UiT The Arctic University of Norway

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[EFFECT OF CROWDING ON BEHAVIOUR OF ATLANTIC SALMON (*Salmo salar*) IN AQUACULTURE]

[Using behaviour as a tool to assess welfare in Atlantic salmon aquaculture production]

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Abstract

Aquaculture productions are increasing worldwide and producers are concerned about the welfare of farmed fish. The importance of good welfare cannot be overemphasized throughout the production cycle as each stage has risks and as such must be closely monitored. In this study, a literature review of animal and fish welfare and behaviour was done with special emphasis on the behaviour of Atlantic salmon during crowding operations. A review of existing scientific literature was carried out to identify welfare indicators related to crowding and how they are measured. Overall, technological advancements like a video surveillance system and the usage of cameras installed above and underwater have been used to monitor the behaviour of Atlantic salmon during crowding operations, as it is a robust source of collecting information to help us understand the behaviours that Atlantic salmon may exhibit during crowding. Eventually, a behavioural toolbox was suggested so it might be used by fish farmers and technical staff to assess welfare during the crowding of Atlantic salmon in aquaculture.

Keywords: Atlantic salmon, animal welfare, animal behaviour, farmed fish, welfare, crowding, welfare indicators, stress and welfare assessments.

1. Background

The global population is increasing and there is a need to ensure global food security. This, in a context of global warming where draughts and lack of suitable land and freshwater is a fact, can lead to poor farming conditions jeopardizing animal welfare in rearing facilities. Over the last decades, aquaculture production has increased with a focus on improving the health and welfare of farmed fish.

Animal welfare can be defined as the quality of life as perceived by the animals themselves (Stien et al., 2013), and welfare needs can be defined as all the requirements that animals have that influence their qualitative experience of life. Rearing of animals involves a lot of factors, including housing, nutrition, weather conditions, availability of water, land space, and access to healthcare facilities in case of disease outbreak or other health-related problems and most times focus is mainly on the other factors and not fully on the animal involved, and this could pose a problem for the farmer. Animal behavioural displays can arise from specific health status or as a response to the environment they find themselves in, these displays can then be further used to assess the level of welfare that the animal is subjected to, whether it is enough or lacking.

Animal welfare focuses mainly on how animals are treated. It is a very broad and complex topic that varies from species to species, e.g., birds, reptiles, and mammals, as there are several factors influencing every one of them. Each animal husbandry industry has tried to improve the procedures involved in the handling of farmed animals. There are rules and regulations, laws, and policies constantly being reviewed to ensure that management systems are functioning at an optimal level, and it is important to meet all market standards when it comes to animal treatment. (Noble et al., 2018)

Nowadays, the BLUE ECONOMY paradigm relies on the sustainable exploitation of aquatic ecosystems as one of the main drivers of the economy worldwide. Particularly, within the food industry, the BLUE REVOLUTION promotes the migration of food production from land to the aquatic environments and sets aquaculture as one of the main economic drivers for the industry with the highest growth potential.

To promote the sustainable development of aquaculture, regulations and monitoring programs should be carried out, including welfare assessments. These often involve handling animals and can sometimes require that animals should be sampled and thus stressed, or also euthanised.

Within the indicators used to assess fish welfare, one can find direct (“animal based”) or indirect (“environment-based”) examples; additionally, when indicators do not interfere with the workflow in the farms and the outcome is relatively immediate they are called OWIs (Operational Welfare Indicators), whereas if the indicators need from further post-processing, for instance in the laboratory, they are called LABWIs (Laboratory Welfare Indicators) (Noble et al., 2018). In order to avoid impracticalities of non-operational indicators, behaviour analysis comes into place to avoid such shortcomings and improve fish welfare assessments while minimizing stress. Although behaviour can be potentially an OWI, it should be combined with other OWIs and/or LABWIs, as they are both necessary in the overall welfare assessments. The behaviour of the fish is one of the best welfare indicators that the farmer can use to describe what the fish is experiencing in its immediate environment (Noble et al., 2018). Monitoring fish behaviour would provide farmers with welfare indicators that could serve as a warning sign to prevent welfare issues in the nearest future. In most situations, it is a non-invasive measure, and an increasing number of aquaculture production systems are equipped with underwater cameras for monitoring fish behaviour and feeding. Video monitoring is a very important and robust method for data collection (Stien et al., 2013a) and allows for operation standards to be reviewed continuously to ensure that all welfare requirements are met.

Crowding is one of the key management processes used in salmon farming. It involves the gathering of fish in high densities for vaccination, transport, sampling, lice treatments and harvesting. (Noble et al., 2018). When fish are handled in sea cages or nets, cameras are used to monitor their response to their environment, including their response to the handling procedure, light, water quality, swimming behaviour, and also potential stressors. This operation can negatively or positively affect fish welfare depending on how it is done, and there is a need to follow established protocols during this procedure. Crowding densities range from low to high and they refer to the number of fish per unit area or unit space during crowding operations. There is a need to better understand salmon behaviour during crowding and how this understanding can be applied to further develop procedures to assess welfare during crowding operations.

The main objectives of this thesis include the following:

i. Review of the development of animal behaviour science and animal welfare applied to the production of Atlantic salmon to improve welfare assessments of the industry.

ii. Review the fish welfare scientific literature to identify welfare indicators that can be used to assess welfare during crowding operations.

iii. Suggesting a behavioural toolbox to assess welfare during crowding of Atlantic salmon in aquaculture.

To achieve the goals of this study, a literature review has been the approach used to better understand and evaluate how behaviour can be used to monitor welfare during the crowding process in Atlantic salmon production. Several literature searches have been done based on specific keywords related to the topic: farmed and wild salmon, production, welfare, crowding, Atlantic salmon, behaviour, recovery time, monitoring, stress, swimming behaviour, rainbow trout, on databases such as ORIA, Google scholar and Science direct. A total of 3766 articles were found and after going through the contents of the abstract about 100 were further reviewed. The papers retained for the review contained information related to farmed fish, crowding and welfare indicators used to assess welfare.

2. Animal welfare

The concern about animals did not just start today, people living with companion and farm animals during the early nineteenth century observed them and argued that they could feel pain and suffering but it was quite difficult to differentiate animal protection from animal welfare. These observations would later be investigated using a scientific approach to describe what animal feels (Haynes, 2008). Later on, in the first half of the twentieth century, knowledge about biological functioning increased greatly. By the end of this time, scientific disciplines such as ethology and neuroscience started to become accepted within the scientific community. As a result of this, it became clearer that there were methods for evaluating suffering and feelings such as pain, anxiety and pleasure, and other methods of coping with the world (Broom & Johnson, 2019). In recent times, people continue to realize that the deprivation of the possibility of an animal displaying its natural behaviour can lead to poor welfare (Stien et al., 2013) and it is a very sensitive topic that needs to be researched further. The principles of animal welfare have emerged primarily in terrestrial animals, many of which have similar anatomies, physiologies and behaviours shared by humans. Most animal welfare principles are based on the assumptions that these similarities indicate that animals are sentient (i.e., are cognizant and feel comfort and discomfort) and that it is unethical to purposefully, or through neglect, inflict or allow animals to experience discomfort (Broom & Johnson, 2019)

Animal welfare is a complex topic but it can be defined using three different perspectives, (Fraser, 1997).

- The function-based approach uses the animal's biological functioning to define welfare i.e., if the animal is functioning well and has a good growth rate and there is no sign of disease or poor health, then it is said that such animal has good welfare.
- The nature-based definition states that an animal has a high level of welfare if it is given a natural environment and allowed to perform innate species-specific behaviours.
- Feeling-based approach to emotions suggests that an animal has a high level of welfare if it is free from long-lasting negative emotions such as suffering, pain, fear and distress and can also experience pleasure.

The Farm Animal Welfare Council (1996), outlined the five freedom paradigms that animal welfare frameworks should consider and they are listed below:

- Freedom from hunger and thirst (good osmotic regulation in the case of fish)
- Freedom from an environmental challenge (proper water quality, appropriate temperature ranges according to the species, etc.)
- Freedom from pain, injury and disease
- Freedom from behavioural restriction (including lack of space and isolation, depending on species)
- Freedom from fear and distress (avoidance of mental suffering).

Some of the organizations that have accepted the five-freedom paradigm worldwide include RSPCA (Royal Society for the Prevention of Cruelty to Animals), American Society for the Prevention of Cruelty to Animals and World Organization for Animal Health (OIE), UK Animal welfare act 2006, Association of Shelter Veterinarians for companion animals in the shelter and American Humane in the United States.

3. Animal behaviour

Animal behaviour can be simply defined as the natural responses that animals exhibit freely in their natural state. (Shettleworth, 2001). Animals display different behaviours throughout their life cycle, from the moment they are born they rely more on their instincts before starting to react to an external stimulus to be able to survive, feed, reproduce and protect their offspring.

Behaviour is also influenced by the environment that animals find themselves in, and can differ between for example birds, amphibians, reptiles, cattle, bovines and equines. In mammals, since some of them might live in water, or on land, the behavioural differences between aerial, terrestrial, underground and aquatic environments must therefore be specifically studied as it is a very important factor driving the welfare of such animals. In situations whereby animals are not able to live in their natural environment maybe as a result of captivity, laboratory experiments, or loss of habitat, the ability to display their natural behaviours may be lost or difficult to replicate and this can jeopardize their welfare. In terrestrial-farmed animals, examples of natural behaviours e.g., poultry include pecking, flapping of wings, running freely, scratching, resting and sleeping without any form of disturbance (Tiemann et al., 2022).

Fish species like Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), exhibit behaviours such as splashing, swimming speed, escape-related behaviours, bursts, tail-flapping and aggression. Animals can perform complex behaviours by instinct or innate abilities. The presence of awareness or learning is based on evidence of responses which change or adapt to situations and are persistent. (Dawkins, 2003)

4. Fish welfare

Animal welfare was defined as the quality of life as perceived by the animals themselves, (Stien et al., 2013) and welfare needs were defined as all the requirements that animals have that influence their qualitative experience of life. There are many benefits to improving animal welfare in food production systems and fish farming is no different. Fish farmers know this and have directly or indirectly tried to optimize fish welfare over the years; they want their animals to thrive, grow and stay healthy, all of which are usually correlated with good welfare. In addition to good farm husbandry and stock person ethics, animals in Norway and most European countries are protected by laws and regulations, e.g., the Norwegian Animal Welfare Act (2009) that protects all vertebrates. (Cox & Lennkh, 2016). Welfare indicators are measurements that have been used to assess the welfare status of animals and they can be direct (based on the animals themselves) or indirect (the resources or the environment they are subjected to). (Noble et al., 2018; Duncan, 2014; Stien et al., 2013a)

Environmental-based indicators (indirect welfare indicators), can include water quality, temperature, pH, oxygen, salinity, turbidity, heavy metals, and lighting and they can be traditionally measured with the use of handheld instruments. A practical example would be the water temperature and oxygen levels that must be within a certain range for the fish to fulfil their metabolic requirements for thermoregulation and respiration (Martins et al., 2012).

Animal-based welfare indicators focus on the observations of attributes with the animal itself i.e., attributes that are directly linked to the state of the farmed fish. They are also called outcome-based welfare indicators which emphasize that they measure the result of the treatment of the animals themselves. Examples of these indicators include behaviour, surface activity, appetite, growth, disease, glucose, lactate, emaciation state, eye damage and status, scales or blood in the water, sexual maturity stage, deformed opercula, fin status, sea lice and mortality,(Noble et al., 2018).

Laboratory-based welfare indicators (LABWIs) are welfare indicators (WIs) that require access to a laboratory or other analytical facilities to provide useful information (Noble et al., 2018). This is because they are complex to measure and also to ensure the accuracy of the results, to avoid making inaccurate conclusions about the welfare of the fish. An example includes the measurement of plasma cortisol which is used to measure the amount of stress a fish has been subjected to and this is done by analyzing blood samples in the laboratory. Plasma cortisol concentration is a common indicator of stress in fish and the major physiological roles of cortisol are regulation of hydromineral balance and energy metabolism (Wendelaar Bonga, 1997). Panic behaviour and burst swimming activities utilise the white muscles resulting in higher levels of lactate and can also increase the risk of mechanical damage, (Erikson et al., 2016). Elevated lactate levels in other studies suggest high activity levels during crowding (Espmark et al., 2015).

Operational welfare indicators (OWIs) are WIs that can be used for on-farm (Martins et al., 2012; Noble et al., 2018) to assess welfare in real time and allow the farmer to react to a potential problem. These indicators must fulfill the following requirements:

- Provide a valid reflection on fish welfare
- Be easy to use on the farm
- Be reliable
- Be repeatable and comparable
- Be appropriate for purpose indicators for specific rearing systems or husbandry systems.

Examples of Operational welfare indicators that are currently used on farms include appetite, growth, and surface activity. Loss of appetite, starvation and lack of feeding can be identified by the degree of emaciation in the fish.

Using just one indicator is not enough to assess fish welfare, therefore multiple indicators should be taken into consideration. As such, both animal-based and environmental indicators need to be combined for more suitable ways of measuring the welfare of farmed fish. The FISHWELL handbook recommended that one should consider a toolbox containing a combination of both animal and environmental based indicators that can be easily implemented by fish farmers to assess fish welfare (Noble et al., 2018).

5. Fish behaviour in welfare assessment

Behaviour can be a sign of either good or poor welfare in fish and this is because it reflects how fish responds to their immediate environment (Martins et al., 2012). Although, by merely looking at the fish, it can be difficult to interpret how the fish is feeling, in some studies it has been said that they have a rich body language and this is defined by their response to food, the way they position themselves in the water, the display of different swimming modes, fin displays, gill ventilation frequencies, different skin pigment patterns and colouration (Martins et al., 2012). Fish farmers implemented behaviour as an operational non-invasive key tool for monitoring fish welfare because it can give an immediate indication of the state of the fish in most situations.

Traditional invasive methodologies such as the measurement of cortisol or lactate (i.e., blood indicators) require that the fish is handled or killed before samples for analysis can be obtained. However, the use of these indicators might have some shortcomings:

1. Source of stress: the level of invasiveness is very high and stress the fish, which jeopardizes its welfare.

2. Non-Operational: the fact that traditional measurements need in situ sampling in parallel to farming activities, the setup of sensors or even several operators which altogether might interfere with the normal workflow of the fish farm.

Fish behaviour analysis arises as a key tool to complement and tackle welfare shortcomings deriving from the exclusive use of traditional welfare indicators. Examples of behaviours that fish exhibit include foraging behaviour, exploratory behaviour, swimming behaviour, aggressive/predatory behaviour and sexual display/mating behaviour. Teleost fish exhibit a wide variety of foraging strategies, which can be affected by the farming systems (Turnbull & Kadri, 2007), gender (Øverli et al., 2006), genetic strain or family group. Variations in foraging behaviour are dependent on i) where fish feed (bottom feeders, surface feeders and feeding from the water column), ii) when they feed (e.g., nocturnal vs. diurnal), iii) how they feed (active predators, scavengers or more passive feeders) and iv) what they feed on (animal or vegetable matter or both). This variation should be taken into consideration when interpreting foraging behaviour as it might have implications to assess fish welfare. The techniques for delivering food should be appropriate for the species (A. Alanärä & Brännäs, 1996; Anders Alanärä et al., 1998; Noble et al., 2008). For instance, bottom-feeding flatfish have been shown to have improved welfare (measured as feed intake and feeding motivation) when fed sinking pellets compared with floating pellets (Kristiansen & Fernö, 2007).

Another example would be exploratory behaviour or feed anticipatory activity, and this can be a clear indication of good welfare in addition to their normal schooling behaviour and daily activity (Martins et al., 2012) and a negative response to these indicators may be seen as poor welfare. Freezing behaviour or the absence of movement has also been termed as an indication of poor welfare. This behavioral display could be a strategy for avoiding predation (Vilhunen & Hirvonen, 2003) or it might reflect fear (Sneddon, 2003; Yue et al., 2004). Moreover, escape-type behaviours, hiding, burrowing into the bottom of the holding tank, seeking shelter or increased group "clumping" may also mirror fight-or-flight strategies (Sneddon et al., 2016).

Chaotic swimming out of feeding periods might indicate the presence of a predator inside the cages, whereas swimming tilted might indicate buoyancy issues derived from poor surface accessibility to adapt the air volume held in the swim bladder to swim normally

Pablo Almazán-Rueda and colleagues also validated the use of skin lesion frequency to assess the welfare of African catfish in two studies (P. Almazán-Rueda et al., 2004; 2005) and showed that feeding methods, photoperiod and light intensity affect skin lesions frequency as well as other welfare indicators (such as swimming activity, growth, plasma cortisol and free fatty acids). Similarly, (Martins et al., 2006) used skin lesions as an indicator of aggression to assess the welfare of African catfish subjected to a simulated grading. Considering the time-consuming characteristics of direct or video-recording observations, counting skin lesions may be used as an operational welfare indicator when fish is subjected to handling procedures.

6. Atlantic salmon

The Atlantic salmon (*S. salar*) is an actinopterygian (fin-rayed) fish belonging to the Salmonidae family. Salmonids have an important biological, cultural and economic role and are naturally distributed along both the east and west coasts of the North Atlantic Ocean. The life cycle usually involves spawning in fresh water and eventual migration to the sea, where rapid growth due to rich food resources occurs (Klemetsen et al., 2003). They possess an overall anadromous character, and display long and complex migrations through different habitats. Atlantic salmon are migrants, returning to freshwater during the 12 months preceding spawning, usually between October and December in the Northern Hemisphere.

The Atlantic salmon is iteroparous, meaning it may spawn repeatedly, as opposed to most species of Pacific salmon (*Oncorhynchus* spp.), which are semelparous and die after the first spawning event. The parr–smolt transformation (smolting) and the post-smolt stage (the period just after the smolts have left the rivers) are of particular interest because these periods may be critical for survival in the sea.

The life cycle of Atlantic salmon has seven stages namely, egg, alevin, fry, parr, smolt, post-smolt and adult salmon.

- Egg – Salmon eggs are spherical and slightly translucent with a pink/red colour. They are laid in gravel beds at the bottom of streams and lakes and are protected from being exposed to sunlight and predators while being well-oxygenated. The developing organs can be seen easily through the translucent body as pigmentation development occurs in further stages. Hatching would occur normally within 2-3 months after eggs are laid.
- Alevin –1 inch long and is characterized by the presence of abnormally large eyes that are attached to a bright yolk sac. For about 3-4 months, the newly hatched alevins continue to reside in the gravel nest, feeding on the nutritive yolk-sac

- Fry – This life stage starts once the yolk-sac nutrients are completely absorbed and individuals start feeding actively on external food. Once fry leaves the protection of the gravel beds and grows bigger, predation mortality might increase as they become easier prey for other fish, insects and/or birds.
- Parr – At this stage, individuals start to increase in size up to 6 inches. Vertical markings begin to develop on its body and flanks. In the wild, they feed and grow in freshwater for 1-3 years before they begin their journey toward the ocean.
- Smolt – In this stage, the developing salmon reaches the estuaries-transitional water body between the lower part of a river and the ocean where the salinity gradient increases from the river to the open sea, they turn into silvery white while losing their vertical stripes. In the wild, smolting normally occurs in the spring, under photoperiod and temperature control and is stimulated by endocrinal changes,(Hoar, 1988). The morphological changes include a slimmer body form and alterations in body colouration (darkened fins, dark back, white belly and silver sides) that help to conceal the fish in the pelagic environment.
- Post-smolt Stage from departure from the river (usually in spring/early summer) to the end of the first winter in the sea (sea-winter) or lake.
- Adult salmon- Fish after the end of the first winter in the sea/mature fish which returns to the river to spawn. the body markings of adult Atlantic salmon are very distinctive, presence of an adipose fin and an axillary process at the base of each pelvic fin, and they differ significantly from each other based on species. They have a silvery colouration during ocean life and turn brownish during maturation, with the males also developing reddish hues, (Leclercq et al., 2010). They may spend between 3-7 years in the ocean, after which they migrate upstream to their birthplace for spawning. They stop eating and develop different body colours, males retain bright colours, while females turn darker. After mating, the adult female lays eggs in gravel beds within the spawning sites, and the entire journey is repeated all over again. There are salmon within a population that do not migrate to seawater, and they mature as well. Despite being smaller in size compared to the adult salmons coming from the ocean, they sneak into the spawning event of a pair of salmons so some of the eggs can be fertilized. Even though the female did not select him as a partner, he still has the chance to transfer his genes to the offspring.

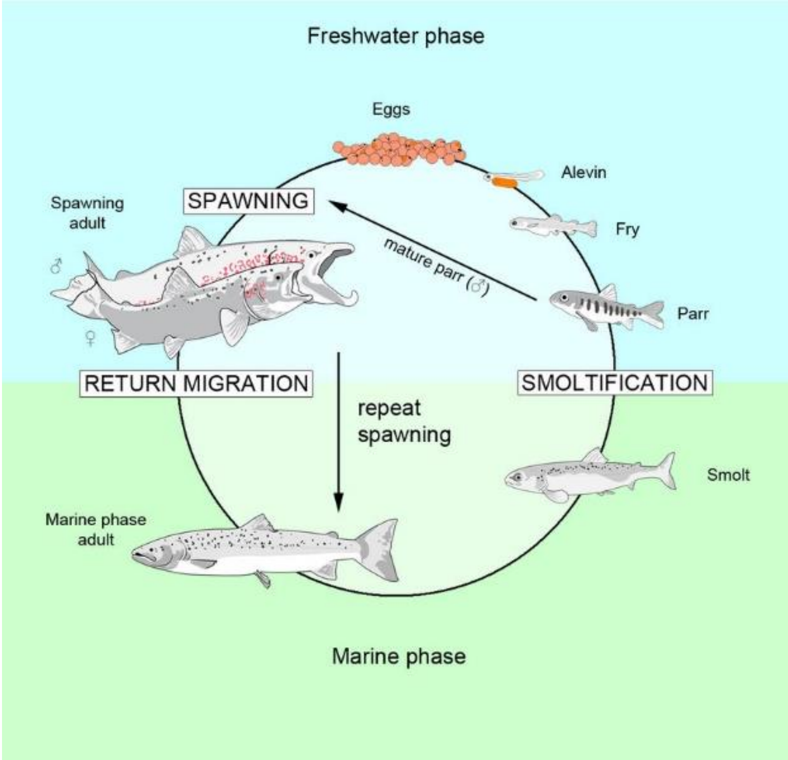

Figure 1 Life cycle of Atlantic salmon (Salmo salar) (Mobley et al., 2021)

7. Atlantic salmon production

Back in the 1960s/1970s, the fish farming of Atlantic salmon started in Norway as the entire life cycle was completed in captivity (*A Brief History of Salmon Farming*, 2003). The nation's wild salmon stocks collapsed due to overfishing, acid rain and damming of waterways, and the farmers in Norway were inspired by the success of their Danish counterparts with trout farming. The enclosed fjords along Norway's coast were ideal for farming salmon in ocean net pens making the industry one to be rivalled across Europe. In the 1980s, the industry dispersed further into the west, Alaska and British Columbia where met with the strong political system that outlawed fish farming in Alaska but was promoted in British Columbia. In the 1980s and 1990s, Norway strengthened its environmental regulations as a response to the problems they were having with fish farms and the lack of ability of the farmers not complying with strict rules of managing the environment. (*A Brief History of Salmon Farming*, 2003). During the 1990s salmon farming increased dramatically around the globe. Stymied by environmentalists from further expansion in Canada, the industry headed to Chile, where farming corporations found cheaper labour and few environmental restrictions. Total global salmon production is nearly 2.5 million tons per annum, which accounts for between 288 and 674 million fish. The main producer is Norway accounting for more than 50% share of the production and followed by Chile with a 27% share (SOFIA, 2020). Throughout the development of the industry, Norway increased its production from about 500 tons in the 1970s to 743 tons in 2008 (Statistics Norway, 2008), and over 1 million tons in 2020 with a market value of over 70 billion NOK (*The Salmon Farming Industry in Norway 2021 Report*) (https://www.kontali.com/b/the-salmon-farming-industry-in-norway-2021-report). The contribution of aquaculture to the global production of fish, reached a record of 49.2% in 2020. Aquaculture of fed aquatic animals continues to outpace that of non-fed aquatic animals. Despite the great diversity in farmed aquatic species, only a small number of "staple" species dominate aquaculture production, particularly grass carp for global inland aquaculture and Atlantic salmon for marine aquaculture. Farmed salmon, which is recognized in the market for its homogenous quality and constant supply, represents more than two thirds of the total market value in the production of aquaculture species. Atlantic salmon (*Salmo salar*) is the most important fish farmed species, followed by rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*).

The expansion of the farmed salmon industry has introduced problems such as sea lice, infections, and parasite transfer and all of these are major environmental and biological impediments to wild salmon and also in some countries the farmed salmon sector's growth. Studies have confirmed that interbreeding between wild and farmed salmon has caused changes in genotypes and a decline in genetic variations in the population of wild salmon, (Heggberget et al., 1993).

8. Crowding

Crowding is one of the operations that occur during the production cycle of Atlantic salmon irrespectively to the rearing system. It is conducted for different reasons such as vaccination, transport and slaughter (Erikson et al., 2016). In tanks, the water flow is stopped until the level of the water decreases enough to be able to harvest the desired fish biomass (Delong et al., 2009). In sea cages, fish are gathered in high densities by using sweep nets or by forcing them into a smaller volume by lifting part or all of the cage (Noble et al., 2018). Crowding is an important operation within the farming process since fish might suffer and experience poor welfare conditions leading to bad health status.

Some of the challenges that may occur during crowding include stress from swimming in high densities in a confined system (e.g., a cage or tank), close against each other and not being able to behave as they would when in the natural habitat. . Crowding time seems to be an important factor of stress and it should not exceed more than two hours (RSPCA, 2021). The oxygen levels in the water may also drop lower than the normal requirements fish would require as their activity level increases, there is also the possibility of inflicting damage on other fish in the tank when they run into each other accidentally and this may lead to loss of skin, fin or even eye damage.

Welfare assessment during crowding must be carried out *in situ* and the procedure must meet operational standards and not interfere with the normal activities on the fish farm. Some of the indicators characterizing fish behavior during crowding include:

- changes in the dorsal skin colour from greyish black to blueish green (Stien et al., 2013b)
- bursts-like swimming close to the surfaces of tanks or cages
- side swimming fish
- fish bringing their head out of the water to gulp or take in air through their mouth
- fish swimming in an unorthodox position or motion-less i.e. fish may just be laying at the bottom of the tank or cage in a withdrawn or exhausted form. (Noble et al., 2018).

Monitoring the aforementioned behavioural indicators can help farmers to anticipate adequate response to prevent/mitigate poor welfare situations.

Erikson et al. (2016), used a remote-operated vehicle (ROV) and cameras placed below and above the cages to monitor behavioural activities during crowding, but neither panic nor aggressive behaviours were observedduring the crowding process. However, cortisol and pH levels (blood LABWIs) and lactate (blood OWI) demonstrated an acute stress response that they did not detect from the behaviour of fish. This fact highlights the need of holistic toolboxes where several indicators might act in synergy to better assess welfare than if they were applied individually. Therefore, operators or fish farmers should be aware that even before panic behaviour is observed, the fish may already be stressed. It is also very important that mortality should be monitored carefully, and all efforts should be taken to avoid negative effects on fish welfare. The spatial distribution of fish is also indicative of welfare status. Shoaling of fish and the vertical/horizontal distribution of fish changes under stress, salmon and tilapia crowd together at the bottom of the tanks when stresses and swimming patterns change (Rey et al., 2019).

The fillet quality of Atlantic salmon has been said to be influenced by several antemortem factors, including handling and crowding, which have been reported to alter rigour Mortis contraction, muscle pH and fillet size (Veiseth et al., 2006). Crowded salmon had lower muscle pH immediately after post-mortem compared with control salmon, due to the anaerobic energy metabolism during the crowding procedure. Something similar was observed in exhaustively exercised rainbow trout (Milligan, 1997; Pagnotta et al., 1994). The muscle pH of control fish was higher than previously reported for unstressed Atlantic salmon, (Einen et al., 1998) and may likely be because muscle pH in the present experiment was measured within 5 min of slaughter compared with 1 hr in the above-mentioned studies. In the stress response of Atlantic salmon, the release of cortisol and catecholamines into the blood is a primary stress response in fish and initiates a series of secondary effects that involve respiratory function, osmotic regulation, and energy metabolism (Iwama et al., 1998). The subjection of crowded salmon to active swimming for 6 h before slaughter delayed the onset of rigor mortis contraction and was also affected by crowding and post-stress swimming activity and the largest degree of contraction was found in crowded salmon. In conclusion, active swimming accelerated the return of plasma cortisol, hydromineral balance and the energy metabolism of adult salmon to pre-stress levels. Moreover, an active swimming period delayed the onset of rigor mortis contraction, which has a positive technological implication for the salmon processing industry. It is important that fish is flexible at the time of filleting and the industry aims to reduce stress in salmon before slaughter to avoid soft flesh, reduce the incidence of gaping, and enable prerigour processing. Espe et al., (2016) also talked about how Atlantic salmon had an accelerated high rate of recovery after swimming during the crowding process in their studies. Increased swimming activity reduces blood cortisol concentration in both juvenile Atlantic salmon (Boesgaard et al., 1993) and rainbow trout, (Postlethwaite & McDonald, 1995).

A picture of the crowding intensity scale (Figure 2, Noble et al., 2018shows how behavioural operational welfare indicators are used to monitor crowding behaviour in aquaculture operations.

Figure 2. Crowding intensity scale and behavioral indicators used to operationalize the welfare assessment of Atlantic salmon during crowding operations. Figure used with permission from (Noble et al., 2018).

9. Welfare of farmed Atlantic salmon and welfare assessment

The welfare needs are basic requirements of Atlantic salmon to fulfil its immediate survival, good health and behavioural needs, all necessary for long-term success, including social contact (Noble et al., 2018). The welfare needs of Atlantic salmon include the following:

- **Feeding and nutrition** – Healthy and nutritious food must be readily available for the fish in all stages of its life to aid growth. Feeding of Atlantic salmon is dependent upon the life stage, i.e., life stage-specific rations that satisfy its requirements. The appetite and motivation to eat also depend on the life stage and the individual's energy reserves.
- **Environmental factors** - Examples include thermal regulation, osmotic balance, good water quality, and respiration. The ability to pump water through respiration over the gills to allow for the uptake of oxygen and the release of carbon dioxide is essential for aerobic metabolism and maintaining pH in the body of the fish.
- **Health and hygiene** – One major concern about fish is their health status which is threatened by diseases and the possibility of having a rearing environment that is free or has a low concentration of such organisms, e.g., viruses and bacteria can help to reduce the risk of poor welfare.
- **Safety and protection** – this refers to the ability of the fish to avoid possible dangers and potential injuries.
- **Behavioural control**- this is the ability to move freely and display natural behaviours.
- **Rest** – the ability to recover from a high level of activities e.g. stress from swimming.
- **Social contact** – Ability to interact with others in the rearing environment for companionship.
- **Sexual behaviour**- Ability to perform sexual displays.

Health and welfare are two important components of sustainable aquaculture production. The report done by the Norwegian Veterinary Institute in 2021 on Fish Health confirms once again that the health and welfare situation for Norwegian farmed fish is not good (Sommerset et al., 2022). Even though many companies use considerable resources and effort to promote more sustainable production, overall figures for fish mortality in the industry were far too high in 2021. Over 50 million fish died during the sea phase; a figure that has not changed significantly in the last five years. An additional 30 million fish are lost in freshwater production. Many companies and individuals are making progress and have achieved good results related to lice control and disease challenges in general. Nevertheless, the aquaculture industry still performs poorly where fish health and welfare are concerned (Sommerset et al., 2022).

Mortality in juvenile fish (larger than 3 grams) was reported to the Norwegian Food Safety Authority to be 33.4 million salmon and 1.9 million rainbow trout in 2021. This level has been relatively stable over the past five years. It is worth noting that the quality of the data for salmonids in the juvenile phase is not as good as for salmon in the on-growing phase, which makes calculating annual mortality percentages even more difficult (Tørud B, 2021),

A welfare assessment system should describe the welfare of farmed fish and allow the farmer to assess the development over time and respond appropriately. Welfare indicators that are relevant for inclusion in an operational welfare assessment system should be science-based, should measure welfare over extended periods, should be measurable on a commercial farm within a realistic framework and should be relevant as a decision support system for the farmer. To fulfil these requirements, the welfare indicators must provide information on potential welfare problems and the causes of impaired welfare (Rey et al., 2019)

Farmed fish welfare can be assessed based on the combination of animal and environmental welfare indicators that can describe the rearing environment, the physical state of the fish, and its behaviour and appearance. One advantage of using animal welfare indicators is that they are largely system and treatment independent and can be used in most situations. Welfare assessment protocols include the monitoring program for physical damage or deformity suggested in the RSPCA welfare standards for farmed Atlantic salmon (RSPCA, 2021), the welfare assessment protocol developed by the Norwegian Veterinary Institute (NVI) (Grøntvedt et al., 2015) and the Salmon Welfare Index (SWIM) (Pettersen et al., 2014; Stien et al., 2013a). These protocols are used to score the welfare of individual fish based on a set of welfare indicators describing their appearance. Each welfare indicator is divided into levels from good to bad welfare and the results are typically represented as the distribution of sampled fish before and after treatment. In the SWIM protocol, the levels are not only ranked from good to bad but also weighted according to their suggested welfare impact on the fish. The welfare of the fish is calculated as an aggregated score from 0 (worst) to 1 (best), (Noble et al., 2018). The protocols can be used as an alarm system that warns the farmer that something is wrong and needs to be investigated, preferably before mortality starts to increase.

The FISH WELL handbook (Noble et al., 2018) combined all these existing protocols and suggested a unified scoring scheme which is primarily aimed at farmers to help them assess welfare and rapidly detect potential welfare problems on the farm. It is an amalgamation of the injury scoring schemes used in the Salmon Welfare Index Model (SWIM) (Stien et al., 2013), the injury scoring scheme developed by the Norwegian Veterinary Institute (NVI) (Grøntvedt et al., 2015)and from other schemes developed by J. F. Turnbull (University of Stirling) and J. Kolarevic and C. Noble (Nofima). The suggested scheme standardized scoring for 14 different indicators to a 0 -3 scoring system. Level 0- little or no evidence of the OWI, Level 1 – minor to level 3, clear evidence of the OWI. The indicators include the following:

- Emaciation
- Skin haemorrhages
- Lesions/wounds
- Scale loss
- Eye haemorrhages
- Exophthalmia
- Opercular damage
- Snout damage
- Vertebral deformities
- Upper jaw deformity
- Lower jaw deformity
- Sea lice infection
- Active fin damage
- Healed fin damage

Measurement of environmental factors as indirect welfare indicators, but most of the literature reviewed relates to the effect of environmental parameters on productivity or survival rather than the welfare of farmed salmon.

To correctly assess any negative effects on fish welfare, one tries to identify the cause of the problem and investigates whether the issue occurred in the rearing environment or before their transfer. If the source of the issue can be found, then a correction needs to be done to reduce the risk of that welfare concern, if such an issue persists, a secondary level of evaluation needs to be done, and the fish would have to be assessed individually and the possibility of getting a fish health personnel to investigate the situation should be considered if the problem continues (Noble et al., 2018).

10. Filling the gap- Specific behavioural toolbox to assess welfare during crowding fish procedures

Crowding of fish involves gathering fish in high densities for various processes like pumping, transportation, vaccination, slaughter and de-lousing. The amount of stress that fish are subjected to during and after this period needs to be closely monitored and analyzed quantitatively or qualitatively and the results should be able to provide an overview of how best to reduce the level of risks associated with the crowding process.

Monitoring fish behaviour during crowding operations would be a robust source of data collection to help understand how to prevent the risks associated with the process. Such qualitative assessment can be converted into quantitative measures via video recordings and software for data collection, analysis and presentation, while image analysis facilitates continuous surveillance of behaviour. In this way, behaviour can be used as a noninvasive tool and as an early sign of identifying potential welfare problems.

Behavioural welfare indicators have the advantage of being fast and easy to observe and therefore are good candidates for use 'on-farm'. Existing behavioural indicators that have been previously explored in other studies include monitoring the swimming behaviour of fish, the presence of fins or white sides on the surface of the water, surface activity, burst, escape related and panic behaviour, (Noble et al., 2018). Surface activity has been used to monitor the swimming activity in water and the desired goal is one in which there is a low amount of stress and no vigorous activity going on, with no dorsal fins or white sides present as well. Overcrowding is not acceptable in aquaculture production as it poses risks to the fish, they are unable to maintain swimming equilibrium, the surface activity is constantly increasing and there is the presence of many dorsal fins and white sides on the surface.

We can anticipate that fish can be stressed during crowding and by the following behaviour we can find out more about the stress levels of individuals and groups: ventilatory activity, individual and group swimming behaviour, and stereotypic and abnormal behaviour (Martins et al., 2012). The prevalence of avoidance behaviour and collision between fish can maybe explain the damages we see on fish after crowding.

Erikson et al. (2016), used a remote-operated vehicle (ROV) and cameras placed below and above the cages to monitor behavioural activities during crowding but they did not observe any panic or burst behaviour.

In the same studies, the ventilation rates of fish were assessed from underwater videos from the GoPro camera that was mounted on the ROV. For each selected fish, the number of operculum intervals was counted within the time each fish was visible within the field of view of the camera. The time interval from the first to the last registered operculum movement was measured, and ventilation rates were calculated as operculum movements (breaths) per min. (Erikson et al., 2016).Based on the literature review done in this study and the potential constraints crowding can have on fish, the following behavioural toolbox is suggested for assessment of Atlantic salmon welfare during crowding: -

- **Surface activity** would include observation of fish with fins out of the water or side swimmers (white sides) on the surface of the water during crowding according to the crowding intensity scale.
- **Swimming speed** can be measured by observing the speed at which the fish is swimming and how often the level of activity increases in the tanks or cages used to hold the fish.
- **Swimming direction** can be measured by closely observing the direction in which the fish is facing or swimming, this can be done in real-time or by analysing video recordings.
- **Escape-related behaviour or bursts** of any kind during crowding can be measured and counted at the exact number of times it happens, during this period fish can run into each other and this can also provide information on how frequent this happens and what part of the body comes into contact with each other. Depending on the level of contact, it might cause injuries like loss of fin, eye damage and skin loss. Thus, observing the number of times that this could happen can help us explain how they acquired it.
- **Collision with another fish**- the amount of times fish come in contact with each other can also be monitored to provide information on why such occurrence happens during the crowd.

- **Number of lethargic fish** observed in the video recordings to document the prevalence of the individuals most affected by the crowding.

- **Ventilatory activity** can be calculated for individual fish using camera systems as a number of operculum movements (breaths) per min.

The major reason for recommending all these behaviours to assess welfare during crowding operations in aquaculture is that they are non-invasive and do not affect the fish in any negative way, nor does it require the handling of fish directly. Therefore, they can be combined with existing and new potential welfare indicators after they have been standardized and cleared for use on the farm.

11. References

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12. Appendix

Author	Year	Keywords	Topic
Eva Veiseth , Svein Olav Fjæra , Bjørn Bjerkeng , Per Olav Skjervold	2006	Atlantic salmon Cortisol Crowding Muscle pH Recovery Rigour Mortis Swimming	Accelerated recovery of Atlantic salmon (Salmo salar) from effects of crowding by swimming(Veiseth et al., 2006)
Tore Håstein, A.David Scarfe V.L. Lund	2014	Animal welfare	Science-based assessment of welfare: Aquatic animals(Office, 2005)
Donald M. Broom Ken G. Johnson	2019	Stress Animal welfare Animal behaviour	Stress and Animal Welfare(Broom & Johnson, 2019) Key Issues in the Biology of Humans and Other Animals
Jennifer L. Bailey , Sigrid Sandve Eggereide	2020		Indicating sustainable salmon farming: The case of the new Norwegian aquaculture management scheme(Bailey & Eggereide, 2020)
Florent Govaerts	2021	Salmon aquaculture	Media representation of salmon aquaculture in France
P. McIntosh, L.T. Barrett, F. Warren-Myers, A. Coates, G. Macaulay, A. Szetey, N. Robinson, C. White, F. Samsing,	2022	Disease Environmental impact Fish farm Salmo salar Satellite image Welfare	Supersizing salmon farms in the coastal zone: A global analysis of changes in farm technology and location from 2005 to 2020

F. Oppedal, O. Folkedal, P. Klebertf, T. Dempster			
Turid Synnøve Aas , Trine Ytrestøyl, Torbjørn Åsgård	2019	Feed ingredients Ingredient origin Annual salmon production Nutrient retention Whole body analysis	Utilization of feed resources in the production of Atlantic salmon (Salmo salar) in Norway: An update for 2016
James Turnbull, Alisdair Bella, Colin Adams, James Bron, Felicity Huntingford	2004	Stocking density; Welfare; Atlantic salmon; Multivariate analysis	Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis
Mark D. Powell, Pat Reynolds, Torstein Kristensen	2015	Bath treatments Water chemistry AGD Paramoeba perurans Sea lice Lepeophtheirus salmonis Atlantic salmon	Freshwater treatment of amoebic gill disease and sea-lice in seawater salmon production: Considerations of water chemistry and fish welfare in Norway
Heidi Moe Føre , Trine Thorvaldsen , Tonje C. Osmundsen , Frank Asche , Ragnar Tveterås, Jan Tore Fagertun a , Hans V. Bjelland	2022	Innovative production technology Development licenses Sustainability measures Marine salmon aquaculture	Technological innovations promoting sustainable salmon (Salmo salar) aquaculture in Norway
Malthe Hvas , Ole Folkedal, David Solstorm , Tone Vågseth , Jan Olav Fosse , Lars Christian Gansel b , Frode Oppedal	2017	Swimming behaviour School structure Push-cage Atlantic salmon Exposed aquaculture Fish welfare	Assessing swimming capacity and schooling behaviour in farmed Atlantic salmon Salmo salar with experimental push-cages
Yajie Liu , Jon Olaf Olaussen , Anders Skonhoft	2011	Wild salmon fisheries Salmon farming	Wild and farmed salmon in Norway—A review

		Management Conflicts Regulations Challenges	
Lena Geitung , Daniel William Wright , Frode Oppedalc , Lars Helge Stienc , Tone Vågsethc , Angelico Madaro	2020	Ballan wrasse Biological control Lumpfish Mariculture Salmon aquaculture	Cleaner fish growth, welfare and survival in Atlantic salmon sea cages during an autumn-winter production(Geitung et al., 2020)
G. Macaulay, S. Bui, F. Oppedal, T. Dempster	2020	Atlantic salmon Behaviour Cages Farmed salmon	Acclimating salmon as juveniles prepares them for a farmed life in sea cages (Macaulay et al., 2020)
C. Klykken, A.K. Reed, A.S. Dalum, R.E. Olsen, M.K. Moe, K.J.K. Attramadal, L. Boissonnot	2022	Farmed Atlantic salmon Nephrocalcinosis Hypercalcemia Hypermagnemia Calcium deposits	Physiological changes observed in farmed Atlantic salmon (Salmo salar L.) with nephrocalcinosis
Kathy Overton, Frode Oppedal, Lars H. Stien, Lene Moltumyr, Daniel W. Wright, Tim Dempster	2019	Aquaculture Cold shock Control Lepeophtheirus salmonis Sea lice Treatment	Thermal delousing with cold water: Effects on salmon lice removal and salmon welfare
Tom Mc Dermott, Jack D'Arcy, Suzanne Kelly, Jamie K. Downes, Bogna Griffin, Robert F. Kerr, Damien O'Keeffe, Micheal O'Ceallachain, Louise Lenighan, Felix Scholz, Neil M. Ruane	2021	Hyposaline Sea lice AGD -Amoebic Gill Disease Atlantic salmon Tarpaulin Efficacy	Novel use of nano-filtered hyposaline water to control sea lice (Lepeophtheirus salmonis and Caligus elongatus) and amoebic gill disease, on a commercial Atlantic salmon (Salmo salar) farm
K.M. Grimsrud , H.M. Nielsen , S. Navrud , I. Olesen	2013	Willingness to pay Animal welfare Atlantic salmon Aquaculture Breeding	Households' willingness to pay for improved fish welfare in breeding programs for farmed Atlantic salmon

Lars Helge Stien , Brit Tørud , Kristine Gismervik , Marianne Elisabeth Lien , Christian Medaas , Tonje Osmundsen , Tore S. Kristiansen , Kristine Vedal Størkersen	2020	Aquaculture Regulation Governance Salmon lice limit Pancreas disease Farm siting	Governing the welfare of Norwegian farmed salmon: Three conflict cases(Stien et al., 2020)
Albert K.D. Imsland, Patrick Reynolds, Morten Lorentzen, Roy Arne Eilertsen, Giulia Micallef, Raymond Tvenning	2020	Lumpfish Growth Operational welfare indicators Feeding Feed blocks	Improving survival and health of lumpfish (Cyclopterus lumpus L.) by the use of feed blocks and operational welfare indicators (OWIs) in commercial Atlantic salmon cages
Malthe Hvas , Jonatan Nilsson , Tone Vågseth , Velimir Nola, Per Gunnar Fjelldal , Tom Johnny Hansen, Frode Oppedal , Lars Helge Stien, Ole Folkedal	2022	Feed intake Feed withdrawal Fish welfare Maturation Skeletal deformities Specific growth rate Starvation	Full compensatory growth before harvest and no impact on fish welfare in Atlantic salmon after an 8-week fasting period
Kristine Vedal Størkersen , Tonje C. Osmundsen , Lars Helge Stien , Christian Medaas , Marianne Elisabeth Lien , Brit Tørud , Tore S. Kristiansen , Kristine Gismervik	2021	Aquaculture Conflicting demands Fish farming Fish health Organization theory Salmon	Fish protection during fish production. Organizational conditions for fish welfare

Table 1- Table showing lists of articles and book chapters reviewed.