UNIVERSITY OF NORDLAND

MASTER THESIS

Horizontal and Vertical Distribution of Atlantic Salmon (Salmo salar) in Semi-closed Cage System

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Abstract

Comparison of horizontal and vertical distribution of Atlantic salmon (*Salmo salar*) were performed in open and closed cage using underwater camera system. The purpose of the present study is to demonstrate the characteristics of Atlantic salmon distribution behaviors in the semi-closed cage systems. Closed cage systems are a very new system to rear Atlantic salmon in Norway or even in the whole world. Postsmolts were distributed in total in three cages and exposed to natural light conditions from June to November. The horizontal and vertical distribution of Atlantic salmon at those three periods showed that the fish used the whole water body, avoiding the cage bottom and the upper 1m. No significant differences were found of water qualities at different deep water layers which means that the water quality in the semi-closed cage systems are stable and constant at all depths. Nevertheless the surface avoidance were observed in the September period when the average water temperature is the highest during the year. Further studies need to be performed to test the direct reasons resulting in the distribution of Atlantic salmon in the semi-closed cage to improve the efficiency of aquaculture operations.

Content

1. Introduction

1.1 The state of aquaculture in the world and Norway

World aquaculture production goes on to grow though at a slowing rate (Krause et al., 2015). And to catch up with world appetites, the fish-farming business will have to keep this trend (Cressey, 2009). Rohana Subasinghe (2014), a senior officer at the FAO, said that the reason is simple that we will not get sufficient fish from the sea in the coming years. According to the current available statistics gathered globally by FAO, world aquaculture production achieved another high of 90.4 million tonnes (live weight equivalent) in 2012 (US$144.4 billion), comprising 66.6 million tonnes of food fish (US$137.7 billion) (FAO, 2014). Farmed food fish output from Norway attained 1,321,119 tonnes in total occupying 2 percent of world total production in 2012 (FAO, 2014). As published by the Norwegian Seafood Federation and the Norwegian Seafood Council, 38 million meals of Norwegian seafood are served worldwide everyday. Twelve millions of these come from aquaculture, and of this eleven million are salmon meals. Dating back to 1970, the first Salmon farm was built by Norwegian pioneers at Hitra; between 1972 and 1975, production from Norwegian aquaculture increased by 40 per cent annually; at 1980, production in the industry reaches 8000 tonnes, compared with 500 tonnes ten years earlier; after the last round in 1989, the aquaculture industry extends along the entire coast and the production in the industry is 170,000 tonnes; in 2000, Norway exports 343,000 tonnes of salmon (FHL & NSC, 2011). In 2013, the Norwegian fish industry had a first-hand value of NOK 40 billion, up 35 percent from 2012 (Statistics Norway, 2014). The total production was 1.25 million tonnes (Figure.1). However, the parasite, salmon lice creates increased mortality, bad welfare and uneconomical growth rate in fish, and is one of the primary problems in modern-day Norwegian aquaculture production (Revie, Dill, Finstad, & Todd, 2009), together with escapes and environmental pollution (Thorvaldsen, Holmen, & Moe, 2015). Scientific and public focus have concentrated on the sustainability and environmental effect of the aquaculture industry (Rillahan, Chambers, Howell, & Watson Iii, 2009). So to know how to built an effective and environmental-friendly model of aquaculture is the future.

Figure 1. Sales of salmon. Quantity and first-hand value. 1997-2013

1.2 Closed cage

Closed cage systems in sea water is a new area in the salmon aquaculture industry. Thus, both knowledge about the physical and chemical environment in closed cage systems in sea water and how these environmental factors affect growth, fish health and horizontal/vertical distribution for Atlantic salmon are scarce.

Salmon lice and escapes lead to a substantial economic loss to the industry. The use of closed cage systems, pumping deep seawater (25 m) into the cages has so far been a successful way to prevent salmon lice (Arve Nilsen, 2014). Different closed-cage system are being tried out in the Norwegian salmon industry. They are more expensive than open cages both in building investments and operational cost due to the need of pumping water into the cages. It is therefore of necessity to explore the production success both regarding fish growth, welfare, diseases and behavior in closed cages before they are commercialized. So far the use of closed cage has been effective to keep the sea-lice away by pumping deep water into the cage, and physical walls protecting against escapes, and it can be an environmental friendly method if the waste water is filtrated for particles. In some areas where the summer water temperature is high, the use of colder deep water (>25 meter) being pumped into the closed cage can be positive for both growth and fish health during summer time. We need to know more about the closed system before it can be commercialized.

1.3 Open cage

In caged Atlantic salmon production, the diel rhythm in light intensity generated a rhythm in the swimming depth, with salmon descending at dawn and ascending at dusk (Oppedal, Juell, & Johansson, 2007). Recent studies have pointed out that the distribution of artificial light intensity in an open sea cage is an essential factor for the swimming depths and schooling densities detected (F. Oppedal, Juell, Tarranger, & Hansen, 2001). It is reported that in early summer, both rainbow trout and Arctic char reared individually in 8m deep cages displayed a preference for about 13.5℃, within a temperature range from 3 to 18℃ (Sutterlin & Stevens, 1992). And it has been suggested that to maintain temperatures that are optimal for metabolic processes by thermoregulation, fish will migrate vertically. In general, the behavior of Atlantic salmon in the open sea-cage was in accordance with seasonal changes in temperature and diel changes in light (F. Oppedal, 2002). Other factors such as dissolved oxygen (DO) (Kramer, 1987), carbon dioxide (CO_2) (Nilsson et al., 2012), pH (European Inland Fisheries Advisory Commission Working Party on Water Quality Criteria for European Freshwater, 1969), fish size (Werner, 1974) , fish density (Bohnsack, 1989), water current (Johansson, Juell, Oppedal, Stiansen, & Ruohonen, 2007a) and so on are all affecting the behavior of salmon fish. It is well known that majority fish are ectothermic ("cold-blooded"), the internal temperature changing with that of the surrounding environment, having no means of controlling body temperature. Temperature is one of the essential water quality parameters due to data such as conductivity, pH, and DO concentrations are correlated with water temperatures (Kerry Weber). An increase in temperature accelerates metabolic rate and results in a concomitant rise in oxygen consumption and activity as well as production of CO_2 (Luo et al., 2015).

1.4 Temperature and caged fish cultivating.

The water temperature in coastal areas are controlled by all kinds of environmental variables including 1)daily and seasonal meteorological changes; 2)amount of mixing caused by wind, storms and tides; 3)seawater depth; and 4)incoming water sources such as precipitation, tributaries, man-made canals (Lluch Cota, Wooster, & Hare, 2001). The water temperature normally increases during the daytime while decreasing at night because of the lack of sunlight. It is well known that both daylight hours and sun intensity are higher in the summer than in the winter, giving rise to a higher water temperature in the summer and water temperature is influenced by water depth (Jacobson, 1948). This results in stratification, deeper water is less chilled by the cold air above the water surface in the winter, while opposite when in the summertime surface water being more heated by the sun than deeper water. The body temperature of individual are closely related to the speed of biochemical reactions in biology (Gillooly, Brown, West, Savage, & Charnov, 2001). Previous studies had reported that the higher the temperature, the faster the biochemical reactions (Luo et al., 2015). Concerning the survival of individual organisms, the upper and lower deadly temperatures determine the whole temperature gradient, and in this range, the species will be found in greatest abundance where the growth and reproduction temperature requirements are met (PANKHURST, 1997).

The metabolic rate, chemical reaction and oxygen consumption almost doubled for a 10°C rise in temperature and the solubility of dissolved gases (especially oxygen) decrease, thus cool water holds higher dissolved oxygen than warm water (Claireaux, Webber, Lagardère, & Kerr, 2000). Aquatic animals are sensitive to rapid temperature fluctuations (Montgomery & Macdonald, 1990). Fish, therefore, need to be acclimatized gradually when transferring them from one location to another. Broadly speaking, in the terms of temperature tolerance, fish can be divided into four categories: tropical water, warm water, cool water and cold water (Eaton et al., 1995). There is an optimum temperature for the speed of growth and reproduction for each fish species. This optimal temperature range, also called the standard environmental temperature (SET), may differ in each development stage of the fish (Brett, 1956).

1.5 Lights and caged fish cultivating

Fish behavior is usually strongly affected by light conditions, and it has been declared that artificial light may modify the behavior of Atlantic salmon in sea cages (F. Oppedal, 2002). The diel light cycle generally has a dominant effects on fish behavior and activity, making up a predictable restriction (Helfman, 1993; Anras et al., 1997).

Vast majority pelagic fish are visual predators, and their behavior is strongly influenced by the diel light cycle (Masson, Angeli, Guillard, & Pinel-Alloul, 2001). As numerous fish species are more active during daytime, numerous animals have an internal biological clock, named a circadian rhythm, which is dominated by the light/dark cycle every 24 hours (Brown, Hastings, & Palmer, 2014). The direct receptor of light is the eye, and the visual systems in fish are complex. Scientific studies on salmon have suggested that it takes half an hour for the eye to regulate bright light, and an hour to regulate dim light(Byron, 2011).

But how does light influence fish behavior? Early studies indicated that marine fish larvae have diel vertical migration behaviors, which have been linked largely to following optimum light conditions, suitable prey concentrations and predator avoidance (Lampert, 1989). Absolute majority species seem to ascend towards the surface at night, however some species disperse at night and do not display diel variations or else form aggregations during the daytime (Sabatés, Olivar, Salat, Palomera, & Alemany, 2007). Farmed salmon held at high densities in sea-cages often form a circular school in the cage at daytime,while the schooling groups dispersing gradually after sunset, in accordance with a decrease in swimming speed(F. Oppedal, 2002). The timing for the animal to experience and evaluate the day length was set by a difference in light intensity between day and night (F. Oppedal, 2002).

In closed cage systems, the light intensity in the cage is lower than in the same size open sea cage because it uses a closed net bag that built an isolation between fish and the water outside the cage.

1.6 Dissolved oxygen concentration and caged fish cultivation

Water quality has its own physical, chemical and biological processes (Wildish, Keizer, Wilson, & Martin, 1993). In an open net cage photosynthesis and the mixing of atmospheric oxygen is the major sources of new oxygen to the water (Davis, 1975). The local topography (Wiebke Ziebis & Forster, 1996), the existence of a pycnocline (Johansson, Juell, Oppedal, Stiansen, & Ruohonen, 2007b), the mesh size of the net walls (Zhao, Li, Dong, Gui, & Teng, 2007), net fouling (Cronin, Cheshire, Clarke, & Melville, 1999) and cage configuration (Kennedy, Mulvey, & Rowlings, 1998) are all influencing oxygen supplement and other parameters of water quality in caged farming system. Compared to open cage the closed cage system have oxygen supply equipment due to the low water exchange between the inside and outside of the closed cage. And the water turbidity is higher in closed cage compared with open cage. It takes much more time for feces to leave the closed cage than open cage.

1.7 The aim of the study

The aim of my work was to study the horizontal and vertical distribution of Atlantic salmon in the closed and open cage systems.

The thesis has been done in cooperation with Aquaculture Innovation and Norsk Havbrukssenter, both are commercial companies. Ideally the study should have been done in duplicate cages, but only one closed cage and one open cage was available at two of three periods when the practical work of the thesis was to be performed. This makes statistical challenges when data is to be statistically analyzed .This work, therefore, must be looked upon as the first approach to looking at the horizontal and vertical distribution of salmon in semi-closed cages. Human factors such as camera management, fish counting will increase random error causing the reliability of the data. The fish distribution was studied using an underwater camera, which was the technology available. Preferably more advanced equipment (sonar, echo sounder) would have been better. The present study is based on underwater camera systems focusing on the distribution of salmon fish in the closed cages. But there are some drawbacks of monitoring fish behavior by using underwater camera systems: both systematic error and random error are big, which may cause the reliable problems of the results.

2.Materials and Methods

2.1 Study site

The study was performed at Toft, Brønnøysund (65°28′30″N 12°12′43″E), in Northern Norway between June 2014 and November 2014 (Figure 2.). All fish used in the study was originally obtained from a commercial fish farm SBH (Sinkaberg Hansen), Norway).

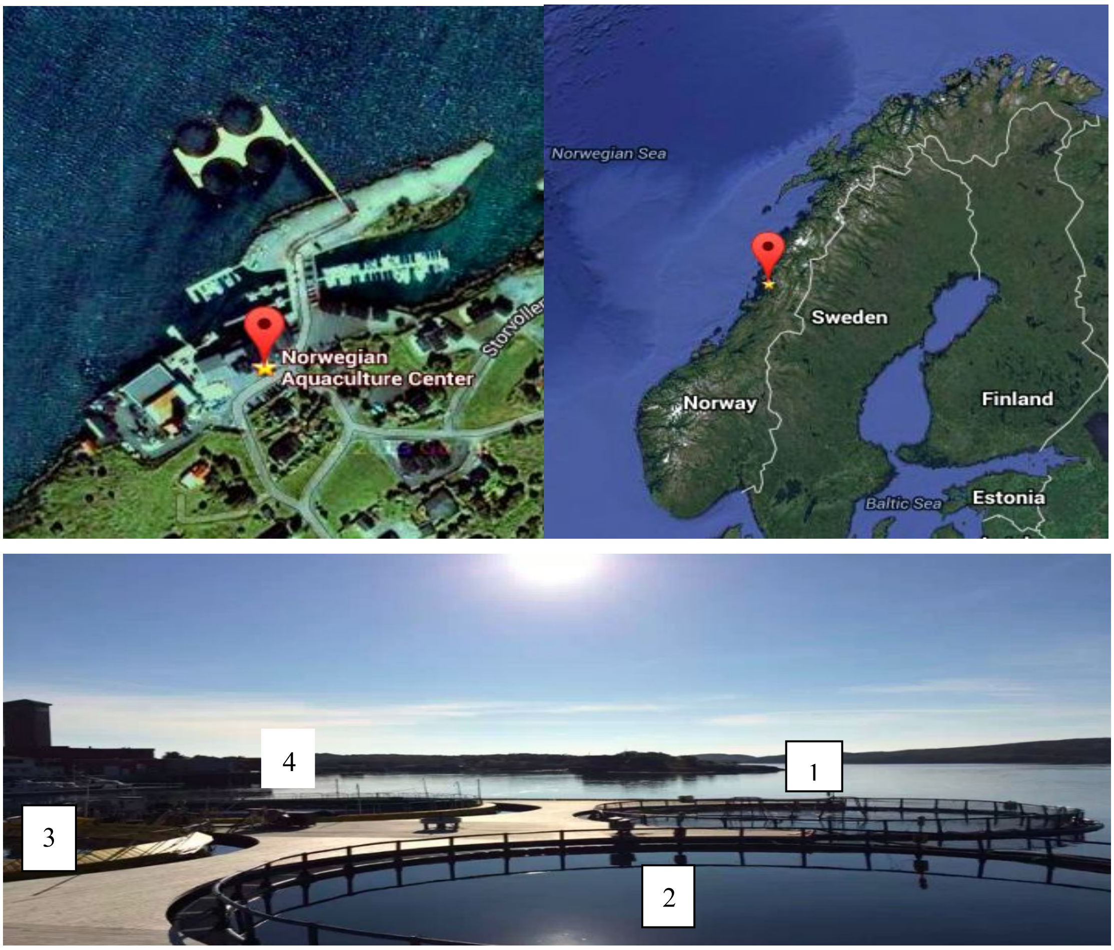


Figure 2. The location of experimental site and experimental cages

2.2. Cage structures

Both the semi-closed cage and the open cage were 70m circumference circular with a diameter of 21.5m (Aqua Group, Norway). The basic brackets of the cages are the same, but the nets are very different. Open cages used regular open nets (Egersund net) about 18 meters deep and an estimated volume of 5590m^3 while the closed cage have 12m deep with an estimated volume of 3000m^3. They also have the other basic parts of a traditional cage (Figure 3): a jumping net above the surface fixed to the net bag to prevent fish escaping; cage collar for spreading out the net bag and give buoyancy to keep the bag in the correct position in the water column; and a mooring system(Lekang, 2013).

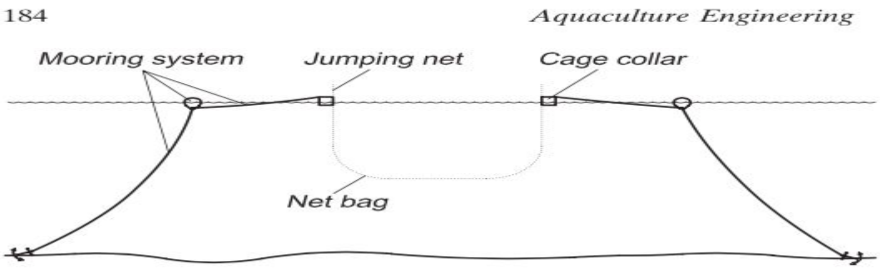


Figure 3. Major components in a traditional open sea cage farm.

The semi-closed cages look a little different (Fig.4).

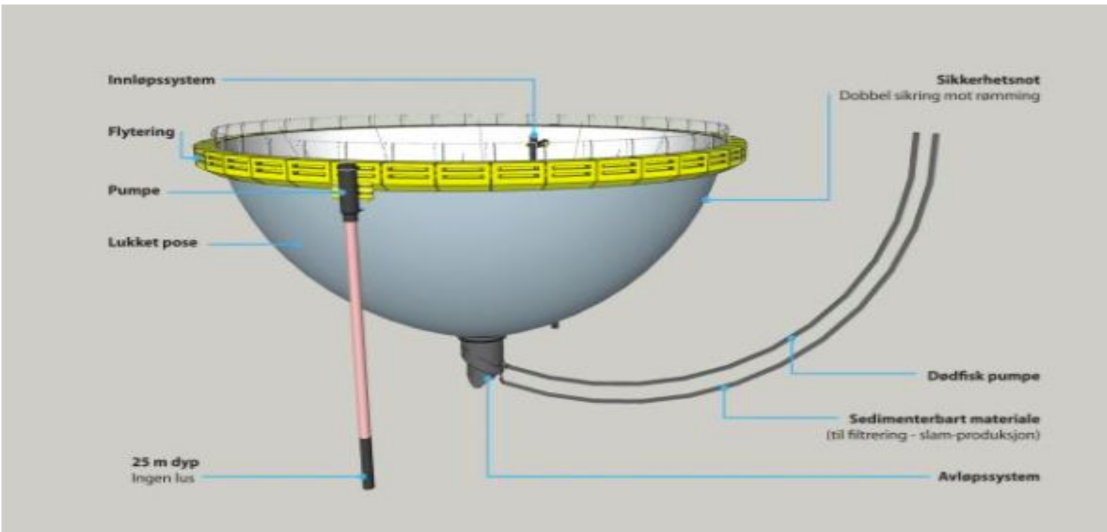


Figure 4. The main structures of semi-closed cage.

The semi-enclosed cages consisted of a specially developed float collar consisting of elements (supplied by Polyform AS) which are assembled to the desired diameter and a bag of dense, flexible tarpaulin wall (supplied by Rantex AS). Water intake in semi-enclosed cages was 25 meters deep, the water was sourced through a strainer tube and transported into the cage. The systems tested in 2014 had pumps with a maximum theoretical capacity of 40 m^3 / min with a maximum 1 meter lift height; in

November 2014 intending to provide a practical capacity of up to 24-36 m^3 / min (Arve Nilsen, 2014). The drain is located in the center at the bottom of the cage bag. Dead fish were also removed in the same pattern. In the floor drains there were two levels, where dead fish were collected first and taken up with a separate lift-up, while sludge particles fell further down to the bottom and to be pumped up into separate extraction. Each cage was set up with a control cabinet where also data-collecting sensors was hooked up (IQ Sensor Net). The system was provided via wireless mobile networks, allowing monitoring and controlling to be performed via remote computers.

2.3. Environmental conditions in the semi-closed cages

Water circulation in the cages is a function of bag design, a design of drainage and inlet and flow that goes through the cage. The manufacturer has set a provisional target of about 20 cm / sec as the current speed, which will provide a smolt (60 to 120 grams) a swimming speed of 0.8 to 1 BL/S (body length per second), and approximately 0.3 to 0, 4 BL/S for a harvestable fish of 4.5 to 5 kg (Arve Nilsen, 2014). An emergency unit and a tank for liquid oxygen was placed on the floating dock and connected to the closed cages. Oxygen system consisted of a supply hose from the control cabinet, manifold with input supply hose and 12 outputs holed diffusion pipes. Oxygenation net was located at about 9 meters deep in the cages. The oxygen was supplied to the cages with two independent hoses. At low consumption only one line would open so that line two act as "back-up". The oxygen sensor was located at 3 meters deep about 3 feet from the cage wall. The open cage had no extra oxygenation, and was dependent on natural water exchange.

2.4. Monitoring equipment

Every cage was equipped with an underwater camera video system which was applied to observe the feeding activities of the fish under the water. The cameras were hanged by a rope and could be moved both horizontal and vertical in the cage. A remote computer system controlled the movement of the camera and the pictures were seen on the computer screen. The water quality data such as pH, temperature, oxygen and salinity were collected everyday by smart roll MP Handheld Instrument (In-Situ-inc, TORMATIC AS) together with CO_2 to be measured by Oxyguard CO2 Portable Analyzer during the experimental periods. The data was transmitted wireless to an iPod with a separate application from In-Situ Inc. And data was stored in comma-separated values (CSV) format and sent as an email to a computer and stored in separate folders for each single day. CSV data can be used directly or transferred to Excel. The pH level in closed aquaculture systems will mainly be controlled by the CO2 concentration. Measurement of CO2 with Oxyguard is highly related to the flow rates. Thus, CO2 values must be manually on the gauge display and noted in a log together with the other water quality data. Water quality was regularly measured at 1, 3, 5 and 10 meters. Besides, temperature, oxygen and salinity were logged daily with an Akva company sensor at 4 m depth. The SmartEye Twin 360 Camera System gives sharp, color and monochrome video underwater pictures. Standard configuration comprises upper camera and lower camera in high-resolution color, however for looking down into deep and dark cages monochrome is used to get very high light sensitivity; using one joystick, both cameras are synchronized for full 360^{o} vertical movement, and no external moving parts will prevent leaks (AKVA-GROUP, 2015). Additionally, the feeding response and condition of the fish can be clearly observed. It is a highly useful camera that is linked to the base via CAP (Cage Access Point) wireless video transmitter. And below is the parameter details about the camera. As we can see, this camera has a 72^{o} underwater angle of view.

Camera Specifications

Cameras	SmartEye	SmartEye 360 Twin	
Type:	Colour	Upper: Colour	Lower: Monochrome
Resolution:	480 TVL	480 TVL (Center)	570 TVL (Center)
Light sensitivity:	0.01(@F0.8)	1.5lx (@ F1.2)	0.0003lx (@ F1.4)
CCD sensor:	1/3" Sony	1/3" Sony	1/2" Sony
El. iris/Lense:	Auto / 3.6mm	Auto / 2.8mm	Auto / 3.5mm
Angle of view:	92° (72° under water)	90° in air, 76° in water	
Materials:	Delrin/Acrylic/Bronze	Delrin/Acrylic/Bronze	
Cable:	50m (165') Urethane *	50m (165') Urethane *	
Depth rating:	200m (655') limited by cable	200m (655') limited by cable	
Power:	12VDC/400mA	11-30VDC / 1.2A	
Size - H x Ø:	216mm x 165mm (8.5"x 6.5" diam.)	361mm x 171mm 14.2 x 6.7" diam.)	
Weight:	2.6kg (5.7 lbs.) without cable	5.4kg (12 lbs) (bouyancy ≈ -0.5kg) without cable	

* Longer cables are available on request.

Figure 5. Camera specification of Smart-Eye 360 Twin

2.5. Experimental fish and experimental setup

The study was performed during 3 periods: June, September and November. In June, two semi-closed cages with fish were available, one cage with 10800 fish (average 3.57kg) and the other cage with 33194 fish (average 1.14kg). In August, the fish in the first cage was slaughtered, whereas the second cage were split into one open and one semi-closed cage. In September and November, the open cage contained 15529 fish with an average of 2.5kg and 3.95kg respectively. Equivalent the semi-closed cage contained 17665 fish with an average of 2.12kg and 3.33kg respectively.

The camera was moved at different location in the cage and three pictures taken at each location and angle. The camera was located in the center (A) and closer to the edge (B) at different depths (1, 3, 6 and 9m in closed cage ; 1, 3, 6, 9, 12 and 15m in open cage) (Figure 6.a). The “center” includes the underwater body that lies inner the circle connected by lots of “B” points and “B” is defined as the middle of semi-diameter of the cage surface. The rest part of the caged water column is called “edge”. The camera was directed from A towards B, and from B towards A at every location. In June we also tried out other angles directing upwards, downwards as well as north, west, east and south. The camera was moved around in the cage to exact positions and angles to take pictures (Figure 6c). At each location pictures were taken at every 90° moving the camera in a horizontal way, as well as upwards and downwards. That gave 6 different angles for every camera position (Figure 6b.). But those pictures did not give any new information and was rejected from the data material. Analyzing the data for June, it was decided that only two angles (A towards B, and B towards A) was necessary, which were used in September and November.

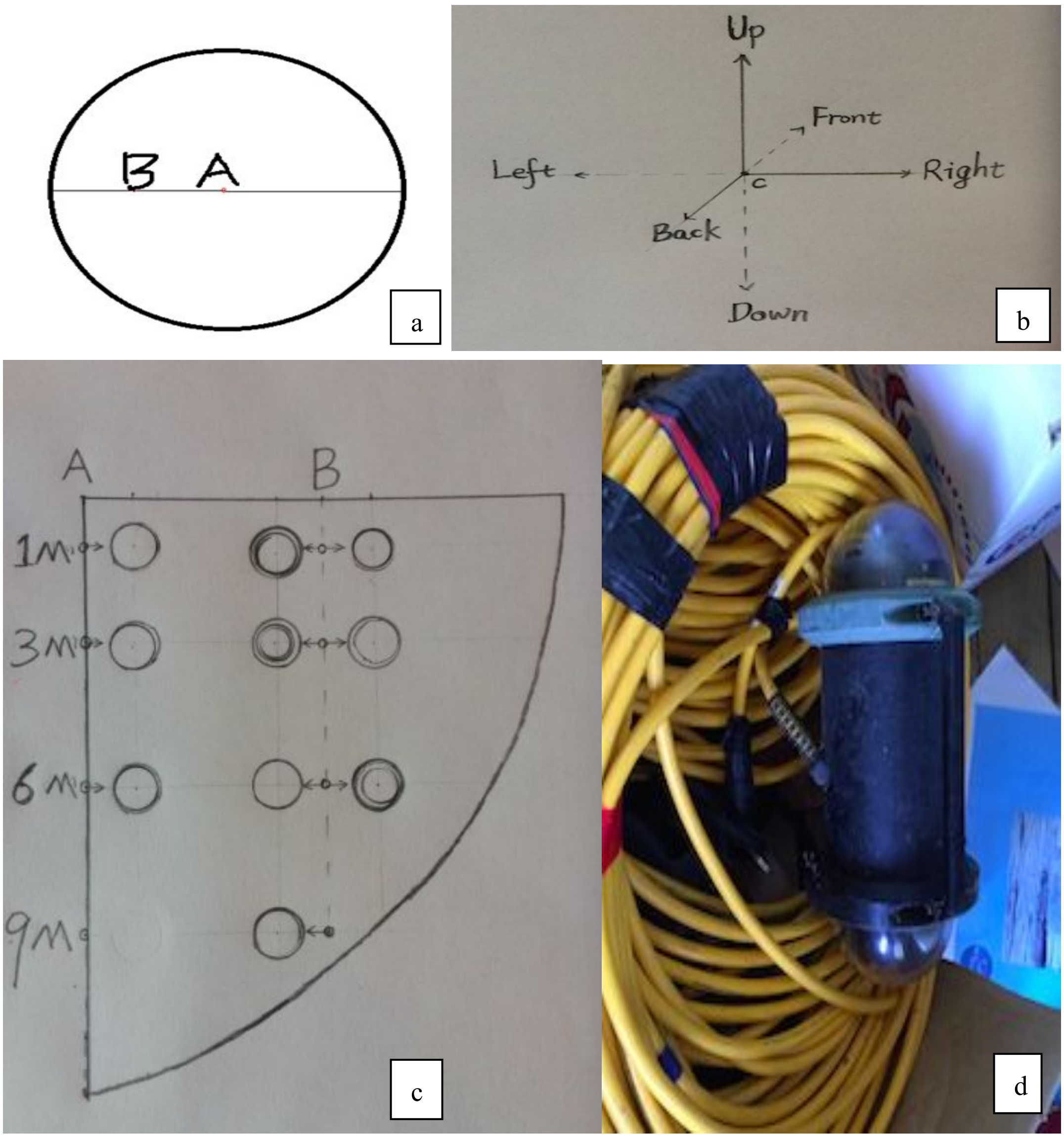


Figure 6. Experimental position in the cage and the underwater camera Smart-Eye 360.

As the pictures can be seen in front of the computer screen, we took three pictures every position by using the Snipping Tool (win7 system software) with an interval of three seconds. As the camera has its angle of view (76^{o} in water), only parts of the underwater space where the fish existed can be observed.

The fish were fed two meals everyday (08:00-09:00 and 15:00-16:00) by a central feed system (Akvasmart CCS Feed System) with feeding times and meal duration regulated every season according to different day length and increased feed amount required with biomass increase. Feeding affected fish distribution most significant in summer, with fast upwards swimming when feeding initiated and a gradual descent during the process of feeding (Fernö, Huse, Juell, & Bjordal, 1995). The feeding time were avoided by taking pictures one hour after the feeding activities completed.

After all the original data were acquired, the pictures were analyzed using a digital image processing software (AxioVision, Carl Zeiss). Only fish where you could see the head clearly was counted. Fish showing only the tail part, or fish seen very diffuse was not counted (Figure 7.). The average fish number was used since three pictures were taken in one exact position and direction, and we call it the number of fish per location (NFloc.). Ten locations were recorded in closed cage and seventeen locations in open cage were recorded each day. Calculate the mean value of the number of fish per location at different depths (1, 3, 6 and 9m in closed cage ; 1, 3, 6, 9, 12 and 15m in open cage) in one cage at one of three periods and we call it the M_iNFloc. (i means the depth). Sum all the M_iNFloc up getting the total number of fish counted in one cage called NFcage. From this number the percentage of fish in each depth was calculated:

$$\text{Percentage} = \frac{\text{MiNFloc}}{\text{NFcage}} \bullet 100\%$$

The similar methods were used to make the horizontal fish distribution figures (Figure 9.).

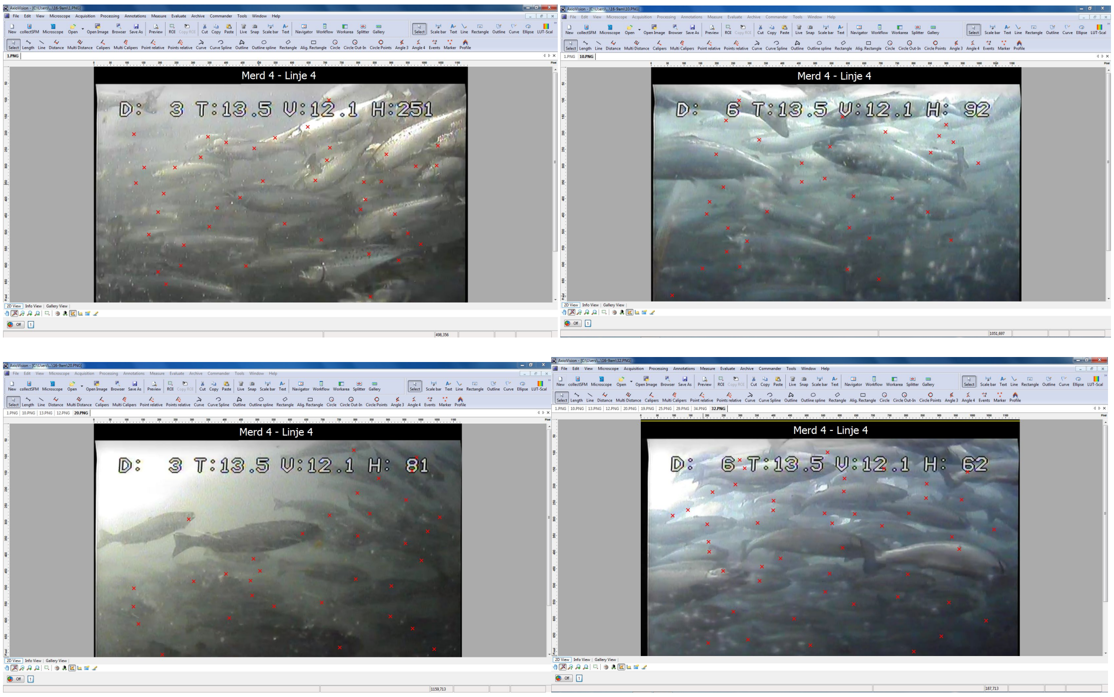


Figure 7. Fish counting using AxioVision digital image processing software

Additionally, the jump roll of Atlantic salmon in cages were measured manually on morning, afternoon and evening in June for ten minutes in each cage. A jump roll refers to the fish jump out of the water surface with the whole body exposing to the air and then back to the water column. A timer was used when counting the fish jump roll with my eyes focusing on the water surface. The original data of jump roll were calculated to jump roll per minute in the morning, afternoon and evening. Firstly, calculate the mean valuer of jump roll per minute in the different period of the day in one cage in June. Secondly, sum the mean values up getting the total jump roll. Finally, calculate the percentage of jump roll in the morning, afternoon and evening making figures (Figure 10.).

2.6. Statistical analysis

Statistical analysis was performed by using SPSS statistics (IBM SPSS Statistic 19, SPSS Inc. US). The student t-test was used to show the differences in fish distribution between cages and depths. One Way-ANOVA was used to test if there was an effect of depth on fish distribution. And the homogeneity of variance was test using the Levene's Test for all the data. Data were expressed as mean±standard errors and the significance was normally set at < 0.05 level. If the difference was significant after the One Way-ANOVA test, the Post Hoc Tests-LED continued with a multiple comparisons. Correlation (Pearson) test of different parameters of water quality and fish distribution was performed.

The individual difference between days within the same period had also been measured by the statistical analysis and the difference between days were not significant within the same period. There were also no remarkable discrepancy found between the morning and afternoon within the same day during all the periods. The data are therefore merged in the presentation.

3.Results

3.1 Fish material data

The total fish , biomass and average weights of farmed Atlantic salmon used in this study are listed below. It is divided into three periods. In total 40 000 individual fish were counted during the study.

Table 1. Period I (24-30,June) of fish biomass

Cage	Total fish (n)	Biomass (kg)	Average weights (kg)	Volume (m^3)	Stocking density (kg/m^3)
Closed cage3(C3)	10800	38567	3.57	3000	12.86
Closed cage4(C4)	33194	37958	1.14	3000	12.65

Table 2. Period II (16-22, September) of fish biomass

cage	Total fish (n)	biomass (kg)	average weights (kg)	volume (m^3)	stocking density (kg/m^3)
Open cage1(C1)	15529	38823	2.50	5590	6.95
Closed cage2(C2)	17665	37450	2.12	3000	12.48

Table 3. Period III (1-5, November) of fish biomass

cage	Total fish (n)	biomass (kg)	average weights (kg)	volume (m^3)	stocking density (kg/m^3)
Open cage1(C1)	15529	61340	3.95	5590	10.97
Closed cage2(C2)	17665	58824	3.33	3000	19.61

3.2 Vertical fish distribution

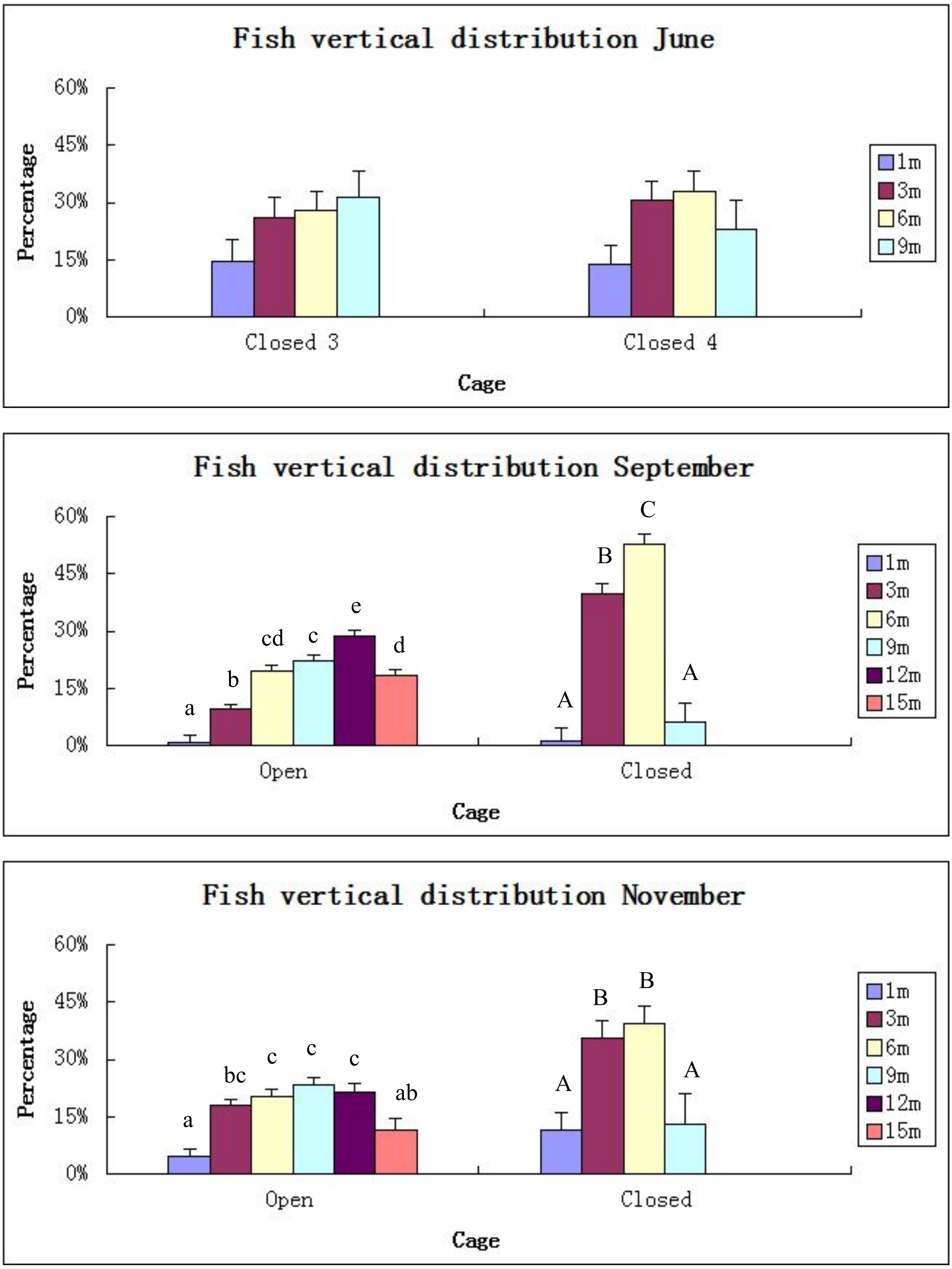


Figure 8. fish distribution in June, September and November. Bars indicate mean value with standard errors. Lowercase letters mean significant difference at different depths within the open cage. Uppercase letters mean significant difference at different depths within the semi-closed cage. A description of significant levels is found in Appendix 1.

3.2.1 June

In June the majority of the fish were distributed at 3, 6 and 9m while less fish were found in the surface water layer in both closed cage 3 and cage 4. No significant difference in percentage of fish between different depths were found in the cages. Neither was there any differences in fish distribution between the two different cages (Figure 8).

3.2.2 September

Significant difference of fish number was found between different depths both in the open cage ($p< 0.05$) and in the closed cage ($p< 0.05$) (Figure 8). The lowest recorded fish number was at 1m depth in the open cage (3.7±5.0) and in the closed cage (1.3±4.1). The highest recorded fish number was at 12m depth in the open cage (100.1±37.3) and at 6m depth in the closed cage (48.7±14.3).

In the open cage, the fish number increased significantly from 1m to 3m ($p< 0.05$) and from 3m to 6m ($p< 0.05$) and from 9m to 12m ($p< 0.05$). Finally the fish number decreased significantly from 12m to 15m ($p< 0.05$).

In the closed cage, there is a significantly higher concentration of fish at 3m and 6m compared with 1m and 9m. The increase in fish number are significant from 1m to 3m and from 3m to 6m. Low fish number are recorded at 1m deep and decreased significantly at 9m ($p< 0.05$). The difference of fish number between 1m and 9m are not significant.

The difference between open and closed cage at 1m and 3m depth is not significant. And the difference between open and closed cage at 6m and 9m are significant ($p< 0.05$, $p< 0.05$).

3.2.3 November

The fish distribution in this period is much like the fish distribution in Septembr. Significant difference of fish number was found between different depths both in the open cage ($p< 0.05$) and in the closed cage ($p< 0.05$). The lowest recorded fish number was at 1m depth both in the open cage (12.0±15.6) and in the closed cage (12.1±9.3). The highest recorded fish number was at 9m depth in the open cage (59.3±35.5) and at 6m depth in the closed cage (41.6±11.8).

In the open cage, the fish number increased significantly from 1m to 3m ($p< 0.05$) and decreased significantly from 12m to 15m ($p < 0.05$). The fish are even distributed in the middle part of open cage and the fish number at 15m is significantly different with 6m, 9m and 12m, but similar with 3m'.

In the closed cage, the difference between 1m and 3m are significant ($p< 0.05$). Low fish number are recorded at 1m deep, while there is no significant increase of fish from 3m to 6m, but a significantly decrease from 6m to 9m ($p< 0.05$). The difference of fish number between 1m and 9m are not significant.

3.3 Horizontal fish distribution

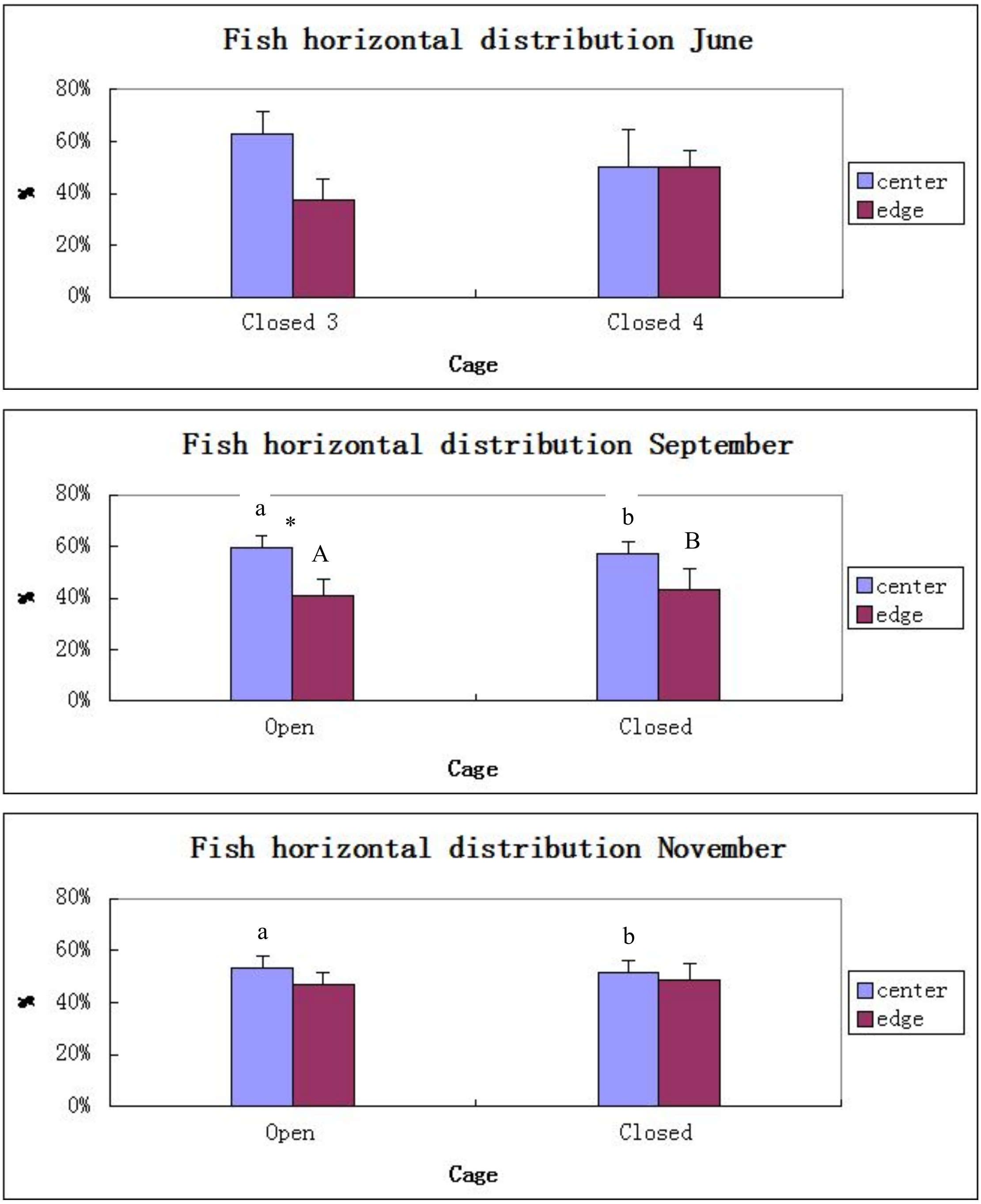


Figure 9. Horizontal fish distribution in September. Bars indicate mean value with standard errors. Asterisks indicate significant difference between locations in the same cage. Different letters indicate significant difference between either the center or the edge between two cages in the same month. A description of significant levels is found in Appendix 2.

3.3.1 June

Summing up the whole fish distribution in June, it is clear that no significant fish number difference was found between center part and edge within the two semi-closed cages. No differences between center part or between edge were seen between the two semi-closed cages.

3.3.2 September

In the open cage, there is significant difference of fish number between center part and edge part of the cage ($p < 0.05$). In the closed cage, no significant difference of fish distribution was found between center part and edge part of the cage.

In the open cage there is significant more fish in the center compared with semi-closed cage ($p< 0.05$). And opposite semi-closed cage have significant more fish in the edge ($p< 0.05$).

3.3.3 November

In November there is no difference in horizontal distribution of fish between center and edge in either of the cages. The open cage has significantly more fish in the center compared with semi-closed cage, but the differences are small ($p< 0.05$).

3.4 Changes of the surface fish active state - Jump roll.

The measurements were taken place both in the morning, afternoon and evening during June period. Jump roll is the parameter that we collect. There is no significant difference of jump roll between morning and afternoon in cage 3 while a significant difference of jump roll was found between morning and afternoon ($p < 0.05$) in cage 4. The jump roll between morning and afternoon in cage 3 and cage 4 are both significantly different ($p < 0.05$, $p < 0.05$). And the difference of jump roll between afternoon and evening is significant in cage 3 ($p < 0.05$) while it is not significant in cage 4.

Table 4. Effect of time on fish activity (jump roll) in the Atlantic salmon cage 3 in June. a, b and c: significant at 5%, 1% and 0.1% respectively.

Post Hoc Tests-LSD (Closed C3)		
(I) time	(J) time	effect of time
morning	afternoon	ns
morning	evening	0.009^{b}
afternoon	evening	0.007^{b}

Table 5. Effect of time on fish activity (jump roll) in the Atlantic salmon cage 4 in June. a, b and c: significant at 5%, 1% and 0.1% respectively.

Post Hoc Tests-LSD (Closed C4)		
(I) time	(J) time	effect of time
morning	afternoon	0.001^{b}
morning	evening	0.000^{c}
afternoon	evening	ns

There was no clear differences between the time of the day regarding numbers of jump roll. In the morning, cage 4 (smaller fish) had significant more jump roll, whereas in the evening cage 3 (larger fish) had significant more jump roll activity.

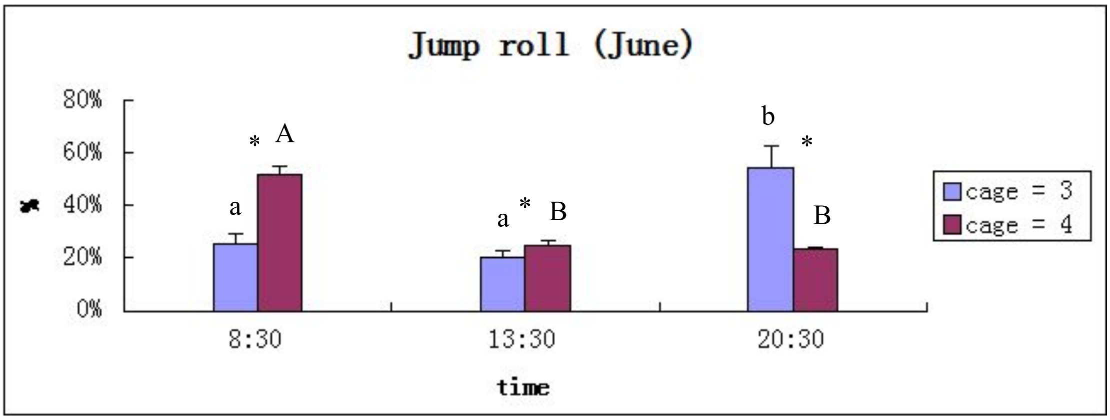


Figure 10. Jump roll of Atlantic salmon in closed cage in June. Bars indicate mean value with standard errors. The level of significant between cages is shown by asterisks (* = $p<0.05$). Lowercase letters mean significant difference at different time of the day within cage3. Uppercase letters mean significant difference at different time of the day within cage 4.. A description of significant levels is found in Appendix 3.

3.5 Weather and day length

The weather were sunny during the first period (28-30 June). The day length in June are at the maximum with approximately 22 hours of light (Figure. 11). The weather in September (16-22 September) varied with the first three days` sunny, then, cloudy for the fourth day and rainy for the last three days. The daylight from sunrise to sunset was approximately 13 hours. During the November (1-5 November), the first three days were rainy and the rest were sunny and day-length nearly 7.5 hours. The sunrise and sunset of study periods are listed in Table. 6.

Table 6. The weather in June, September and November within the experimental time

Period	Date	Weather
I	28-Jun	Sunny
I	29-Jun	Sunny
I	30-Jun	Sunny
II	16-Sep	Sunny
II	17-Sep	Sunny
II	18-Sep	Sunny
II	19-Sep	Cloudy
II	20-Sep	Rainy
II	21-Sep	Rainy
II	22-Sep	Rainy
III	1-Nov	Rainy
III	2-Nov	Rainy
III	3-Nov	Rainy
III	4-Nov	Sunny
III	5-Nov	Sunny

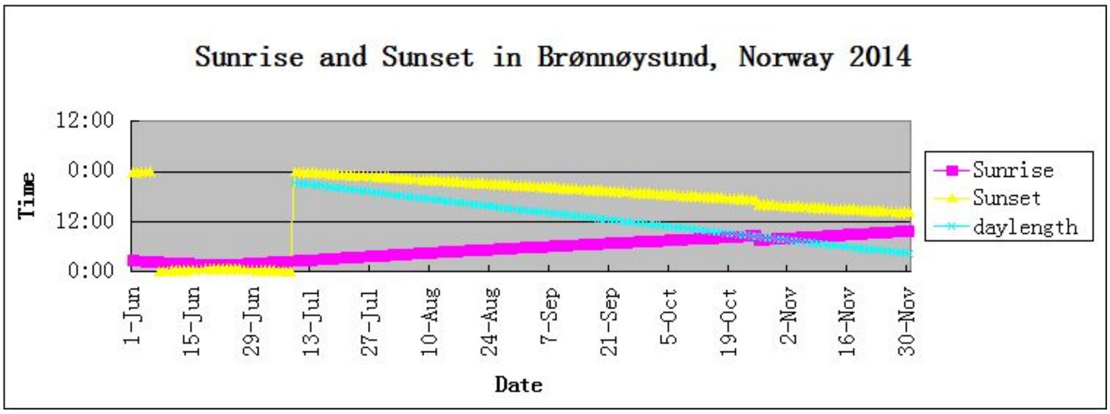


Figure 11. The sunrise and sunset data in Brønnøysund, Norway 2014(www.timeanddate.no)

Table 7. The sunrise and sunset of study periodsI, II and III (hh:mm)

date	28-Jun	29-Jun	30-Jun	**16-Sep**	**17-Sep**	**18-Sep**	**19-Sep**	**20-Sep**	**21-Sep**	**22-Sep**	1-Nov	2-Nov	3-Nov	4-Nov	5-Nov
Sunrise	1:59	2:01	2:04	**6:35**	**6:38**	**6:41**	**6:44**	**6:47**	**6:50**	**6:53**	8:02	8:06	8:09	8:13	8:16
Sunset	0:30	0:28	0:25	**19:36**	**19:32**	**19:28**	**19:24**	**19:21**	**19:17**	**19:13**	15:46	15:43	15:39	15:36	15:32
daylength	22:31	22:27	22:21	**13:01**	**12:54**	**12:47**	**12:40**	**12:33**	**12:27**	**12:20**	7:44	7:37	7:30	7:23	7:16
average	22:26			**12:40**							7:30				

3.6 Temperature

The history temperature (2013-2014) in open cage at 4m deep and in the sea at 25m deep were acquired (Figure 12.). The sea water temperature in there aquaculture area fluctuates between 4℃ to 15℃ all the year round with the coldest day in March and the hottest in September.

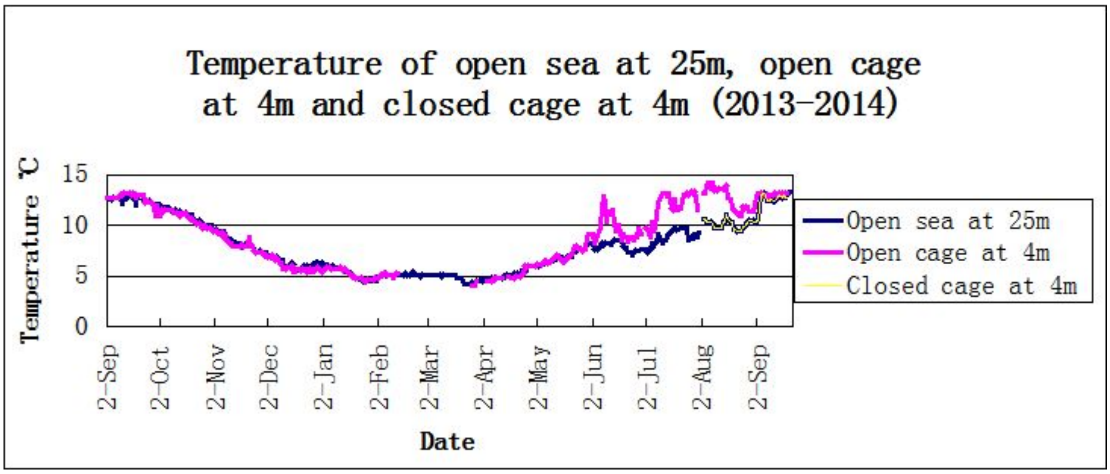


Figure 12. The data of water temperature in Toft, Norway 2013-2014

3.7 Water quality of temperature, DO and PH.

3.7.1 Temperature.

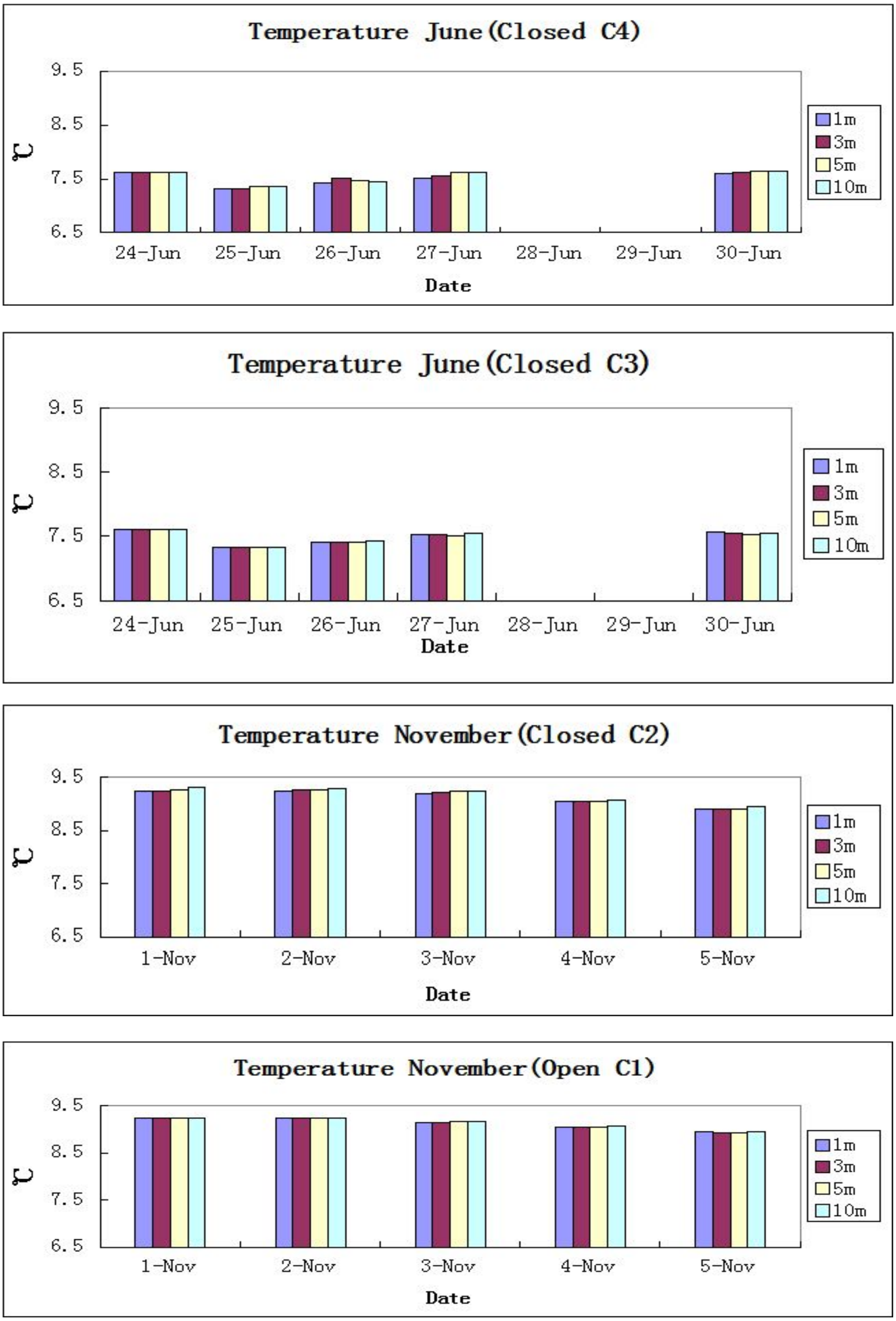


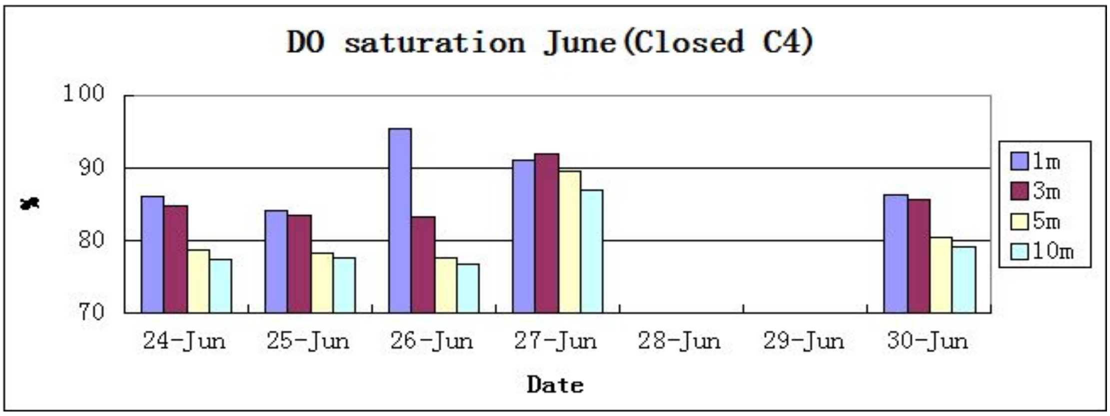
Figure 13. Temperature in cages, C1 is open cage andC2, C3, C4 are closed cages.

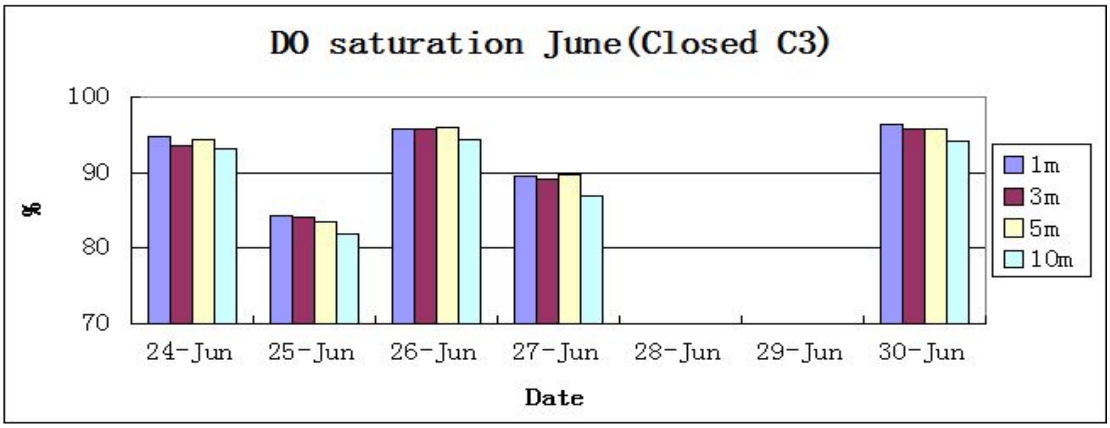
The sea water temperature was similar at all measured depths in the closed cage and open cage. The temperature in June in both cage 3 and cage 4 are pretty stable and no significant fluctuation were found among different depths. There seems to be no difference of temperature between two cages, but some small day to day variation.

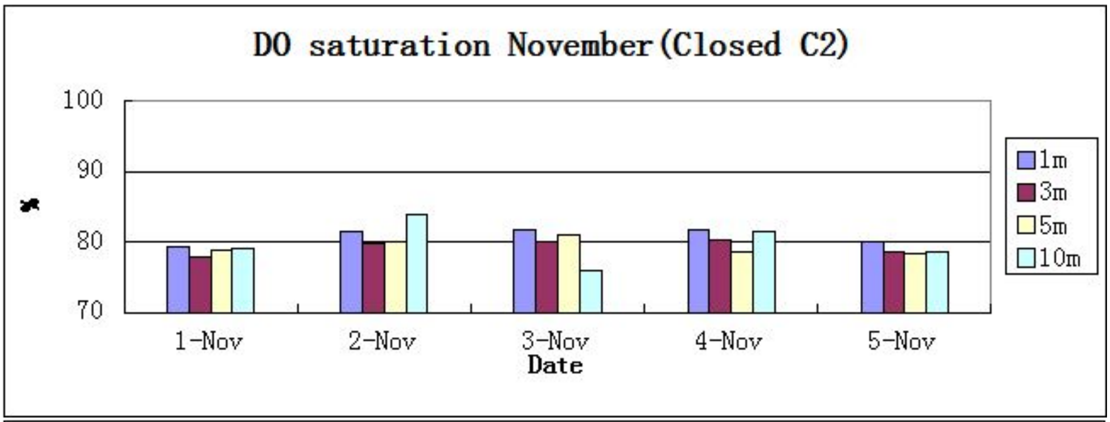
No temperature data is available In September due to the lack of measured equipment which was used by the company employees in another place.

The temperature in November in cage 1 and cage 2 are also steady with pretty small fluctuation (~0.3 ℃) found among different depths. There also seems to be no difference of temperature between two cages with no small day to day variation.

3.7.2 Dissolved oxygen saturation.







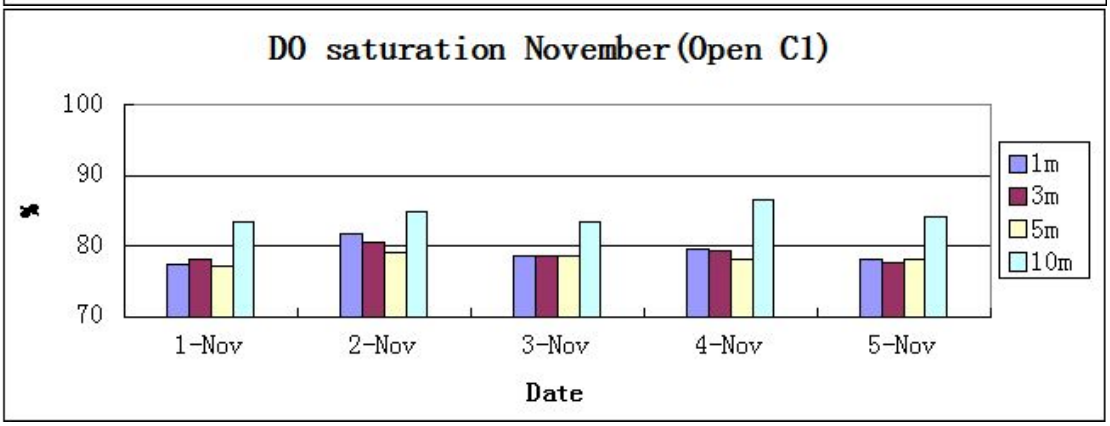


Figure 14. DO saturation in semi-closed cage and open cage.

In cage 3 in June, the DO saturation decreases with increasing depth not significantly but fluctuated significantly between different days. However, there is a large fluctuation in cage 4 in June with the biggest decrease of approximately 20 percent between upper water layer and bottom water layer. The average DO saturation in cage 3 and cage 4 are 91.43% and 83.73%, respectively in the same short period.

The DO saturation in November varies between 75% and 85%, and has lower fluctuations than seen in June. The highest levels are measured at 10m in open cage.

3.7.3 pH.

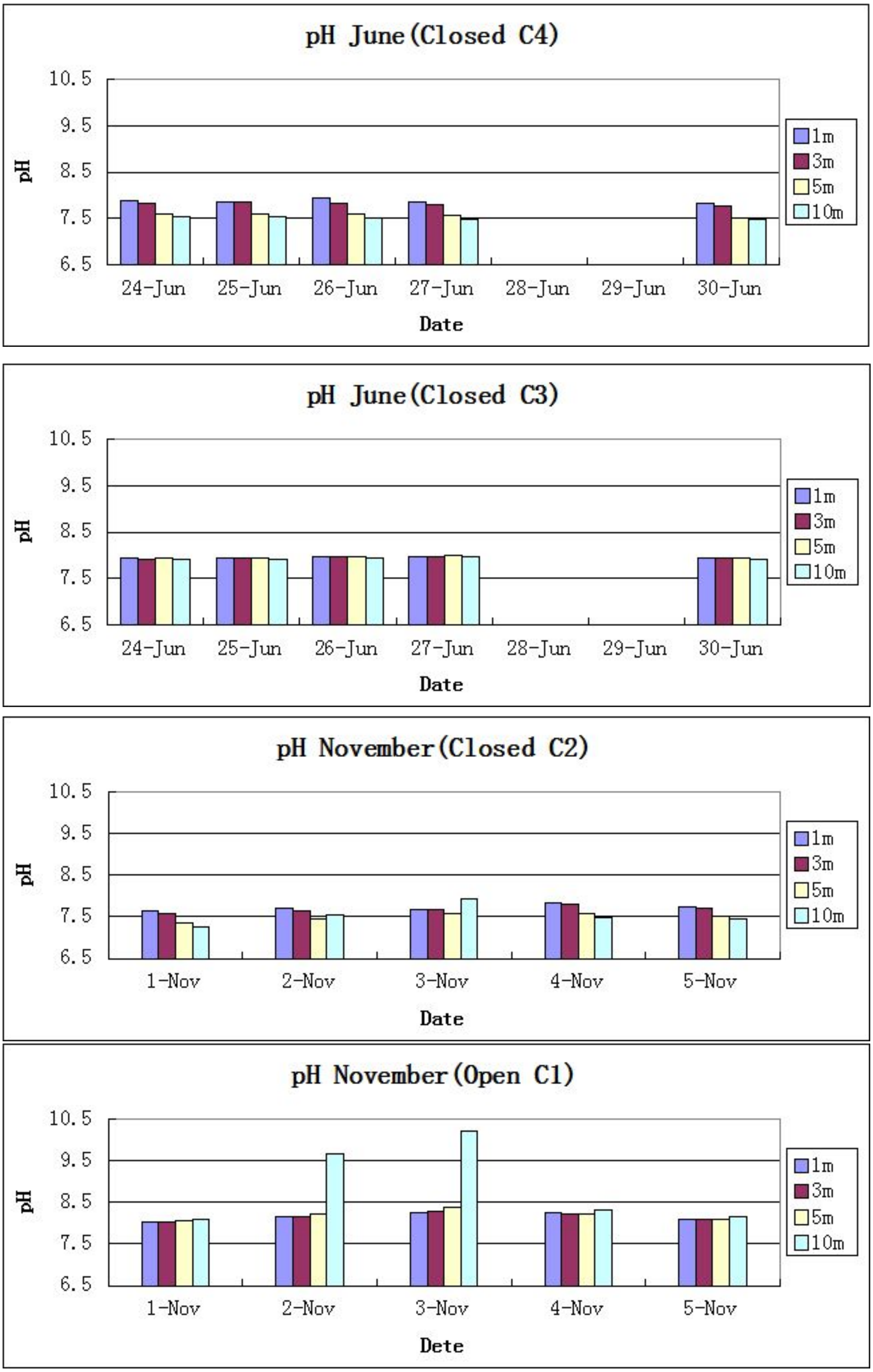


Figure 15. pH in semi-closed and open cage .

In June Only small variation in pH were see between depths and days in cage 3. While in cage 4, there was a trend towards decrease in pH with depth.

In November the pH in closed cage are stable at around 7.5 at all depths. In the open cage the pH is higher, especially at two dates at 10m.

3.7.4 CO_2

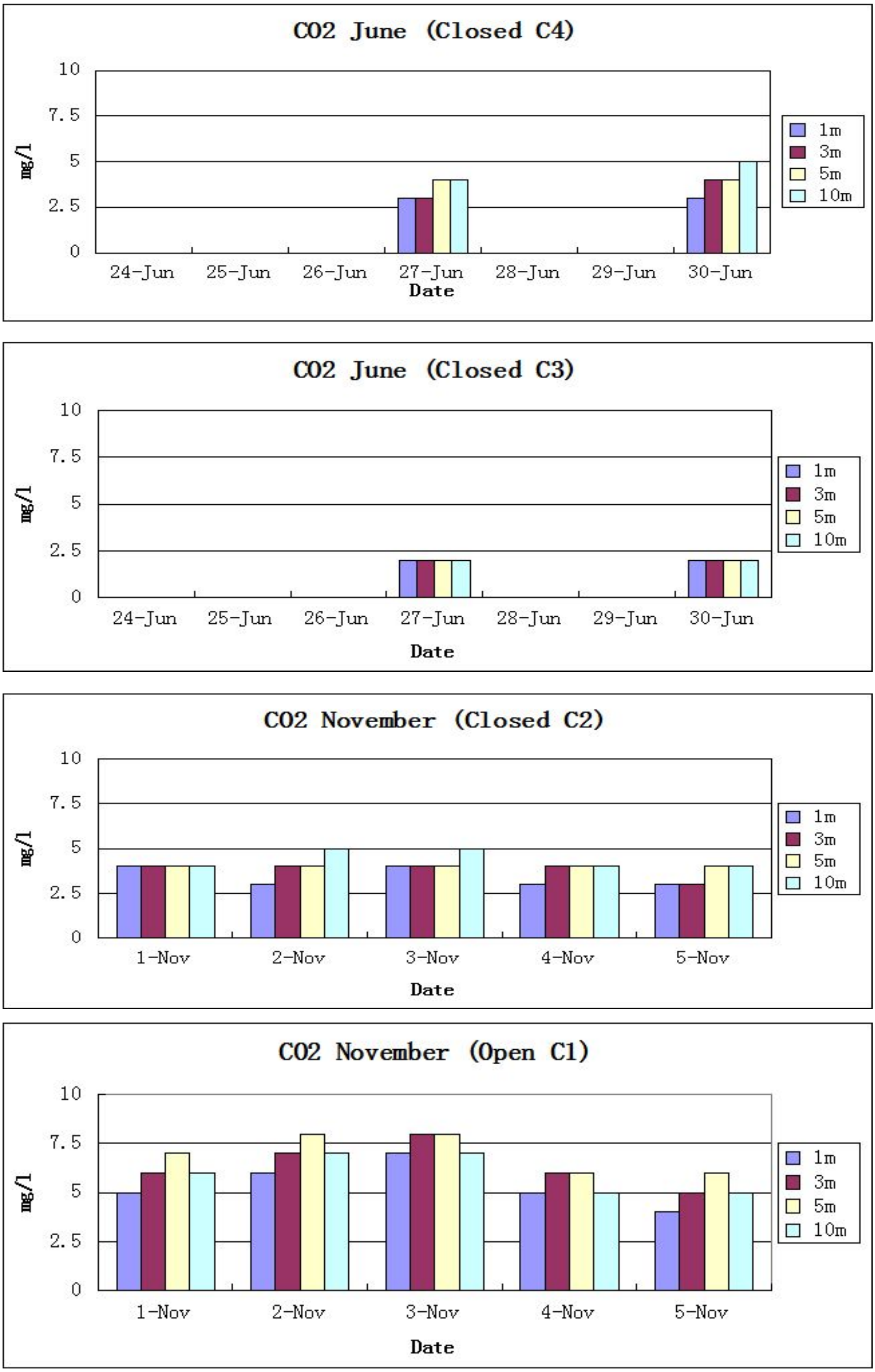


Figure 16. CO_2 in semi-closed and open cage .

The concentration of CO_2 in closed cage is pretty same at different depths within the same cage in June and November. But in the open cage in November the middle layer water in the cage have a little higher concentration of CO_2 than in the upper and bottom of the cage.

In addition, the salinity was pretty stable at all time and all depths with an average of 32.8‰ during all the experimental periods.

3.8 Correlation between water quality and average fish distribution.

A Pearson correlation was performed between various parameters of water quality and average fish number collected in the whole experimental periods. The average fish number only correlate with depth with a low correlation(0.313, $p < 0.05$), and the best correlation was found between pH and CO_2(-0.920, $p < 0.01$).

Table 8. Correlation between fish number and water quality for the whole periods.

	Correlation coefficient (Pearson)
Average/depth	.313*
Temperature/DO	-.726**
DO/CO_2	-.586**
pH/CO_2	-.920**

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

Our results expand the knowledge regarding how the Atlantic salmon is distributed in the semi-closed cage system and the environmental condition in this systems. During the three research periods, different fish size, fish density and biomass were used (Table 1). Ideally fish size and density should have been similar in both cages being compared at each period. But this study only had access to two semi-closed cages in June, while only had access to one semi-closed and one open cage in September and November, which made the task difficult since the open cage has different net depths and volumes. In addition, to use the underwater camera system to observe the fish distribution was not optimal, especially difficult to study the fish when dark. It would be better to use some other equipment to keep track of individual fish as a research method if possible. There were also problems with equipment for measuring water quality in September. The equipment was not available due to technical problems. Moreover, the quantifying of fish was both challenging and time-consuming which need to be taken into consideration.

In our study the fewer variables, the better when performing an experiment while, in fact, there are many variations of environmental conditions in this study. For example, the initial fish states between experimental group and control group are not similar; the growth rates of the caged fish are different and the stress caused by parasites infection all have an effect on the vertical and horizontal distribution of caged Atlantic salmon more or less. However, some results are supported by previous studies and the others may just for consideration purpose when studying in semi-closed cage system of Atlantic salmon farming. All in all, there are a lot of abiotic and biotic variables that may have influenced fish distribution, which was unmeasured or unobserved due to limited practical reasons. So the study concentrates on using the collected data furthest to illustrate the characteristics of horizontal and vertical distribution of caged Atlantic salmon.

4.1 Vertical distribution of caged Atlantic salmon

It is said that the feeding regime based on pellet distribution at the cage surface may have resulted in the general attraction towards surface and low occupation of the deeper parts of the cages (Skulstad et al., 2013). In order to avoid the effect caused by feeding activities, observing the fish distribution at the time one hour after the feeding is completed. The fish were reared in larger numbers at high densities, to observe the distribution of all individuals was therefore not possible because individuals are difficult to follow. In a study conducted by Vijayan & Leatherland (1988), high stocking density evokes density-dependent behavioral changes. And those changes are mostly due to alterations in social dominance hierarchies and to aggressive and or disturbing interactions, whether enhanced or lowered (Bégout Anras & Lagardère, 2004). The fish density are comparable between closed cages in June and September whereas in November the density is higher due to the fish growing. However, in the open cages the fish density was lower than in the closed cage due to larger cage column. This difference in stocking density may have affected the fish distribution in the study.

Caged salmon fish both in the closed and open cages are swimming against the water currents in a circle at different depths. Grouping swimming (Martins et al., 2012) model was observed. Every individual has to adjust itself to minimize the risk of physical injuring such as collisions. Thus, the interaction between the fish individuals may have some effects on fish vertical distributions (Shin & Cury, 2001). There is an obvious phenomenon that caged Atlantic salmon swim in a group in different speed at different times or precisely under different environmental conditions. Also, some research have reported that water current may have a big effects on fish grouping swimming forms (Johansson et al., 2014), which may result in different types of vertical distributions. Some fish prefer to stay behind the inlet water that may correlate with the preference of low water current speed, which has not previously been described for caged Atlantic salmon in the semi-closed system.

Fish distributed in different depths of water column and the results shows that in June fish were pretty even distributed in the whole cages. Furthermore, fish seems to avoid top and bottom in semi-closed cage in September and November. That implicated that the fish is not using the whole water body. Again, that implicated higher fish density in the middle part of the cage. Fish density is important Juell & Fosseidengen (2004). If the fish spread in the whole cage, avoiding extra oxygen, might open up to increased fish density, that is more fish in the cage. (Johansson, Ruohonen, Juell, & Oppedal, 2009) suggests that spatial variation was strong correlated with environmental preferences. Caged Salmon fish seems to have lived in the suitable environmental preference ranges that provide them carefree swimming style especially in the open cage in June. And the carefree swimming style defined as the fish is swimming at a very low speed that almost stay at the same position all the time with their fins moved in a low frequency.

4.2 Horizontal distribution of caged Atlantic salmon

In this study, the results showed that more fish in the "center" compared with "edge" both in open and semi-closed cage. This result does not fit to the research reported that Salmonids typically form a circular swimming pattern and avoid both the innermost part of the cage volume and the cage corners in the daytime (Tim Dempster, Juell, Fosseidengen, Fredheim, & Lader, 2008). It is not a clear observation. The differences are not clear in three cages. Many a factors, which may have an influence on the vertical distribution of Atlantic salmon, may also affect the fish horizontal distributions. I have not been able to find anyone studying horizontal distribution in cages. The result might be something with the method used for measuring fish distribution. Alternatively, it can also mean that fish avoid the "edge" because more predators outside cage. If so the fish in a closed cage do not see them. Perhaps they get hurt when they have contacted with net or wall in the cage. No such kind of previous studies have been found yet.

Our semi-closed cage system, with water inlet in the surface areas of the cage edge and outlet in the bottom of the cage center, produced various flows, which enabled caged salmon to use different spacial areas characterized by different water speed. In the present study, the surface activities were recorded at different period of daytime in June only. There is a significant difference in jump roll between cage 3 and cage 4 in June, which may indicate that horizontal movement do happen actively especially in the upper layer of the caged water column.

4.3 Environmental drivers and swimming depths

It has been suggested that large temporal and spatial variation have been studied in floating marine cages, which means that the environmental requirements must be preferentially taken into account if you are willing to optimize the caged farming system (Johansson et al., 2006). Diel rhythms in the swimming depth of Atlantic salmon farmed in cages have been linked to feed attraction (Frenzl et al., 2014), perceived predation risk (Solberg, Zhang, & Glover, 2015), diel variations in light intensity (Stien et al., 2014) combined with temperature conditions (Johansson et al., 2009). In this study no significant correlations were found between water temperature and fish swimming depth in semi-closed cages, besides, the dissolved oxygen saturation and pH were also not significant correlated with fish swimming depth. This result may be partly due to the distinct environment conditions characterized by a low fluctuation of water parameter factors, which is included in the optimized environmental requirement (shown in the results part).

Identifying a single factor such as optimal temperature in a small size experimental cage may be difficult for fish because many an other environmental factors can not be excluded definitely. Additionally, the interactions between fish individuals are also complicated. However, there is no doubt that temperature do affect the fish behavior and spacial distribution. A previous review of environmental drivers of Atlantic salmon behavior in sea-cage suggested that temperature profiles vary from being negatively correlated with depth in summer to positively correlated with depth in winter, with transitional periods where profiles are more variable, but often with highest temperatures at mid-cage depths in fjords (Frode Oppedal, Dempster, & Stien, 2011). Jobling (1994) concluded the literature and suggested that the optimal growth rate of Atlantic salmon were observed between the temperature of 12 and 17℃.

In this study, the temperature was homogeneous at all depths both in the semi-closed cages and open cage within the same day, and also within the same period. It is known from the literature that fish have thermal preference range (Johansson et al., 2009). This might explain that fish even distributed at all depths, just avoiding the bottom and the top. The reason for avoiding the top can be the light. Many a research have suggested that Atlantic salmon are positively photo-tactic and show a strong attraction to light sources (Frenzl et al., 2014). In the open cage of Atlantic salmon study, Oppedal (2001) had reported that in winter they had a shallower vertical distribution and swam with a lower fish density than in summer (F. Oppedal et al., 2001). In addition, there is also research reported that when allowed to swim to greater depths in a 20 m deep cage, the relationship between light intensity and mean swimming depth showed a light intensity preference (Huse & Holm, 1993). For the bottom, it can not be lack of oxygen or other water quality aspects. Perhaps close to bottom swimming activity is disturbed. There are some variation in water quality between cages, depths and season. There are variation in DO saturation, but not affecting fish vertical distribution, as supported by no correlation between DO saturation and fish vertical distribution. The oxygen level were above 75% at all times. Previous study with full-feeding Atlantic salmon reared in seawater at 16 °C concluded that under the condition of 70% dissolved oxygen saturation levels initiated reduced appetite; 60% additionally led to sharp anaerobic metabolism and enhanced skin lesions; 50% additionally caused acute stress responses, decreased feed conversion and growth; and 40% additionally initiated vitiated osmoregulation and mortality (Anon, 2008). A recent research have reported that if the oxygen concentration decreased below 7.0 mg/l O2 the growth rate fell, and that below 6.0 mg/l O2 rainbow trout fed less at a temperature of 15 °C (Pedersen, 1987). Dissolved Oxygen Percent (%) Saturation

Sheet was list in appendix IV. Similar, also pH was stable with only minor variation. PH has been concluded as a possible directing factor for swimming depth in this study. It has also been correlated with the concentration of CO_2 in the water column (Tseng et al., 2013). For fish in closed cages, it is particularly important to identify values of CO2 gasses that can cause stress and a number of injuries on the organisms by prolonged overexposure (Enzor, Zippay, & Place, 2013). It is suggested that the CO2 at very high values may in the worst case lead to acute mortality (Briffa, de la Haye, & Munday, 2012). In normal operation the values should be below 10 mg / l, though fish in shorter periods can tolerate up to 20 mg / l without detectable damage (Gilmour, 2001). In this project we have detected that pH range nearly from7 to 8 in the semi-closed cage during the whole experimental time which will not significantly affect the fish welfare. When the pH drops below 7.0, even down to 6.7 in the closed cages, will increases the risk of discovering organs damage, and we believe it must be a good safety margin which limits set for pH. It may also be that decreasing pH is related to other changes in water chemistry which also has a negative impact on fish welfare. In this study, it seems to be that no relationship were found between fish swimming depth and pH values, which may result from that the fish reared in the semi-closed cage have a stable and an acceptable pH environment. This result has not previously been reported for farmed Atlantic salmon and indicated that the vertical distribution of salmon fish may be even and not being affected by the water quality (observed in the study).

4.4 Jump roll.

In our study, the jump roll were observed in the morning, afternoon and night in the semi-closed cage in June. Significant difference of fish jump roll were concluded after analyzing the data but what factors impact on the behaviors is still not clear. No research in this kind of areas have been reported before and related articles were difficult to be found. Small fish seems more active in the morning in cage 3 while large fish seems more active in the night in cage 4. In the wild, Salmon breed in freshwater rivers and mature in the ocean. There is a physiological change

(Smoltification) of the fish when they swim downstream to the sea and an freshwater adaptation period when they swim back upstream to the spawning grounds (Folmar & Dickhoff, 1980). The jump activities happened when they travel upstream to overcome obstacles (Young, Björnsson, Prunet, Lin, & Bern, 1989). The reason that salmon jump is still not clear. An explanation for this could be either that the salmon are trying to get rid themselves of parasites or that it plays a role in assisting the salmon to hold buoyancy (Pinder & Eales, 1969; T Dempster, Kristiansen, Korsøen, Fosseidengen, & Oppedal, 2011). Those jump behaviors might be used to relate the feeding operation in the semi-closed salmon cage farm but there is still a long way to go.

4.5 Further research

The purpose of this study is to analyze the horizontal and vertical distribution of Atlantic salmon in the semi-closed cage system. In the present study, recorded fish activities were based on group-level measurements. Studies of individual behavior may result in a better understanding of the motivational mechanism and environmental cues that control the behavior of the fish in such semi-closed cage environments. Further research of Atlantic salmon in the semi-closed cages should take the limits described above into consideration in order to minimize the number of variables to make the errors at the lowest levels. On the other hand, other measurement equipment such as echo sounder and telemetry can be used to detect the fish distribution if possible. All in all, this initial investigation was very revealing and a more sophisticated model that accounts for fish distribution targeted to ensure the welfare of the fish and optimize the spacial utilization.

5. Conclusion

This study is a first approach to study horizontal and vertical distribution of Atlantic salmon fish reared in semi-closed sea cage. The study found diverging results, firstly, fish in semi-closed cage was evenly distributed in the whole cage in June, whereas in September and November the fish were mainly distributed in the middle part of the cage avoiding top and bottom. Secondly, small difference of fish number was found between center and edge part of the cage during the whole experimental period. Thirdly, it seems like that there was no clear tendency between the time of the day regarding numbers of jump roll. In the morning smaller fish are more active while in the evening larger fish are more active. The study, furthermore, describes data on the detail parameters of water quality in the semi-closed systems, showing that the water quality in the experimental semi-closed cage do have no significant difference between depths.

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Appendix

Appendix 1: Test results of vertical distribution.

Type	month	C3			C4			P	Test
		#	mean	SD	#	mean	SD		Effect of depth or cage
Average	June	37	25.0923	18.07339				0.238	One-Way ANOVA
Average	June				31	28.1317	18.12566	0.052	One-Way ANOVA
percentage(1m)	June	9	14.7407	12.06323	9	15.3426	13.11332	0.921	T-Test
percentage(3m)	June	10	26.3333	15.39521	10	34	13.00261	0.245	T-Test
percentage(6m)	June	12	28.3889	17.26375	8	36.5417	19.56182	0.339	T-Test
percentage(9m)	June	6	31.9583	27.5338	4	25.4167	24.95681	0.713	T-Test

Type	month	C1			C2			P	Test
		#	mean	SD	#	mean	SD		Effect of depth or cage
Average	September	77	59.8139	38.01379				0.000	One-Way ANOVA
Average	September				141	28.4374	22.4797	0.000	One-Way ANOVA
percentage(1m)	September	9	3.6667	4.91031	36	1.3241	4.08131	0.146	T-Test
percentage(3m)	September	16	33.125	19.32945	49	36.6395	12.05808	0.502	T-Test
percentage(6m)	September	16	68.5417	21.64182	43	48.6512	14.37774	0.003	T-Test
percentage(9m)	September	15	77.8444	27.33707	13	5.7436	6.30425	0.000	T-Test

means the number of date samples

Appendix 1: Test results of vertical distribution.

Type	month	cage	(I) depth	(J) depth	Mean Difference (I-J)	Std. Error	Sig.	Test-LED
Average	9	1	1	3	-29.45833*	10.40579	0.006	Post Hoc Tests
Average	9	1	1	6	-64.87500*	10.40579	0	Post Hoc Tests
Average	9	1	1	9	-74.17778*	10.52992	0	Post Hoc Tests
Average	9	1	1	12	-96.47222*	11.01246	0	Post Hoc Tests
Average	9	1	1	15	-60.40741*	11.77281	0	Post Hoc Tests
Average	9	1	3	1	29.45833*	10.40579	0.006	Post Hoc Tests
Average	9	1	3	6	-35.41667*	8.82961	0	Post Hoc Tests
Average	9	1	3	9	-44.71944*	8.97556	0	Post Hoc Tests
Average	9	1	3	12	-67.01389*	9.53707	0	Post Hoc Tests
Average	9	1	3	15	-30.94907*	10.40579	0.004	Post Hoc Tests
Average	9	1	6	1	64.87500*	10.40579	0	Post Hoc Tests
Average	9	1	6	3	35.41667*	8.82961	0	Post Hoc Tests
Average	9	1	6	9	-9.30278	8.97556	0.304	Post Hoc Tests
Average	9	1	6	12	-31.59722*	9.53707	0.001	Post Hoc Tests
Average	9	1	6	15	4.46759	10.40579	0.669	Post Hoc Tests
Average	9	1	9	1	74.17778*	10.52992	0	Post Hoc Tests
Average	9	1	9	3	44.71944*	8.97556	0	Post Hoc Tests
Average	9	1	9	6	9.30278	8.97556	0.304	Post Hoc Tests
Average	9	1	9	12	-22.29444*	9.67235	0.024	Post Hoc Tests
Average	9	1	9	15	13.77037	10.52992	0.195	Post Hoc Tests
Average	9	1	12	1	96.47222*	11.01246	0	Post Hoc Tests
Average	9	1	12	3	67.01389*	9.53707	0	Post Hoc Tests
Average	9	1	12	6	31.59722*	9.53707	0.001	Post Hoc Tests
Average	9	1	12	9	22.29444*	9.67235	0.024	Post Hoc Tests
Average	9	1	12	15	36.06481*	11.01246	0.002	Post Hoc Tests
Average	9	1	15	1	60.40741*	11.77281	0	Post Hoc Tests
Average	9	1	15	3	30.94907*	10.40579	0.004	Post Hoc Tests
Average	9	1	15	6	-4.46759	10.40579	0.669	Post Hoc Tests
Average	9	1	15	9	-13.77037	10.52992	0.195	Post Hoc Tests
Average	9	1	15	12	-36.06481*	11.01246	0.002	Post Hoc Tests

T-test between depths within the same cage

Appendix 1: Test results of vertical distribution.

Type	month	cage	(I) depth	(J) depth	Mean Difference (I-J)	Std. Error	Sig.	Test-LED
Average	9	2	1	3	-35.31538*	2.42512	0	Post Hoc Tests
Average	9	2	1	6	-47.32709*	2.49575	0	Post Hoc Tests
Average	9	2	1	9	-4.41952	3.57477	0.218	Post Hoc Tests
Average	9	2	3	1	35.31538*	2.42512	0	Post Hoc Tests
Average	9	2	3	6	-12.01171*	2.30853	0	Post Hoc Tests
Average	9	2	3	9	30.89587*	3.44667	0	Post Hoc Tests
Average	9	2	6	1	47.32709*	2.49575	0	Post Hoc Tests
Average	9	2	6	3	12.01171*	2.30853	0	Post Hoc Tests
Average	9	2	6	9	42.90757*	3.49673	0	Post Hoc Tests
Average	9	2	9	1	4.41952	3.57477	0.218	Post Hoc Tests
Average	9	2	9	3	-30.89587*	3.44667	0	Post Hoc Tests
Average	9	2	9	6	-42.90757*	3.49673	0	Post Hoc Tests
Average	11	1	1	3	-33.08577*	7.85942	0	Post Hoc Tests
Average	11	1	1	6	-39.45419*	7.85942	0	Post Hoc Tests
Average	11	1	1	9	-47.22222*	7.96492	0	Post Hoc Tests
Average	11	1	1	12	-42.18519*	8.35367	0	Post Hoc Tests
Average	11	1	1	15	-17.2963	10.15332	0.092	Post Hoc Tests
Average	11	1	3	1	33.08577*	7.85942	0	Post Hoc Tests
Average	11	1	3	6	-6.36842	7.75248	0.414	Post Hoc Tests
Average	11	1	3	9	-14.13645	7.85942	0.075	Post Hoc Tests
Average	11	1	3	12	-9.09942	8.25314	0.273	Post Hoc Tests
Average	11	1	3	15	15.78947	10.07077	0.12	Post Hoc Tests
Average	11	1	6	1	39.45419*	7.85942	0	Post Hoc Tests
Average	11	1	6	3	6.36842	7.75248	0.414	Post Hoc Tests
Average	11	1	6	9	-7.76803	7.85942	0.326	Post Hoc Tests
Average	11	1	6	12	-2.73099	8.25314	0.741	Post Hoc Tests
Average	11	1	6	15	22.15789*	10.07077	0.03	Post Hoc Tests
Average	11	1	9	1	47.22222*	7.96492	0	Post Hoc Tests
Average	11	1	9	3	14.13645	7.85942	0.075	Post Hoc Tests
Average	11	1	9	6	7.76803	7.85942	0.326	Post Hoc Tests
Average	11	1	9	12	5.03704	8.35367	0.548	Post Hoc Tests
Average	11	1	9	15	29.92593*	10.15332	0.004	Post Hoc Tests

T-test between depths within the same cage

Appendix 1: Test results of vertical distribution.

Type	month	cage	(I) depth	(J) depth	Mean Difference (I-J)	Std. Error	Sig.	Test-LED
Average	11	1	12	1	42.18519*	8.35367	0	Post Hoc Tests
Average	11	1	12	3	9.09942	8.25314	0.273	Post Hoc Tests
Average	11	1	12	6	2.73099	8.25314	0.741	Post Hoc Tests
Average	11	1	12	9	-5.03704	8.35367	0.548	Post Hoc Tests
Average	11	1	12	15	24.88889*	10.46106	0.019	Post Hoc Tests
Average	11	1	15	1	17.2963	10.15332	0.092	Post Hoc Tests
Average	11	1	15	3	-15.78947	10.07077	0.12	Post Hoc Tests
Average	11	1	15	6	-22.15789*	10.07077	0.03	Post Hoc Tests
Average	11	1	15	9	-29.92593*	10.15332	0.004	Post Hoc Tests
Average	11	1	15	12	-24.88889*	10.46106	0.019	Post Hoc Tests
Average	11	2	1	3	-25.43177*	3.43652	0	Post Hoc Tests
Average	11	2	1	6	-29.43177*	3.43652	0	Post Hoc Tests
Average	11	2	1	9	-1.81481	4.92521	0.714	Post Hoc Tests
Average	11	2	3	1	25.43177*	3.43652	0	Post Hoc Tests
Average	11	2	3	6	-4	3.38976	0.243	Post Hoc Tests
Average	11	2	3	9	23.61696*	4.8927	0	Post Hoc Tests
Average	11	2	6	1	29.43177*	3.43652	0	Post Hoc Tests
Average	11	2	6	3	4	3.38976	0.243	Post Hoc Tests
Average	11	2	6	9	27.61696*	4.8927	0	Post Hoc Tests
Average	11	2	9	1	1.81481	4.92521	0.714	Post Hoc Tests
Average	11	2	9	3	-23.61696*	4.8927	0	Post Hoc Tests
Average	11	2	9	6	-27.61696*	4.8927	0	Post Hoc Tests

T-test between depths within the same cage

Appendix 2: Test results of horizontal distribution.

Type	month	center			edge			P	Test	Eq Var.
		#	mean	SD	#	mean	SD			
Average	June	20	27.0667	17.16129	7	16.1429	16.5785	0.156	t-test	Y
Average	June	17	29.0539	19.978	7	28.9286	10.50567	0.988	t-test	Y
Average	September	59	64.565	40.46456	18	44.2407	23.21055	0.046	t-test	Y
Average	September	41	33.8699	24.84506	20	25.6333	21.58674	0.210	t-test	Y
Average	November	62	45.7849	32.41551	24	40.1528	21.68304	0.435	t-test	Y
Average	November	42	29.6905	17.98545	18	28.1852	15.00075	0.757	t-test	Y

Type	month	cage 3			cage 4			P	Test	Eq Var.
		#	mean	SD	#	mean	SD			
Average	June	20	27.0667	17.16129	17	29.0539	19.978	0.747	t-test	Y
Average	June	7	16.1429	16.5785	7	28.9286	10.50567	0.110	t-test	Y
Average	September	59	64.565	40.46456	41	33.8699	24.84506	0.000	t-test	N
Average	September	18	44.2407	23.21055	20	25.6333	21.58674	0.015	t-test	Y
Average	November	62	45.7849	32.41551	42	29.6905	17.98545	0.002	t-test	N
Average	November	24	40.1528	21.68304	18	28.1852	15.00075	0.052	t-test	Y

means the number of date samples

Effect of horizontal location or cages

Appendix 3: Test results of jump roll.

Type	month	cage 3			cage 4			P	Test
		#	mean	SD	#	mean	SD		
jump roll	6	11	15.22	8.089				0.005	One way ANOVA
jump roll	6				11	69.5	29.091	0.000	One way ANOVA

Type	month	cage	(I) daytime	(J) daytime	Mean Difference (I-J)	Std. Error	P	Test
jump roll	6	3	1	2	2.527	3.383	0.744	Post Hoc Tests
jump roll	6	3	1	3	-13.740*	3.383	0.009	Post Hoc Tests
jump roll	6	3	2	1	-2.527	3.383	0.744	Post Hoc Tests
jump roll	6	3	2	3	-16.267*	3.783	0.007	Post Hoc Tests
jump roll	6	3	3	1	13.740*	3.383	0.009	Post Hoc Tests
jump roll	6	3	3	2	16.267*	3.783	0.007	Post Hoc Tests

Type	month	cage	(I) daytime	(J) daytime	Mean Difference (I-J)	Std. Error	P	Test
jump roll	6	4	1	2	50.900*	8.191	0.001	Post Hoc Tests
jump roll	6	4	1	3	53.600*	8.191	0	Post Hoc Tests
jump roll	6	4	2	1	-50.900*	8.191	0.001	Post Hoc Tests
jump roll	6	4	2	3	2.7	9.158	0.953	Post Hoc Tests
jump roll	6	4	3	1	-53.600*	8.191	0	Post Hoc Tests
jump roll	6	4	3	2	-2.7	9.158	0.953	Post Hoc Tests

Type	month	cage 3			cage 4			P	Test
		#	mean	SD	#	mean	SD		
jump roll	6	5	12.16	4.217	5	98	15.174	0	t-test
jump roll	6	3	9.63	1.976	3	47.1	5.828	0	t-test
jump roll	6	3	25.9	6.811	3	44.4	2.96	0.012	t-test

means the number of date samples
Effect of cages or daytime

Appendix 4

Dissolved Oxygen Percent (%) Saturation Sheet

Temp (C)	D.O. (mg/L) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15*
0	7%	14%	21%	27%	34%	41%	48%	55%	62%	68%	75%	82%	89%	96%	103%
1	7%	14%	21%	28%	35%	42%	49%	56%	63%	70%	78%	85%	92%	99%	106%
2	7%	14%	22%	29%	36%	43%	51%	58%	65%	72%	80%	87%	94%	101%	109%
3	7%	15%	22%	30%	37%	45%	52%	60%	67%	74%	82%	89%	97%	104%	112%
4	8%	15%	23%	31%	38%	46%	53%	61%	69%	76%	84%	92%	99%	107%	115%
5	8%	16%	24%	31%	39%	47%	55%	63%	71%	78%	86%	94%	102%	110%	118%
6	8%	16%	24%	32%	40%	48%	56%	64%	72%	80%	88%	97%	105%	113%	121%
7	8%	17%	25%	33%	41%	50%	58%	66%	74%	83%	91%	99%	107%	116%	124%
8	8%	17%	25%	34%	42%	51%	59%	68%	76%	85%	93%	101%	110%	118%	127%
9	9%	17%	26%	35%	43%	52%	61%	69%	78%	87%	95%	104%	113%	121%	130%
10	9%	18%	27%	35%	44%	53%	62%	71%	80%	89%	98%	106%	115%	124%	133%
11	9%	18%	27%	36%	45%	54%	64%	73%	82%	91%	100%	109%	118%	127%	136%
12	9%	19%	28%	37%	46%	56%	65%	74%	84%	93%	102%	112%	121%	130%	139%
13	10%	19%	29%	38%	48%	57%	67%	76%	86%	95%	105%	114%	124%	133%	143%
14	10%	19%	29%	39%	49%	58%	68%	78%	87%	97%	107%	117%	126%	136%	146%
15	10%	20%	30%	40%	50%	60%	70%	79%	89%	99%	109%	119%	129%	139%	149%
16	10%	20%	30%	41%	51%	61%	71%	81%	91%	102%	112%	122%	132%	142%	152%
17	10%	21%	31%	41%	52%	62%	73%	83%	93%	104%	114%	124%	135%	145%	155%
18	11%	21%	32%	42%	53%	63%	74%	85%	95%	106%	116%	127%	138%	148%	159%
19	11%	22%	32%	43%	54%	65%	76%	86%	97%	108%	119%	130%	140%	151%	162%
20	11%	22%	33%	44%	55%	66%	77%	88%	99%	110%	121%	132%	143%	154%	165%
21	11%	22%	34%	45%	56%	67%	79%	90%	101%	112%	124%	135%	146%	157%	169%
22	11%	23%	34%	46%	57%	69%	80%	92%	103%	115%	126%	138%	149%	161%	172%
23	12%	23%	35%	47%	58%	70%	82%	93%	105%	117%	129%	140%	152%	164%	175%
24	12%	24%	36%	48%	60%	71%	83%	95%	107%	119%	131%	143%	155%	167%	179%
25	12%	24%	36%	49%	61%	73%	85%	97%	109%	121%	133%	146%	158%	170%	182%
26	12%	25%	37%	49%	62%	74%	87%	99%	111%	124%	136%	148%	161%	173%	185%
27	13%	25%	38%	50%	63%	75%	88%	101%	113%	126%	138%	151%	164%	176%	189%
28	13%	26%	38%	51%	64%	77%	90%	102%	115%	128%	141%	154%	166%	179%	192%
29	13%	26%	39%	52%	65%	78%	91%	104%	117%	130%	143%	156%	169%	183%	196%
30	13%	27%	40%	53%	66%	80%	93%	106%	119%	133%	146%	159%	172%	186%	199%
31	13%	27%	40%	54%	67%	81%	94%	108%	121%	135%	148%	162%	175%	189%	202%
32	14%	27%	41%	55%	69%	82%	96%	110%	124%	137%	151%	165%	179%	192%	206%
33	14%	28%	42%	56%	70%	84%	98%	112%	126%	140%	154%	168%	182%	196%	209%
34	14%	28%	43%	57%	71%	85%	99%	113%	128%	142%	156%	170%	184%	199%	213%
35	14%	29%	43%	58%	72%	87%	101%	115%	130%	144%	159%	173%	188%	202%	216%
36	15%	29%	44%	59%	73%	88%	103%	117%	132%	147%	161%	176%	191%	205%	220%
37	15%	30%	45%	60%	75%	89%	104%	119%	134%	149%	164%	179%	194%	209%	224%
38	15%	30%	45%	61%	76%	91%	106%	121%	136%	151%	166%	182%	197%	212%	227%
39	15%	31%	46%	61%	77%	92%	108%	123%	138%	154%	169%	184%	200%	215%	230%

*** If D.O. is greater than 15 mg/L then use the formula on pg. 175 in your Stream Keepers Field Guide:**

$$\frac{\text{Actual Dissolved Oxygen (mg/L)}}{\text{Max Oxygen Concentration at Water Temp}}$$