# Deep submergence in large Atlantic Salmon (*Salmo salar L.*); does experience with submergence increase fish coping ability?


For the Fulfilment of the Master of Science in Aquaculture and Seafood

By

Nikolai Eide

Department of Biological Sciences
University of Bergen, Norway

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Supervisors: Ole Folkedal[1] & Ivar Rønnestad[2]
1: Institute of Marine Research (IMR)
2: University of Bergen (UiB)

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Author: Nikolai Eide

University of Bergen

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# Abstract

Submergence of sea-cages holding Atlantic salmon is a possible solution to surface-related welfare hazards to the fish such as sea lice infestation and periodic events of algal or jellyfish blooms. It is now known that submerged salmon should be provided with air, such as in an air-filled dome, to allow re-filling of the swim bladder and thereby prevent negative buoyancy and exhaustive compensatory behaviour. Less is known about whether salmon undergoing submergence will benefit from previous experience with submergence and artificial air supply, or how well salmon cope with deep submergence (>20 m depth). In the present study, the coping ability of large salmon (>2 kg) during deep submergence (>40 m depth) was tested in a small common garden sea cage. Three groups of fish were stocked into the common garden cage: two groups had experienced >6 months of submergence previously, either with access to an air dome or bubbles for re-filling, while the third group was naïve to submergence and methods of re-filling while submerged, and functioned as a control group for the effect of experience. Two 1-week trials were followed by a third, 3-week, trial. All groups were given access to air bubbles and an air-filled dome, although the dome size and position relative to the cage net roof was altered between trials due to technical challenges. Prior to experimental start, all fish were given a welfare assessment and external tags to discriminate between fish of different experience groups in camera observations. Fish swimming speed was observed during cage descent, during submergence and cage ascent, while body tilt angle and interactions with the dome or bubbles were recorded. At the end of each trial, the fish were sacrificed, their welfare was assessed, and their swim bladders were measured for air and water content. All fish survived the submergence challenge, although an increase in snout damage occurred for all groups. Successful air filling in the dome was only observed when the dome was vertically aligned with the net roof. Fish with previous exposure to the dome were the most frequent users of the dome in the common garden cage. Similarly, fish experienced with bubbles visited the bubbles immediately and were the most frequent users. Other than that, no systematic difference between groups of different background was found over the trials. The measured swim bladder content of air or water was low overall, indicating low success in swim bladder re-filling. Fish swimming speed increased during the descent (+53.3 ± 3.2%), was 69.2 ± 3.8 % higher than at surface levels by the end of each submergence period, and further increased 26.4 ± 5.0 % when the cage was ascended to surface. A gradual increase in mean body tilt angle up to 7.7 ° was found during the 3-week trial. All over, the results suggest that large salmon, experienced with submergence or not, can cope with short-term submergence to a depth which is highly relevant for industry applications, such as avoiding periodic algal or jellyfish blooms.

# Abbreviations

| | |
|---|---|
| ANOVA. | Analysis of variance |
| BI. | Bubble interactions |
| BL. | Body length |
| CI. | Confident interval |
| DF. | Degrees of freedom |
| DI. | Dome interactions |
| DS. | Deformity score |
| FES. | Fin erosion score |
| GCS. | General condition score |
| IMR. | Institute of Marine Research |
| LR $Chi^2$. | Likelihood Chi-square |
| mL. | Millilitres |
| OWI. | Operational welfare indicators |
| P. | Pressure |
| PA. | Production Areas |
| ppt. | Parts per thousand |
| Q. | Quantile |
| SBCG. | Swim bladder content: Gas |
| SBCW. | Swim bladder content: Water |
| SD. | Standard deviation |
| SE. | Standard error |
| SLC. | Scale loss score |
| SS. | Sums of squares |
| SSA. | Swimming speed: Ascent |
| SSD. | Swimming speed: Descent |
| SSS. | Swimming speed while submerged |
| SWS. | Snout wound score |
| T. | Temperature |
| TA. | Tilt angle |
| V. | Volume |
| W. | Weight |

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# 2. Introduction

## 2.1. Norwegian salmon farming

Since the first pioneers started farming Atlantic salmon (*Salmo salar*) in the late 1960s, the Norwegian salmon industry has been world-leading. (Olaussen, 2018). Throughout the following decades, production volume increased from 600 tonnes in 1974 to approximately 160,000 tones in 1990 (Olaussen, 2018; Stefansson et al., 2016). However, in the early 1990s, a major challenge hit the industry. Several diseases lead to a crisis affecting both fish welfare and economy, and driving research that resulted in successful introduction of preventive vaccines (Lillehaug et al., 1992; Stefansson et al., 2016). With preventive actions taken, the rise in productional volumes continued through the 1990s with about 260,000 tonnes in 1995, reaching a peak of 1,350,000 tonnes in 2019 (Stefansson et al., 2016; Statistics Norway 2020). Aquaculture production surpassed that of wild caught fisheries in export value in 2006 (Statistics Norway, 2021), leading to a new era in Norwegian fisheries and aquaculture. In 2020, the Norwegian salmon industry exported fish worth more than 70 billion NOK (Statistics Norway, 2021), the second most valuable year for the industry.

The Norwegian Government's goal is to increase the seafood export value 5-fold by the 2050. With quotas regulating the wild caught fisheries, the 5-fold target is mainly based on aquaculture growth. This indicates a bright future for the industry if current and upcoming challenges can be solved (Olafsen et al., 2012; The ministry of trade and fishery, 2021).

## 2.2. Challenges

The industry is facing several challenges that constrain further expansion, reduce profits and impact societal reputation (Olaussen, 2018). The two major concerns for the industry today are the salmon lice (*Lepeophtheirus salmonis*) and escaped farmed salmon, both of which affect fish welfare and wild salmonid populations (Olaussen, 2018).

Escaped farmed salmon are often a result of extreme weather, equipment failures, or human errors (Thorvaldsen et al., 2015) and raise both genetic and ecologic concerns (Gross, 1998). Genetic interaction between farmed and wild Atlantic salmon lowers the overall heterogeneity of the allelic frequencies in wild fish, as well as it leads to introgression and interbreeding disrupting the gene-pools (Crozier, 1993). From an ecological perspective, escapees can reduce

the wild salmonids adaptiveness by increasing the competition for space and food (Gross, 1998).

Salmon lice (*Lepeophtheirus salmonis*) is a major and persistent challenge for the salmon industry (Forseth et al., 2017). Increase in the abundance of salmon lice hosts is correlated with increased lice numbers in the sea (Dempster et al., 2021; Sommerset et al., 2021). This is particularly problematic for wild smolt migrating from the rivers to their offshore habitat (Liu et al., 2011). Increased lice infestation on wild post-smolt salmonids has contributed to a decline in wild salmon stocks, and threatens the survival rates for both outgoing and returning salmonids (Forseth et al., 2017; Liu et al., 2011). While the allowed number of salmon lice is regulated by law (The Aquaculture Act, 2021; Regulation on salmon lice control, 2013, §8), delousing treatments increase mortality rates and reduce growth and welfare in farmed salmonids, as well as causing higher production costs (Grefsrud et al., 2021). Estimations from 2018 indicated that the economic cost directly related to treatment and prevention of salmon lice was >5 billion NOK (Iversen et al., 2019). This alone accounts for about 4 NOK/kg of the salmon production cost. Salmon with comorbidities are especially prone to negative outcomes following lice treatment, which over the later years have predominantly been conducted by mechanical and thermal methods which require more handling than previous methods (e.g. chemotherapeutants) that the lice have developed resistance to (Grefsrud et al., 2021). Furthermore, the continuous increase in salmon lice is also forcing the industry to invest in, and develop, preventive technology against lice infestation (Iversen et al., 2020).

Due to unacceptably high numbers of salmon lice in some regions of Norway, the Norwegian Government has introduced a new legislation model to ensure sustainable growth of the salmon industry (Olaussen, 2018). The new regulation system is known as the “Traffic light system” and is used as a model to control further industry growth based on numbers of salmon lice (Ådlandsvik, 2015). The core of the legislation is to secure a low salmon lice infestation pressure on wild migrating smolt. The system divides the Norwegian coast into 13 different production areas (Figure 1). The border between each area is based upon natural water currents where the probability of salmon lice migration between them is lowest. By evaluating the mortality risk of wild salmon smolt caused by lice infestation, each area is given a colour code every second year. Green indicates an area where <10% of the wild migrating smolt is estimated to die on their migratory path to the ocean. If the numbers are between 10-30%, a yellow light is given. Mortality rates over 30% are indicated by a red light. A green light gives the farmers

a chance to increase their production by 6%, a yellow light allows no further growth, while a red light forces the farmers to reduce their production by 6%.


***Figure 1*:** The 13 productional areas (PA) in Norway. PA1: Swedish border – Jæren, PA2: Ryfylke, PA3: Karmøy – Sotra, PA4: Nordhordaland – Stadt, PA5: Stadt – Hustadvika, PA6: Nordmøre – South Trøndelag, PA7: Northern Trøndelag with Bindal, PA8: Helgeland – Bodø, PA9: Vestfjorden and Vesterålen, PA10: Andøya – Senja, PA11: Kvaløya – Loppa, PA12 – Western Finnmark, PA13: Eastern Finnmark. (Vollset et al., 2019).

There has been a paradigm shift in treatment against salmon lice whereby the industry has moved from chemotherapeutant treatments to non-medical treatment methods (Overton et al., 2019). The evolutionary capacity of salmon lice is well documented, possessing favourable evolutionary traits such as short reproduction frequencies and high fertility, as one mature female can produce up to 1000 eggs (Heuch et al., 2000; Samsing et al., 2016; Brooker et al., 2018). These properties, together with a high availability of hosts, has resulted in a great potential to mutate and become resistant to chemotherapeutants (Ljungfeldt et al., 2017). Ljungfeldt et al., (2017) also raised concerns regarding the capacity of salmon lice to become resistant towards non-medicinal treatments. In that manner, an optimal solution to the salmon lice challenge is by preventing physical proximity between the parasite and its host (Stien et al., 2012).

### 2.2.1. Salmon lice biology

Salmon lice is an ectoparasitic stenohaline copepod that lives on salmonid hosts (Costello, 2006), dispersed mainly in the upper 20 m of the water column (Asplin et al., 2013). The parasite attaches its host where it grazes on skin, mucus and underlaying tissues by the use of its rasping mouth organ. The grazing can include epithelium losses, increased mucus discharge, bleedings, tissue necrosis and loss of microbial and physical protective function for the host (Costello, 2006; Johnson et al., 2004; Tully & Nolan, 2002). Infected salmonids may experience reduced appetite, food conversion efficiency and growth, and increased stress in conjunction with open wounds which in its turn leads to higher susceptibility for secondary infections (Costello, 1993; Costello, 2006; Mustafa et al., 2000). Norwegian regulations states that average salmon lice levels in a sea cage cannot exceed 0.5 mature females per salmon, and for 6 weeks during wild smolt migration in the spring the levels are reduced to 0.2. If lice levels exceeds the threshold, treatment procedure are initiated (Dean et al., 2021). Thus, salmon lice thresholds are regulated at substantial lower levels than what is lethal for the farmed fish *per se* (Wagner et al., 2008), and the sea lice itself is predominately a hazard for the wild salmonids (Grimnes & Jakobsen, 1996).

The salmon lice goes through eight developmental stages, with a developmental rate that is greatly influenced by temperature (Hamre et al., 2019; Heuch et al., 1995). Stage 1 and 2 includes the planktonic nauplius larvae stage, while the organism becomes infective as a copepodite at stage 3. Development of stages 4-8 (chalimus I and II, pre adult I and II and adult) occurs while the copepod is attached to the salmonid host (Hamre et al., 2013; Hamre et al., 2019; Johnson & Albright, 2011, Schram, 1993). Fertilized adult females carries the eggs in a paired string until hatching occurs, following a release from the mother whereas the larvae's will drift freely under their two first planktonic stages (Hamre et al., 2019).

Due to salmon lice's poikilotherm nature, temperature is an important developmental regulator of their reproductive output. Higher temperatures results in a more rapid development of the organism that increases the frequency of egg production, which in its turn forces the farmer to increase the frequency of treatments against salmon lice (Costello, 2006; Samsing et al., 2016). Lower temperatures result in production of larger egg batches (Bjørn & Finstad, 1998; Finstad et al., 2000; Heuch et al., 2000; Johnson & Albright, 1991; Samsing et al., 2016; Tucker et al., 2000).

Lice dispersion in fjords and coastal areas are dependent on currents, fresh water runoff, fjord-coast water exchange, tides and winds (Asplin et al., 2013; Johnsen et al., 2014; Skarhamar et al., 2018). The mean annual freshwater runoff in Norway is calculated to be 12 000 $m^3/s$, making fjords are the main source of freshwater in the Norwegian Coastal Current (Sætre et al., 2007). This runoff is at its maximum in May-June and minimum in February-March which impacts the stratification of the water layers. Other factors that influence the swimming behaviour and amount of sea lice in fjords and coastal areas are salinity and light, which is closely related to freshwater runoff, circulation and stratification in the upper layers of the waters (Johnsen et al., 2016; Johnsen et al., 2014; Myksvoll et al., 2020).

### 2.2.2. Fish welfare

The purpose of the Norwegian Animal welfare act is to promote good animal welfare and respect for animals, and states that animals kept in captivity shall have a suitable living environment and be handled in a way that promotes good welfare throughout their life cycle (Lovdata, 2021). The concept of animal welfare is commonly divided in to three main aspects: the animal's biological functioning with regards to health and physiological coping abilities (function-based definition), the animal's ability to perform natural behaviour (nature-based definition), and the animals subjective experience of its situation (feeling-based definition) (Duncan and Fraser, 1997; Huntingford et al., 2006). The concern for animal welfare is first and foremost based on the assumption that animals can experience sentient states of both good and bad welfare, and a common core for the abovementioned definitions is the animal's coping ability with its environment (Dawkins, 1990; Korte et al., 2007). A better definition may thus be that "good animal welfare is characterized by a broad predictive physiological and behavioural capacity to anticipate environmental challenges", saying that the animal should be challenged sufficiently to learn from its environment and adjust its physiology and predict forthcoming events in a beneficial way (Korte et al., 2007).

In the sea phase of farmed Atlantic salmon, it is evident from persisting high mortality rates that the coping ability of the fish is commonly compromised (Grefsrud et al., 2018; Bang-Jensen et al., 2020; Sommerset et al., 2021). Mechanical damage from delousing, gill infections, several common diseases and poor smolt quality are reported as important drivers of reduced welfare and mortality (Bleie & Skrudland, 2014; Stien et al., 2019; Sommerset et al., 2021).

The welfare status of farmed salmon is regarded as positively correlated with their production performance, saying that promoting good welfare is economically beneficial for fish farmers (Føre et al., 2018). A shift from a traditional experience-based to a knowledge-based production regime is regarded important to solve key challenges by use of emerging technologies (Føre et al., 2018), for example, rearing technologies that prevent sea lice infestation and thus need for delousing (e.g. Geitung et al., 2019), advancements in vaccine technology (Ma et al., 2019), or management procedures that reduce transmission (Ådlandsvik, 2015).

In 2020, 54% of all the welfare incidents (significant mortality or damage) reported to the Norwegian Food Safety Authority were linked to non-medicinal delousing. A total of 2983 non-medicinal treatments were conducted the same year. The average annual mortality rate in salmon farming between 2016-2020 was 15.5%, and it is believed that both increased handling due to salmon lice infestation, and salmon lice itself, are important contributors to high mortality rates, both direct and indirectly (Sommerset et al., 2021). These welfare challenges have increased the demand for preventive solutions against lice amongst other factors, and has triggered innovation in novel farming technologies (Olaussen, 2018).

## 2.3. Motivation for submerged production

One solution to minimize the spatial overlap between the sea lice copepodites and farmed salmon is to create a physical barrier or other spatial decoupling (Oppedal et al., 2020). Over the recent years, a number of lice-preventing technologies have been developed and tested. Some of these inventions includes semi-closed cages (i.e. skirts (Stien et al., 2012; 2018) or snorkel cages (Stien et al., 2016)), submergence of cages (Dempster et al., 2008; 2009; Korsøen et al., 2009; Sievers et al., 2018; Glaropoulos et al., 2019), with an air dome (Korsøen et al., 2012; Oppedal et al., 2020; Warren-Myers et al., 2022), or with access to air bubbles (Unneland Larsen, 2021). Furthermore, other preventing strategies, including deep-lights and/or -feeding, have been tested to manipulate the salmonids to swim deeper and overlap less with the sea lice in the water column (Bui et al., 2019; Geitung et al., 2019).

Submergence of Atlantic salmon sea cages, by fitting a roof on the cage and lowering it below the surface, is a technological solution which should keep the fish away from hazards connected to the surface water layers. So far, the biological research and technological development has mainly been motivated by avoidance of sea lice, yet the strategy may also be useful to avoid

other hazards that are more periodic and acute (Sievers et al., 2021). Relative frequent dire algae blooms have caused massive acute mortalities of farmed salmon in Norway, Canada and Chile, due to toxin accumulation, gill damage or hypoxia (Esenkulova et al., 2021). This is a welfare concern and may result in major economic losses for the farmers, e.g. 14,500 tones Atlantic salmon died during an 3 week long algae bloom in Northern Norway in 2019 (Karlson et al., 2021; Karlsen et al., 2019; Table 1). Similarly, blooms or aggregations of jellyfish poses a similar threat against farmed fish (Mitchell et al., 2021; Tiller et al., 2016).

***Table 2.1:*** The caused fish mortality and cost by a number of selected algae bloom events in Scandinavia as of 1988 and after.

| **Country & date** | **Killed (ton)** | **Cost (USD)** | **Reference** |
|---|---|---|---|
| Sweden, 1988 | 100 | 1 million | Skjoldal & Dundas (1991) |
| Norway, 1988 | 800 | 9 million | Skjoldal & Dundas (1991) |
| Norway, 1989 | 750 | 9 million | Johnsen & Lein (2011); Kaartvedt et al., (2011) |
| Norway, 1991 | 742 | 3.5 million | Aure & Rey (1992); Rey (1991) |
| Norway, 1998 | 350 | 1.4 million | Aure et al., (2001; 2002) |
| Norway, 2001 | 1,100 | 3.5 million | Naustvoll et al., (2002) |
| Norway, 2007 | 135 | *No estimate* | Johnsen et al., (2010) |
| Norway, 2019 | 14,500 | 100.0 million | Karlsen et al., (2019) |
| Denmark, 2019 | 400 | 1.4 million | Karlson et al., (2021) |

Furthermore, high surface water temperatures may during the summer reduce the availability of preferred water temperature and result in low oxygen concentrations for Atlantic salmon (Johansson et al., 2006; Oppedal et al., 2011; Stehfest et al., 2017). Conversely, in winter months, warmer conditions are regularly observed at lower depths, triggering increased appetite in fish (Brett, 1979; Bui et al., 2020). By holding salmonids deeper in the water column, submerged cages could thereby also play an role in counteracting the effects of suboptimal surface temperatures in winter or summer months, possibly leading to a more optimal condition for survival and growth rates (Warren-Myers et al., 2022).

While little is known empirically about the vertical distribution of pathogens, evolutionary theory predicts that all else being equal, infection pressure will be highest at the depth at which most potential hosts reside, as this will maximise the probability of transmission (Bonneaud &

Longdon, 2020). Other parasites than sea lice may also be most abundant near surface, and e.g. tapeworms can be transferred because of close proximity to birds. Violent weather conditions where wave and surface water current forces can be a threat to both fish and farming constructions can cause damage to farm structures and fish, leading to mortalities and escapees (e.g. Tveit, 2011; Cherry, 2020). Ice bergs, and build-up of ice layers on constructions, and ship traffic are other plausible treats to fish farms which are closely connected to the large surface structures of conventional sea cages (Bjelland et al., 2016).

### 2.3.1. Biological challenges with submergence

Through evolution, salmonids have adapted to a life style where they migrate vertically in the water columns in search for food while avoiding predators (Westerberg, 1982). It is assumed that Atlantic salmon rolling and jumping at the water surface is largely driven by their need for gulping air into their open swim bladder for buoyancy regulation (Furevik et al., 1993). Surface behaviours may also be driven by behavioural needs linked with or preparatory for e.g. homing, whereas lice infestation levels in farmed salmon are found positively correlated with their level of surface activity (Furevik et al., 1993).

Due to the salmons need for buoyancy regulation by gulping air into its swim bladder, it has been concluded that submerged salmon should have air available when submerged for periods more than two weeks (Korsøen et al., 2009; Sievers et al., 2018). It has been successfully demonstrated that salmon can maintain buoyancy when air is available in submerged dome structures (Oppedal et al, 2020; Warren Myers et al., 2022), and there are compelling evidence that air bubbles can also be utilized for buoyancy regulation in submerged rainbow trout (*Oncorhynchus mykiss*) (Yu et al., 2022) and Atlantic Salmon (Unneland Larsen, 2021).

### 2.3.2. Fish welfare in submerged cages

Submergence of sea caged Atlantic salmon without access to air for swim bladder re-filling has repeatedly shown that the swim bladder empties over 2-3 weeks (Dempster et al., 2009; Korsøen et al., 2009; Sievers et al., 2018). The salmon will, within a few days, begin to compensate for negative buoyancy by increased swimming speeds, and later, by swimming with a tilted body angle to generate lift (like an airplane landing), as most pronounced during darkness/night (Dempster et al., 2008; 2009; Korsøen et al., 2009; Sievers et al., 2018). The need for re-filling can be assessed by activity levels when submerged salmon are given access to the surface, where groups of negatively buoyant salmon show high surface activity/re-filling

behaviour in the hours following resurfacing. (Tim Dempster et al., 2008, 2009; Glaropoulos et al., 2019; Korsøen et al., 2009).

While submergence with increasingly negative buoyancy for up to 3 weeks did not affect central welfare parameters in Atlantic salmon (Dempster et al., 2008, 2009; Glaropoulos et al., 2017), long-term submergence for 6 weeks under a continuous light regime resulted in poorer growth (Sievers et al., 2018). The same was observed with 6 weeks submergence under a natural light regime, where also snout wounds occurred, as well as vertebral compressions due to severely tilted swimming (Korsøen et al., 2009). Such negative welfare impact was avoided when submerged salmon successfully refilled their swim bladder in an air-filled dome over 5-7 weeks and under a natural light regime (Oppedal et al., 2020).

Growth rate are considered an important long-term welfare indicator in farmed fish (Huntingford et al., 2006), where a recent study of a full submerged production with air dome showed poor growth rates compared with salmon reared in surface control cages (Warren-Myers et al., 2021). While the submerged fish maintained neutral buoyancy, their rearing environment between 15 and 35 m depth at a fjord site with strong vertically stratification of temperature and oxygen was suboptimal for periods, which suggests a trade-off between physical environmental conditions and sea lice exposure (Warren-Myers et al., 2021). Commercial scale testing of submergence with air-dome at a vertical homogenous costal site was successful in maintaining normal growth and welfare of salmon while avoiding sea lice infestation, suggesting the technology as highly feasible (Olufsen & Tjølsen, 2020).

## 2.4. Buoyancy, swim bladder re-filling behaviour and hydrostatic pressure

Submergence may, by default, reduce the ability of salmon to express natural behaviour, as surface access is restricted and a higher hydrostatic pressure is exerted on the fish. Access to air for successful swim bladder re-filling is regarded as the key factor for successful submerged farming (Korsøen et al., 2009; Oppedal et al., 2020; Sievers et al., 2021), whereas the effect of depth and thus hydrostatic pressure has received less scientific attention.

The swim bladder is the primary organ for adjusting buoyancy in most teleosts. Situated dorsally in the abdominal cavity, the swim bladder is positioned below the fish's centre of gravity, which forces the fish to continuously adjust its body position to keep the belly facing

downwards (Alexander, 1966). By regulating the swim bladder gas content according to swimming depth, fish save energy by avoiding behavioural compensation (e.g. by continuous or fast swimming) to prevent sinking (Alexander, 1966).

Based on the anatomy of the swim bladder, the teleost species with swim bladder can be divided into two groups, the fish with open bladder (physostomous) and closed bladder (physoclist), where Atlantic salmon has an open swim bladder (Fánge, 1953). The ductus pneumaticus which connects the open swim bladder with the oesophagus is characteristic for this type and re-filling occurs by gulping air through the ductus pneumaticus – often in combination with a jump or a roll in the water surface (Furevik et al., 1993; Kryvi & Poppe, 2016). Gradually, salmon will passively deplete air from the swim bladder if surface access is denied, as indicated by a linear extinction of echo backscatter over 3 weeks in submerged salmon (Dempster et al., 2009).

The majority of all scientifically reported experimental trials on submergence of Atlantic salmon has been carried out within the minimum depth span (roof depth) between 1-15 m (Unneland Larsen, 2021; Sievers et al., 2021; Warren-Myers et al., 2021). The salmon lice and other surface related problems may occur deeper (Forseth et al., 2017), calling for a deeper submergence. Deeper submergence will exert higher pressure on the fish and little is known about the effects of change in pressure on swim bladder function during the submergence procedure, the pressure *per se* when submerged and maintaining buoyancy by gulping air under high pressure. The first known report of salmon submergence tested 30 m depth in 3-week trials using a very small sea cage (4 × 4 m and 2 m deep) and a small air dome, where the fish swam tilted and most fish died (Fosseidengen et al., 1982). However, commercial scale testing of submergence with air dome to 30 m depth have been successfully tested regarding both technology and biology (Olafsen & Tjølsen, 2020), suggesting that salmon cope well with deep submergence as long they are provided with a suitable rearing environment.

Hydrostatic pressure decreases with 1 standard atmospheric pressure (atm) for every 10 meters depth (Mallen & Roberts, 2020). The gas volume inside the swim bladder is consistent with Boyle's law: where the pressure of gas is equal to the ambient hydrostatic pressure (Alexander, 1959; Macaulay et al., 2020). Vertical movement in the water column thereby affects the gas volume in the swim bladder, and hence fish density, by the change in hydrostatic pressure (Watanabe et al., 2008). Likewise, submerged air for swim bladder re-filling (e.g., in a dome) will be compressed with depth, and the fish will fill its swim bladder with air pressure according

to the depth of air available, and will therefore super inflate its bladder vs. the pressure for the natural surface re-filling (i.e. the mass of gas will be higher for the same volumes at increasing depths). A large salmon will have a deeper maximum neutral buoyancy depth compared to a younger and smaller salmon, due to greater lipid reserves in the body (Macaulay et al., 2020). Lipids have a lower density than water and will thereby favour buoyancy, which was tested by Yu et al. (2022) who found that submerged rainbow trout (*Oncorhynchus mykiss*) could partially compensate the loss of buoyance by the saturated fatty acid content in the muscles. Macaulay et al. (2020) investigated the maximum neutral buoyancy depth (MNBD) for Atlantic salmon. The study calculated the mean MNBD for a 2.4 kg salmon to be approximately 24 m, Similarly, the study found the MNBD of salmon weighing 175g to be 20.8 m.

## 2.5. Swim bladder re-filling options when submerged

While air domes have been demonstrated to be functional for successful swim bladder filling in submerged Atlantic salmon (Oppedal et al., 2020; Olafsen & Tjølsen, 2020; Warren-Myers et al., 2021), they are technically advanced and documented use has been restricted to a position in the net roof, being the shallowest available depth for the fish. One alternative is to deliver a stream of air bubbles from a submerged hose, reducing technical difficulties and allowing a more flexible positioning and vertical distribution of air within the sea cage (Korsøen et al., 2013; Unneland Larsen, 2021; Yue et al., 2022).

Korsøen et al. (2013) were the first to study the feasibility of air bubbles for re-filling of swim bladder, where Atlantic salmon submerged for 11 days was provided with air bubbles for 7 hours a day for 7 days. The salmon were unsuccessful in re-filling from bubbles, even though fish were observed swimming into the bubbles. More recently, it has been shown that small post-smolts (130-300 g) were able to maintain buoyancy when submerged with air bubbles over 2-week periods (Unneland Larsen, 2021), while Yu et al. (2022) showed that rainbow trout could partly maintain buoyancy when submerged with bubbles in aquariums. Air bubbles may also aid in supporting negatively buoyant salmon as salmon tend to position themselves into the vertical water lift created by bubbles (Unneland Larsen, 2021).

Yet, the use of air bubbles as source for swim bladder re-filling is not an established method and there are still several questions remaining regarding the efficiency of the method considering size of both fish and bubbles, acclimation to utilize bubbles in naïve fish, and the efficiency and preference for air re-filling in bubbles vs. in domes.

## 2.6. Learning capacity of salmon

Submerged air filling in salmon, by either air in domes or bubbles is a subject of learning, which is found to occur within 2-7 days in small salmon (Unneland Larsen, 2021; Nilsson et al. unpublished data; Macaulay et al., 2020). Several studies show that salmon have capacity to learn and habituate to changes and recurring events in their environment. For example, Bratland et al. (2010) showed that Atlantic salmon post smolt were efficient in habituating to an initially frightening stimulus (a flashing light) and formed a positive association towards the event when being rewarded with feed. Macaulay et al. (2020) showed that smolts accustomed to fill air at surface domes during the freshwater production phase had a more efficient transition to swim bladder filling in domes after sea transfer. Salmon acclimation to better cope with submergence has been indicated over repeated submergence by Glaropoulos et al. (2017).

Unpublished data show that large salmon (3kg+) naïve to submergence failed in re-filling their swim bladders when submerged with air available in either domes or bubbles (Oppedal and Folkedal pers. comm.). It is not known whether age and thus fish size is important for the coping ability of naïve salmon when submerged, i.e. whether the learning process will be too slow to prevent detrimental welfare effects of negative buoyancy. This raises a concern for submerging large salmon, for example as a periodic measure to escape from transient surface related hazards.

## 2.7. Aim of the study

There is a lack of knowledge about how previous experience with submergence and air filling in domes or air bubbles affects salmon coping ability when submerged. Based on anecdotal evidence, large salmon are considered less able to accustom themselves to a novel rearing environment, including swim bladder re-filling using a submerged air supply. Therefore, I conducted a study to test whether large farmed salmon would benefit from previous experience with submergence and swim bladder re-filling using either air-domes or bubbles, by comparing them to salmon naïve to submergence. The fish were periodically submerged to 40 m, a relevant depth for industry applications such as avoidance of surface-related hazards and salmon lice.

The current study will address the following questions:

1. Whether large salmon (>2 kg) experienced with submergence and different air supply methods will perform better than salmon naïve to submergence when challenged in a novel submerged rearing environment. More specifically, will experienced fish respond differently than naïve to submergence and be more effective in swim bladder re-filling?

2. How salmon will cope with the hydrostatic pressure of deep submergence. This includes observing if there is a change in welfare parameters, and how behavioural parameters are affected by change in hydrostatic pressure when descending to submergence depth, being kept submerged for a period of time, and then being ascended to surface.

# 3. Materials and methods

## 3.1. Location and experimental design

The study was conducted over three separate trials at the Smørdalen sea cage research facility (61°N) of the Institute of Marine Research (IMR), Norway. One submersible square sea cage (5 × 5 m and 5 m depth and a 1.5 m deep cone at the bottom; approximate volume = 130 $m^3$) was used (Figure 2). For each trial Atlantic salmon were caught with a hauling net and randomly netted out from three neighbouring cages (33 per cage, in total 99 fish) representing fish that had been reared over >6 months under control conditions, or in submerged cages with either air dome or air bubbles. The sampled fish were anaesthetized (0.1 g $L^{-1}$, FinquelVet, Western Chemical Inc, Washington DC, USA), individually measured for weight and length and scored according to the Fishwell protocol (Noble et al., 2018), and tagged with a colour code Floy tag (Floy T-bar Anchor, 80 mm, Floy Tag & Mfg., Inc, Seattle, USA) at the base of the dorsal fin to visually separate fish from different treatment groups. The fish were given 3 days to acclimate to the experimental cage and were exposed to air bubbles the last acclimation day to lessen a potential adverse response to bubbles when submerged. Submergence of the cage to 40 m (roof depth) was done by lowering the cage 5 m every 10 minutes. The cage was then kept at 40 m for 1 week for trials 1 and 2, and 3 weeks for Trial 3, where the fish were given continuous access to air in both an air-filled dome and air bubbles, and continuous illumination from a submerged lamp. The same protocol as during submergence was used for resurfacing. At resurfacing all fish were netted out, sacrificed with an overdose of Finquel (1 g $L^{-1}$) and again scored according to the Fishwell protocol. Approximately 50% of the fish group were netted out before surface access, i.e. before swim bladder re-filling at surface. This was done to enable assessment of swim bladder gas and potential water content to the closest ml as measured using a needle and syringe.

Trial 1 was conducted from December 10$^{th}$ – 20$^{th}$, 2021, 7 months after the fish were transferred to sea as smolts. Trial 2 was conducted from January 12$^{th}$ – 25$^{th}$, 2022, and the Trial 3 from February 14$^{th}$ – March 7$^{th}$, 2022.

## 3.2. Experimental fish and treatment groups

The salmon (MOWI strain) were transferred and stocked in groups of ~2700 fish over 9 sea cages (12 × 12 m and 14 m depth; approximate volume = 2000 $m^3$) at the Smørdalen sea cage

facility May 13th, 2021. After a 2-week acclimation period, triplicate treatment cage groups were reared from May 25 as either controls in a standard surface cage (Control group), submerged with a hexangular dome (Ø = 3 m) filled with compressed air and positioned at surface (Dome group), or submerged and given air bubbles released at 12 m depth (Bubble group). For submerged cages, a net roof was sewn into the cage walls at approximate depth of 2 m, which fully hindered access to the surface, while the net roof had a cone shape from 2 m to the surface air dome for the Dome group. Based on these different rearing conditions, the different groups had different experience with being submerged and with access to air during submergence. For sampling of fish, the fish in the dome cages had been given surface access over 24 h every second month, while the fish in the bubble cages had been given surface access over 1-5 days every 4-5 weeks.

For each trial the 33 fish per treatment group was caught from one of the treatment cages with a different cage per trial. The size of the fish varied with treatment group and over time as the fish grew (Table 3.1).

The welfare scoring of individual fish before and after each submergence trial included: general morphological appearance, emaciation, deformities, gill cover damage, scale loss, snout wounds, skin bleeding, skin wounds, fin wounds, fin erosion, eye status and eye bleeding.

**Table 3.1** – Numbers of fish and mean values ± standard deviation for length (cm), weight (g), conditional factor (K-factor), scale loss score, snout wound score and fin erosion score for experimental groups at sampling before each trial.

| Treatment group | Number of fish | Length (cm) | Weight (g) | K-factor | Scale loss | Snout wound | Fin erosion |
|---|---|---|---|---|---|---|---|
| 1 Bubble | 33 | 50.7 ± 2.67 | 2105 ± 366 | 1.60 ± 0.17 | 0.82 ± 0.47 | 1.03 ± 0.53 | 1.30 ± 0.50 |
| 1 Dome | 32 | 52.6 ± 4.79 | 2204 ± 619 | 1.47 ± 0.26 | 0.94 ± 0.44 | 0.66 ± 0.48 | 1.38 ± 0.55 |
| 1 Control | 32 | 54.8 ± 4.73 | 2506 ± 662 | 1.48 ± 0.11 | 0.97 ± 0.39 | 0.61 ± 0.50 | 1.48 ± 0.51 |
| 2 Bubble | 33 | 55.8 ± 4.12 | 2750 ± 535 | 1.57 ± 0.13 | 1.00 ± 0.00 | 0.67 ± 0.65 | 2.12 ± 0.33 |
| 2 Dome | 33 | 57.8 ± 4.46 | 2834 ± 636 | 1.45 ± 0.15 | 1.00 ± 0.00 | 0.30 ± 0.47 | 2.06 ± 0.35 |
| 2 Control | 33 | 59.8 ± 3.65 | 3168 ± 633 | 1.47 ± 0.13 | 1.00 ± 0.00 | 0.27 ± 0.52 | 2.09 ± 0.38 |
| 3 Bubble | 33 | 58.6 ± 5.69 | 3027 ± 872 | 1.46 ± 0.13 | 1.09 ± 0.38 | 0.64 ± 0.55 | 2.09 ± 0.29 |
| 3 Dome | 33 | 61.0 ± 3.51 | 3251 ± 569 | 1.42 ± 0.11 | 1.00 ± 0.00 | 0.61 ± 0.61 | 2.09 ± 0.29 |
| 3 Control | 33 | 61.6 ± 4.72 | 3303 ± 850 | 1.38 ± 0.10 | 1.33 ± 0.48 | 0.64 ± 0.70 | 2.09 ± 0.38 |

## 3.3. Cage design

The sea cage was of standard type and modified by two 5 × 5 m net roofs (same mesh size as cage, 20 × 20 mm). One roof was sewn into the surface construction rope of the cage to restrict the cage volume for the fish, and the other was sewn into the top construction rope of the cage as an extra safety measure against fish escape during submergence (Figure 2). For the first trial, an air-dome (Ø = 60 cm, air height 10 cm) was sewn into the centre of the lower roof. For the second and third trials, a larger dome was used (Ø = 90 cm, air height 10 cm). The dome was attached to 4 straps that passed through the lower roof and was weighed down within the cage, making depth adjustment of the dome possible (Figure 2). Air bubbles (Ø ≈ 2 cm) were released from a hose in a mid-radial position at the cage bottom. Air for bubbling and dome filling was connected to separate hoses (Ø = 19 mm) with valves at surface to control the air pressure as provided by a compressor (Kaeser, Coburg, Germany). Regulation of cage depth (submergence and resurfacing) was carried out by hand with ropes (Ø = 16 mm) attached to the cage top corners. To maintain the cage at a horizontal position and prevent deformation it was attached plastic rings to slide along corner ropes (Ø = 20mm) from the cage floating construction (steel construction with gangway). The ropes were weighed down with steel weights (300 kg per corner) at 70 m depth. To further prevent cage deformation, the cage bottom was attached to a rigid 5 × 5 m frame of circular steel pipes (Ø = 40 mm).

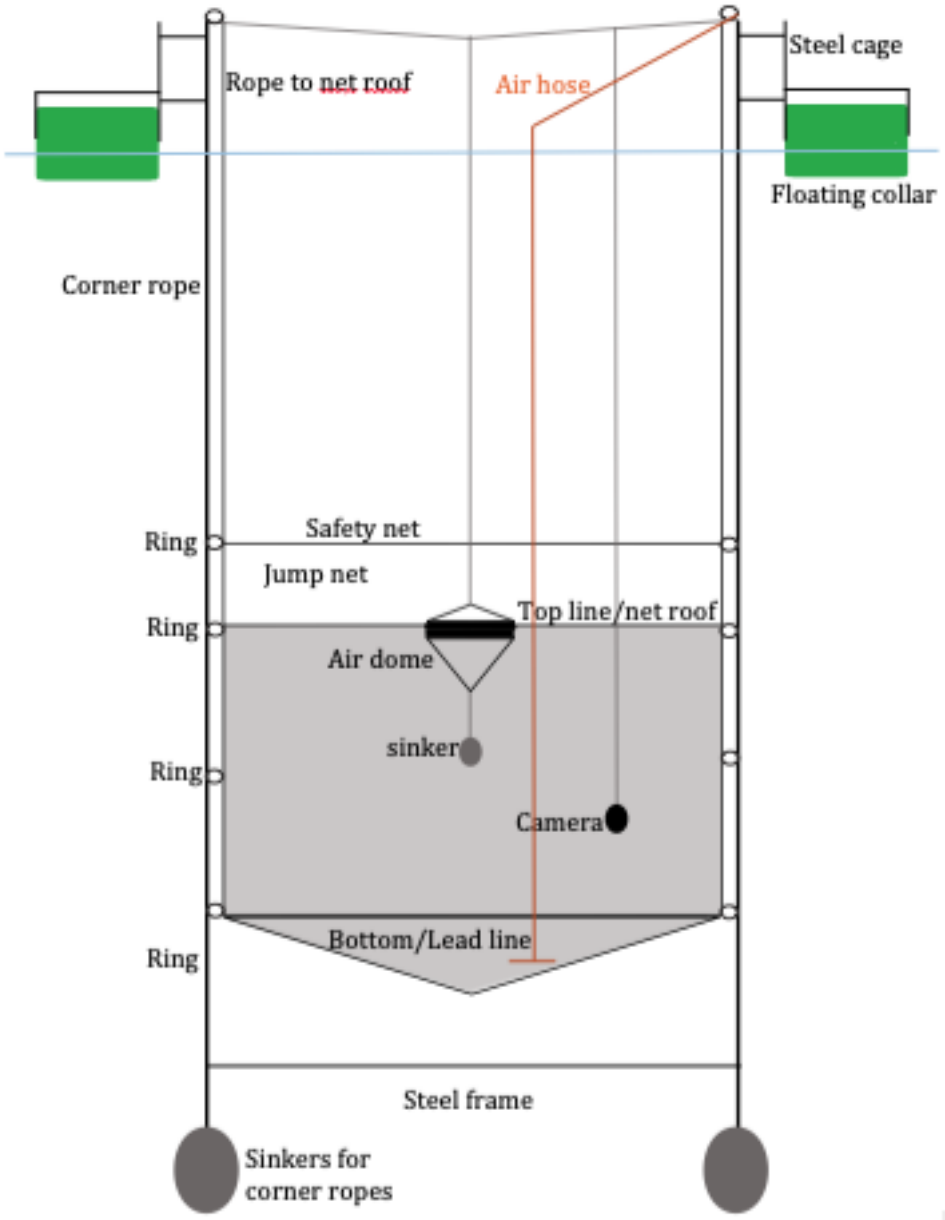


***Figure 2:*** Outline of the experimental setup of the submerged cage. Shadow area illustrating the cage volume. Cages is situated in submerged position at 40m depth. The air hose is placed in such way that it fills the air dome.

## 3.4. Environmental variables and feeding

The water environment was monitored at a reference point close to the cage using a CTD (SD240, SAIV AS, Bergen, Norway) connected to a winch system (AP85, Argus Remote Systems, Bergen, Norway) which profiled the water column (0.5 m steps) daily down to 50 m depth. During acclimation to the experimental cage, the fish were given a natural photo regime, which the fish also were given before the experimental start. When submerged, the entire cage volume was continuously illuminated by a submerged lamp (BlueLed 100W, AkvaGroup ASA, Bryne, Norway) positioned one meter above the cage roof.

The fish were fed once per day (10 min) using pellets (4.5 mm Skretting Optiline pellets, Skretting AS, Stavanger, Norway), and shortly prior to the resurfacing/ascending procedure in Trial 3. Feeding occurred by dropping pellets through a 30 m long hose (Ø = 30 cm) from surface to 10 m above the centre of the cage.

## 3.5. Behavioural observations

A camera with pan and tilt function (Orbit GMT, Scale AQ, Trondheim, Norway) was positioned within the cage in a mid-radial position at the opposite side from the air bubbles (Figure 2). The vertical position of the camera was changed by pulling and lowering an attached rope in order to observe fish residing at different depth positions. Camera observation of individual fish swimming speed (n = 15 fish per treatment group as observed from read out of Floy tag colour code) was carried out before submergence, for each depth interval during submergence and resurfacing, and at hours 0800, 1400 and 2000 every day the fish were submerged. At 1400 each day when submerged, the body tilt angle was observed for 15 fish per treatment group. A static camera position at mid cage depth was maintained for observations of both swimming speed and tilt. The frequency of interaction with bubbles and the air dome were observed daily over one 5 min period and one 60 min period, respectively, after the 1400 observation time point, and group tag colour code were observed. After Trial 3, all fish were examined for feed/pellets in the gut and intestine to investigate whether appetite was present.

## 3.6. Statistical analysis

All numerical data was prepared in Microsoft Excel Version 16.53 (Copyright 2021, Microsoft, Washington, USA), and all statistical analyses and figures were carried out in RStudio (Version 1.3.1073, RStudio, Inc, Boston, MA, USA) using the R programming language (Version 4.1.2, R core team, Vienna, Austria) and the following R packages; dplyr (Wickham et al., 2018), ggplot2 (Wickham, 2016), ggeffects (Lüdecke et al., 2021), patchwork (Pedersen, 2020), mgcv (Wood, 2021), gratia (Simpson & Singmann, 2022), and tidymv (Coretta et al., 2021).

Statistical models were fitted to test for effects on response variables for swimming speed, tilt angle, dome and bubble interactions, swim bladder content of air and water, and fish welfare scores. All response variables were tested for homogeneity of variance and normality using the Levene test and Shapiro-Wilk test, respectively, and if necessary, response variables were transformed to improve conformity with linear model assumptions (e,g, logarithmic and square

root transformations). The suitability of the model was then assessed by inspecting residual plots. If linear models still resulted in a poor fit, or if the data type was unsuitable (e.g. count data or binomial data, or a non-linear relationship),a generalized linear model (GLM) or generalized additive model (GAM) was fitted instead. To estimate the statistical significance of effects on response variables (swimming speed, body tilt angle, welfare score, swim bladder content), type II analysis of variance tables were generated using the car package. The alpha level was set at $p = 0.05$. All statistical results are reported in Appendix I.

# 4. Results

## 4.1. Environmental conditions

During the experimental period, vertical stratification of oxygen, salinity and temperature in the water column were present (Figure 3). At 40 m depth, the temperature remained at stable levels between 9-10°C, and salinity in the range between 30-35 ppt. Stable and high oxygen saturation levels (>80% sat.) were recorded in Trial 1 and 2, whereas a drop in saturation (72%) occurred prior to and was maintained during Trial 3.

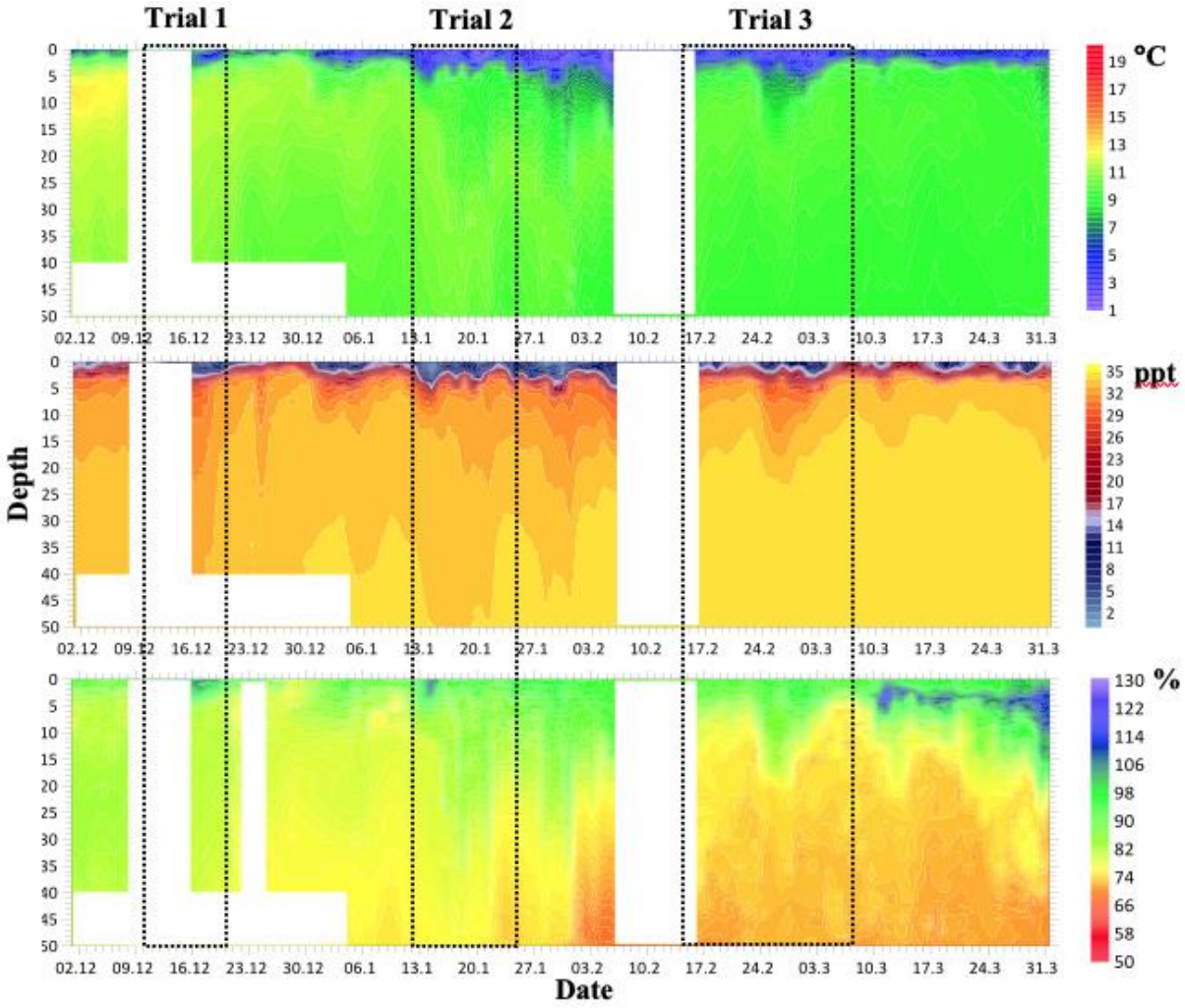


***Figure 3:*** Environmental parameters of water temperature (top), salinity (middle) and oxygen (bottom) levels for 0-50m depth from December 2nd, 2021, to March 31st 2022 for the respective trials (marked with dashed rectangles). White periods indicate missing data due to technical error of the winch that profiled the CTD.

## 4.2. Fish growth, mortality and welfare

At the beginning of the study, the Control fish were significantly heavier and longer (2396 ± 98 g and 54,7 ± 0,72 cm (mean± SE)) than both the Dome (2146 ± 97 g and 54,0 ± 0,69 cm) and the Bubble fish (1985 ± 92g and 51,4 ± 0,81cm), and this continued to be the case throughout the three trials ($p < 0.05$, Model: Weight; Length, Appendix I, Table 4.1). The mean weight of each group decreased through each trial, affecting the K-factor negatively (Figure 4). Significant differences were found between the groups and trials for K-factor, length and weight ($p < 0.05$; Model: K-factor; Weight, Length, Appendix I). Furthermore, a significant difference in weight, length and K-factor were found between start and end of each trial. A slight increase in mean length from start to end was observed in in all trials and groups (0,66, 0,57, 0,27 cm in Trial 1, 2, and 3, respectively), except in the Control (-0.1 cm) and Dome (-0.9 cm) group in Trial 1 and 3 respectively. Predicted values with a 95% confident interval of both K-factor and weight are provided in Appendix I. One fish died during the acclimation period in Trial 2, while no mortality occurred during submergence.

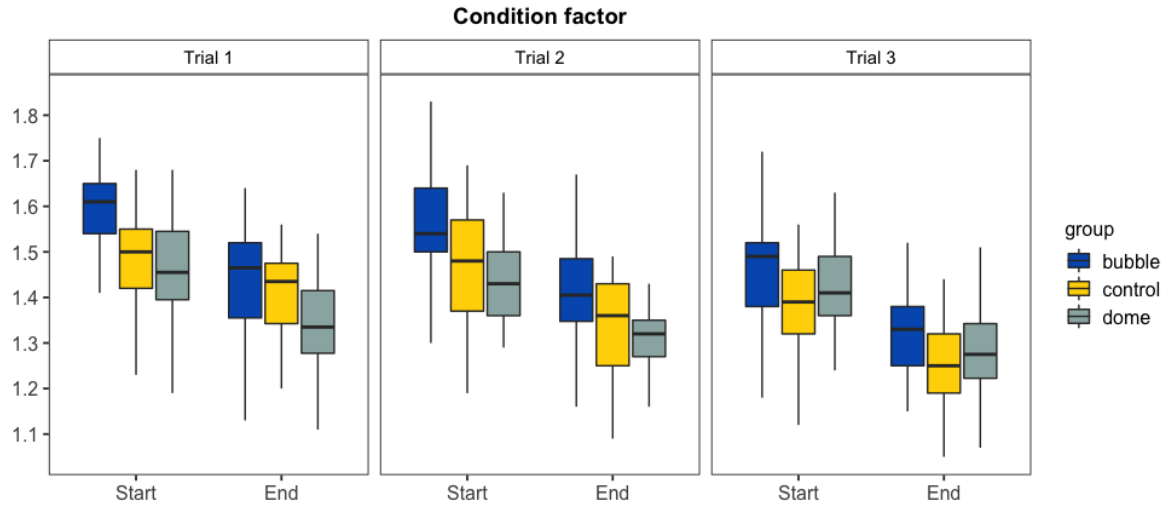


***Figure 4:*** Condition factor (K-factor) prior to and post submergence in Trial 1 (left), Trial 2 (middle), and Trial 3 (right) for all groups. Boxes show the median (middle line), interquartile range (top and bottom of the box), and 1.5 times the interquartile range (whiskers).

The individual fish scores for emaciation, body wounds, fin wounds, gill injury and skin bleeding were not different between groups at the start of any trial and did not change with submergence (Appendix figures 8-12, Appendix II). Neither did the general status assessment of the fish, scale loss, or deformity ($p < 0.05$, Model: GCS; SLS; DS, Appendix I). The snout wound score was worse for all groups in all trials after submergence (scoring level 1.47 ± 0.10

(mean ± SE); Table 4.1) compared with the start score (0.60 ± 0.10), while no significant differences was found between groups over the trials (1.43 ± 0.10, 1.55 ± 0.09, 1.43 ± 0.11 for Trial 1,2 & 3, respectively; $p < 0.05$, Model: SWS, Appendix I). Some increase in scale loss from start to end of the trials was found, but not of significant difference (Table 4.1; Appendix figure 4, Appendix II). Although morphologic examination showed no difference in fin *wounds* (Appendix figure 12, Appendix II), a significant increase in fin *erosion* were found after each trial ($p < 0.05$, Model: FES, Appendix I; Appendix figure 5, Appendix II; Table 4.1).

**Table 4.1** – Trial, numbers of fish and mean values ± standard error (SE) for length (cm), weight (g), conditional factor (K-factor), scale loss score, snout wound score and fin erosion score for experimental groups at sampling after trials.

| Trial and Treatment | Number of fish | Length (cm) | Weight (g) | K-factor | Scale loss | Snout wound | Fin erosion |
|---|---|---|---|---|---|---|---|
| 1 Bubble | 33 | 51.4 ± 0.81 | 1985 ± 92 | 1.44 ± 0.05 | 1.06 ± 0.04 | 1.44 ± 0.10 | 1.74 ± 0.08 |
| 1 Dome | 32 | 54.0 ± 0.69 | 2146 ± 97 | 1.33 ± 0.02 | 1.16 ± 0.07 | 1.50 ± 0.12 | 1.88 ± 0.06 |
| 1 Control | 32 | 54.7 ± 0.72 | 2396 ± 98 | 1.45 ± 0.04 | 1.00 ± 0.05 | 1.35 ± 0.09 | 1.77 ± 0.08 |
| 2 Bubble | 32 | 56.2 ± 0.79 | 2506 ± 77 | 1.42 ± 0.03 | 1.00 ± 0.00 | 1.63 ± 0.09 | 2.09 ± 0.07 |
| 2 Dome | 33 | 58.7 ± 0.78 | 2672 ± 106 | 1.30 ± 0.02 | 1.03 ± 0.03 | 1.45 ± 0.09 | 2.03 ± 0.11 |
| 2 Control | 33 | 60.2 ± 0.62 | 2947 ± 101 | 1.33 ± 0.02 | 1.09 ± 0.05 | 1.56 ± 0.10 | 2.09 ± 0.05 |
| 3 Bubble | 33 | 59.5 ± 1.01 | 2829 ± 133 | 1.31 ± 0.02 | 0.97 ± 0.07 | 1.45 ± 0.11 | 2.00 ± 0.04 |
| 3 Dome | 33 | 60.1 ± 1.94 | 2941 ± 120 | 1.23 ± 0.04 | 1.15 ± 0.08 | 1.52 ± 0.11 | 1.94 ± 0.06 |
| 3 Control | 33 | 62.4 ± 0.81 | 3079 ± 131 | 1.25 ± 0.02 | 1.03 ± 0.03 | 1.33 ± 0.11 | 1.94 ± 0.04 |

## 4.3. Behaviour during descending

***Swimming speed***

Shortly before submergence, the groups swam at mean speeds between 0.34 to 0.51 BL $s^{-1}$ and no statistical difference between groups were found for any of the trials ($p < 0.05$, Model: SSD, Appendix I). For all trials the fish responded to submergence at the two first depth steps; 5 and 10m (Figure 5) by a significant increase in swimming speed ($p < 0.05$, Model: SSD, Appendix I). This increase was strongest for Trial 3, whereas the speed then declined over the further depth steps to 40 m where the swimming speed was lower than the two previous trials (Figure 5). Shortly after the full descent to 40 m depth, the speed for all fish per trial was 0.64 ± 0.04, 0.72 ± 0.03, and 0.58 ± 0.02 BL $s^{-1}$ (mean ± SE) for Trial 1, 2, and 3 respectively, and significantly higher ($p < 0.05$, Model: SSD, Appendix I) than shortly before submergence.

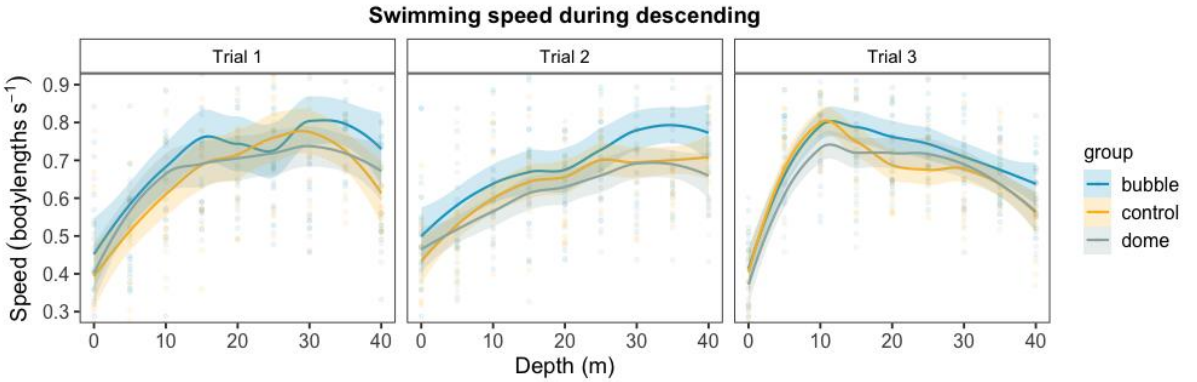


***Figure 5:*** Mean swimming speed (body lengths $s^{-1}$) during submergence procedure for the three trials and for all groups. For illustration, time and group effects are fitted by a loess model fit. Shading indicates 95% confidence interval. Background points illustrates the individual data points of sampling.

***Group and individual behaviour***

For all trials the fish swam polarized in a circular school before submergence and maintained this behaviour during the descending procedure, except from 25-30 m in Trial 1 and 15-25 m in Trial 2 when the fish swam unstructured, concurrent with variations in swimming speed (Figure 5). Unstructured swimming during Trial 2 was possibly triggered by acute stress to sudden change of light conditions when lowering the lamp.

## 4.4. Behaviour while submerged

### 4.4.1. Swimming speed

The swimming speed during submergence was affected by trial, treatment group, and time submerged ($p < 0.05$; Model: SSS, Appendix I). The mean swimming speed for all groups combined in Trial 1 was 0.69 ± 0.010 $BLS^{-1}$ (mean BL $s^{-1}$ ± SE). During Trial 1 all groups swam at similar and increasing speeds the first 70 h, before the speeds levelled out and the Bubble and Control group departed with higher speed from that of the Dome group at ~120 h (Figure 6). On average for the full submergence period, the Bubble group showed a higher speed than the Control and Dome groups (0.72 ± 0.012 vs. 0.68 ± 0.010 and 0.66 ± 0.008 respectively).

For Trial 2, the Control group maintained its initially higher swimming speed compared to the two other groups until ~100 h submerged, where a slight increase was observed over the last three days (Figure 6). The Bubble group showed an increase during Trial 2 and swam at similar speed as the Control group at the end. The Dome group increased its speed along with the

Bubble fish over the ~72 first hours and declined towards initial levels at the end where the speed was significantly lower ($p < 0.05$) than in the other groups (0.53 ±0.003 vs. 0.64 ±0.006 (Bubble group) and 0.61 ± 0.007 (Control group); Figure 6).

In comparison to Trial 1 and 2, each lasting one week, the swimming speed the first week of Trial 3 was again highest in the Bubble group (0.65 ± 0.002) compared to the Dome and the Control group (0.56 ± 0.001 and 0.54 ± 0.001, respectively) (Figure 6). Mean swimming speeds in Trial 3 were 0.59 ± 0.004, 0.57 ± 0.004, 0.57 ± 0.004 BL $s^{-1}$ for the Bubble, Control and the Dome group respectively. Both the Control and the Dome group swam at relatively low speeds until ~250 hours. After this all groups increased their speeds, which was most pronounced for the Control (Figure 7).

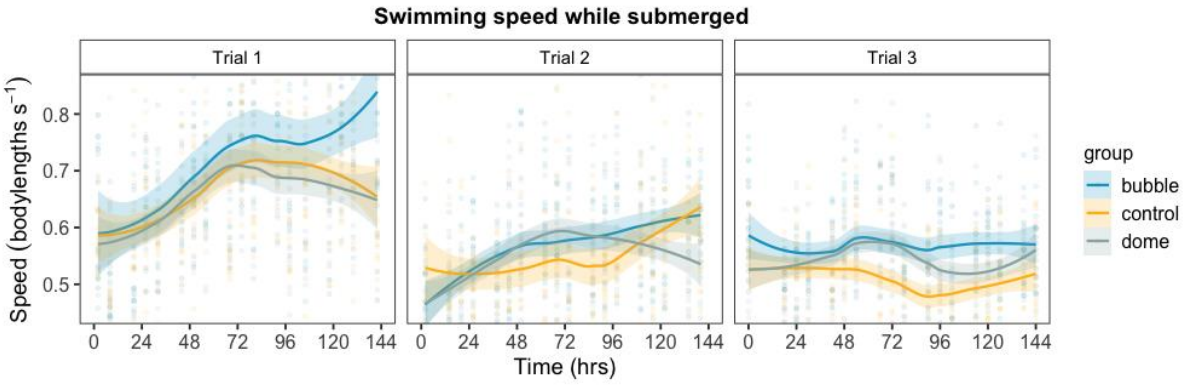


***Figure 6*:** Mean swimming speed (body lengths $s^{-1}$) for Bubble, Control and Dome group the first 144 hours submerged period for Trial 1 (top), Trial 2 (middle), and Trial 3 (bottom). For illustration, time and group effects are fitted by a loess model fit. Shading indicates 95% confidence interval. Background points illustrates the individual data points of sampling.

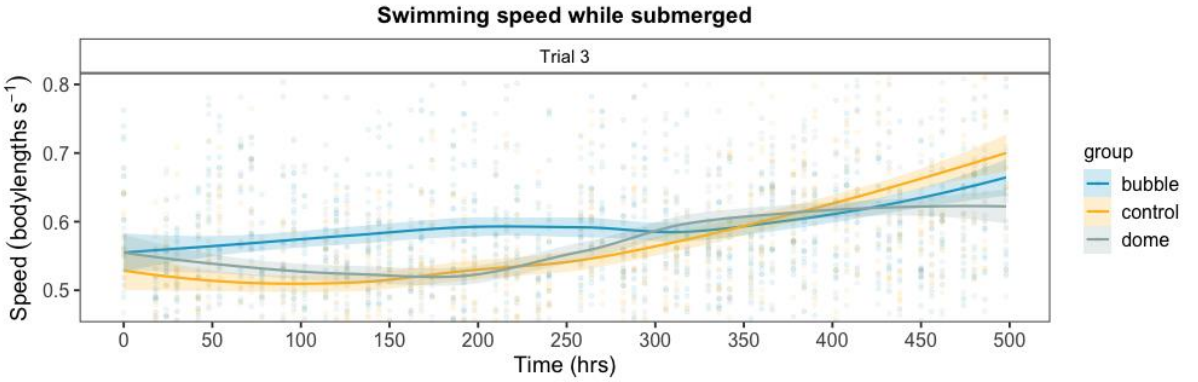


***Figure 7*:** Mean swimming speed (body lengths $s^{-1}$) for Bubble, Control and Dome group during Trail 3. For illustration, time and group effects are fitted by a loess model fit. Shading indicates 95% confidence interval. Background points illustrates the individual data points of sampling.

### 4.4.2. Body tilt angle during submergence

The fish body tilt angle varied extensively between individual fish within all groups and did not show any common trend over trials 1 and 2 or the first week of Trial 3 (Figure 8). Negative tilt angle in trials 1 and 2 was a result of downwards swimming in the water column, while positive tilt where of individuals that swam upwards, while no fish were observed to swim in a horizontal direction with a noticeable tilt of their body.

A significant increase in tilt angle was observed over the full period of Trial 3 ($p < 0.05$; Model: $TA_3$, Appendix I). A trend of increasing positive tilt angle was observed for all groups (7.7 ± 1.63° (mean ± SE)) and was strongest for the Control group (8.5 ± 1.83°), although not significantly stronger than the other groups at the end (Figure 8; $p < 0.05$; Model: $TA_3$, Appendix I). Dome and Control group had a tilt angle of 7.8 ± 1.5° and 6.7 ± 1.6°, respectively, after 21 days submerged. Still, a relative high variation was seen between individual fish in tilt angle, where it became apparent during week 2 and 3 that individuals swam with a tilted body angle.

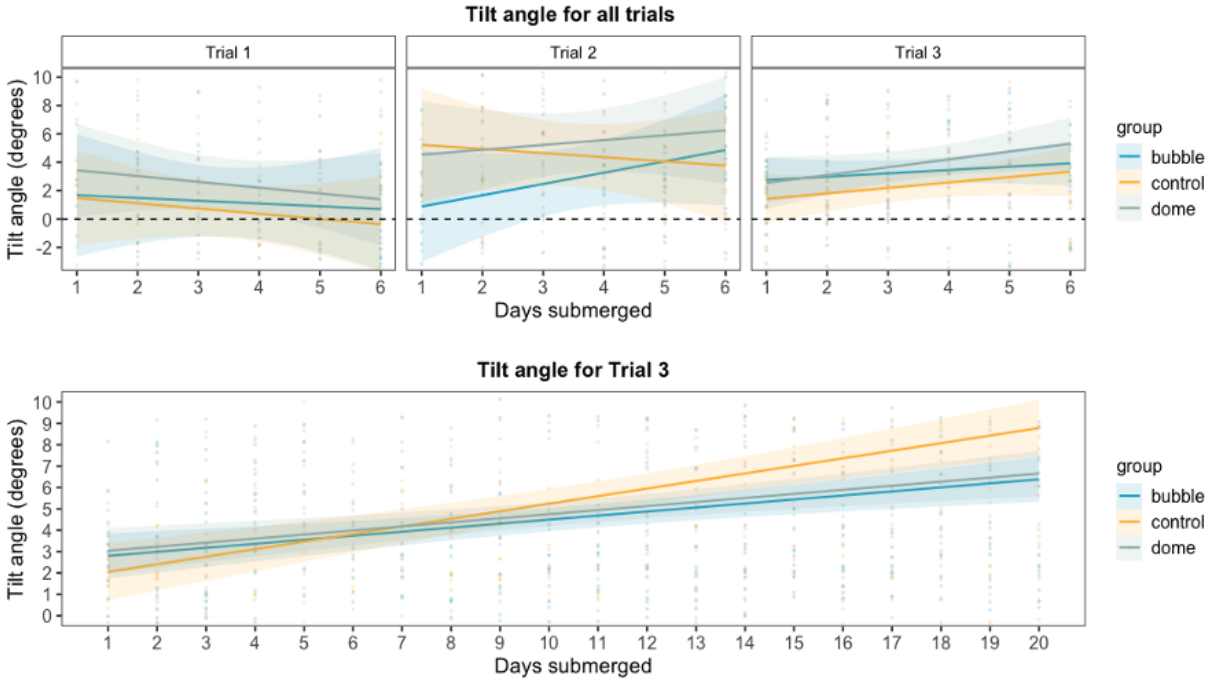


***Figure 8:*** Tilt angle during first 6 days for all trials (top) and full Trial 3 (bottom) for all groups. For illustration, time and group effects are fitted by a linear model fit. Shading indicates 95% confidence interval. Background points illustrates the variation of sampling. Black dotted line illustrates horizontal plane.

### 4.4.3. Fish interaction with air supply for swim bladder re-filling

#### *4.4.3.1. Air bubble interaction*

The cumulative recorded bubble interaction was highest for the Bubble group over all trials (59.4% of all interactions), while the Control and the Dome group, both unexperienced with air bubbles, showed similar levels (23.3% and 17.3% respectively; Figure 9). A significant difference in bubble interactions were seen between the groups in each trial ($p < 0.05$; Model: BI, Appendix I).

The Bubble group were observed interacting with the bubbles every day and showed the highest number of interactions in Trial 1 (28 interactions in total, counting for 66.6% of total recorded interactions; Figure 9). The Control group interacted with the air bubbles in 5 of 7 days, resulting in a total of 11 interactions (counting for 22.9% of all interactions) with the bubbles throughout Trial 1. The Dome group showed interactions in all days beside Day 3, resulting in 9 interactions (18.8% of all interactions) in total.

As in Trial 1, the Bubble group were observed interacting with the bubbles every day in Trial 2 with a total of 20 interactions, counting for 47% of all recorded bubble interactions in Trial 2. Fish from the Dome group had a total of 8 recorded interactions throughout the trial (19%). Fish from Control group showed some more interest in the air bubbles counting for 14 of the 42 recorded interactions (33.3%).

Relative to Trial 1 and 2, few bubble interactions were observed in Trial 3. The Bubble group had clearly the most interactions, with a total of 31 out of 43 interactions (72%). The Bubble and Dome groups had 6 interactions each in the whole submerged period (14%). Day 5-7 had no recorded interactions at all, whereas Day 9 was the day with highest number of total interactions with bubbles.

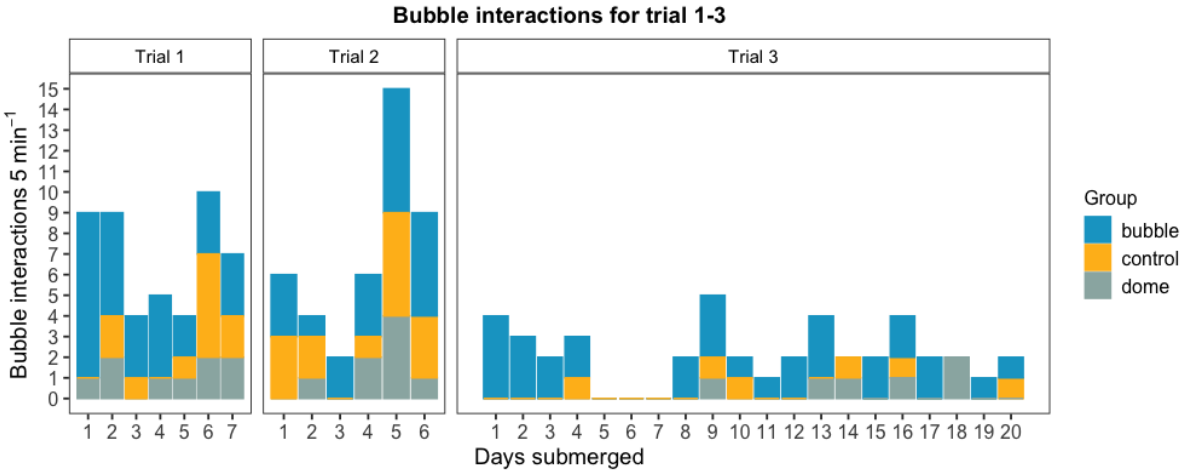


***Figure 9:*** Bubble interactions (5min$^{-1}$ a day) for each fish group in Trial 1 (left), Trial 2 (middle), and Trial 3 (right).

### *4.4.3.2. Dome interaction*

No dome interactions were observed in Trial 1 and Trial 3. For Trial 2, a total of 32 interactions were observed during the whole trial (Figure 10), where most interactions were observed at Day 1 (28 of 32 interactions). Of the 28 observations at Day 1, 15 were of Dome group, and of all interactions observed through Trial 2, the Dome group represented 50%. The Bubble group had in total 12 recorded interactions (37.5%) during Trial 2, where 9 of them were observed at Day 1. The Control group had the lowest number of dome interactions in the trial (12.5%), all of them observed at Day 1.

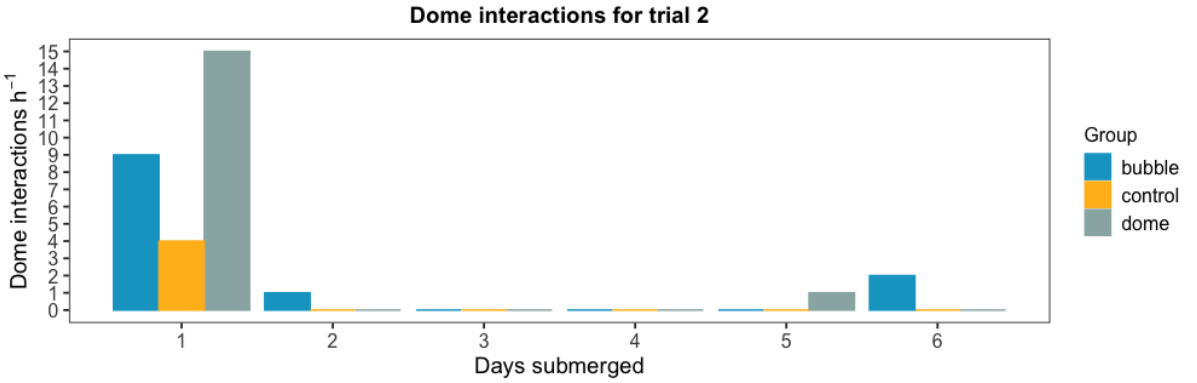


***Figure 10:*** Numbers of daily dome interactions for each fish group in trial 2. Observation time were 1 hour.

The design and positioning of the air dome varied between trials due to technical difficulties. In Trial 1, the small (Ø=60 cm dome) was sewn into the net roof which resulted in a ~45° angle of the net roof towards the dome (Figure 11). This steep angle may have contributed to the lack

of observed dome filling of the fish during Trial 1. Therefore, a 30 cm larger dome was deployed and positioned below the net roof for Trial 2 and 3.

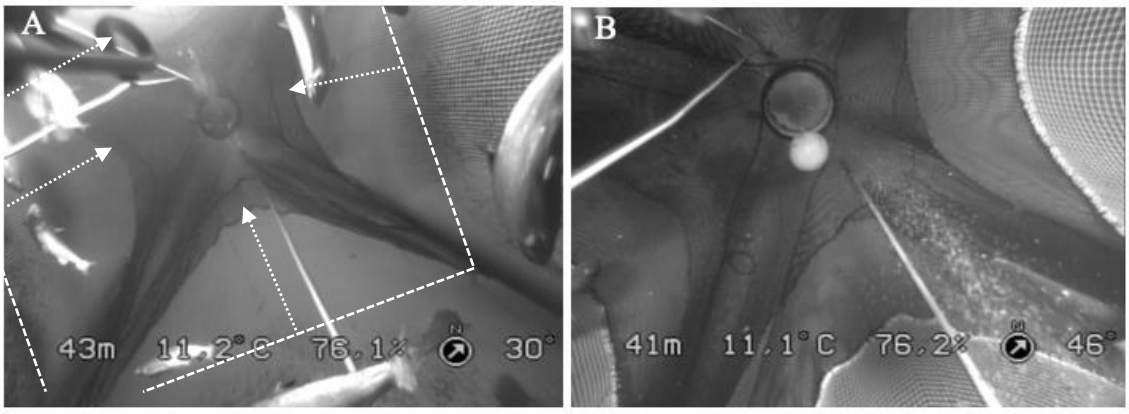


***Figure 11:*** An illustration of the coning shape of the net's roof in Trial 1 (A & B). Circle in top is the installed dome. Arrows indicating the stretch in the net wall. Dotted line indicates where the net roof originally should be situated.

At Day 1 in Trial 2, the dome was well aligned with the net roof and the highest frequency of dome interactions was observed (Figure 12 A). The dome was, however, lifted by air bubbles at Day 2 and a created a slight cone shape (~20° angle of the net roof), but less than in Trial 1 (Figure 11 A & B). To prevent coning of the net roof during Trial 3, a heavier weight was attached to the dome. Regretfully, the net roof got tangled on the straps holding the dome and created a cone in the net roof which provided a ~1 m height above the dome which the fish could swim in (Figure 12 B).

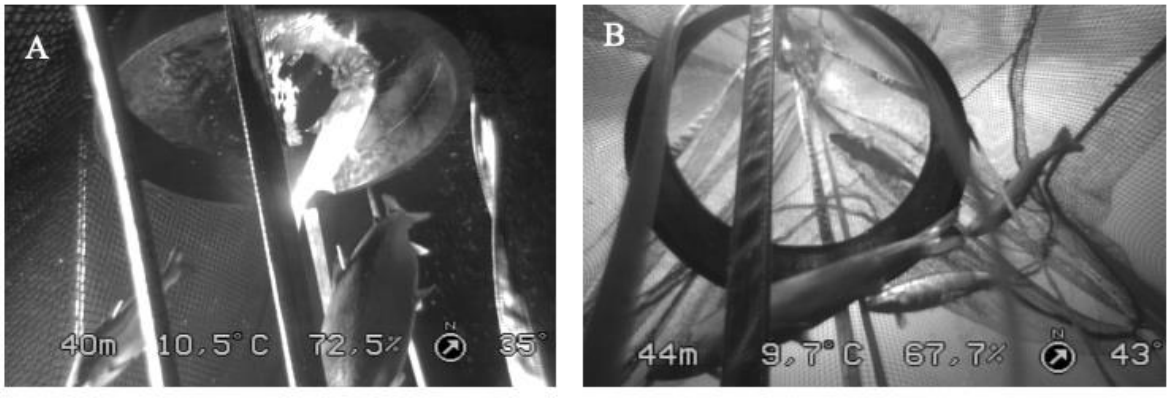


***Figure 12 A:*** Photo showing a fish interacting with a correct situated dome during Day 1 in Trial 2.
***Figure 12 B*:** Photo showing the water pocket above the dome which fish could swim in.

### 4.4.4. Aggressive interaction behaviour

Although not systematically recorded, aggressive interaction between individuals were observed during each trial (Figure 13). This behaviour occurred in a dog-fight-like manoeuvre and occurred among fish that swam in a solitary manner in the upper part of the cage.

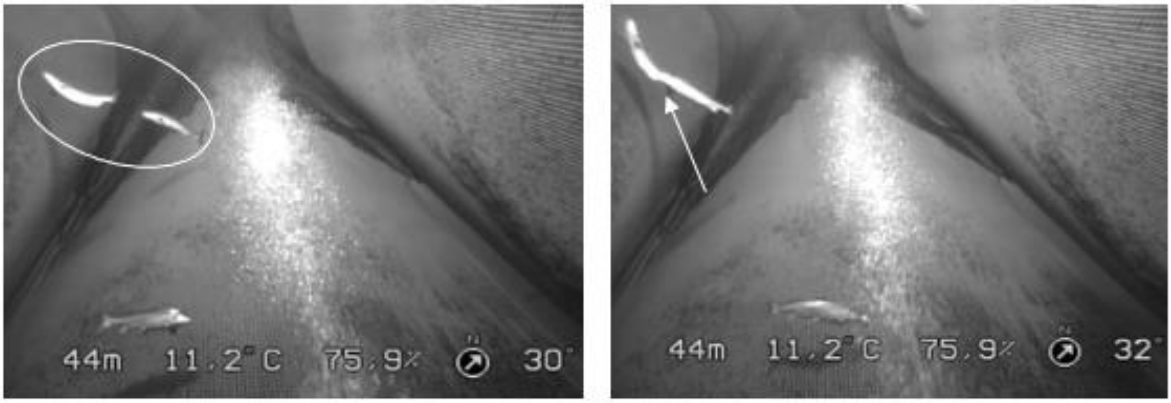


***Figure 13:*** Photos showing an impact of aggressive tail fin biting behaviour. Arrow indicates moment of impact.

## 4.5. Behaviour during ascending

The swimming speed during the stepwise ascent to surface was affected by depth and treatment group ($p < 0.05$; Model: SSA, Appendix I). A significant difference in swimming speed was also observed between each trial and level of depth ($p < 0.05$; Model: SSA, Appendix I). The Bubble group had the highest mean swimming speed ($0.81 \pm 0.04$ BL $s^{-1}$ (mean value ± SE)) in all trials during the ascending procedure, followed by the Control group ($0.76 \pm 0.03$ BL $s^{-1}$) and Dome group ($0.75 \pm 0.03$ BL $s^{-1}$; Appendix table 6, 7, 8, Appendix II).

All treatment groups in Trial 1 showed a decline in swimming speed towards 15 m depth. The groups then, relative rapidly, increased their speed towards the surface, ending at a speed of $1.08 \pm 0.04$, $0.86 \pm 0.04$, and $0.91 \pm 0.11$ BL $s^{-1}$ for the Bubble, Dome and Control group respectively (Figure 14). In contrast to this, an increase in swimming speed towards 15 m was observed in Trials 2 and 3, where the fish in Trial 2 had the highest mean swimming speed throughout the ascending procedure ($0.82 \pm 0.24$, $0.78 \pm 0.18$ and $0.76 \pm 0.20$ BL $s^{-1}$ for the Bubble, Control and Dome group respectively; Appendix Table 7, Appendix II). All treatment groups in Trial 2 showed a linear tendency in swimming speed towards surface, ending with a swimming speed of $0.93 \pm 0.05$, $0.96 \pm 0.04$ and $0.91 \pm 0.04$ BL $s^{-1}$ for the Bubble, Control and Dome group, respectively (Figure 14). In Trial 3, a fluctuating tendency in swimming speed

occurred towards surface. Thus, both the Bubble and the Dome group increased their speed from start to the end. The Control group had a lower speed at the end (0,73 ± 0.03 BL $s^{-1}$) compared to start (0,74 ± 0.04 BL $s^{-1}$) of the ascent procedure. Swimming speeds at surface for Bubble and Dome groups were 0.76 ± 0.04 and 0.74 ± 0.04 BL $s^{-1}$ respectively.

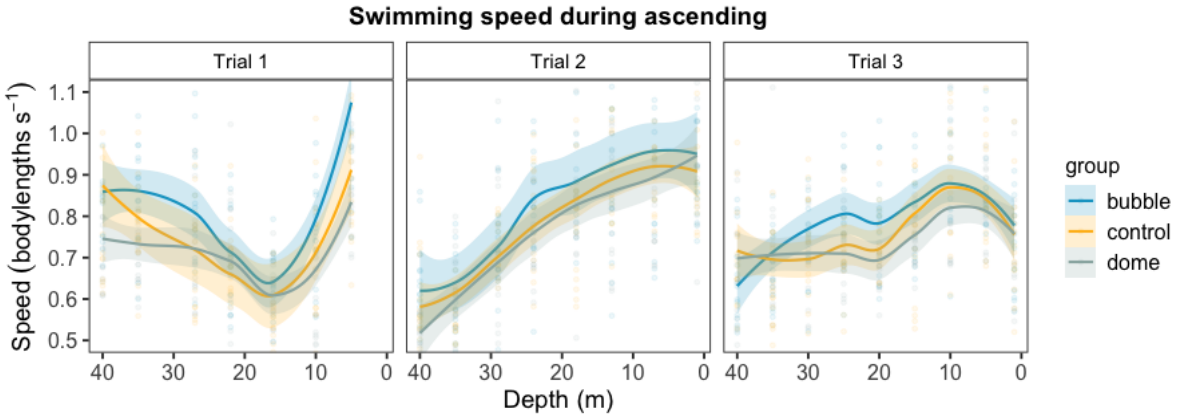


***Figure 14:*** Swimming speed (body lengths $s^{-1}$) for Bubble, Control and Dome group at different depths during ascending procedure in Trial 1 (left), Trial 2 (middle), Trial 3 (right). For illustration, time and group effects are fitted by a loess model fit. Shading indicates 95% confidence interval. Background points illustrates the variation of sampling.

## 4.6. Swim bladder content

Swim bladders in 42, 45 and 98 fish were examined for gas and water content shortly after ascending the fish in Trial 1, 2 and 3, respectively. The results after 186 swim bladders were investigated showed that in total 68 fishes had swim bladders that contained both water and gas.

### 4.6.1. Gas content volume at surface

A large variation in individual fish swim bladder gas volume was measured, irrespective of group or trial. Of all the 186 investigated swim bladders, 161 contained gas, counting for 86.6% of the fish. A significant difference in gas volume were seen between the trials ($p < 0.05$; Model: SPCG, Appendix I).

Of the investigated swim bladders in Trial 1, 90.7% (38 out of 42 fish) had gas present (88.2%, 90.9%, 92.8% for the Bubble, Dome and Control group respectively; Figure 15). Mean gas content analysed at surface just after ascending for all groups were 3.61 ± 1.10 mL (mean ± SE, surface gas volume). One fish had a relative high content of gas (26 mL), affecting the mean

gas volume for the Bubble group. Possible surface re-filling behaviour of a fish was however observed, and when excluding this outlier, the mean gas volume of the Bubble group was 2.98 ± 0.51 mL. This decreased the mean gas volume content for all groups (3.04 ± 0.81 mL). For the Dome and Control group, the mean gas volume for the measured fish was 2.75 ± 0.89 and 3.39 ± 1.04 mL, respectively.

A total of 84.4% (38 out of 45 fish) of the investigated swim bladders contained gas in Trial 2 (86.7%, 80.0%, and 86.7% of swim bladders for the Bubble, Control and Dome group respectively; Figure 15). The mean gas content for all groups combined were 7.92 ± 1.25 mL, ranging from 0 - 36 mL (Figure 15). Some fish gulping air at surface prior to netting out were observed. When removing these outliers (20, 25 and 36 mL) from the equation, the mean gas volume decreases for all groups combined (6.16 ± 1.33 mL). The mean gas volume was highest for the Bubble group (6.37 ± 1.27 mL), followed by the Dome group (6.11 ± 1.06 mL) and Control group (6.00 ± 1.68 mL) in Trial 2.

Of 98 investigated fish in Trial 3, a total of 85.7% of swim bladders contained gas (84 out of 98), proportioned as 78.8%, 87.8% and 90.9% for the Bubble, Control and Dome group, respectively (Figure 15). The combined mean gas content for all fish in Trial 3 was 1.93 ± 0.29 mL. The mean gas volume for the Bubble group was 1.85 ± 0.34 mL. The Control group had a mean gas volume of 1.94 ± 0.36 mL, while the Dome group had the highest measured mean volume at 2.00 ± 0.37 mL.

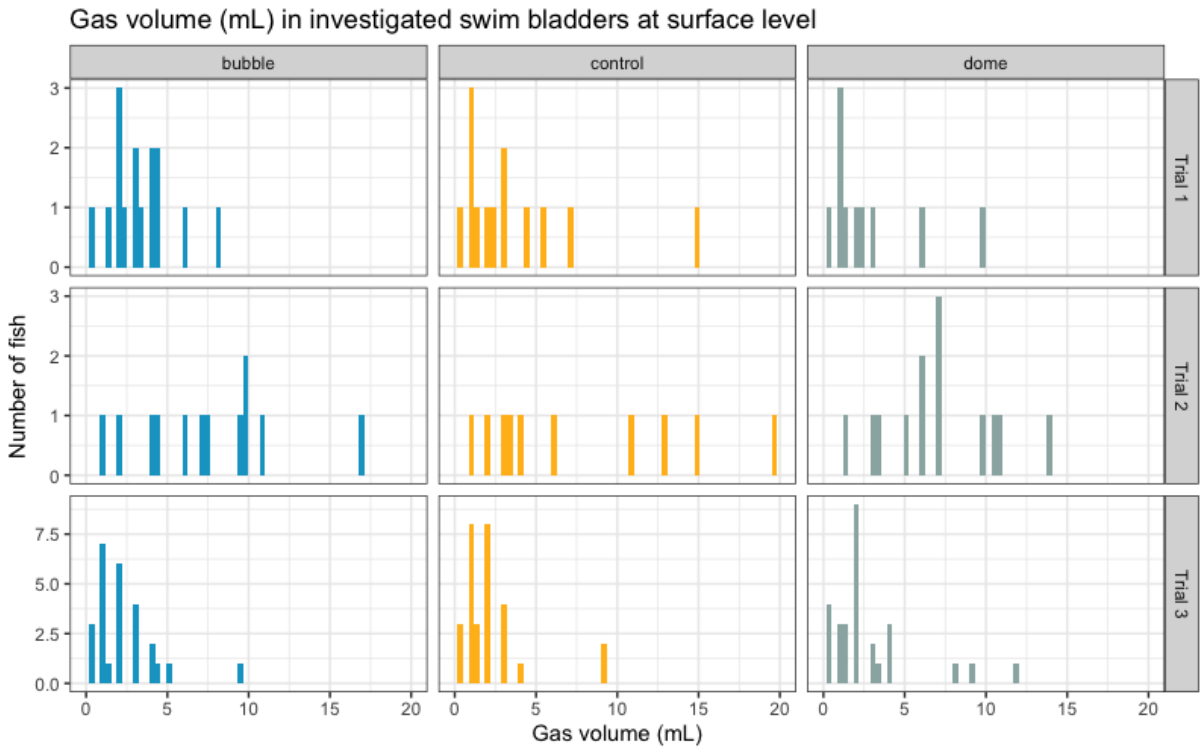


***Figure 15:*** Histograms illustrating the occurrence and amount (mL) of gas as content in investigated swim bladders post Trial 1 (n = 42; 17, 11 and 14 fish in Bubble, Dome, Control group respectively), Trial 2 (n = 45; 15, 15 and 15 fish in Bubble, Dome, Control group respectively), and Trial 3 (n = 99; 33, 33 and 33 fish in Bubble, Dome, Control group respectively) at surface levels.

### 4.6.2. Gas content volume at submergence depth

The hydrostatic pressure is increased by 1 atm for every 10 m depth according to Boyle's law, saying that the measured swim bladder gas content in the current study was compressed 5 to 6 times when the fish resided between 40 and 47 m depth. This results in a lower gas volume at submergence depth compared to surface where the swim bladders gas content was measured. In order to estimate the gas volume at 40m depth, Boyle-Mariotte law (formula 4, Appendix IV) can be used. Since Boyle-Mariotte law only works with constant temperatures, the influence of temperature change on volume property has been further calculated using Charles' Law (formula 5, Appendix IV) and Guy-Lussac's Law (formula 6, Appendix IV). Temperature difference between 40 m depth and surface air (where fish was examined) decreased by 13°, 8°, and 7° C in Trial 1, 2, & 3, respectively. This alone decreased the surface gas volume by 4.8%, 2.9%, and 2.5 % for Trial 1, 2, & 3, respectively, and is taken into account when recalculating gas volume at 40m depth. Combining this with Boyle-Mariotte law, the relative small proportion of the swim bladders with gas, as measured on land, was much lower when submerged due to the hydrostatic pressure at 40m (Figure 16). The mean gas volume at submergence depth were 0.91 ± 0.17 mL, 2.00 ± 0.32 mL, and 0.58 ± 0.07 mL for fish in Trial

1, Trial 2 and Trial 3, respectively. This emphasizes the need for successful swim bladder re-filling during deep submergence.

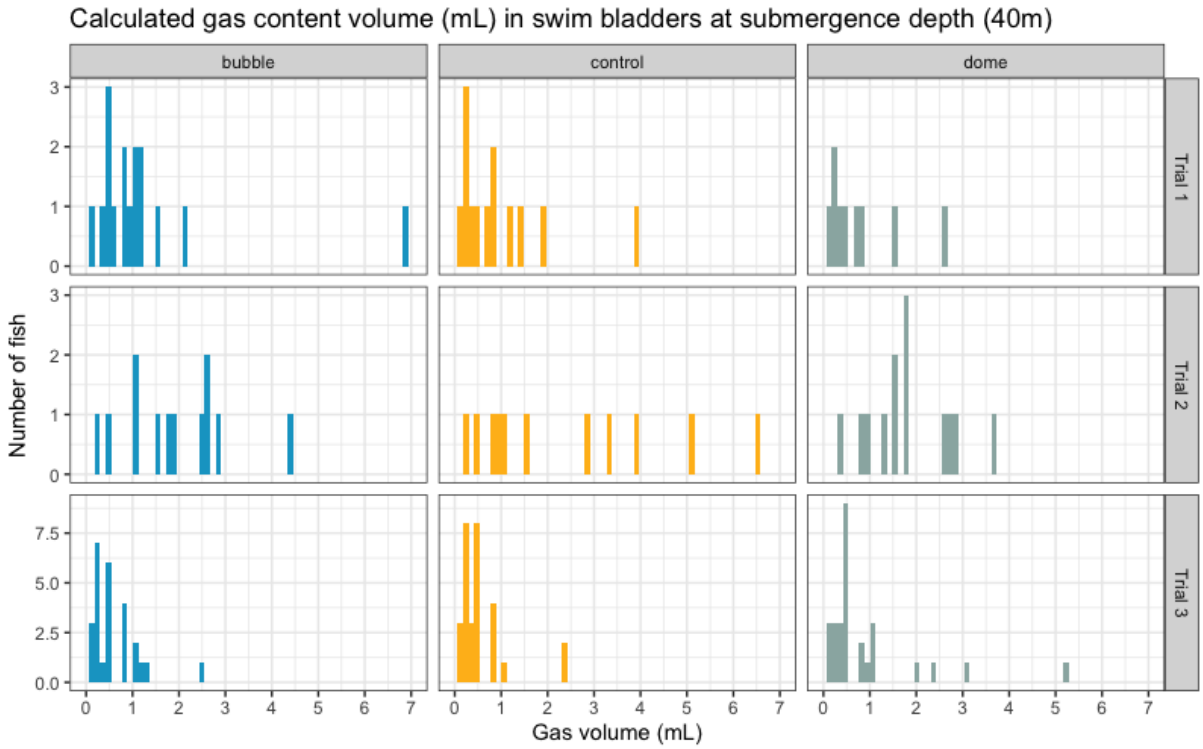


***Figure 16:*** Histograms illustrates the calculated volume (mL) of gas content in investigated swim bladders post Trial 1 (n = 42; 17, 11 and 14 fish in Bubble, Dome, Control group respectively), Trial 2 (n = 45; 15, 15 and 15 fish in Bubble, Dome, Control group respectively), and Trial 3 (n = 99; 33, 33 and 33 fish in Bubble, Dome, Control group respectively) at submergence depth (40m).

### 4.6.3. Water content volume in swim bladders

Water content in the measured swim bladders varied between the trials but did not differ significantly between trials. Of all the 184 examined swim bladders, 87 (47.3%) contained water.

For Trial 1, 42 fish were examined for water content and 76.1% of all investigated swim bladders contained water (59%, 73% and 92% for the Bubble, Dome and Control group respectively; Figure 17). Mean water content for all groups were 0.76 ± 0.14 mL (mean ± SE). The Control group had the highest water content (1.11 ± 0.26 mL), followed by the Dome group (0.77 ± 0.22 mL) and the Bubble group (0.46 ± 0.20 mL).

Water was found in 28 of 45 fish (62.2%) in Trial 2, distributed as 53.3% of fish in the Bubble group, 60% in the Dome group and 73.3% in the Control group (Figure 17). The mean value of

water content in the swim bladder for all groups were 0.50 ± 0.07 mL. The Control group had the highest mean water amount of the measured fish (0.60 ± 0.15 mL), followed by the Bubble and Dome group (0.37 ± 0.09 mL and 0.33 ± 0.08 mL respectively).

In Trial 3, water was found in 27.2% of investigated swim bladders (27 of 98), distributed as 30.3%, 30.3%, and 21.2% in the Bubble, Control and Dome group respectively (Figure 16). The mean water content for all groups combined were 0.60 ± 0.14 mL. The highest mean volume was found in the Control group (0.77 ± 0.33 mL). Dome and Bubble group had a mean volume of 0.53 ± 0.20 mL and 0.48 ± 0.16 mL respectively.

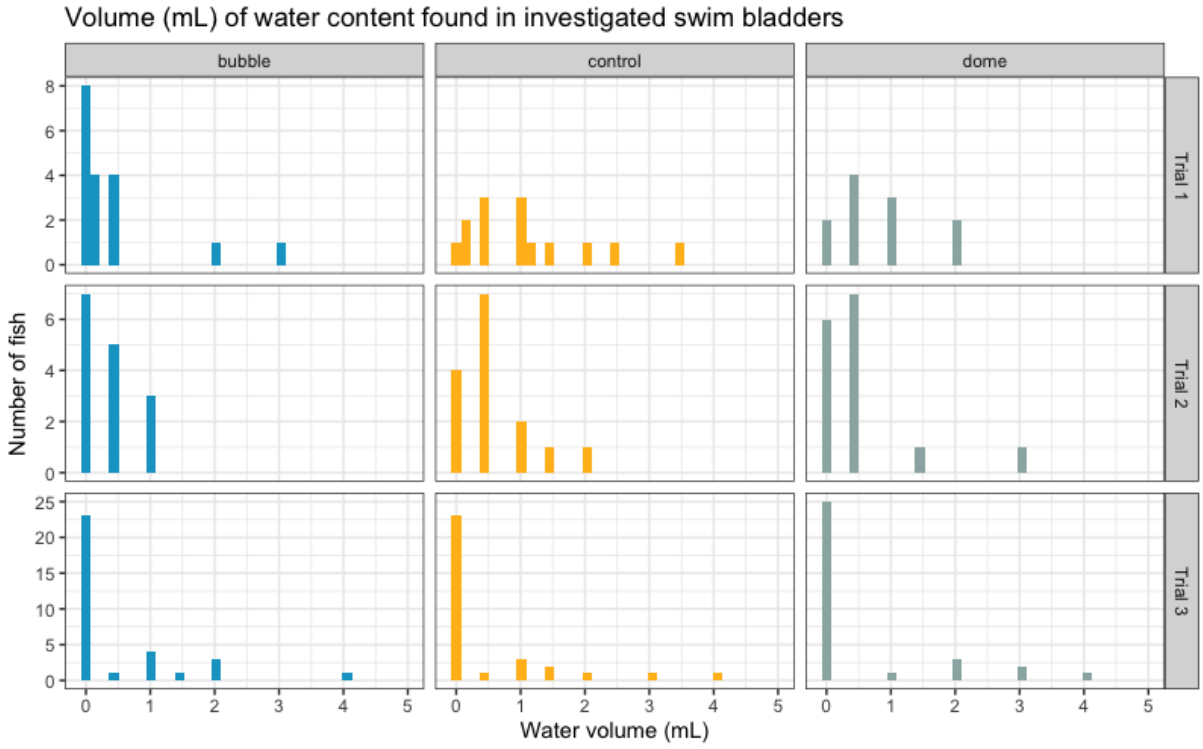


***Figure 17:*** Histograms illustrates the occurrence and amount (mL) of water as a content in investigated swim bladders post Trial 1 (n = 42; 17, 11 and 14 fish in Bubble, Dome, Control group respectively), Trial 2 (n = 45; 15, 15 and 15 fish in Bubble, Dome, Control group respectively), and Trial 3 (n = 99; 33, 33 and 33 fish in Bubble, Dome, Control group respectively) at surface levels.

## 4.7. Feed in gut

Feeding occurred 0.5 h prior to the ascending procedure in Trial 3. Results from intestinal inspection showed that 64.3% of all fish had feed in the gut. The Bubble group had the highest amount of feed in the gut (75.8%), followed by the Dome (63.6%) and the Control (45.5%) group. No significant difference was found between the groups ($p < 0.05$; Model: FG, Table 50, Appendix I). When investigating if there is a correlation between feed in gut and K-factor,

no significant difference was neither found ($p < 0.05$; Model: FG, Table 49, Appendix I; Appendix figure 14, Appendix II).

# 5. Discussion

In the current study the coping ability during deep submergence (40 m) in a small sea cage was compared between large Atlantic salmon that had experienced with submergence, either with air bubbles or air dome, and salmon naïve to submergence. Air supply for re-filling of the fish swim bladder was available for the fish in both bubbles and an air-filled dome, where the design and positioning of the dome varied between the three trials lasting 1- (Trial 1 and 2) and 3 weeks (Trial 3). The fish were only observed in the dome when it was aligned with the net roof during Trial 2, signalling that dome positioning is key. Although the fish with dome experience showed the highest frequency of visiting the dome, the recorded data is somewhat limited and it is therefore difficult to conclude on whether the Dome fish benefitted from their previous experience or not. The fish accustomed to bubbles showed a higher bubble interaction frequency in all trials, but this was not reflected in behavioural parameters or swim bladder gas content, suggesting that the fish did utilized the bubbles for efficient swim bladder re-filling. Nevertheless, and given low success with swim bladder re-filling, the fish swimming speed and body tilt angle, gas- and water content in the swim bladder after submergence, and welfare scoring indicate that all groups did cope with a short period of deep submergence, regardless of previous experience. Fish in all groups showed similar weight loss during submergence, and similar levels of increased snout wound score, signalling that the submergence treatment was a demanding challenge. Although not significant, the Control group swam with a higher tilt angle and higher speed at the end of Trial 3, and Control fish had feed in their gut, which indicate some benefit of previous submergence. The fish responded to descend and ascend of the cage by changes in swimming speed that most likely were driven by acute stress than change of pressure *per se*. For short-term deep submergence the present study indicates that prior experience with submergence is not essential in large salmon.

## 5.1. Experimental design and setup, and ethical considerations

Due to its pioneering-like experimental setup considering the submergence depth and size of the fish, necessary adjustments in regard to design and sampling were made along the way. An experimental design with triplicate cages in parallel would have been ideal but was predominately restricted by the availability of sea cages and required observation tools. Another reason was to minimize risks to animal welfare by limiting the number of experimental fish, as the current design was evaluated to impose a high risk for poor fish welfare, as also pointed out by the Norwegian Ethics committee (Food Authorities). A cautious approach resulted in trials

separated in time with increasing fish size and longer time in the different treatments (submerged or control), allowing effects on fish to be assessed before proceeding to the next submergence.

The sea cage used in this study is the smallest size available for experimental production of salmon, and smaller than the fish were accustomed to. A larger cage would potentially allow the fish to compensate differently, for example by faster swimming speed, and also allow a larger air dome and a larger stream or "curtain" of bubbles to be provided. The group size of fish was chosen from experience as the minimum number to secure normal group behaviour of schooling in a small sea cage, but created a different social environment than in the cages that the fish were taken from. In general, it is thought that any possible effects of a reduction in cage and group size will be negative for the fish performance, suggesting that the coping ability of the fish would have been better in a larger cage.

The observation frequency of fish swimming behaviour (3 times per day) was higher compared with previous submergence studies. This was predominately due to the high welfare risk for the fish with the current experimental design, and thus to enable early detection of behavioural anomalies pre-defined as humane endpoints (e.g. ≥5 fish having maintaining contact with the net structure, or 5 fish accumulated mortality), and thus rapid evacuation of the fish to surface. Observations of dome and bubble interactions were less frequent (once per day). The chosen time for dome interactions covered 1 h per day, while the bubble interaction frequency only 5 daily minutes. From the findings in this study it is clear that a 1 h daily observation period for bubble interactions would have been more beneficial and provided stronger data for this specific behaviour.

The use of common garden and tagged fish was predominantly chosen to reduce the number of trials and thereby the number of experimental fish. This can lead to copying behaviour (social learning) (Laland et al., 2011) and fish of different background acting together as a group may have given less clear results than if the groups were tested separately.

For testing the effects of hydrostatic pressure per se, the study would have benefitted from additional groups tested at different depth positions. This was, however, not possible within the current availability of fish and resources.

Regretfully, the design and positioning of the air dome was not the same for any trial, where re-filling was only observed during Trial 2 and mainly the first day when the dome was vertically aligned with the net roof. This hampered testing under successful swim bladder re-filling to address the aim of whether previous experience with submerged air filling in a dome is beneficial. The observed re-filling rate being higher in the Dome group during Trial 2 does indicate that experienced fish are faster in utilizing an air pocket, which here was presented in a much smaller dome than the fish previously had been accustomed to use (Ø = 0.9 vs. 3 m). However, whether this potentially faster rate of learning observed in experienced fish during Trial 2 would prevent negative consequences to fish welfare relative to in unexperienced fish remains unknown. To address this question fully it would require additional testing without common garden to rule out the possibility of naïve fish learning by observation of experienced fish.

The feeding regime in this study differed from what the fish were accustomed to (2 × 1.5h daily feeding periods) as a relatively small quantity of food was presented to the fish over one daily meal which lasted for 10 minutes. From the present design it is difficult to conclude whether the loss of weight and condition under submergence was due to reduced appetite or poor feed availability. Considering the proportion of fish with feed in their intestines after 3 weeks of submergence after Trial 3, appetite was present in most fish.

The rate of bubble interactions did vary within and between trials, which can be ascribed to adjustment of air pressure in the bubble hose to prevent the dome from ascending. Although the air pressure was not quantified, the pressure was lowest during Trial 3 concurrent with the lowest rate of fish – bubble interactions, signalling that the fish prefer a higher amount of bubbles.

The measuring of tilt angle in Trial 1 and 2 included fish swimming upwards and downwards in the water column, which resulted in a negative tilt angle for some groups. Based on a high number of fish that changed their vertical position in Trial 1 and 2, the observation method for Trial 3 was altered with regards to only include fish with a horizontal swimming direction to better enable detection of swimming with a tilted body posture.

The environmental monitoring was not optimal, as the winch that profiles the CTD failed for periods during Trial 1 and 3.

## 5.2. Environmental conditions

The trials were carried out over a relative long period, from December 2021 to March 2022. A thermo- and halocline fluctuated between 2-7m of the water column for all trials. The water temperature and salinity levels remained very stable and similar to what the fish had available between 0 and 15 m depth in the cages they were sampled from. Similarly, the oxygen levels at the submergence depth (40 – 47 m) were similar to the surface-based cages for Trial 1 and 2, but lower during Trial 3. Moreover, some between-trial variation was found in environmental parameters. The relatively low oxygen levels (72%) observed at 40 m during Trial 3 with a water temperature of ~10°C, is considered mildly sub-optimal for salmon growth and performance (Stien et al, 2013). This risk for a sub-optimal deep environment was also shown in the recently reported study by Warren-Myers et al. (2022), where submerged salmon at the very same experimental site as for this study were negatively affected by the deeper environment compared with the surface control cages.

## 5.3. Welfare parameters

No fish died during submergence in this study. One fish died prior to submergence in Trials 2, most likely as a result from handling during tagging and welfare scoring. Hence, the fish were given an additional stressor to the submergence. Moreover, the fish were netted two times (into and out of the experimental cage) after the initial welfare scoring, which might have imposed physical damage and negatively affected the scoring levels (Folkedal et al., 2016).

Due to the sub-optimal feeding regime a small decrease in weight and a relatively small increase in length were seen for each trial, which influenced the condition factor negatively. Considering the studies from Dempster et al. (2008, 2009) and Glaropoulos et al. (2019) which found that short term or repeated submergence did not have an influence on growth, it is highly plausible that the feeding regime itself contributed to the weight decline rather than the submergence in this study. Unneland Larsen (2021) also found that salmon (340g) showed normal growth when being submerged for up to 20 days under a standard feeding regime.

The initial K-factor for all trials were high and well over what is considered as positive performance and good health for Atlantic salmon (K-factor > 1.1) (Stien et al., 2013). The mean decrease in condition factor for all groups over all trials were -9.3%, varied between -2 to -13.4% between groups and trials. The Dome group had the highest mean decrease in K-factor (-11.1%), whereas the Control group showed the least decrease in K-factor (-7%). This may be ascribed to the observed differences between groups in condition factor at the start of each trial. Long term submergence (41 days) without swim bladder re-filling did also decrease the condition factor in Atlantic salmon (Korsøen et al., 2009).

All fish was scored according to the FISHwell protocol before and after submergence, and the most prominent differences was a significant increase in snout wound score. To a lesser degree, the fin erosion was also worse after submergence. The observed severity of snout wounds and fin erosion is similar to that reported after long term submergence without swim bladder re-filling (Korsøen et al. 2009; Sievers et al., 2018), and after long term submergence with successful swim bladder re-filling in an air dome (Warren-Myers et al., 2022). Although the fish were not observed to have physical contact with cage structures, snout damage and fin erosion are both identified to be caused by fish interacting with the cage roof (Korsøen et al., 2009). Aggressive interactions, as observed in this study, might also have contributed to fin erosion (Figure 13). Wounds are a potential gateway for pathogens as well as contribute to osmoregulatory disturbance and increased metabolic rate during wound healing (Stien et al., 2013), and should be monitored closely during submergence.

## 5.4. Behavioural observations

### 5.4.1. Swimming speed

Previous studies investigating swimming speed during submergence have shown that there is a negative correlation between size and swimming speed (Unneland Larsen, 2021; Glaropoulos et al., 2019; Korsøen et al., 2009). The observed swimming speeds increased with 27.3%, 15.7% and 40.8% over Trial 1, 2, and 3, respectively, and the highest mean swimming speed was 0.84 BL $s^{-1}$ (Bubble group in Trial 1). Swimming speed of large submerged Atlantic salmon have previously been found to have an increase of 64% over 36 days with no air-supplement available (Sievers et al., 2018), and between 50-100% over 7 days with air-supplement turned on and of every 7 day (Korsøen et al. 2012). Increased swimming speed causes hydrodynamic lift and is considered an effective behavioural compensation of negative buoyancy and hence

to prevent sinking (Dempster et al. 2008). Increased swimming speed in negative buoyant salmon is assumed to increase the energy expenditure leading to higher metabolic rates and negative growth effects (Korsøen et al., 2009, Sievers et al., 2018). Providing air for swim bladder re-filling is thereby of great importance for inducing neutral buoyancy and maintain normal swimming speeds (Sievers et al., 2021). Compared with Trial 1 and 3, the relatively lower swimming speed during Trial 2 concurrent with observed dome interactions and a higher swim bladder gas content suggests that the fish did re-fill their swim bladders during this specific trial. However, the gas volumes at resurfacing were modest, and there was a large variation between the examined individuals, signalling that re-filling was sub-optimal even for Trial 2. The duration of submergence should explain the difference between trials in swimming speed. This considering the relative high increase in swim speed through the three-week Trial 3 and low swim bladder gas content compared with the one-week Trial 1, and no apparent success with swim bladder re-filling in any of the trials.

### 5.4.2. Tilted swimming

Tilted swimming behavior were observed in all trials. Data from Trial 3 showed a positive mean tilt angle of 7.7 ± 1.63° (mean ± SE) at the end of the trial, whereas the highest tilt angle recorded was 32°. This is in line with the observation of Sievers et al. (2018) who found a linear increase in tilt angle over time, resulting in a tilt angle of 16.3 ± 4.3° after 36 days submerged without air-supplement, which emphasizes that swim bladder re-filling most likely was unsuccessful during Trial 3.

Overt tilted swimming has been observed after weeks of submergence without swim bladder re-filling, and thereby weeks after the onset of increased swimming speeds, and signals that the swim bladder gas content is low (Sievers et al., 2021). Swimming with a positive tilted body angle is believed to generate hydrodynamic lift in the water (Dempster et al., 2008, 2009). Unneland Larsen (2021) observed a very high swimming speed in small negatively buoyant fish (~2.8 Bl $s^{-1}$) and a mild decreased in speed when tilted swimming occurred after two weeks. Such was, however, not observed in negatively buoyant large salmon that maintained a relatively stable swimming speed while submerged but increased their tilt angle (Korsøen et al., 2009; Sievers et al., 2018). In other words, the behavioral compensatory mechanisms may differ with fish size, and importantly, with swim bladder gas volume. Observations from this study showed that fish swimming with relative low speeds (0.4 BL $s^{-1}$) had a relatively severe

tilt angle (~20°) (Figure 21). This observation is in line with the swimming speed and tilt angle observed by Korsøen et al. (2009) of large salmon during the night, when the swimming speed was about 50% of that observed during day light. The current study used continuous artificial light, but the small size of the cage (5 × 5 m) may have hampered high swimming speeds relative to what observed at daytime in the larger cages (12 × 12 m), as used in the abovementioned experiments. This suggest a context dependent use of behavioral compensation for negative buoyancy and that the present results of such are not directly relevant for larger cages. The tilt angles recorded during Trial 1 and 2 supports this, as the individual variation was very high compared with the above-mentioned experiments and ranged from negative to positive. The fish were here observed to change their swimming depth, rather than swimming horizontally with a tilt angle. Dempster et al. (2008) speculated that negatively buoyant salmon have a strategy of swimming towards the net roof and slowly spiral downwards and utilize faster swimming on the way back up. The present indication of change in individual swimming depth supports this.

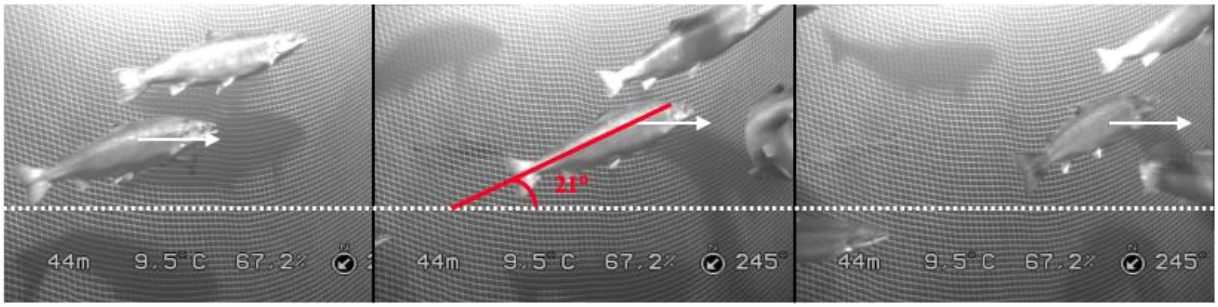


***Figure 18:*** Photos illustrating a short timeline of tilted swimming behaviour. Red line indicates degree of tilt (21°). White dotted line indicates a horizontal plane, white arrows indicate direction of swimming. Recording camera in a fixed positioned.

### 5.4.3. Swim bladder re-filling

As previously explained, the experimental design of the air dome differed between the three trials. Observations shows that the fish used the dome when positioned in level with a flat net roof, but not with a steep angle of the roof or when the dome was positioned beneath the roof. This is a highly interesting observation for how air domes should be positioned within submerged cages. The only position that was found to be at least partly functional was similar to the reported experiments with a uccessful dome-filling where the dome was positioned and attached as a part of the net roof, and without severe coning of the roof (Korsøen et al., 2012; Oppedal et al., 2020; Warren-Myers et al., 2022). The present indication that salmon will not utilize the dome if they can swim above it (Trial 3) has implications for possible positioning of

air domes at the depth where the fish preferable reside within sea cages. Although the air in the dome must have been highly visible and very accessible for the fish, the failure of using it may be explained by an unnatural air position (i.e. not at the highest point of the highest accessible space for the fish). However, it cannot be ruled out that a larger dome or learning from an earlier stage (e.g. Macaulay et al. 2020) would help. Usage of the dome in the rather confined dome position of Trial 1 is considered less plausible for the fish to adapt to, as very few fish were observed to explore the top and thus highly coned shaped volume of the cage.

Fish previously experienced with dome re-filling (Dome group) showed a higher interaction frequency with dome than the other groups in Trial 2. Additionally, the Dome group had the lowest mean swimming speed during the trial, being somehow indicative of successful swim bladder re-filling. However, the swim bladder gas content of the Dome group was not significantly higher than in the other groups, and there were Dome fish that lacked gas in the swim bladder. The successful re-filling event was, however, on Day 1 of submergence, and if the swim bladders were not filled after this, leakage may explain the lack of swim bladder gas content. Although the total volume of a fully inflated swim bladder was not tested for in this trial, these fish may be in the higher end of the gas content spectrum for fish of this size (3.1 kg), as indicated by sampling from the same control cages in a parallel study. The mean swim bladder gas content fish in the parallel study (n = 89, 3.1 kg mean weight) which resided and was captured between 2-7 m depth was 12.1 ± 1.4 ml (mean ± SE) and ranged from 0 to a maximum of 65 ml ($10^{th}$ percentile ≤ 2 ml, $90^{th}$ percentile ≥ 29 ml) (Folkedal et al., in prep). If swim bladders were inflated at 40 m depth, one would have expected that the fish would release air from their swim bladders during ascending procedure in order to avoid getting super-inflated as the gas volume increases with lower depths. This would presumably been observed from the fish during re-surfacing of submerged dome cages (O. Folkedal. Pers. Comm.). This was, however, not observed when ascending the fish in the current trials.

The Bubble group showed the most interactions with the bubbles during all trials and used the dome when it was situated correctly. The Bubble group had, however, the highest mean swimming speed in all trials. This might indicate the lowest success in re-filling of the three groups, but the mean swim bladder gas content was, however, not the lowest for the Bubble group, except for in Trial 3. The condition factor was the highest among the Bubble fish, which should in theory contribute positively to flotation by a higher fat content (Macaulay et al., 2020). A possible explanation to the difference in condition is spinal deformations due to long term

negative buoyancy (Korsøen et al., 2009) in the Bubble group before the fish were included in the present experiment, and thus that a different body shape that could explain their difference in swimming speed (Folkedal et al., in prep).

The observed effect of bubble interactions on swim bladder re-filling on gas content seem to be negligible. Little is known about swim bladder re-filling from air bubbles, and few studies are conducted in this area of research. Unneland Larsen (2021) showed that small Atlantic salmon (up to 130-300g) submerged over two weeks could maintain normal swimming behaviour when air bubbles were provided in sea cages. Similar bubble size (Ø = 2 cm) as Unneland Larsen (2021) was used in the present study, but over a much smaller volume, and the difference between bubble and fish size was much larger in the present study. It is thus a need for further investigation of the importance of bubble size, as well as the air pressure and thereby quantity of bubbles.

Water in the swim bladder was found for most individuals after Trial 1 and 2, while only in ~25% of the fish after Trial 3. To my knowledge, Unneland Larsen (2021) is the first author to report prevalence of water in the swim bladder, where it was emphasized that submergence itself, rather than failed swim bladder refilling with artificial air supply, was the reason. The present study did not control for any effect of bubbles or any possible effect of gulping of water instead of bubbles. The current study is the first to quantify the amount of water, showing that it was a relatively small amount of water compared to the full bladder volume, and also to demonstrate that water can occur after only one week of submergence. Unneland Larsen (2021) indicated that the salmon could evacuate the water after being re-surfaced. A speculation towards explaining less fish with water after Trial 3, being the longest time of submergence in the present study, could be that salmon may evacuate water over time even when submerged. Alternatively, bubble interactions do generate water in the swim bladder and the lower gas pressure and less bubble interactions during Trial 3 can explain the difference. The observation of higher water content in the Control group in all trials suggest an effect of previous submergence. Further studies around on the topic of swim bladder water content are required to understand the mechanisms of both water intrusion and evacuation, and to which degree it is problematic for the fish.

### 5.4.4. Learning

It has previously been showed that groups of Atlantic salmon have a good capacity to learn (e.g. Bratland et al. 2010; Macaulay et al. 2020). Macaulay et al. (2020) showed that fish accustomed to dome-re-filling during the freshwater life-stage were more efficient in dome-re-filling at the early sea phase. In this study, an aim was to investigate whether previous experience with artificial air supply would increase the rate of learning towards utilizing such vs. in naïve fish during a submergence challenge. The lack of successful dome filling other than one day during Trial 2 makes it difficult to investigate a rate of learning for dome use. The higher frequency of Dome fish in the air dome vs. other groups during this specific observation do support the finding of Macaulay et al. (2020) by indicating that experience with dome-filling was positive towards re-filling in a novel dome and rearing environment. The data for bubble interactions was more suitable for investigation of a learning/adaptation process. The fish in the Bubble group showed the highest interaction frequency for all trials, and especially during the three initial days of each trial. This suggest an effect of being accustomed to bubbles in the Bubble group, while adaptation occurred over the initial days in the Dome and the Control group. This is like the observation of Unneland Larsen (2021) of a three-day accommodation period, where a transition from fright to attraction towards bubbles occurred. Whether the fish were attracted to bubbles for swim bladder re-filling or other purposes such as utilizing the mild vertical water flow created by bubbles is not known. Considering the results from bubble interactions, fish from all three groups had interacted with the bubbles already at Day 2 in Trial 1 and 2, and Day 9 in Trial 3.

## 5.5. Hydrostatic pressure and buoyancy

The present study with submergence to 40 m depth represents the deepest reported submergence depth of sea caged Atlantic salmon. Previous reported experimental studies where salmon was submerged down to a maximum of 15 m depth (net roof) (Warren-Myers et al., 2022), have little focus on potential effects of hydrostatic pressure (Sievers et al., 2021). In comparison, the forced hydrostatic pressure on the fish in this study was more than doubled from previous experiments. However, the hydrostatic pressure per se at 40 m should not be a big physical challenge for salmon. Tag data from wild Atlantic salmon shows that they mainly roam the surface depth layer (0 -5 m), but occasionally perform dives towards the sea bottom (Godfrey et al., 2014), and to extreme depths of more than 1000 m when crossing ocean channels and the shelf edge (Einarsson et al., 2018; Lacroix & Bradford, 2013). Moreover, in deep sea cages (50

m) the farmed salmon may voluntarily positioned themselves towards the cage bottom (N. Eide pers. obs.). Forced submergence is, however, different as it is involuntary, and the duration of the "dive" is much longer than a voluntary deep dive in nature which typically last for a few hours (Lacroix and Branford, 2013).

When the fish were submerged in the present study there was a swimming speed increase of ~60, ~30, and ~120% from surface and down to 10 m depth for Trial 1, 2, and 3 respectively, and similar levels when fully descended to 40 m depth. The observed variations in speed between depths and trials when descending the fish were most likely caused by stress from the submergence procedure itself, making it hard to disentangle the effect of the stepwise higher hydrostatic pressure. Previous submergence studies have shown that salmon did not show an immediate increase when submerged to 3 m depth (Dempster et al., 2009) while an increase in speed occurred faster when submerged to 5 m (Dempster et al., 2008) and 10 m depth (Korsøen et al., 2009). In small salmon submerged to 10 m, swimming speed increase of 50 – 100% were found one day after submergence (Glaropaulos et al., 2019), while small salmon that was merely restricted surface access by a net roof at 1 m and maintained their swimming depth showed a less immediate but a gradual increase over days and weeks as the fish became negatively buoyant (Unneland Larsen, 2021). The currently observed behaviour shortly after submergence and the initial days of any trial do not support that the fish compensated much stronger with deep submergence than what reported in previous studies of shallow submergence, although other compensational mechanisms may have been in play. Such may include the abovementioned frequent change of swimming depth.

The observed gas content of the swim bladder was very low only after one week at 40 m, whereas previous studies have reported a gradual decline in echo strength over a 3-week submergence period (Dempster et al., 2009; Korsøen et al., 2009). Although echo sound was not measured in the present study and the precise relationship between echo strength and swim bladder content is not known in Atlantic salmon, it may be speculated that the higher pressure exerted on the fish in the current study affected the rate of swim bladder gas leakage.

The currently used fish had a relatively high condition factor, which should have provided an advantage, although small, compared to leaner fish. Large fish normally contains higher lipid stores than of small ones, and hence have a higher buoyancy as shown by a deeper maximum neutral buoyancy depth (Macaulay et al., 2020). Lie & Huse (1992) found that whole fish fat in

fasted Atlantic salmon was reduced by ~1% over 35 days in cold water. Due to its very low compressibility and relatively low density (<0.93 kg/L), lipids decrease the fish overall body density and hence increases the static lift, with only very low effects on lipid density by depth (1% change over 100 bar) (Campbell & Dower, 2003; Macaulay et al. 2020). Thus, Macaulay et al (2020) found that maximum neutral buoyancy depth (MNBD) was 13% shallower in small fish (175g) than of large fish (2400g) (MNBD at 21.2m vs. 24.4m, respectively). Considering this, the reduction in lipid content following the weight loss of fish in the present study would have a rather small effect on the buoyant force on the fish.

The mean weight reduction in Trial 3 (submerged for 21 days), were 244g for all groups combined. In a study done by Mørkøre et al., (2008) where Atlantic salmon (initial mean weight 2949g) were starved for 35 days, no significant reduction in lipid content ($p < 0.001$) of body weight was found at the end. The weight at day 35 for the starved fish group were 2861, resulting in a decrease of 88g. The fish examined by Mørkøre et al. (2008) had surface air for swim bladder re-filling available. Comparing these results to the weight reduction in Trial 3 (initial mean weight 3194g), a relative larger weight reduction was seen in Trial 3 (which were fed each day). This implies that insufficient swim bladder re-filling increases the metabolism by increased swimming speed and indicates the importance of swim bladder re-filling on the basis of metabolism isolated.

# 6. Conclusion and further perspectives

This study has demonstrated the coping ability of large Atlantic salmon (>2 kg) during deep short-term submergence (>40 m) in a small common garden sea cage (5 x 5 x 7 m) with fish experienced with submergence, either with air bubbles or dome, or being naïve to submergence. The experimental setup which intended to provide the fish with air for swim bladder re-filling did largely fail, as the dome was only used for a short period when aligned with the net roof, while the air bubbles attracted the fish but seemingly without successful swim bladder re-filling. In other words, it was difficult to answer whether the previous use of air dome or bubbles affected the fish buoyancy regulation when submerged. However, a positive effect of experience was observed towards the use of air dome when successfully situated and the group with dome experience showed the lowest swimming speed. Similarly, the interaction with air bubbles was highest for the fish experienced with bubbles. Although the fish had all over little success in swim bladder re-filling, they all coped with the challenge of being submerged to below 40 m depth in the small sea cage. Negative effects of increased snout and fin damage was found, and the fish showed compensatory behavior by increase in swimming speed and body tilt angle. The levels of these parameters were similar to that observed in long-term studies of submergence without air supply in large salmon (Korsøen et al., 2009; Sievers et al., 2018), and indicate that the fish were not overloaded, even when submerged for 3 weeks. The current setup was evidently not fit for permanent submergence, but the results should be regarded as a positive outcome towards the coping ability large salmon have for short-term deep submergence, especially as the naïve fish did not differ much from the experienced fish. In a production perspective, the possible negative effects of submerging the fish, including reduced or impaired growth as measured in the current study, should be traded off with the possible dire outcomes of keeping the fish at surface during e.g. an algae bloom. The tested depth and duration (1-3 weeks) should be highly relevant for such an application. This study used the deepest submergence depth yet for Atlantic salmon and did not find any apparent negative effects of hydrostatic pressure. A larger cage volume than used for the current experiment would most likely better facilitate technical air supply installations for swim bladder re-filling and compensatory behavior towards negative buoyancy. Overall, this study demonstrates that large salmon can cope with short-term and deep submergence to avoid harmful surface related hazards.

The results from this study suggests that future research on this topic should be carried out in larger sea cages with industrial feeding regime. This would be beneficial in order to investigate fish coping and welfare, including growth and other production performance traits in deep submersible systems for future submergence and avoidance of lice and dire algae and jellyfish blooms.

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# Appendices

## Appendix I – Statistical analysis

*All significant levels presented in this appendix are indicated indicated with asterisks, p < 0.05: (*); p < 0.01: (**); p < 0.001: (***); p < 0.0001 (****). Abbreviations: SS = Sums of squares, DF = Degrees of freedom, CI = Confident interval, LR Chi$^2$: likelihood Chi-square, Q = Quantile.*

### Sampling analysis – Model: K-factor.

***Appendix table 1**:* Test results from a three-way ANOVA log-model. Multiple $R^2$: 0.285; Adjusted $R^2$: 0.264.

| | SS | DF | F-value | p-value |
|---|---|---|---|---|
| Trial | 0.633 | 2 | 27.7 | < 0.0001**** |
| Time | 1.368 | 1 | 119.6 | < 0.0001**** |
| Group | 0.405 | 2 | 17.7 | < 0.0001**** |
| Trial:Time | 0.020 | 2 | 0.9 | 0.425 |
| Trial:Group | 0.099 | 4 | 2.2 | 0.073 |
| Time:Group | 0.035 | 2 | 1.5 | 0.221 |
| Trial:Time:Group | 0.036 | 4 | 0.8 | 0.536 |
| Residuals | 6.519 | 570 | | |

*Model predictions*

| Trial | Group | Time | Predicted K | 95% CI |
|---|---|---|---|---|
| 1 | Bubble | Start | 1.59 | [1.54, 1.65] |
| 1 | Bubble | End | 1.41 | [1.36, 1.47] |
| 1 | Control | Start | 1.48 | [1.43, 1.54] |
| 1 | Control | End | 1.44 | [1.38, 1.49] |
| 1 | Dome | Start | 1.45 | [1.40, 1.51] |
| 1 | Dome | End | 1.33 | [1.28, 1.38] |
| 2 | Bubble | Start | 1.56 | [1.51, 1.62] |
| 2 | Bubble | End | 1.40 | [1.35, 1.46] |
| 2 | Control | Start | 1.46 | [1.41, 1.51] |
| 2 | Control | End | 1.34 | [1.29, 1.39] |
| 2 | Dome | Start | 1.44 | [1.39, 1.49] |
| 2 | Dome | End | 1.30 | [1.25, 1.35] |
| 3 | Bubble | Start | 1.45 | [1.40, 1.50] |
| 3 | Bubble | End | 1.30 | [1.25, 1.35] |
| 3 | Control | Start | 1.38 | [1.33, 1.43] |
| 3 | Control | End | 1.24 | [1.20, 1.29] |
| 3 | Dome | Start | 1.21 | [1.36, 1.46] |
| 3 | Dome | End | 1.26 | [1.22, 1.31] |

## Sampling analysis – Model: Weight

***Appendix table 2:*** Test results from a three-way ANOVA log-model. Multiple $R^2$: 0.272; Adjusted $R^2$: 0.250.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 11.723 | 2 | 83.7 | < 0.0001**** |
| Time | 0.540 | 1 | 7.7 | 0.006 ** |
| Group | 2.053 | 2 | 14.7 | < 0.0001**** |
| Trial:Time | 0.026 | 2 | 0.2 | 0.833 |
| Trial:Group | 0.345 | 3 | 1.2 | 0.296 |
| Time:Group | 0.031 | 2 | 0.2 | 0.801 |
| Trial:Time:Group | 0.032 | 4 | 0.1 | 0.977 |
| Residuals | 39.922 | 570 | | |

*Model predictions*

| **Trial** | **Group** | **Time** | **Predicted K** | **95% CI** |
|---|---|---|---|---|
| 1 | Bubble | Start | 2072.45 | [1893.52, 2268.29] |
| 1 | Bubble | End | 1897.64 | [1736.12, 2074.18] |
| 1 | Control | Start | 2407.17 | [2199.34, 2634.64] |
| 1 | Control | End | 2343.21 | [2131.49, 2575.97] |
| 1 | Dome | Start | 2091.65 | [1908.39, 2292.51] |
| 1 | Dome | End | 2070.24 | [1888.85, 2269.05] |
| 2 | Bubble | Start | 2693.02 | [2460.51, 2947.50] |
| 2 | Bubble | End | 2461.74 | [2246.05, 2698.14] |
| 2 | Control | Start | 3101.54 | [2833.76, 3394.63] |
| 2 | Control | End | 2890.85 | [2641.26, 3164.03] |
| 2 | Dome | Start | 2755.87 | [2517.94, 3016.29] |
| 2 | Dome | End | 2596.46 | [2372.29, 2841.82] |
| 3 | Bubble | Start | 2876.06 | [2627.75, 3147.84] |
| 3 | Bubble | End | 2701.48 | [2468.24, 2956.76] |
| 3 | Control | Start | 3194.80 | [2918.97, 3496.70] |
| 3 | Control | End | 2988.11 | [2730.12, 3270.48] |
| 3 | Dome | Start | 3200.35 | [2924.04, 3502.78] |
| 3 | Dome | End | 2989.22 | [2727.32, 3276.28] |

## Sampling analysis – Model: length.

***Appendix table 3:*** Test results from a three-way ANOVA log-model. Multiple $R^2$: 0.396; Adjusted $R^2$: 0.376.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 1.903 | 2 | 149.6 | < 0.0001**** |
| Time | 0.021 | 1 | 3.4 | 0.07 |
| Group | 0.408 | 2 | 32.1 | < 0.0001**** |
| Trial:Time | 0.001 | 2 | 0.1 | 0.944 |
| Trial:Group | 0.013 | 3 | 0.5 | 0.723 |
| Time:Group | 0.004 | 2 | 0.3 | 0.738 |
| Trial:Time:Group | 0.003 | 4 | 0.1 | 0.978 |
| Residuals | 3.627 | 570 | | |

*Model predictions*

| **Trial** | **Group** | **Time** | **Predicted K** | **95% CI** |
|---|---|---|---|---|
| 1 | Bubble | Start | 50.67 | [49.31, 52.07] |
| 1 | Bubble | End | 51.20 | [49.85, 52.59] |
| 1 | Control | Start | 54.58 | [53.12, 56.09] |
| 1 | Control | End | 54.63 | [53.10, 56.22] |
| 1 | Dome | Start | 52.39 | [50.96, 53.86] |
| 1 | Dome | End | 53.86 | [52.39, 55.37] |
| 2 | Bubble | Start | 55.66 | [54.16, 57.20] |
| 2 | Bubble | End | 55.97 | [54.45, 57.54] |
| 2 | Control | Start | 59.65 | [58.05, 61.30] |
| 2 | Control | End | 60.05 | [58.43, 61.70] |
| 2 | Dome | Start | 57.62 | [56.07, 59.21] |
| 2 | Dome | End | 58.48 | [56.91, 60.09] |
| 3 | Bubble | Start | 58.32 | [56.76, 59.93] |
| 3 | Bubble | End | 59.21 | [57.62, 60.84] |
| 3 | Control | Start | 61.43 | [59.78, 63.12] |
| 3 | Control | End | 62.21 | [60.54, 63.93] |
| 3 | Dome | Start | 60.96 | [59.33, 62.64] |
| 3 | Dome | End | 61.88 | [60.19, 63.61] |

## Sampling analysis – Model: GCS (General condition score)

***Appendix table 4:*** Test results from a three-way ANOVA log-model. Multiple $R^2$: 0.105; Adjusted $R^2$: 0.078.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 0.082 | 2 | 4.0 | 0.020 * |
| Time | 0.030 | 1 | 2.9 | 0.088 |
| Group | 0.252 | 2 | 12.2 | < 0.0001**** |
| Trial:Time | 0.013 | 2 | 0.6 | 0.523 |
| Trial:Group | 0.228 | 4 | 5.5 | 0.0002 *** |
| Time:Group | 0.034 | 2 | 1.7 | 0.190 |
| Trial:Time:Group | 0.050 | 4 | 1.2 | 0.307 |
| Residuals | 5.877 | 570 | | |

*Model predictions*

| **Trial** | **Group** | **Time** | **Predicted K** | **95% CI** |
|---|---|---|---|---|
| 1 | Bubble | Start | 1.00 | [0.97, 1.04] |
| 1 | Bubble | End | 1.08 | [1.05, 1.12] |
| 1 | Control | Start | 1.00 | [0.97, 1.04] |
| 1 | Control | End | 1.00 | [0.96, 1.04] |
| 1 | Dome | Start | 1.00 | [0.97, 1.04] |
| 1 | Dome | End | 1.00 | [0.97, 1.04] |
| 2 | Bubble | Start | 1.09 | [1.05, 1.13] |
| 2 | Bubble | End | 1.11 | [1.08, 1.15] |
| 2 | Control | Start | 1.00 | [0.97, 1.04] |
| 2 | Control | End | 1.00 | [0.97, 1.04] |
| 2 | Dome | Start | 1.00 | [0.97, 1.04] |
| 2 | Dome | End | 1.00 | [0.97, 1.04] |
| 3 | Bubble | Start | 1.00 | [0.97, 1.04] |
| 3 | Bubble | End | 1.00 | [0.97, 1.04] |
| 3 | Control | Start | 1.00 | [0.97, 1.04] |
| 3 | Control | End | 1.02 | [0.99, 1.06] |
| 3 | Dome | Start | 1.00 | [0.97, 1.04] |
| 3 | Dome | End | 1.00 | [0.97, 1.04] |

## Sampling analysis – Model: SLC (Scale loss score)

***Appendix table 5:*** Test results from a three-way ANOVA linear model. Multiple $R^2$: 0.114; Adjusted $R^2$: 0.088.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 1.315 | 2 | 7.1 | 0.0009 *** |
| Time | 0.249 | 1 | 2.7 | 0.100 |
| Group | 0.724 | 2 | 3.9 | 0.020 * |
| Trial:Time | 1.459 | 2 | 7.9 | 0.0004 *** |
| Trial:Group | 0.503 | 4 | 1.4 | 0.244 |
| Time:Group | 1.027 | 2 | 5.6 | 0.004 ** |
| Trial:Time:Group | 1.482 | 4 | 4.0 | 0.003 ** |
| Residuals | 52.427 | 570 | | |

*Model predictions*

| **Trial** | **Group** | **Time** | **Predicted K** | **95% CI** |
|---|---|---|---|---|
| 1 | Bubble | Start | 0.82 | [0.71, 0.92] |
| 1 | Bubble | End | 1.06 | [0.96, 1.16] |
| 1 | Control | Start | 0.97 | [0.87, 1.07] |
| 1 | Control | End | 1.00 | [0.89, 1.11] |
| 1 | Dome | Start | 0.94 | [0.83, 1.04] |
| 1 | Dome | End | 1.16 | [1.05, 1.26] |
| 2 | Bubble | Start | 1.00 | [0.90, 1.10] |
| 2 | Bubble | End | 1.00 | [0.89, 1.11] |
| 2 | Control | Start | 1.00 | [0.90, 1.10] |
| 2 | Control | End | 1.09 | [0.99, 1.19] |
| 2 | Dome | Start | 1.00 | [0.90, 1.10] |
| 2 | Dome | End | 1.03 | [0.93, 1.13] |
| 3 | Bubble | Start | 1.09 | [0.99, 1.19] |
| 3 | Bubble | End | 0.97 | [0.87, 1.07] |
| 3 | Control | Start | 1.33 | [1.23, 1.44] |
| 3 | Control | End | 1.03 | [0.93, 1.13] |
| 3 | Dome | Start | 1.00 | [0.90, 1.10] |
| 3 | Dome | End | 1.19 | [1.08, 1.29] |

## Sampling analysis – Model: SWS (Snout wound score)

***Appendix table 6:*** Test results from a three-way ANOVA square root model. Multiple $R^2$: 0.399; Adjusted $R^2$: 0.381.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 1.938 | 2 | 5.6 | 0.004 ** |
| Time | 54.072 | 1 | 313.0 | < 0.0001 **** |
| Group | 2.271 | 2 | 6.6 | 0.002 ** |
| Trial:Time | 4.267 | 2 | 12.4 | < 0.0001 **** |
| Trial:Group | 0.754 | 4 | 1.1 | 0.360 |
| Time:Group | 1.416 | 2 | 4.1 | 0.017 * |
| Trial:Time:Group | 0.627 | 4 | 0.9 | 0.459 |
| Residuals | 98.469 | 570 | | |

*Model predictions*

| **Trial** | **Group** | **Time** | **Predicted K** | **95% CI** |
|---|---|---|---|---|
| 1 | Bubble | Start | 1.03 | [0.84, 1.22] |
| 1 | Bubble | End | 1.44 | [1.25, 1.63] |
| 1 | Control | Start | 0.61 | [0.41, 0.80] |
| 1 | Control | End | 1.37 | [1.16, 1.57] |
| 1 | Dome | Start | 0.66 | [0.46, 0.85] |
| 1 | Dome | End | 1.50 | [1.30, 1.70] |
| 2 | Bubble | Start | 0.67 | [0.47, 0.86] |
| 2 | Bubble | End | 1.63 | [1.43, 1.82] |
| 2 | Control | Start | 0.27 | [0.08, 0.47] |
| 2 | Control | End | 1.58 | [1.38, 1.77] |
| 2 | Dome | Start | 0.30 | [0.11, 0.50] |
| 2 | Dome | End | 1.45 | [1.26, 1.65] |
| 3 | Bubble | Start | 0.64 | [0.44, 0.83] |
| 3 | Bubble | End | 1.45 | [1.26, 1.65] |
| 3 | Control | Start | 0.64 | [0.44, 0.83] |
| 3 | Control | End | 1.33 | [1.14, 1.53] |
| 3 | Dome | Start | 0.61 | [0.41, 0.80] |
| 3 | Dome | End | 1.56 | [1.37, 1.76] |

## Sampling analysis – Model: FES (Fin erosion score)

***Appendix table 7:*** Test results from a three-way ANOVA log model. Multiple $R^2$: 0.329; Adjusted $R^2$: 0.309.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 29.006 | 2 | 104.9 | < 0.0001 **** |
| Time | 1.194 | 1 | 8.6 | 0.003 ** |
| Group | 0.045 | 2 | 0.2 | 0.850 |
| Trial:Time | 7.395 | 2 | 26.7 | < 0.0001 **** |
| Trial:Group | 0.635 | 4 | 1.1 | 0.333 |
| Time:Group | 0.173 | 2 | 0.6 | 0.536 |
| Trial:Time:Group | 0.272 | 4 | 0.5 | 0.742 |
| Residuals | 78.794 | 570 | | |

*Model predictions*

| **Trial** | **Group** | **Time** | **Predicted K** | **95% CI** |
|---|---|---|---|---|
| 1 | Bubble | Start | 1.30 | [1.18, 1.43] |
| 1 | Bubble | End | 1.74 | [1.61, 1.86] |
| 1 | Control | Start | 1.48 | [1.36, 1.61] |
| 1 | Control | End | 1.77 | [1.63, 1.90] |
| 1 | Dome | Start | 1.38 | [1.25, 1.50] |
| 1 | Dome | End | 1.88 | [1.75, 2.00] |
| 2 | Bubble | Start | 2.12 | [1.99, 2.25] |
| 2 | Bubble | End | 2.09 | [1.96, 2.22] |
| 2 | Control | Start | 2.09 | [1.96, 2.22] |
| 2 | Control | End | 2.09 | [1.96, 2.22] |
| 2 | Dome | Start | 2.06 | [1.93, 2.19] |
| 2 | Dome | End | 2.03 | [1.90, 2.16] |
| 3 | Bubble | Start | 2.09 | [1.96, 2.22] |
| 3 | Bubble | End | 2.00 | [1.87, 2.13] |
| 3 | Control | Start | 2.09 | [1.96, 2.22] |
| 3 | Control | End | 1.94 | [1.81, 2.07] |
| 3 | Dome | Start | 2.09 | [1.96, 2.22] |
| 3 | Dome | End | 2.00 | [1.87, 2.13] |

## Sampling analysis – Model: DS (Deformity score)

***Appendix table 8:*** Test results from a three-way ANOVA log model. Multiple $R^2$: 0.084; Adjusted $R^2$: 0.057.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 0.761 | 2 | 4.8 | 0.008 ** |
| Time | 0.000 | 1 | 0.0 | 0.969 |
| Group | 2.572 | 2 | 16.3 | < 0.0001 **** |
| Trial:Time | 0.036 | 2 | 0.2 | 0.796 |
| Trial:Group | 0.686 | 4 | 2.2 | 0.070 |
| Time:Group | 0.023 | 2 | 0.1 | 0.863 |
| Trial:Time:Group | 0.048 | 4 | 0.2 | 0.962 |
| Residuals | 44.947 | 570 | | |

*Model predictions*

| **Trial** | **Group** | **Time** | **Predicted K** | **95% CI** |
|---|---|---|---|---|
| 1 | Bubble | Start | 0.04 | [0.01, 0.09] |
| 1 | Bubble | End | 0.06 | [0.02, 0.11] |
| 1 | Control | Start | 0.00 | [0.00, 0.02] |
| 1 | Control | End | 0.00 | [0.00, 0.02] |
| 1 | Dome | Start | 0.00 | [0.01, 0.01] |
| 1 | Dome | End | 0.00 | [0.01, 0.01] |
| 2 | Bubble | Start | 0.04 | [0.01, 0.09] |
| 2 | Bubble | End | 0.03 | [0.01, 0.08] |
| 2 | Control | Start | 0.01 | [0.00, 0.04] |
| 2 | Control | End | 0.02 | [0.00, 0.05] |
| 2 | Dome | Start | 0.00 | [0.01, 0.01] |
| 2 | Dome | End | 0.00 | [0.01, 0.01] |
| 3 | Bubble | Start | 0.01 | [0.00, 0.03] |
| 3 | Bubble | End | 0.00 | [0.00, 0.02] |
| 3 | Control | Start | 0.00 | [0.01, 0.01] |
| 3 | Control | End | 0.00 | [0.01, 0.01] |
| 3 | Dome | Start | 0.00 | [0.01, 0.01] |
| 3 | Dome | End | 0.00 | [0.01, 0.01] |

## Sampling analysis – Model: SSD (Swimming speed: Descent)

***Appendix table 9:*** Test results from a three-way ANOVA log model. Multiple $R^2$: 0.170; Adjusted $R^2$: 0.158.

| | SS | DF | F-value | p-value |
|---|---|---|---|---|
| Trial | 0.048 | 2 | 2.1 | 0.121 |
| Time | 2.106 | 1 | 184.5 | < 0.0001 **** |
| Group | 0.248 | 2 | 10.9 | < 0.0001 **** |
| Trial:Time | 0.331 | 2 | 14.5 | < 0.0001 **** |
| Trial:Group | 0.028 | 4 | 0.6 | 0.659 |
| Time:Group | 0.011 | 2 | 0.5 | 0.624 |
| Trial:Time:Group | 0.027 | 4 | 0.6 | 0.666 |
| Residuals | 13.667 | 1197 | | |

*Model predictions*

| Trial | Group | Time | Predicted K | 95% CI |
|---|---|---|---|---|
| 1 | Bubble | 0 | 0.54 | [0.50, 0.59] |
| 1 | Control | 0 | 0.49 | [0.45, 0.54] |
| 1 | Dome | 0 | 0.52 | [0.48, 0.57] |
| 1 | Bubble | 10 | 0.61 | [0.58, 0.65] |
| 1 | Control | 10 | 0.56 | [0.52, 0.59] |
| 1 | Dome | 10 | 0.58 | [0.55, 0.62] |
| 1 | Bubble | 20 | 0.68 | [0.65, 0.71] |
| 1 | Control | 20 | 0.63 | [0.60, 0.66] |
| 1 | Dome | 20 | 0.64 | [0.61, 0.67] |
| 1 | Bubble | 40 | 0.84 | [0.78, 0.90] |
| 1 | Control | 40 | 0.78 | [0.72, 0.84] |
| 1 | Dome | 40 | 0.77 | [0.71, 0.83] |
| 2 | Bubble | 0 | 0.53 | [0.49, 0.58] |
| 2 | Control | 0 | 0.50 | [0.45, 0.55] |
| 2 | Dome | 0 | 0.50 | [0.45, 0.55] |
| 2 | Bubble | 10 | 0.60 | [0.57, 0.64] |
| 2 | Control | 10 | 0.56 | [0.52, 0.59] |
| 2 | Dome | 10 | 0.55 | [0.52, 0.58] |
| 2 | Bubble | 20 | 0.67 | [0.64, 0.70] |
| 2 | Control | 20 | 0.62 | [0.59, 0.65] |
| 2 | Dome | 20 | 0.60 | [0.57, 0.63] |
| 2 | Bubble | 40 | 0.82 | [0.76, 0.89] |
| 2 | Control | 40 | 0.75 | [0.70, 0.81] |
| 2 | Dome | 40 | 0.72 | [0.66, 0.77] |
| 3 | Bubble | 0 | 0.62 | [0.57, 0.67] |
| 3 | Control | 0 | 0.63 | [0.58, 0.68] |
| 3 | Dome | 0 | 0.58 | [0.53, 0.63] |
| 3 | Bubble | 10 | 0.65 | [0.61, 0.69] |
| 3 | Control | 10 | 0.64 | [0.60, 0.68] |
| 3 | Dome | 10 | 0.60 | [0.57, 0.64] |
| 3 | Bubble | 20 | 0.68 | [0.65, 0.71] |
| 3 | Control | 20 | 0.65 | [0.62, 0.68] |
| 3 | Dome | 20 | 0.63 | [0.61, 0.66] |
| 3 | Bubble | 40 | 0.74 | [0.68, 0.80] |
| 3 | Control | 40 | 0.67 | [0.62, 0.72] |
| 3 | Dome | 40 | 0.69 | [0.64, 0.75] |

## Sampling analysis – Model: SSS (Swimming speed while submerged)

***Appendix table 10:*** Test results from a three-way ANOVA log model. Multiple $R^2$: 0.231; Adjusted $R^2$: 0.228.

| | SS | DF | F-value | p-value |
|---|---|---|---|---|
| Trial | 9.241 | 2 | 320.3 | < 0.0001 **** |
| Time | 6.580 | 1 | 456.2 | < 0.0001 **** |
| Group | 1.126 | 2 | 39.0 | < 0.0001 **** |
| Trial:Time | 1.611 | 2 | 55.8 | < 0.0001 **** |
| Trial:Group | 0.092 | 4 | 1.6 | 0.171 |
| Time:Group | 0.590 | 2 | 20.4 | < 0.0001 **** |
| Trial:Time:Group | 0.383 | 4 | 6.6 | < 0.0001 **** |
| Residuals | 65.297 | 4527 | | |

*Model predictions*

| Trial | Group | Time | Predicted K | 95% CI |
|---|---|---|---|---|
| 1 | Bubble | 0 | 1.31 | [1.29, 1.34] |
| 1 | Control | 0 | 1.31 | [1.29, 1.34] |
| 1 | Dome | 0 | 1.31 | [1.28, 1.34] |
| 1 | Bubble | 120 | 1.16 | [1.14, 1.17] |
| 1 | Control | 120 | 1.20 | [1.18, 1.22] |
| 1 | Dome | 120 | 1.23 | [1.21, 1.24] |
| 1 | Bubble | 260 | 0.97 | [0.92, 1.02] |
| 1 | Control | 260 | 1.07 | [1.02, 1.12] |
| 1 | Dome | 260 | 1.13 | [1.08, 1.18] |
| 1 | Bubble | 500 | 0.66 | [0.55, 0.78] |
| 1 | Control | 500 | 0.85 | [0.73, 0.96] |
| 1 | Dome | 500 | 0.97 | [0.85, 1.08] |
| 2 | Bubble | 0 | 1.43 | [1.40, 1.46] |
| 2 | Control | 0 | 1.45 | [1.43, 1.48] |
| 2 | Dome | 0 | 1.40 | [1.37, 1.43] |
| 2 | Bubble | 120 | 1.29 | [1.27, 1.31] |
| 2 | Control | 120 | 1.32 | [1.30, 1.34] |
| 2 | Dome | 120 | 1.34 | [1.32, 1.36] |
| 2 | Bubble | 260 | 1.12 | [1.06, 1.18] |
| 2 | Control | 260 | 1.17 | [1.11, 1.23] |
| 2 | Dome | 260 | 1.28 | [1.22, 1.34] |
| 2 | Bubble | 500 | 0.84 | [0.71, 0.98] |
| 2 | Control | 500 | 0.91 | [0.78, 1.05] |
| 2 | Dome | 500 | 1.17 | [1.03, 1.30] |
| 3 | Bubble | 0 | 1.36 | [1.34, 1.37] |
| 3 | Control | 0 | 1.46 | [1.44, 1.47] |
| 3 | Dome | 0 | 1.41 | [1.39, 1.42] |
| 3 | Bubble | 120 | 1.34 | [1.32, 1.35] |
| 3 | Control | 120 | 1.41 | [1.40, 1.42] |
| 3 | Dome | 120 | 1.38 | [1.37, 1.39] |
| 3 | Bubble | 260 | 1.31 | [1.30, 1.32] |
| 3 | Control | 260 | 1.35 | [1.34, 1.35] |
| 3 | Dome | 260 | 1.34 | [1.33, 1.35] |
| 3 | Bubble | 500 | 1.27 | [1.26, 1.29] |
| 3 | Control | 500 | 1.24 | [1.23, 1.26] |
| 3 | Dome | 500 | 1.28 | [1.26, 1.29] |

## Sampling analysis – Model: SSA (Swimming speed: Ascent)

***Appendix table 11:*** Test results from a three-way ANOVA reciprocal root transformation model. Multiple $R^2$: 0.252; Adjusted $R^2$: 0.240.

| | SS | DF | F-value | p-value |
|---|---|---|---|---|
| Trial | 0.035 | 2 | 1.3 | 0.263 |
| Time | 2.408 | 1 | 183.1 | < 0.0001 **** |
| Group | 0.401 | 2 | 15.2 | < 0.0001 **** |
| Trial:Time | 1.726 | 2 | 65.6 | < 0.0001 **** |
| Trial:Group | 0.067 | 3 | 1.3 | 0.282 |
| Time:Group | 0.005 | 2 | 0.2 | 0.835 |
| Trial:Time:Group | 0.050 | 3 | 1.0 | 0.430 |
| Residuals | 13.965 | 1062 | | |

*Model predictions*

| Trial | Group | Time | Predicted K | 95% CI |
|---|---|---|---|---|
| 1 | Bubble | 0 | 1.13 | [1.08, 1.17] |
| 1 | Control | 0 | 1.20 | [1.15, 1.24] |
| 1 | Dome | 0 | 1.21 | [1.16, 1.26] |
| 1 | Bubble | 10 | 1.13 | [1.10, 1.16] |
| 1 | Control | 10 | 1.19 | [1.16, 1.22] |
| 1 | Dome | 10 | 1.21 | [1.18, 1.24] |
| 1 | Bubble | 20 | 1.13 | [1.11, 1.15] |
| 1 | Control | 20 | 1.18 | [1.16, 1.20] |
| 1 | Dome | 20 | 1.20 | [1.18, 1.23] |
| 1 | Bubble | 40 | 1.13 | [1.09, 1.17] |
| 1 | Control | 40 | 1.17 | [1.13, 1.21] |
| 1 | Dome | 40 | 1.20 | [1.16, 1.24] |
| 2 | Bubble | 0 | 0.99 | [0.95, 1.03] |
| 2 | Control | 0 | 1.00 | [0.96, 1.04] |
| 2 | Dome | 0 | 0.99 | [0.95, 1.03] |
| 2 | Bubble | 10 | 1.06 | [1.03, 1.09] |
| 2 | Control | 10 | 1.07 | [1.05, 1.10] |
| 2 | Dome | 10 | 1.08 | [1.05, 1.11] |
| 2 | Bubble | 20 | 1.13 | [1.11, 1.15] |
| 2 | Control | 20 | 1.15 | [1.13, 1.17] |
| 2 | Dome | 20 | 1.17 | [1.15, 1.19] |
| 2 | Bubble | 40 | 1.27 | [1.23, 1.30] |
| 2 | Control | 40 | 1.31 | [1.27, 1.34] |
| 2 | Dome | 40 | 1.35 | [1.31, 1.38] |
| 3 | Bubble | 0 | 1.08 | [1.04, 1.11] |
| 3 | Control | 0 | 1.10 | [1.07, 1.14] |
| 3 | Dome | 0 | 1.12 | [1.09, 1.16] |
| 3 | Bubble | 10 | 1.11 | [1.08, 1.13] |
| 3 | Control | 10 | 1.13 | [1.11, 1.16] |
| 3 | Dome | 10 | 1.15 | [1.12, 1.17] |
| 3 | Bubble | 20 | 1.14 | [1.12, 1.16] |
| 3 | Control | 20 | 1.16 | [1.14, 1.18] |
| 3 | Dome | 20 | 1.17 | [1.15, 1.19] |
| 3 | Bubble | 40 | 1.21 | [1.18, 1.25] |
| 3 | Control | 40 | 1.22 | [1.18, 1.26] |
| 3 | Dome | 40 | 1.23 | [1.19, 1.26] |

## Sampling analysis – Model: BI (Bubble interactions)

***Appendix table 12:*** Test results from a three-way ANOVA generalized linear model (GLM).

| | DF | LR Chi$^2$ | p-value |
|---|---|---|---|
| Group | 2 | 37.952 | < 0.0001**** |
| Trial | 2 | 42.285 | < 0.0001**** |
| Group:Trial | 4 | 5.919 | 0.205 |

***Appendix table 13:*** Summary of test results from a quasipoisson generalized linear model

| Comparison | Estimate | SE | t-value | p-value |
|---|---|---|---|---|
| (Intercept) | 1.386 | 0.19 | 7.3 | < 0.0001**** |
| Control | -0.934 | 0.36 | -2.6 | 0.011 * |
| Dome | -1.135 | 0.39 | -2.9 | 0.004 ** |
| Trial 1:Trial 2 | -0.182 | 0.30 | -0.6 | 0.540 |
| Trail 1:Trial 3 | -0.948 | 0.26 | -3.6 | 0.001 *** |
| Control:Trial 1:Trial 2 | 0.578 | 0.50 | 1.1 | 0.255 |
| Dome:Trial 1:Trial 2 | 0.219 | 0.57 | 0.4 | 0.704 |
| Control:Trial 1:Trial 3 | -0.708 | 0.58 | -1.2 | 0.224 |
| DomeTrial1:Trial 3 | -0.507 | 0.60 | -0.9 | 0.396 |

## Sampling analysis – Model: DI (Dome interactions for Trial 2)

***Appendix table 14:*** Test results from a three-way ANOVA generalized linear model (GLM).

| | DF | LR Chi$^2$ | p-value |
|---|---|---|---|
| Group | 2 | 0.996 | 0.6076 |

***Appendix table 15:*** Summary of test results from a quasipoisson generalized linear model.

| Comparison | Estimate | SE | t-value | p-value |
|---|---|---|---|---|
| (Intercept) | 0.693 | 0.82 | 0.9 | 0.409 |
| Control | -0.099 | 1.63 | -0.7 | 0.511 |
| Dome | 0.288 | 1.08 | 0.3 | 0.793 |

## Sampling analysis – Model: $TA_{1\text{-}3}$ (Tilt angle for all trials)

***Appendix table 16:*** Test results from a three-way ANOVA linear model. Multiple $R^2$: 0.069; Adjusted $R^2$: 0.058.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 1511 | 2 | 13.5 | < 0.0001 **** |
| Day | 1625 | 1 | 29.0 | < 0.0001 **** |
| Group | 133 | 2 | 1.2 | 0.304 |
| Trial:Day | 309 | 2 | 2.8 | 0.064 |
| Trial:Group | 264 | 4 | 1.2 | 0.318 |
| Day:Group | 118 | 2 | 1.0 | 0.350 |
| Trial:Day:Group | 340 | 4 | 1.5 | 0.194 |
| Residuals | 82082 | 1466 | | |

*Model predictions*

| | **Min** | **1Q** | **Median** | **3Q** | **Max** |
|---|---|---|---|---|---|
| Residuals | -46.9 | -3.7 | -0.9 | 3.1 | 49.8 |

## Sampling analysis – Model: $TA_3$ (Tilt angle Trial 3)

***Appendix table 17:*** Test results from a two-way ANOVA linear model. Multiple $R^2$: 0.078; Adjusted $R^2$: 0.073.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Trial | 1786 | 1 | 64.8 | < 0.0001 **** |
| Day | 107 | 2 | 2.0 | 0.142 |
| Group | 133 | 2 | 1.2 | 0.304 |
| Day:Group | 180 | 2 | 3.3 | 0.038 * |
| Residuals | 24612 | 893 | | |

*Model predictions*

| | **Min** | **1Q** | **Median** | **3Q** | **Max** |
|---|---|---|---|---|---|
| Residuals | -22.2 | -3.4 | -0.9 | 2.7 | 26.8 |

## Sampling analysis – Model: SBCG (swim bladder content: Gas)

***Appendix table 18:*** Test results from a two-way ANOVA linear model. Multiple $R^2$: 0.171; Adjusted $R^2$: 0.133.

| | **SS** | **DF** | **F-value** | **p-value** |
|---|---|---|---|---|
| Group | 0.146 | 2 | 0.1 | 0.885 |
| Trial | 19.900 | 2 | 16.7 | < 0.0001 **** |
| Group:Trial | 1.671 | 4 | 0.7 | 0.592 |
| Residuals | 105.491 | 177 | | |

*Model predictions*

| | **Min** | **1Q** | **Median** | **3Q** | **Max** |
|---|---|---|---|---|---|
| Residuals | -1.8 | -0.4 | -0.0 | 0.5 | 2.0 |

## Sampling analysis – Model: SBCW (Swim bladder content: water)

***Appendix table 19:*** Test results from a three-way ANOVA generalized linear model (GLM).

| | **DF** | **LR Chi$^2$** | **p-value** |
|---|---|---|---|
| Group | 2 | 3.683 | 0.159 |
| Trial | 2 | 1.353 | 0.509 |
| Group:Trial | 4 | 0.614 | 0.962 |

*Model predictions*

| | **Min** | **1Q** | **Median** | **3Q** | **Max** |
|---|---|---|---|---|---|
| Residuals | -1.5 | -1.0 | -0.9 | 0.2 | 5.7 |

***Appendix table 20:*** Summary of test results from a quasipoisson generalized linear model.

| **Comparison** | **Estimate** | **SE** | **t-value** | **p-value** |
|---|---|---|---|---|
| (Intercept) | -0.836 | 0.50 | -1.7 | 0.099 |
| Control | 0.945 | 0.62 | 1.5 | 0.127 |
| Dome | 0.636 | 0.69 | 0.9 | 0.357 |
| Trial 1:Trial 2 | -0.167 | 0.78 | -0.2 | 0.831 |
| Trail 1:Trial 3 | 0.112 | 0.61 | 0.2 | 0.855 |
| Control:Trial 1:Trial 2 | -0.452 | 0.98 | -0.5 | 0.645 |
| Dome:Trial 1:Trial 2 | -0.274 | 1.04 | -0.3 | 0.793 |
| Control:Trial 1:Trial 3 | -0.479 | 0.76 | -0.6 | 0.521 |
| DomeTrial1:Trial 3 | -0.544 | 0.84 | -0.6 | 0.520 |

## Sampling analysis – Model: FG (Feed in gut)

***Appendix table 21:*** Test results from a two-way type II ANOVA of a generalized linear model (GLM) of feed in gut over groups in Trial 3.

| | **DF** | **LR Chi$^2$** | **p-value** |
|---|---|---|---|
| Group | 2 | 5.723 | 0.057 |
| *Model predictions* | | | |
| **Group** | **SE** | **Predicted** | **95% CI** |
| Bubble | 0.41 | 0.76 | [0.58, 0.87] |
| Control | 0.35 | 0.48 | [0.32, 0.65] |
| Dome | 0.38 | 0.69 | [0.51, 0.82] |

***Appendix table 22:*** Test results from a two-way type II ANOVA of a generalized linear model (GLM) of feed in gut compared with K-factor over groups in Trial 3.

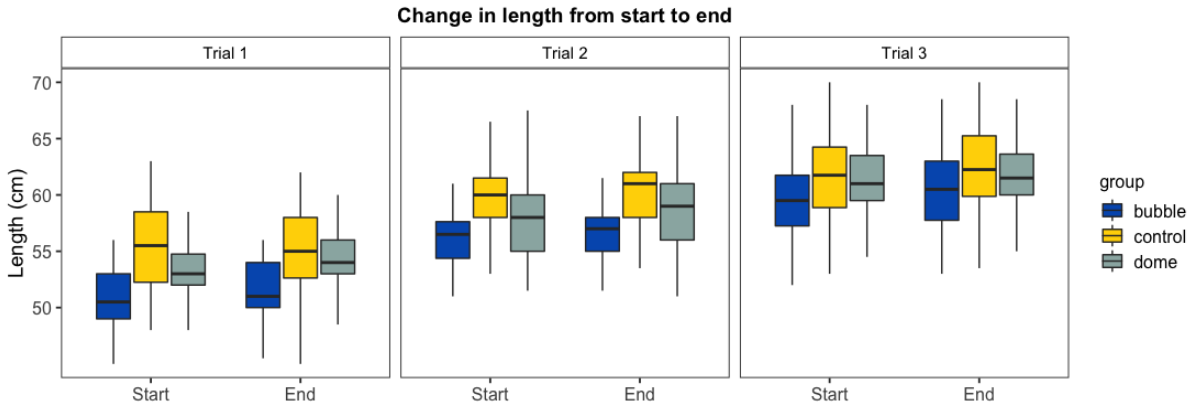
| | **DF** | **LR Chi$^2$** | **p-value** |
|---|---|---|---|
| K | 1 | 1.350 | 0.245 |
| Group | 2 | 4.547 | 0.103 |
| K:Group | 2 | 0.948 | 0.622 |

***Appendix table 23:*** Summary of test results from a binomial GLM between K and Feed.

| **Comparison** | **Estimate** | **SE** | **t-value** | **p-value** |
|---|---|---|---|---|
| (Intercept) | 1.471 | 4.50 | 0.3 | 0.744 |
| K | -0.254 | 3.42 | -0.1 | 0.941 |
| Control | -6.873 | 6.52 | -1.1 | 0.292 |
| Dome | -4.827 | 6.29 | -0.8 | 0.443 |
| K:Control | 4.540 | 5.10 | 0.9 | 0.373 |
| K:Dome | 3.542 | 4.89 | 0.7 | 0.469 |

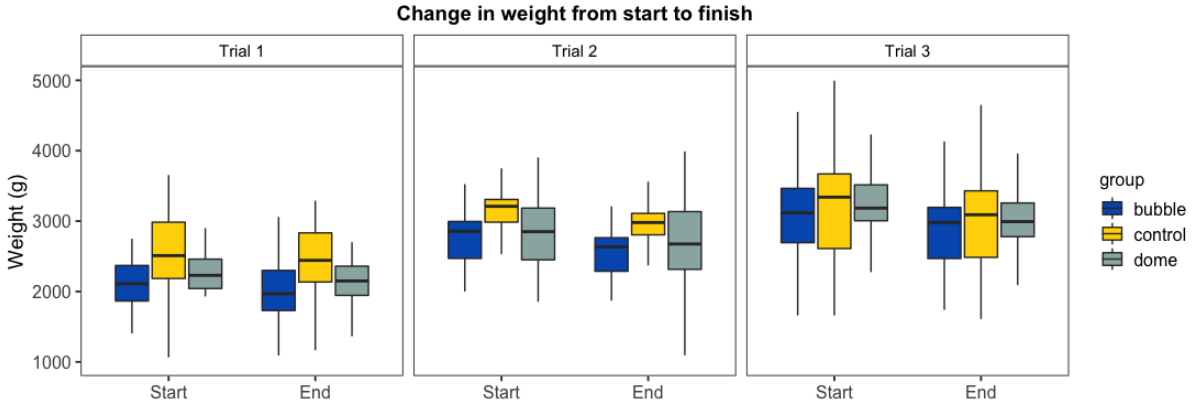
## Appendix II – Figures and tables

***Welfare protocol: Length.***



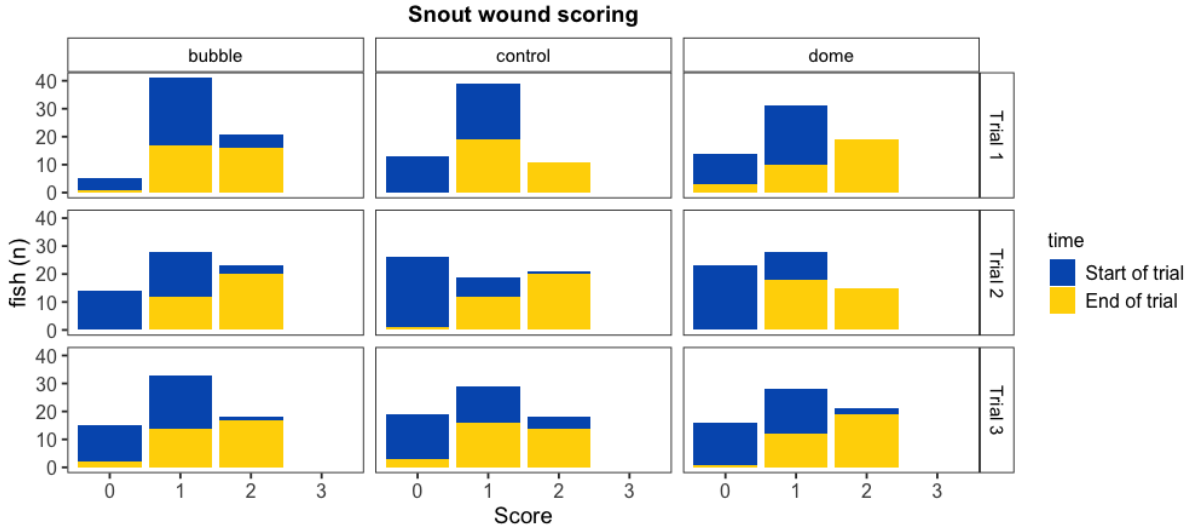

***Appendix figure 1:*** Change in length for each group from start to end of submergence period in Trial 1, Trial 2, and Trial 3. Boxes show the median (middle line), interquartile range (top and bottom of the box), and 1.5 times the interquartile range (whiskers).

***Welfare protocol: Weight.***



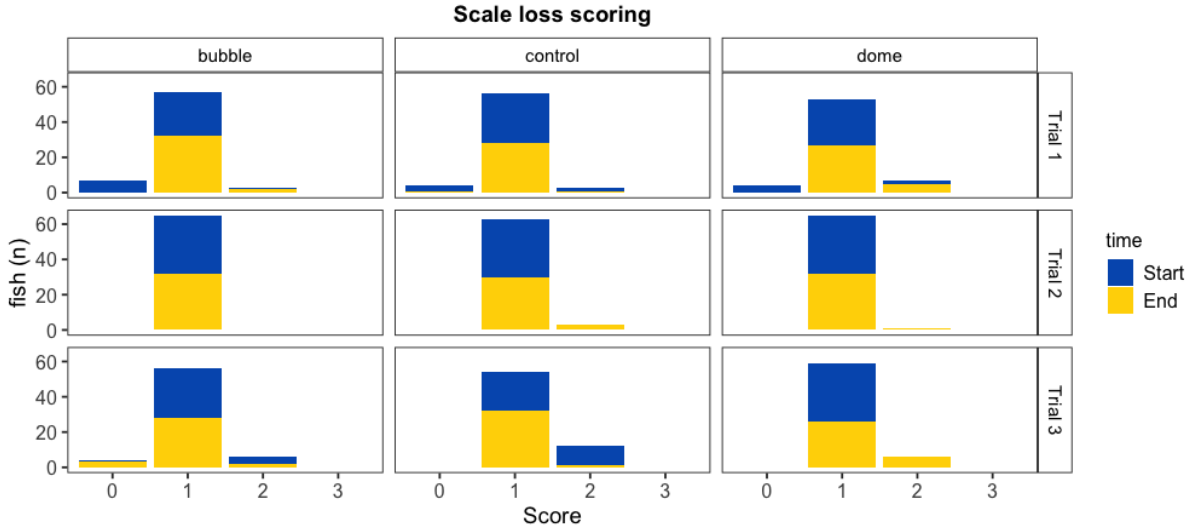

***Appendix figure 2:*** Change in weight for each group from start to end of submergence period in Trial 1, Trial 2, and Trial 3. Boxes show the median (middle line), interquartile range (top and bottom of the box), and 1.5 times the interquartile range (whiskers).

***Welfare protocol: Snout wounds***



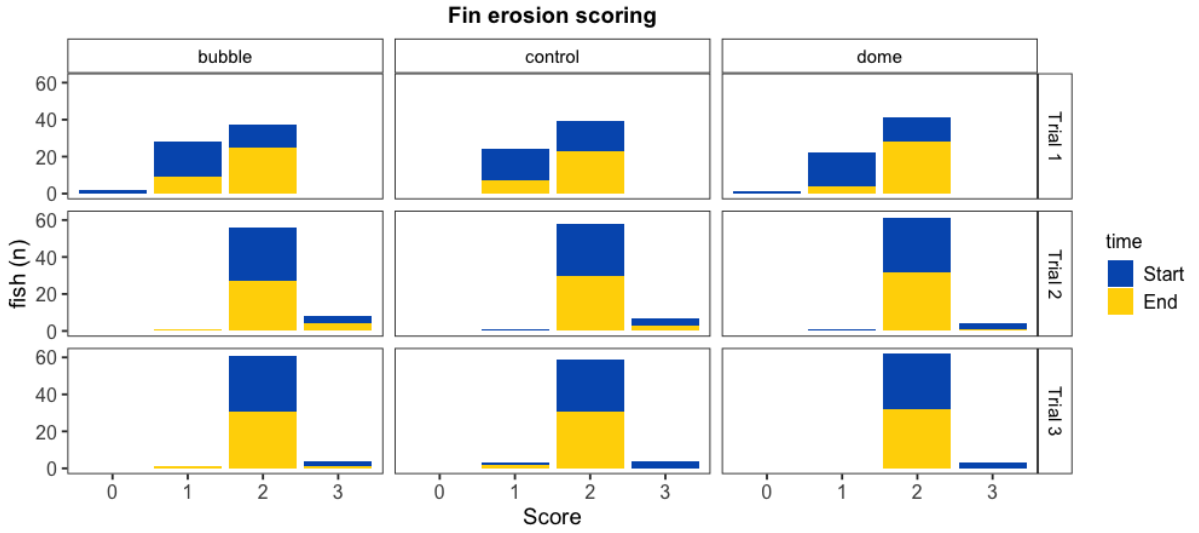

***Appendix figure 3:*** Changes in welfare scoring in regards to snout wound from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare protocol: Scale loss***



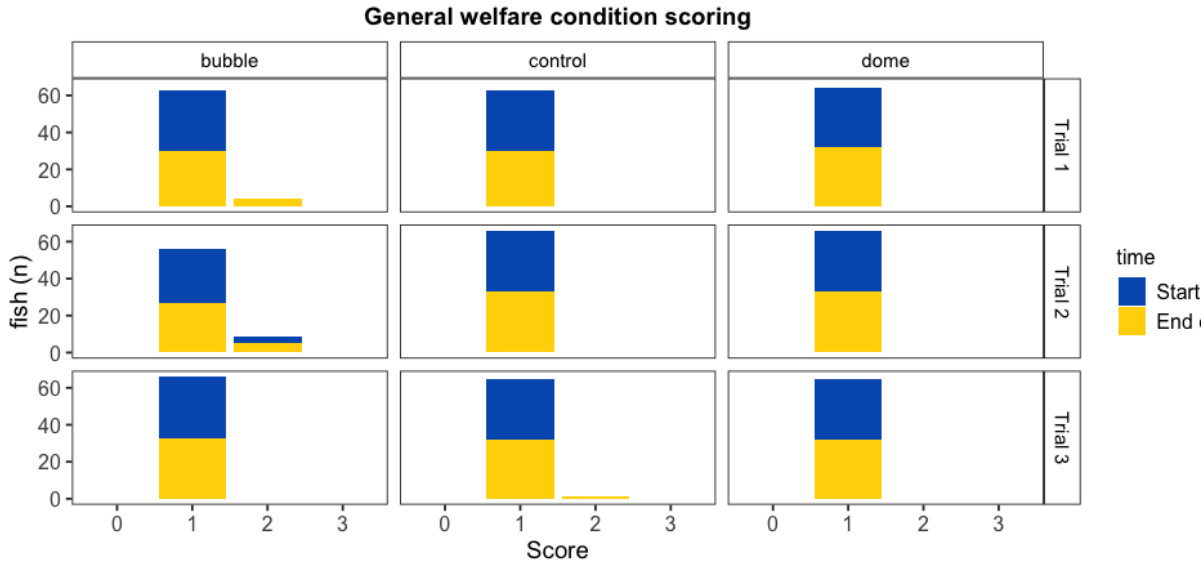

***Appendix figure 4:*** Changes in welfare scoring in regards to scale loss from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare protocol: Fin erosion***



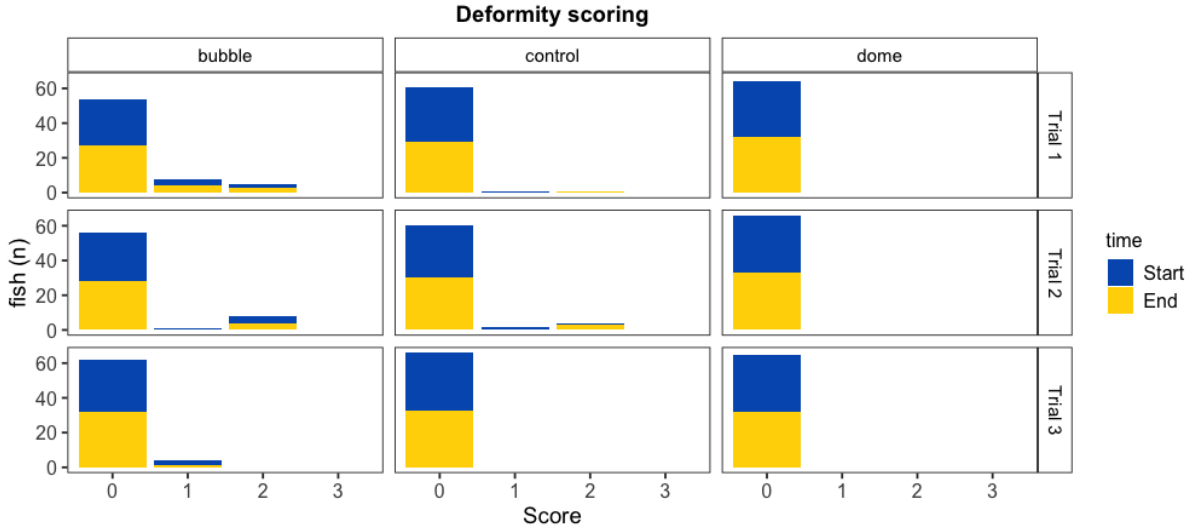

***Appendix figure 5:*** Changes in welfare scoring in regards to fin erosion from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare protocol: General condition***



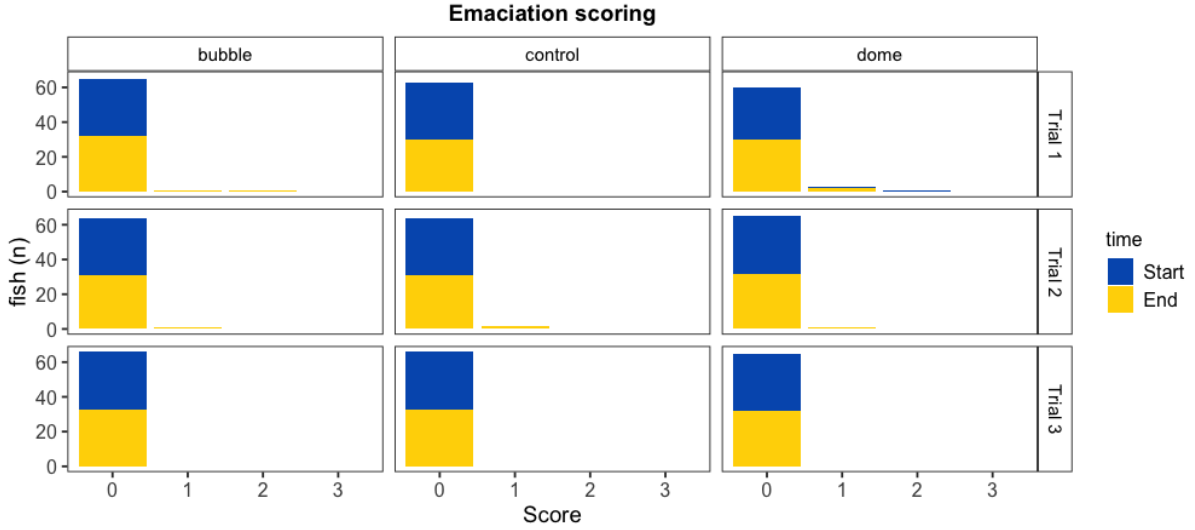

***Appendix figure 6:*** Changes in welfare scoring in regards to general condition from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare protocol: Deformity.***



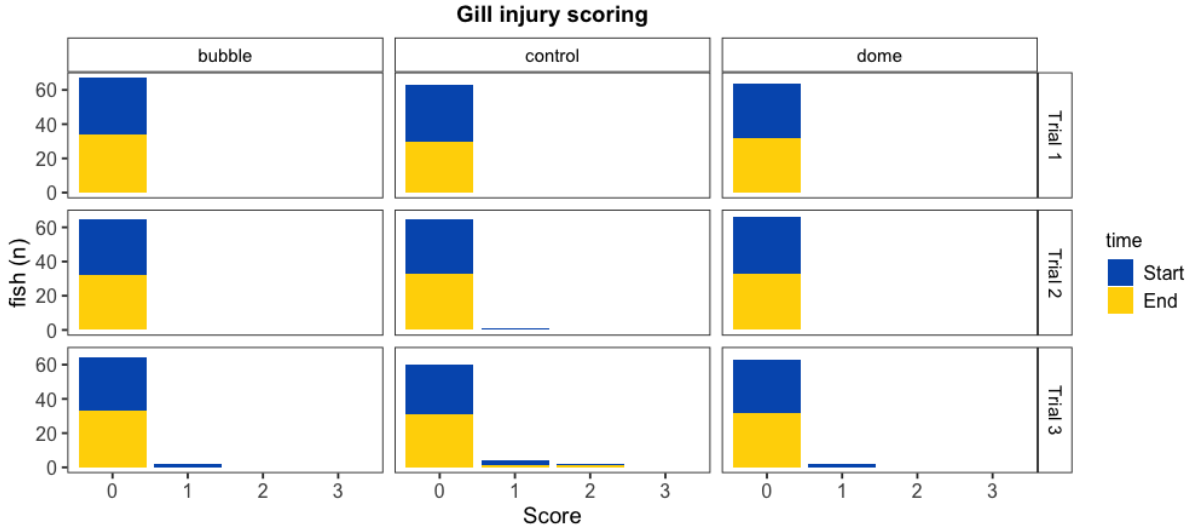

***Appendix figure 7:*** Changes in welfare scoring in regards to deformity from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare emaciation: Emaciation.***



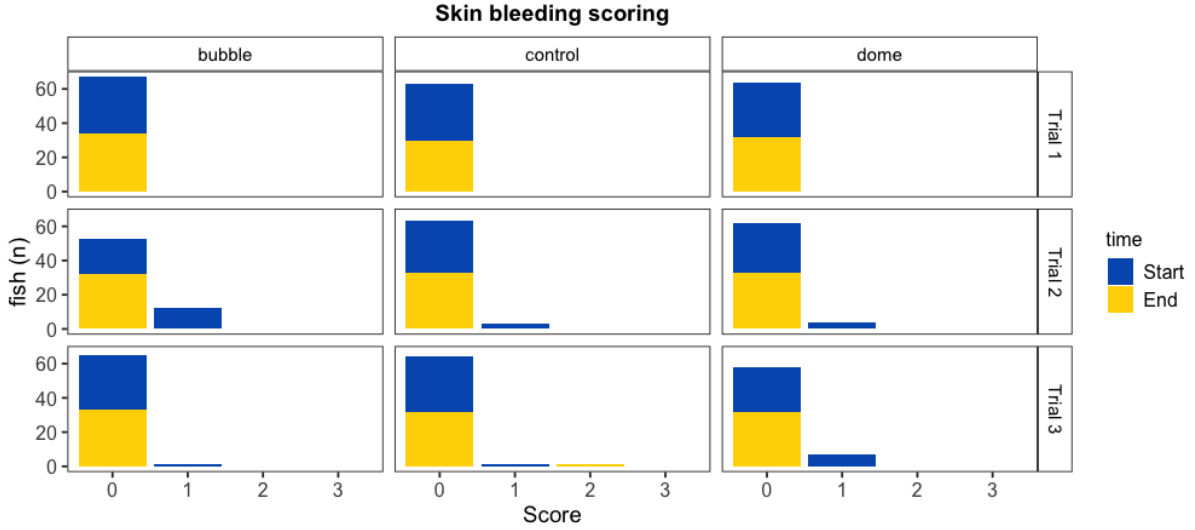

***Appendix figure 8:*** Changes in welfare scoring in regards to emaciation from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare protocol: Gill injury.***



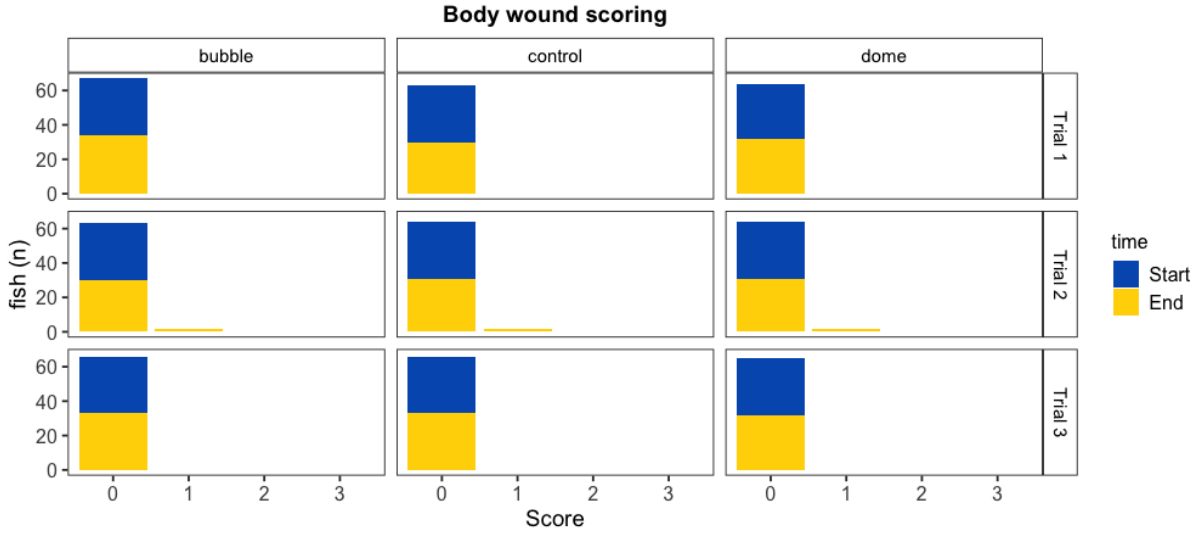

***Appendix figure 9:*** Changes in welfare scoring in regards to gill injury from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare protocol: Skin bleeding.***



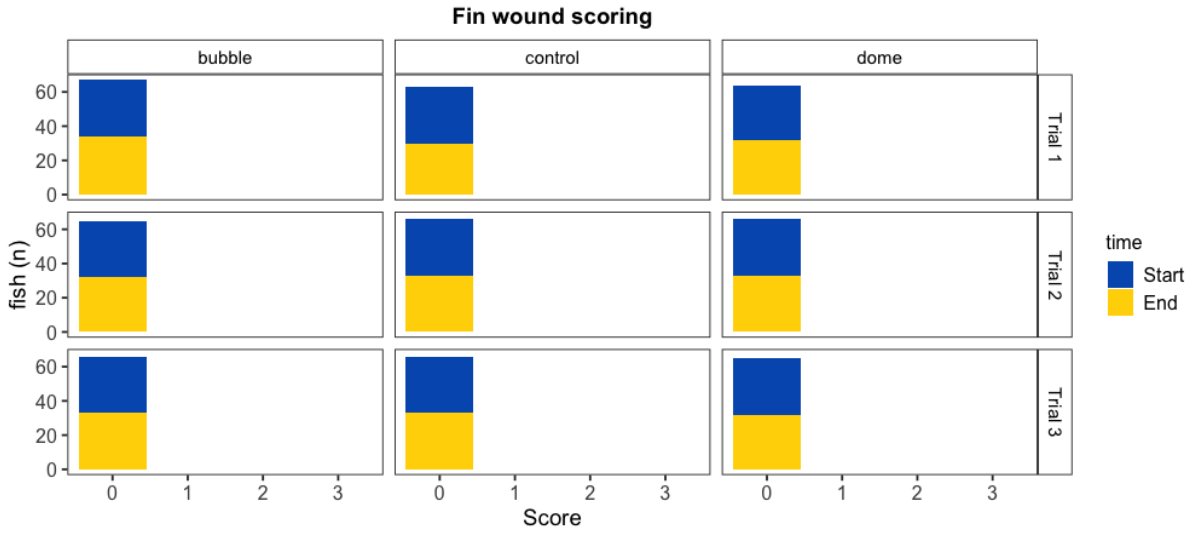

***Appendix figure 10:*** Changes in welfare scoring in regards to skin bleeding from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare protocol: Body wound.***



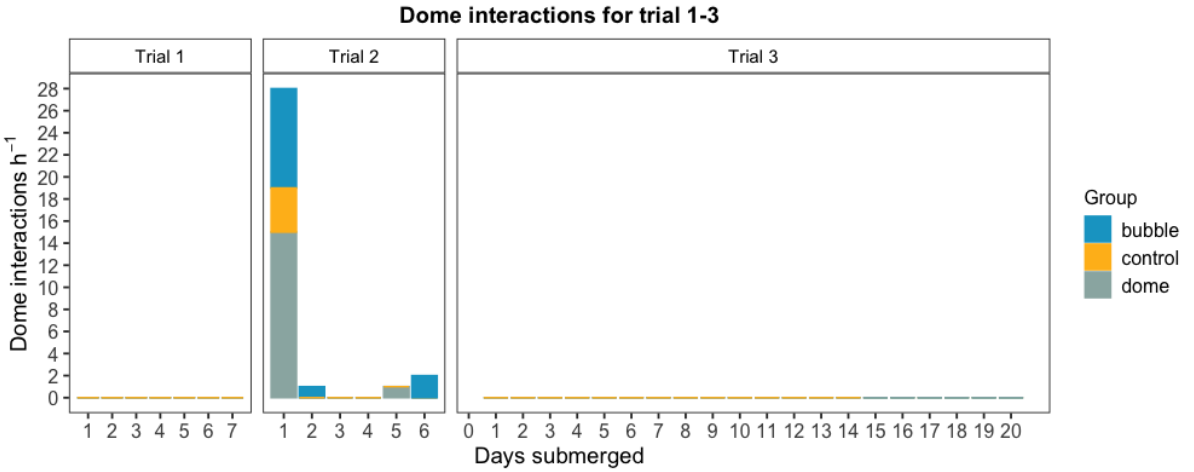

***Appendix figure 11:*** Changes in welfare scoring in regards to body wound from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Welfare protocol: Fin wound.***



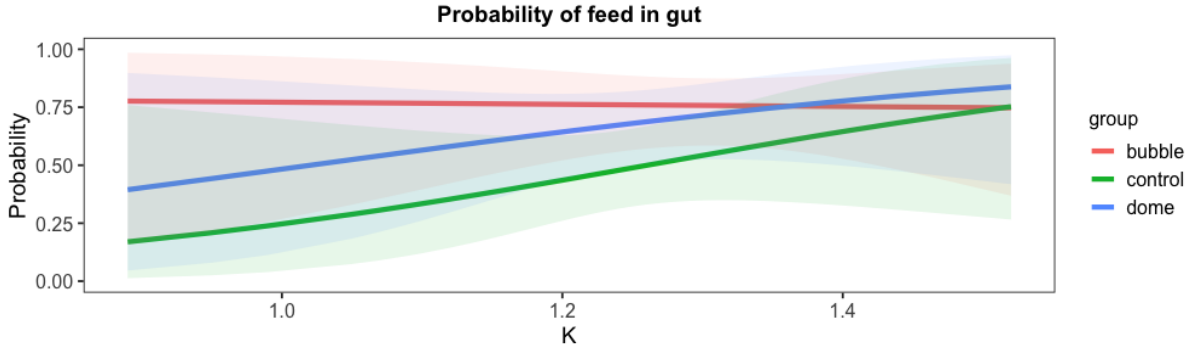

***Appendix figure 12:*** Changes in welfare scoring in regards to fin wound from start to finish for Trial 1, Trial 2 and Trial 3. A shift from left to right of yellow bar indicates poorer welfare at the end of the Trial.

***Dome interactions: All trials.***




***Appendix figure 13:*** Dome interactions (60 min a day) for each treatment group in Trial 1 (left), Trial 2 (middle), and Trial 3 (right).

***Feed in gut: Probability***




***Appendix figure 14:*** Probability of feed in gut over K-factor for the three treatment groups after Trial 3. Shadowed area illustrates 95% confidence intervals.

## Appendix III – Tables

### Mean values of K-factor, scale loss, snout wound and fin erosion before and after trials:

**Appendix table 1** – Comparison of mean values ± standard error (SE) on K-factor, scale loss, snout wound and fin erosion for experimental groups before and after submergence.

| Trial and Treatment | K-factor | | Scale loss | | Snout wound | | Fin erosion | |
|---|---|---|---|---|---|---|---|---|
| | *start* | *end* | *start* | *end* | *start* | *end* | *start* | *end* |
| 1 Bubble | 1,60 ± 0,05 | 1,44 ± 0,05 | 0,82 ± 0,04 | 1,06 ± 0,04 | 1,03 ± 0,10 | 1,44 ± 0,10 | 1,30 ± 0,08 | 1,74 ± 0,08 |
| 1 Dome | 1,47 ± 0,02 | 1,33 ± 0,02 | 0,94 ± 0,07 | 1,16 ± 0,07 | 0,66 ± 0,12 | 1,50 ± 0,12 | 1,38 ± 0,06 | 1,88 ± 0,06 |
| 1 Control | 1,48 ± 0,04 | 1,45 ± 0,04 | 0,97 ± 0,05 | 1,00 ± 0,05 | 0,61 ± 0,09 | 1,35 ± 0,09 | 1,48 ± 0,08 | 1,77 ± 0,08 |
| 2 Bubble | 1,57 ± 0,03 | 1,42 ± 0,03 | 1,00 ± 0,00 | 1,00 ± 0,00 | 0,67 ± 0,09 | 1,63 ± 0,09 | 2,12 ± 0,07 | 2,09 ± 0,07 |
| 2 Dome | 1,45 ± 0,02 | 1,30 ± 0,02 | 1,00 ± 0,00 | 1,03 ± 0,03 | 0,30 ± 0,09 | 1,45 ± 0,09 | 2,06 ± 0,11 | 2,03 ± 0,11 |
| 2 Control | 1,47 ± 0,02 | 1,33 ± 0,02 | 1,00 ± 0,00 | 1,09 ± 0,05 | 0,27 ± 0,10 | 1,56 ± 0,10 | 2,09 ± 0,05 | 2,09 ± 0,05 |
| 3 Bubble | 1,46 ± 0,02 | 1,31 ± 0,02 | 1,09 ± 0,07 | 0,97 ± 0,07 | 0,64 ± 0,11 | 1,45 ± 0,11 | 2,09 ± 0,04 | 2,00 ± 0,04 |
| 3 Dome | 1,42 ± 0,04 | 1,23 ± 0,04 | 1,00 ± 0,07 | 1,15 ± 0,08 | 0,61 ± 0,11 | 1,52 ± 0,11 | 2,09 ± 0,06 | 1,94 ± 0,06 |
| 3 Control | 1,38 ± 0,02 | 1,25 ± 0,02 | 1,33 ± 0,03 | 1,03 ± 0,03 | 0,64 ± 0,11 | 1,33 ± 0,11 | 2,09 ± 0,04 | 1,94 ± 0,04 |

### Mean swimming speed while submerged:

**Appendix table 2:** Mean values of swimming speed (BL $s^{-1}$ ± SE) while submerged for all groups and trials

| | Trial 1 | Trial 2 | Trial 3 |
|---|---|---|---|
| Bubble group | 0.72 ± 0.012 | 0.57 ± 0.007 | 0.59 ± 0.004 |
| Dome group | 0.66 ± 0.008 | 0.55 ± 0.006 | 0.57 ± 0.004 |
| Control group | 0.68 ± 0.010 | 0.55 ± 0.008 | 0.57 ± 0.004 |

**Swimming speeds during descending**:

**Appendix table 3:** Mean swimming speeds (BL $s^{-1}$ ± SE) during descending procedure in trial 1

| Depth | Bubble (BL $s^{-1}$) | Dome (BL $s^{-1}$) | Control (BL $s^{-1}$) |
|---|---|---|---|
| 0 | 0,48 ±0,05 | 0,39 ±0,03 | 0,41 ±0,05 |
| 5 | 0,52 ±0,05 | 0,58 ±0,06 | 0,48 ±0,03 |
| 10 | 0,72 ±0,06 | 0,70 ±0,05 | 0,64 ±0,07 |
| 15 | 0,74 ±0,04 | 0,63 ±0,03 | 0,72 ±0,06 |
| 20 | 0,76 ±0,04 | 0,74 ±0,05 | 0,68 ±0,03 |
| 25 | 0,71 ±0,03 | 0,72 ±0,04 | 0,78 ±0,05 |
| 30 | 0,77 ±0,06 | 0,69 ±0,04 | 0,79 ±0,06 |
| 35 | 0,94 ±0,06 | 0,79 ±0,04 | 0,73 ±0,03 |
| 40 | 0,67 ±0,05 | 0,65 ±0,04 | 0,61 ±0,04 |

**Appendix table 4:** Mean swimming speeds (BL $s^{-1}$ ± SE) during descending procedure in trial 2

| Depth | Bubble (BL $s^{-1}$) | Dome (BL $s^{-1}$) | Control (BL $s^{-1}$) |
|---|---|---|---|
| 0 | 0,51 ±0,04 | 0,48 ±0,02 | 0,44 ±0,03 |
| 5 | 0,56 ±0,05 | 0,47 ±0,03 | 0,52 ±0,03 |
| 10 | 0,65 ±0,04 | 0,60 ±0,02 | 0,61 ±0,03 |
| 15 | 0,70 ±0,04 | 0,59 ±0,03 | 0,66 ±0,03 |
| 20 | 0,62 ±0,03 | 0,65 ±0,03 | 0,62 ±0,04 |
| 25 | 0,76 ±0,03 | 0,64 ±0,03 | 0,71 ±0,02 |
| 30 | 0,79 ±0,05 | 0,70 ±0,04 | 0,73 ±0,05 |
| 35 | 0,80 ±0,06 | 0,72 ±0,04 | 0,63 ±0,02 |
| 40 | 0,77 ±0,03 | 0,65 ±0,03 | 0,74 ±0,03 |

**Appendix table 5:** Mean swimming speeds (BL $s^{-1}$ ± SE) during descending procedure in trial 3

| Depth | Bubble (BL $s^{-1}$) | Dome (BL $s^{-1}$) | Control (BL $s^{-1}$) |
|---|---|---|---|
| 0 | 0,38 ± 0.04 | 0,34 ± 0.03 | 0,36 ± 0.02 |
| 5 | 0,74 ± 0.03 | 0,67 ± 0.04 | 0,76 ± 0.02 |
| 10 | 0,79 ± 0.04 | 0,78 ± 0.03 | 0,83 ± 0.03 |
| 15 | 0,79 ± 0.03 | 0,69 ± 0.04 | 0,75 ± 0.03 |
| 20 | 0,76 ± 0.03 | 0,71 ± 0.02 | 0,66 ± 0.02 |
| 25 | 0,74 ± 0.03 | 0,76 ± 0.03 | 0,70 ± 0.02 |
| 30 | 0,72 ± 0.03 | 0,64 ± 0.02 | 0,66 ± 0.03 |
| 25 | 0,66 ± 0.02 | 0,67 ± 0.02 | 0,68 ± 0.02 |
| 40 | 0,65 ± 0.03 | 0,56 ± 0.02 | 0,54 ± 0.02 |

**Swimming speeds during ascending**:

**Appendix table 6:** Mean swimming speeds (BL $s^{-1}$ ± SE) at different depths during ascending procedure in Trial 1.

| Depth (m) | Bubble (BL $s^{-1}$) | Dome (BL $s^{-1}$) | Control (BL $s^{-1}$) |
|---|---|---|---|
| 40 | 0,76 ± 0.04 | 0,86 ± 0.04 | 0,89 ± 0.12 |
| 35 | 0,85 ± 0.03 | 0,70 ± 0.02 | 0,77 ± 0.03 |
| 27 | 0,82 ± 0.04 | 0,73 ± 0.04 | 0,71 ± 0.03 |
| 22 | 0,73 ± 0.04 | 0,71 ± 0.04 | 0,70 ± 0.02 |
| 16 | 0,61 ± 0.03 | 0,61 ± 0.02 | 0,57 ± 0.02 |
| 10 | 0,80 ± 0.05 | 0,62 ± 0.03 | 0,72 ± 0.04 |
| 5 | 1,08 ± 0.05 | 0,86 ± 0.03 | 0,91 ± 0.06 |
| *Mean* | 0,81 ± 0.04 | 0,73 ± 0.03 | 0,75 ± 0.04 |

**Appendix table 7:** Mean swimming speeds (BL $s^{-1}$ ± SE) at different depths during ascending procedure in Trial 2.

| Depth (m) | Bubble (BL $s^{-1}$) | Dome (BL $s^{-1}$) | Control (BL $s^{-1}$) |
|---|---|---|---|
| 40 | 0,64 ± 0.03 | 0,53 ± 0.02 | 0,61 ± 0.03 |
| 35 | 0,59 ± 0.02 | 0,58 ± 0.03 | 0,56 ± 0.02 |
| 29 | 0,74 ± 0.04 | 0,71 ± 0.04 | 0,71 ± 0.02 |
| 24 | 0,84 ± 0.04 | 0,74 ± 0.02 | 0,81 ± 0.03 |
| 18 | 0,91 ± 0.05 | 0,84 ± 0.03 | 0,78 ± 0.03 |
| 13 | 0,88 ± 0.05 | 0,87 ± 0.05 | 0,95 ± 0.05 |
| 7 | 1,02 ± 0.11 | 0,87 ± 0.04 | 0,90 ± 0.02 |
| 1 | 0,93 ± 0.05 | 0,96 ± 0.04 | 0,91 ± 0.04 |
| *Mean* | 0,82 ± 0.05 | 0,76 ± 0.04 | 0,78 ± 0.03 |

**Appendix table 8:** Mean swimming speeds (BL $s^{-1}$ ± SE) at different depths during ascending procedure in Trial 3.

| Depth (m) | Bubble (BL $s^{-1}$) | Dome (BL $s^{-1}$) | Control (BL $s^{-1}$) |
|---|---|---|---|
| 40 | 0,64 ± 0.03 | 0,70 ± 0.06 | 0,74 ± 0.04 |
| 35 | 0,68 ± 0.02 | 0,70 ± 0.04 | 0,64 ± 0.03 |
| 30 | 0,80 ± 0.06 | 0,71 ± 0.03 | 0,73 ± 0.03 |
| 25 | 0,79 ± 0.03 | 0,74 ± 0.03 | 0,71 ± 0.02 |
| 20 | 0,79 ± 0.03 | 0,67 ± 0.01 | 0,75 ± 0.02 |
| 15 | 0,80 ± 0.03 | 0,74 ± 0.02 | 0,75 ± 0.04 |
| 10 | 0,94 ± 0.04 | 0,88 ± 0.05 | 0,96 ± 0.05 |
| 5 | 0,88 ± 0.03 | 0,84 ± 0.03 | 0,86 ± 0.03 |
| 1 | 0,76 ± 0.04 | 0,74 ± 0.04 | 0,73 ± 0.03 |
| *Mean* | 0,79 ± 0.03 | 0,75 ± 0.03 | 0,76 ± 0.03 |

## Appendix IV – Formulas

**Standard deviation (formula 1)**

Used to estimate the dispersion of data values from the mean value.

$$SD = \sqrt{\frac{\sum_{i=1}^{n} x_i - \bar{x}}{n-1}}$$

Where $n$ = number of data points, $x_i$ = Each of the values of data, $\bar{x}$ = the mean value of $x_i$

***Standard Error (formula 2)***

Used to estimate how much discrepancy that is likely in a sample's mean compared to the populations mean.

$$SE = \frac{\sigma}{\sqrt{n}}$$

Where $\sigma$ is standard deviation and $n$ is the number of samples.

**Fulton's condition factor (formula 3)**

Used to estimate the condition factor (K-factor), expressed as the relationship between weight and length (Kleiven 2018).

$$K = \frac{100 \bullet W}{L^3}$$

where $W$ is weight and $L$ is length.

**Boyle-Mariotte law (formula 4)**

Used to estimate the gas volume in swim bladder at 40m depth.

$$P_1 \bullet V_1 = P_2 \bullet V_2 \quad \Rightarrow \quad V_1 = \frac{P_2 \bullet V_2}{P_1}$$

Where $P$ is pressure and $V$ is volume (Bonnar, 1956).

**Charles' Law (formula 5)**

Used to estimate the expansion of a gas when there is a difference in temperature.

$$\frac{V_1}{T_1} = \frac{V_2}{T_2}$$

Where V is volume and T is temperature

**Gay-Lussac's Law (formula 6)**

Used to estimate the expansion of a gas when there is a difference in pressure

$$\frac{P_1}{T_1} = \frac{P_2}{T_2}$$

Where P is pressure and T is temperature