Refilling behaviour of Atlantic Salmon *(Salmo salar)* in submerged sea cages with air-dome

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1. Abstract:

Production of Atlantic salmon in submerged sea cages may solve surface related challenges like sea lice and toxic algae blooms but previous trials have faced problems linked to buoyancy. Korsøen et al. (2012) showed that salmon adapt rapidly to refilling in air-dome, using plexi-glass (1m x 1m, with height of 0,3m), but not by normal refilling behaviour. This study suggests Atlantic salmon refill nearly singularly by rolling in an eight squared air-dome with diameter of 2,5m and height 0,1m during submergence. Leaping was also observed in the air-dome, but at a limited level. This study suggest that Atlantic salmon were able to maintain neutral buoyancy by refilling every other day in the air-dome, with swimming speeds ranging from 1,2 - 0,5 Bl s^{-1} throughout the experiment. This study also revealed that refilling activity in an air-dome varies diurnally, with numerous collinear factors as potential drivers for this.

The activity in the air dome was observed using a camera attached to the inner side of the dome. Sixteen samples were made in the period from September 2019 – June 2020. Three steel cages of (12 m x 12 m x 15 m) with 6000-6500 salmon and one air dome per cage were used.

2. Introduction

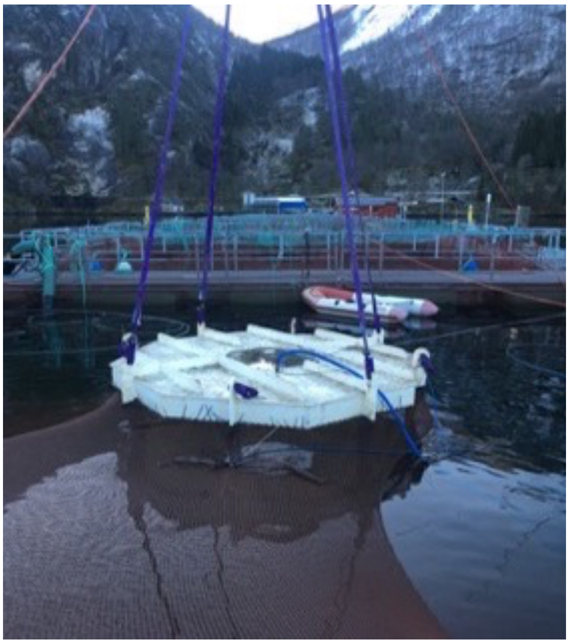


Figure 1 - Picture of air-dome used in this experiment. The eigth-squared dome attached to roof netting (red netting) being placed in sea-cage. The blue hose is connected to a compressor and fills dome with air. There is a glass-window in the center where light gets through.

Submerged sea-cages are gaining interest in the salmon aquaculture industry in light of their potential to solve surface related problems but have thus far been plagued with challenges concerning fish welfare. These challenges are primarily linked to buoyancy, which was described by Archimedes to equal the weight of the displaced mass of water. It is therefore by definition equal to the volume (V) of the fish, multiplied by the specific weight of the water (p, density) and the acceleration of gravity (g):

$$B = V \times \rho\ (water) \times g$$

$$W = V\ \times \rho\ (fish) \times g$$

Since tissue is heavier than water, fish will sink standing still. Most fish species have therefore developed a swim bladder which creates a static lift (Kryvi and Poppe 2016). When standing still, neutral buoyancy is then achieved when the lifting force (B) is equal to the downward force (W). As the swimming speed increase, dynamic lift is generated, allowing a reduction of the swim bladder's volume to maintain the vertical position. Negative buoyancy occurs when the downforce weight of the fish is greater than the buoyancy. Problems linked to negative buoyancy have been observed in all previous trials, when salmon had no access to air, during submergence resulting in modified swimming behaviour (Korsøen et al. 2009; Tim Dempster et al. 2009). Specifically, the fish compensates for negative buoyancy with increased swimming speed and/or by tilting their body towards the surface to maintain vertical positioning. Atlantic salmon have a physostomous swim bladder, which they refill by swallowing air at surface (Kryvi and Poppe 2016). In wildlife, salmon spend most of their lives in the upper 15 m of the water column, close to the surface (Juell 1995) and are therefore not ideally adapted for a submerged life. One possible solution to facilitate refilling during submergence and avoid negative buoyancy is the provision of a submerged air-dome which the fish can refill within (Fig. 1, Korsøen et al. 2012). At a small scale, salmon adapted rapidly to refilling in an air-dome (Korsøen et al. 2012), can the same principle work at commercial scale?

2.1. Motivation to submerge – surface related problems

The salmon aquaculture industry in Norway produced about 1,3 million tonnes of salmon in 2018, worth 64,5 billion NOK (Statistics Norway 2019). Still, too many fish die during production, and many of these are linked to treatment against the parasitic sea lice (Overton et al. 2019). Other surface related problems, like toxic algae blooms also challenge fish welfare and have pushed several salmon farmers to the edge of bankruptcy (Vigsnæs and Alnes 2019). In addition, storms and foul weather cause considerable damage to sea-cages each year, and will only become a larger issue as farming moves to more exposed sites (Holmer 2010). All of these surface related challenges can be mitigated, or potentially avoided entirely, by submerging sea-cages (Tim Dempster et al. 2009).

2.1.1. Sea lice

The ectoparasitic sea louse *(Lepeophteirus Salmonis)* is a key obstacle for further growth of the salmon industry. In 2017 production costs linked to treatment against the parasite exceeded NOK 5 billion (Iversen et al. 2017). Sea lice also impair host fitness and welfare (Noble et al. 2018), and as a result are the main indicator for the traffic light system in Norway which regulates salmon farming (Grefsrud et al. 2019). The infective stage of sea lice are typically found in the upper 10m of the water column in the marine environment, below the halocline (Heuch, Parsons, and Boxaspen 1995; Hevrøy et al. 2003). For caged salmon, the probability of lice infestation decreases exponentially with increasing swimming depth (Oppedal et al. 2017). Submerged sea-cages can therefore dramatically reduce the probability of lice infestation and may solve one of the main challenges in salmon aquaculture in Norway.

The current trend is toward an integrated management approach against sea lice with synchronized treatments, biological control (cleaner fish), immunological interference (immunostimulants), mechanical delousing systems, selective breeding for louse resistance and regulatory approaches (zones with synchronized production and fallowing) (Torrissen et al. 2013). In addition, new production systems and methods have been developed based on the principle of reducing the encounter probability between sea-lice and salmon by either installing a barrier around the upper 5-15m of the cage or the use of submerged artificial lights and -feeding to attract the fish to greater depth (Oppedal et al. 2017). Methods involving submergence of salmon to greater depth, have shown variation in results due to environmental changes and behavioural preferences (Oppedal, Dempster, and Stien 2011;

Oppedal et al. 2011). Knowledge regarding behaviour of Atlantic salmon during submergence is therefore valuable in developing these methods.

2.1.2. Water quality

Submergence can enable a better production environment to be accessed by moving the fish away from poor surface conditions (Tim Dempster et al. 2009). In extreme cases of poor surface conditions, like toxic algae blooms, entire production cycles can be wiped out, and cause major economic losses for salmon farmers (Vigsnæs and Alnes 2019). In less extreme cases, salmon may experience reduced appetite and elevated mortalities from e.g. jellyfish, or infestation from the bacteria *Moritella viscosa* with surface temperatures below 7 ºC (Lunder 1992).

Environments within sea-cages variates the most with depth, and preferred swimming depth of salmon is the result of active trade-offs among environmental influences and an array of internal motivational factors such as feed and perceived threats (Oppedal, Dempster, and Stien 2011). Salmonid farming sites in fjord systems compared to coastal areas are less likely to experience upwelling events caused by winds, but are more likely to experience greater seasonal variations in water quality. Such seasonal changes in vertical stratification, including salinity levels, temperature, oxygen and water currents have important implications for the production performance and welfare of farmed salmon (Oppedal, Dempster, and Stien 2011). By moving salmon to depths where the environment varies less, or conditions are more optimal for production, fish welfare can be improved.

The preferred temperature of Atlantic salmon is 16-18 °C (Johansson et al. 2006; 2009). Optimizing thermal exposure can improve circulation, food intake, digestion and ultimately, growth. With climate change, many of the optimal salmonid farming regions today will be exposed to a range of higher surface water temperatures, likely above thresholds during the summer months (>20 °C) (Oppedal, Dempster, and Stien 2011). The ability to submerge cages to depths with cooler temperatures will therefore be beneficial for fish welfare, and potentially enable farming in locations which otherwise would be impractical. Submerged sea-cages may also reduce specific environmental impacts related to salmon farming in sea-cages, such as escapes during storms (Tim Dempster et al. 2009).

2.2. Refilling behaviour

Atlantic salmon refill their swim-bladder by gulping air at the surface, either via rolling or leaping (Furevik et al. 1993). Rolling is when fish breaks surface with the head followed by the dorsal side upwards, making a bow, aiming down towards the water. The roll looks like a whale breaking the surface for breathing. Occasionally rolling can be observed by fish barely breaking the surface with the jaw, followed by the dorsal fin and part of the back (Furevik et al. 1993). Rolling activity can vary between days, but has previously been observed to be relatively constant throughout the year (Furevik et al. 1993). Leaping starts at 1-3 m depth, with the fish swimming horizontally, followed by upwards acceleration at a 30-40 angle to the horizontal plane until the fish bursts through the water's surface. During a leap, the swimming speed of the fish increases 5-10 times above the normal cruising speed (Furevik et al. 1993). Earlier studies in standard production cages suggest that surface activity varies with numerous factors, and that rolling and leaping are not necessarily driven by all of the same motivational factors.

Glaropoulos et al. (2019) studied submerged salmon with weekly surface access; in their study, refilling mainly consisted of rolling with 9,2 ± 1,2 rolls fish^{-1} h^{-1} and 2,7 ± 0,3 leaps fish^{-1} h^{-1} immediately after surfacing. Activity then decreased with time, and after 60 minutes the activity was down to 0,4 ± 0,1 rolls fish^{-1} h^{-1} and 0,7 ± 0,2 leaps fish^{-1} h^{-1}. The fish in the control cages, which had continuous access to the surface throughout the whole experiment, had a maximum weekly average of 1,01 ± 0,02 refills fish^{-1} h^{-1} (Glaropoulos et al. 2019). Salmon have therefore shown to regulate buoyancy on a daily basis, and to refill rapidly when being negative buoyant.

Physostomous fish gradually lose air through the pneumatic duct (Korsøen et al. 2009). When salmon get startled, they release air from their swim bladder and dive away from the danger, inducing an increased demand for refilling afterwards due to negative buoyancy. This have been observed by increased refilling activity after stressful situations like delousing operations, and long term submergence (Glaropoulos et al. 2019; Furevik et al. 1993). In periods without ability to refill the swim bladder, salmon will suffer from negative buoyancy and develop modified swimming behaviours, reduced growth and poor feed utilisation (Korsøen et al. 2009; Tim Dempster et al. 2009). In previous studies of submerged salmon, when the fish had no access to air, increased swimming speeds (1,3-1,4 times faster than the control cages) and a distinct 'tail-down, head-up' (tilted) swimming behaviour were observed (Korsøen et al. 2009; Glaropoulos et al. 2019). Korsøen et al. (2009) found that continuous submergence without access to surface/air for longer than 2 weeks, reduced welfare and performance of Atlantic salmon. Without facilitating refilling during submergence, the cage must be resurfaced within two weeks to avoid severe consequences to production and welfare.

Swimming speeds typically average from 0,2 to 1,9 Bl s^{-1} in a cage environment (Juell 1995). This depends on several factors like currents, feeding and light/vision (schooling). Feeding have shown to be a key activity stimulator as there is an increase in activity and thus swimming speed with both feeding and the expectation of it (Oppedal, Dempster, and Stien 2011). During night-time; schooling groups have been observed to disperse gradually after sunset and this shift is preceded by reduced swimming depth and speed (Juell 1995).

2.3. Testing different dome sizes

Making the air-dome as small as possible is preferrable considering dimensioning of downward forces needed to keep the air-dome in place. The air-dome should on the other hand meet demand from the fish regarding behaviour and thus fish welfare. In one unpublished study, refilling activity in the air-dome increased with dome diameter (Nilsson et al., n.d.). In addition, they observed that fish go through a learning process, and that it is possible to reduce the diameter of dome over time as the salmon learn to use it. The results suggested, however, that domes smaller than two meters in a 12m x 12m cage with 10 000 salmon were not sufficient to meet the refilling demand and resulted in poor fish welfare. Correct dimensioning of dome to meet refilling demand is therefore critical.

In addition, type of refilling behaviour in an air-dome may vary with the height and diameter of the dome, but this is unknown. Rolling, which doesn't involve any remarkable acceleration, distance or height, is considered to be feasible in an air-dome. Leaping, on the other hand, requires considerably more space and energy expenditure than rolling and is often observed with high lice numbers and during acute stress events (Furevik et al. 1993). The question, whether salmon should be able to exhibit same behaviour as with full surface, should be discussed considering future design of air-domes.

2.4. What affect the surface activity?

Regulating buoyancy is linked to optimizing utilization of energy and maintaining vertical position in the water (Kryvi and Poppe 2016). Salmon therefore continuously exhibit trade-off between swimming depth, swimming speed, feeding and refilling to optimize its energy utilization. Further development of submerged cages depend on understanding these variations of behaviour and securing fish welfare in submerged cages (Tim Dempster et al. 2009; Korsøen et al. 2009). The life of a farmed salmon can be considered one-sidedly, swimming in circles getting food from the same source every day. It is therefore possible to identify factors which affect the fish. Smørdalen, which is a typical fjord farming site have a strong pycnocline (Nilsson et al., n.d.), which is the region in the water column of rapid density change (Johansson et al. 2006).

Surface activity have been described as hunger-dependent during feeding and related to stress (Juell 1995; Furevik et al. 1993). Feeding also affect the preferred vertical distribution (Frenzl et al. 2014) and have been suggested as a way to guide the fish towards the air-dome (Oppedal, Dempster, and Stien 2011). Artificial light may also attract the fish to the air-dome (Wright et al. 2015), but light attractiveness fades when overriding motivational factors, like stratified temperatures, are present (Oppedal, Dempster, and Stien 2011). Temperature arise as maybe the most important environmental factor considering vertical distribution (Johansson et al. 2006). Salmon have shown a deeper vertical distribution during winter-time or periods with cold surface temperatures and lowering of activity level (Oppedal, Dempster, and Stien 2011). When salmon dive to greater depth, pressure increases from surrounding water, shrinking the swim bladder. To compensate for lost lift, swimming speed must increase to maintain vertical positioning. Salmon may therefore do a trade-off between swimming faster at preferred depth and refill the swim bladder. The lipid content of salmon is positive correlated with fish size (Glaropoulos et al. 2019), which mean that larger salmon are less dense than smaller salmon and thus may be less reliant on their swim bladder for maintaining neutral buoyancy (Macaulay et al. 2020). Large salmon may therefore refill less than smaller salmon, and therefore be better adapted to a submerged cage.

2.5. Submerged cages with surface access

Submergence with frequent access to full surface is the simplest way to submerge, but have shown to cause negative buoyancy, increased swimming speeds and reduced growth (Glaropoulos et al. 2019; Korsøen et al. 2009). The previous studies show that salmon become negative buoyant within a week or two, and develop compensatory behavioural responses and reduced welfare (Glaropoulos et al. 2019; Korsøen et al. 2009). Submerged salmon are therefore depending on accessing surface weekly, increasing risk for lice infestation severely. The whole point with submergence is then potentially lost. There is, however, with this method less probability for lice infestation, and larger fish may enable longer periods of submergence without surface access. The latest stage of production, where salmon have increased fatty tissue, may therefore be the best adapted to submergence with frequent access to surface.

However, an air-dome is a possible solution to keep salmon submerged continuously (Korsøen et al. 2012). Fifteen salmon, with average weight of 3,3 kg refilled rapidly in a small air dome (1m x 1m) containing 120 L of air. The salmon were observed to swallow air by lifting their upper jaw above the surface, followed by rapid swimming downwards, resulting in normal buoyancy and swimming speeds (Korsøen et al. 2012). Refilling by rolling and leaping in an air-dome have therefore not previously been observed. In periods with no air in the air-dome; increased swimming speeds (1,5 – 2 times faster) were observed. After a week without access to air, salmon refilled 4-14 times per day within the first 24 h with restored surface in air-dome. After two weeks with air in the dome the average refilling activity was 0,4 – 1,4 refills fish^{-1} day^{-1} (Korsøen et al. 2012). The study showed that salmon adapted rapidly to refill in an air-dome, but due to limited space refilling behaviour was completely different from that in standard cages.

A later trial using the same dome size (120 L) in a 12m x 12m cage with 5000 individuals, had less success, resulting in reduced welfare. The reason suggested for this was that the air-dome was too small to meet the demand from the fish group (Bakketeig et al. 2013).

2.6. Aim of study

Despite the wealth of information available regarding the behaviour of Atlantic salmon in sea cages, little is known regarding their surface-access requirements and swim bladder refilling behaviour. Given the critical importance of buoyancy regulation to the health and welfare of aquatic organisms, a better understanding of the factors influencing refilling behaviour are necessary if submerged cages are to become a viable option for commercial salmon production. So far, the surface behaviour of Atlantic salmon in submerged sea cages with air-dome is unknown. The aim of this study can therefore be divided into two parts:

First, describe refilling behaviour of Atlantic salmon in submerged sea-cages equipped with air-dome and determine how often salmon refill, and how this varies diurnally and seasonally.

Second, compare and contrast the behaviour of fish in submerged cages to those in standard production cages (3).

By improving understanding of refilling activity this study will help to optimize future application, design and dimensioning of submerged farming of Atlantic salmon.

3. Materials & Methods

3.1. Experimental set-up

The experiment was conducted at the Institute of Marine Research field station, Smørdalen, in Masfjorden, western Norway (~60° N) from September 2019 to June 2020. Approximately 35 505 Atlantic salmon (*Salmo salar,* Aquagen strain) were distributed amongst six cages, 3 submerged and 3 control (Figure 3). The salmon was put at sea in June 2020, and had three weeks acclimation with full surface, before they were submerged.

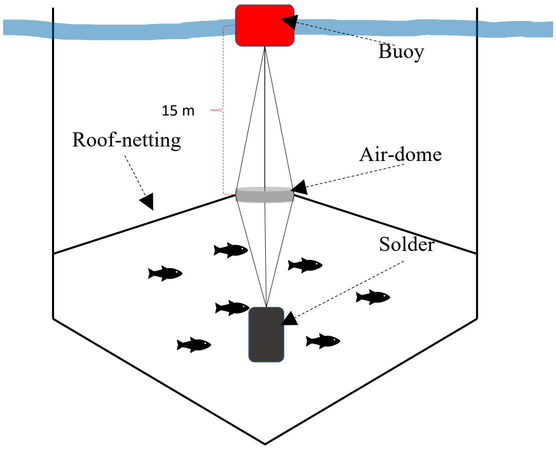


Figure 2 - Sketch of submerged cage with air-dome. Aspect ratio is not correct.

Three submerged cages of approximately 2000 m^3 (12 m x 12 m x 15 m) were used (Fig. 3). The submerged cages had an air-dome installed into the roof netting, which consisted of the same material as the net-pen and was sewn into the net wall (Fig. 2). The air-dome was placed in centre of the cage at 15m depth, slightly beneath the pycnocline. The bottom of the submerged cages was therefore at 30 meters depth. The submerged salmon had no access to air other than within the air-dome. Each submerged cage had a standard compressor (230 V, 2,2 kW, 8 bar) on site with an air hose connected to the dome, ensuring it was continually filled with air. This was done by setting the compressor to maintain a certain pressure in air-dome creating a 0,1m air column. Each dome was held up by a buoy and stabilized by six rigid wires attached to a weight which hung beneath holding the air-dome steady state (Fig. 2). Each cage had a lift-up system to remove the dead fish. In between the submerged cages, there were three standard (12 m x 12 m x 15 m) control cages with continuous surface access (Fig. 4). The surface activity of fish in the control cages was monitored in another parallel study.

Feeding was stopped at least 15 minutes before observing surface activity. Feeding regime varied with season, and thus changed throughout the experiment. In the beginning, feeding was between 06:45 – 15:00 for samples 1 to 11, then from 08:00 – 15:00 for samples 12 to 15, and finally from 05:30 – 15:00 on sample 16. The total amount of feed given over 335 days (from late June 2019 – late May 2020) were 42 603 kg to cage 2, 37 495 kg to cage 4 and 31 357 kg to cage 6. Feeding was adjusted to observed appetite, but always made sure to be surplus to not hinder growth.

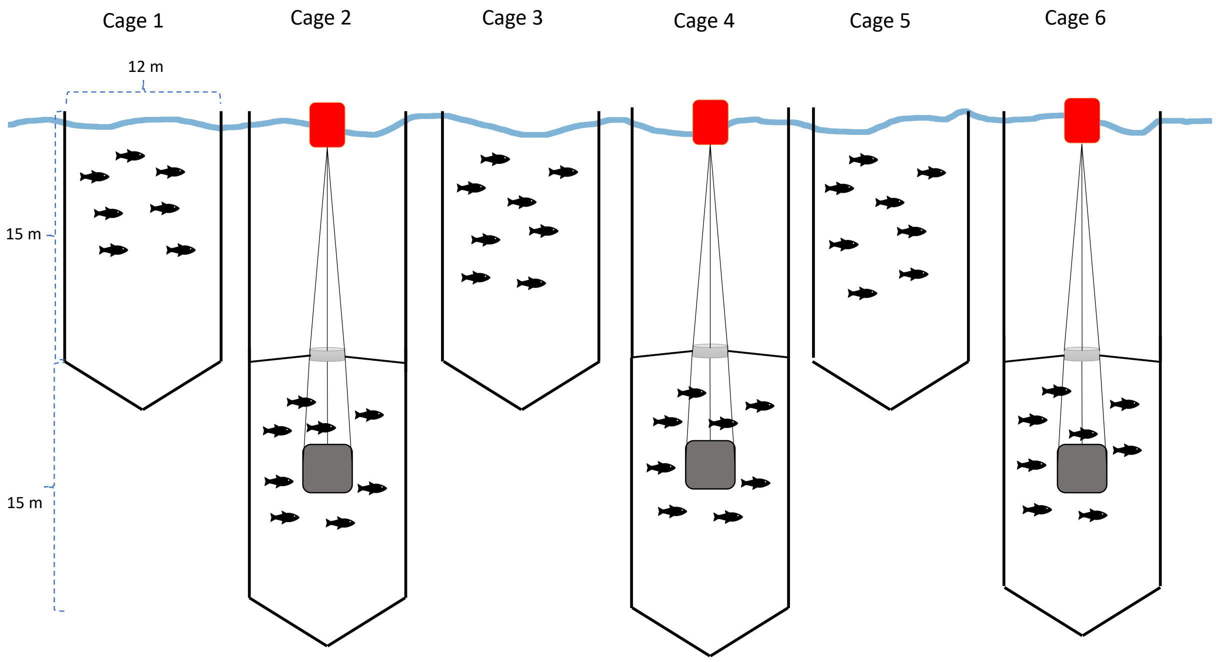


Figure 3 - Experimental setup with submerged cages with air-dome are given by even numbers and controls by odd-numbers

3.2. Observations and sampling regime

Observations of surface within the air-dome were made using a waterproof infrared camera (WCAM-50IR, Smartprodukter Norge AS, Ulsteinvik, Norway) attached to the inner side of the air-dome in each cage. Swimming speeds and schooling behaviour were observed by an adjustable, sub-surface camera (360º pan/tilt Orbit Subsea camera, www.orbitgmt.com). In periods where the camera in dome was not working, the orbit camera was used for all obervations. When using the orbit camera, observations were made 4-5 meters below the dome, with camera facing upwards. Observations from orbit was depending on available light.

Each observation window consisted of a 5-minute recording of water's surface within the air-dome. Five minutes recordings were chosen to optimize value effort, as Furevik et al. (1993) demonstrated that surface activity during 5-min observation windows were highly correlated with the activity measured during 1-hour observation periods (r=0,98, P<0,001, n=12). One complete sample consisted of 9 observation windows per 24-hours for each cage, one every 3 hours. Feeding was stopped at least 15 minutes before each observation window. In total, sixteen samples were made in time period from August 2019 to June 2020 (Table 1).

In addition, environmental data were measured for each sample. As an indicator of buoyancy state, swimming speeds were monitored once each sample at 12:00 pm using the orbit camera. Swimming speeds were calculated in bodylengths per seconds (Bl s^{-1}) by recording the time taken for 30 fish in each cage to swim its own bodylength passed a reference point. Schooling percentage was rated in percentage (0 - 100%), whereas 0% was no schooling behaviour, and 100% was all synchronised.

Table 1 – Sampling regime.

Sample nr	Day	Month	Year	Type	Comment
1	9	September	2019	Standard	
2	11	September	2019	Standard	
3	23	September	2019	Standard	
4	10	October	2019	Standard	
5	21	October	2019	Standard	
6	10	November	2019	Standard	Dome out of air in cage 2
7	22	November	2019	Light-trial	Light on cage 2
8	23	November	2019	Light-trial	Light on cage 4
9	24	November	2019	Light-trial	Light on cage 6
10	21	January	2020	Standard	
11	30	January	2020	Standard	
12	31	January	2020	Light-trial	Lights on all cages
13	1	February	2020	Standard	
14	6	April	2020	Standard	Lights on (21:00-06:00)
15	6	May	2020	Standard	
16	11	June	2020	Standard	

Table 2 - Distribution of 30 000 fish. Control cages got in average approximately 1000 fish less than submerged

	Submerged				Control			
Parameter	**Cage 2**	**Cage 4**	**Cage 6**	**Mean**	**Cage 1**	**Cage 3**	**Cage 5**	**Mean**
n fish at start	6315	6539	6355	6403	6237	3359	6700	5432

3.3. Environmental variables & Artificial light

At a reference point positioned at the outer end of the sea cage facility, a vertically profiling CTD (SD204, SAIV AS, Bergen, Norway, www.saivas.no) connected to an automatic winch (HF5000, Belitronics, Lunde, Sweden) was used to measure salinity, temperature and oxygen levels from 0 – 40 meters depth throughout the experimental period. One profile was taken every 15 minutes. Current speed estimates were collected from weather forecast data (https://www.yr.no/place/Ocean/60.87156_5.52970/), while sunrise and sunset were ascertained from (https://www.timeanddate.com/astronomy/@3146284) for each sample. Light intensities were measured by using a LI-1500 (1.0.0) light sensor placed at surface on site for each sample, measuring light intensities every hour.

As a solution to help salmon find the way to the dome during night-time, the idea to use artificial light came up. The idea was then to facilitate an illumination effect, which would make the dome visible for salmon during darkness, and to avoid salmon swimming straight upwards hitting the net-roof in darkness, searching for the air-dome. Artificial light is also a well-used method to avoid maturation in salmon farming, and it would therefore be interesting to see what effects artificial light would have on surface activity during submergence with an air-dome. The artificial light used (400W, Akvagroup blue LED light) were standard commercial anti-maturation lights. One light per cage were lowered to its maximum depth - 10m, in centre above the air-dome using ropes. The distance to the air-dome was then approximately 5 m.

Two set-ups for light-tests were used: First setup, used for sample 7, 8 and 9, consisted of turning on light in one cage at the time (table 4). Prior to this, the light pollution was measured among neighbouring cage. The light was lowered to 10m depth, then the vertical and horizontal light pollution was measured using the light sensor (LI-1500, 1.0.0, Table 3).

The vertical measurements showed 80-90% decrease in light intensity per meter moved away from the light source: Light was placed at 10 m depth and showed 29,8 µmol photons m^{-2} s^{-1}, at 11 meters the light intensity dropped to 5,2 µmol photons m^{-2} s^{-1} and at 12 m depth light intensity was 0,7 µmol photons m^{-2} s^{-1}. At dome depth (15 m) the light intensity was measured to 0,01 µmol photons m^{-2} s^{-1}.

For the horizontal measurements the light sensor was lowered to 10 meters depth and recorded measures at 0-6 m, 8 m (closest end of neighbour control cage) and at 18-20 m (closest end of neighbour submerged cage). Vertically the light sensor was lowered to 15 meters depth, then measured every meter to surface.

Table 3 - Light pollution measured in micormol photons per square meter per second was recorded before first light trial to determine light pollution to neighbouring cages.

Measure point (m)	Sample 1 - 05.11.2019 (µmol photons m-2 s-1)	Sample 2 - 23.11.2019 (µmol photons m-2 s-1)
0	29,8	247,5
1	7,8	8,9
2	2,5	6,5
3	1	1,8
4	0,5	1,1
5	0,44	0,6
6	0,02	0,03
8	0	0
Neighbour cage (18~20 m)	0	0

Table 4 - Light trial set-up 1. Sample 7, 8 and 9 were conducted three days in a row. Possible light pollution on the control-submerged cages may have interfered with the behavioural response.

Set-up	**Sample**	**Cage 2**	**Cage 4**	**Cage 6**
1	7	Light	Control	Control
	8	Control	Light	Control
	9	Control	Control	Light
2	11	Control	Control	Control
	12	Light	Light	Light
	13	Control	Control	Control

Second set-up, used for sample 12, consisted of two control samples and one sample with use of artificial light (Table 4). One light per cage were placed at 10m depth, in centre above the air-dome. Different from set-up 1, all lights were turned on at the same time, eliminating the light pollution.

Artificial light was introduced permanently for submerged salmon during night-time (21:00-06:00) from March 2020 (Table 1). Standard samples for this trial did not include use of artificial light, so lights had to be turned off before sampling after sample 13. In sample 14, the lights were not turned off during night-time due to failure in communication, and the night-observations were therefore with use of artificial light.

3.4. Behavioural classifications

Rolling is described by Furevik et al. (1993) to be when the fish breaks surface with the head followed by the dorsal side upwards, making a bow, aiming down towards the water. The roll looks like a whale breaking surface for breathing. Occasionally rolling can be observed by fish barely breaking the surface with the dorsal fin and part of the back (Furevik et al. 1993). Leaping is described by Furevik et al. (1993) to start at 1-3 m depth, with the fish swimming horizontally, followed by upwards acceleration at a 30-40 angle to horizontal plane. The swimming speed increased 5-10 times from the normal cruising speed before the fish broke the surface (Furevik et al. 1993).

Two examples of the two refilling behaviours observed with camera in air-dome is shown chronological from left to right in Figure 4: rolling (Fig. 4A) and leaping (Fig. 4B).

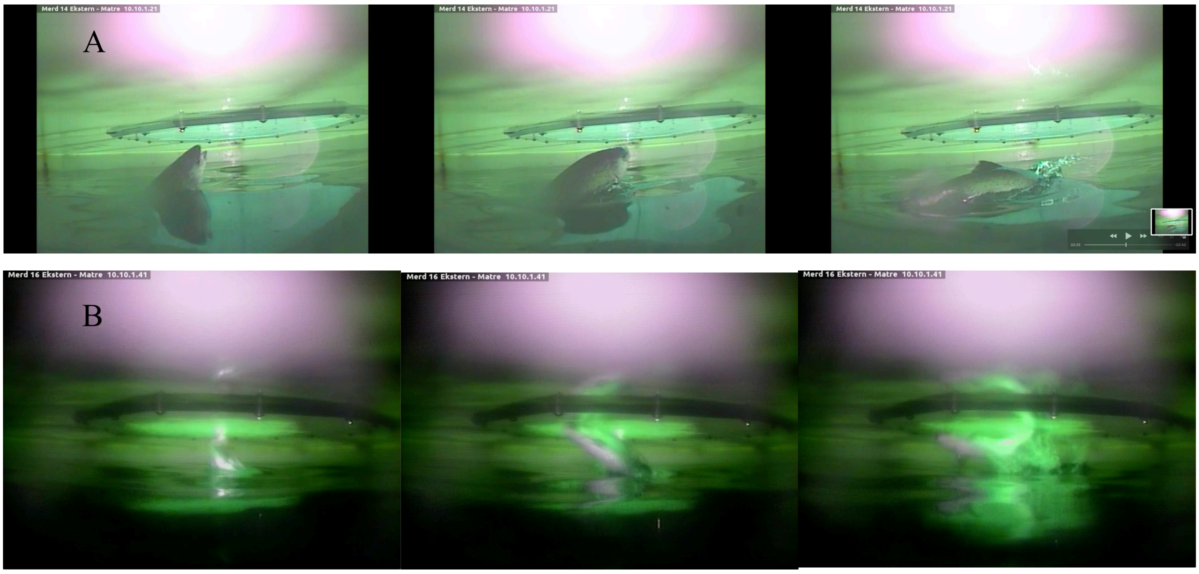


Figure 4 - A) A roll observed from left to right: The fish breaks surface with the head followed by the dorsal side upwards, making a bow, aiming down towards the water. B) Leaping observed from left to right. The fish breaks surface with high speed, hits the roof of dome, turning sideways and falls down again.

3.5. Statistical analyses

During data exploration, following protocol described in Zuur, Ieno, and Elphick (2010), outliers and distinct patterns in refilling activity were identified. One biased observation was removed to avoid statistical problems for interactions.

Refilling activity and swimming speeds were series of repeated observations for each sample. All statistical tests were performed in Microsoft Office 365 excel, and R (RStudio version 1.2.5001). Figures and tables were also made in Microsoft Office 365 excel, and R (RStudio version 1.2.5001).

To compare refilling activity between the three submerged cages, a single factor ANOVA was used on the refilling activity per fish from each cage.

To compare growth performance of the submerged fish to those in control, a t-test was used to compare if average weight of submerged and control fish were the same throughout the experimental period. Correlation was used to find trends of refilling activity with key factors, average weight of fish and days into the experiment.

To compare refilling activity with and without artificial light, a t-test was used to see if refill activity during standard samples close to light trials and samples with artificial light were the same. The same method was done to see if refilling activity varied with hours since feeding. The t-test then compared if refilling activity during hours since feeding (09:00-15:00) were the same as hours after feed had ended (18:00-06:00). A t-test was also used to see if swimming speeds were the same from start to end of the experiment. The t-test compared the measured swimming speeds from the three first samples and the three last. This however only compared two points and did not show a decrease throughout the period. To account for this, the correlation with days into the experiment was calculated.

4. Results

Total of 420 observations were made throughout the experiment, where 25 were not successful due to poor visibility in dome-camera or technical breakdown.

Table 5 - Production data on number of fish per cage, number of dead fish, growth and average swimming speed. Initial values are from fish were put at sea in June 2019. Final values are from last data collection in June 2020.

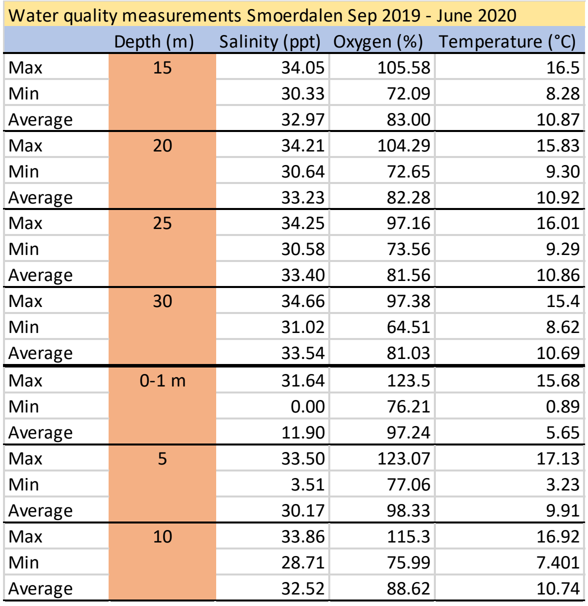
	Submerged				Control			
Parameter	**Cage 2**	**Cage 4**	**Cage 6**	**Mean**	**Cage 1**	**Cage 3**	**Cage 5**	**Mean**
n fish at start	6315	6539	6355	6403	6237	3359	6700	5432
n fish dead	839	709	774	774	474	447	439	453
Dead (%)	13%	11%	12%	12%	8%	13%	7%	8%
Initial weight (g)	254	206	201	220	239	239	213	230
Final weight (g)	3649	2886	3050	3195	5181	5955	5576	5571
Average swim speed (Bl s-1)				0,668				0,703

From September 2019 to June 2020 average weight of submerged salmon increased from 467,6 ± 76,02 g (mean ± SE) to 3195 ± 401,6 g, and had significant less growth compared to the control fish which increased average from 577 ± 177 g to 5571 ± 1614 g *(n=21, t-Stat (4,61) > t-Critical (3,84),* $P(T \leq t)$ *= 0,00016).* Average mortality rate in submerged cages was 12%, while the control cages averaged 8% (Table 5). Salmon in submerged cages swam approximately with same swimming speed as the control with average swimming speed of 0,668 Bl s^{-1} but was in average 0,035 Bl s^{-1} slower (Table 5). The salmon were observed to swim extremely tight in all cages. Observed schooling percentage increased from 90% to 99% throughout the experimental period.

4.1. Environmental data

Light intensities averaged at the test facility in Smørdalen from 0 – 1779,9 µmol photons m^{-2} s^{-1}. Highest light intensity was measured 12:00 p.m. in June (sample 16), probably due to direct sunlight. Daylength ranged from shortest 6,5 hours (sample 9, late November) to longest lasting 19 hours (sample 16, early June). The salinity at 15 m and 30 m depth differed in average 0,57 ppt throughout the experiment (Table 6). Currents ranged from 1- 5 cm/s and oxygen levels variated throughout the experiment from 100 % to below 75 % saturation (15 – 30 m depth). Down at 30 m; oxygen levels were recorded as low as 64,5 % saturation, whereas lowest oxygen at 15m was about 75% saturation. The average oxygen level was 82 % from 15-30 m

Table 6 - Summary of water quality measured during the experimental period in Smørdalen. Submerged cages were placed at 15 - 30 m depth, whereas control cages 0 - 15 m



Water quality measurements Smoerdalen Sep 2019 - June 2020

	Depth (m)	Salinity (ppt)	Oxygen (%)	Temperature (°C)
Max	15	34.05	105.58	16.5
Min		30.33	72.09	8.28
Average		32.97	83.00	10.87
Max	20	34.21	104.29	15.83
Min		30.64	72.65	9.30
Average		33.23	82.28	10.92
Max	25	34.25	97.16	16.01
Min		30.58	73.56	9.29
Average		33.40	81.56	10.86
Max	30	34.66	97.38	15.4
Min		31.02	64.51	8.62
Average		33.54	81.03	10.69
Max	0-1 m	31.64	123.5	15.68
Min		0.00	76.21	0.89
Average		11.90	97.24	5.65
Max	5	33.50	123.07	17.13
Min		3.51	77.06	3.23
Average		30.17	98.33	9.91
Max	10	33.86	115.3	16.92
Min		28.71	75.99	7.401
Average		32.52	88.62	10.74

depth (Table 6). Average temperature differed 0,18 °C from 15 m to 30 m depth. At 15 meters depth temperature decreased from 16,5 ºC to 8,3 ºC during the experimental period (Figure 5). Refilling activity decreased when temperatures went below 10 ºC.

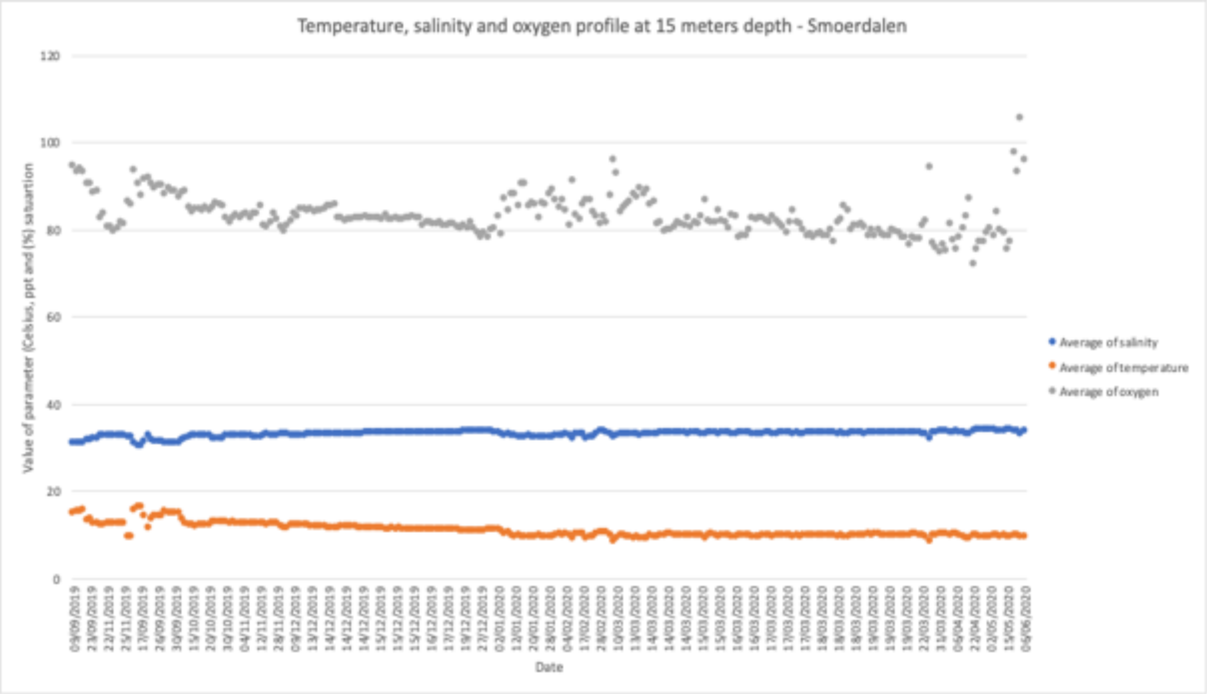


Figure 5 - Temperature, salinity and oxygen measurements at 15m depth throughout the experimental period in Smørdalen. Grey dots are oxygen saturation (%), blue dots are salinity measured in parts per trillion (ppt) and orange dots are temperature measured in celcius degrees.

4.2. Refilling behaviour

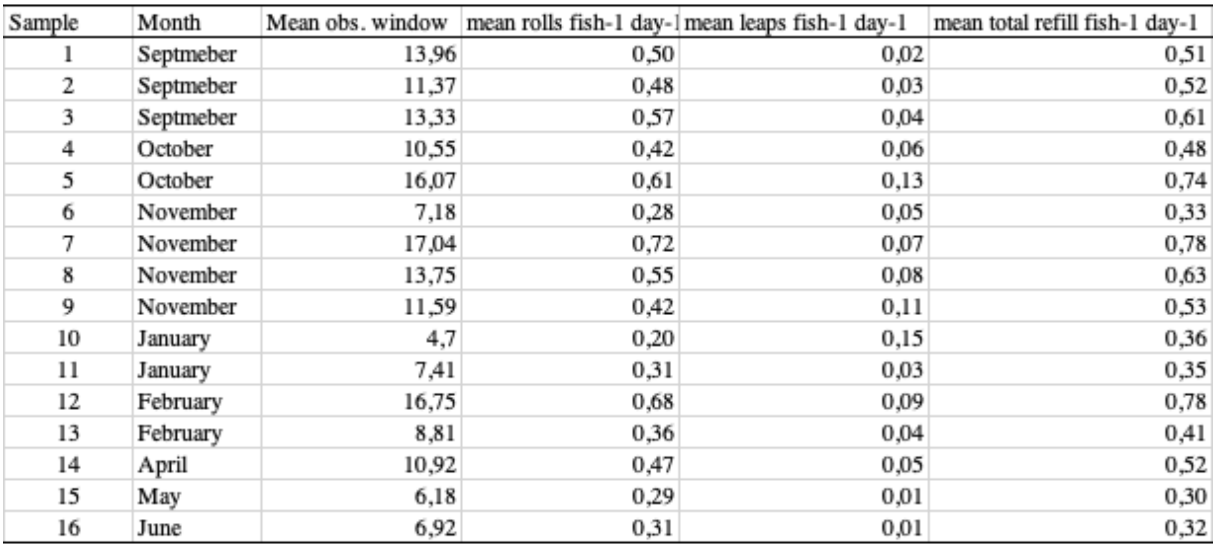
Two types of refilling behaviour were observed in submerged cages with air-dome through the experimental period: rolling and leaping. No fish were observed "grasping" for air by tilted swimming at the surface as previously observed by Korsøen et al. (2009). A third surface behaviour, possibly described earlier by Korsøen et al. (2012) as a surface searching behaviour, was observed whereby 5-10 salmon were hovering at the water's surface within the dome during night-time. This behaviour was evaluated to not be a refilling activity, because only the dorsal fin and parts of the back were constantly above water. The hovering behaviour was observed during night-time in all cages and samples except during when artificial light was in use.

The roll was recognised to be as Furevik et al. (1993) described it; fish breaks surface with the head followed by the dorsal side upwards, making a bow, aiming down towards the water (fig. 4A). Some variation in execution, where the salmon barely broke surface before aiming down towards the water was observed. Then only the anterior part; jaw and nose, and the dorsal fin broke surface.

Leaping was also observed, and recognised by its speed, distance and by the fish hitting the roof and wall of dome (Fig. 4B). More than half of the 420 observation windows did not contain a single leap. Observed with orbit camera; leaping started from right under the dome (approximately 0,5m - 1m), accelerating often back and forth, before aiming upwards and breaking the surface. Leaping was often repeated, e.g., 3 leaps in a row, by the same salmon before diving down to the school again. How often salmon hit the roof or wall of dome while leaping was not registered but was frequently associated with leaping. The whole body of the salmon did not always break the surface during a leap.

4.3. Refilling activity

Table 7 - Refilling activity per sample and month. The column mean observation window show the average refills observed per observation window in that sample. The mean rolling and leaping activity is showing the calculated level of activity per fish per day.



Sample	Month	Mean obs. window	mean rolls fish-1 day-1	mean leaps fish-1 day-1	mean total refill fish-1 day-1
1	Septmeber	13,96	0,50	0,02	0,51
2	Septmeber	11,37	0,48	0,03	0,52
3	Septmeber	13,33	0,57	0,04	0,61
4	October	10,55	0,42	0,06	0,48
5	October	16,07	0,61	0,13	0,74
6	November	7,18	0,28	0,05	0,33
7	November	17,04	0,72	0,07	0,78
8	November	13,75	0,55	0,08	0,63
9	November	11,59	0,42	0,11	0,53
10	January	4,7	0,20	0,15	0,36
11	January	7,41	0,31	0,03	0,35
12	February	16,75	0,68	0,09	0,78
13	February	8,81	0,36	0,04	0,41
14	April	10,92	0,47	0,05	0,52
15	May	6,18	0,29	0,01	0,30
16	June	6,92	0,31	0,01	0,32

A total of 4123 rolls and 480 leaps were observed throughout the experiment. The total average refilling activity was 0,50 ± 0,02 refills fish^{-1} day^{-1}. Average rolling activity was 0,46 ± 0,14 rolls fish^{-1} day^{-1} and average leaping activity 0,05 ± 0,03 fish^{-1} day^{-1} (Table 7). Rolling was significantly more common than leaping *(n = 16, t-Stat(11,5) > t-Critical (4,07),* $P(T \leq t) = 7{,}51 \times 10^{-9}$*, α = 0,001)* and dominated refilling activity. Thus, there was a strong correlation between rolling and total refilling activity (corr = 0,98, Fig. 6).

The refilling activity varied between samples ranging in average from highest 0,74 refills fish^{-1} day^{-1} in October to lowest 0,30 refills fish^{-1} day^{-1} in May, without use of artificial light. Total refilling activity correlated weakly negative (corr = -0,15) with number of days into the experiment and weight (corr = -0,1) showing no clear pattern given season or fish size throughout the experiment (Fig. 7). The vast majority (n = 411) of 5-minutes observation windows included fewer than 40 total refilling events. However, unusually high refilling activity (up 60 refills) was observed in 3 observation windows during autumn 2019 (Fig. 7), and in 5 observation windows during light trials (sample 7 and 9). After November 2019, no observations of unusual high refilling activity were made. This trend occurs together with the drop of water temperature which declined below 10ºC between sample 9 and 10. No observation window exceeded 30 refills with water temperatures below 10 ºC. Maximum refilling activity was observed at 12,4 ºC during period with use of artificial light. At maximum temperature (15,5 ºC) one observation window exceeded 50 refills, whereas an additional 4 exceeded 30 refills.

The average refill activity per cage ranged from 0,47 refills fish^{-1} day^{-1} in cage 2 to 0,55 refills fish^{-1} day^{-1} in cage 4. A single factor analysis of variance on the average refilling activity per fish showed that differences between cages were not significant (*F(2, 417) = 0,78 (F-Critical = 3,01), P = 0,455 (α = 0,05)).*

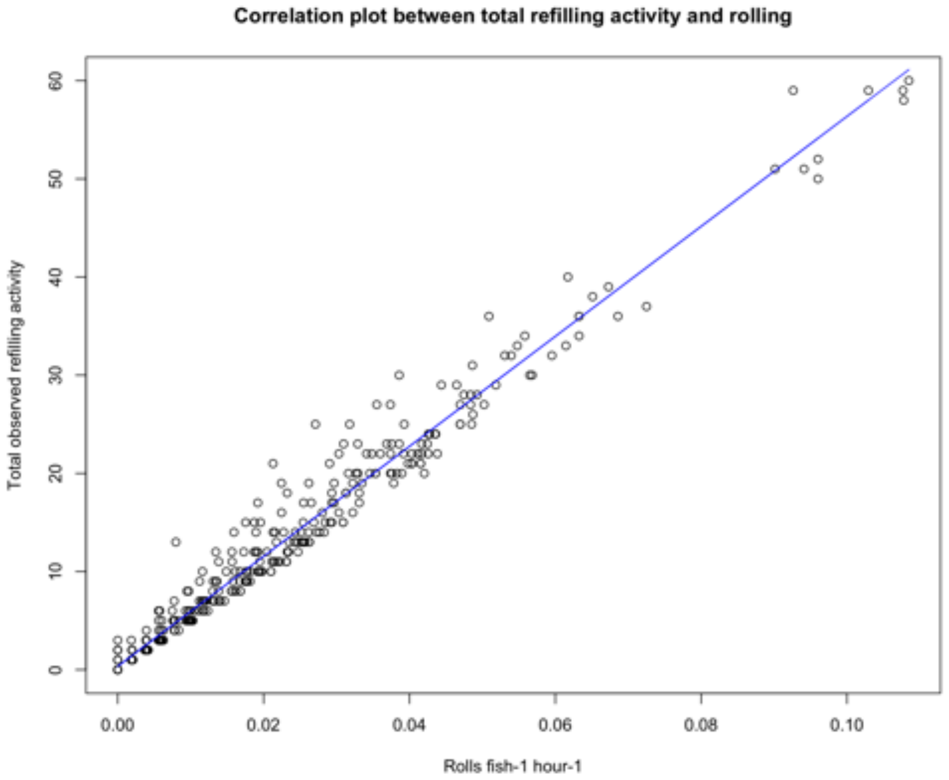


Figure 6 - Rolling activity per fish per hour on x-axis, total observed refilling on y-axis show that rolling and total refilling correlates strongly (corr = 0,98). Each dot represent an observation (n = 420).

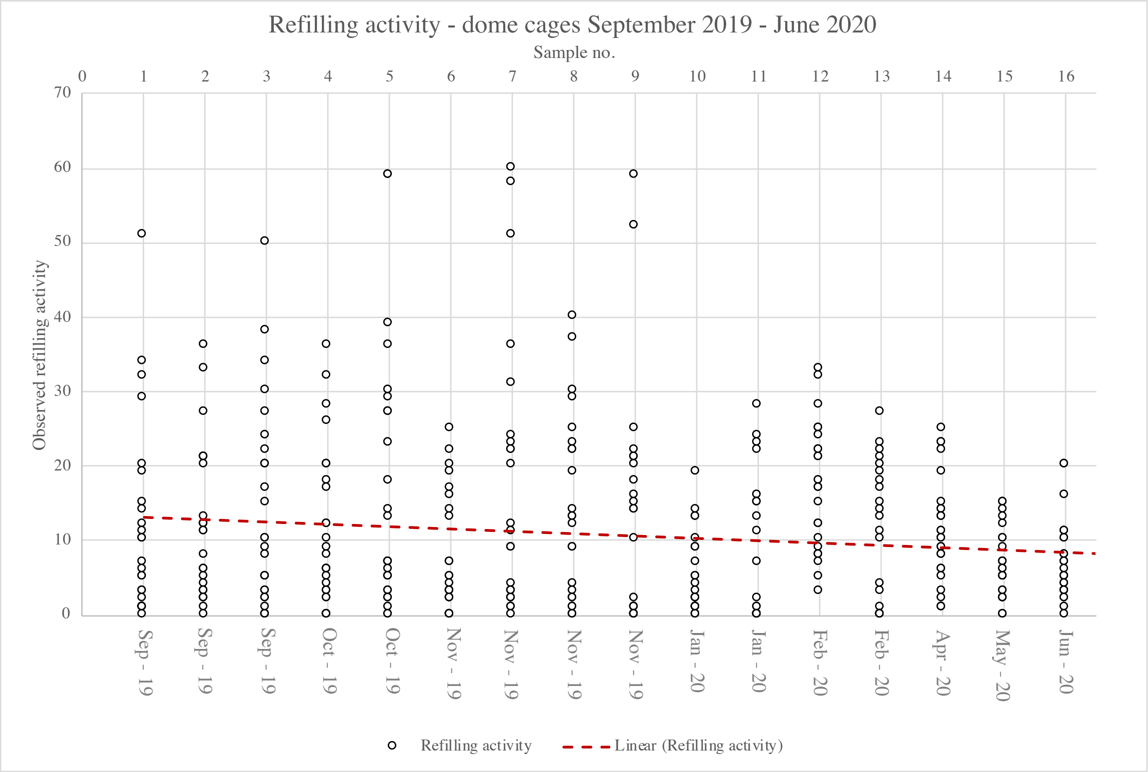


Figure 7 - Observed refilling activity) given time(bottom x-axis, month – year) and sample number (upper x-axis). Each dot represents an observation (n=420). Red dotted line shows the linear regression based upon observations. There were no observations above 30 refills after sample 9, except for during sample 12, which was with use of artificial light.

4.4. Diurnally trends

Refilling activity peaked midday and was lowest at night-time between 21:00 – 06:00 (Fig. 8). Both rolling and leaping displayed this trend given time of day, which was consistent through the whole experimental period, but became less clear after sample 9 where activity decreased especially midday (< 30 refills per observation window, Fig. 7). Daylength changed from 6 hours and 50 minutes in January (sample 10) to 19 hours and 10 minutes in June (sample 16), whereas average refilling activity were approximately the same with 0,32 to 0,36 refills fish^{-1} day^{-1}, showing that daylength neither seemed to drive peaks of surface activity nor the general number of refills per day. Same level of average refill activity, without an observation window with over 30 refills, was also observed in November, during sample 6 with 0,33 refills fish^{-1} day^{-1} and daylength of 7 hours and 50 minutes (Table 7, Fig. 7). Compressor in cage 2 did however fail during sample 6, which led to no air in the dome from 12:00. Salmon were, however, observed to refill air-column below 10 cm.

Refilling activity was also to be influenced by time since feeding (Fig. 9). Shortly after feeding (0,25 hours), refilling activity was significantly higher than 3,25 - 15,25 hours after feeding (Fig. 10) *(Welch two sample t-test:* $P(T \geq t) = 2,2 \times 10^{-16}$ *,* $\alpha = 0,001$*)*. Feeding regime followed available light, thus correlated negative (corr = -0,31) with light intensity. For the samples not exposed to artificial light, the refilling activity was significantly higher during hours with natural light at surface (light-intensities > 1 µmol photons m^{-2} s^{-1}) *(n = 120, t-Stat(9,72) > t-Critical(3,37),* $P(T \leq t) = 8,46 \times 10^{-17}$*,* $\alpha = 0,001$*).*

Light intensity above 1,0 µmol photons m^{-2} s^{-1} was observed 12 times during 06:00; in September, April, May and June (Sample 2, 3, 14, 15 and 16). The five observations with ≥ 20 observed refilling events at 06:00 were observed in September where feeding started 06:45.

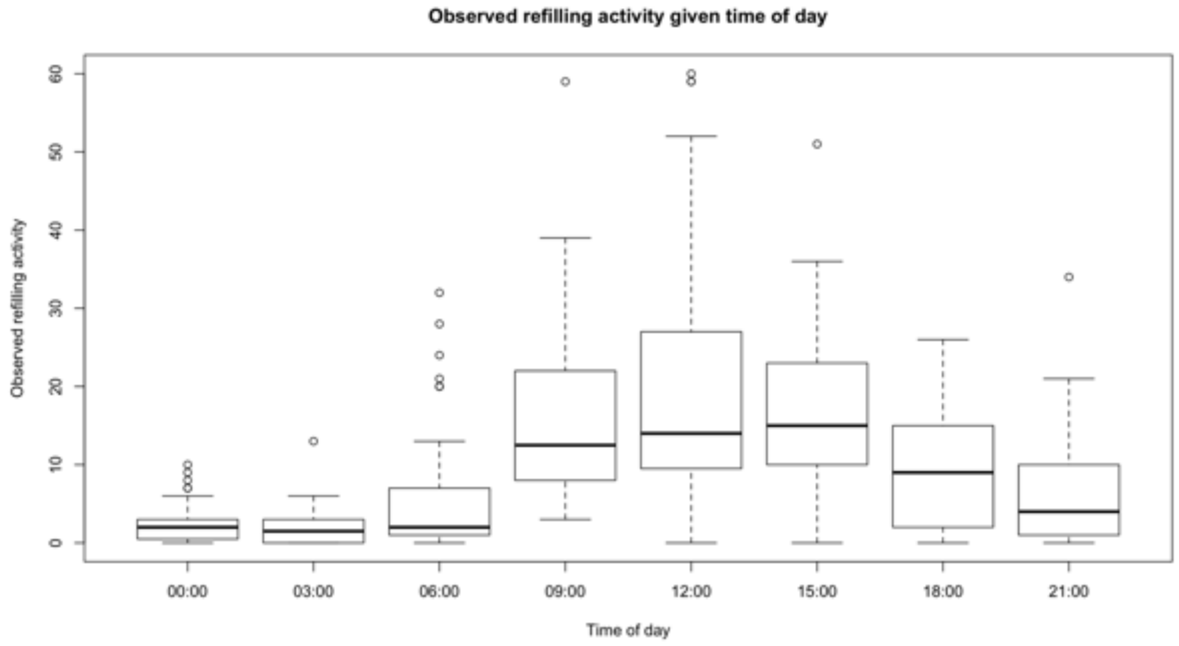


Figure 8 - Observed refilling activity given time of day during samples without use of artificial light. The results

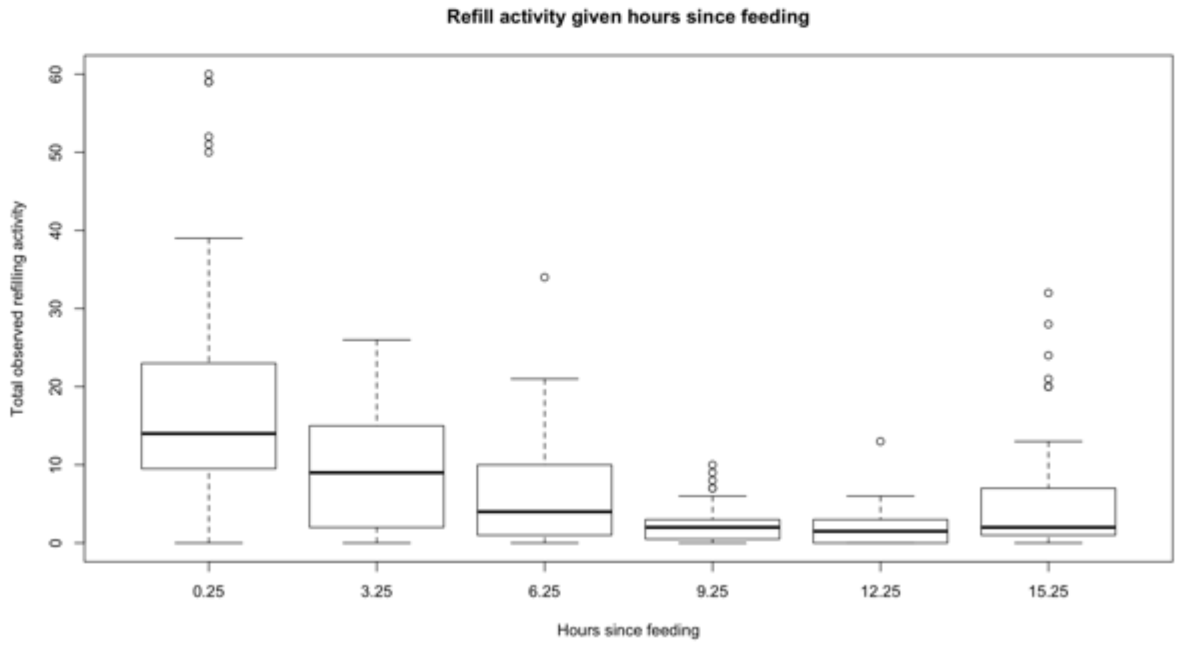


Figure 9 - Refilling activity given hours since feeding. Hours 09:00-15:00 = 0.25, 18:00 = 3.25, 21:00 = 6.25, 00:00 = 9.25, 03:00 = 12.25 and 06:00 = 15.25.

4.5. Swimming speed

Swimming speed correlated negatively with days into the experiment (corr = -0,64) and decreased significantly *(t-Test: Paired two sample for means, n = 120,* $P(T \geq t) = 3{,}22 \times 10^{-39}$*,* $\alpha < 0{,}001$*)* from September 2019 with average 1,03 ± 0,23 Bl s^{-1} to June 2020 with average 0,63 ± 0,1 Bl s^{-1}. Swimming speed also showed a negative correlation of -0,73 with fish-weight (Pearson product-moment correlation). Fastest swimming speed was observed September 2019 with observations up to 1,2 Bl s^{-1} (sample 2) and slowest in May 2020 with below 0,5 Bl s^{-1} (sample 14). No extraordinary swimming behaviour was observed. Analysis of variance (Single factor: ANOVA) showed that there were no significant differences between cages in swimming speed (*F(2, 537) = 0,604, (F-Critical = 6,997), P = 0,546)*.

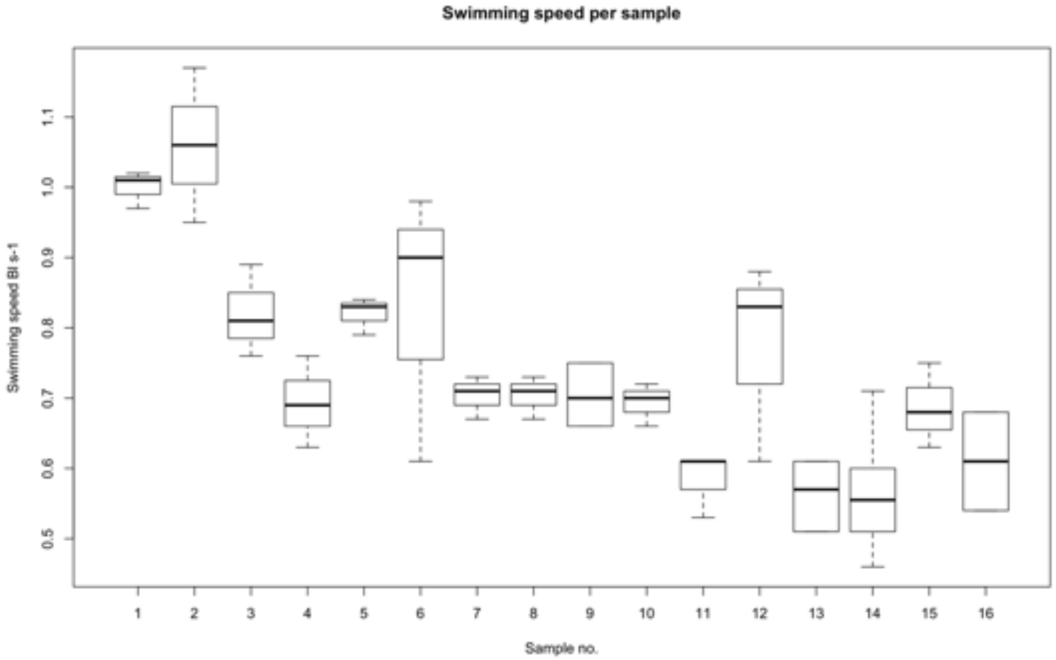


Figure 10 - Swimming speed, in bodylength per second, given sample.

4.6. Light trials

The addition of artificial light increased refilling activity significantly *(*$P(T \leq t) = 2{,}7 \times 10^{-5}$*,* $\alpha = 0{,}001$*)*, mainly observed during night-time (00:00-03:00) (Fig. 11) making the diurnal trend less clear. However, even with artificial light, refilling activity was still highest during daytime (09:00-15:00). In particular, artificial lights had an effect on leaping activity, which increased significantly from an average of 0,89 ± 1,8 leaps per observation without lights to 2,86 ± 2,5 leaps per observation window with lights present *(n = 46,*

$P(T \leq t) = 0{,}0001$). The average refill activity with artificial light range from 0,53 refills fish^{-1} day^{-1} to 0,78 refills fish^{-1} day^{-1}.

With use of artificial light, the refill activity given hours since feeding pattern weakened (Fig. 11). The night-time activity (at 9,25 and 12,25 hours) did not drop to zero with use of artificial light and the small increase at 15,25 hours are not present (Fig. 12).

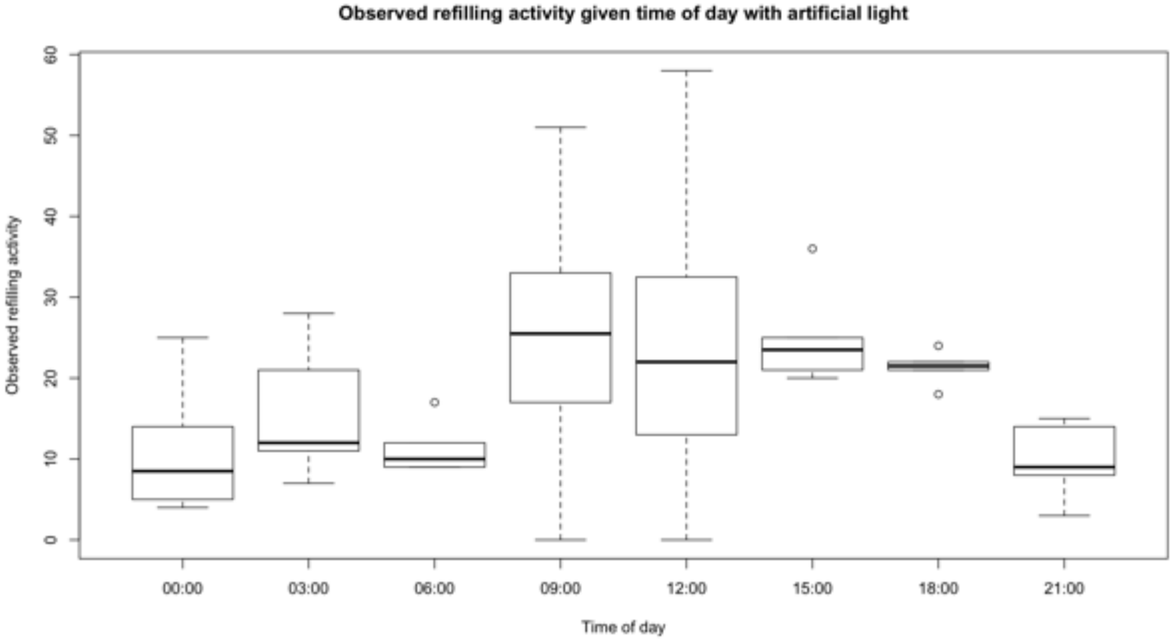


Figure 11 - Refilling activity given time of day during use of artificial lights.

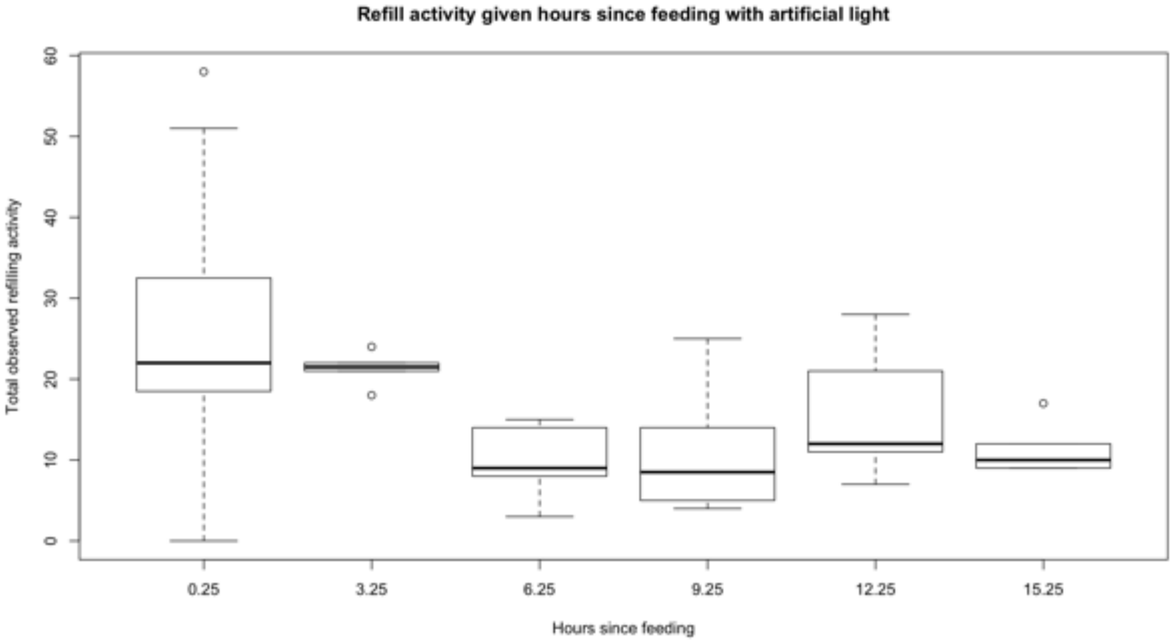


Figure 12 - Refilling activity given hours since feeding during use of artificial lights.

5. Discussion

Generally, the observations indicate that submerged salmon in all three cages were synchronised in both refilling behaviour and activity in an eight squared air-dome (Ø = 2,5 m, h = 0,1m) at 15m depth, suggesting a common environmental signal that drove their behaviour. The submerged salmon were able to maintain neutral buoyancy with swimming speeds ranging from 1,03 ± 0,23 Bl s^{-1} to 0,63 ± 0,1 Bl s^{-1} throughout the whole experiment.

However, the submerged salmon had concerningly less growth and higher mortality compared to the control group (Table 5), which indicate lower welfare.

5.1. Experimental set-up and observations

A lot of crushed pellet was observed in the opening of the 12m pipe which led the feed down to the fish in all submerged cages. This may affected the growth of submerged salmon, but no evidence for this was measured. A severe amount of pellet was observed to be pumped up by the lift-up, indicating that feeding was surplus and successfully led down to the submerged salmon. Based on these observations, submerged cages might require a higher featured feeding method compared to standard cages to ensure desired growth and quality of production.

Submerged salmon were observed to swim extremely tight, especially as they grew bigger. Stocking densities exceeding 26,5 kg/m^3 have shown to reduce feed intake, growth and feed utilization (Oppedal et al. 2011). The submerged cages did not exceed such limits following average fish weights, number of fish and a volume of 2000 m^3. The roof netting hung from the dome to the sides the net-pen, and the six rigid wires together with the solder led to some lost volume in the submerged cages. This may have increased the stocking density beyond thresholds at specific times and places in submerged cages, leading to reduced feed intake.

With merely 6% of the observation windows not successful, the experimental set-up was considered to have worked well. At the same time, there is room for improvement. Six of the not successful observations were due to loss of electrical power, whereas the electrical supplier performed maintenance work. Four observation windows were lost due to compressor failure, which led to no air in the air-dome. The rest of were due to growth of algae on camera, which led to very low visibility during night-time. A lot of extra time were spent looking at blurry recordings. No observation longer than 5-minutes was made to evaluate if the length of the observation windows correlated in air-domes. To ensure improved data quality, observations over one hour should be made in air-domes to see if 5-minutes observation window correlates, following Furevik et al. (1993) method on this.

The placement of camera in air-dome made some dead-angles, which may have led to missing out on some activity. How this affected the results is unknown, but considered to be limited whereas observation windows made using the orbit camera did not differ severely from the ones made with the camera in dome.

5.2. Refilling behaviour

While both of the refilling behaviours typically observed in standard cages were seen, the dominance of rolling suggests that the salmon were not able to behave normally within the confines of the air-dome structure. Compared to the control cages, where rolling and leaping occurred at similar levels, leaping in the air-dome was relatively vague with merely 10% of the total refill activity. It is therefore suggested that the air-dome did not provide sufficient space for leaping behaviour, whereas several samples went nearly without observing a single leap (Table 7).

The leaping behaviour was mostly recognised by its speed, distance and hitting the roof and wall of air-dome (Fig. 4B). Based on some of the impacts with the dome, leaping was considered as possibly harmful, but no casualties were observed due to this. Further studies should investigate the specific consequences of leaping and dome height to determine how air-domes can meet the behavioural demand from the salmon.

Observations made using the orbit camera showed salmon accelerating back and forth under the air-dome. This behaviour was linked to leaping as the acceleration which takes place before the leap (Furevik et al. 1993). Some salmon were also observed to complete a leap after accelerating back and forth. The limited area beneath the dome seemed to be an obstacle for leaping behaviour, pushing the salmon to increase the angle to the horizontal plane to avoid crashing into the net-wall/roof. This may have pushed the salmon to leap with greater vertical speed, increasing the risk of hitting the roof of dome. Salmon may have experienced increased stress levels as a result from this limited environment. Increased stress levels due to limitations of environment have shown to reduce appetite (Tim Dempster et al. 2009; Noble et al. 2018), which may be a reason for the reduced growth observed in the submerged cages. Specific consequences due to limited leaping behaviour is however unknown and should be studied further to evaluate the importance of facilitating this behaviour.

Some leaping was, however, observed showing that it was possible in an air-dome. Similar to observations in standard cages, leaping were performed multiple times in a row by the same fish (Glaropoulos et al. 2019; Furevik et al. 1993; T. Dempster et al. 2011). This indicate that some salmon may have learned to leap in an air-dome while others not. Individual differences in ability to perform surface activity in air-domes have been observed before, and to be decreasing with size of air-dome (Nilsson et al., n.d.). It is therefore likely that a greater air-dome may lead to greater level of leaping activity.

The rolling behaviour in an air-dome was recognised to be performed in the same manner as in standard cages, which is different from what Korsøen et al. (2012) observed in a smaller air-dome. Supported by Nilsson et al., (n.d.) findings, this study suggest that increasing the size of air-dome further may facilitate normal surface behaviour.

The salmon was observed to barely break surface with only the jaw and nose, followed by the dorsal fin barely "touching" the surface. This behaviour have been observed before in a smaller dome (Korsøen et al. 2012), indicating that this behaviour may be linked to exploring or learning to use the air-dome. Similar behaviour have also been observed in standard cages as variations of rolling behaviour (Furevik et al. 1993). Nevertheless, with the increasing the diameter of dome, from 1m to 2,5m, both rolling and leaping were observed, though in different levels of ratio aspects compared to standard cages (Glaropoulos et al. 2019; Yuen, Oppedal, and Oldham, n.d.) and with leaping as potentially harmful for the salmon.

The surface hovering behaviour, which also possibly have been described earlier by Korsøen et al. (2012) seemed to be linked to the disperse schooling behaviour which occurs during night-time/darkness in net-pens (Juell 1995; Johansson et al. 2006). The hovering behaviour was not observed during light-trials which strengthens the evidence for this theory, whereas artificial light have shown to remove disperse schooling during night-time (Sievers et al. 2018; Oppedal, Dempster, and Stien 2011).

5.3. Refilling activity

5.3.1. General trend

The refilling activity in submerged cages was considered to keep a persistent level, with variation between samples. This differs from refilling activity in the control cages, which decreased throughout the experiment and showed to vary with several environmental factors. The submergence of salmon beneath the pycnocline, are therefore suggested to cause less change in refilling activity. Changes in surface activity have also earlier been linked to environmental change (Oppedal, Dempster, and Stien 2011). Submerged salmon therefore tend to have a more stable pattern in refilling activity compared to standard cages. However, tops of refill activity seemed to decrease with temperature and fish weights. Observation windows with high refilling activity were non-existent when temperatures went below 10ºC and average fish weights above 2,5 kg. Fish are generally more active at higher temperature due to increased metabolism and physiological function (Jobling 1981; Johansson et al. 2009; Juell 1995). The lipid content of salmon is showed to increase with fish weight, making the body density less dense with increased weight (Macaulay et al. 2020). With decreasing temperature and increasing fish weights, the tops of activity are therefore suggested to decline, while the general level of activity remained (Table 7, Figure 7), allowing swimming speeds to decrease.

The refilling activity averaged in total with 0,5 ± 0,024 refills fish^{-1} day^{-1} ranging from average 0,3 – 0,78 refills fish^{-1} day^{-1}, indicate that submerged salmon refill every other day, which is less compared to the control which ranged from the highest 4,7 refills fish^{-1} day^{-1} to lowest 1,3 refills fish^{-1} day^{-1} (Yuen, Oppedal, and Oldham, n.d.). However, the refilling activity was similar to Korsøen et al. (2012) findings which averaged 0,45 – 1,4 refills fish^{-1} day^{-1}. One important factor for comparison with Korsøen et al. (2012) is the consideration of observation period diurnally, whereas they did not monitor refilling activity during night-time. Average refilling activity using only observation windows during daytime (09:00-18:00) ranged from 0,24 – 3,1 refills fish^{-1} day^{-1} in this study. Comparing results with Korsøen et al. (2012) suggest that both refilling behaviour and level of refilling activity tends towards normal behaviour with increased size of air-dome.

In evaluating continuously submergence, it is important to consider the cost benefits. Results from this study show a concerning picture considering growth compared to the control cages. Future submergence of salmon may probably go deeper than 15m to completely avoid sea lice. With increased depth, the pressure from surrounding water increases, shrinking the swim bladder. How smolts will cope with greater depth compared to larger salmon is unknown. The maximum neutral buoyancy depth (hereinafter referred to as MNDB) of farmed Atlantic salmon were studied in four different size groups, using an increased excess mass test. The hypothesis stated that larger salmon, due to less body density (more fatty tissue), allows a greater MNDB than smaller salmon (Macaulay et al. 2020). Their results suggested an average MNBD of farmed Atlantic salmon in seawater of 22,8 m, ranging from 18,3-31 m. They also found a significant difference between fish sizes of 175 g to 2400 g (175 g mean MNBD = 21,2 m, 2400 g mean MNBD = 24,4 m). They therefore point out size group and body density as important predictors for MNBD. One may therefore expect that 2400 g salmon refill less than 175 g due to less body density and also use less energy staying at preferred depth due to higher MNDB. Following the theory of MNDB, one might suggest that salmon greater 2,5 kg are more fitted for a submerged life.

5.3.2. Diurnal trend

Salmon exhibited a diurnal rhythm in refilling activity, which was present throughout the whole experiment. Refilling activity were at its highest during daytime, peaking at noon, indicating a pattern given light intensity. The refilling activity decreased to a very low level during night-time when no light was available. One theory for this was the lack of vision during night-time, whereas the dome was not visible during darkness. A diurnal rhythm following light intensities was also observed in the control cages, whereas activity peaked at dusk and dawn (Yuen, Oppedal, and Oldham, n.d.). This differs from the trend at the submerged cages, indicating an effect caused by the depth. Considering the physiological aspect, there is no indication, however, that light intensity should directly affect the need for buoyancy regulation. It is therefore logical to suspect factors, collinear to- or light dependent, possibly caused this trend. The results point out two factors: feeding and schooling pattern, which varied with light intensity and thereby possibly affected the diurnal rhythm in refilling activity.

Feeding events took place during periods when light was available. Surface activity have previously been described as hunger dependent, and increasing during feed events (Juell 1995). Swimming speeds have also been observed to increase during feeding, inducing release of air from swim bladder (Oppedal, Dempster, and Stien 2011). In addition salmon have also been observed to release gas bubbles and dive after eating pellets during feeding (Bui et al. 2013). Feeding may therefore lead to negative buoyancy, which stimulates to increased refill activity (Glaropoulos et al. 2019; Korsøen et al. 2009). The observed refilling activity gradually decreased with hours since feeding. Then, right before feeding started again, refilling activity increased. Feeding is therefore suggested to increase refilling activity in submerged cages together with light, which also became available at dawn. Light have also showed to increase swimming speed and thus activity level of salmon (Frenzl et al. 2014), which may have caused the salmon to release air from the swim-bladder. A combination of the two factors is therefore suggested to stimulate refilling activity during submergence. The combinational factor can also explain peaks of refilling activity whereas salmon are observed to release air and thus assembling negative buoyancy. Since negative buoyant salmon have shown to refill rapidly (Glaropoulos et al. 2019) and salmon tend to maintain neutral buoyancy to optimise fitness (Kryvi and Poppe 2016), feeding and increased level of activity are suggested to drive peaks of refilling activity.

Observations windows during night-time included a low amount of refill events, and all of the observed surface hovering behaviour. This indicate that the submerged salmon dispersed and changed schooling pattern during night-time. Intense refilling activity at dusk, which allows salmon to swim slower at neutral buoyancy, have been observed and linked to this schooling pattern in standard cages (Tim Dempster et al. 2009). The intense refilling activity right before dusk was however not observed in the submerged cages, which could indicate that submerged salmon maintained nearly neutral buoyancy at all times, and swam at same depth, releasing no air after feeding. It could also indicate that the school got split in two during night-time where one part swam slow, and the other swam with modified swimming behaviour due to negative buoyancy, as observed in (Korsøen et al. 2009). Looking at the refilling activity at dawn, swimming speeds and observations of the school at dawn the last option is considered unlikely due to relatively low refilling activity at dawn. This study has, however, no evidence to confirm the night-schooling behaviour since observations of schooling only were made during daytime due to light dependency.

5.4. Artificial light

Refilling activity in an air-dome increased with use of artificial light placed above the dome. Previous studies have shown that salmon are attracted to artificial light and have pointed out use of artificial light as a strategy to attract salmon to an air-dome (Wright et al. 2015). The idea behind the use of artificial light above the air-dome, was to create an illumination effect, which would make the dome visible during night-time and thus available for the salmon 24 hours per day. Increased refilling activity indicate that use of artificial lights stimulated increased refilling activity in the air-dome during night-time and ruled out the hovering behaviour completely, which became non-existent during light-trials. This indicate that artificial light also changed the schooling pattern in addition to increasing refilling activity, especially during night-time. The diurnal rhythm became less clear and thus artificial light disrupted the pattern given by the combinational effect from feed and natural light. However, the addition of artificial light did not transform this pattern completely whereas refilling activity still peaked midday, when time since feeding was shortest and daylight was present. Artificial light also stimulated peaks of refilling activity, when water temperature was below 10ºC, with over 30 refills per observation window. The highest refill activity per observation window was also observed during light trial. Swimming speed was also observed to increase with artificial light, which suggest that the general activity level increased. Increased surface activity and swimming speeds have previously been linked to stress (Furevik et al. 1993; Juell 1995). Whether use of artificial light caused increased refilling activity due to an illumination effect, or increased stress levels is unknown and should be investigated further.

6. Conclusion and implications

The results of this study suggest that Atlantic salmon refill mainly by rolling, every other day, in a submerged sea cage with air-dome (Ø = 2,5 m, height = 0,1m). The salmon were able to maintain buoyancy in all three submerged cages with normal swimming speeds. Refilling activity varied diurnally, peaking at noon and lowest at night-time. Seasonal variation was weak, except for a decrease in tops of activity as temperature decreased and fish weights increased.

While submerged cages may solve some of the problems linked to the surface environment, more research is needed to determine the exact effects of this production method. Surface behaviour is considered to be an important factor considering welfare. The submerged sea cages with air-dome did not succeed enabling normal surface behaviour of Atlantic salmon in this trial. However, the air-dome fulfilled its purpose by facilitating refilling during submergence, making it possible to keep salmon submerged continuously. Challenges regarding fish welfare was, nevertheless, still present. All the submerged cages had significantly less growth compared to the control cages. This study points out limited leaping behaviour in air-dome, together with low quality of feeding and high stocking density as possible factors to have caused the reduced welfare and growth. The influence of reduced leaping on growth have never been observed before. It is therefore important to underline that limited leaping behaviour only may have caused the reduced growth. Further experiments should therefore focus on determine the importance of surface behaviour in air-domes to develop knowledge regarding production of Atlantic salmon in submerged cages.

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