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Novel welfare determination of farmed Atlantic salmon exposed to different photo period regimes prior to sea transfer using digital phenotyping and machine learning

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It was difficult to strike a balance between studies and motherhood, just want to apologize to my first-born daughter, Olivia, for not being able to be there whenever she needed.

Lastly, I want to dedicate this thesis to my newborn, Ellinor.

ABSTRACT:

The well-being of fish must be a priority as fish have cognitive abilities and good welfare is a must for optimum production. The purpose of the research was to determine welfare of Atlantic salmon exposed to different light regimes prior to seawater transfer: 8 hours of light:16 hours of darkness, 12 hours of light:12 hours of darkness, 24 hours of light:0 hours of darkness for six weeks, thereafter all treatment groups were exposed to a continuous light regime of 24 hours light:0 hours darkness for six weeks. The PIT-tagged fish were sampled for analysis prior to sea water transfer in June 2021, 3 months after transfer in November 2021 and before harvesting in April 2022. Fish were weighed, measured for fish length, and individually photographed at all three samplings. The images were then analyzed using ImageJ software, where the wound area and affected area surrounding the wounds were calculated. Welfare scoring was also done, including scale loss, looser fish (emaciation), jaw deformity, skin hemorrhages, eye hemorrhages, cataract, dorsal fin damage(active), dorsal fin damage (healed), and cataract. The results from ImageJ were compared with the result from machine learning algorithm.

Prior to sea water transfer the fish had no wounds, whereas in November 9.45 % of the fish population had wounds. In April, 48.4% of the fish had wounds. The results showed that a relatively high variation in biometric traits is expected among individual salmon farmed in seawater, independent of light treatment in freshwater. Nevertheless, 24-hour light treatment in freshwater appeared to be preferable for body growth and condition factor in the long-term in seawater, without compromising fish welfare due to skin issues, including ulcers. Salmon exposed to 12 hours light and 12 hours darkness in freshwater appeared to have highest prevalence of dorsal fin damages. However, calculated growth in seawater (TGC and SGC) was similar for all light treatments in freshwater. Image analyses, and particularly machine learning based on images, show a promising potential for efficient determination of fish welfare issues such as skin ulcers in Atlantic salmon.

Keywords*:* *Salmo salar*, welfare, light regimes, wounds, ImageJ

1. INTRODUCTION:

The aquaculture sector is one of the world's fastest expanding food production industries. According to FAO (2022), the global production of aquatic animals in 2020 was predicted to be 178 million tons, with catch fisheries accounting for 51% (90 million tons) and aquaculture accounting for 49% (88 million tons). This resulted in a USD 406 billion sale price (141 billion for capture fisheries and 265 billion for aquaculture). Norway is the major producer of Atlantic salmon and one of the greatest producers of aquatic food. According to the Norwegian Seafood Council (5 January 2022), the country exported 3.1 million tons of seafood worth 120.8 billion Norwegian Kroner (NOK) in 2021, with salmon accounting for 81.4 billion NOK, followed by cod (Nok 9.8 billion NOK) and mackerel (9.8 billion NOK) (5.9 billion NOK). These facts also illustrate that aquaculture, particularly salmon farming, is important to the Norwegian economy.

Given the cognitive awareness exhibited by salmon, it is crucial to provide them with opportunities for a positive and contented existence. The welfare of fish must be considered in this era of exponential industrialization and production of farmed Atlantic salmon. High stocking densities, sea lice and disease susceptibility, as well as poor water quality are among factors that can have a negative impact on salmon welfare (Santurtun et al., 2018), and potentially also production procedures in the freshwater and seawater phase. If the well-being of salmon is compromised, it has an impact not only on the welfare level but also on the production efficiency and product quality. It is also known that fish, like other vertebrates, have significant cognitive powers and can suffer pain, hence they are properly protected by Norwegian laws and legislations (Hvas et al., 2021). In a salmon production cycle, suffering may occur, but now along with the consumer's awareness, the focus on fish welfare is highly prioritized in addition to being directly linked with the sustainability of salmon farming.

The ability to improve fish welfare requires focus on methods for measuring the welfare. Skin is an important organ of the fish that acts as a barrier against the outer environment. Therefore, any kind of lesions or wounds in the skin can directly or indirectly affect productivity and can become an economic threat to the farmers and cause welfare issues in fish (Sveen et al., 2019). According to Fish Health Report (2022), the mortality rate in aquaculture farming industry is increasing and wounds is one of the major causes for this.

Atlantic salmon life cycle is quite dependent upon internal and external factors. In intensive aquaculture, exposure of salmon to various regimes of photoperiod is done with many objectives (Myklatun et al., 2023). The same authors suggest that long daylength is used while salmon are in fresh water for maximization of growth and change in daylength is again done to trigger salmon to smoltify. Since light is an essential component in salmon production, it would be very interesting to study its effect in growth and susceptibility to wounds.

To access and optimize the welfare of salmon (and other species), welfare indicators (WI) are used. These WI can be either direct, animal-based, or indirect, resource-based (Noble et al., 2018). According to Pavlidis (2022), animal-based indicators are used to measure the aftermath of resources and conditions applied to fish farming, whereas resources or input based indicators include the environment and resources that fish is exposed to. Operational Welfare Indicators (OWIs) are determined visually on site, and they are traditional and generally dependent upon human factors as different people may have different ways of judging. Moreover, only a certain number of fish can be analyzed as the assessment of OWIs is time consuming. However, using image analyses such as ImageJ can be more precise, and enables welfare assessment of a larger fish population than manual scoring. Machine learning is currently an important aspect of salmon farming, and it is commonly utilized in fast pathogen identification, including sea-lice detection (Guragain et al., 2021), biomass detection, and other growth metrics. Therefore, building a machine learning model to develop an algorithm that can identify wounds in fish could be advantageous. Furthermore, comparing it to ImageJ results could reveal its precision.

2. OBJECTIVES:

Main objective:

-To study the welfare of farmed Atlantic salmon exposed to different photoperiod regimes prior to smoltification using novel digital phenotyping.

Specific objectives:

- To calculate wound area dimensions using ImageJ.
- To study the effects of different light treatments in freshwater on subsequent wound development in seawater.
- To study the correlation between wounds and other production parameters.
- To compare results from Image J with the results from Machine learning.

3. THEORITICAL BACKGROUND:

3.1 Fish welfare:

Atlantic salmon, *Salmo salar,* is one of the most successful species in modern aquaculture. It is uncomplicated to cultivate, grows quickly, has a significant commercial market value, and adapts well to a variety of farming conditions. Salmon is anadromous and is traditionally farmed in open sea-cages along the seacoast after smoltification in freshwater (Hvas et al., 2021).

Many arguments about fish pain perception have raged for a long time. Some studies suggest that fish have cognitive abilities, i.e., that they can feel pain and have sensory systems that enable fish to gather information and make intelligent decisions (Nilsson et al., 2018). In addition to this, the myriad surroundings and constraints with which fish interact necessitate equally diverse cognitive talents, and the answers that fish have devised are quite remarkable (Salena et al., 2021). There has been a substantial body of research demonstrating that fish exhibit all the characteristics that are associated with intellect in mammals, as well as tension, fear, and pain. Farmed fish are under our control their entire lives, and despite there are welfare criteria in place, the purpose of welfare consideration is generally to maximize production and minimize losses, rather than to assure good welfare. So, this trend must be changed to address both welfare and sustainability (Brown, C., & Dorey, C. 2019). Fish require sufficient care in captivity even more than they do in their natural habitat because they are in captivity against their will. Therefore, the well-being of farmed fish must be a priority along the whole production chain (Kristiansen & Bracke, 2020).

According to the handbook *Welfare Indicators for Farmed Atlantic Salmon*, it is very difficult to find the universal definition of welfare because different approaches have different definitions of it. However, we can combine all those approaches in defining welfare. The first approach says that if an animal is healthy with good body growth and performance, it is said to be in good welfare. Second approach says that an animal is considered to be in very good welfare if it is given natural environment and opportunity to perform innate species-species behaviors. The third approach focuses on emotions and explains that if an animal is free from persisting negative emotions like fear, pain and distress and feel pleasure, then it is in good welfare (Noble et al, 2018).

According to a survey, Norwegian consumers will happily pay higher price if the Atlantic salmon is selected for traits related to fish welfare (Grimsrud et al., 2013). This also expresses how important is wellbeing of fish to the consumers.

The five freedoms given by FAWC (1996) is the milestone in defining animal welfare and is extensively used in fish welfare too which spells out that the best method to attain good welfare and health of fish in aquaculture is by respecting, maintaining and improving rights of fish (Mustapha, 2014).

FIVE FREEDOMS FAWC (1996)

1. Freedom from hunger and thirst
2. Freedom form discomfort,
3. Freedom from pain,
4. Freedom to express normal behavior
5. Freedom from fear and distress

(Mustapha, 2014)

3.2 Skin:

Skin plays a key function in defense mechanisms and is an important organ for assessing the health and wellbeing of farmed fish. (Sveen et al., 2016)

The skin of a fish contains three layers: epidermis, dermis, and hypodermis, epidermis is the exterior layer, which contains primarily epithelial cells alongside a few mucous cells (Elliott, 2011; Sveen et al., 2016) and the dermis, the inner layer, typically consists of a matrix of fibers with few cells, but it can include scales, nerves, arteries and veins, deposits of fat, and pigment cells (Elliott, 2011).

Since skin of the fish is mucus-coated and studded with scales, any sort of cut, abrasion, or even a minor scale loss can contribute to welfare issues or subsequent development of wounds and/or pathogen attack. The observer can easily identify epidermal damage, and skin condition is a key component of OWI. Additionally, a study done by Noble and his colleagues in 2018 revealed the presence of nociceptors near the epidermis can make the fish more vulnerable towards lesions.

3.3 Wounds:

Sores and wounds are the leading causes of fish mortality during the production cycle, and they also decrease the market value of the fillet/fish product, causing significant financial losses. According to The Health Situation in Norwegian Aquaculture (2018), skin sores caused by bacteria are widespread in Northern Norway, and in the winter, bacteria develop in these wounds, which cause 30% mortality in Northern Norway (Cargill, 2018).

3.3.1 Causes of wounds:

Many factors can cause wounds in fish, but basically, there are two types of wounds, 1. mechanically induced because of farming activities like handling, delousing, storms and because of predators (Sveen et al., 2019), and 2. lesions because of pathogens (e.g., fish sea-lice) which may further develop into wounds (Sveen et al., 2019~~-~~; Gupta et al., 2022). The presence of lice can also cause wounds, especially around mouth (Gupta et al., 2022). Wounds on skin may not be the only cause of fish mortality but these can be harbor of pests and diseases, and they can further expand into deeper lesions and directly counteract with welfare of fish (Hjeltnes et al., 2016; Sveen et al., 2020). Furthermore, non-medicinal delousing, like thermal and mechanical delousing, can cause wounds and crushing injuries, as well as damage to the operculum or even skin ulceration (if the water temperature is low) or loss of mucous layer. Thermal delousing often involves exposing fish to water temperatures of 29-34 ^{0}C for approximately 30 seconds, which can be extremely unpleasant for the fish (Moltumyr et al, 2020).

3.3.2 Effects of wounds:

Being an anadromous fish, salmon live in fresh water until the parr stage and then migrates to sea water following smoltification. The fish is hypertonic in freshwater, and water flows into the fish to maintain osmotic equilibrium. As a result, there is a requirement for water excretion via diluted urine, as well as enriching the body as much as possible with salt from the environment and feed. In the sea water phase, however, fish is hypotonic to water, which means that water is constantly streaming out of fish to maintain osmotic equilibrium. As a result, in order to survive, salmon must drink a lot of water and excrete salt (Biomar, n.d.).

Any kind of rupture in the skin or wounds will lead to a faster flowing of water which makes it difficult for fish to maintain osmotic balance (Biomar, n.d.).

Skin injury and wounds are common in intense production systems, and disruptions such as mucus removal, scale loss, or wounds are inversely correlated with barrier effectiveness and disease resistance (Sveen et al., 2020).

3.3.3 Wound healing:

Wounds in Atlantic salmon, like those in other species, undergo re-epithelialization, inflammation, cell proliferation with granulation tissue development, and tissue remodeling. Small shallow wounds on salmon skin may recover in a few hours, but numerous environmental and internal factors, like temperature, wound size, stress, and nutrition, govern the re-epithelization process. An initial barrier of epidermis with mucous secretion is developed to safeguard the wound from external influences. Although the renewal of the epidermis is a principal element of the healing process, the renewal of the dermis is also needed in deeper wounds (Sveen et al., 2019).

3.4 Condition factor:

The condition factor is a measure that depicts the interaction of biotic and abiotic elements in physiological circumstances of fish. It expresses a length-weight relationship which compares the “condition of fish”. It is predicated on the assumption that heavier fish of a certain length are in better physiological condition. Furthermore, it can be used to track feeding intensity, age, and growth rates in fish (Getso et al.,2017; Seher and Suleyman, 2012).

The condition factor is a widely accepted metric for determining nutritional level; the greater the score, the rounder the fish, and vice versa (Stien et al., 2013). Emaciation is generally represented by a number less than 0.9. Its number varies throughout the year and is higher during the hot seasons than during the cold seasons. It is also generally lower during smoltification and then increases again, following sea transfer (Enda et al., 2000; Noble et al., 2018).

Condition Factor (K) = 100W/L3 where W is weight (g) and L is total length (cm).

While the freshwater phase, salmon feed consumption, growth rate, and condition factor are all directly associated with longer daylength. Conversely, the condition factor decreases slightly in the penultimate month of the freshwater phase, indicating that the fish has undergone parr-smolt metamorphosis (Jørgensen & Jobling 1994).

3.5 Growth rate, SGR and TGC:

Growth of an organism is a biological process, and it is influenced and dependent on biotic and abiotic factors. It is also a parameter which is of major concern in fisheries and There are different models which help to quantify the growth. Specific Growth Rate (SGR) and Thermal Growth Coefficient (TGC) are two of them.

SGR is built up on natural algorithm where body weight of two different time period is calculated (Bureau et al., 2000), hence is a coefficient which depicts the increase in fish weight per day in percentage.

$SGR = (Ln\ (Wt) - Ln(W0)) * 100 / t(d)$, where,

- Wo[g]= the weight in grams at the beginning
- Wt [g]= the weight in grams at the end
- t[d]= period, expressed in number of days
- Ln = natural logarithm

(Terms used in aquaculture,2021)

TGC also measures the growth of fish in a specific period but here temperature is also considered. A TGC model is a combination of cubic root of weight and linear function with temperature (Iwama and Tautz, 1981). TGC is considered as a very reliable predictor of growth in Atlantic Salmon where temperature is also an essential component. It has been reported that mean TGC is 2.5 in many studies but for Norwegian salmon industry, this is slightly higher, (more than 2.5) (Thorarensen & Farrell, 2011).

$TGC = [(W2(1/3) – W1(1/3)) / {}^{o}D] \times 1000$, where,

- W2 = weight (g) at time 2 (end of period)
- W1 = weight (g) at time 1 (beginning of period)
- ºD = Degree-days, sum of daily temperatures in °C between t1 and t2 (or duration in days x average temperature in period)

3.6 Welfare scoring:

There are 14 indicators that are scored from 0-3: i) emaciation(looser fish), ii) skin hemorrhages, iii) lesions/wounds, iv) scale loss, v) eye hemorrhages, vi) exophthalmia, vii) opercular damage, viii) snout damage, ix) vertebral deformities, x) upper jaw deformity, xi) lower jaw deformity, xii) sea lice infection, xiii) active fin damage, xiv) healed fin damage (Noble et al.,2018). This external welfare scoring is an assessment of morphological parameters where 0-3 ranking system is used, with 0 denoting no impairment/abnormality/deformity, can be called as healthy, and 3 denoting significant impairment/abnormality/deformity (Noble et al., 2018; Timmerhaus et al., 2021). The score system was designed primarily for controlled and field-based trials, where the mentioned damages used as welfare indicators were frequent in production systems (Timmerhaus et al., 2021).

In another scoring systems/Salmon Welfare Scorecard, issues included are enclosure, feed and feeding, genetics, health planning and treatments, slaughter, key welfare indicators, mortality, sea lice infection, predator management, stocking density, training and husbandry, transport and transfer, and water quality (Compassioninfoodbusiness, n.d.).

3.7 Light: An important input:

Atlantic salmon needs to undergo morphological and physiological changes while transforming from parr to smolt. These changes are meant to acclimatize salmon to being transferred to sea water from fresh water. Smoltification is a process which involves change in hormones and salmon undergo morphological and physiological and behavioral changes and these changes assist salmon for the migration from fresh water to sea and survival (Stefansson et al, 2020).

The process is associated with hormonal alteration, osmoregulation, morphology, and behavior (Johansson et al., 2016). In order to be transformed to smolt, the minimum weight should be 35g. However, salmon remain smolt only for a short period of time because the smolt window last only for 300-400 DGR. If the smolt is not transferred at the right time, consequences occur. If the salmon are transferred too early, mortality can occur, while if transferred too late, salmon can desmoltify. According to Fjelldal et al., (2018), domesticated salmon can smoltify and desmoltify, and again desmoltified salmon can resmoltify within a very short period of time.

Transfer of salmon from freshwater to sea water has been and is an important yet stressful phase. Adapting a natural summer- winter-spring daylength pattern has been used since long ago for smoltification but in recent years, keeping the fish in continuous daylight throughout the freshwater phase (LL)and addition of salt mixture in feed is common (Jansen, 2020).

The traditional pattern of nature mimicking involves keeping parr in 24-hour daylight (LL) and then giving a winter signal by less than 12 hour of day light for 6 weeks, again exposing to 24-hour daylight for another 6 weeks and the process is known as light stimulated smolting (Striberny et al., 2021).

Under the natural regime of photoperiods and water temperatures, salmon parr undergoes smoltification only partially and they change into smolts in spring season when they are about 16 months old; these are called 1 + smolt. The parr which did not undergo transformation live in fresh water and undergo smoltification in the second spring season when they are approximately 28 months old, are called 2 + smolt (Khaw et al., 2021). In the current salmon industry, salmon are transferred to sea during the whole year, and fish that have been kept in freshwater for less than a year are termed 0+ salmon that commonly are transferred to sea during the Autumn period (August-November).

3.8 Image analyses and Machine learning:

ImageJ is a free Java software which is used to analyze photos. It can calculate the dimensions of selected area very easily.

According to the Oxford’s dictionary, machine learning is the use and development of computer systems that are able to learn and adapt without following explicit instructions, by using algorithms and statistical models to analyze and draw inferences from patterns in data.

ImageJ has been used extensively in analyzing the images in biological sciences (Elliott et al., 2022), however use of machine learning is quite new yet is being explored. Both methods, to quantify the images and extract the results, have its own advantages and disadvantages.

Machine learning, which is primarily a category of artificial intelligence, has gained significant attention in the realm of technology as a vital element of digitalization solutions (Ray, 2019). Machine learning (ML) is the study of the statistical models and methods that computers use to do specific jobs without being explicitly programmed. Using test input-output pairs, supervised learning is used to train a function that maps an input to an output. To infer a function, it uses labeled training data comprised of a set of training instances. Supervised machine learning refers to algorithms that need assistance from outside sources. The input dataset is used to build the train and test datasets. The train dataset's output variable must be predicted or categorized. To predict or categorize data from the test dataset, all algorithms use patterns they have learnt from the training dataset (Mahesh, 2020).

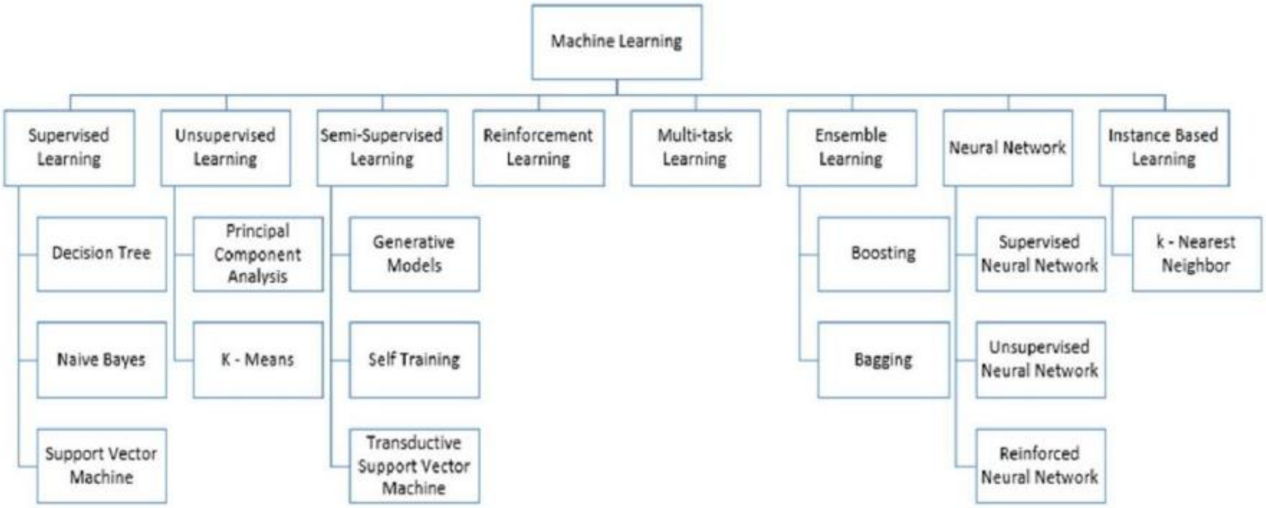


Figure 1. Types of machine learning models (Mahesh, 2020)

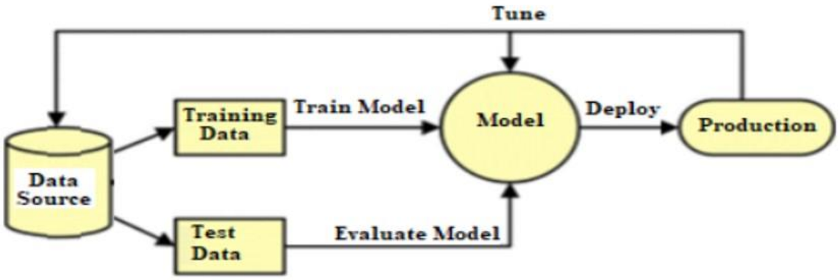


Figure 2. Supervised machine learning model workflow (Mahesh, 2020)

The assessment's credibility is based on the expertise of the analysts, the sort of scoring system used, and the quality of the samples. The outcome from these kinds of studies is susceptible to both human bias and errors. So, in this era of technology, machine-based measures are used to reduce chances of human error and help generate impartial conclusions and reliable data (Wolf and Maack 2017; Penttienen et al., 2018; Sveen et al., 2020).

4. MATERIALS AND METHODS:

The fish material used were 945 Atlantic salmon (*Salmo salar* L.), originating from the Mowi strain, hatched February 2020. In March 2021, fish (approximately 50 g) were individually tagged with passive integrated transponders (PIT-tag) and distributed among circular 1200 L tanks with 500 fish per tank. For a duration of six weeks, from 22.03. - 03.05. 2021, the fish in duplicate tanks were subjected to three different photoperiodic light regimes: 8 hours of light:16 hours of darkness, 12 hours of light:12 hours of darkness, 24 hours of light:0 hours of darkness. The water temperature was maintained at 8 °C. Subsequently, for the following six weeks, the fish were exposed to a continuous light regime of 24 hours of light:0 hours of darkness. The fish in all tanks were fed ad libitum with extruded salmon pellet feed (Nutra Olympic 3 mm, Skretting AS).

On 23.06.2021, fish were transferred to the seawater facility of ARST. Fish from the three light treatments groups were kept in one sea-cage and fed standard extruded feed (Skretting AS, pellet size and composition adjusted according to fish growth). Dead fish were removed from the sea cage daily and moribund fish were euthanized by a lethal dose of benzocaine (150 ppm). Fish that had either died or were humanely euthanized were identified using a PIT-tag reader. Following identification, a visual examination was conducted to determine the potential cause of demise. Subsequently, measurements of individual fork length and body weight were taken and recorded. Given the significant fish loss and the resultant welfare concerns for the remaining live fish, it was decided to end the experiment in April 2022, nearly six months earlier than planned.

4.1 Fish sampling

Fish were sampled for analyses prior to seawater transfer in June 2021 (07.06.-11.06.2021, and 14.06.2021), in November 2021 (2-5.11.2021) and in April 2022 (19-22.04.2022). Fish were starved for 24h before they were anesthetized with benzocaine (60 ppm). Thereafter the body weight and body (fork) length were recorded electronically using Fishreader and the software ZeusCapture (Trovan Ltd,Germany.). Additionally, images were taken of each individual fish by a mobile phone for subsequent determination of ulcers and welfare indicators.

4.2 Analyses of images:

A) B)

Figure 3. Annotation of A) affected area and B) wound area in Atlantic salmon image using ImageJ

The images from the month June showed that the fish had no wounds. The images from November and from April were analyzed using the software ImageJ developed by Wayne Rasband and Contributors (National Instititute of health, USA). Wound surface area in square cm was calculated along with the affected area. First, scale was set in the software, as most of the photographs included a scale. One of the photographs was used to set the scale. The scale was 71.02 pixels/cm. After that, every photo was zoomed, and wounds were annotated with the help of polygon dots. The affected surface area, determined as scale loss around wound, was also annotated. While annotating the wounds, fresh wounds along with the healed ones were also considered

In this experiment for the scoring, categories that were kept in focus for operational welfare indicators are as follows; looser fish(emaciation), jaw deformity, scale loss, skin hemorrhages, eye hemorrhages, cataract, dorsal fin damage(active), dorsal fin damage (healed) and cataract.

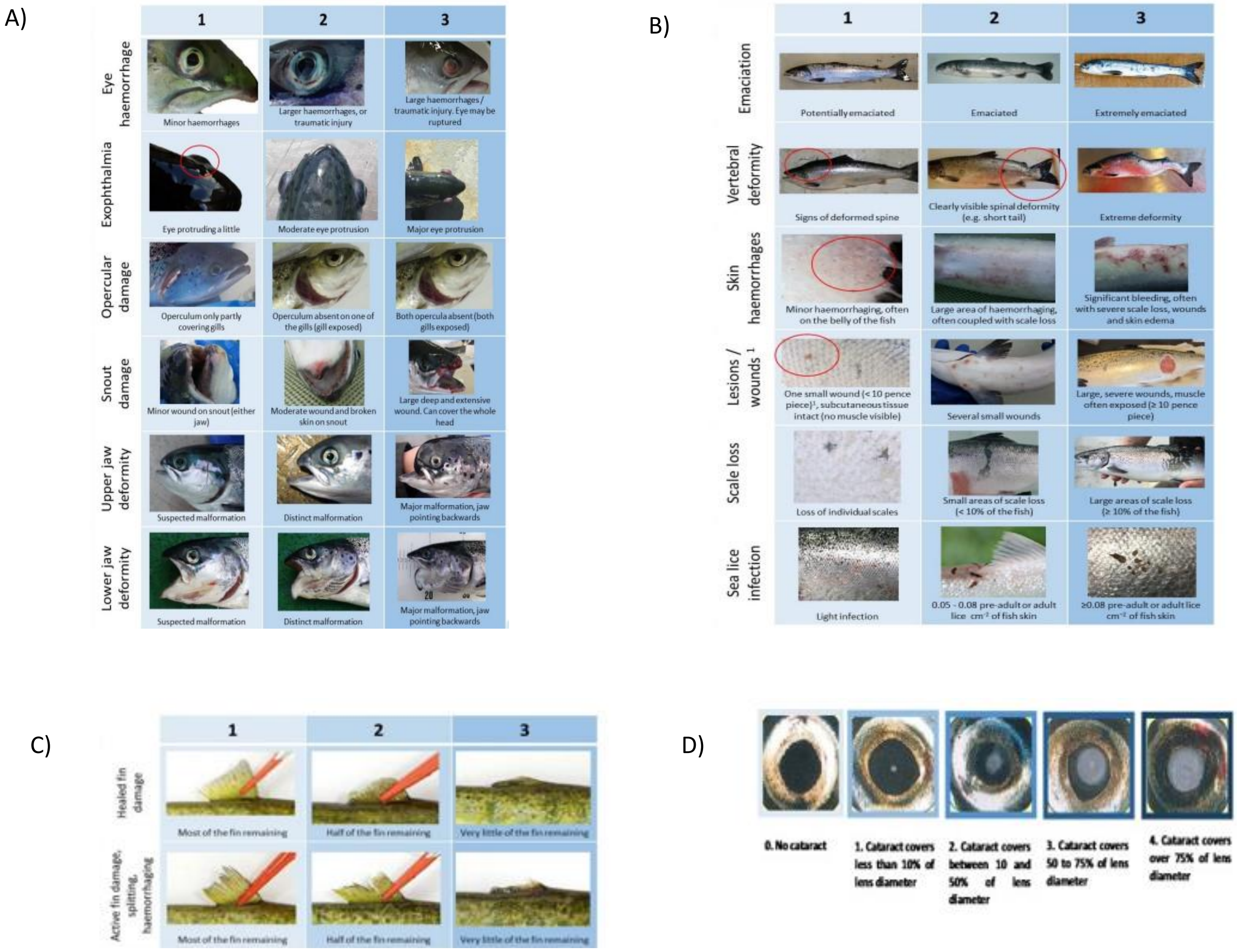


Figure 4. A-C) Morphological scheme for classifying external injuries (Operational welfare Indicators, OWIs). Level 0: There is little or no evidence of this OWI (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI (Noble et al. 2018) and D) morphological scheme for diagnosing and classifying eye cataracts in Atlantic salmon (Wall & Bjerkås 1999).

In addition to this, machine learning algorithm was developed to detect and quantify the wound and area of the wounds from the images in April. For this, 50 images were entered into CVAT software and annotated using the polygon tool. Computer Vision Annotation Tool (CVAT) is an image annotation tool which is developed by Intel for the annotation of images.

These 50 annotated images were used to train a machine-learning model. Thereafter, all the other images were read by the algorithm and was able to detect as well as quantify the wounds. Then, using these 50 images a model was trained using Yolov5(released in 2020 by Ultralytics) for prediction of wounds. After training, the model validation and testing was done. The model created was able to detect the wounds present in the images.

4.3 Calculations and statistics:

Condition factor was calculated as:

Condition Factor (CF) = $100W/L3$ where W is weight (g), and L is total length (cm).

SGR was calculated as, $SGR = (\ln(W_t) - \ln(W_0)) * 100/t(d)$, where,

- $W_0[g]$= the weight in grams at the beginning
- $W_t\,[g]$= the weight in grams at the end
- $t[d]$= period, expressed in number of days
- Ln = natural logarithm

TGC was calculated as: $TGC = [(W2(1/3) - W1(1/3)) / {}^{o}D] \times 1000$, where,

- W2 = weight (g) at time 2 (end of period)
- W1 = weight (g) at time 1 (beginning of period)
- ºD = Degree-days, sum of daily temperatures in °C between t1 and t2 (or duration in days x average temperature in period)

Statistical analyses (ANOVA and Pearson correlation) were done using SAS, version 9.4 for Windows (SAS Institute Inc). The level of significance was set at 5 % ($P < 0\cdot05$)

5. RESULTS:

5.1 Biometric traits

The body weight in June averaged 160g (SD 41g, range 19-320 g). In November, the average body weight had increased to 689 g (SD 163 g; range 248 – 1182 g), while the average weight was 1248 g at the final sampling in April 2022 (SD 287 g; range 569 – 2472 g) (Figure 5).

The body length in June averaged 22 cm (SD 2.0; range 16 – 29 cm). In November, the average body length had increased to 36.5 cm (SD 2.9 cm; range 27 – 44 cm), while the average body length at the final sampling in April 2022 was 44.8 cm (SD 3.2; range 35 – 54 cm) (Figure 6).

The condition factor in June averaged 1.42 (SD 0.11; range 1.09 – 1.87). In November, the condition factor was 1.41 on average (SD 0.11; range 1.06 – 1.79), while the average condition factor at the final sampling in April 2022 was 1.37 (SD 0.10; range 0.87 - 1.66) (Figure 7).

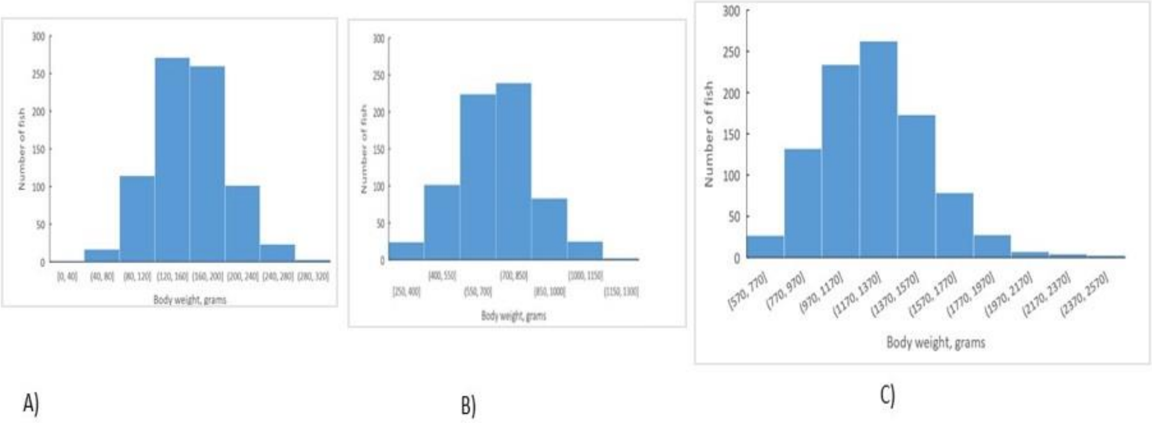


Figure 5. Body weight (g) distribution of Atlantic salmon sampled for analyses in A) June 2021 (n=789), B) November 2021 (n=698) and C) April 2022 (n=945).

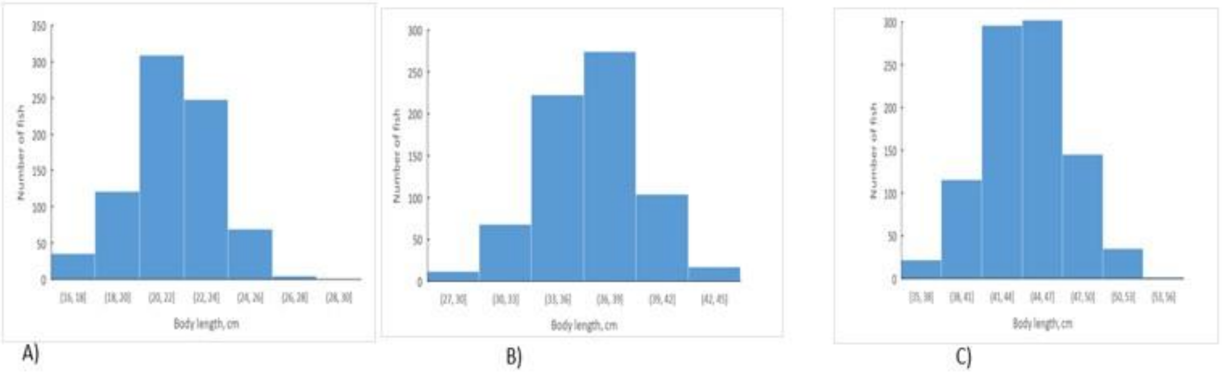


Figure 6. Body length (cm) distribution of Atlantic salmon sampled for analyses in A) June 2021 (n=789), B) November 2021 (n=698) and C) April 2022 (n=945)

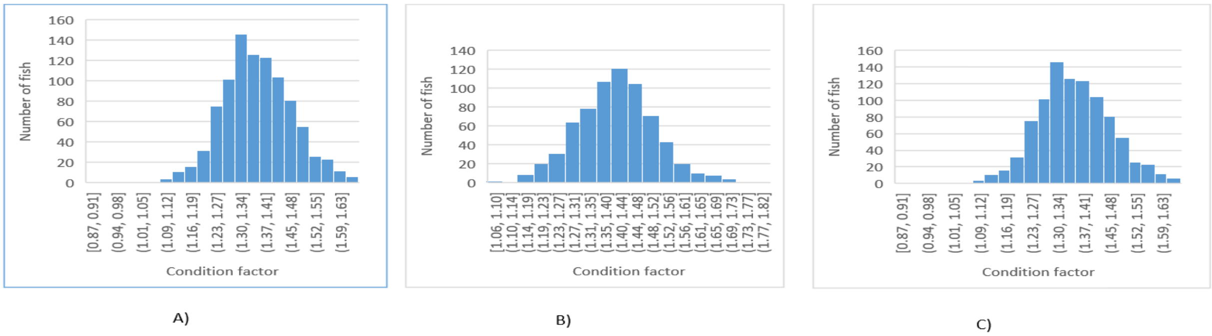


Figure 7 Condition factor distribution of Atlantic salmon sampled for analyses in A) June 2021 (n=789), B) November 2021 (n=698) and C) April 2022 (n= 945)

Figure 8: Images illustrating salmon with (A) highest and (B) lowest CF in April

5.2 Photoperiod

5.2.1 Photoperiod vs biometric traits

The body weight of the salmon exposed to 24 h lightness was significantly higher in June (172 g) compared with salmon exposed to either 12_12 (156 g) or 8_16 (155 g). Similarly, in November, the body weight of salmon exposed to 24_0 was numerically higher compared with salmon exposed to 12_12 (694 g) or 8_16 (688 g) but not significantly different, while in April the body weight of salmon exposed to 24 h light was significantly higher again (1270 g) when compared to salmon exposed to 12_12 light treatment (1217 g) (Figure 9).

In June, the body length of salmon exposed to 24 h light was significantly higher (23 cm) compared with 12_12 (22 cm) and 8_16 (22 cm). However, in November, no significant differences were observed between the 3 light treatments (BL = 37 cm). Similarly, no significant differences in BL because of light were observed in April (Figure 9).

The condition factor of salmon exposed to 8_16 was significantly higher (1.43) compared to salmon exposed with 24 h light (1.4). However, in November, the CF of salmon exposed to 24 h light was significantly higher compared with 12_12 (1.40) and 8_16 (1.39). In April, the CF of salmon exposed to 24 h light was again significantly higher (1.38) than salmon exposed to 8_16 (1.35) (Figure 9).

In the period between June and November, the fish exposed to all three light treatments had similar body weight increase (0.54), and the results were not significantly different from each other. Similarly, in the November - April period, the body weight increase of 24_0 was slightly higher (0.55), but the results were not significantly different from each other. (Figure 10)

The length increase in the period of June and November was significantly higher in the fish exposed to 8_16 light treatment (14.8 cm) and 12_12 light treatment (14.7 cm) than in 24_0. The length increase in June-November period was significantly higher in fish exposed to 24_0 light treatment (8.3 cm) than 12:12 treatment (7.9 cm) (Figure 10).

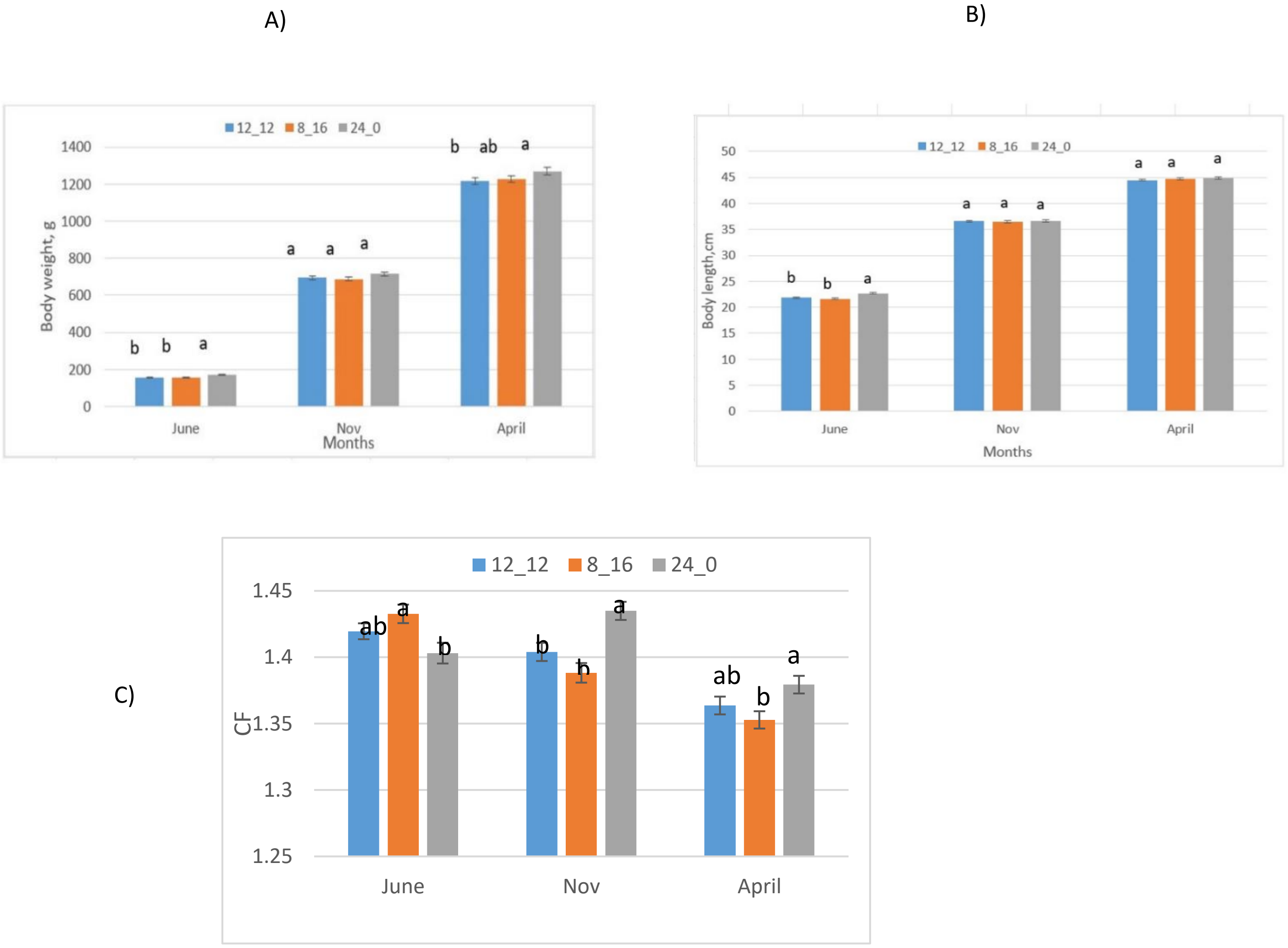


Figure 9:Body weight (A), body length (B) and condition factor (C) of Atlantic salmon exposed to different photo period regimes prior to sea transfer; 12 hours darkness and 12 hours light, 8 hours darkness and 16 hours light, 24 hours light. Results are presented as LSMeans ± SE. Different letters above the error bars indicate significant differences between light treatment within sampling time ($P<0.05$).

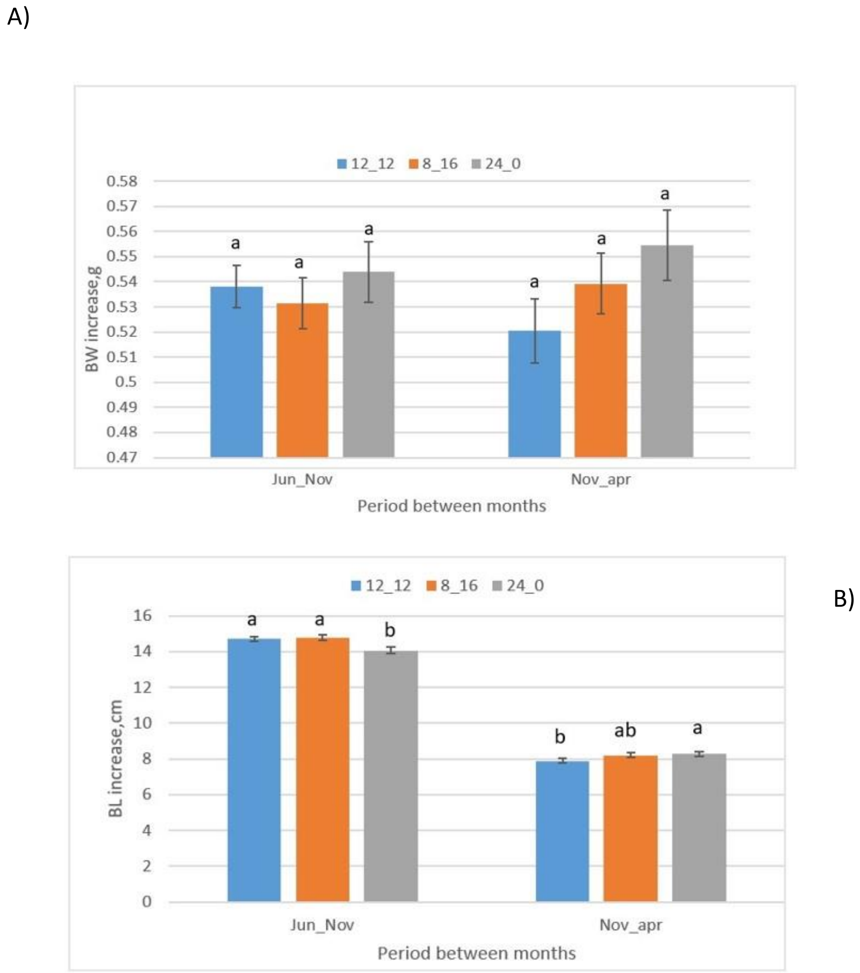


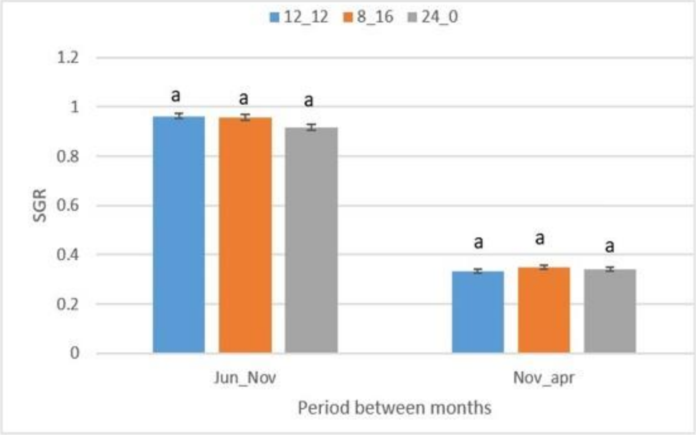
Figure 10. Body weight increase (A) and Body length increase (B) of Atlantic salmon exposed to different photo period regimes prior to sea transfer; 12 hours darkness and 12 hours light, 8 hours darkness and 16 hours light, 24 hours light. Results are presented as LSMeans ± SE. Different letters above the error bars indicate significant differences between light treatment within sampling time ($P<0.05$).

5.2.2 SGR and TGC:

The SGR between June and November was found to be numerically higher for salmon exposed to light treatment 12_12 (0.97) compared with 24_0 (0.92). However, there was no significant differences in SGR under different light treatments in the period between November and June (Figure 11).

There was no significant difference between the TGC of salmon exposed to the different light treatments between the period of June and November. Similar result was observed in the period between November and April. But in general, TGC was numerically higher in 12_12 in June-Nov and 8_16 in Nov_Apr (Figure 11).

A)



B)

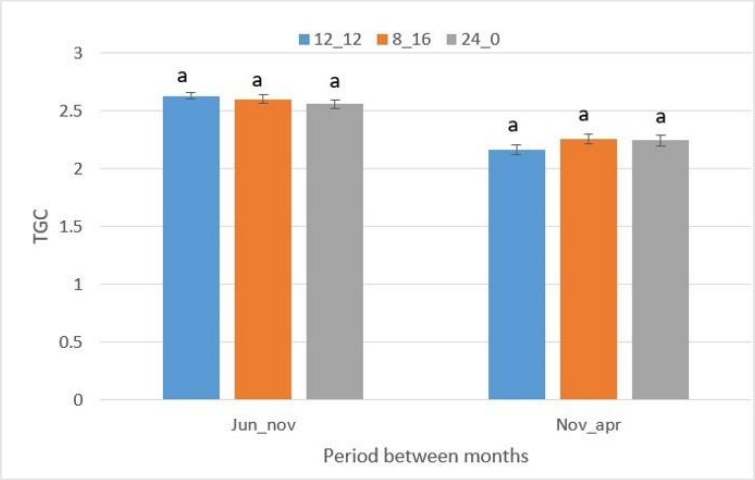


Figure 11. SGR (A) and TGC (B) of Atlantic salmon exposed to different photo period regimes prior to sea transfer; 12 hours darkness and 12 hours light, 8 hours darkness and 16 hours light, 24 hours light. Results are presented as LSMeans ± SE. Different letters above the error bars indicate significant differences between light treatment within sampling time ($P<0.05$).

5.2.3 Wounds:

Among 945 fish in June, no fish had wounds.

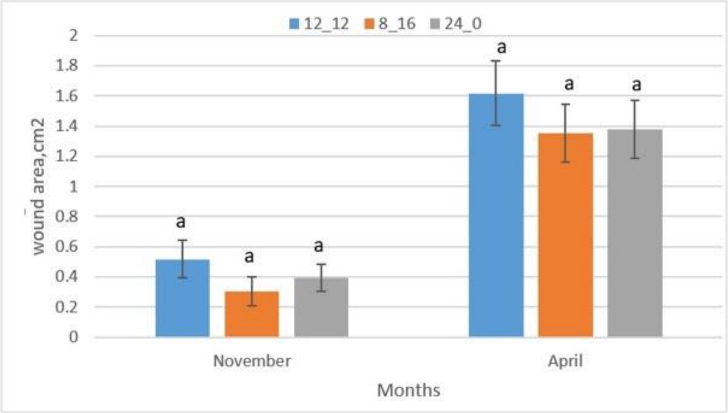
Among 945 fish in November, 90.7 % of fish had no wounds and only 9.3 % of the total fish had wounds. The maximum of wound and affected area for November was found to be 21.0 cm^2 and 41.7 cm^2 respectively. Similarly, the average cm^2 of wound and affected area in fishes were found to be 0.3 cm^2 and 0.8 cm^2.

Among 945 fish in April, 48.3 % of the fish had wounds and 51. 8% did not have any. The maximum cm^2 of wound and affected area calculated among total was 36.2 cm^2 and 49.5 cm^2. Similarly, average cm^2 of wound and affected area was found to be 1.4 and 3.2 cm^2.

This shows that the wound intensity had increased along with time.

Figure 12. Image illustrating the salmon with highest wound area in April.

A)



B)

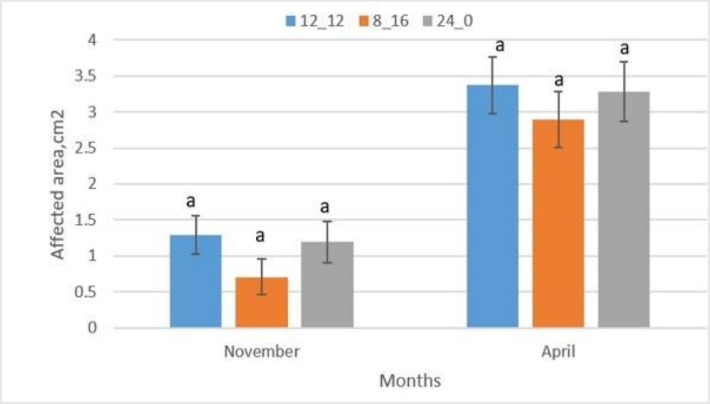


Figure 13. Wound area (A) and affected area (B) of Atlantic salmon exposed to different photo period regimes prior to sea transfer; 12 hours darkness and 12 hours light, 8 hours darkness and 16 hours light, 24 hours light. Results are presented as LSMeans ± SE. Different letters above the error bars indicate significant differences between light treatment within sampling time (P<0.05).

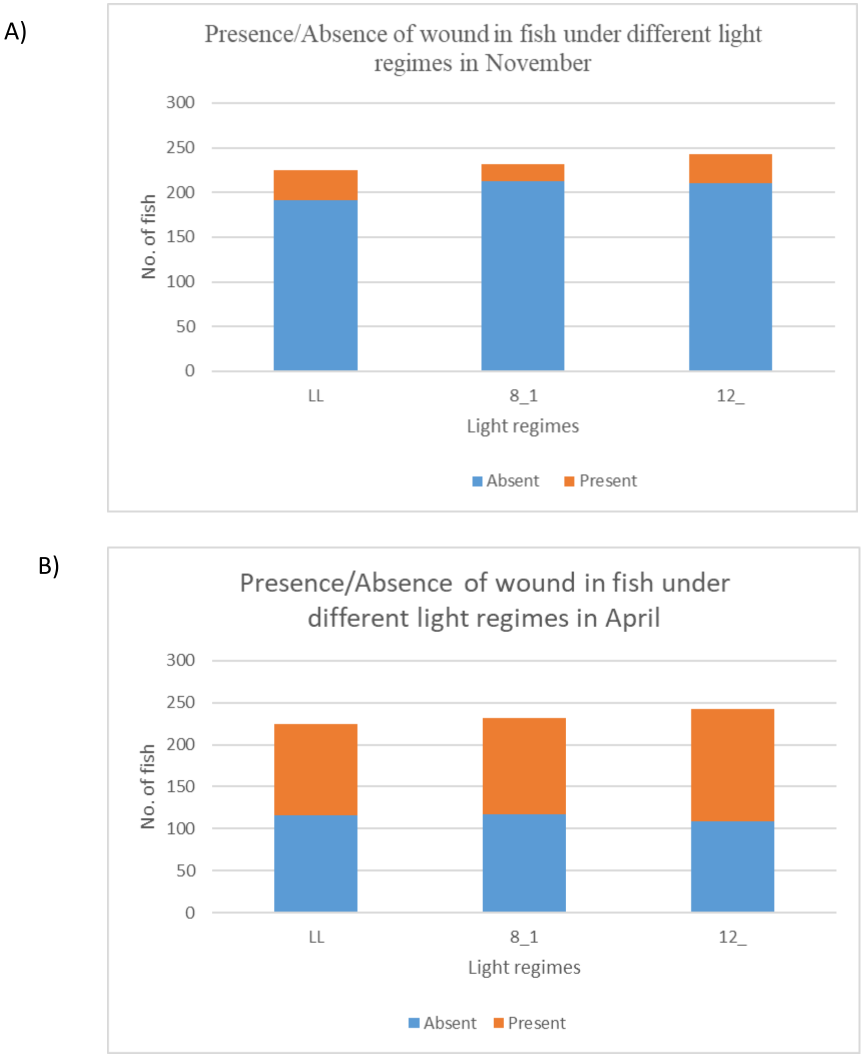


Figure 14. Presence/absence of wounds of Atlantic salmon in November (A) and April (B) exposed to different photo period regimes prior to sea transfer; 12 hours darkness and 12 hours light, 8 hours darkness and 16 hours light, 24 hours light.

The wound area was found to be highest in the salmon exposed to 12_12 light treatment (0.52 cm^2, SD = 0.13) than 8_16 and 24_0 in November. However, there was no significant differences between them. Similarly, in April too, the salmon exposed to 12_12 has higher wound area (1.62 cm^2) than exposed to other two treatments but there was no significant difference between them (Figure 14).

In November, the salmon exposed to 12_12 treatment had higher wound affected area (1.3 cm^2; SD =0.27) than other two light treatments. However, they were not significantly different to each other. Similar result was observed in April too where the salmon exposed to 12_12 had higher affected area (3.37 cm^2; SD = 0.4) but they were not significantly different (Figure 14).

Out of 945 fish, there were no light treatment records of 245 fish.

In November month, fish with LL treatment were more susceptible to wounds, followed by 12_12. However, In the month of April, fish with 12_treatment were found more susceptible to wounds followed by 8_16. However, 8_16 and LL treatment do not have much difference.

In both months, 12_12 treatment has a higher average of wounds, followed by LL. Similarly, the affected area of wound under different light treatments has similar results as in wound area under different light regimes.

5.2.4 Welfare

In November, no looser fish were observed for the salmon exposed to 12_12 and 8_16, while a looser fish score of 0.004 was recorded for salmon exposed to 24_0 treatment. However, the results were not significantly different from each other. No jaw damage was seen in the salmon exposed to 8_16 light treatment, but salmon exposed to 12_12 had a jaw damage score of 0.004 and fish exposed to 24_0 had score of 0.004. Similar with the results on looser fish, jaw damage did not differ significantly between light treatment. The scale loss welfare score of salmon exposed to 8_16 treatment was significantly higher (1.24) compared with salmon exposed to 12_12 light treatment. The eye bleed welfare score of salmon exposed to 12_12 treatment was numerically higher (0.03) than that for the other two treatments, but the results were not significantly different from each other. Salmon exposed to 24_0 treatment had significantly higher welfare score of cataracts (0.67) than salmon exposed to 8_16 treatment. The dorsal fin damage score of salmon exposed to 12_12 treatment was numerically highest (1.2) among the three treatments; however, the results were not significantly different from each other. The dorsal fin healed score was found to be significantly higher for salmon exposed to 12_12 treatment (1.35) as compared to salmon exposed to 8_16 (1.07) or 24_0 (1.1) (Figure 15).

In April, the looser fish score of fish exposed to 24_0 light treatment was numerically higher (0.13) than the other two. However, the results were not significantly different from each other. The jaw damage score of fish exposed to 8_16 was higher (0.22) than the other two light treatments, but the results were not significantly different. The bleeding score of the skin of fish exposed to 24_0 was significantly higher than the fish exposed to 8_16 light treatment. On the other hand, the scale loss score of fish exposed to 24_0 was numerically higher than the other two treatments. However, no significant differences were observed. The eye bleed score of fish exposed to all three light treatments was nearly zero. The cataract score of fish exposed to 24_0 was slightly, but not significantly higher than the other two light treatments. The dorsal fin damage score of fish exposed to 8_16 was significantly higher than that of 12_12. The dorsal fin damage score of fish exposed to 12_12 was significantly higher than the other two treatments (Figure 15).

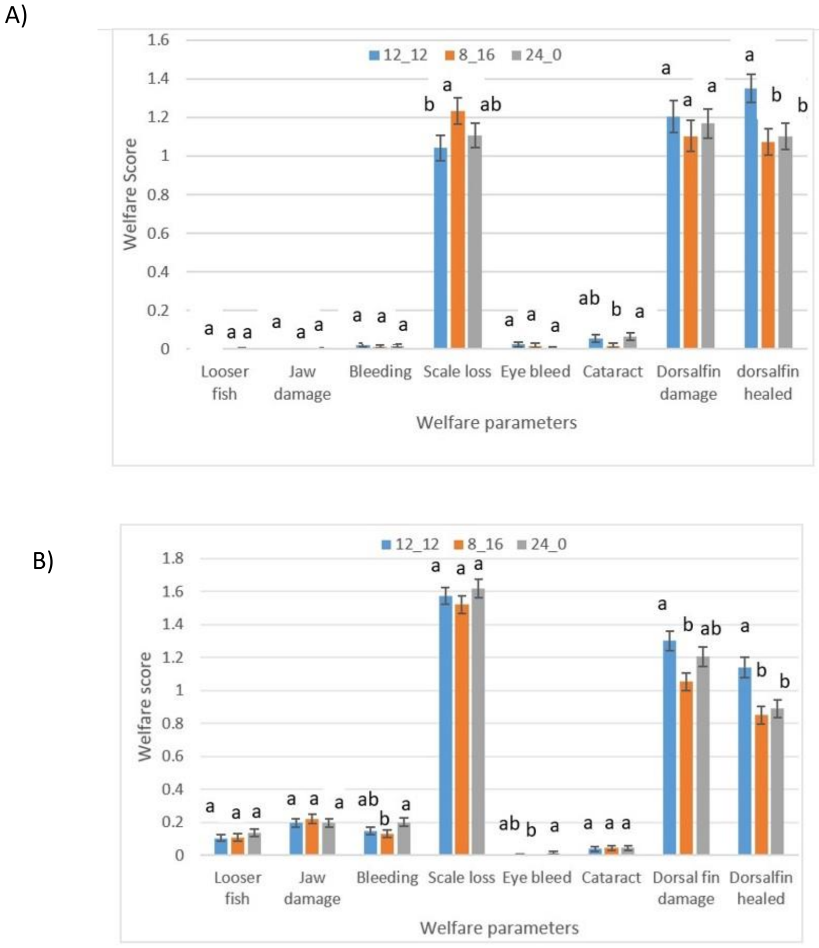


Figure 15. Welfare score in November (A) and in April (B) of Atlantic salmon exposed to different photo period regimes prior to sea transfer; 12 hours darkness and 12 hours light, 8 hours darkness and 16 hours light, 24 hours light. Results are presented as LSMeans ± SE. Different

letters above the error bars indicate significant differences between light treatments within sampling time (P<0.05).

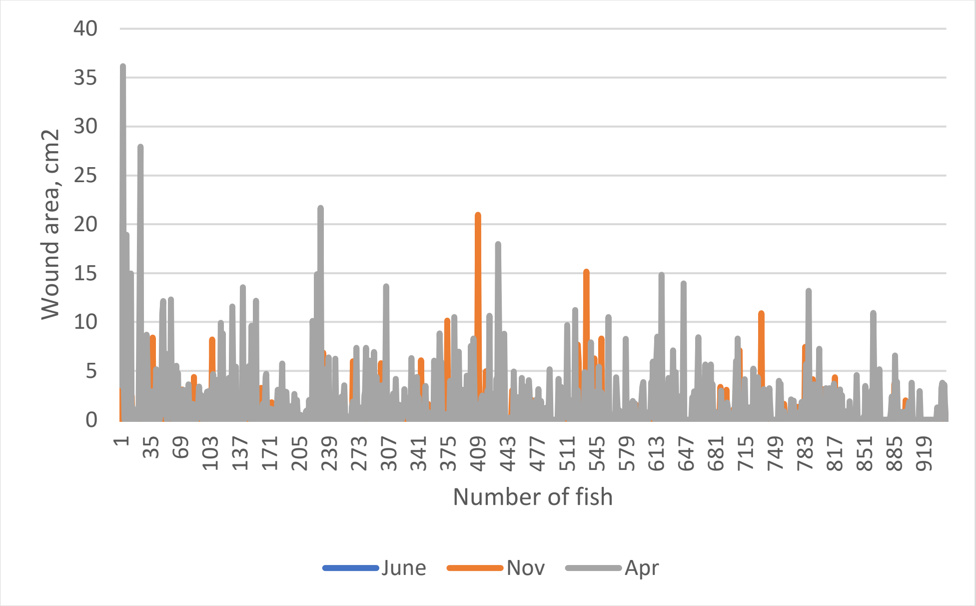


Figure 16: Trend of wound development in June, November and April.

5.3 Wound vs body weight vs other parameters:

In order to investigate the relationship between wounds and growth parameters, i.e., body weight, length and CF of Atlantic salmon, regression analysis was performed.

The results showed weak correlations between body weight and score for wounds for the month of November with multiple R values of 0.013, although the parameters being highly significant (p value = 0.00038)

Similarly, for the month of April, it was again found to be very weak correlations between body weight and wounds with multiple R value of 0.004 and parameters being significant (p-value = 0.0407). It was found to be a weak positive correlation between wound and body length in the month of November where multiple R value was 0.0036 and the regression being non-significant (0.0655).

In April, there was a non-significant correlation between wounds and body length (R = 0.001; p =0.3010). In November, it was found to be weak positive correlation between wounds and CF with an R value of 0.0119 and p = 0.00078. In April, it was found to be weak positive correlation between wounds and condition factor with an R of 0.0105 and p = 0.0015.

5.4 Results from ImageJ vs Machine Learning (ML):

While comparing the results from ImageJ and Machine Learning, the average wound area was higher in the fish exposed to 12_12, however, results were not significantly different from each other. However, in ML, the 24_0 light treatment showed higher wound area than the other two, but the results were not significantly different from each other.

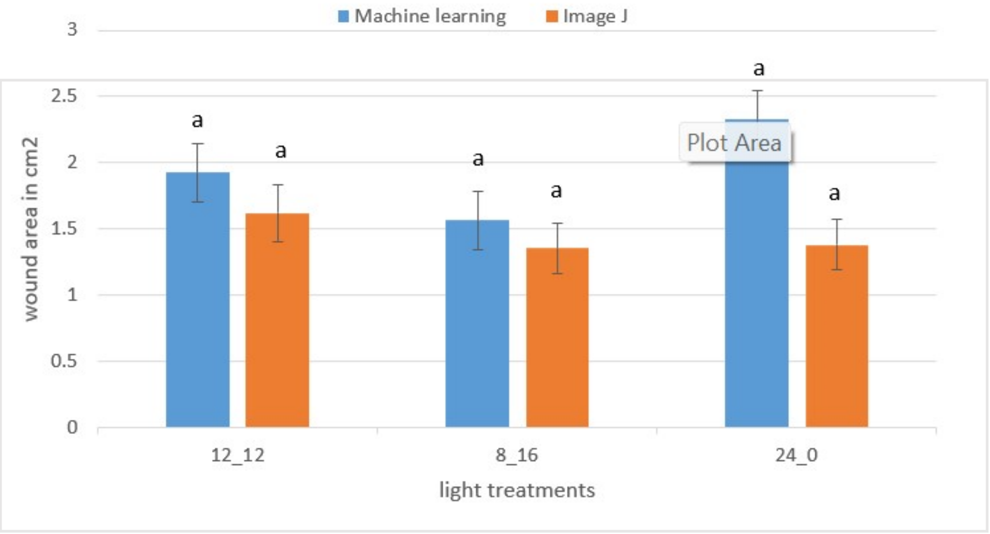


Figure 17. Wound area comparison of results from ImageJ and Machine learning of Atlantic salmon exposed to different photo period regimes prior to sea transfer; 12 hours darkness and 12 hours light, 8 hours darkness and 16 hours light, 24 hours light.Results are presented as LSMeans ± SE. Different letters above the error bars indicate significant differences between lightreatment within sampling time (P<0.05) .

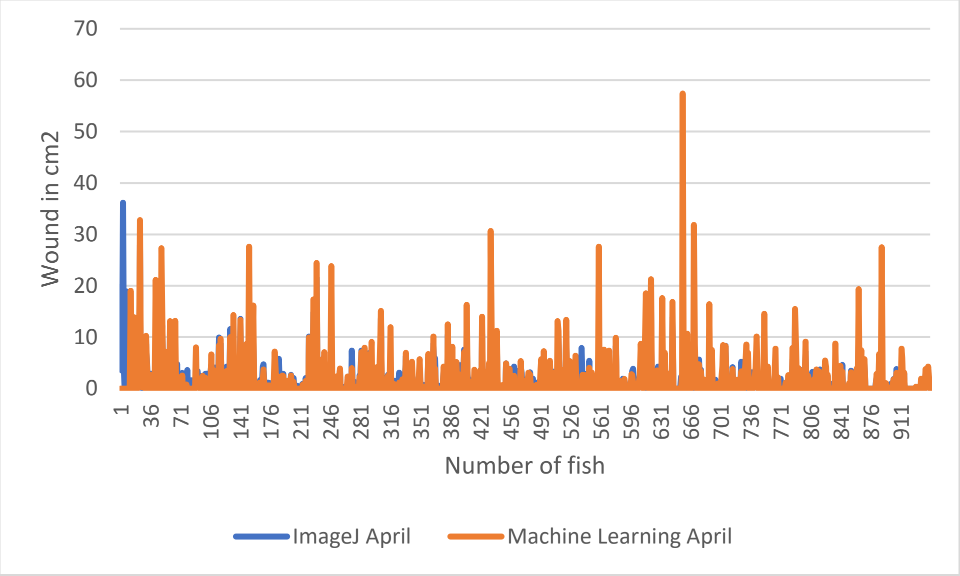


Figure 18. Comparison of results from ImageJ and Machine learning

Both ImageJ and machine learning were able to predict the wounds. Image J predicted wounds in 549 fish images while machine learning detected wounds in 578 fish images.

6. DISCUSSION:

This study assessed development of biometric traits and salmon welfare parameters with time from June, which was before the sea transfer, until April the year after, with a sampling also performed in the center of the trial. The mean body weight was found to be 160 g in June, 689 g in November and 1248 g in April. These results tally with the experiment done by Mørkøre and Rørvik (2001) where the average body weight was noted to be 200g in 1 + smolt and 0.43 g in 0 + smolt in July.

The results showed that salmon exposed to 24 h light treatment in freshwater had significantly higher body weight in June and April, and numerically higher body weight in November compared with the other light treatments. Similarly, 24 h light treatment showed good results for body length in the month of June (where in November and April no significant difference). Moreover, these results are supported by Striberny et al. (2021), who found that salmon exposed to continuous light (LL, 24_0) had a higher weight gain in fresh water. However, the author reported higher SGR for a continuous light treatment + winter signal (12_12) in the sea water phase.

The condition factor in June was significantly higher of salmon exposed to 8_16 light treatment compared with the salmon exposed to 24 hours light, but during the grow out period in seawater the variation between the fish groups changes significantly. In both November and April, the salmon exposed to 24 hours lightness in freshwater had the numerically highest condition factor. In November the 24_0 group differed significantly from the other fish groups, while in April the condition factor of the 24-hour light treatment only differed significantly compared with 8_16 treatment. 24-hour light treatment group differed significantly and again in November and April, 24-hour light showed better result than others. The 24_0 light treatment could be better for fish overall growth, and this is supported by the experiment done by Myklatun et al. (2023) where they used two different treatments for production of 0+ salmon, 1. Use of continuous light+also fed super smolt (containing free tryptophan) (LLS) 2. Use of continuous light and then reduce it to 12 hours for 6 weeks and increase again to continuous light(LD-LL). They found out that salmon receiving LLS had faster growth than others in fresh water and salmon with the 12_12 treatment had faster growth after the transfer in the sea. This is also supported by another experiment of Døskeland et al. (2016), showed that the 24_0 light treatment resulted in better physical and skeletal growth in lower temperature (4.3 degree Celsius) than 12_12 light treatment. The result showed that there was no effect of light in TGC and SGR except in the month of June where SGR of salmon exposed to 12_12 was significantly higher than other two treatments. TGC even though the value has reduced, the average value is nearly equal to the Norwegian salmon farming average, (average value is 2.4-2.5 or higher (Thorarensen & Farrell, 2011). The reduction in the SGR is normal as suggested by study done by Mørkøre & Rørvik (2001) where they agree that SGR decreases during late autumn and winter. However, in the experiment of Døskeland et al. (2016), 24_0 light treatment with low temperature also resulted in increment of SGR by 30 %.

CF is considered a very important parameter for the determination of wellbeing of fish. The relation between length and weight is very crucial in fish development because it is considered as a biological parameter and explains about wellbeing of fish and entire population (Sarkar et al., 2009; Ndiaye et al.,2015). In my experiment, there was a slight decrease in CF in November (after the sea transfer) which means that the fish has undergone parr-smolt transformation thus causing the body of salmon to elongate (Jørgensen & Jobling, 1998). As shown in the result, the body weight and body length has increased with time, while the CF has decreased slightly. Study done by Le Cren (1951) and Ndiaye et al. (2015) supports this result where they confirm that the condition factor could vary during different life-event like growth, smoltification and sexual maturity and with the increase in length, the CF decreases.

While analyzing the effect of various light treatments on susceptibility of fish to prevalence of wounds and wound intensity, they were not significantly affected by freshwater light treatment, although the wound area and affected area were numerically higher in 12_12 light treatment followed by 8_16 and 24_0 light treatment . Hence, freshwater light treatment may not have any noticeable relation to the wound intensity. Also, in terms of susceptibility, in the month of November, fish with 24_0 treatment were most susceptible to wounds, whereas in April, fish with 12_12 treatment were the most susceptible and was Similarly, it was found that in both the November and April months, fish with 12_12 treatment had more wounds. The reason why fish with 12_12 treatment had more wounds area and susceptibility is because fish do smolt better in 12_12 treatment, however, show very weak growth (Pino Martinez et al., 2021). No wounds were present in June, during freshwater phase but after sea water transfer, fish suffered with wounds. The cause of no fish having wounds before sea transfer, but after sea transfer, may be because, they undergo variety of changes, including environment changes, handling stress during transfer, and exposure to pathogens, which could trigger wounds. The development of wounds may also be associated to even a minor skin injuries or loss of scales that can further progress to secondary infections and increase in surface area of the wounds, affecting health, product quality and sometimes mortality (Jensen et al., 2015).

Smoltification is a complex process and involves a stressful phase for the fish. In the post smolt phase, the skin is more vulnerable to environmental alterations (Sveen et al., 2016), and involves immunological changes. Hence, because of changes in gene expression, fish become more vulnerable to infections and diseases (Johansson et al., 2016). Parr is more likely to respond to stressful situations than smolts, according to experiments done by Carey and McCormick (1998), revealing that the stress hormone plasma cortisol and plasma thyroxine levels of parr and smolts are quite different from each other. In parr, these stress markers are lower than in smolts, even in stressful situations. Moreover, these stress markers are higher and do not return to normal concentrations as quickly as they do in parr.

Even if a wound is present in the body, this may not drastically affect the overall weight gain or other parameters in the fish (Molumyr et al., In 2022).

Both ImageJ and machine learning were able to predict the wounds. Image J predicted wounds in 549 fish images while machine learning detected in 578 fish images. However, both models had certain advantages and disadvantages.
ImageJ was easy to learn and analyze the images, however, it was more labor intensive. Analyzing approx. 1200 images of 945 fish only of April month took lots of time. It was a tedious yet very easy tool. On the other hand, machine learning requires knowledge about computer programming language and the correct way of coding, which could only be done and achieved by a professional programmer. In my point of view, ImageJ is more accurate than Machine Learning (at least for the beginner level) and results from ML carries potential yet require further in-depth verification.

7. CONCLUSION:

A relatively high variation in biometric traits is expected among individual salmon farmed in seawater, independent of light treatment in freshwater. Nevertheless, 24-hour light treatment in freshwater appeared to be preferable for body growth and condition factor in the long-term in seawater, without compromising fish welfare due to skin issues, including ulcers. Salmon exposed to 12 hours light and 12 hours darkness in freshwater appeared to have highest prevalence of dorsal fin damages. However, calculated growth in seawater (TGC and SGC) was similar for all light treatments in freshwater. Image analyses, and particularly machine learning based on images, show a promising potential for efficient determination of fish welfare issues such as skin ulcers in Atlantic salmon.

REFERENCES:

Ángeles Esteban, M. (2012). An overview of the immunological defenses in fish skin. International scholarly research notices, 2012.

Arnesen, A. M., Johnsen, H. K., Mortensen, A., & Jobling, M. (1998). Acclimation of Atlantic salmon (Salmo salar L.) smolts tocold'sea water following direct transfer from fresh water. Aquaculture, 168(1-4), 351-367.

Aunsmo, A., Krontveit, R., Valle, P. S., & Bohlin, J. (2014). Field validation of growth models used in Atlantic salmon farming. Aquaculture, 428, 249-257.

Baxter, A. (1998). Condition Factor, K, for Salmonid Fish

Biomar. (n.d.). SKIN HEALTH AND WOUND PROBLEMS.

Blackwel B.G., Brown M.L., Willis D.W. (2000). Relative weight (Wr) status and current use in fisheries assessment and management. Reviews in Fisheries Science, 8, 1-44

Bureau, D. P., Harris, A. M., Bevan, D. J., Simmons, L. A., Azevedo, P. A., &

Carey, J. B., & McCormick, S. D. (1998). Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. Aquaculture, 168(1-4), 237-253.

Cargill.(2018). EWOS Dermic launched.

Cho, C. Y. (2000). Feather meals and meat and bone meals from different origins as protein sources in rainbow trout (Oncorhynchus mykiss) diets. Aquaculture, 181(3-4), 281-291.

Compassioninfoodbusiness. (n.d.). Improving the welfare of farmed Atlantic salmon at rearing.

Costa, R. A., & Power, D. M. (2018). Skin and scale regeneration after mechanical damage in a teleost. Molecular immunology, 95, 73-82.

Døskeland, I., Imsland, A. K. D., Fjelldal, P. G., Stefansson, S. O., Roth, B., Eriksen, K. E., ... & Handeland, S. (2016). The effect of low temperatures and photoperiods on growth and vertebra morphometry in Atlantic salmon. Aquaculture international, 24, 1421-1434.

Elliott, D. G. (2011). THE SKIN| Functional morphology of the integumentary system in fishes

Elliott, K., Berry, J. C., Kim, H., & Bart, R. S. (2022). A comparison of ImageJ and machine learning based image analysis methods to measure cassava bacterial blight disease severity. Plant Methods, 18(1), 1-12.

Endal, H. P., Taranger, G. L., Stefansson, S. O., & Hansen, T. (2000). Effects of continuous additional light on growth and sexual maturity in Atlantic salmon, Salmo salar, reared in sea cages. Aquaculture, 191(4), 337-349.

Getso, B.U., Abdullahi, J.M. and Yola, I.A. (2017). Length-weight relationship and condition factor of Clarias gariepinus and Oreochromis niloticus of Wudil River, Kano, Nigeria. Agro-Science, 16 (1), 1-4. DOI

Gomez, D., Sunyer, J. O., & Salinas, I. (2013). The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. Fish & shellfish immunology, 35(6), 1729-1739.

Gupta, A., Bringsdal, E., Salbuvik, N., Knausgård, K. M., & Goodwin, M. (2022, June). An Accurate Convolutional Neural Networks Approach to Wound Detection for Farmed Salmon. In Engineering Applications of Neural Networks: 23rd International Conference, EAAAI/EANN 2022, Chersonissos, Crete, Greece, June 17–20, 2022, Proceedings (pp. 139-149). Cham: Springer International Publishing.

Guragain, P., Tkachov, M., Båtnes, A. S., Olsen, Y., Winge, P., & Bones, A. M. (2021). Principles and methods of counteracting harmful salmon–arthropod interactions in salmon farming: addressing possibilities, limitations, and future options. Frontiers in Marine Science, 8, 701793.

Hjeltnes B, Bang-Jensen B, Bornø G, Haukaas A, Walde C S (Eds), The Health Situation in Norwegian Aquaculture 2018 Norwegian Veterinary Institute 2019

Hjeltnes, B., Walde, C., Bang Jensen, B., & Haukaas, A. (2016). Fiskehelserapporten 2015. Veterinærinstituttets rapportserie, 3, 2016.

https://polarpedia.eu/en/smoltification/

https://www.biomar.com/en/uk/articles/fishhealth/healthchallanges/skin-health-and-wound-problems https://www.compassioninfoodbusiness.com/media/7436971/improving-welfare-at-rearing-atlantic-salmon.pdf/

https://www.compassioninfoodbusiness.com/media/7436971/improving-welfare-at-rearing-atlantic-salmon.pdf

https://www.fao.org/3/cc0461en/online/sofia/2022/world-fisheries-aquaculture.html

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6119700/

https://www.oecd.org/agriculture/topics/fisheries-and-aquaculture/documents/report_cn_fish_nor.pdf

Hvas, M., Folkedal, O., & Oppedal, F. (2021). Fish welfare in offshore salmon aquaculture. Reviews in Aquaculture, 13(2), 836-852.

Hvas, M., Folkedal, O., & Oppedal, F. (2021). Fish welfare in offshore salmon aquaculture. Reviews in Aquaculture, 13(2), 836-852.

Iversen, M., Finstad, B., McKinley, R. S., Eliassen, R. A., Carlsen, K. T., & Evjen, T. (2005). Stress responses in Atlantic salmon (Salmo salar L.) smolts during commercial well boat transports, and effects on survival after transfer to sea. Aquaculture, 243(1-4), 373-382.

Iversen, M., Mulugeta, T., Gellein Blikeng, B., West, A. C., Jørgensen, E. H., Rød Sandven, S., & Hazlerigg, D. (2020). RNA profiling identifies novel, photoperiod-history dependent markers associated with enhanced saltwater performance in juvenile Atlantic salmon. PLoS One, 15(4), e0227496.

Iwama, G. K., & Tautz, A. F. (1981). A simple growth model for salmonids in hatcheries. Canadian Journal of Fisheries and Aquatic Sciences, 38(6), 649-656.

Jansen, M. (2020). Dietary vs. light stimulated smoltification: effects on smolt development and appetite regulation in pre-and post-smolts (Master's thesis, UiT Norges arktiske universitet).

Jensen, L. B., Wahli, T., McGurk, C., Eriksen, T. B., Obach, A., Waagbø, R., ... & Tafalla, C. (2015). Effect of temperature and diet on wound healing in Atlantic salmon (Salmo salar L.). Fish physiology and biochemistry, 41, 1527-1543

Johansson, L. H., Timmerhaus, G., Afanasyev, S., Jørgensen, S. M., & Krasnov, A. (2016). Smoltification and seawater transfer of Atlantic salmon (Salmo salar L.) is associated with systemic repression of the immune transcriptome. Fish & shellfish immunology, 58, 33-41.

Johansson, L. H., Timmerhaus, G., Afanasyev, S., Jørgensen, S. M., & Krasnov, A. (2016). Smoltification and seawater transfer of Atlantic salmon (Salmo salar L.) is associated with systemic repression of the immune transcriptome. ‘

Jørgensen, E. H., & Jobling, M. (1994). Feeding and growth of exercised and unexercised juvenile Atlantic salmon in freshwater, and performance after transfer to seawater. Aquaculture International, 2, 154-164.

Karlsen, C., Ytteborg, E., Timmerhaus, G., Høst, V., Handeland, S., Jørgensen, S. M., & Krasnov, A. (2018). Atlantic salmon skin barrier functions gradually enhance after seawater transfer. Scientific reports, 8(1), 1-12.

Khaw, H. L., Gjerde, B., Boison, S. A., Hjelle, E., & Difford, G. F. (2021). Quantitative Genetics of Smoltification Status at the Time of Seawater Transfer in Atlantic Salmon (Salmo Salar). Frontiers in genetics, 12, 696893.

Kristiansen, T. S., & Bracke, M. B. (2020). A brief look into the origins of fish welfare science. The welfare of fish, 1-17.

Mahesh, B. (2020). Machine learning algorithms-a review. International Journal of Science and Research (IJSR).[Internet], 9, 381-386.

Moltumyr, L., Nilsson, J., Madaro, A., Seternes, T., Winger, F. A., Rønnestad, I., & Stien, L. H. (2022). Long-term welfare effects of repeated warm water treatments on Atlantic salmon (Salmo salar). Aquaculture, 548, 737670.

Mørkøre, T., & Rørvik, K. A. (2001). Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (Salmo salar) transferred to seawater as 0+ smolts or 1+ smolts. Aquaculture, 199(1-2), 145-157

Mustapha, M. K. (2014). Aquaculture and Fish Welfare: Are the Rights of Fish Compromised?/Akwakultura I Dobrostan Ryb: Czy Prawa Ryb Sa Szanowane?. Zoologica poloniae, 59(1-4), 49. Mustapha, M. K. (2014). Aquaculture and Fish Welfare: Are the Rights of

Fish Compromised?/Akwakultura I Dobrostan Ryb: Czy Prawa Ryb Sa Szanowane?. Zoologica poloniae, 59(1-4), 49.

Mustapha, M. K., Oladokun, O. T., SALMAN, M., Adeniyi, I. A., & Ojo, D. (2014). Does light duration (photoperiod) have an effect on the mortality and welfare of cultured Oreochromis niloticus and Clarias gariepinus?. Turkish Journal of Zoology, 38(4), 466-4

Myklatun, L. E., Fraser, T. W. K., Fjelldal, P. G., Pedersen, A. Ø., & Hansen, T. J. (2023). Long term effects of smolt production strategy and early seawater phase rearing environment on mortality, growth, sexual maturation, and vertebra deformities in farmed Atlantic salmon (Salmo salar L.). Aquaculture, 569, 739346.

Ndiaye, W., Diouf, K., Samba, O., Ndiaye, P., Panfili, J., Marbec, U. M. R., ... & Bataillon, P. E. (2015). The length-weight relationship and condition factor of white grouper (Epinephelus aeneus, Geoffroy Saint Hilaire, 1817) at the south-west coast of Senegal, West Africa. International Journal of Advanced Research, 3(3), 145-153.

Nilsson, J., Stien, L. H., Iversen, M. H., Kristiansen, T. S., Torgersen, T., Oppedal, F., ... & Noble, C. (2018). Welfare Indicators for farmed Atlantic salmon–Part A. Knowledge and theoretical background. 2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare. 351pp, 10-145

Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H., ... & AS, N. (2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare.

Pavlidis, M. (2022). Welfare of farmed fish: moral considerations, science, and problems of implementation. Bulletin of the European Association of Fish Pathologists, 174-177.

Penttinen, A.-M., Parkkinen, I., Blom, S., Kopra, J., Andressoo, J.-O., Pitkänen, K., Voutilainen, 587 M.H., Saarma, M., Airavaara, M., 2018. Implementation of deep neural networks to count 588 dopamine neurons in substantia nigra. European Journal of Neuroscience 48, 2354-236

Pino Martinez, E., Balseiro, P., Pedrosa, C., Haugen, T. S., Fleming, M. S., & Handeland, S. O. (2021). The effect of photoperiod manipulation on Atlantic salmon growth, smoltification and sexual maturation: A case study of a commercial RAS. Aquaculture Research, 52(6), 2593-2608.

Ray, S. (2019, February). A quick review of machine learning algorithms. In 2019 International conference on machine learning, big data, cloud and parallel computing (COMITCon) (pp. 35-39). IEEE.

Salena, M. G., Turko, A. J., Singh, A., Pathak, A., Hughes, E., Brown, C., & Balshine, S. (2021). Understanding fish cognition: a review and appraisal of current practices. Animal Cognition, 24, 395-406.

Salinas, I., Zhang, Y. A., & Sunyer, J. O. (2011). Mucosal immunoglobulins and B cells of teleost fish. Developmental & Comparative Immunology, 35(12), 1346-1365.

Santurtun, E., Broom, D. M., & Phillips, C. J. C. (2018). A review of factors affecting the welfare of Atlantic salmon (Salmo salar). Animal Welfare, 27(3), 193-204.'

Sarkar, U.K., Deepak, P.K. and Negi, R.S. (2009): Length-weight relationship of clown knifefish Chitala chitala (Hamilton 1822) from the River Ganga basin, India. J. Appl. Ichthyol., 25(2): 232-233. Sarkar, U.K., Khan, G.E., Dabas, A., Pathak, A.K., Mir, J.I., Rebello, S.C., Pal, A. and Singh, S.P. (2013): Length weight relationship and condition factor of selected freshwater fish species found in River Ganga, Gomti and Rapti, India. J. Environ. Biol., 34: 951-956

Sawaki, R., Sato, D., Nakayama, H., Nakagawa, Y., & Shimada, Y. (2019). ZF-AutoML: An Easy Machine-Learning-Based Method to Detect Anomalies in Fluorescent-Labelled Zebrafish. Inventions, 4(4), 72.

Seher D. and Suleyman C.I. (2012). Condition factors of seven cyprinid fish species from Çamligöze Dam Lake on central Anatolia, Turkey. African Journal of Agricultural Research, 7 (31), 4460-4464

Sissener, N. H., Hamre, K., Fjelldal, P. G., Philip, A. J. P., Espe, M., Miao, L., ... & Remø, S. C. (2021). Can improved nutrition for Atlantic salmon in freshwater increase fish robustness, survival and growth after seawater transfer. Aquaculture, 542, 736852.

Stefansson, S. O., Björnsson, B. T., Ebbesson, L. O., & McCormick, S. D. (2020). Smoltification. In *Fish larval physiology* (pp. 639-681). CRC Press.

Stien, L. H., Bracke, M. B. M, Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P. J., Vindas, M. A., Øverli, Ø. & Kristiansen, T. S. (2013) Salmon Welfare Index Model

(SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. Reviews in Aquaculture 5, 33-57.

Striberny, A., Lauritzen, D. E., Fuentes, J., Campinho, M. A., Gaetano, P., Duarte, V., ... & Jørgensen, E. H. (2021). More than one way to smoltify a salmon? Effects of dietary and light treatment on smolt development and seawater growth performance in Atlantic salmon. Aquaculture, 532, 736044.

Sveen, L. R., Timmerhaus, G., Krasnov, A., Takle, H., Handeland, S., & Ytteborg, E. (2019). Wound healing in post-smolt Atlantic salmon (Salmo salar L.). Scientific reports, 9(1), 1-16

Sveen, L. R., Timmerhaus, G., Torgersen, J. S., Ytteborg, E., Jørgensen, S. M., Handeland, S., ... & Takle, H. (2016). Impact of fish density and specific water flow on skin properties in Atlantic salmon (Salmo salar L.) post-smolts. Aquaculture, 464, 629-637.

Sveen, L. R., Timmerhaus, G., Torgersen, J. S., Ytteborg, E., Jørgensen, S. M., Handeland, S., ... & Takle, H. (2016). Impact of fish density and specific water flow on skin properties in Atlantic salmon (Salmo salar L.) post-smolts. Aquaculture, 464, 629-637.

Sveen, L., Karlsen, C., & Ytteborg, E. (2020). Mechanical induced wounds in fish–a review on models and healing mechanisms. Reviews in Aquaculture, 12(4), 2446-2465. Sveen, L., Karlsen, C., & Ytteborg, E. (2020). Mechanical induced wounds in fish–a review on models and healing mechanisms. Reviews in Aquaculture, 12(4), 2446-2465.

Sveen, L., Timmerhaus, G., Johansen, L. H., & Ytteborg, E. (2021). Deep neural network analysis-a paradigm shift for histological examination of health and welfare of farmed fish. Aquaculture, 532, 736024.

Terms used in aquaculture. (29 April 2021).Fish Farm Feeder https://www.fishfarmfeeder.com/en/blog/common-terms-in-aquaculture/

Timmerhaus, G., Lazado, C. C., Cabillon, N. A. R., Reiten, B. K. M., & Johansen, L. H. (2021). The optimum velocity for Atlantic salmon post-smolts in RAS is a compromise between muscle growth and fish welfare. Aquaculture, 532, 736076.

Turnbull, J. Assessing and Safeguarding the Welfare of Farmed Fish. In American Fisheries Society 150th Annual Meeting. AFS.

Turnbull, J., Bell, A., Adams, C., Bron, J., & Huntingford, F. (2005). Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. Aquaculture, 243(1-4), 121-132

Villarreal, C. A., Thorp, J. E., & Miles, M. S. (1988). Influence of photoperiod on growth changes in

Wickens, S. M. (2021). Welfare indicators for farmed rainbow trout: Tools for assessing fish welfare—FISHWELL handbook. *Animal Welfare*, *30*(2), 225-225.

Wolf, J.C., Maack, G., 2017. Evaluating the credibility of histopathology data in environmental 657 endocrine toxicity studies. Environmental Toxicology and Chemistry 36, 601-611.

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