October (Table 2.3), and CTD measurement (Conductivity-Temperature-Density) were taken at each location, which provided a vertical profile of each location. Due to habitat requirements of *C. intestinalis*, the results were based on temperature, salinity and fluorescence. Fluorescence is an indicator of the algal concentration. Individuals were present at both ropes and plates, except Kvitsøy (5b). At the top layer (5 m) individuals were only found on the underside of the plate. Settled individuals on plates were scraped off and put into buckets marked with depth of plate (5 to 25 m). During retrieval of the April plates from 10 m depth in Saltkjelen (4b), several individuals fell off. The total biomass for each plate was measured with a hand held weight (Twine digital scale, 25 kg) and the total biomass was recorded. The biomass of *C. intestinalis* was estimated as kg wet weight pr 0.04 m^2 if not otherwise stated. The total number of individuals on each plate was counted, and 30 individuals from each depth and time of deployment were brought back to the laboratory at the Department of Biology in Bergen for further analysis. In Ulvesundet (1b) and Hjeltefjorden (2b) a monoculture of *C.*

intestinalis was found. In Saltkjelen (4b) there was a monoculture of blue mussels (*Mytilus edulis*) at 5 metres, while a monoculture of *C. intestinalis* were found at depths below 10 metres. Kvitsøy (5b) was dominated by *Ascidella aspersa*, with few individuals of *C. intestinalis.*

2.2.3 Analysis

The individuals were preserved in a freezer (-18° C) until further analysis. The total weight was measured for each bag, and afterwards the wet weight of each individual was estimated. The excess water was divided by the number of individuals, which gave an approximation of the total wet weight of each individual. Individuals were retrieved from the freezer, and dried for four days in aluminium beakers (Appendix A1). Before the dry weight was estimated, the individuals were kept in a desiccator with silica gel until ambient temperature was obtained. The dry weight was measured with a Sartorius scale, type ME2355. To find the amount of organic matter in *C. intestinalis,* a total of nine individuals were kept to find the ash weight. Ash weight was found using an incinerator (Naberthern IFM 3201), with a maximum temperature of 500 ºC.

2.3 Statistics

Statistics was conducted in R (version 3.0.2) (www.r-project.com) and Libre office calc (version 4.3.5). The temperature plots are represented by a daily mean. The maturation index at each date was found using mean and ± standard deviation. A correlation between the dry weight and maturation index was found for each month, illustrated by a figure with a linear trend line. The correlation coefficient (R^2) says something about how much correlation there is between dry weight and maturation index. The values are between -1 and 1, where 1 indicates a high correlation.

A oneway ANOVA (Analysis of variation) was conducted to see if there was a statistically significant difference between the biomass and depth at each location, biomass and time of deployment, as well as dry weight for the 30 individuals at different depths and time of

deployment. All these were graphically presented in box plot. In box plots the middle line is the median, while the lower part of the box are the lower quartile, the upper part of the box are the upper quartile. The vertical lines with a horizontal line on the end are the maximum and minimum values, while the circles are representing out layers.

3. Results

3.1. Hydrography

3.1.1 Temperature

The temperatures in all locations were lowest at time of deployment. In Tveitnes (3b) and Saltkjelen (4b) there was a slow increase in temperature until the end of July, when a rapid increase in temperature occurred. In Ulvesundet (1b), Hjeltefjorden (2b) and Kvitsøy (5b) an increase in temperature over time occurred, with high fluctuations during the summer months. All locations had a slow decrease in temperature after the beginning of September (Figure 3.1)

The temperature in Ulvesundet (1b) and Hjeltefjorden (2b) increased from 6.2 and 6.5 ºC at time of first deployment to 8 ºC in mid-May. A continuous increase in temperature occurred until the beginning of June, when the temperature fluctuated with up to 10 ºC during summer. The maximum temperature occurred in the beginning of August. In Ulvesundet (1b) the temperature reached 18.7 ºC while Hjeltefjorden (2b) reached 18.6 ºC (Figure 3.1.A and 3.1.B). Tveitnes (3b) increased from 4.3 ºC at the time of first deployment to 8 ºC in mid-May. A further increase in temperature occurred until the end of July, when a rapid increase of 9 ºC was recorded. The highest temperature recorded was 16.4 ºC in August (Figure 3.1.C). The temperature in Saltkjelen (4b) increased from 4.6 ºC at time of first deployment, to 8 ºC in the beginning of May. A further increase in temperature occurred until the end of July, when a rapid increase of 10.5 ºC was observed. The highest temperature recorded in Saltkjelen was 18.5 ºC in August (Figure 3.1.D). The temperature in Kvitsøy (5b) was 6.3 ºC at time of first deployment, and increased to 8 ºC in the end of May. Fluctuations were found in temperature during the summer months, with a difference of 13 ºC in a short period of time. The highest temperature recorded in Kvitsøy was 19.9 ºC in the beginning of August (Figure 3.1.E)
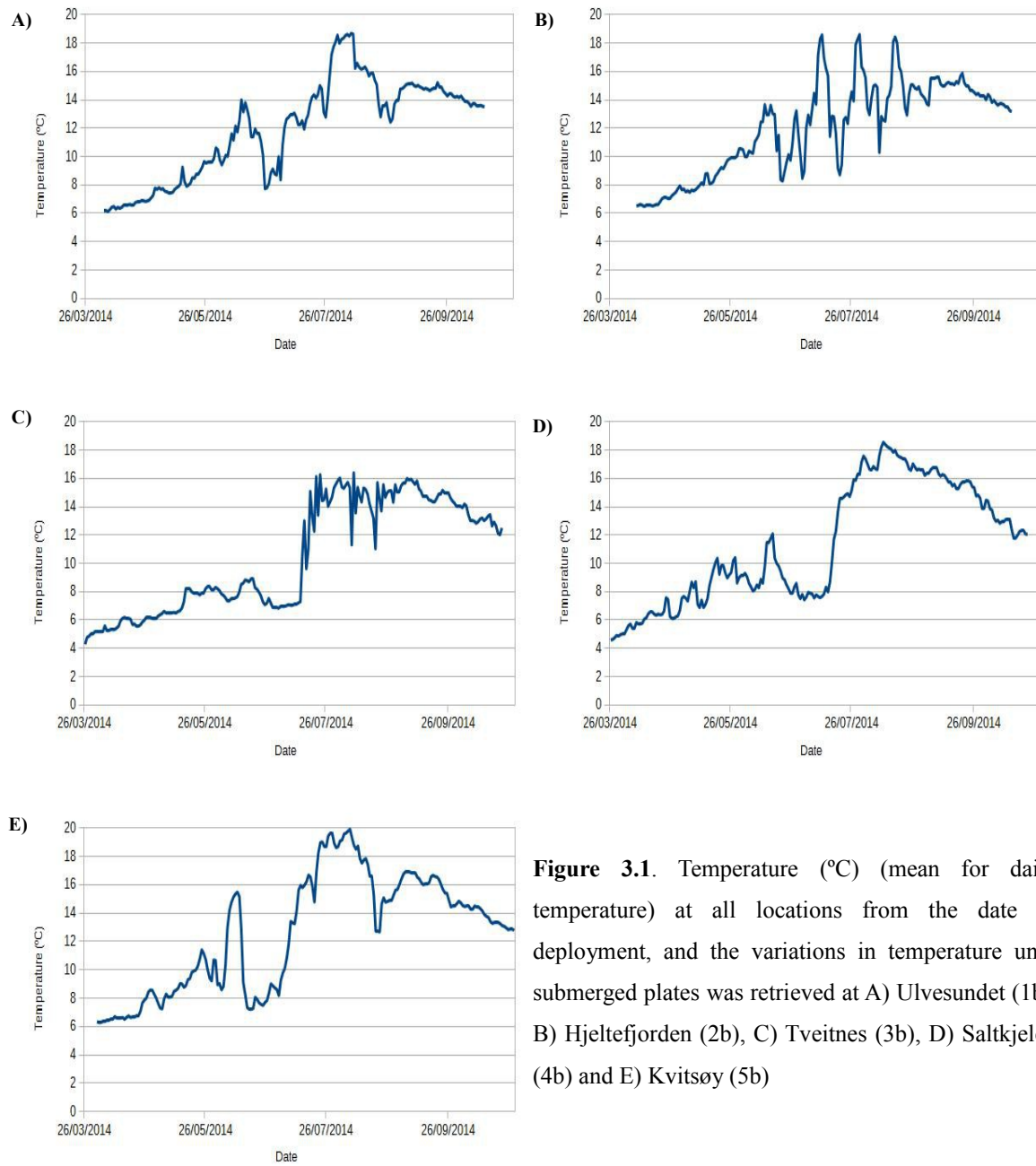
Figure 3.1. Temperature (ºC) (mean for daily temperature) at all locations from the date of deployment, and the variations in temperature until submerged plates was retrieved at A) Ulvesundet (1b), B) Hjeltefjorden (2b), C) Tveitnes (3b), D) Saltkjelen (4b) and E) Kvitsøy (5b)

3.1.2 Vertical profile

3.1.2.1 Temperature

The temperature in all locations was lowest temperature at 5 m. The lowest temperature found in Ulvesundet (1b) was 12.9 ºC, with only small variations with increasing depth. The lowest temperature found in Hjeltefjorden (2b) was 12.5 ºC, with an increasing temperature with increasing depth. The lowest temperature found in Tveitnes (3b) was 11.5 ºC at 5 m, with an increasing temperature with increasing depth. The lowest temperature found in Saltkjelen (4b) was 11.7 ºC at 5 m depth, with an increase in temperature with increasing depth. The temperatures in Kvitsøy (5b) were stable at 12.6 ºC between 5 and 10 m, with only a small increase of 0.1 ºC at 15 m (Figure 3.2).

Figure 3.2: The vertical profile of temperature (ºC) was measured by CTD in October 2014 when retrieval of submerged plates at 1b) Ulvesundet, 2b) Hjeltefjorden, 3b) Tveitnes, 4b) Saltkjelen and 5b) Kvitsøy.

3.1.2.2 Salinity

The salinities (‰) at both Tveitnes (3b) and Saltkjelen (4b) were lower than in the other three locations (Figure 3.3). The salinities in Ulvesundet (1b) and Kvitsøy (5b) showed small variations with depth, ranging from 31.55-31.73 and 31.34-31.42. In Hjeltefjorden (2b) there was a slight variation of 1.2 in salinity from the shallowest to the deepest depth measured. At 5 m, salinity was 31.1, while it was 32.3 at 25 m. In Tveitnes (3b) salinity ranged from 26.7 at 5 m depth to 31.5 at 25 m. The salinity in Saltkjelen (4b) ranged from 27.7 at 5 m to 31.6 at 25 m depth.

Figure 3.3: The salinity was measured by CTD in October 2014 when retrieval of submerged plates at 1b) Ulvesundet, 2b) Hjeltefjorden, 3b) Tveitnes, 4b) Saltkjelen and 5b) Kvitsøy.

3.1.2.3 Fluorescence

Fluorescence decreased with increasing depths at all locations, except in Ulvesundet (1b), where there was a slight increase from 5 to 15 m, before decreasing at 20 m (Figure 3.4). Fluorescence in Ulvesundet (1b) was lowest at 25 m (0.29 λ g/L), and highest at 15 m (0.37 λ g/L). In Hjeltefjorden (2b), fluorescence decreased from 0.38 λ g/L at 5 m to 0.12 λ g/L at 25 m. The highest fluorescence in Tveitnes (3b) was found at 5 m (1.25 λ g/L). A decrease in fluorescence was found with increasing depth, and the lowest fluorescence was found at 25 m (0.14 λ g/L). The highest fluorescence in Saltkjelen (4b) was at 5 m (1.23 λ g/L). A decrease in fluorescence was found with increasing depth, and the lowest fluorescence was found at 25 m (0.2 λ g/L). The highest fluorescence in Kvitsøy (5b) was found at 5 m (0.95 λ g/L). The lowest fluorescence was found at 10 m (0.72 λ g/L) and 15 m (0.73 λ g/L).
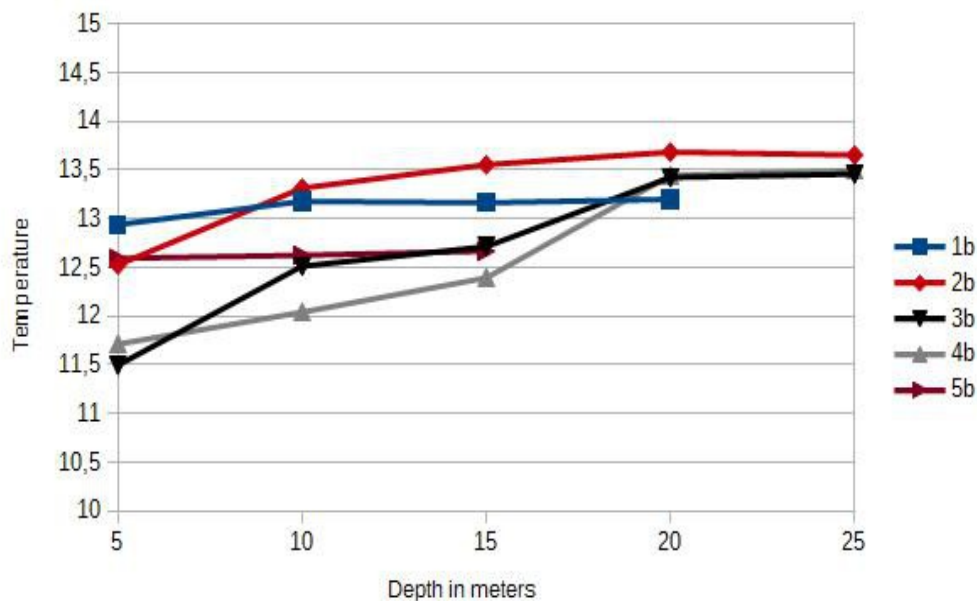

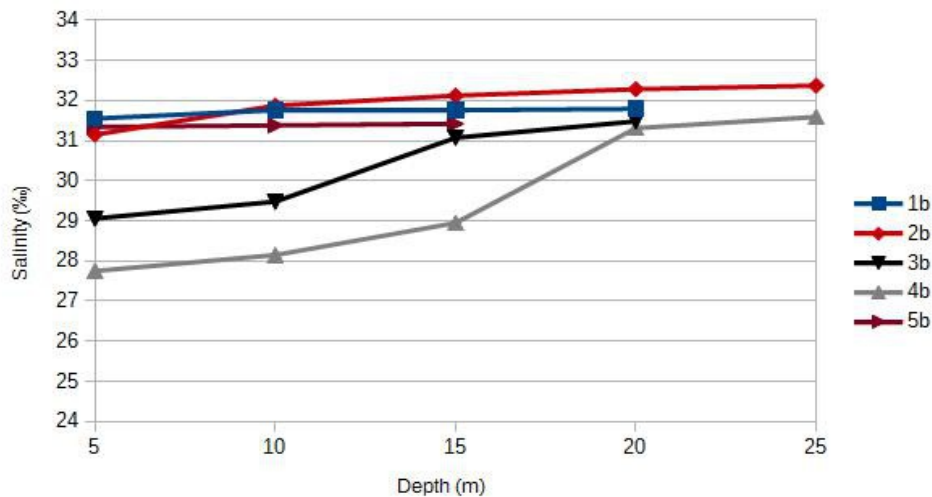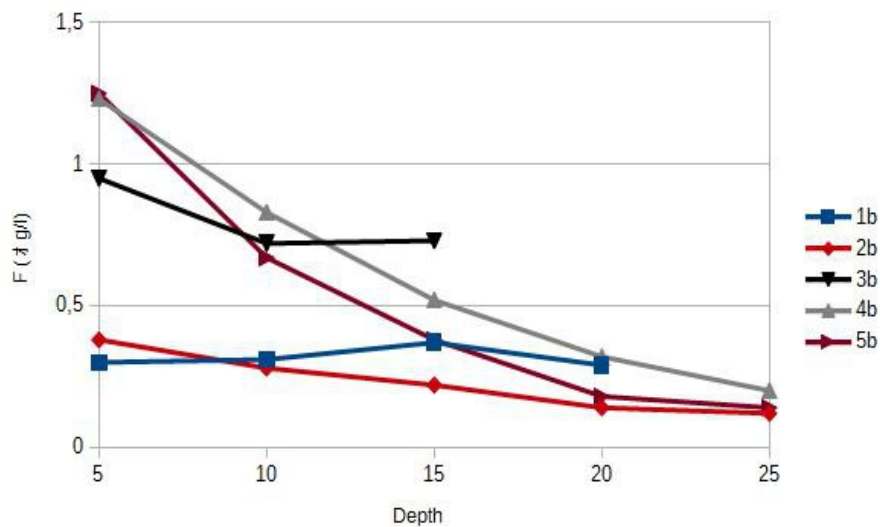**Figure 3.4**: The fluorescence (λ g/L) was measured by CTD in October 2014 when retrieval of submerged plates at 1b) Ulvesundet, 2b) Hjeltefjorden, 3b) Tveitnes, 4b) Saltkjelen and 5b) Kvitsøy.

3.2 Reproduction study

3.2.1 Maturation index

High indexes (mean, ± SD) were found in Steinstø (3a) from the beginning of sampling. A high index was found in Ulvesundet (1a) and Øystese (4a) in the end of April. A rapid increase in maturation index occurred over a short period of time in Ulvesundet (1a), while Øystese (4a) increased continuous over time. Low indexes were found in Kvitsøy (5a) until the beginning of May (Figure 3.5)

At the start of sampling, Ulvesundet (1a) showed a low index (1.8, ± 0.76). The highest indexes were found in the end of April (5.83, ± 0.7) (Figure 3.5.A). In the beginning of sampling, a high maturation index (4.43, ± 1.14) was found in Steinstø (3a). The highest maturation index was found in the end of May (6.1, ± 0.84) (Figure 3.5.B). A low maturation index was found at the start of sampling (2.8, ± 1.63) in Øystese (4a). The highest maturation index in the end of May (5.83, ± 1.82) and the beginning of June (5.8, ± 0.76) (Figure 3.5.C). The lowest maturation index was found in the beginning of sampling in Kvitsøy (5a) (1, ± 0). The highest maturation index was found in the end of June (5.13, ± 1.04) (Figure 3.5.D).
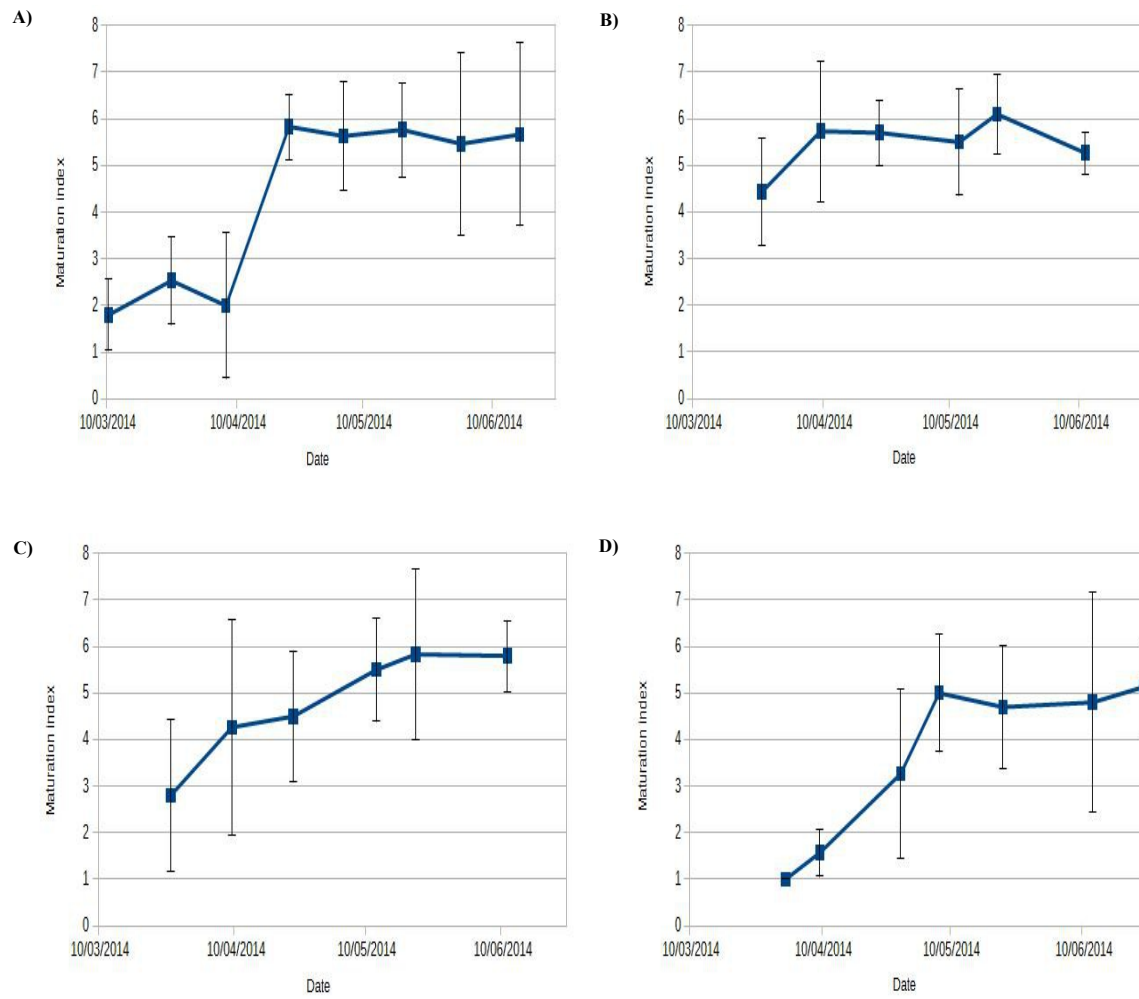
Figure 3.5: Maturation index (mean, ± SD, n=30) from first sampling (10th of March) until last sampling (24th of June) at location A) Ulvesundet (1a), B) Steinstø (3a), C) Øystese (4a) and D) Kvitsøy (5a). Vertical lines represents the standard deviation (± SD).

3.2.2 Size of broodstock animals

A high dry weight (mean) was found in Ulvesundet (1a) and Steinstø (3a), compared to Øystese (4a) and Kvitsøy (5a) (Figure 3.6). High dry weight was found in Ulvesundet (1a) at all sampling dates, and ranged between 2.0 and 4.9 g (Figure 3.6.A). High dry weight occurred in Steinstø (3a) at all sampling dates, and ranged between from 2.67 to 3.61 g (Figure 3.6.B). Lower dry weight was found in Øystese (4a), and ranges between 0.85 to 2.18 g (Figure 3.6.C). The lowest dry weight was found in Kvitsøy (5a), and ranged between 0.84 and 1.28 g (Figure 3.6.D)
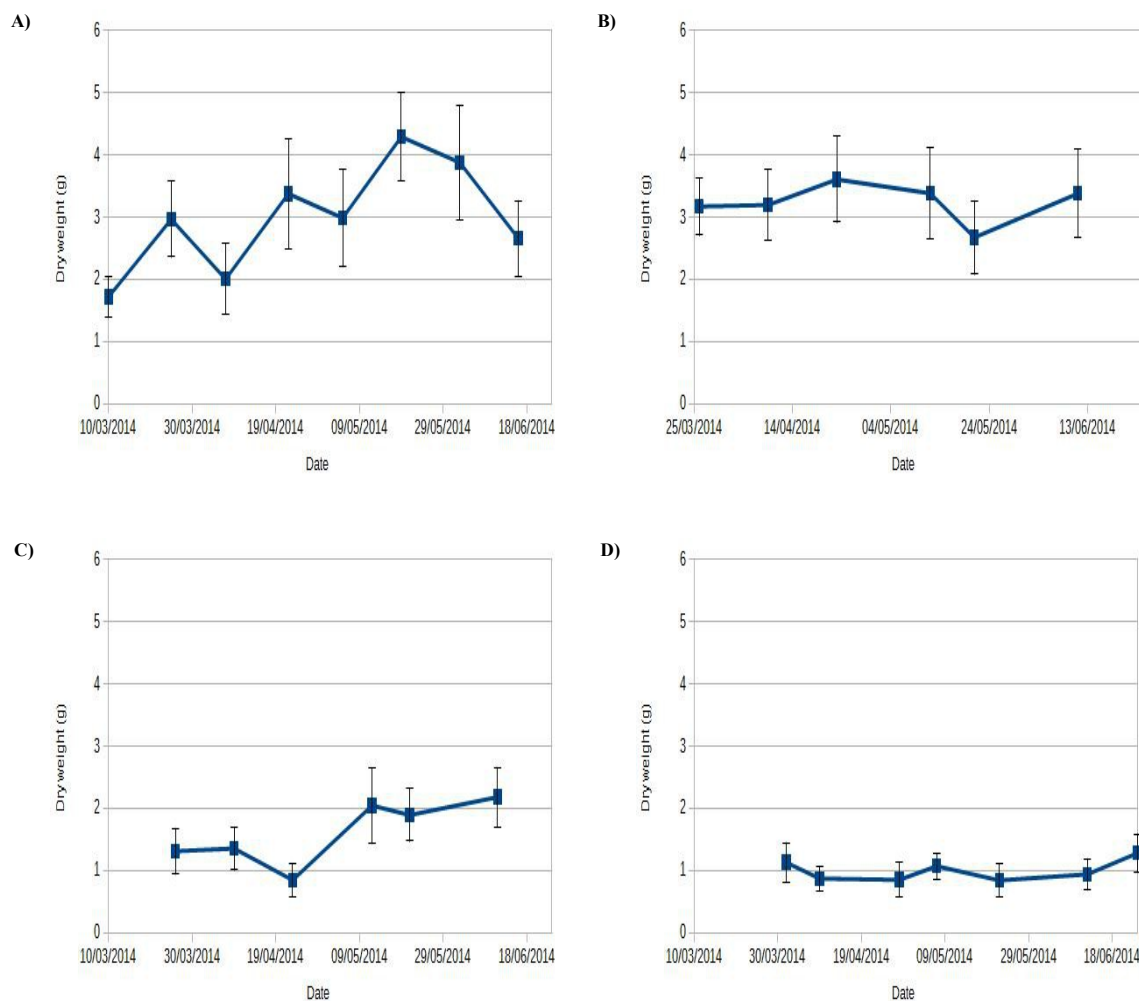
Figure 3.6: Dry weight (mean ± SD, n=30) in grams at location A) Ulvesundet (1b), B) Steinstø (3b), C) Øystese (4b) and D) Kvitsøy (5b) from first sampling (25th of March) until last sampling (24th of June). Vertical lines represents the standard deviation (± SD).

3.2.3 Correlation between maturation index and dry weight

A linear regression was conducted in order investigate the correlation between dry weight and maturation index during the sampling months. The highest correlation was found in April (R^2=0.290), while the lowest correlation was found in June (R^2=0.035). No direct correlation was found between dry weight and maturation index.
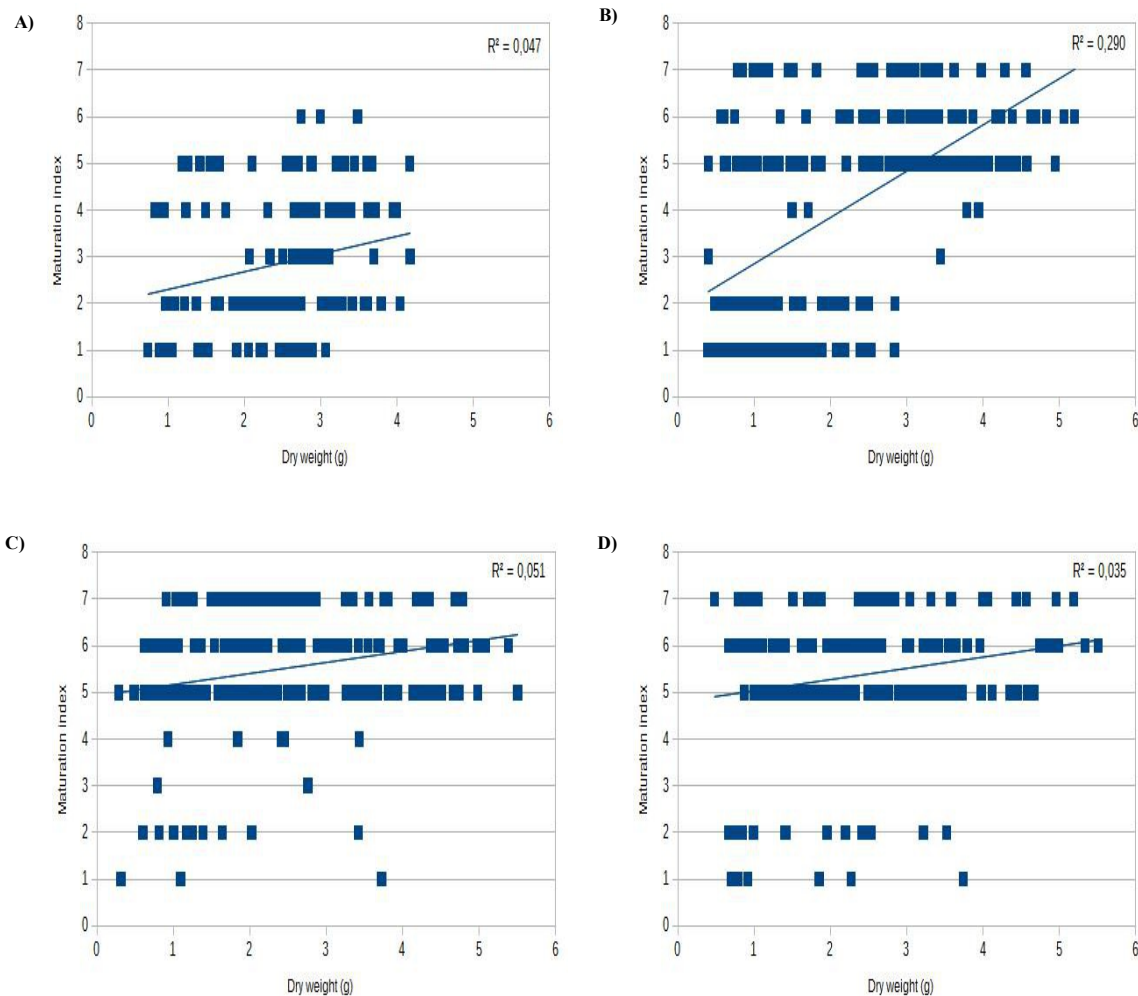
Figure 3.7: The correlation between dry weight (g) and the maturation index for individuals collected in A) March (n=120), B) April (n= 270), C) May (n=240) and D) June (n=180) The line represents a trend line between dry weight and maturation index, and the R^2 represents the correlation coefficient.

3.2.4 Egg analysis

The number of eggs (mean, ± SD) and dry weight of individuals (n=17) were investigated to find correlation (Figure 3.8), and no correlation was found (R^2=0.083). The number of eggs varied between individuals, and ranging between 1512 and 61601 (11416, ± 14817).
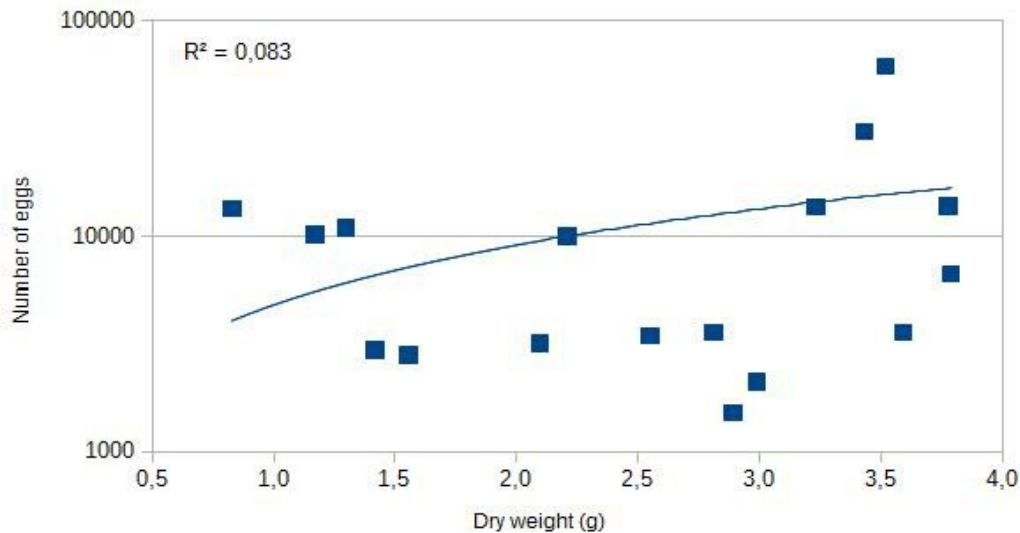
Figure 3.8: The number of eggs and the dry weight (g) of individuals in a logarithmic scale. Each point represents an individual with number of eggs and dry weight (g) (n=17). The line represents the linear correlation between number of eggs and dry weight.

3.3 Biomass study

3.3.1 Integrated biomass

The median biomass per plate, integrated over time and depth showed that high values of biomass were found at all sites, except Kvitsøy (5b) (Figure 3.9). The largest median biomasses (5.09 kg and 4.55 kg) found were in Ulvesundet (1b) and Saltkjelen (4b). The intermediate median biomasses (2.96 kg and 0.97 kg) were found in Hjeltefjorden (2b) and Tveitnes (3b). The lowest median biomass (0.23 kg) was found in Kvitsøy (5b).
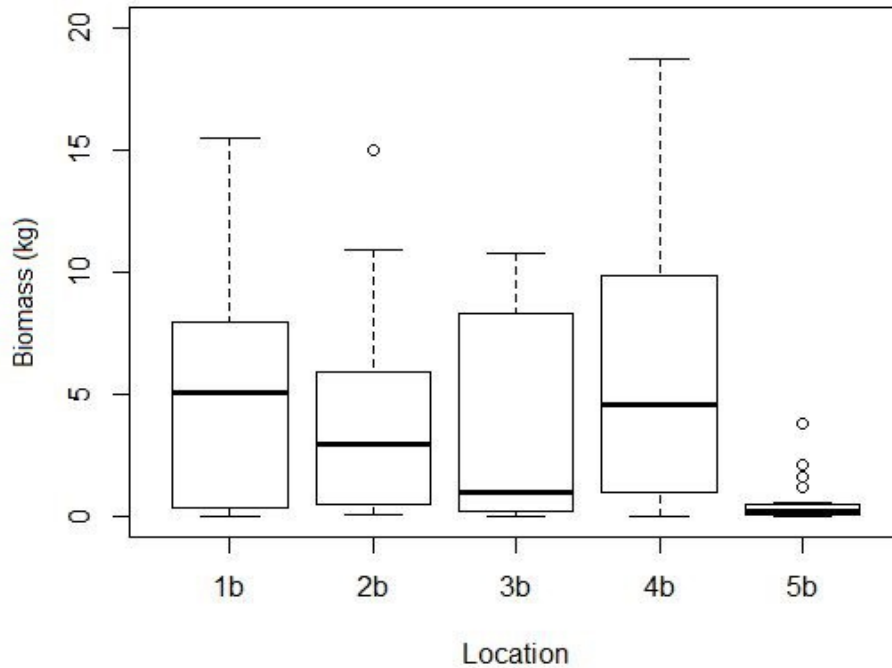
Figure 3.9: Integrated biomass over time and depth per plate at 1b) Ulvesundet, 2b) Hjeltefjorden, 3b) Tveitnes, 4b) Saltkjelen and 5b) Kvitsøy.

3.3.2 Vertical distribution

A oneway anova was conducted to find if the biomass was significant (P< 0.05) different between depth. A statistical significance was found in Ulvesundet (1b, p=5.24×10^{-5}), Tveitnes (3b, p=0.0001) and Saltkjelen (4b, p=9.1×10^{-5}). No statistical significance was found in Hjeltefjorden (2b, p=0.0660) and Kvitsøy (5b, p=0.1605). The highest median biomass (11.46 kg) was found at 15 m in Ulvesundet (1b). The lowest median biomass was found at 5 m (1.21 kg) and 25 m (0.00 kg) (Figure 3.10.A). The highest median biomass (8.97 and 8.39 kg) occurred at 5 and 10 m in Hjeltefjorden (2b). The lowest median biomass (1.01 kg) was found at 25 m (Figure 3.10.B). The highest median biomasses (10.48 and 8.42 kg) were found at 10 and 15 m in Tveitnes (3b). The lowest median biomasses (0.23 and 0.77 kg) were found at 5 and 25 m (Figure 3.10.C). The highest median biomasses (16.97 and 10.47 kg) were found at 15 and 10 m in Saltkjelen (4b). The lowest median biomass (1.93 kg) was found at 25 m. No biomass was found at 5 m (Figure 3.10.D). The highest median biomass (0.39 kg) was found at 15 m in Kvitsøy (5b). The

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lowest median biomass (0.11 kg) was found at 5 m (Figure 3.10.E).

Big variations were found between the number of individuals between depth, where the highest number of individuals occurred between 10 and 20 m in all locations, and ranged between 41 and 497.6 (Table 3.1). In Hjeltefjorden (2b) a high number of individuals at 5 m was found in addition to 10 to 20 m.

Table 3.1: Mean of number of individuals with standard deviation (±) at each plate (0.04 m^2) at location 1b) Ulvesundet, 2b) Hjeltefjorden, 3b) Tveitnes, 4b) Saltkjelen and 5b) Kvitsøy.

Depth (m)	1b	2b	3b	4b	5b
5	16.6 ± 14.7	218.0 ± 16.9	12.0 ± 0.0	0.0 ± 0.0	33.3 ± 16.1
10	136.0 ± 13.86	207.5 ± 38.8	215.5± 30.4	360.3 ± 60.0	41 ± 22.1
15	270.6 ± 67.3	226.5 ± 16.26	199 ± 4.2	497.6 ± 64.4	51.8 ± 7.7
20	175.3 ± 92.4	215.0 ± 4.2	62 ± 31.1	213.3 ± 104.0	X
25	0.0 ± 0.0	113 ± 24.7	38 ± 7.07	119.6 ± 5.85	X
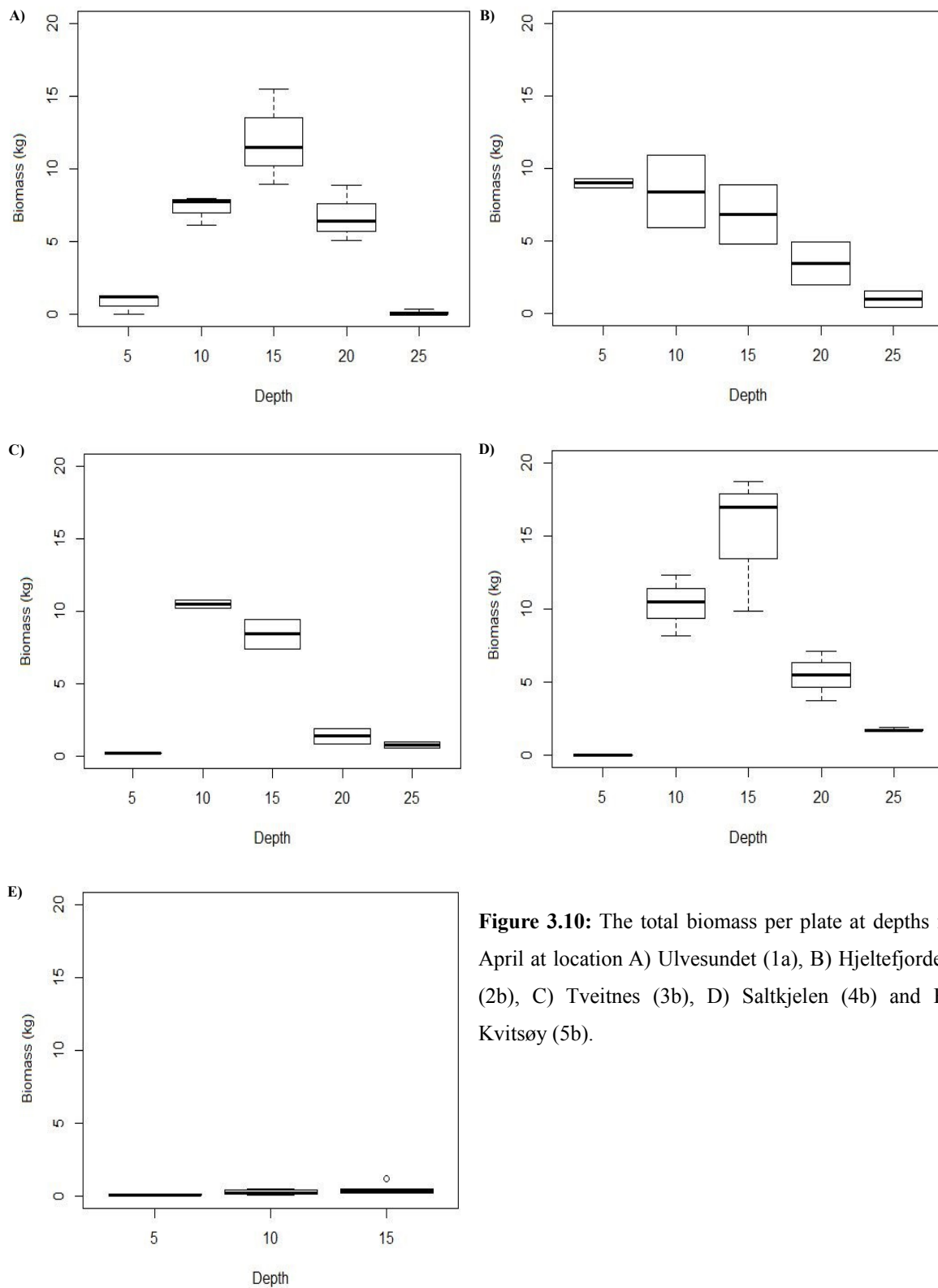
Figure 3.10: The total biomass per plate at depths in April at location A) Ulvesundet (1a), B) Hjeltefjorden (2b), C) Tveitnes (3b), D) Saltkjelen (4b) and E) Kvitsøy (5b).

3.3.3 Time of deployment

In all locations except Kvitsøy (5b), the lowest biomass occurred on the plates submerged in June. In Kvitsøy (5b), the lowest biomass was found on the plates submerged in March. There was no significant difference in biomass between the different times of deployment in Ulvesundet (1b, p=0.6598), Hjeltefjorden (2b, p=0.2432), Tveitnes (3b, p=0.5609) and Saltkjelen (4b, p=0.8383). In Kvitsøy (5b), the biomass difference was significant (p=0.0316).

In Ulvesundet (1b) the highest median biomass was found in April (6.78 kg) and May (5.49 kg), while the lowest median biomass was found in June (0.98 kg) (Figure 3.11.A). In Hjeltefjorden (2b) the highest median biomass was found in April (6.81 kg), while the lowest median biomass was found in June (1.01 kg) (Figure 3.11.B). In Tveitnes (3b) the highest median biomass in March (2.39 kg). The lowest median biomass was found in June (0.21 kg) (Figure 3.11.C). The highest median biomass in Saltkjelen (4b) was found in March (5.36 kg) and April (5.46 kg), and the lowest median biomass was found in June (1.47 kg) (Figure 3.11.D). In Kvitsøy (5b) the highest median biomass was found in May (0.59 kg). The lowest biomass median was found in March (0.05 kg) (Figure 3.11.E).

The highest total biomass (28.95 kg) in March was found in Saltkjelen (4b), while the lowest total biomass (0.18 kg) was found in Kvitsøy (5b) (Figure 3.12.A). The highest total biomass (33.34 kg) found in April where found in Saltkjelen (4b), and the lowest total biomass (1.17 kg) was found in Kvitsøy (5b) (Figure 3.12.B). The highest total biomass (32.29 kg) in May was found in Hjeltefjorden (2b), while the lowest total biomass (2.67 kg) was found in Kvitsøy (5b) (Figure 3.12.C). The highest total biomass (19.18 kg) in June was found in Saltkjelen (4b), and the lowest total biomass (2.26 kg) was found in Kvitsøy (5b) (Figure 3.12.D).

In all locations the highest biomass per plate was found at 10 and 15 m at all time of deployments (Figure 3.12). In addition to 10 and 15 m, a high biomass per plate was found at 5 m in Hjeltefjorden (2b) in April and at 5 m in Kvitsøy (5b) in May.
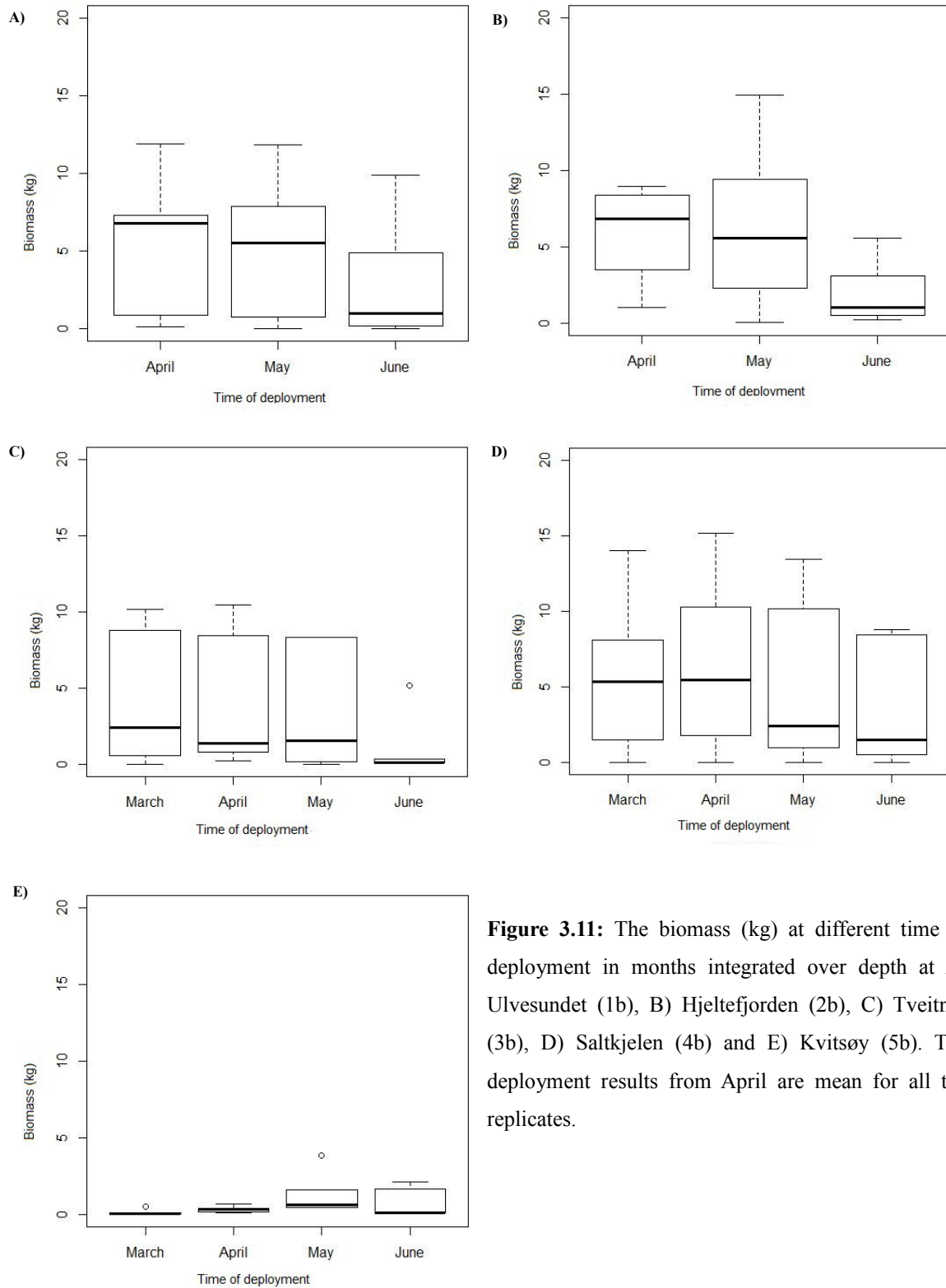
Figure 3.11: The biomass (kg) at different time of deployment in months integrated over depth at A) Ulvesundet (1b), B) Hjeltefjorden (2b), C) Tveitnes (3b), D) Saltkjelen (4b) and E) Kvitsøy (5b). The deployment results from April are mean for all the replicates.
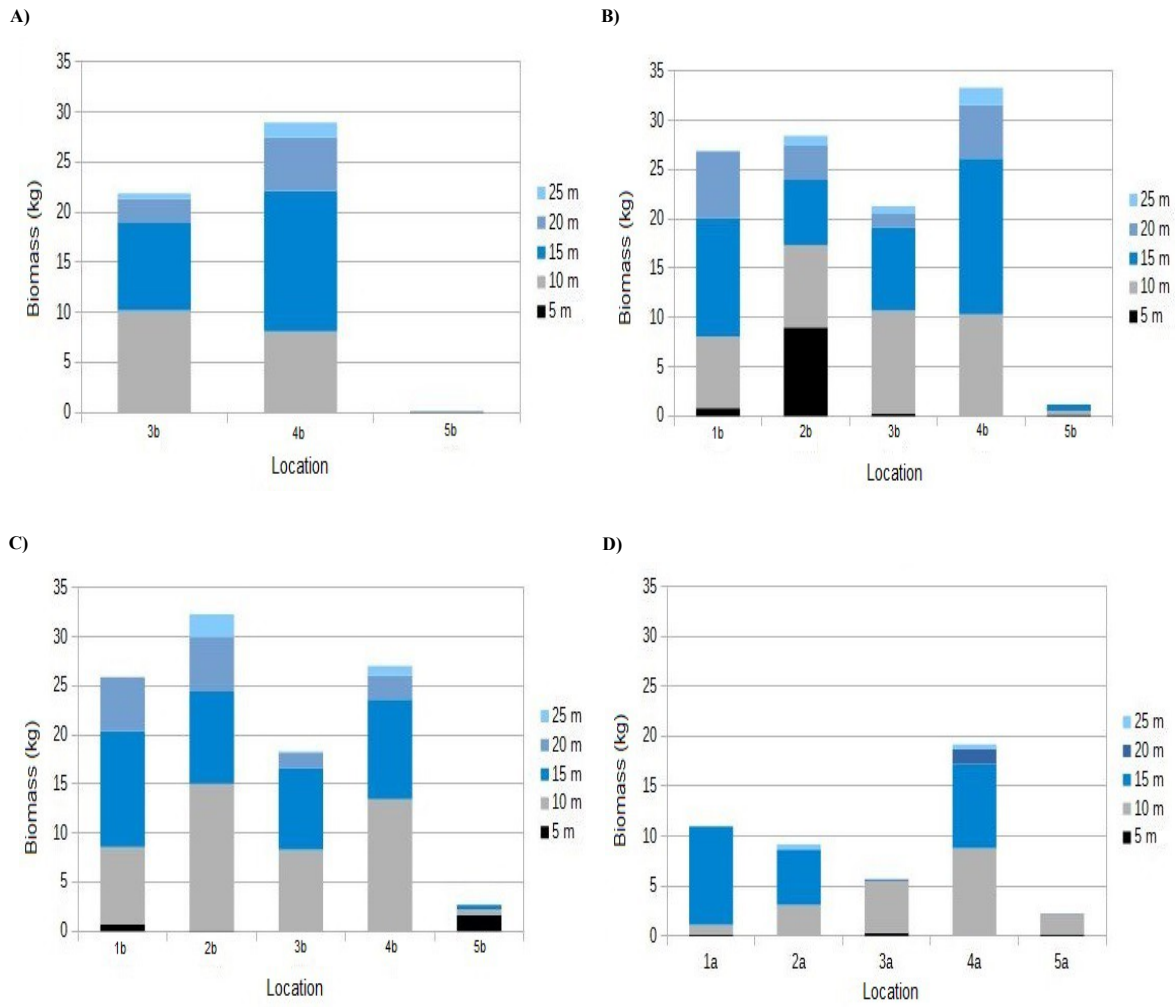
Figure 3.12: The total biomass (kg) and time of deployment for A) March, B) April, C) May and D) June at 1b) Ulvesundet, 2b) Hjeltefjorden, 3b) Tveitnes, 4b). Saltkjelen and 5b). Kvitsøy. Colours are representing depth of biomass.

3.3.4 Individual growth

Based on results from vertical distribution (Figure 3.10), the highest biomass is found between 10 and 20 m. Dry weight for individuals from these depths were used for further analysis. Due to the low biomass in Kvitsøy (5b), this location was excluded.

3.3.4.1 Vertical distribution

The median dry weight integrated over time for individuals (n=120) at depth (m) varied between depth (Figure 3.13). The highest median dry weight in Ulvesundet (1b) was found at 10 m (1.81 g). The lowest median dry weight was found at 20 m (1.18 g) (Figure 3.13.A). The highest median dry weight in Hjeltefjorden (2b) was found at 10 m (1.11 g), while the lowest median dry weight was found at 20 m (0.57 g) (Figure 3.13.B). The highest median dry weight in Tveitnes (3b) was found at 10 m (1.07 g). The lowest median dry weight was found at 20 m (0.44 g) (Figure 3.13.C). The highest median dry weight in Saltkjelen (4b) was found at 15 m (0.75 g), while the lowest median dry weight was found at 20 m (0.52 g) (Figure 3.13.D)

3.3.4.2 Time of deployment

The median dry weight of individuals (n=360) integrated over depth varied between the time of deployment. The highest median dry weight in Ulvesundet (1b) was found on plates deployed in April (1.67 g), with a decrease in median dry weight in May (1.2 g) and June (0.78 g) (Figure 3.14.A). The highest median dry weight in Hjeltefjorden (2b) was found in May (1.14 g), while the lowest median dry weight was found in June (0.71 g) (Figure 3.14.B). The highest median dry weight in Tveitnes (3b) was found in March (1.24 g). The lowest median dry weight was found in June (0.72 g) (Figure 3.14.C). The highest median dry weight in Saltkjelen (4b) was found in May (0.71 g). The lowest median dry weight was found in June (0.49 g) (Figure 3.14.D).
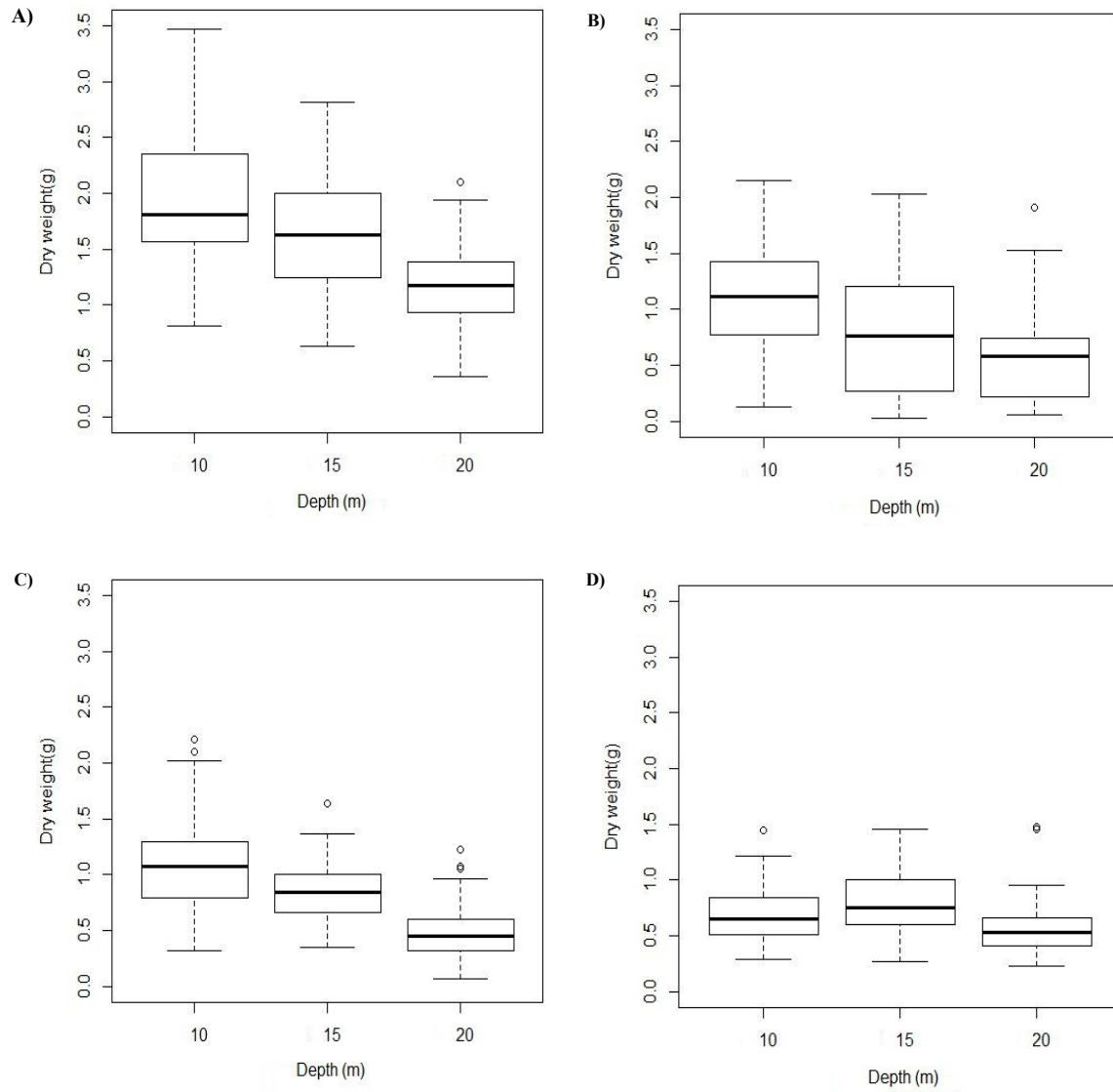
Figure 3.13: Dry weight (g) at the 3 depths with the highest biomass (Figure 3.10) integrated over time for A) Ulvesundet (1b), B) Hjeltefjorden (2b), C) Tveitnes (3b) and D) Saltkjelen (5b).
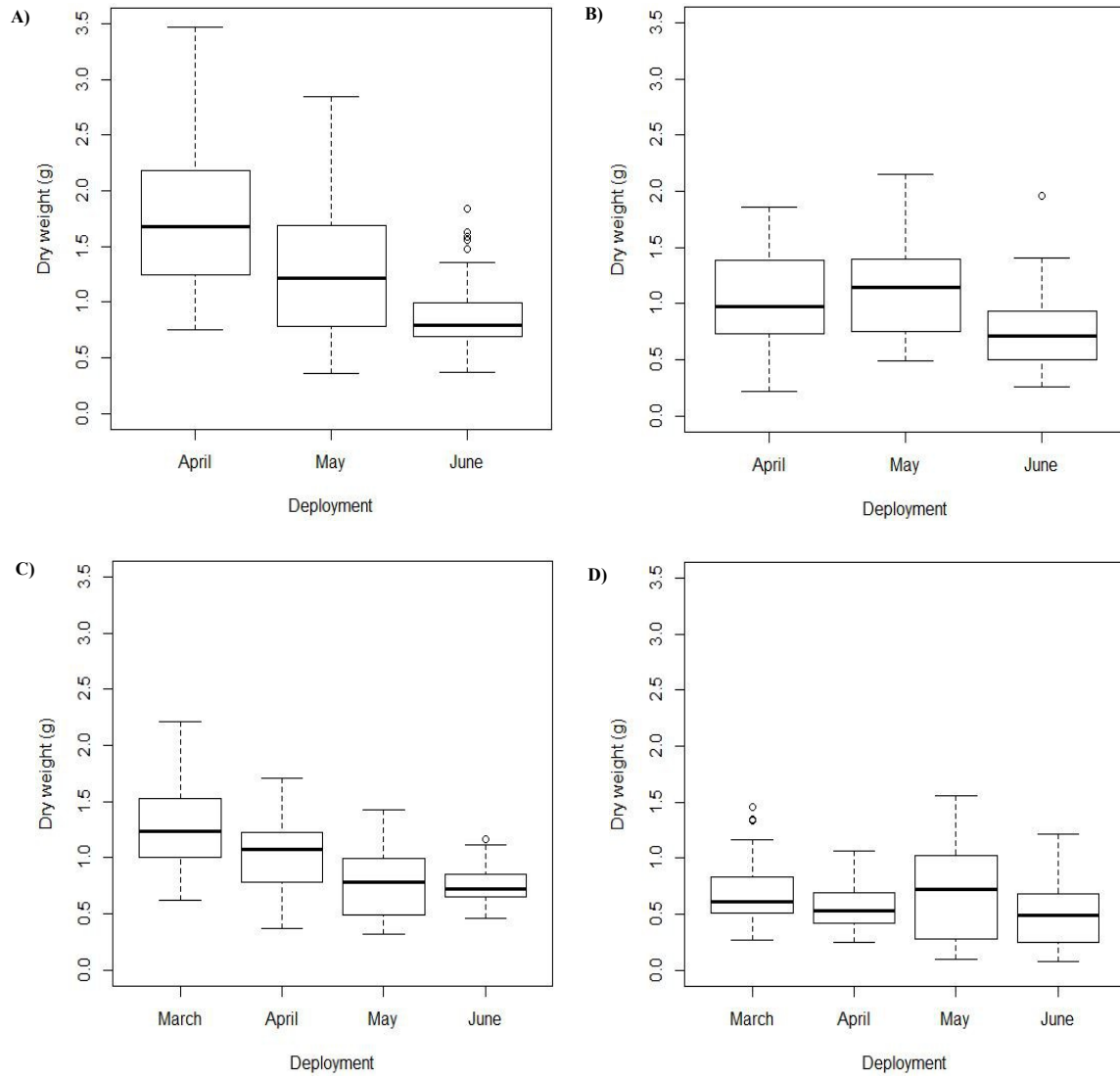
Figure 3.12: Dry weight (g) for individuals for time of deployment at A) Ulvesundet (1b), B) Hjeltefjorden (2b), C) Tveitnes (3b) and D) Saltkjelen (5b).

3.3.4.3 Ash free dry weight

Estimated ash free dry weight was based on a total of nine individuals. The dry weight (mean=0.56, SD ± 0.22) are found to be 5.17 % of the wet weight (mean=11.07, SD ± 4.85) and the ash free dry weight was 2.31 % of the wet weight (Table 3.2).

Table 3.2: Mean for ash free dry weight in both grams and percent of the nine individuals.

Individual	Wet weight	Dry weight	Ash weight	Ash free dry weight	Dry weight of wet (%)	Ash free dry weight of dry weight(%)	Ash free dry weight of wet weight(%)
1	9.730	0.506	0.282	0.225	5.205	44.357	2.309
2	5.500	0.327	0.156	0.172	5.950	52.455	3.121
3	10.660	0.560	0.301	0.259	5.253	46.169	2.425
4	21.160	0.978	0.573	0.404	4.620	41.362	1.911
5	14.880	0.719	0.441	0.278	4.834	38.636	1.868
6	11.390	0.702	0.407	0.295	6.166	41.992	2.589
7	9.520	0.455	0.246	0.209	4.775	45.957	2.194
8	11.730	0.514	0.307	0.207	4.383	40.357	1.769
9	5.080	0.272	0.140	0.132	5.352	48.681	2.605
Mean value	11.07	0.56	0.32	0.24	5.17	44.44	2.31
Standard deviation	4.85	0.22	0.14	0.08	0.59	4.38	0.43

4. Discussion

4.1 Reproduction study

4.1.1 Maturation index

Variations were found among the locations examined. In Steinstø (3a), an overall high maturation index of 4.43 was found from the beginning of collection in late March, when temperatures were approximately 4.5 ºC. In Ulvesundet (1a) and Øystese (4a) sperm was found in the beginning of sampling, while a high maturation index was found in late April, when temperatures were 6.8 ºC and 7.5 ºC. In Kvitsøy (5a), sperm was found from the beginning of April, while a high maturation index was found in the beginning of May, when the temperature was approximately 8.0 ºC. Results from my study showed that Ulvesundet (1a), Steinstø (3a) and Øystese (3a) had eggs in the gonoduct earlier than late April and May, when temperatures were lower than 8.0 ºC. In Kvitsøy (5a) eggs were found in the gonoduct from the beginning of May. In Saltkjelen (4b) and Kvitsøy (5a) the temperature reached 8.0 ºC in the start of May. In Ulvesundet (1b) and Tveitnes (3b) the temperature reached 8.0 ºC in mid May.

Dybern (1965) and Gulliksen (1972) found that spawning is limited to May and June, but can sometimes occur as early as late April. Studies conducted by Gulliksen (1972) were based on settlement on eternite plates, where the highest occurrence of settlement was found between 16th of May to 22nd of June. Dybern's (1965) methods were based on a maturation index table, as well as plankton samples to detect larvae of *C. intestinalis*. Another study conducted on populations in Norway showed that the main spawning occurred from the beginning of May to July (Os 1974). These results were based on the maturation index table, as well as plankton samples. Results from the plankton samples showed that the highest peak of larvae occurred in July (Os 1974). Spawning has been found to be temperature dependent (Dybern 1965; Carver et al. 2003). In Scandinavian populations, a lower limit for spawning is set to be 8.0 °C (Dybern 1965; Gulliksen 1972). When temperatures are suitable (> 8.0 ºC), *C. intestinalis* can produce offspring as long as the gonoduct contains eggs or sperm, even though the quantities are low (Dybern 1965). In

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Steinstø (5a), a high maturation was found in late March, when the temperature was approximately 6.5 ºC. In Ulvesundet (1a) and Øystese (4a) a high maturation index was found in late April, when the temperature was approximately 6.7 ºC. This indicates that the individuals in Ulvesundet (1b), Steinstø (3a) and Øystese (4a) are ready for spawning when temperatures are suitable. In Kvitsøy (5a), a high maturation index was found in the beginning of May, which corresponds with results found by Dybvern (1965). *C. intestinalis* is a protandric hermaphrodite, which means that they produce sperm before they start producing eggs (Carver et al. 2006). This can explain why sperm was found before eggs in Ulvesundet (1a), Øystese (4a) and Kvitsøy (5a).

4.1.2 Size of broodstock animals

The dry weights in Ulvesundet (1a) and Steinstø (3a) were high on all sampling dates, and was ranging between 2.0 to 4.9 g. Lower dry weight was found in Øystese (4a) and Kvitsøy (5a), ranging between 0.84 to 1.28 g. All locations showed a high maturation index during the sampling period. The spawning of *C. intestinalis* is size dependent, and the minimum size for maturation can differ between populations (Yamaguchi 1975; Millar 1952; Dybern 1965). The minimum size of maturation in the Scandinavian populations was found to be 5 cm by Dybern (1965). Os (1974) found that the smallest individuals that contained sperm had a length of 8.5 cm, while the smallest individuals that contained eggs had a length of 11 cm. This indicates that there are variations between populations. Petersen et al. (1997) found that individuals that were 3.1 cm had a dry weight of 0.007 g. This indicates that all individuals in my study are big enough for spawning. However, no correlation was found between dry weight and maturation index. Although no correlation was found, the mean dry weight was higher in Steinstø (3a), where the maturation index was high from the beginning of sampling.

4.1.3 Egg analysis

Big variations in number of eggs per individual was found, and ranged between 1512 and 61601. The mean was found to be 11416, with a standard deviation of 14817. No correlation was found between dry weight and number of eggs in the gonoduct. Yamaguchi (1975) estimated that *C. intestinalis* spawned between 2000 and 3000 eggs per spawning, with spawning every second or third night interval (Yamaguchi 1975). Carver et al. (2003) claimed that *C. intestinalis* can spawn 500 eggs daily during the spawning period. Based on my results, big variations are found between individuals. This can be due to spawning occurring before broodstock animals were collected, as well as the size of the individuals.

C. intestinalis can release eggs in groups connected with mucus, or as separate eggs (Svane & Havenhand 1993), and these eggs are very sticky (Szewzyk et al. 1991). These observations were confirmed in the egg analysis; some eggs were clustered while others were separated. Several animals have clustering eggs, and there can be several beneficial factors. For instance, the clustering of eggs can lead to an avoidance of cannibalism on own eggs (Faraji et al. 2002). *C. intestinalis* can not sort particles and reject unsuitable materials (Carver et al. 2006), so releasing eggs in clusters could be an advantage. Also, by releasing eggs in clusters, a reduction in the area of dispersal can occur (Svane & Havenhand 1993). This indicates that there are variations in spawning of clustered eggs and separate eggs, while the separate eggs might have a larger area for dispersal by following the currents in the water.

4.2 Biomass study

4.2.1 Integrated Biomass

The biomass for plates integrated over time and depth was high in all locations except Kvitsøy (5b). The median biomass ranged between 0.23 in Kvitsøy (5b) and 5.09 kg in Ulvesundet (1b). Salinities in all locations were within the habitat requirements for *C. intestinalis*. Temperature increased continuously in all locations, before decreasing slightly

in September. Temperature is an important environmental factor that influence the recruitment of *C. intestinalis* (Vercaemer et al. 2011). *C. intestinalis* often favours settlement in new territory (Aldred & Clare 2014), and PVC plates have shown several good results of settlement of *C. intestinalis* (Vercaemer et al. 2011). Growth is temperature dependent, and high temperatures cause more rapid growth (Dybern 1965; Petersen & Riisgård 1992). Gulliksen (1972) found that the highest biomass occurred in outer Borgenfjord compared to the inner part of Borgenfjord. The highest biomass Gulliksen (1972) found was in outer Borgenfjord approximately 140 kg/m^2 at 8 m. This are low values compared to my study findings. In addition, my study showed high biomass at all locations, except Kvitsøy (5b). Studies conducted in 2012 in Kvitsøy (5b), showed a high biomass of *C. intestinalis* (Pers. comm. C. Troedesson 2015), whereas the biomass in Kvitsøy (5b) was comparatively low. *C. intestinalis* has been shown to have a sporadic population outbreak, and these outbreaks are not linked to changes in environmental conditions (Keough 1983; Cayer et al. 1999). The high biomass found in Kvitsøy (5b) in earlier years, could be due to these sporadic outbreaks. In addition, the high amount of *A. aspersa* were found in Kvitsøy (5b), which indicates competition for settlement space between *C. intestinalis* and *A. aspersa*. Results from my reproduction study showed that Kvitsøy (5a) had matured eggs in the gonoduct later than the other locations. This indicates that other biofouling species, such as *A. aspersa,* can outcompete *C. intestinalis* for settlement space. In addition, the plates in Kvitsøy (5b) were submerged in a different way than in the other locations, which could explain why biomass was lower in Kvitsøy (5b). Tveitnes (3b) and Saltkjelen (4b) are located near salmon farming sites, and a higher noise level can be expected at these locations, which can reduce the settlement of *C. intestinalis* (McDonald et al. 2014).

4.2.2 Vertical distribution

All the individuals at 5 m were located on the underside of the plate, while *C. intestinalis* was found on both sides of the plates that were submerged at 10 m or deeper. In Hjeltefjorden (2b), a higher settlement was found at 5 m in April. In Ulvesundet (1b), Hjeltefjorden (2b), Tveitnes (3b) and Kvitsøy (5b) little or no biomass was found at 5 m. In

Saltkjelen (4b) the plates at 5 m were dominated by blue mussels (*Mytilus edulis*). Results from my study showed differences in the settlement depths. The highest median biomass per plate (0.004 m²) was found at 10 and 15 m in all locations, ranging between 0.22 kg in Kvitsøy (5b) to 16.97 kg in Saltkjelen (4b). The highest biomass per plate was 16.97 kg in Saltkjelen (4b), which gives a total wet weight biomass of 424.25 kg/m². Of the total body weight, approximately 5 % is dry weight (Table 3.2). This means that of 16.97 kg gives approximately 0.848 kg dry weight per plate, or 21.2 kg/m². The highest mean number of individuals per plate (0.04 m²) was 497.6, or 12437 individuals/m².

In boreal regions, *C. intestinalis* is often found at depths of 15-30 m (Dybern 1965; Svane & Havenhand 1993). This can be explained by *C. intestinalis'* preference of shaded areas, where its larvae become photonegative at the end of the larval stage (Howes et al. 2007; Gulliksen 1972; Schmidt & Warner 1984). This corresponds with my study findings, where the highest biomass was found between 10 and 20 m and the individuals settled at the underside of the plate at 5 m. However, Gulliksen (1972) found the highest biomass between 4 and 8 m, with a dry weight ranging between approximately 1 and 7 kg/m², and no settlement below 14 m. This is not consistent with my study findings, where some settlement was found at all depths, and the highest biomass occurred at 10 and 15 m. In Hjeltefjorden (2b), a high biomass was found at 5 m, which corresponds with the findings of Gulliksen (1972). Gulliksen (1972) found a lower biomass compared to my study, where the highest dry weight was 7 kg/m². Populations in Scandinavia can be very dense, and several thousand individuals can be found at one square meter (Svane & Havenhand 1993), which corresponds with my study.

The temperature was increasing after time of deployment in all locations, and showed high temperatures during the summer. A slow decrease in temperature was found from the beginning of September. Temperature is an important factor for the growth, and Petersen & Riisgård (1992) found a linear relationship between the maximum filtration rate and an increased temperature. In my study, the temperatures were high during summer, which can lead to an increased growth. Some differences in temperature between depths are expected, where 10 m and 15 m probably have a higher temperature compared to 25 m. This means

that a more rapid growth is expected at 10 and 15 m during summer months. This indicates that temperatures can explain the higher biomass found at 10 and 15 m.

Petersen et al. (1995) found a logarithmic relationship between increased algal concentration and growth rate. Fluorescence measurements in my study, revealed that the fluorescence was highest in Tveitnes (3b), Saltkjelen (4b) and Kvitsøy (5b). The high fluorescence in my study found in Tveitnes (3b) and Saltkjelen (4b) can be explained by the salmon farming sites that are located near the submerged PVC plates. Waste from these aquaculture sites can lead to eutrophication (Skogen et al. 2009), which provide an increase in algal concentration for *C. intestinalis*. The highest fluorescence was found at 5 m, indicating high algal concentration at this depth. A decrease in fluorescence was found with increasing depth for all locations, except Ulvesundet (1b), where the fluorescence were more stable at all depths measured. The fluorescence at 10 and 15 m was still relatively high, which could explain the high biomass found at these depths. The results from my study indicates that depth is an important factor for recruitment of *C. intestinalis*.

4.2.3 Time of deployment

No significant difference in biomass was found between the different deployment times for any of the locations except Kvitsøy (5b). The highest median biomass found at these locations ranged between 0.59 and 6.81 kg. The highest biomass was found on plates deployed from March to May. This indicates that there are big variations between the locations. A significant difference in biomass and time of deployment was found in Kvitsøy (5b), where the highest median biomass was 0.59 kg in May. In Kvitsøy (5b), high amounts of *A. aspersa* was found. Bolton & Havenhand (1996) found that *C. intestinalis* sperm could be activated by the presence of egg water from *A. aspersa*, while *A. aspersa* did not become activated by presence of *C intestinalis* egg water. This can indicate that *A. aspersa* easier can outcompete *C. intestinalis* for settlement space.

Temperature is an important factor for spawning. All locations reached 8 ºC during May. Dybern (1965) and Gulliksen (1972) found that spawning did not occur until mid May-

June, when temperatures are suitable (> 8 ºC). This means that if spawning occurred in May-June, a more equal biomass would be expected at all deployments. As the highest biomass was found between March-May, it is expected that spawning occurred before mid-May. This indicates that the time of deployment is an important factor to obtain the highest biomass.

Wieczorek & Todd (1997) found an increase in settled individuals with biofilm on the settle surface, and the highest numbers were found for biofilms that were 12 days old. This could indicate that PVC plates that were submerged before spawning occurred, had a biofilm that was favoured by *C. intestinalis,* which increases the biomass. Keough & Raimondi (1996) found that this response was connected to the type of bacterial film. Studies by Szewzyk et al. (1991) found that *C. intestinalis* showed no preference for surfaces with biofilms compared to hydrophobic or hydrophilic surfaces. Hence, there is an uncertainty about *C. intestinalis* and preference of biofilms.

4.2.4 Individual growth

4.2.4.1 Vertical Distribution
Results from my study showed that the highest median dry weights ranged between 0.75 g in Saltkjelen (4b) and 1.81 g at Ulvesundet (1b). The highest median biomass occurred at 10 m in Ulvesundet (1b), Hjeltefjorden (2b) and Tveitnes (3b), while the highest median dry weight occurred at 15 m in Saltkjelen (4b). The lower dry weight in Saltkjelen (4b) could be explained with the high number of individuals at this location, where competition for feed could occur. The highest fluorescence was found in the top layers (5 m) with a decrease with increasing depth. Petersen et al. (1995) found a logarithmic correlation between the growth and algal concentration, which can explain the variations in dry weight between depth. In addition to algal concentration, these depths have reduced light intensity (Pinet 2009), which is preferred by *C. intestinalis.* Kvitsøy (5b) was excluded from these analyses, due to low amount of *C. intestinalis* and the maximum depth of 15 m. Previous studies in Kvitsøy (5b) showed that the highest dry weigh occurred at 5 m, with a decrease in dry weight with increasing depth (Pers. comm. C. Troedesson 2015). Temperatures is an

important factor for growth, and a higher temperature are expected from 5 to 15 m, which can explain the higher dry weight that was found at these depths.

4.2.4.2 Time of deployment

Results from my study showed that the individuals that settled in June had a lower dry weight in all locations, ranging between 0.49 and 0.78 g. The highest median dry weight occurred between March and May. The dry weight ranged between 0.71 g in Saltkjelen (4b) on plates deployed in May, and 1.67 g in Ulvesundet on plates deployed in April. The lower median dry weight in Saltkjelen (4b) could be explained by the high densities found in this location. Variations were found between locations, where Ulvesundet (1b) and Tveitnes (3b) showed high median dry weight in March and April. The highest dry weight found in Hjeltefjorden (2b) and Saltkjelen (4b) were on plates deployed in May. This indicates that spawning could occurred earlier than May-June, before the temperature reached 8 ºC, My results indicate that the spawning probably occurred earlier than May-June, which would give the individuals that settled earlier than June a longer time period to obtain high dry weight. This indicates that the time of deployment was an important factor for obtaining the highest dry weight per individual.

4.2.4.3 Ash free dry weight

Results from ash free dry weight showed an ash free dry weight of 38.6 to 52.4 % of dry weight. These results correspond with Os' (1974) findings, where the ash free dry weight was estimated to be between 30.8 and 47.2 % of dry weight. The amount of ash free dry weight can be due to NaCl from seawater (Os, 1974). Other studies conducted on ash free dry weight showed that ash weight is ~ 61 % of dry weight (Pers. comm, C. Troedesson 2015), which correlates with my study findings.

5. Discussion of methods

5.1 Reproduction study

5.1.1 Maturation index

The methods used for the reproduction study are well known from Dybern's (1965) table on maturation index, and the use of PVC plates to look at differences between settlement time. Results have shown that individuals with a high maturation index, are ready for spawning. Even though eggs were observed early, there are an uncertainties about eggs spawning earlier. Several studies showed that eggs can be present before the temperature is suitable for spawning, but spawning does not occur until the temperature is suitable. Temperature loggers were placed at locations for the biomass study, and some differences could occur between these locations, and the locations that were used for sampling of broodstock animals in their natural habitat. The settlement on PVC plates can indicate if the spawning occurred before the temperature reached 8 ºC, with variations in biomass between different deployment times and dry weight. In addition to this, big variations are found between populations, which can explain the variations in maturation. A more accurate method for detecting spawning, would be plankton samples to find larvae of *C. intestinalis*. However, Dybvern (1965) and Os (1974) studies tried plankton sampling, and only small amounts of larvae were found. There are several studies conducted on spawning and length. In this study, no length measurements were found, which could have been an important factor. The dry weight was measured, and will have some correlation with the size of an individual.

5.1.2 Egg analysis

The method used for counting eggs were inefficient. The biggest challenge was the clustering of eggs, where many of the samples were ruined when stored in a refrigerator. Only half of the eggs that were collected were useful, and only 17 individuals were examined. Several studies have examined the amount of eggs in each spawning, and estimated the total fecundity of *C. intestinalis* (Yamaguchi 1975). These studies were

conducted *in vitro*, where mature individuals were kept in tanks until spawning. After hatching, larvae were collected and counted. This can give a more precise estimation of total number of eggs. Konno et al. (2010) used the same method for collecting eggs as this study, with collecting eggs directly from the gonoduct. Differences between Konno et al. (2010) and this study is that Konno let the eggs hatch in a water tank, and collected larvae, while in my study eggs were counted using a stereo microscope, which was inefficient. In addition, some eggs laid in clusters, which made it difficult to count all the eggs in one cluster.

5.2 Biomass study

As *C. intestinalis* favours new territory, PVC plates or eternite plates have showed high recruitment of *C. intestinalis* (Vercaemer et al. 2011; Petersen & Svane 1995), and showed good results in this study. In Kvitsøy (5b) the plates were deployed in a different way than in the other locations (with maximum depth of 15 m), which can have affected the results from this study. Growth of *C. intestinalis* is heavily influenced by the food availability, while the fluorescence was only examined when plates were retrieved. This means that CTD measurement should been taken more often in order to see if there are fluctuations in fluorescence throughout the year.

Ash free dry weight was only measured for nine individuals to give an indicator of ash free dry weight in *C. intestinalis*. A more accurate measure of ash free dry weight would require the use of similar sized individuals from each location. Time constraints was the main reason for not doing this during this study.

6. Conclusion

1. Large variations in maturation index were found between the locations examined, where Steinstø (3a) showed a high maturation index of 4.43 from the beginning of sampling in late March. In Ulvesundet (1a) and Øystese (4a) a high maturation index was found from in the middle of April. In Kvitsøy (5a) a high maturation index in the beginning of May. All locations showed eggs in the gonoduct before temperature was suitable (> 8 ºC) for spawning, except Kvitsøy (5a).

2. No correlation between dry weight and maturation index was found, but individuals in Steinstø (3a) had a higher dry, ranging between 2.67 g and 3.61 g weight compared to Øystese (4a) and Kvitsøy (5a).

3. The number of eggs in the gonoduct varied considerably, ranging between 1512 and 61601 eggs in one individual. No correlation was found between the number of eggs and dry weight.

4. The highest integrated biomass over depth and time of deployment showed variations between locations. The highest median biomass was 5.09 kg in Ulvesundet (1b). The lowest median biomass was 0.23 kg in Kvitsøy (5b).

5. The highest biomass occurred at 10 and 15 m in all locations. The highest median biomass was 16.97 kg at 15 m in Saltkjelen (4b). In addition, Hjeltefjorden (2b) showed high biomass at 5 m as well as at 10 and 15 m. The lowest median biomass was found at 5 m at all locations. This indicates that depth is an important factor to obtain the highest possible biomass.

6. The highest biomass occurred on plates submerged between March and May. The highest median biomass found was 6.81 kg on plates deployed in April, in Hjeltefjorden (2b). The lowest biomass found occurred in June in all locations, except Kvitsøy (5b). The lowest biomass in Kvitsøy (5b) was in March (0.05 kg). This indicates that spawning could have occurred earlier than May-June.

7. The individuals settled at 10 and 15 m had the highest dry weight in all locations, and the highest dry weight ranged between 0.75 g at 15 m in Saltkjelen (4b) and 1.81 g at 10 m in Ulvesundet (1b). This indicates that depth is an important factor

to obtain the highest dry weight.

8. The individuals settled before June showed a higher dry weight compared to the individuals that settled in June. The highest median dry weight was ranging between 0.71 g on plates deployed in May in Saltkjelen (4b) and 1.67 g on plates deployed in April in Ulvesundet (1b). This indicates that spawning could have occurred earlier than May-June. In addition to this, the time of deployment seemed to be an important factor to obtain the highest dry weight per individual.

9. The ash free dry weight showed normal values compared to other studies, and with a mean of 44.4 % of dry weight.

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8. Appendix
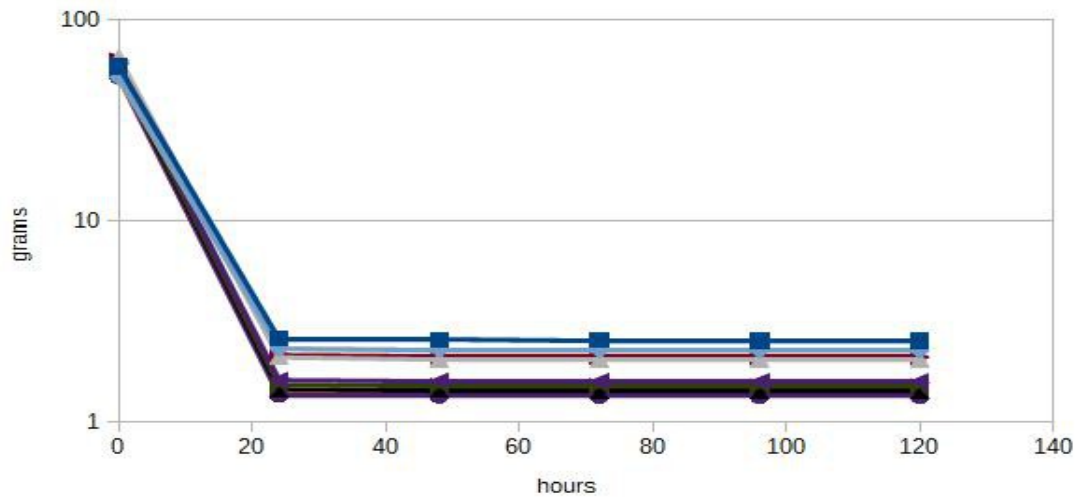
8.1 Appendix A

Figure 8.1: Weight after drying at 60 ºC to obtain stable weight for large individuals (> 50 g wet weight)
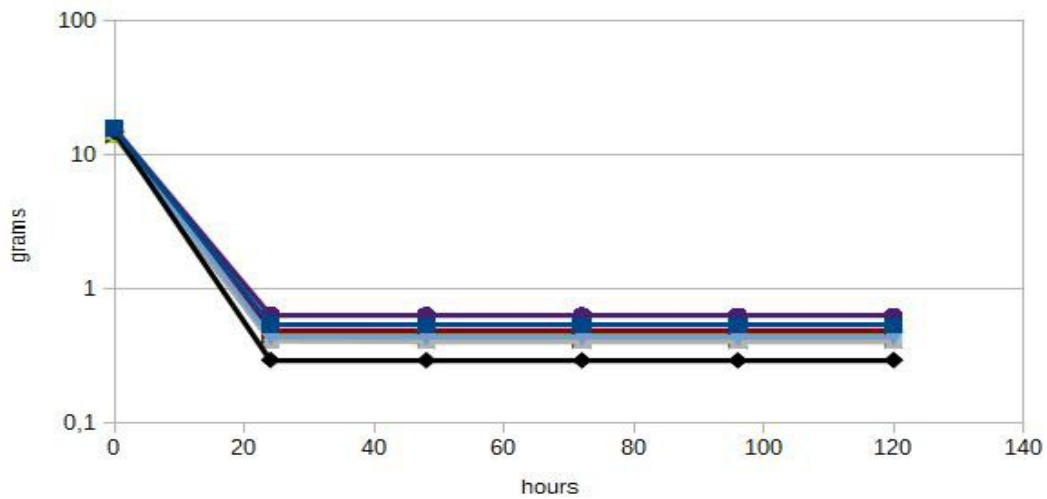

Figure 8.2: Weight after drying at 60 ºC to obtain stable weight small individuals (< 50 g dry weight)

8.2 Appendix B

Table 8.1: The wet weight biomass at depth and time of deployment in Ulvesundet (1b)

	5 m	10 m	15 m	20 m	25 m
April(1)	1.21	7.75	11.46	6.39	Bottom
April(2)	1.26	7.97	15.49	8.87	Bottom
April(3)	0.00	6.13	8.97	5.09	0.36
May	0.72	7.85	11.82	5.49	0.00
June	0.14	0.98	9.86	4.87	0.00

Table: 8.2: The wet weight biomass at depth and time of deployment in Hjeltefjorden (2b)

	5 m	10 m	15 m	20 m	25 m
April(1)	9.32	10.89	8.85	4.93	1.56
April(2)	8.63	5.90	4.78	2.01	0.47
April(3)	2.46	2.96	0.31	0.50	0.47
May	0.05	14.97	9.42	5.54	2.31
June	0.22	3.09	5.57	1.01	0.50

Table: 8.3: The wet weight biomass at depth and time of deployment in Tveitnes (3b)

	5 m	10 m	15 m	20 m	25 m
March	0.00	10.19	8.76	2.39	0.55
April(1)	0.23	10.17	7.39	1.93	0.97
April(2)	0.24	10.79	9.46	0.85	0.57
May	0.00	8.31	8.35	1.54	0.13
June	0.31	5.16	0.07	0.11	0.07

Table 8.4: The wet weight biomass at depth and time of deployment in Saltkjelen (4b)

	5 m	10 m	15 m	20 m	25 m
March	0.00	8.12	14	5.36	1.47
April(1)	0.00	8.19	9.86	3.74	1.68
April(2)	0.00	12.28	18.71	7.14	1.68
April(3)	0.00	10.47	16.97	5.50	1.93
May	0.00	13.47	10.16	2.41	0.98
June	0.00	8.78	8.44	1.47	0.49

Table 8.5: The wet weight biomass at depth and time of deployment in Kvitsøy (5b)

	10 m	15 m	15 m	10 m	5 m
March	0.52	0.04	0.04	0.07	0.05
April(1)	0.13	0.19	0.28	0.41	0.08
April(2)	0.21	0.53	0.5	0.53	0.11
April(3)	0.09	0.23	1.2	0.23	0.17
May	0.45	3.82	0.46	0.59	1.62
June	0.12	1.63	0.04	2.12	0.10