Identification of Potential Welfare Indicators for Commercially Farmed King Salmon (Hāmana, *Oncorhynchus tshawytscha*): A Scoping Review to Inform the Development of a National Code of Welfare

A thesis presented in the partial fulfilment of the
requirements for the degree of

Master of Science
in
Physiology

At Massey University, Manawatu,
New Zealand

Izabella Maree Norris
2022

Abstract

Aquaculture – the cultivation of marine plants and animals – is one of New Zealand's fastest growing primary industries. A major contributor to the growth of New Zealand's aquaculture industry is the farming of King salmon (*Oncorhynchus tshawytscha*). Parallel to the growth of the aquaculture industry is an increasing interest in the welfare of farmed fish from people in the industry, government, and public. Under the Animal Welfare Act 1999, fish are considered sentient beings capable of experiencing positive and negative emotions. However, unlike other animals under the Act, fish do not have a Code of Welfare to formally guide farmers to meet their obligations as animal carers under the Act. In order to develop a Code of Welfare for farmed fish, there needs to be an understanding of current and future state of the New Zealand salmon farming industry along with an evaluation of potential areas within production systems that may influence fish welfare, and identification of potential indicators of farmed salmon welfare.

The New Zealand salmon farming industry use freshwater and marine-based operations to farm King salmon. Juvenile salmon are reared in freshwater hatcheries until they have reached an acceptable weight and/or stage of smoltification before being transferred into grow-out cages. Smolt are grown out in net cages, situated within either freshwater hydro-canals or coastal bays of the South Island, until they reach a harvest weight of ~4kg. At harvest, farmed salmon are stunned and slaughtered using methods such as Aqui-S and carbon dioxide immersion, manual and automatic percussion, and electrical stunning. Brain spiking is also used to euthanise broodstock prior to stripping.

The Five Domains Model was used as a guide to evaluate areas of potential welfare impacts within New Zealand salmon farming systems. In terms of nutritional impacts (Domain 1), management of feed withdrawal regimes and factors associated

with underfeeding may negatively impacts salmon welfare. Appropriate water quality parameters are crucial for the maintenance of adequate environmental living conditions (Domain 2). Water quality parameters that may influence salmon welfare include water flow rate, temperature, oxygen saturation, carbon dioxide concentration, ammonia concentration, and salinity. Impacts on salmon health (Domain 3) may arise from the contraction of infectious bacterial diseases, physical injuries, and the development of spinal deformities. Lastly, in Domain 4, salmon welfare may be impacted through interactions with humans (e.g., handling stress during handling events), other non-human animals (e.g., aggressive interaction with conspecifics or presence of predators), and the environment (e.g., limited ability to exercise agency within barren and confined environments).

A scoping review of globally published literature relevant to measures of salmon welfare was conducted to identify potential indicators of farmed salmon welfare. The scoping review identified a total of 112 potential animal- and resource-based indicators of farmed salmon welfare from 60 articles. There was a clear focus on the use of survival-critical indicators reflecting welfare impacts in Domains 1-3 (nutrition, health, and physical environment). A limited number of situation-related indicators (Domain 4) were identified. Of the identified indicators, a large proportion were classified as animal-based indicators sampled post-mortem. These indicators can only provide evidence of a previous experience in the animal assessed. To create a reliable and holistic assessment of farmed salmon welfare, contextual information is required for appropriate application and interpretation of welfare indicators with regards to affective experiences and a range of indicators across Domains 1-4 should be used in combination to capture all possible welfare impacts. The 112 unique welfare indicators identified and the understanding of their collective value to the welfare of commercially farmed salmon in New Zealand presented in this review provides industry, government, and policy makers the necessary information to develop regulations and on-farm welfare assessments.

Acknowledgements

Firstly, I would like to express my utmost gratitude to my amazing supervisors: Dr Nikki Kells and Associate Professor Ngaio Beausoleil. With your guidance and dedication, this thesis, who I am as an academic, and who I am as a person have all developed in ways that I could not have ever imagined. It has been an absolute privilege to have learnt from you both and I look forward to continuing to learn more.

To Dr Nikki Kells – Your dedication to your students is not something to be taken for granted. That level of support you provided me is something I feel very privileged to have experienced. Thank you for always having your door open for me. The countless times that I have interrupted your focus for a doorway conversation or to join me sitting at your table to figure something out on my laptop, along with the thought, effort, and time you have put into this thesis along with me, has not only helped create depth in my thesis but also my thought process. I cannot thank you enough.

To Associate Professor Ngaio Beausoleil – Throughout this journey you have pushed me to do my absolute best which is far beyond what I previously thought I was capable of. Your belief in me and enthusiastic, unwavering support has taught me to own my worth – which also something that you model very well. Working with you has provided endless amounts of inspiration and I cannot thank you enough for providing me with more opportunities to grow.

To Marie McAninch – Your support as a mentor was invaluable. We have spent many hours on Zoom. Thank you for taking time out of what I know to be a very very busy schedule! Your support as a mentor helped me not only form important connections, but also allowed me to see different perspectives within industry.

To Professor Naomi Cogger – Thank you for your invaluable time and feedback towards the scoping review of this thesis. Your honesty and knowledge helped to keep my review on-track and taught me skills that I will continue to use as an academic.

To Mark Preece and Brian Blanchard – Thank you for welcoming me onto your farms and sharing your invaluable knowledge and experience as key members in the salmon farming industry.

To my mum and dad, family – I would not be where I am today without the love and support that I continuously receive from you all. It is not possible to pay you back for all you have done for me, but I will continue to make the most of the opportunities that you have gifted me.

To Maddy – Thank you for always being there for me through my really high, highs and my really low, lows. I cannot thank you enough for your support, your wise words and for providing me with a scenery change and escape from reality when I needed it the most. Mr Declan Power you also get an honourable mention here. Thank you for all the hugs and memes to keep me in good spirits.

To the Science of Sentence Structure – Morgan and Sophia, you both welcomed me with open arms and made me feel like I instantly belonged. I cannot thank you both enough for the endless space to rant and bounce ideas. I am very incredibly grateful for your support through all of my highs and lows. I cannot wait to continue the post grad journey with you lot.

To Dr Kat Littlewood – You have provided me with endless support, honesty, opportunities, excitement, and invaluable advice. I am very excited to see what future brings!

To the flat of 2021 – Katya, Tom, Jacob and Cjay, what a time we had. I may have just lost my sanity without you lot providing me a space to relax, laugh and vent. Thank you for the unforgettable memories, hugs, and endless good times.

To the Ministry for Primary Industries – I would like to thank you for the support your scholarship programme not only provided for my project but also myself as an academic.

To the Universities Federation for Animal Welfare – I would like to thank you for not only your support in funding this research, but also for your continuous kind words and patience throughout this journey.

This research was also funded by the Massey University Foundation and Massey University School of Veterinary Science. Thank you for your support.

Table of Contents

List of Figures

List of Tables

Thesis Objectives and Outline

The overall objective of this research was to identify measures that may be used as welfare indicators to evaluate the welfare of commercially farmed salmon in New Zealand. Identification of salmon welfare indicators and understanding of their relevance to the welfare of commercially farmed salmon in New Zealand will aid industry representatives and policy makers in making informed decisions during the development of welfare assessments and regulations.

This thesis contains three chapters. The first chapter outlines the evolution of the salmon industry in New Zealand, features of the production systems, the current regulatory environment, what animal welfare is and how it can be assessed, why the consideration of fish welfare is important, and the potential welfare issues in New Zealand salmon production systems. The second chapter presents the first step in developing a systematic approach to assessing fish welfare which takes the form of a scoping review. This chapter identifies specific measures relevant to the welfare assessment of farmed salmon and is written in the format of a manuscript for publication. Because of this, some information presented in Chapter one is duplicated in Chapter two. Chapter three presents a general discussion of the implications of the welfare indicators identified in the scoping review. Appendices can be found at the end of the thesis outlining information relevant to the scoping review and the accomplishments that I have achieved throughout the course of my Masters degree.

Chapter 1 – A focused review on King salmon (Hāmana, *Oncorhynchus tshawytscha*) welfare in Aotearoa New Zealand aquaculture systems

1. Salmon farming industry in Aotearoa New Zealand

King salmon (Hāmana, *Oncorhynchus tshawytscha*) farming is a significant contributor to the Aotearoa New Zealand aquaculture industry's economic success. King salmon were first successfully introduced into New Zealand rivers in the early 1900s and are currently the only salmon species farmed on a commercial scale in the country (Symonds et al., 2019). The commercial production of King salmon in New Zealand has grown rapidly in the past 50 years, with six major farming operations across the country's South Island producing 75% of the world's King salmon product (Aquaculture New Zealand, 2020). Of the three main species farmed in New Zealand aquaculture systems – King salmon, Greenshell™ mussels (Kūtai, *Perna canaliculus*), and Pacific oysters (Tio, *Crassostrea gigas*) – King salmon is the most valuable per farmed hectare (New Zealand Government, 2018) and contributes approximately 40% ($254 million) to the aquaculture industry's annual revenue (Aquaculture New Zealand, 2020).

In response to an increase in global demand for marine-based protein, aquaculture industries are expanding both nationally and internationally to meet supply demands. In New Zealand, the aquaculture industry as a whole is projected to increase from a $600 million industry to a $3 billion industry by 2035 (New Zealand Government, 2018). As an economically important species, the production of King salmon is expected to increase in both product quantity and value to help satisfy this goal (Casanovas et al., 2021). However, in order to increase the production of King salmon, careful and effective management of fish and their environment is required to ensure the protection and enhancement of fish welfare.

All animal-based industries have the potential to influence the welfare of animals, both positively and negatively. Thus, there is an expectation that industries scrutinise their practices to maintain and improve the welfare of the animals under their care (Barreto et al., 2022). The salmon farming industry in New Zealand is at the start of this process. The first step is to understand fish management systems and consider features of, and points within, the production cycle at which animals may be particularly vulnerable to welfare impacts.

1. 1. Features of salmon farming systems in Aotearoa New Zealand

Hatchery phase: Egg to smolt

The natural life history of King salmon in New Zealand follows a similar pattern of morphologically distinct life stages displayed by other salmonid species seen in Figure 1. In attempts to reflect the natural life history of salmon, farming operations in New Zealand utilise a production cycle of a similar natural pattern (Figure 2). As such, the lifecycle of all farmed salmon in New Zealand begins within land-based, freshwater hatcheries. Fertilised eggs, collected from the previous generation of salmon, are incubated in hatching trays at various temperatures to facilitate control over hatching time. Incubation temperature, as well as other water quality parameters have the ability to influence the viability of eggs and the survival and health of hatched young.

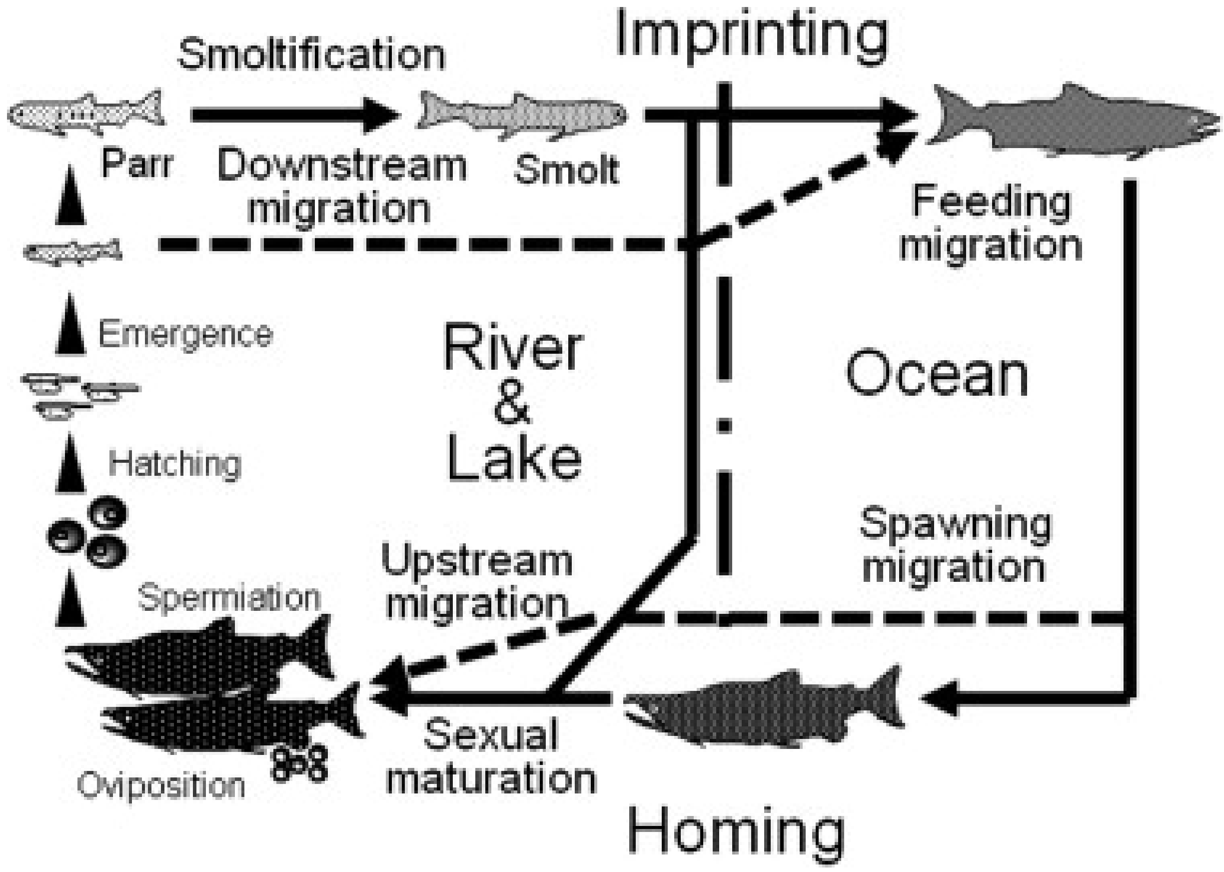

Figure 1. Natural lifecycle of Pacific salmon. Reprinted from General and Comparative Endocrinology, 170, Ueda, Hiroshi., Physiological mechanism of homing migration in Pacific salmon from behavioral to molecular biological approaches, 222-232, Copyright (2011), with permission from Elsevier.

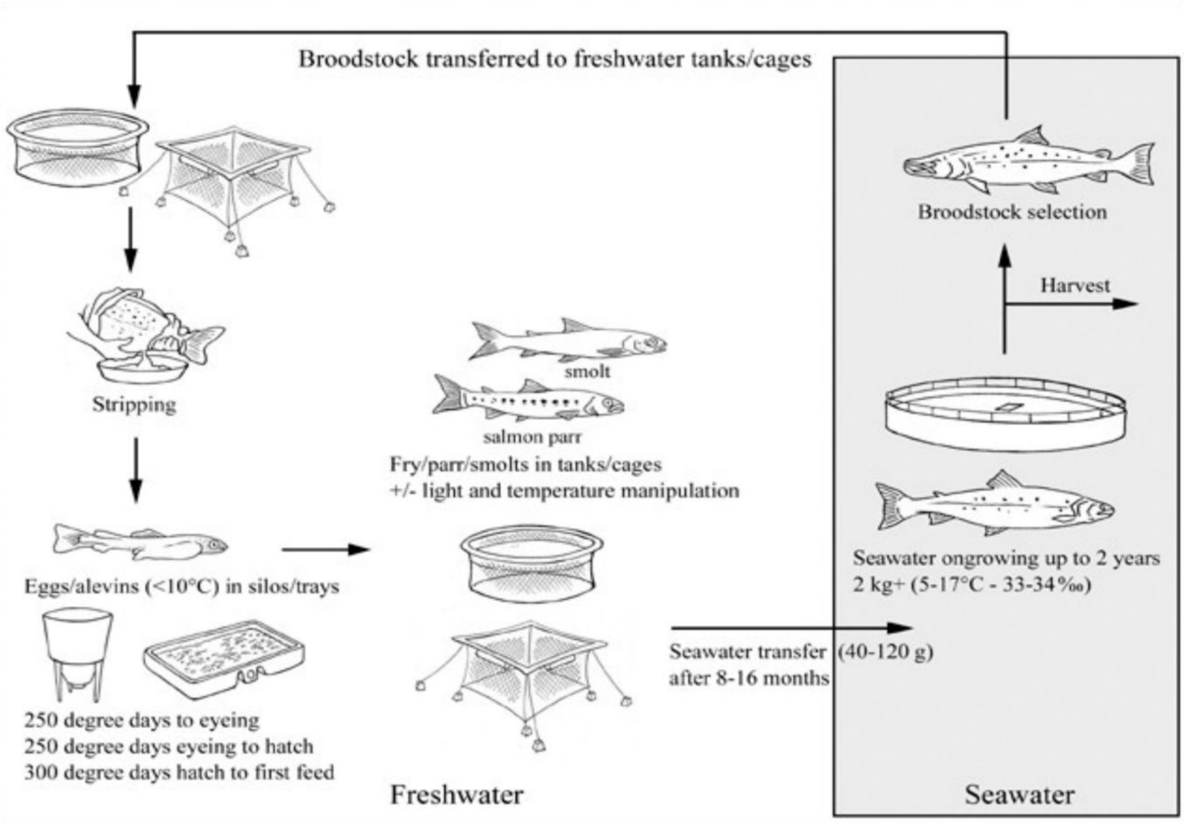

Figure 2. Production cycle of Atlantic salmon. This production cycle is similar to that of King salmon in New Zealand. Reprinted from Food and Agriculture Organisation of the United Nations (FAO) (2022).

Newly hatched young (termed 'alevins') descend through perforated hatching trays onto holding trays. Here, alevins metabolise their attached yolk sacs before transfer into first feed tanks as 'fry/parr'. Post-first feeding, fry/parr are size-graded manually by stockmen and transferred into freshwater circular tanks or flow-through raceways (Figure 3) to mature for 8-13 months (M. Preece, personal communication, May 18, 2021; B. Blanchard, personal communication, May 27, 2021). Size-grading is performed to prevent aggressive interactions between large- and small-sized fish as well as to maintain uniform growth rates between different schools (groups) of salmon (M. Preece, personal communication, May 18. 2021). Stocking density, territory maintenance and distribution of feed can influence aggressive interaction between fry/parr. At this life-stage, fry/parr exhibit territorial behaviour (Haworth, 2010).

Figure 3. Concrete flow-through raceway in Norway. Similar style of flow-through raceways are used in New Zealand hatcheries. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature. Welfare of Farmed Fish in Different Production Systems and Operations by Hans van de Vis, Jelena Kolarevic, Lars H. Stein et al. Copyright (2020).

In freshwater rearing tanks and flow-through raceways, juvenile salmon welfare can be influenced by water quality, stocking density, and the physical features of their holding spaces. In New Zealand, flow-through raceways are typically rectangular canals, or circular tanks, constructed out of concrete (Haworth, 2010). Each canal, or tank, has a single inlet and outlet, allowing water to only pass through the system once. Inlet water can be either passed through by gravity or mechanically pumped straight from a natural source of water (e.g., river), or may be filtered through a pump. The source of inlet water and the rate at which water flows through the canals can influence water temperature, oxygen saturation levels and the rate of elimination of wastes such as carbon dioxide and ammonia (van de Vis et al., 2020). This open system design (i.e., continuous entry and exit of new water) may expose salmon to environmental pathogens and, without careful regulation, subpar water quality (van de Vis et al., 2020).

Smoltification

Within the 8-13 month maturation phase, photoperiod, temperature and feeding rate can influence the onset and rate of smoltification, i.e., the maturation of freshwater parr to saltwater-adapted smolt. The process of smoltification is regulated by the thyroid gland which facilitates morphological, physiological, and behavioural changes associated with adaptations for life at sea. Secretion of triiodothyronine from the thyroid gland regulates the secretion of prolactin, adrenocorticotropin and growth hormone from the pituitary gland, as well as the secretion of corticosteroids from the interrenal gland (McCormick et al., 2000). This prepares juveniles to move towards saltier, hyperosmotic water. Fluctuations in the above mentioned hormones regulate increases in salinity tolerance. To maintain electrolyte balance in body fluids, growth hormone and cortisol act together to increase the density and ion secretion activity of chloride cells found in the gills (Richman III et al., 1987). This change in chloride cell activity is important for osmoregulation – the maintenance of cellular fluid composition and volume (Evans, 2011). Behavioural changes associated with smoltification include increased preference for hyperosmotic water, decreased territorial behaviour, and increased schooling behaviour (Yamauchi et al., 1985).

Readiness for transfer of smolt to grow-out cages is determined by scale colouring, bodyweight, and time spent in the rearing facility. Changes in scale colouring, from a brown body to predominantly silver with black spots, is associated with readiness for grow-out as a morphological signal of smoltification. Links have also been made by stockman in the local industry between readiness for grow-out and the weight of smolt (M. Preece, personal communication, May 18, 2021). At a particular weight (100-120 grams), it is assumed that smolt are physiologically adapted and ready for saltwater transfer. In wholly freshwater operations, smolt can be transferred, on the basis of time spent in rearing facility as there is no requirement for them to be adapted to saltwater. However, in marine-based operations, juveniles that have not properly undergone smoltification will be ill-adapted to a hyperosmotic environment, compromising their growth and survival rates. Early transfer into saltwater has been associated with growth retardation and mortality in King salmon (Iremonger, 2008).

Transport

Transfer of smolt to grow-out cages involves the use of specialised trucks and, in marine-based operations only, barges. Prior to transport, juvenile salmon are fasted for three days to two weeks. Fasting limits ammonia secretion during their confined transport at high stocking densities (Hvas et al., 2020). At high levels (0.1 mg L^{-1}; Noble et al., 2018), ammonia can negatively impact fish growth, health, and welfare. To further prevent reductions in water quality, transport trucks are equipped with automated aeration systems responsible for regulated distribution of gases during transportation (van de Vis et al., 2020). Oxygen is released from the bottom of the tank and an agitator is used to eliminate carbon dioxide from the system. Such aeration systems are required to deliver oxygen to the salmon to prevent hypoxia and the build-up of carbon dioxide.

To reach grow-out cages in marine-based operations, trucks are loaded and transported on barges. Here, trucks are connected to a deck hose which flushes seawater through the transport tanks, facilitating the acclimation of salmon to hyperosmotic water. While helpful for acclimatisation, flushing seawater through the tanks poses a risk of pathogen contamination throughout the journey. It can be assumed that during transportation, smolt may be in states of distress, which is correlated with reduced resilience (Hvas et al., 2020). On land-transportation can last anywhere from thirty minutes to four hours in both freshwater and marine-based operations, plus an extra four hours of barge transportation for marine-based operations, at the end of which salmon are released straight into their grow-out cages. Direct emersion into a novel environment may potentially be stressful for the juvenile fish.

Grow-out phase: Smolt to post-smolt

King salmon in New Zealand are either grown-out (i.e., farmed until harvest weight) in freshwater or marine net cages for approximately 16-20 months (NZKS, 2017; B. Blanchard, personal communication, May 27, 2021). Nets are constructed out of nylon mesh, allowing for water to enter and exit net cages following the environmental water current rate and direction (NZKS, 2017). Mesh size (i.e., number of openings per unit measurement of material or diameter of mesh openings) can influence water flow rate within net cages, subsequently affecting oxygen saturation levels and the rate of waste removal. Smaller mesh sizes (12.5 mm bar length) are associated with the accumulation of more waste in comparison to larger mesh sizes (35 mm bar length) (NZKS, 2017). Mesh sizing varies according to the growth stage of salmon schools. Younger salmon schools are held in net cages with a small mesh size to prevent their escape, while larger, older salmon are transferred to net cages of a larger mesh size that do not facilitate escape, but instead prevent potential water quality deterioration (NZKS, 2017).

New Zealand's largest salmon producers operate marine-based systems in the Marlborough Sounds and Stewart Island. A smaller marine-based system is operated in Akaroa Harbour (Symonds et al., 2018). Sea cages are positioned within coastal bays with varying environmental water flow rates and temperatures. Similar to cages with small mesh sizes, sites with low water flow rates may impair dissolved oxygen distribution and waste removal from net cages. King salmon show preferences for water temperatures between 12-18°C (Haworth, 2010). Sites with temperatures above 18°C can enhance salmon susceptibility to disease and likelihood of mortality (Noble et al., 2018). For this reason, sites classified as low-flow, high temperature are only suitable, and used, for salmon farming in colder months of the year.

Freshwater net cages are located in man-made hydro-canals found in Te Manahuna (Mackenzie Basin) of the central South Island (Symonds et al., 2018). Within the hydro-canals, net cages are anchored to the canal sides and receive water from Lakes Tekapo, Pukaki, and Benmore (B. Blanchard, personal communication, May 27, 2021). The purpose of the hydro-canals is to produce water currents strong enough to facilitate the production of hydroelectricity, subsequently subjecting salmon to relatively high water flow rates. As such, it is likely that waste removal, relative to low water flow sites in marine-based systems, is not a large issue. However, the canals do receive water run-off from the glaciers of Aoraki (Mount Cook) (B. Blanchard, personal communication, May 27, 2021). There is potential for water temperatures to fall to 7°C, which is outside the King salmon's optimal range of 12-18°C (Harworth, 2010).

In New Zealand, farmed salmon are at risk of predation by seals, dolphins, sharks, and birds. Both freshwater and marine-based operations employ 'bird nets' to reduce predation of stock by birds. Similarly, marine-based operations also employ nets around the perimeter of net cages to reduce the risk of marine predators. However, there are instances where predators are able to penetrate such protections to access stock.

Both freshwater and marine net cages are open systems which are susceptible to contamination by environmental pollutants. As climate-change is causing global increases in water temperatures, the risk of a disease outbreak is increasing (Brosnahan et al., 2019). A solution to climate change related issues that is currently being explored in New Zealand is open ocean farming. Open ocean farming moves grow-out operations away from warmer coastal areas into the open sea. Here, there is greater exposure to strong wind and waves which may impact salmon welfare and the structural integrity of current nylon net cages (Hvas et al., 2021). To combat weather extremes, submersible cages with inflexible net walls are currently being developed overseas. Submersible cages are designed with copper netting, which allow for enhanced water flow (in comparison to nylon netting) and structural rigidity to withstand strong waves (Chu et al., 2020; Drach et al., 2013). Positioning salmon farms off the coast presents an issue of reduced ability to perform manual monitoring of fish by stockmen. Monitoring of fish welfare at sea would require partial reliance on video systems and electronic sensors. Electrical feeding systems may also have to be operated off-site by stockmen.

Another farming system currently being considered for commercial operation in New Zealand is the recirculating aquaculture system (RAS). RAS are considered closed systems in which water flows through the system in a loop-like fashion (van de Vis et al., 2020). Water from housing tanks is drained into the pipes of a series of water treatment compartments (Mota et al., 2019; Figure 4). The compartments mechanically filter out solids, remove ammonia through a biofilter, remove carbon dioxide and oxygenate water, as well as disinfect water via ultra-violet and ozone treatment (Mota et al., 2019). These systems allow farmers to control and manipulate the environmental conditions used to produce salmon. RAS operations are currently being developed in New Zealand for their benefits related to improved production and environmental sustainability. There is potential for RAS systems in New Zealand to reduce inlet water requirements and effluent loads. These systems also allow for greater control over risk factors known to affect open, outdoor systems such as exposure to pathogens, predators, and pollution.

However, for RAS to be successful and maintain adequate fish welfare, RAS operations designs need to be tailored to species-specific welfare needs and risk management plans must be put in place to mitigate issues arising from technological malfunction. Inadequate system design and technological breakdown can lead to reductions in water quality. There are also potential risks to biosecurity associated with the addition of new fish into a farming unit and use of contaminated feed and top-up water (Noble et al., 2018; van de Vis et al., 2020). If a pathogen were to enter the closed system, the pathogen itself and attempts of eradicating the pathogen may reduce water quality. This is because chemical treatments and pathogens themselves can impair biofilter functionality and promote pathogen circulation within the system (Noble et al., 2018).

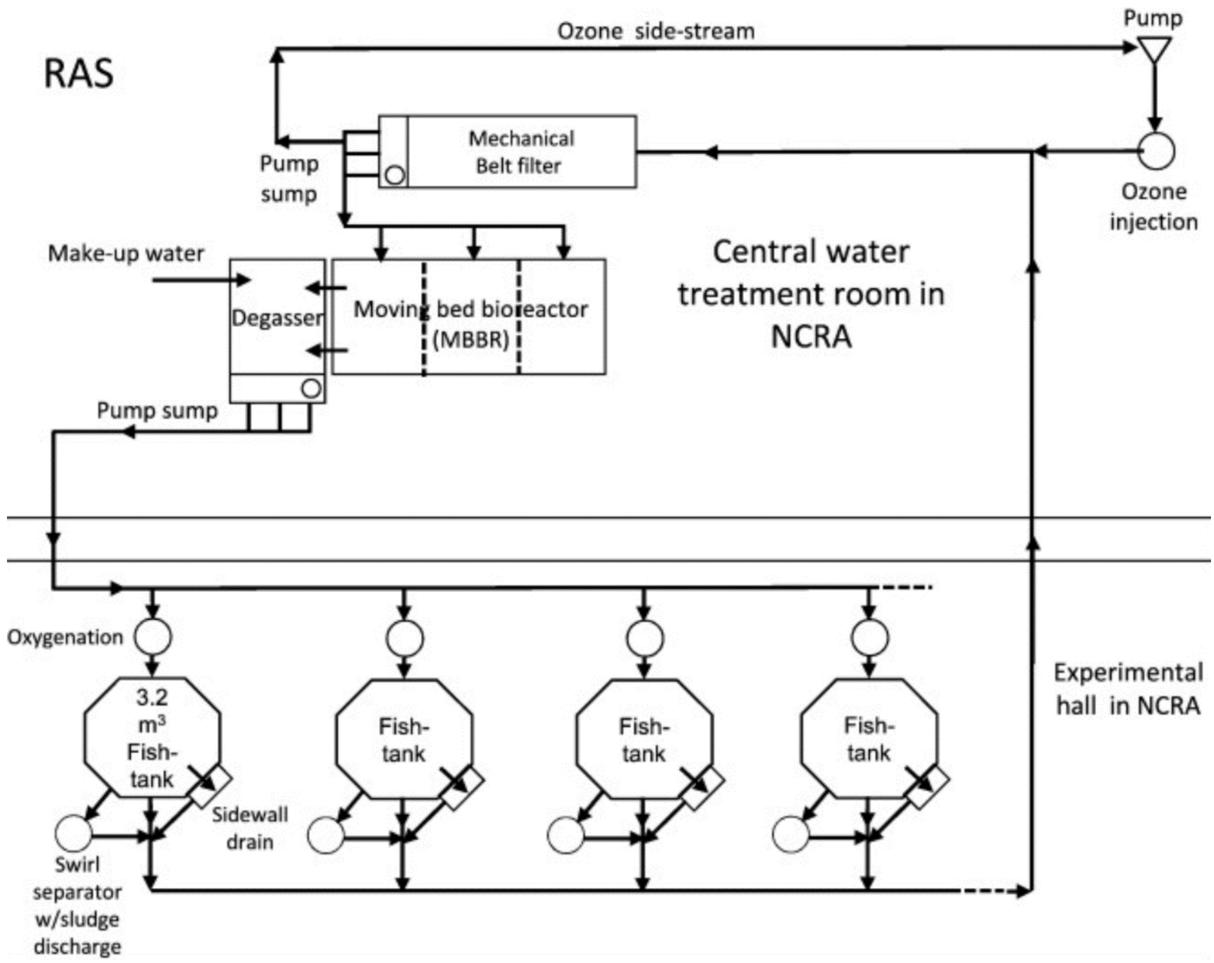

Figure 4. Schematic view of a recirculating aquaculture system. Biosolids from water exiting the fish tanks are separated by a swirl separator. Water is then passed through a biofilter to remove ammonia. Ozone treatment is used to disinfect water before entering the biofilter again. Water from the biofilter enters a degasser to remove carbon dioxide, and then oxygenated before re-entering the fish tanks. Reprinted from Aquaculture, 498, Mota, V. C., Nilsen, T. O., Gerwins, J., Gallo, M., Ytteborg, E., Baeverfjord, G., Kolarevic, J., Summerfelt, S. T., & Terjesen, B. F., The effects of carbon dioxide on growth

performance, welfare, and health of Atlantic salmon post-smolt (Salmo salar) in recirculating aquaculture systems, 578-586, Copyright (2019). Elsevier requires no direct permission for open access article content for thesis reuse.

Feeding Regimes

Fish feeding regimes vary depending on growth rate protocol (i.e., fast or slow growth) and lifecycle stage. Feeding regime factors include feeding intervals, pellet sizes, diet composition, and distribution of feed. In New Zealand, King salmon are fed specialised pellet diets developed by Skretting (NZKS, 2017). Juvenile salmon are fed pellets of a small size that are high in protein to serve their relative high growth rates. Juvenile salmon are typically hand fed (i.e., stockmen throw handfuls of feed into rearing tanks) up to six times a day. In contrast, adult salmon are fed a diet relatively higher in fat for energy maintenance (M. Preece, personal communication, May 18, 2021). Adult salmon are typically fed twice a day by either a mechanical feeder (in marine-based operations) or hand-fed by stockmen (in freshwater operations). In freshwater operations, the cessation of feeding is determined by a reduction in head turning movements (i.e., feeding behaviour). In marine-based operations, feeding stops when feed pellets fall below 5 metres in the water column. The diet composition and feeding rates employed by farming operations can influence aggressive interactions between fish as well as their growth and health status (Noble et al., 2018).

Spawning phase: Broodstock

Broodstock (individuals selected for breeding purposes) are grown-out in a similar fashion to fish grown-out for harvest. Following the grow-out phase in net cages, broodstock populations are transported back to land-based, freshwater hatcheries at 2-3 years of age (M. Preece, personal communication, May 18, 2021; B. Blanchard, personal communication, May 27, 2021). Broodstock are housed in freshwater raceways (or tanks) under controlled light and temperature protocols. Stockmen control light and temperature conditions to manipulate the timing of spawning (i.e., the release of sperm and eggs by salmon). Broodstock are hand sorted to evaluate readiness for egg/sperm removal. Prior to egg and sperm removal, salmon are humanely killed by the application of an Iki gun (i.e., method of brain spiking) or manual percussion to the head (B. Blanchard, personal communication, May 27, 2021). Upon egg and sperm removal, identification tags (microchips inserted into body cavity when juveniles reach 10g liveweight) are recovered from broodstock and used for traceability of offspring.

Stunning and slaughter

The lifecycle of farmed salmon (with the exception of broodstock) ends at harvest. Prior to presentation for stunning and slaughter, salmon are crowded within their cages and drawn onto a barge with an in-built stunning and slaughter system using an automated vacuum pump. This process of crowding and pumping can last up to two hours.

Stunning and slaughter methods should, ideally, result in rapid loss of consciousness and/or death. This prevents fish from being sensible to the experiences of pain and distress associated with the slaughter process (van de Vis et al., 2014). The stunning and slaughter methods known to be used commercially in New Zealand include Aqui-S and carbon dioxide (CO_2) immersion, percussion, electrical stunning, and brain spiking.

Aqui-S and carbon dioxide stunning

Aqui-S and carbon dioxide immersion may be applied as a stunning method after crowding within a specialised pontoon before salmon are vacuum pumped onto barges. The combination of Aqui-S and carbon dioxide is used to adequately stun fish before manual slitting of the throat arteries onboard the barge. In New Zealand, Aqui-S is an approved drug for use on commercial farms to anesthetise fish. According to the manufacturer, fish should be immersed in water containing Aqui-S at concentrations between 15-20 mg/L to achieve anaesthetisation (Aqui-S™, n.d.). Aqui-S contains 540 g/L of the active ingredient iso-eugenol, derived from clove oil (Zahl et al., 2012). Iso-eugenol acts in a similar way to clove oil (eugenol) as an anaesthetic by decreasing the generation of membrane action potentials in nerve axons, resulting in general depression of the central nervous system (Zahl et al., 2010). After presumed loss of consciousness (indicated by loss of movement), salmon are immersed in carbon dioxide saturated water.

Carbon dioxide stunning is a well-established method for stunning and/or killing farmed fish. The method is applied by first bubbling carbon dioxide into a tank of water until the desired water pH, or carbon dioxide concentration, is achieved – a pH of 5.5-6.0 or 200-450 mg CO_2/L (van de Vis et al., 2020). This is followed by immersing the fish in the CO_2 saturated water for up to 10 minutes or until the cessation of movement (Gräns et al., 2016). The elevated CO_2 levels elicit a strong physiological stress response – release of cortisol and catecholamines – and results in the acidification of blood plasma (Hayashi et al., 2004). Combined, these effects impair carbon dioxide excretion through the gills, which has negative downstream effects on neurological and physiological systems, eventually leading to narcosis and death (Hayashi et al., 2004).

The proposed benefits of a dual stunning method are two-fold. First, there is potential for the animal to regain consciousness after the use of an anaesthetic dose of Aqui-S (Zahl et al., 2012). To ensure stunning is irreversible, salmon are immersed in carbon dioxide saturated water to prolong stunning effect. Second, carbon dioxide stunning has been shown to be a very stressful method of stunning (Erikson, 2011). Immersion of conscious salmon into CO_2 saturated water elicits strong aversion behaviour before unconsciousness is achieved (Erikson, 2011). Thus, the use of an anaesthetic before CO_2 stunning aims to mitigate unnecessary stress before the animal becomes unconscious.

Percussion

Percussion, also referred to as ‘concussion’, requires fish to be removed from the water and a blow to be delivered to the head to produce rapid insensibility (van de Vis et al., 2020). The blow to the fish’s head can be applied manually using a wooden or polypropylene tool called a ‘priest’, or mechanically using a pressurised bolt fired by a pneumatic gun or piston in an automated commercial system (Lambooij et al., 2010; Figure 5). The blow to the head results in a differential acceleration of the brain within the skull (Robb et al., 2000). The rapid change in pressure within the skull causes cerebral haemorrhaging, disrupting normal blood flow and brain function (Lambooij et al., 2010). If a sufficient force is applied, the gross trauma to the brain can result in rapid unconsciousness and death (Roth et al., 2007). Automatic and manual percussion methods are utilised in New Zealand. Automatic percussion is used as a primary stunning method. Where automatic stunning is unsuccessful in rendering a fish unconscious, and in instances of euthanasia, manual stunning is performed.

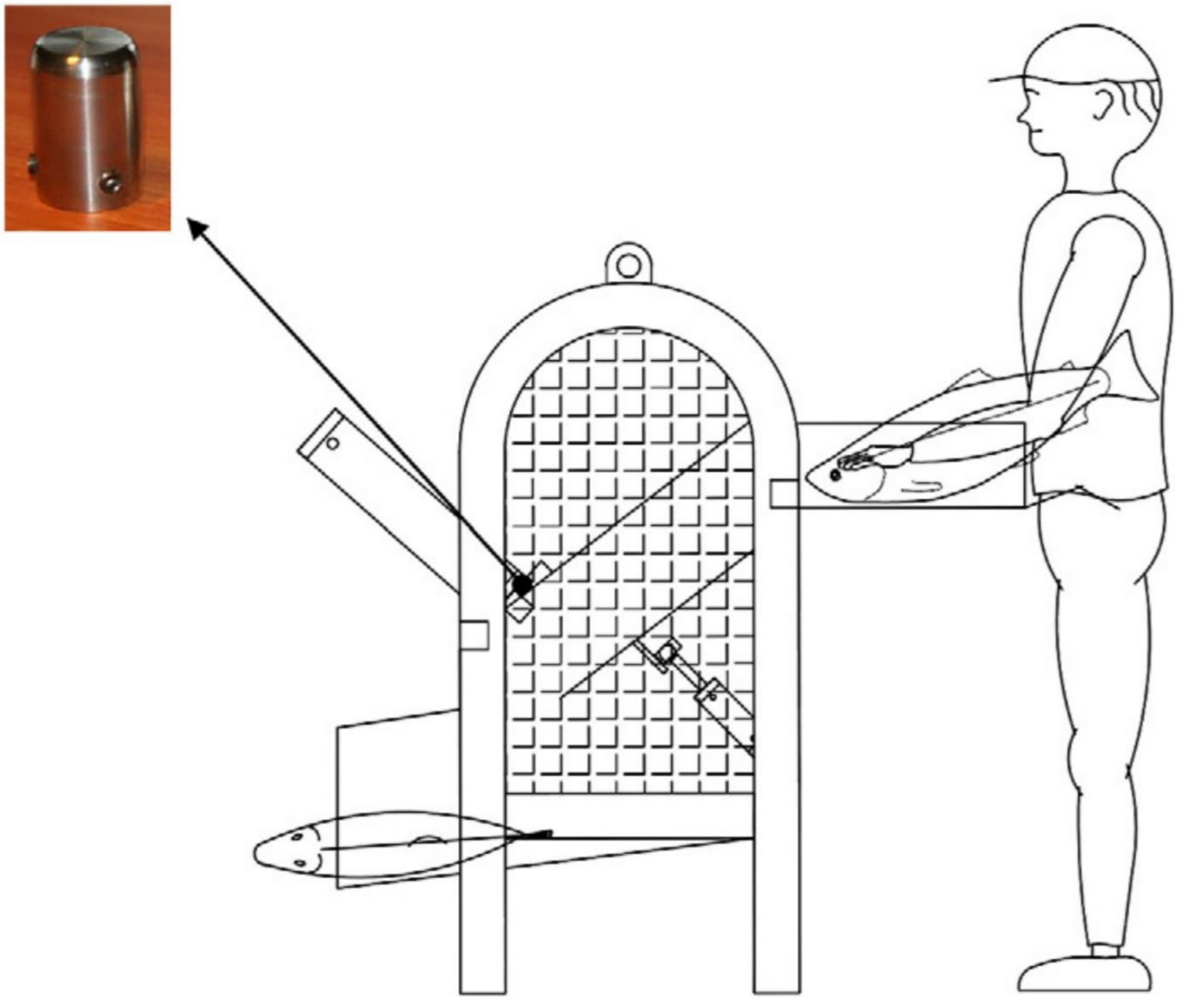

Figure 5. Schematic diagram of a manually fed automated commercial stunner. Fish are manually fed into the stunning machine to ensure an upright position is maintained. The position of fish within the stunner activates a trigger system, firing a piston connected to a bolt (top left picture). Reprinted from Aquaculture, 300, E. Lambooij, E. Grimsbø, J.W. van de Vis, H.G.M. Reimert, R. Nortvedt, B. Roth, Percussion and electrical stunning of Atlantic salmon (Salmo salar) after dewatering and subsequent effect on brain and heart activities, 107-112, Copyright (2010), with permission from Elsevier.

Electrical stunning

There are two electrical stunning methods which can be used to produce unconscious fish: (1) wet stunning or (2) dry stunning. Wet stunning occurs by passing an electrical current through submerged electrode plates, creating an electrical field in the water which stuns fish (Robb et al., 2002). While dry stunning occurs after de-watering and requires the electrodes to make direct contact with the fish (Lambooij et al., 2010; Figure 6). To stun fish, both methods require a sufficient current to pass through the brain (van de Vis et al., 2020). The electrical current depolarises neurons and triggers action potentials which disrupt the normal neural functioning of the brain, inducing a seizure-like state (Grimsbø et al., 2016). In a seizure-like state, fish are considered to be unconscious and therefore insensible to noxious stimuli (Grimsbø et al., 2016).

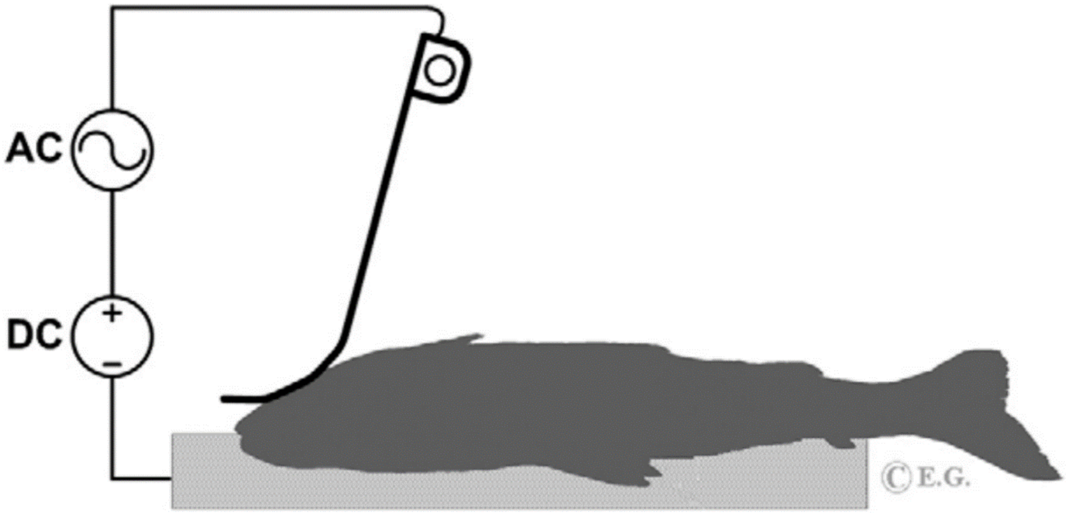

Figure 6. Schematic diagram of a head-to-body dry electrical stunner. Fish are fed into the stunner, ensuring one electrode is contacting the head while the length of the body contacts the other electrode. Reprinted from Aquaculture, 300, E. Lambooij, E. Grimsbø, J.W. van de Vis, H.G.M. Reimert, R. Nortvedt, B. Roth, Percussion and electrical stunning of Atlantic salmon (Salmo salar) after dewatering and subsequent effect on brain and heart activities, 107-112, Copyright (2010), with permission from Elsevier.

Brain spiking

Brain spiking, also known as iki-jime, is used to humanely kill broodstock in New Zealand. This method requires a spike to be inserted, either by hand or pneumatic gun, into the brain and rotated (Poli et al., 2005; Robb & Kestin, 2002; Robb et al., 2000). The rotation of the spike aims to physically destroy the cerebellum and/or medulla oblongata to result in immediate brain death. Death using the spiking method is dependent on the positioning and trajectory the spike (Robb et al., 2000). This method of humane killing is likely reserved for broodstock due to the relative smaller numbers of fish to process in comparison to stock destined for food consumption.

1. 2. Regulatory environment of farmed fish

Fish welfare is legally protected in Aotearoa New Zealand. The Animal Welfare Act (1999) sets out a legal framework which regulates human-animal interactions to manage the potential welfare compromise of animals legally recognised as sentient (i.e., animals which have the capacity to suffer). Under the long title of the Act there are no qualifiers regarding the degree of sentience that an animal must demonstrate to be offered legal welfare protection. Sentience under the Act, at present, may be considered as an 'all-or-nothing' quality, i.e., an animal is either sentient (legally protected) or not sentient (not legally protected). Fish, past the larval stage, are legally recognised as sentient beings and, thus, afforded legal welfare protection ("Animal Welfare Act," 1999). However, unlike other farm animals (e.g., dairy cattle, beef cattle, and broiler chickens) that are protected by the Act, farmed fish do not have a dedicated Code of Welfare.

Codes of Welfare are formal guidance documents relevant to the enforcement of the Act. The broad purpose of the Codes is to provide animal carers detailed information, in the form of minimum standards and best practice recommendations, regarding their conduct towards their animals. In other words, Codes provide animal carers detailed guidance with regards to meeting their necessary legal obligations under the Animal Welfare Act. Of the current 19 Codes of Welfare in New Zealand (for a full list of Codes see Wells & Rodriguez-Ferrere, 2018), fish are only acknowledged in two Codes: Code of Welfare for Commercial Slaughter (NAWAC, 2018a) and the Code of Welfare for Transport (NAWAC, 2018b). Specific Codes for fish are yet to be developed. Information specific to fish in the two above Codes is substantially less detailed than the information provided for other animals such as large mammals, small mammals, and birds. For example, commercial slaughter minimum standards relevant to mammals and birds require these animals to be stunned prior to a killing method being applied (NAWAC, 2018a). This is done to ensure that animals are insensible to any unnecessary pain and distress caused by a method of killing. However, there is no such requirement when slaughtering fish. To uphold the purpose of the Act, formal guidance should also be provided to cover all aspects of fish farming relevant to fish welfare.

A systematic approach should be taken to develop fish welfare regulations in Aotearoa New Zealand. Fish welfare regulations established in other countries (e.g., Norway) cannot simply be directly implemented for use in Aotearoa New Zealand. International fish welfare regulations were developed for each country's specific species, production systems and environmental conditions. A large proportion of other jurisdictions farm Atlantic salmon (*Salmo salar*), whereas the New Zealand industry farms King salmon (Haworth, 2010). Also, some of the production systems utilised in New Zealand were adopted from international industries that are subject to different environmental conditions (B. Blanchard, personal communication, May 27, 2021). As such, the global body of literature regarding salmon welfare largely relates to Atlantic salmon subject to varying environmental conditions. It is possible that there may be more, or different, welfare impacts than what is currently reported in the literature. Therefore, there is a need to understand potential areas for welfare impacts to develop appropriate and practical systems for monitoring and improving where necessary, fish welfare in New Zealand.

2. Fish welfare should be protected

2. 1. What is animal welfare?

Should we consider the welfare of fish? To answer this question there needs to be an understanding of the concept of animal welfare and the approaches utilised to define 'good' welfare. Three main orientations towards defining good welfare exist: biological functioning, natural living, and affective state. The biological functioning orientation focuses on optimising the physical health and productivity of animals

(Fraser, 2009). In contrast, the natural living orientation favours animals residing in 'natural' environments which facilitate the performance of species-specific behaviours (Fraser, 2009). The approach taken towards animal welfare in this thesis is an affective state orientation. Under the affective state orientation animal welfare is characterised as a dynamic state within the animal that reflects the summation of all of an animal's mental experiences, both positive and negative, at a certain point in time (Fraser, 2009). The affective state orientation is favoured here for its holistic approach to animal welfare as the approach integrates elements from both the natural living and biological function approaches. This is because an animal's mental experiences result from the sensory processing of information regarding the animal's internal functional state (e.g., biological mechanisms) and external environment (e.g., ability to perform species-specific behaviour) (Mellor & Beausoleil, 2019). Sensory inputs are cognitively processed by the brain, generating mental experiences that may be valenced, i.e., experienced as positive or negative (Harvey et al., 2020). Good welfare is achieved under this approach when negative mental experiences are minimised, and positive mental experiences are maximised.

An animal's welfare state is considered to exist on a continuum from very good to very poor (Mellor et al., 2020). Negative experiences, such as thirst, hunger, pain, and fear, negatively impact an animal's welfare and pushes an animal's welfare state towards the poor side of the continuum. In contrast, the provision of positive experiences, such as playful interactions with conspecifics or the opportunity to explore, act to push an animal's welfare state in the opposite direction. However, rectifying or preventing negative experiences is not enough to achieve 'good' welfare. It can only ever, at best, achieve neutral welfare. Without first minimising an animal's negative experiences, the provision of opportunities for positive experiences does not always lead to a positive welfare state (Mellor et al., 2020). Opportunities for positive experiences may be provided to the animal, but without first ensuring that negative experiences are avoided, or alleviated, the animal may not be able, or want, to utilise the provided opportunities. For example, a social animal who is in pain may be provided with close proximity to conspecifics or given toys to play with, but the animal may not interact with these opportunities because the unpleasant experience of pain is limiting their ability and/or motivation to engage with them. Therefore, the sum of this example is that the social animal's welfare state is poor, despite the provision for positive experiences.

2. 2. Fish have a welfare to be considered

The affective state orientation requires an animal to have the capacity to have some positive and negative experiences. In other words, to have a welfare to be considered, an animal must have the basic capacity for sentience (Feinberg & Mallatt, 2016). Sentience in this context stipulates that an animal has the capacity to suffer (e.g., experience states of pain, fear, and anxiety) as well as experience pleasurable states (e.g., excitement and contentment) (DeGrazia, 2020). If animal welfare is considered a state within the animal, how can it be objectively determined that an animal possesses the capacity for sentience, i.e., have a welfare to be considered?

The concept of sentience is deemed 'The Hard Problem' (Feinberg & Mallatt, 2016). This is because it is impossible to objectively know and describe *what* animals, or even other human beings, experience. It is possible to explain mental experiences in terms of physical properties (e.g., chemical, and neural pathways), but there will always be an 'explanatory gap' where the personal, subjective experience itself remains unknown (Feinberg & Mallatt, 2016). In the search for sentience, anthropomorphism can be pervasive and limits the consideration of sentience to animals who possess a brain with a similar neuroanatomy to humans (Broom, 2014; Brown, 2015). On its own, this claim proposes that only animals with a brain neuroanatomically similar to humans possess the ability to experience the world in a meaningful, complex way that matters to them. It is an argument that prohibits the affordance of animal welfare to those which are perceived as lesser or simple in comparison to the human species. Considering Nagel's take on bats, an animal who experiences the world in a different light to us does not negate their capacity to experience feelings (Allen & Bekoff, 2007; Feinberg & Mallatt, 2016). Although the biological systems utilised by non-human species may appear to be different or simple, with neurological and behavioural evidence it can be observed that there is 'something it is like to be' an animal and the way it perceives the world has an influence on its welfare (Allen & Bekoff, 2007; Feinberg & Mallatt, 2016). The presence of any subjective experience, regardless of its level of complexity, is the minimal requirement for sentience (Feinberg & Mallatt, 2016). Therefore, neurological and behavioural evidence can be utilised to understand if fish possess the capacity to be sentient.

The recognition of sentience requires the coherence of various lines of evidence, or criteria, related to the experience of valenced mental states. Evidence of the unpleasant experience of pain is often used to support an animal's capacity for sentience. Pain is defined as "an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues…it changes the animal's physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery" (Molony & Kent, 1997). Pain has both sensory (e.g., detection of noxious stimuli by nervous system) and emotional (e.g., negatively valenced mental state) components. It is assumed that if an animal has the capacity to experience the emotional component of pain, then the animal is likely to have the basic capacity for sentience (suffer). Criteria by which pain, and therefore sentience, may be recognised include those utilised by Birch et al. (2021):

1. "Possession of nociceptors;

2. Possession of integrative brain regions;

3. Connections between nociceptors and integrative brain regions;

4. Responses affected by potential local anaesthetics or analgesics;

5. Motivational trade-offs that show a balancing of threat against opportunity for reward;

6. Flexible self-protective behaviours in response to injury and threat;

7. Associative learning that goes beyond habituation and sensitisation;

8. Behaviour that shows the animal values local anaesthetics or analgesics when injured."

The first four criteria present evidence of nociception, i.e., the sensory component of pain. Alone, these criteria do not provide enough evidence of the emotional component of pain but do provide baseline evidence for the capacity to sense noxious stimuli that may matter to the animal. Sneddon (2003b) early research on Rainbow trout (*Oncorhynchus mykiss*) provides evidence supporting criteria 1 (possession of nociceptors). Nociceptors are high-threshold receptors required for the detection of noxious stimuli. During the application of noxious stimuli to the head of Rainbow trout, Sneddon (2003b) used electrophysiological recordings to identify three types of nociceptors: polymodal, mechanothermal, and mechanochemical nociceptors. The recordings demonstrated nociceptor excitation in response to thermal, mechanical, and chemical noxious stimuli. The identification of nociceptors suggests that fish possess neural structures that are able to detect noxious stimuli that may be associated with pain.

The fish brain possesses neural structures which are capable of integrating multiple sources of sensory information (Criteria 2). The fish optic tectum has been suggested to play a role in the generation of mental experiences. The optic tectum is involved in sensorimotor integration of all sensory modalities, with the exception of olfaction (Feinberg & Mallatt, 2016; Kotrschal & Kotrschal, 2020; Woodruff, 2017). The optic tectum is laminated; comprised of six closely stacked layers that receive and integrate different sensory inputs. The importance of receiving input from more than one sensory modality is the ability to provide a complete representation of a fish's environment (Feinberg & Mallatt, 2016). The optic tectum has reciprocal neural connections with a neural substrate termed the 'pallium'. The fish pallium, similar to the bird pallium, is considered analogous to that of the neocortex in humans, such that the fish pallium is proposed to play a role in the generation of emotions in fish. Reciprocal communication between different brain areas and different sensory neurons allows for multiple types of sensory input to be integrated, interpreted, and experienced as mental experiences (Feinberg & Mallatt, 2016).

Neural connections also exist between nociceptors and integrative brain regions in fish (Criteria 3). Similar to Sneddon (2003b), Dunlop and Laming (2005) performed an electrophysiological study on Rainbow trout and Goldfish (*Carassius auratus*). Neuronal responses to a pin-prick stimulus were recorded. Analysis of the recordings showed that pain fibres (Aδ- and C-fibres) were activated in the cerebellum, tectum, and telencephalon (location of the fish pallium). Similar work was performed by Nordgreen et al. (2007) demonstrating the activation of the telencephalon in Atlantic salmon in response to electrical shocks applied to the tail. Demonstration of such connectivity between nociceptors and integrative brain regions suggests the potential ability for conscious interpretation of sensory information.

Following the identification of nociceptors in trout mentioned above, Sneddon (2003a) also demonstrated that trout performed flexible self-protective behaviours (Criteria 6) which reduced with analgesic administration (Criteria 4). Following the injection of a noxious chemical to the lips, trout performed self-protective behaviours such as rubbing their lips against the tank surface and rocking side-to-side (Sneddon, 2003a). These behaviours demonstrate an awareness of the location of injury and attempts to subside, or cope with, the potential painful experience. These protective behaviours ceased when morphine, a central analgesic, was administered to the trout (Sneddon, 2003a). This modification of behaviour demonstrates that morphine is able to attenuate the potential experience of pain. However, because morphine has general depressive effects on behaviour, further behavioural evidence is warranted. This is because caution should be applied when interpreting a reduction in behaviour in response to analgesics as a reduction in the experience of pain.

Evidence of the emotional experience of pain can be better provided by criteria 5 (motivational trade-offs) and 7 (associative learning). Dunlop et al. (2006) used spatially cued avoidance responses to a potentially noxious stimulus to demonstrate the significance of an electrical shock to Rainbow trout. Trout initially demonstrated shock-avoidance behaviour, however, in the presence of a conspecific, this shock-avoidance behaviour reduced. The reduction in shock-avoidance behaviour in the presence of a conspecific demonstrates trout making a motivational trade-off between the value of remaining near a conspecific and the aversiveness of an electrical shock (Criteria 5). Motivational trade-offs require the weighing and ranking of the unpleasantness of various experiences. This action reflects cognitive processing, interpretation, and memory of other experiences in order to make a trade-off. Thus, suggesting high brain processing and that the experience matters to the animal. Wong et al. (2014) used a form of a motivational test paired with a learning and memory component. A conditioned place avoidance paradigm was used to understand Zebrafish (*Danio rerio*) aversion to various anaesthetics. Zebrafish were conditioned to associate anaesthetic exposure (noxious stimuli) with their preferred side of a light/dark box. Fish spent less time in their 'preferred side' when an anaesthetic was present, suggesting aversion to the anaesthetic. After conditioning, when the anaesthetic was removed from the 'preferred side', Zebrafish demonstrated complete avoidance of their previously preferred side despite the lack of present exposure to the noxious stimulus. Wong et al. (2014) therefore demonstrated that Zebrafish are willing to trade-off between residing in their preferred side and exposure to an aversive anaesthetic (Criteria 5), that they are able to form a learned association between a noxious stimulus and location (Criteria 7). Associative learning tests, such as conditioned place aversion, test the animal's response to a cue that is associated with a past experience. In this example, the conditioned place aversion tests reflect the fish's aversion to a noxious stimulus that it has learned to expect. The expression of aversion without direct exposure to the anaesthetic suggests previous exposure events were significant enough to influence the animal's future behaviour.

By themselves, each line of evidence/criteria does not unequivocally signify sentience, or the experience of pain. However, coherence of several lines of evidence produces greater confidence in the assumption of sentience. Although to the authors knowledge, research is yet to be conducted demonstrating evidence of criteria 8, there exists, as demonstrated above, scientific evidence of the ability of fish to fulfil a majority of Birch et al.'s (2021) criteria. Therefore, it can be assumed that fish are sentient beings that have a welfare to be considered and thus, such evidence forms the basis of the inclusion of fish in New Zealand's animal welfare legislation.

2. 3. Assessing fish welfare

To protect fish welfare, there is a need to be able to assess the affective states an animal is likely to be experiencing. The Five Domains Model is one framework which allows for the systematic and comprehensive assessment of an animal's welfare state (Mellor et al., 2020). The Model is predicated on the affective state orientation and integrates different orientations. The structure of the Model recognises that an animal's welfare state can be influenced by mental experiences arising from the interpretation of sensory inputs from both the animal's internal and external environment. The Model organises observable evidence (also known as welfare indicators) of welfare impacts in one of four physical/functional domains related to the animal's nutritional status (Domain 1), physical environmental conditions (Domain 2), health status (Domain 3), and its behavioural interactions with the environment, other non-human animals, and humans (Domain 4) (Mellor et al., 2020). Evidence of welfare impacts in these first four domains is used to carefully infer the likely mental experiences assigned to Domain 5 (Mental state) (Mellor et al., 2020). All positive and negative mental experiences in Domain 5 are integrated to determine the animal's overall welfare status. Thus, the Five Domains Model provides a framework to organise information collected from the animal and its environment to holistically understand the animal's welfare state.

The assessment of animal welfare is reliant on the use of welfare indicators. Welfare indicators are measurements and observations of an animal's biological function, physical environment and behaviour used to infer their likely mental experiences. Welfare indicators can be broadly separated into two categories: animal-based and resource-based indicators. Animal-based indicators are measurements and observations made from the animal itself that are considered 'outputs' of an animal's welfare (Wemelsfelder & Mullan, 2014). For example, vocalisations due to a traumatic injury may be used as an indicator of the unpleasant experience of pain.

This animal-based indicator is considered an output because the animal is performing a behaviour *as a result* of an experience. In contrast to animal-based indicators, resource-based indicators are measurements and observations made from the animal's surrounding external environment and the management applied to the animal (Hampton et al., 2016). For example, a barren environment as a resource-based indicator may provide evidence of the potential experience of boredom. Identification of suitable welfare indicators allows observers to make inferences about the likely mental experience of the animal in question.

3. Potential welfare issues in salmon farming systems

The purpose of this section is to identify areas of potential welfare concern for farmed fish in New Zealand to guide the development of a Code of Welfare for farmed fish. It is outside the scope of this thesis to perform practical welfare assessments on New Zealand salmon farms. Therefore, a theoretical assessment, using scientific peer-reviewed literature, was undertaken to identify potential welfare issues within New Zealand's farming systems, as well as potential risks associated with climate change. Due to the limited amount of welfare specific, scientific peer-reviewed literature on New Zealand King salmon, where necessary, information regarding potential welfare issues was drawn from international literature related to the farming of Atlantic salmon.

The Five Domains Model was used to identify potential welfare issues associated with the various phases of salmon production, including issues associated with transport and pre-slaughter harvesting. Descriptions of the general features of the salmon production process are provided in 1. 1. Features of salmon farming systems in Aotearoa New Zealand. Welfare impacts which can occur across each stage of production and production system are stated generally. Welfare impacts that may arise in a specific stage of production or production system, where they may not occur in other stages or systems, will be stated explicitly.

Domain 1: Nutrition

Feed withdrawal

Prior to transport of juveniles and harvest of adult fish, farmers may abstain from feeding fish for up to two weeks. The impact of feed withdrawal on fish is dependent on nutritional status, energy reserves and environmental conditions such as temperature (Noble et al., 2018). Hvas et al. (2020) suggest that feed withdrawal, lasting for up to 4 weeks, has minimal effects on salmon welfare. This was evidenced by a lack of mortalities, no significant changes in body condition, and immediate vigorous feeding behaviour upon refeeding. However, this vigorous feeding behaviour is likely to indicate that fish were significantly hungry during the withdrawal period, negatively impacting their welfare. At the beginning of the trial there was also an initial reduction in specific metabolic rate. This reduction is an adaptive response which allows fish to preserve their energy. However, a reduction in specific metabolic rate may impair the fish's ability to respond to other challenges during the withdrawal period, thus posing a risk to their welfare state. Also, if feed withdrawal were to result in emaciation, the fish's thin appearance would be indicative of diminished energy reserves which are needed to sustain fish until their next feed, or death if they are destined for slaughter (Hardy, 1995).

Underfeeding

Factors, such as underfeeding, associated with feeding regimes that are ill-adapt to the requirements of salmon can influence their welfare. In the case of underfeeding, identifying satiation of all individuals within a farming unit is difficult due to the aquatic medium and number of individuals within a farming unit. Group measures such as a reduction in head turning, and resource measures such as pellets not eaten by fish visible below 5 metres in water depth, are used as indicators of satiation at a farm level. Use of such measures assume that upon their observation, all salmon within the farming unit are satiated and feeding has ceased. Due to the likelihood of individual variation in nutritional status and energy reserves, it is likely that some individuals, after the cessation of feed provision, remain hungry until their next feed. The provision of food may be considered a limited resource for salmon. Competition for food resources may occur. Such competition is known to increase aggressive interactions (Domain 4) and fin damage (Domain 3) (Noble et al., 2018). In addition, prolonged underfeeding may result in emaciation. Due to their small size, poor ability to swim and low position within the social group, emaciated fish are likely to be out competed for food by larger conspecifics. Being outcompeted may result in further reductions in feed intake and eventual death (Noble et al., 2018). Therefore, underfeeding can negatively impact salmon welfare.

Domain 2: Physical environment

Water quality parameters which can influence fish welfare across the entire production system include water flow rate, temperature, oxygen saturation, salinity, carbon dioxide saturation, and ammonia levels.

Water flow rate

The rate of water flow plays a role in the maintenance of water quality. Sufficient water flow, in relation to the stocking density of salmon, is required to supply oxygenated water and remove solid and dissolved wastes such as carbon dioxide and ammonia (van de Vis et al., 2020). The effects of low oxygen levels and elevated levels of carbon dioxide and ammonia are discussed below. The rate of water flow through flow-through raceways and net cages in New Zealand, are dependent on the water currents of rivers, hydro-canals, and coastal bays. Additional oxygen may have to be supplied by an aerator where natural currents are not sufficient to maintain optimal water quality. The bubbling action of aerators may also help with the dispersal of waste products and temperature regulation.

The rate of water flow also influences swimming performance. In cages, fish adjust their swimming behaviour according to changes in water flow and net deformation to maintain their position in the water column (Hvas et al., 2021). In open ocean farming systems, exposure to high waves and strong currents may impair the fish's ability to maintain their position in net cages and can alter the schooling behaviour of salmon (Hvas et al., 2021; Noble et al., 2018). In all production systems (flow-through raceways, tanks, and hydro-canals), salmon form schools and swim in a circular motion. This behaviour is easily achievable under moderate current flow (0.8 body lengths per second) (Solstorm et al., 2016). At high current speeds (e.g., 1.5 body lengths per second), swimming behaviour changes, such that no forward movements are made and fish increase swimming speeds to hold their position against the current (Hvas et al., 2021; Solstorm et al., 2016). Exposure to strong currents for prolonged periods can lead to salmon become physiologically fatigued and unable to hold their position. Fatigued salmon may become displaced and pushed against the net walls, potentially resulting in injury (Domain 3) (Hvas et al., 2021).

Water temperature and oxygen saturation

As poikilotherms, salmon body temperatures fluctuate as water temperatures change (Huntingford & Kadri, 2014). Due to this, water temperature influences fish metabolic rate and oxygen requirements (Noble et al., 2018; Stien et al., 2013). Increases in water temperature increase the metabolic rate of fish and thus their oxygen requirements. If oxygen levels in the water significantly decline but temperatures remain high, fish will be unable to meet their increased oxygen requirements which can induce a physiological stress response (Noble et al., 2018; Sundh et al., 2010).

Each production stage of salmon has different optimal temperature ranges, outside of which fish welfare may be compromised. Elevated temperatures are associated with increased spinal deformities as a result of altered gene transcription (Ashley, 2007). Thermal stress is also known to increase fish susceptibility to infection and mortality and reduce appetite because of metabolic overload (Ashley, 2007; Huntingford et al., 2006; Stien et al., 2013). The subsequent increase in metabolic rate results in an adaptive response which reduces the feed intake of fish to preserve energy.

Fluctuations in water temperature are likely to have a greater impact on fish in systems where heat cannot easily be controlled (Johansson et al., 2006). These include net cages in hydro canals where temperature is dependent on water supplied by lakes and glaciers, as well as net cages used in coastal bays and open ocean. Unlike on-land freshwater systems where temperature can be controlled through the use of heaters, the temperature within freshwater and seawater net cages cannot be controlled as they are under environmental influence. To mitigate the effects of high temperatures on fish metabolic rates, the use of an aerator at the bottom of net cages is recommended to increase the supply of oxygen (van de Vis et al., 2020).

Carbon dioxide and ammonia concentration

Carbon dioxide and ammonia are normal waste products of respiration and digestion. Buildup of these compounds can occur in systems with low water flow rates, limiting waste removal. At high concentrations, both compounds are toxic to salmon (Noble et al., 2018; van de Vis et al., 2020). Ammonia is excreted by the gills and can react with water to form ammonium ions. If ammonium is not cleared from the water efficiently it can impair ammonia excretion from the gills leading to build up in the blood and ammonia intoxication. Ammonia intoxication is associated with increased gill membrane permeability, impairing osmoregulation (Eddy, 2005).

Carbon dioxide can dissolve in water forming carbonic acid. Carbonic acid decreases water pH. If water CO_2 concentrations increase and water pH decreases, blood concentrations follow, i.e., blood CO_2 concentrations will increase and blood pH will decrease (Noble et al., 2018). Elevated concentrations of blood CO_2 can impair haemoglobin oxygen carrying capability and subsequently respiration. Low blood pH can also result in a loss of calcium ions in the gills, impairing osmoregulation by increasing the permeability of gill membranes to water and other ions (Wendelaar Bonga & Lock, 1992).

Salinity

Osmoregulation is necessary for the maintenance of cellular fluid composition and volume. The relative osmotic concentrations of body fluids and environmental water influence the movement of water and ions across the gills, where solutes move down concentration gradients from areas of high concentration to areas of low concentration (Evans, 2011). During osmoregulation in hyperosmotic, freshwater environments, water is gained and ions are lost. However, in hypoosmotic marine environments, water is lost and ions are gained. Prior to smoltification, juvenile salmon are adapted to freshwater environments. In this life stage, chloride cells in the gills actively uptake ions and the kidneys excrete water. During smoltification, chloride cells begin to function as ion excreters to prepare salmon for a hypoosmotic environment (Evans, 2011). Osmoregulation is impaired in salmon whose chloride cells are ill-adapted for a hyperosmotic marine environment. Inadequate osmoregulation can lead to the build-up of ions such as chloride, sodium and ammonia, which can be toxic to fish, and also lead to dehydration if physiological changes in the gut fail to occur (Evans, 2010).

The development of salinity tolerance associated with the transport of smolt to sea cages is an issue in New Zealand. A 25% mortality rate, 5% of which is attributed transport stress and maladaptation of smolt to increased salinity, across the entire production system is accepted by industry (Fischer & Appleby, 2017). During transport to sea cages, seawater is pumped through transport tanks to acclimatize smolt to a marine environment. If smolt are not yet mature enough, they will be unable to osmoregulate adequately during the transport journey and upon entering the sea cage.

Domain 3: Health

Infectious Diseases

In comparison to international farming operations (e.g., Norway Atlantic salmon farms), infectious disease burden in New Zealand farming systems is relatively low. However, in open systems such as flow-through raceways and net cages, environmental pathogens still present a risk to fish health. Diggles et al. (2002) provide a comprehensive list of previously detected diseases in New Zealand aquaculture systems, as well as a list of yet-to-be-detected diseases that pose a risk to New Zealand aquaculture should they enter New Zealand fish populations. According to experts within industry, infectious diseases that are currently present in New Zealand systems include enteric red mouth disease and vibriosis (M. Preece, personal communication, May 18, 2021; B. Blanchard, personal communication, May 27, 2021).

Enteric red mouth disease is a bacterial infection of both freshwater and marine salmon. The disease is caused by the gram-negative rod-shaped Enterobacterium, *Yersinia ruckeri (Diggles et al., 2002)*. The bacteria remain undetected in the intestines of host fish until periods of stress occur. Growth of the bacteria is facilitated by a reduction in the performance the fish's immune system. Carrier fish, once stressed, can become shedders of the bacteria, passing the bacteria onto conspecific through their faeces. Enteric red mouth causes the congestion of blood-vessels throughout the peritoneum, and petechial haemorrhages, affecting the liver, pancreas, swim bladder, lateral muscles and adipose tissues associated with the pyloric caecae. Enteric red mouth is characterised by reddening of the throat and mouth, blood spots in the eyes, erosion of the jaw and palate, as well as anorexia and lethargy (Diggles et al., 2002).

Similar to Enteric red mouth, Vibriosis is another bacterial infection that affects both freshwater and marine salmon. Vibriosis is caused by bacteria from the genus *Vibrio* such as *Vibrio anguillarum* (Diggles et al., 2002). Vibriosis can be transmitted horizontally through the water where Vibrio bacteria enter fish by penetrating the skin. Transmission can also occur vertically from parent to offspring, via egg contamination by parent fish. The severity of infection, and onset of disease outbreak, is influenced by water temperature and stress of the fish. Vibriosis associated with *V. anguillarum* is characterised by haemorrhagic septicaemia associated with cardiac myopathy, and renal and splenic necrosis (Diggles et al., 2002). Clinical signs of vibriosis include pinpoint haemorrhages of fins and skin, distended abdomen, dark lesions that ulcerate and severe eye damage (Diggles et al., 2002).

As climate change progresses causing water temperatures to rise, the likelihood of potential disease outbreaks also increases. The reasoning for this is two-fold. First, elevated water temperatures enable pathogens, such as New Zealand rickettsia-like organism, *Tenacibaculum maritimum*, and *Vibrio spp.*, to grow and proliferate in the environment as well as in host species (Brosnahan et al., 2019). Second, high water temperatures (e.g., 18°C and above) may lead to heat stress, a welfare problem in its own, which can impair immune responses to pathogen insult (Brosnahan et al., 2019; Noble et al., 2018). Together, these factors increase the likelihood of potential disease outbreaks as climate change persists.

Injuries

Fish are susceptible to injury at various points in the production chain. Injuries can be linked management factors such as poor feeding regimes, crowding, handling, pumping and transport. Highly stocked and underfed salmon are competitive and may become aggressive in the presence of food (Hvas et al., 2020). Aggressive behaviours include attacks directed towards the fins of conspecifics, resulting in fin damage. During crowding and pumping for transport and harvest, injuries can arise from collisions with other fish, as well as with the interior walls and corners of pump pipes (Lines & Spence, 2012). The risk of collision injuries is also present during transport if driving becomes erratic or if oceans swells are strong. During netting of fish for handling, crushing and bruising injuries can result from fish becoming layered on top of one another (Lines & Spence, 2012). Fish positioned lower in the net have a higher chance of becoming crushed as gravity pulls down the weight of multiple layers of fish, effectively compressing lower fish against the bottom of the net (Lines & Spence, 2012). Deformation of net cages is also known to produce skin lesion injuries as fish attempt to escape the net material (M. Preece, personal communication, May 18, 2021; B. Blanchard, personal communication, May 27, 2021).

Spinal deformities

Spinal deformities are common among farmed salmon populations in New Zealand. Perrott et al. (2018) reported 40% of a population of New Zealand farmed salmon presented evidence of spinal deformities at harvest. Spinal deformity types can be broadly characterised as compression or fusion of vertebral bodies, curvature of the spine, or dislocation of vertebral bodies (Munday et al., 2018; Perrott et al., 2018). The most common abnormality presented at harvest in New Zealand is lordosis, kyphosis and/or scoliosis, a type of curvature of the spine (Perrott et al., 2018). Higher prevalence’s of spinal deformities have been associated with fish of high growth rates, suggesting maturation protocol may influence deformity development (Perrott et al., 2018). Similarly, high temperature during early development of King salmon has been associated with the development of spinal deformity (Munday et al., 2018). Deformed fish can often be observed swimming above their school and are noticeably of a smaller size than their conspecifics (B. Blanchard, personal communication, May 17, 2021). The affective experience resulting from spinal deformities is unknown, however, several welfare issues may arise. For example, deformed fish have poor locomotor skills which may impact their ability to maintain their position in the school and compete for food (Noble et al., 2018). The presence of spinal deformities has also been linked to a reduced tolerance of stressful interactions (Noble et al., 2018).

Domain 4: Behavioural interactions

Interactions with humans

Interactions with humans have been evidenced to be aversive to farmed salmon. Throughout the production cycle, salmon may be handled by stockmen on several occasions (e.g., during size-grading and vaccination treatments at fry, smolt, and post-smolt stages, and broodstock fertility checks). Such close proximity to humans and handling events elicits both physiological and behavioural stress responses in salmon. Salmon subjected to acute handling stress (e.g., net chasing/capture and handling out water) demonstrated elevated levels of plasma cortisol and glucose (Carey & McCormick, 1998; Fast et al., 2008). Behavioural stress responses to human interaction include erratic swimming behaviour and rapid congregation at the bottom of tank (Madaro et al., 2015). On-farm, salmon are also reported to disperse when stockmen approach net cages and tanks (B. Blanchard, personal communication, May 27, 2021). Aversiveness to human interaction is known within the farming industry with some operations establishing no-handling policies under particular environmental conditions (e.g., during periods of high and low water temperatures as well as during high water flow conditions) (B. Blanchard, personal communication, May 27, 2021). Additional stress associated with handling under these conditions may result in increased mortality rates (Madaro et al., 2015). Therefore, fish handling practices have the potential to negatively influence salmon welfare.

Interactions with other animals

Stocking densities have the potential to influence the welfare of farmed salmon. Elevated stocking densities normally occur at crowding during handling events, transport and prior to slaughter. Elevated stocking densities (e.g., above 35kg m^{-3} (Turnbull et al., 2005) and above 50 kg m^{-3} (Calabrese et al., 2017)) have been shown to negatively influence body condition, fin and eye condition, physiological stress markers and feed utilisation. Reduced feed utilisation, and subsequent impacts on growth, is attributed to decreased availability of feed at high densities, preservation of energy reserves to maintain physiological processes, and increased complex social interactions (Calabrese et al., 2017). Increased frequency of aggressive interactions is suggested to be a major underlying factor for negative welfare impacts at high densities. Turnbull et al. (2005), Oppedal et al. (2011), and Calabrese et al. (2017) found increases in fin splitting and erosion, and cataracts, likely from aggressive acts of biting and chasing. Interestingly, low and high stocking densities are both associated with reduced welfare of salmon (Oppedal et al., 2011). Whereas intermediate densities are suggested to provide optimal welfare conditions. Stocking density also influences reactiveness of salmon during handling events (Calabrese et al., 2017). Therefore, management of stocking densities has the potential to negatively impact salmon welfare.

Despite the employment of predator nets, the presence of predators around rearing facilities has the potential to influence the welfare of farmed salmon. Flight responses have been shown to occur in response to both visual and odour detection of predators (Hawkins et al., 2007; Johnsson et al., 2001). In response to simulated predator attacks, salmon demonstrate immediate increases in swimming activity and position themselves in lower parts of the water column to escape injury (Johnsson et al., 2001). Elevated predator risk can also induce cardioventilatory responses, such that simulated predator attacks induce elevated heart and ventilatory rates in salmon (Johnsson et al., 2001). Therefore, interactions with animals, other than conspecifics, such as predators may also negatively influence the welfare of farmed salmon.

Interactions with the environment

The welfare of salmon may be influenced by limited expression of agency (i.e., expression of voluntary, goal-direct behaviour) resulting from interactions with rearing environments. Particular aspects of rearing environments that may influence salmon agency, and thus welfare, include barren and confined tanks or net cages. Barren environments are stimuli-deprived environments that lack environmental complexities such as physical enrichments (e.g., variation in physical structures and substrates) and/or sensorial enrichments (e.g., variation in visual, auditory, and chemical stimuli) that facilitate agency-related, species-specific behaviours (Näslund & Johnsson, 2016). In comparison to salmon reared in environmentally rich environments, salmon reared in structurally barren environments had higher plasma cortisol levels and higher levels of aggression resulting in high levels of fin deterioration (Näslund et al., 2013). The provision of structural enrichment by Näslund et al. (2013) also facilitated the expression of shelter-seeking behaviour – a life stage-specific behaviour of juvenile salmon. Similarly, confined environments can negatively impact salmon welfare. Within confined environments, such as tanks and net cages, salmon are unable to swim away from areas of poor water quality. In varying environmental conditions, given the space, salmon swim to preferred depths in the water column (Noble et al., 2018). This allows salmon to move to areas of optimal water quality, as well as avoid algae and jellyfish blooms. Inability to escape may result in negative welfare impacts detailed in Domain 2: Physical environment and Domain 3: Health. The effects of confinement maybe exacerbated at high stocking densities, further restricting the movement of salmon. Therefore, in barren and confined environments, the expression of agency is impeded, negatively influencing farmed salmon welfare.

4. Potential issues associated with stunning and slaughter methods

In this section the welfare impacts of stunning and slaughter methods used in New Zealand are discussed. Prior to death, fish are conscious and sensible to any pain and distress caused by the act of slaughter (Lines & Spence, 2012). For slaughter to be humane, i.e., inflict minimal negative welfare impacts, the general term of reference requires that slaughter methods result in rapid and irreversible loss of consciousness (Lines & Spence, 2012; van de Vis et al., 2014). Where a slaughter method does not render a fish immediately unconscious, a stunning method must first be applied in such a way that the fish is made insensible and must remain insensible until death (Lines & Spence, 2012; van de Vis et al., 2014). Methods which cause the unnecessary suffering of fish are of legal welfare concern.

The legal recognition of the capacity of fish to suffer places a legal duty on animal carers to protect fish from suffering at slaughter (Brown, 2015). Specific detail for safeguarding the welfare of fish at slaughter is provided in the Code of Welfare for Commercial Slaughter (NAWAC, 2018a). Under part 6.1 of the Code, the welfare of farmed fish, and fish caught and held for killing at a later time, is protected by Minimum Standard No. 21 (NAWAC, 2018a). In this section, relevant minimum standards and general information provided in the Code will be evaluated with regards to the general term of reference and best practice recommendations derived from empirical studies will be given. The stunning and slaughter methods discussed below include iso-eugenol and carbon dioxide stunning, percussion, electrical stunning, and brain spiking (descriptions of method application and mode of action are provided in earlier sections - Stunning and slaughter).

4. 1. Aqui-S

There is currently no minimum standard pertaining to the use of anaesthetics for the slaughter of fish. The general information section of the Code does, however, briefly state that "an appropriate dose of iso-eugenol or other appropriate euthanising drug" can be used to kill fish (NAWAC, 2018a). In Atlantic salmon, Aqui-S appears to elicit a physiological stress response similar to that of MS-222, an anaesthetic known to be aversive, relative to other anaesthetics (Zahl et al., 2010). However, the physiological stress response to Aqui-S does not appear to reach the same level of magnitude as MS-222 and other drugs such as benzocaine, and can therefore be recommended as a preferable drug for slaughter in terms of aversiveness (Zahl et al., 2010).

Induction of unconsciousness by iso-eugenol immersion is dependent on dosage and exposure time. Iversen et al. (2003) found that arrested opercular activity occurred at 12 minutes at 100 mg/L in Atlantic salmon. At 17 mg/L, swimming activity ceased between 4-9 minutes, with no significant signs of recovery within a 10 minute recovery period in sea water (Erikson, 2011). Young et al. (2019) were able to produce unconscious King salmon within less than 1 minute of exposure at 40 mg/L and death within 3 minutes. From this data it is recommended that fish are exposed to iso-eugenol concentrations above 40 mg/L for at least 12 minutes to produce unconscious, and potentially dead fish. To ensure fish remain unconscious until death, the animal should be bled out within 10 minutes of last exposure to Aqui-S (Erikson, 2011).

4. 2. Carbon dioxide

Similar to the use of anaesthetics, there is no minimum standard pertaining to the use of CO_2 as a slaughter method. The method is, however, mentioned in the general information section where the use of CO_2 alone is not supported (NAWAC, 2018a). Rather, CO_2 stunning should be preceded by the use of iso-eugenol containing products. This is because CO_2 stunning alone has been shown to be extremely aversive for fish (Bowman et al., 2020; Erikson, 2011). Immersion in CO_2 saturated water elicits violent erratic behaviour for up to 2-4 minutes before the cessation of swimming (~10 minutes) in Atlantic salmon (Erikson, 2011; Robb et al., 2000). Electroencephalogram (EEG) recordings have also shown that visually evoked responses (VERs; indication of consciousness) remain present in Rainbow trout (*Oncorhynchus mykiss*) for up to 3.5 minutes after ventilation ceases and 6.5 minutes after equilibrium is lost (Bowman et al., 2020). This data suggests that despite the cessation of movement, it is possible that fish are sensible to the unpleasant experience of pain inflicted by the act of bleeding out after stunning (Erikson, 2011).

To ensure fish remain unconsciousness until death, the animal must be exposed to adequate concentrations of CO_2. The narcotic effects of CO_2 must take effect before the anaesthetic effects of Aqui-S subside (Erikson, 2011). Water pH between 5.0-5.6 is recommended, as this range has been shown to produce unconscious fish within 8-10 minutes (Bowman et al., 2020; Erikson, 2011; Robb et al., 2000). Therefore, if the narcotic effects of CO_2 take place before anaesthesia wears off, unconsciousness may be prolonged until death occurs from CO_2 exposure or bleeding out.

4. 3. Percussion

Manual percussion where fish are not physically restrained is prohibited by minimum standard No.21 (c) (NAWAC, 2018a). The current restraint suggestions include the use of a non-slip surface, a funnel or wedged holding block, or by hanging fish from their operculum. Restraint is necessary for manual percussion as removal from water elicits escape behaviours (Lambooij et al., 2010). Movement of the fish during stunning is likely to lead to mis-stuns, thereby injuring and causing pain to fish, as well as resulting in multiple blows to the head to produce unconsciousness (Robb et al., 2000). During manual percussion it is recommended best practice that fish are restrained using a funnel or wedged holding block to limit body movement. Hanging fish by their operculum is, however, not recommended as fish have nociceptors in this area (Cooke & Sneddon, 2007). Hanging fish by their operculum is likely to cause unnecessary pain as the force of gravity pulls their body down, stimulating nociceptors.

Several other factors must be considered when using percussion. When a percussive stun is applied correctly, with sufficient force, and with an appropriate hammer head, fish welfare is unlikely to be significantly impacted as the loss of consciousness or death may be relatively rapid. Robb et al. (2000) found that the application of a pressurised bolt on the skull mid-dorsally, in line with the posterior margin of the eyes, rendered Atlantic salmon rapidly unconscious, evidenced by the immediate loss of VERs. However, mis-placed stuns prolonged the loss of consciousness by up to 334 seconds. At forces between 78.68-99.9 N (or 8.1-10 bars), Lambooij et al. (2010) reported the appearance of theta and delta waves, followed by an isoelectric line within 51 seconds of stun application, as well as no response to noxious stimuli immediately following the stun. This is supported by the behavioural analysis of stunned Atlantic salmon by Roth et al. (2007), who found that at forces above 72 N rendered salmon unconscious within 1 minute and prevented recovery, as judged by the presence of an eye roll. At a suggested stunning force above 72 N, Roth et al. (2007) also recommend the use of a flat circular hammer as the kinetic energy transfer required to change the internal pressure within the skull is more efficient in comparison to the use of a cone or spike shaped hammer.

4. 4. Electrical stunning

The Code acknowledges that electrical stunning is not always effective in killing fish, and that where reversible stunning is used, fish must be bled out before they regain consciousness (NAWAC, 2018a). The duration of unconsciousness is dependent on the frequency, current magnitude and duration of the stun (Grimsbø et al., 2016). Robb et al. (2002) found that dry stunning Rainbow trout at a current of 100 mA at 50 Hz for 1 second was sufficient to stun fish. Robb and Roth (2003) found that wet stunning Atlantic salmon using an electrical field of 50 V/m at 50 Hz for 3 seconds produced the longest recovery period, in comparison to 200 V/m for 1 second and 25 V/m for 12 seconds. Dry stunning of Atlantic salmon at 668 mA_{rms} and ~107 V_{rms} for ~0.5 seconds is recommended by Lambooij et al. (2010) to produce unconscious fish, however, fish may regain consciousness before death due to bleeding out. Increasing current magnitude and duration of application both increase the duration of the stun and at high enough levels result in death (Robb et al., 2002). However, there appears to be a threshold above which fish are not effectively stunned as increases in frequency decrease the period of unconsciousness. It is recommended by Grimsbø et al. (2016) that fish are electrically stunned using frequencies between 70-100 Hz. From this data, it is recommended that fish are stunned at 100-668 mA for 1 second (dry stunning), or 50 V/m for 3 seconds (wet stunning), both using 70-100 Hz.

Brain death from bleeding out occurs between 4-7 minutes (Robb et al., 2000). Therefore, the duration of unconsciousness must last longer than this period. The recovery times recorded for trout and salmon species after electrical stunning range between 0.73-7.38 minutes (Robb et al., 2002; Robb & Roth, 2003). At the present investigated currents, frequencies and stun durations, where reversible stunning is used, it is possible that fish may regain consciousness before brain death occurs (Lambooij et al., 2010). Therefore, it is recommended that electrical stunning variables be used at levels which result in death of the animal. Otherwise, where irreversible stunning is not achievable, fish should be stunned or killed using a percussive or brain spiking method to ensure insensibility before bleeding out.

4. 5. Brain spike

The brain spiking method is only humane if the spike is inserted at an appropriate point on the head. Therefore, minimum standard No. 21(e) requires the slaughter person to be competent and experienced to ensure fish welfare is not compromised by incorrect spike placement (NAWAC, 2018a). Correctly placed spikes which destroy the optic lobe or anterior cerebellum are reported to result in an immediate loss of VERs (Robb et al., 2000). Misplaced spikes (e.g., spikes which hit the back of the brain or miss the brain completely) do not result in immediate loss of consciousness. Robb et al. (2000) reported that the loss of VERs in salmon stunned with misplaced spikes took up to 300 seconds, during which salmon showed signs of aversion, interpreted as the experience of pain. Misplaced spikes are likely to result in painful injury and be felt for a relatively lengthy period before unconsciousness is achieved. It is recommended best practice that the slaughter person refer to brain placement diagrams before performing the method (Diggles, 2016). For example, to access the brain of Chinook salmon shown in Figure 3, the spike should enter the skull mid-dorsally, and rostral of the eyes (Digfish Services, 2013). External spike placement and x-ray photographs of various fish species can be found at www.ikijime.com.

The brain spiking method is not a consistently accurate method. Under a commercial farm setting, continuous manual spiking of large batches of fish is likely to increase the incidence of mis-stuns due to stockmen fatigue (Robb et al., 2000). Also, the brain of fish such as salmon are relatively small in comparison to the tuna species, for which this method was established (Poli et al., 2005). The performance of aversive behaviour upon removal from the water is also likely to make locating and accurately spiking small brains difficult (Robb et al., 2000). Therefore, it is recommended that under commercial farm settings, only small batches of fish are stunned at a time and that these fish are restrained using a non-slip mat or funnel/wedged holding blocks.

5. Conclusion

King salmon are an economically important species to New Zealand’s aquaculture industry. The current regulatory environment supports the protection of salmon welfare in New Zealand. However, current welfare regulations can be further bolstered through the identification of welfare-relevant areas of salmon farming that should be considered during the development of welfare regulations and on-farm welfare assessments. Therefore, this chapter systematically considered potential welfare impacts relevant to nutrition, living conditions, health, and behavioural interactions that may influence salmon welfare in the production systems used to farm King salmon in New Zealand.

Chapter 2 – Identification of potential welfare indicators for commercially farmed King salmon (Hāmana, *Oncorhynchus tshawytscha*) in Aotearoa New Zealand: A scoping review to inform the development of on-farm fish welfare assessments

1. Introduction

Aquaculture – the cultivation of aquatic plants and animals – is one of Aotearoa New Zealand's, and the world's, fastest growing primary industries. A major contributor to the growth of New Zealand's aquaculture industry is the farming of King salmon (Hāmana, *Oncorhynchus tshawytscha*). The species was introduced into New Zealand rivers in the early 1900s and is now the only salmon species commercially farmed for human consumption in the country (Haworth, 2010). Since the establishment of New Zealand's first salmon farm in 1983, numerous freshwater and marine-based farming operations span the country's South Island, producing 15,512 tonnes (harvested greenweight) of salmon 2021[1]. New Zealand aquaculture production is expected to increase to a $3 billion industry by 2035 (Stenton-Dozey et al., 2021), requiring the physical expansion of production systems and an increase in the number of individuals harvested per year (Casanovas et al., 2021). The expansion and intensification of national and international aquaculture industries has attracted increased interest in the welfare of farmed fish from people in industry, government, and the public (Barreto et al., 2022).

If fish welfare is to be protected, there is a need to be able to assess the affective states an animal is likely to be experiencing. This is because, contemporarily, an animal's welfare state is characterised as a dynamic state within the animal, representing the summation of all of its mental experiences at a given point in time (Beausoleil & Mellor, 2017). These mental experiences are generated from the detection and interpretation of sensory information from the animal's internal and external environment and can be experienced as either positive or negative (Beausoleil & Mellor, 2017). Various aquaculture husbandry practices and physical environments have the potential to influence the welfare of farmed salmon.

---

[1] http://www.salmon.org.nz/new-zealand-salmon-farming/production/

Conditions that can potentially influence welfare include, but are not limited to, disease, traumatic injury, spinal deformities, poor water quality, aggressive conspecifics, handling-related stress, under-nutrition, poor living environment (in terms of enrichment), and painful death (Noble et al., 2018). Therefore, there is scope for farmed fish to experience a variety of welfare impacts across multiple dimensions of their lives.

The Five Domains Model is one approach used to comprehensively explore the multiple dimensions (or Domains) of animal welfare. The Model organises welfare indicators into one of four physical/functional domains to provide evidence of related survival-critical and situation-related affects in Domain 5 (Mellor et al., 2020). Welfare indicators are measurements and observations of an animal's biological function, physical environment and behaviour used to infer the likely mental experiences of the animal (Mellor et al., 2020). Survival-critical affects arise from internal imbalances influenced by an animal's nutrition (Domain 1), physical environmental (Domain 2), and health status (Domain 3). The resultant survival-critical affects motivate the animal to perform behaviours that correct internal imbalances, ensuring their survival (Mellor, 2016). Situation-related affects reflect the animal's perception of its external circumstances, influenced by an animal's interactions with humans, other animals, and its physical environment (Domain 4). Welfare indicators provide evidence of welfare compromise or enhancement in the first four domains which is used to infer the (likely mental experiences in Domain 5.

Welfare indicators can be broadly separated into two categories: animal-based and resource-based indicators. Animal-based indicators are measurements and observations made from the animal itself that are considered 'outputs' of an animal's welfare state (Wemelsfelder & Mullan, 2014). For example, vocalisations due to a traumatic injury may be used as an indicator of the unpleasant experience of pain. This animal-based indicator is considered an output because the animal is performing a behaviour *as a result* of an experience. Animal-based indicators can also be categorised according to their time of sampling/observation (i.e., either ante- or post-mortem) as this influences their ability to provide information about the temporal relationship of the observation of an indicator and the occurrence of a mental experience. In contrast to animal-based indicators, resource-based indicators are measurements and observations made from the animal's surrounding external environment as well as the management applied to the animal (Hampton et al., 2016). For example, a barren environment as a resource-based indicator may provide evidence of the potential experience of boredom. Resource-based indicators are considered indirect, input measures of welfare that require coherence with animal-based indicators to provide solid evidence of an affective outcome (Beausoleil & Mellor, 2017).

There is an expectation of animal-based industries and welfare regulatory bodies to evaluate the potential impacts of farming practices on the welfare of the individuals in these production systems. For the salmon farming industry, one of the first steps is to systematically develop welfare assessment tools for farmed King salmon in a New Zealand context. This requires a suite of potential welfare indicators to be identified and understood in terms of what they can tell us about fish welfare. Handbooks detailing operational welfare indicators for Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) currently exist (Noble et al., 2018). However, there are no such handbooks available that are directly address King salmon farmed in a New Zealand context.

Previously, literature mapping has been conducted to identify welfare indicators relevant to sheep (Llonch et al., 2015) and cattle (Palmer, 2017). Systematic literature reviews have also been used to identify fish behaviours relevant to welfare (Macaulay et al., 2021), emerging methods for indicator collection (Barreto et al., 2022), effects of structural environmental enrichments on welfare (Näslund &

Johnsson, 2016), and welfare statements relevant to Atlantic salmon welfare (Stien et al., 2013). To the author's knowledge a scoping review which examines globally published research relevant to measures of salmon welfare to inform the development of welfare assessments specific to King salmon in a New Zealand context has yet to be conducted. Therefore, the objective of this paper was to perform a scoping review to identify potential welfare indicators which may be used to evaluate the welfare of commercially farmed King salmon in New Zealand. As such, the primary research question driving the scoping review was:

"What animal-based and resource-based measurements and observations can be potentially used as welfare indicators for commercially farmed King salmon?"

2. Methods

2. 1. Review protocol

The review's protocol was guided by the Preferred Reporting Items for Systematic Reviews and Meta-analysis Protocols – Extension for Scoping Reviews checklist (PRISMA-ScR; Tricco et al., 2018). Scoping reviews are conducted using an *a priori* protocol to provide a structured plan for the searching, analysing, and reporting of information relevant to the review's objectives and questions. An overview of this review process is provided Figure 7. The use of an *a priori* protocol is key to ensuring that the scoping review is repeatable, transparent, and minimises reporting bias (Peters et al., 2020; Tricco et al., 2018).

Prior to the commencement of the formal review process, the protocol was peer-reviewed by animal welfare scientists from the Animal Welfare Science and Bioethics Centre, as well as a veterinary epidemiologist from EpiCentre, at Massey University, New Zealand.

2. 2. Eligibility criteria

The development of the eligibility criteria utilised the population, concept, and context (PCC) framework recommended by Peters et al (2020). The PCC framework defines the focus and scope of articles retrieved in this review (Table 1). The focus population included two salmon species – King salmon and Atlantic salmon. A non-systematic pre-screening of literature demonstrated that there is limited explicit research on the welfare of King salmon. The body of literature related to salmon welfare largely relates to Atlantic salmon. Discussion with industry expert (M. Preece, personal communication, May 18, 2021) indicated that Atlantic salmon are similar to King salmon in key respects. For this reason, Atlantic salmon were also included as a relevant population for this review.

A restriction was placed on the lifecycle stage of the animal to include only salmon in the fry to post-smolt stages. The restriction was based on the stipulation in the New Zealand Animal Welfare Act (1999) that fish are only considered sentient beings capable of experiencing positive and negative experiences after the larval lifecycle stage. That is, welfare considerations do not apply to fish in the egg and larval stages and articles relating to only these lifecycle stages were not included in this review.

The overarching concept of the review was identification of measurements and observations that could be used as indicators of farmed salmon welfare. Therefore, articles were included if they had a focus on animal-based and resource-based measurements and observations relating to nutritional, environmental, health, or behavioural factors of fish. The context of this review included articles which described some facet of the welfare of salmon in an aquaculture or laboratory setting, with no restriction on production system used (e.g., flow-through raceways or recirculating aquaculture systems). There were also no geographical or date restrictions placed on eligible articles. However, only articles published in English were eligible.

Table 1. Eligibility criteria defined using the Population, Concept, and Context framework.

Criteria	Determinant
Population	Fish of the King and Atlantic salmon species in the fry to post-smolt lifecycle stages
Concept	Animal-based and resource-based measurements and observations that may be used as indicators of farmed salmon welfare
Context	Aquaculture and laboratory setting; all study countries; all aquaculture production systems; all publication years; articles published in English

2. 3. Search strategy

The first step of the search strategy involved the development of an appropriate search string. To ensure the number of irrelevant articles was limited, the search string was developed with the assistance of animal welfare scientists, a veterinary epidemiologist and a librarian with experience navigating scientific literature. An initial search string using terms related to '*salmon*', '*aquaculture*', '*indicators*', and '*animal welfare*' was piloted in the Web of Science. To check the search string, a non-systematic search of the literature was conducted. The search found relevant papers that were not identified by the pilot search string. Therefore, to optimise the final search results, key words relating to different aspects of animal welfare such as '*health*', '*nutrition*', and '*behaviour*' were also included in the final search string presented in Table 2.

Table 2. Search string used to retrieve records related to the welfare of salmon from Web of Science, Scopus and Discover.

(salmon OR "King salmon" OR "Atlantic salmon" OR "Oncorhynchus tshawytscha" OR "Salmo salar" OR "Chinook salmon" OR Salmonidae) AND (assess OR indicator* OR index OR indice* OR monitor* OR evaluat* OR measur* OR observ*) AND ("animal welfare" OR welfare OR "fish welfare" OR "animal wellbeing" OR wellbeing OR well-being OR "animal well-being" OR "farmed fish welfare" OR "finfish welfare" OR health OR disease* OR injur* OR lesion* OR deform* OR nutrition OR feed OR malnutrition OR diet OR behavior* OR behaviour*) AND (aquaculture OR cultivat* OR farm OR on-farm OR "fish farm" OR "salmon farm")*

Using the finalised search string, articles were retrieved from multiple online databases. To minimise bias associated with locating relevant studies in a single database, and to ensure all possibly relevant articles were retrieved, the final search string was run through three online databases provided by Massey University: Discover, Scopus, and Web of Science. The search was conducted in April 2021 and allowed for retrieval of all published and unpublished work up to April 28th, 2021. There were no limitations set on article type, allowing for the retrieval of all study types including experimental and observational studies, as well as allowing for the retrieval of all information source types, including journal articles, reviews, conference papers, book chapters, government reports and news articles. No restrictions were placed on the year or country of publication. At this stage of the review, there were no restrictions on publication language. Articles published in a language other than English were removed at later stages in the review process. All retrieved articles were exported to an Endnote X9 library (The EndNote Team, 2013) and duplicate articles were removed.

2. 4. Selection of sources of evidence

To be included, articles had to pass through two levels of screening: title and abstract screening, followed by full-text screening. Article screening ensures that the final set of articles retained for data extraction are relevant to the scoping review's objectives. Before beginning the formal full-text screening process, a convenience sample of 30 articles was independently reviewed by both reviewers to ensure consistency between them. During the calibration process, disagreement between the reviewers was resolved by discussion and the formal full-text screening process began once inter-rater agreement met a threshold of 80%.

The primary reviewer (IN) performed the title and abstract screening of all retrieved articles. Where difficulty arose in determining relevance, animal welfare scientist supervisors (NK and NB) were consulted. At this level, relevance was determined using the criteria presented in Table 3. Articles were considered eligible for the second level of screening if all four inclusion criteria were met affirmatively. Upon completion of title and abstract screening it was apparent that it was not possible for all papers to be reviewed in the time available. Therefore, a decision was made to limit the data extraction to papers published after 2014 (n = 802; Figure 7).

Table 3. Inclusion and exclusion criteria for title and abstract screening.

Criteria	Include	Excluded
Publishing language	Published in English	Not published in English
Publication type	Journal articles, conference papers, book chapters and government reports	Newspaper articles

Criteria	Include	Excluded
Species of interest	Abstracts which explicitly mention results relevant to King and/or Atlantic salmon Abstracts which did not explicitly state the species of fish studied were also included as a precaution	Explicit mention of results only relevant to fish species other than King and/or Atlantic salmon e.g., articles that studied other fish species such as Gilt-head seabream (*Sparus aurata*)
Focus on aspects relevant to fish welfare	Focus on aspects of welfare that fish may experience as described in the introduction e.g., articles investigating the effect of induced hypoxia on fish behaviour and physiology were included	Focus on aspects of fish not relevant to welfare e.g., articles describing the effect of a carotenoid diet on salmon flesh pigmentation were excluded

2. 4. 1. Data set reduction analysis following title and abstract screening

A decision was made to apply a limit on publication date because it was likely to have the least effect on the variables of interest compared to placing a restriction on study type or further limiting the fish species studied. To check the influence of publication date on article retention at first screening, the titles and abstracts of articles published within six time periods over the last 11 years were analysed for keywords relating to different aspects of welfare (nutrition, environment, health, behaviour, and mention of the term 'welfare'). The analysis was performed by sequentially adding one year at each step, i.e., period 2015-2012, then repeat for 2014-2021 up to 2010-2021 when it had become clear the that the impact of adding years beyond 2015 was negligible (see Appendix B). The analysis demonstrates that at this high level, reducing the data set to articles published between 2015-2021 is likely to produce the same result as including an additional 5 years of published articles. Therefore, in order for the project to remain feasible, only articles published between 2015-2021 were considered for full-text screening.

2. 4. 2. Full-text screening

To advance the identification of articles relevant to the review, full-text screening of retained articles was divided between two reviewers (IN and another expert in the subject). The sample of articles to be screened in full were split between the reviewers according to their assigned record number in EndNote X9 – odd record numbers were given to the first reviewer and even record numbers to the second. Article relevance was determined using the criteria in Table 4. Reviewers progressed articles for data extraction if they were able to satisfy all five main criteria. Articles that did not satisfy all five criteria were excluded from the data extraction process. To maintain feasibility of the project, an arbitrary number of 60 articles (10%) from the set retained after the second screen were chosen to progress to data extraction. A random number generator (https://www.calculator.net/random-number-generator.html) was used to produce 60 numbers that corelated to the 'record number' of articles held in EndNote X9.

Table 4. Inclusion and exclusion criteria for full-text screening.

Main criteria	Include	Excluded
Access to full-text	Full-text of article attainable through online database	Full-text of article not attainable through online database
Publishing language	Published in English	Not published in English
Study type	Primary study	Secondary study
Species of interest	Abstracts which explicitly mention results relevant to King and/or Atlantic salmon.	Explicit mention of results only relevant to fish species other than King and/or Atlantic salmon
Direct use of measurements/observations that may be used as indicators of salmon welfare	**Sub-criteria**	**Example**
	Articles investigating resource-based indicators had to investigate the effect of the environment on the fish, not vice versa	Articles investigating the effects of fish production on the effluent load in the environment were not included

	Sub-criteria	Example
	Articles investigating the presence of a pathogenic agent in the water environment of fish were included as this is a measure of water quality. Water-borne pathogen load represents a risk factor for clinical disease	Articles investigating the level and/or presence of pathogenic amoeba in salmon farms operating at different salinity levels were included
	Articles were be excluded if they discussed the effects of a treatment on an aspect of fish biology, such as cognition, that cannot be directly linked to an effect on the animal's welfare state	Studies which look at the effect of environmental features on the cognitive ability (such as spatial learning) of the fish were excluded because it is difficult to determine how spatial learning may affect a fish's welfare state

Sub-criteria	Example
In vitro studies were excluded as they do not provide direct evidence of the welfare state in live animals. They are only able to provide background knowledge to understanding the effects of disease or conditions on welfare state	In vitro studies looking at the effects of heavy metals on salmonid kidneys cell were not included
Investigations of therapies, such as vaccines, that do not include measures of fish status were excluded	Studies that explored the effectiveness of sea lice treatment in a laboratory setting which describe the effects the treatment had on the sea lice but did not reference reductions in sea lice burden or another effect on the fish were excluded. However, articles which tested a therapeutic treatment in a population of fish and described the outcomes with regards to the fish were included

2. 5. Data extraction and analysis

A data-charting form was developed to guide the extraction of relevant information pertaining to measurements and observations that could potentially be used to evaluate the welfare of salmon reared in an aquaculture setting. These data were collected from the methods and result sections of relevant articles. Information found in the introduction and discussion sections of articles were collected to understand the aim of the study as well as the study's use of the concept of 'animal welfare'.

Data were extracted by one reviewer (IN) into an Excel spreadsheet developed based on the data-charting form (Appendix A). The spreadsheet guided extraction of relevant article characteristics (e.g., author, title, and year of publication), study characteristics and contextual information (e.g., type of study and study location), population characteristics (e.g., species studied, level of observation, and age of animals) as well as the study's use of the concept of 'animal welfare'. If the terms 'welfare' or 'wellbeing' were featured in an article, it was noted where in the article it featured and whether the terms were defined or described.

When welfare indicators were extracted from each article, it was noted whether the indicators were animal-based or resource-based. Also, if indicators were animal-based, whether they were sampled post-mortem or ante-mortem. The potential welfare outcomes were also extracted if available (e.g., related physical/functional condition and affective experience).

The number and percentage of studies containing the specific characteristics mentioned above are presented in tabular form. Percentage accuracy was also calculated for each online database using the number of relevant articles retrieved after the full-text screen and the number of articles retrieved in the initial database search. The percentage accuracy equation is presented below:

$$Percentage\ accuracy\ (\%) = \left(\frac{number\ of\ relevant\ articles}{number\ of\ retrieved\ articles}\right) \times 100$$

2. 6. Synthesis of results

2. 6. 1. Summarisation of article characteristics

Descriptive summaries and visualisations of the charted data were generated using Excel 2016. A histogram based on the number of articles published per month from 2015 to 2021 was produced to visualise trends in the publication of salmon welfare-related research. A second histogram, standardised to the number of articles published per year, was produced to visualise trends in the publication of research which explicitly mentions 'welfare'.

2. 6. 2. Categorising welfare indicators using the Five Domains Model

The list of measurements and observations extracted from each article was refined into a list of unique over-arching welfare indicators. Measurements and observations which evaluated the same aspect of fish welfare were consolidated. For example, specific histopathological changes observed on the gills such as lamellar fusion, clubbing and micro abscesses were combined into the welfare indicator 'histopathological changes in tissues'. Similarly, specific components added to salmon diets to improve growth performance such as fish oil, spray dried algae and fish meal were combined into the welfare indicator 'diet additives'. The full list of extracted welfare indicators is presented in Appendix C.

Using the latest iteration of the Five Domains Model (Mellor et al., 2020), welfare indicators identified in the review were grouped into one of four physical/functional domains. These indicators may be considered to provide evidence of potential welfare impacts in terms of the fish's nutritional status (Domain 1), physical environmental conditions (Domain 2), health conditions (Domain 3), and its interactions with the environment, other non-human animals, and humans (Domain 4) (Mellor et al., 2020).

In this review, welfare indicators were not grouped into Domain 5, 'Mental state', which represents the likely mental experiences of an animal which arise from impacts in the first four physical/functional domains (Mellor et al., 2020). It is not possible to directly access and measure mental experiences due to their internal, subjective nature. The function of welfare indicators is to provide evidence of impacts in the first four physical/functional domains that can be used to indirectly evaluate the likely mental experiences of an animal (Beausoleil & Mellor, 2017). Therefore, Domain 5 was not used as a category for welfare indicators.

3. Results

3. 1. Selection of sources of evidence

A total of 8981 potentially relevant articles were retrieved from the online database search (Figure 7). Following removal of duplicates, 5944 articles were retained for relevance screening. After screening the title and abstracts of retained articles, 1966 articles were eligible for full-text screening. After limiting to articles published between 2015-2021 (see Appendix B), 802 remained. Full-text screening resulted in a further 208 articles being excluded. Full-text screening thus identified 594 articles relevant to the objectives the review, of which a sub-sample of 60 articles (10%) was randomly selected for data extraction.

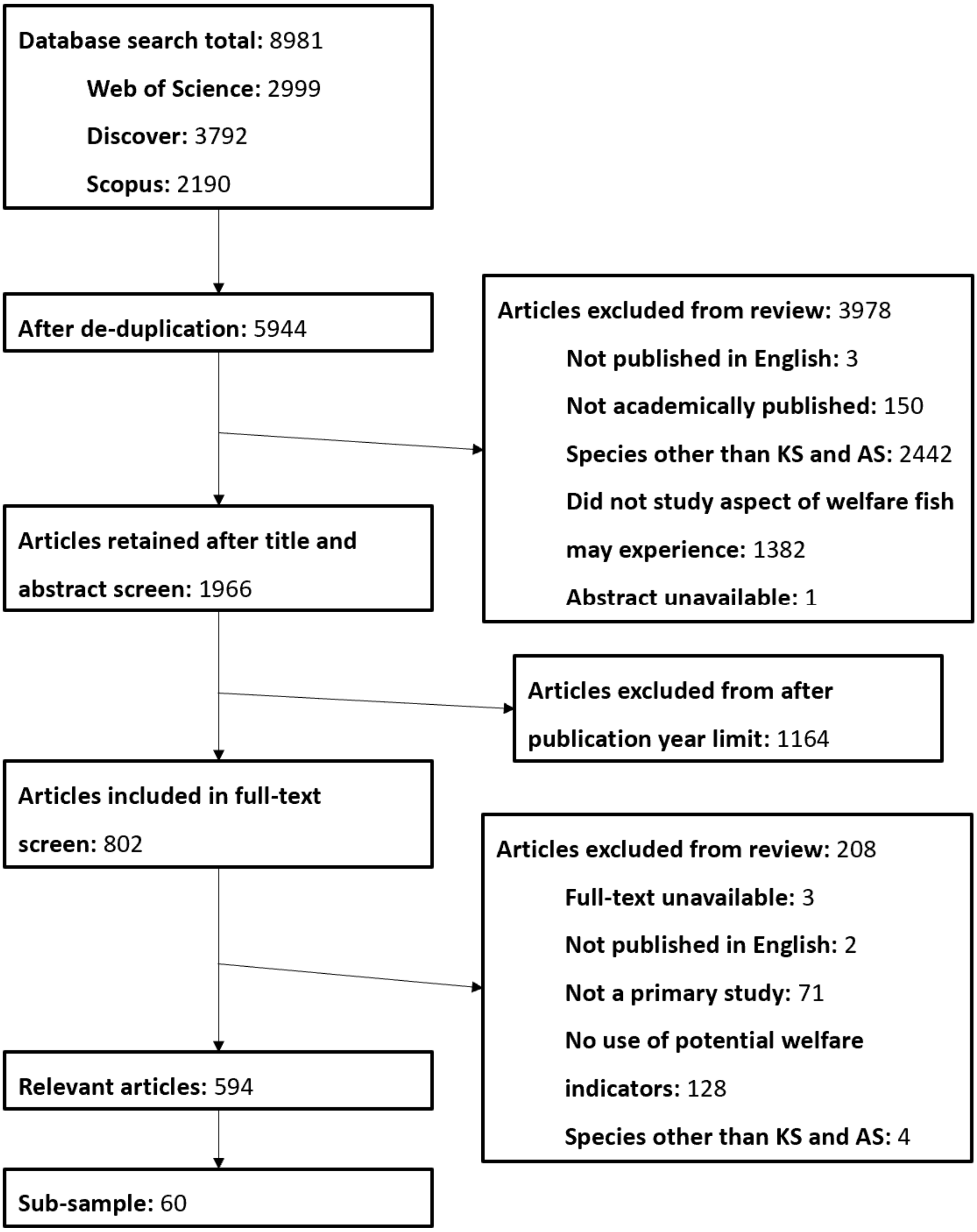

Figure 7. Flowchart describing the inclusion and exclusion of articles through the review process, including reasons for exclusion. King salmon = KS and Atlantic salmon = AS.

The percentage accuracy of each online database was calculated using the number of retrieved and relevant articles from each (Table 5). Discover retrieved the largest number of relevant articles, however it had the lowest calculated percentage accuracy. The highest percentage accuracy was calculated for Scopus, followed by Web of Science.

Table 5. Percentage (%) accuracy calculated from the number of retrieved and relevant articles from each online database.

Database	Retrieved articles	Relevant articles	Percentage accuracy
Web of Science	2999	343	11
Discover	3792	418	11
Scopus	2190	332	15

3. 2. Characteristics of relevant published literature on salmon welfare

Extracted articles characteristics are summarised in Table 6. Due to the sub-sampling approach, all 60 articles were published between the years 2015 and 2021. The number of articles published per month was calculated for each year (Figure 8). There is an increasing trend in the number of articles published per month from 2015 to 2021. The highest number of articles published per month was calculated for 2021, with a marked jump from one article per month in 2020 to two articles per month in 2021. Article retrieval for this review ceased late April 2021. However, if article retrieval concluded at the end of 2021, it is likely that 24 relevant articles would have been published that year.

Of the 60 articles, the largest number of studies were conducted in Norway (22; 36%). Only one study was conducted in New Zealand. One article conducted studies in two countries: Norway and another in Australia. Over half of the articles studied fish under a laboratory setting (39; 65%), while 35% (21) studied fish in an aquaculture setting. A majority of the articles were categorised as experimental studies (43; 70%). Most of the articles studied a population of Atlantic salmon (58; 96%), while only 4% (3) investigated populations of King salmon. One article conducted a study on both Atlantic and King salmon populations. The most frequent level of observation was at a laboratory tank-level (33; 55%), with only 5% (3) at an individual level (Table 6).

The lifecycle stages of fish ranged from fry to post-smolt. Smolt were the most common lifecycle stage studied (19; 32%). However, 24 articles (40%) did not explicitly state the lifecycle stage that was being investigated. Fish in these 24 articles were described as being held in production systems which required them to be past the larval stage (e.g., in a marine environment which requires seawater tolerance developed during smoltification). Therefore, although the articles did not explicitly state the lifecycle stage of fish, they described housing fish in environmental conditions relevant to older lifecycle stages and were thus included in the review.

Other article characteristics collected, such as research aims, are reported in Appendix A. Interestingly, 33 of the 60 articles (55%) had aims related to the investigation of infectious diseases in salmon. The most common infectious disease investigated in these 33 articles was sea lice infection (11; 33%), followed by amoebic gill disease (7; 21%). There were also 16 articles (27%) that had aims related to investigation of the effects of diet additives on salmon growth and health.

Table 6. The number (percentage) of articles included in the review (n = 60) grouped by article characteristic including country, year of publication, type of study, context of investigation, species of fish, lifecycle stage, and population size.

Article Characteristic	**Number (%) of Articles (n = 60)**
Country[1]	
Australia	8 (13)
Canada	11 (18)
Chile	8 (13)
China	2 (3)
Faroe Islands (Denmark)	1 (2)
France	1 (2)
Ireland	1 (2)
New Zealand	1 (2)
Norway	22 (36)

Article Characteristic	Number (%) of Articles (n = 60)
Scotland	4 (7)
Sweden	2 (3)
Year of Publication	
2021	8 (13)
2020	12 (20)
2019	11 (18)
2018	8 (13)
2017	6 (10)
2016	9 (15)
2015	6 (10)
Type of Study	
Experimental	43 (70)
Cross-sectional	1 (2)
Prospective	6 (10)
Retrospective	10 (17)
Context of Investigation	
Aquaculture	25 (42)
Laboratory	35 (58)
Species of Fish[2]	
Atlantic salmon	58 (96)
King salmon	3 (4)
Lifecycle stage	
Unknown	24 (40)
Fry	4 (7)
Parr	3 (5)
Juvenile	3 (5)
Pre-smolt	2 (3)
Smolt	19 (32)
Post-smolt	5 (8)
Levels of Observation	
Farm-level	9 (15)
Cage-level	12 (20)
Aquaculture tank-level	3 (5)
Laboratory tank-level	33 (55)
Individual-level	3 (5)

Note: [1]The total number of articles under the characteristic 'country' is equal to 61. This is because one article by Ruyter et al. (2019) reported conducting one study in Australia and another study in Norway. [2]The total number of articles under the characteristic 'species' is equal to 61 because one article by Zalcman et al. (2021) studied both species.

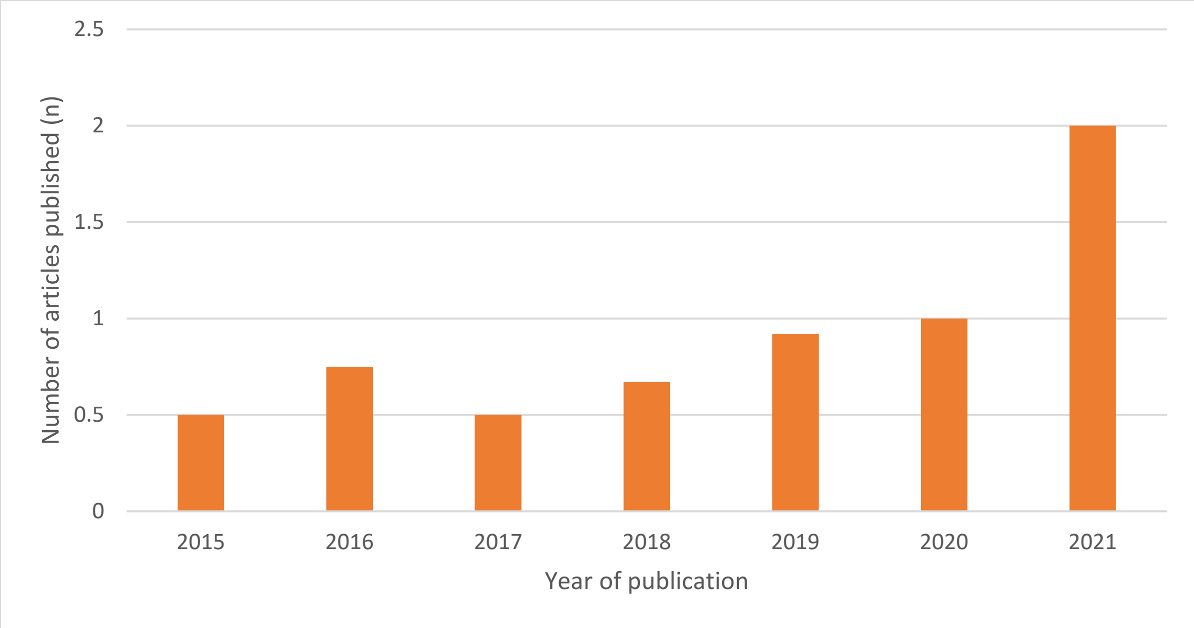

Figure 8. Number of relevant articles (n = 60) published per month by year of publication.

Twenty-six articles (43.3%) mentioned the terms 'welfare' or 'wellbeing'. The frequency distribution of articles which mention the percentage of articles published each year that mention welfare/wellbeing is presented in Figure 9. The distribution is skewed to the left, with sixty-three percent (5 out of 8) of articles published in 2021 mentioning welfare. From 2018 onwards over half the published articles mentioned 'welfare', with a trend that rises yearly. Half of the articles (13; 50%) only mentioned welfare in the introduction and/or methods sections. The other half mentioned welfare either in the discussion alone or the discussion as well as other sections. Of the 26 articles that mentioned the term 'welfare' or 'wellbeing', none characterised or described their conceptualisation of animal welfare.

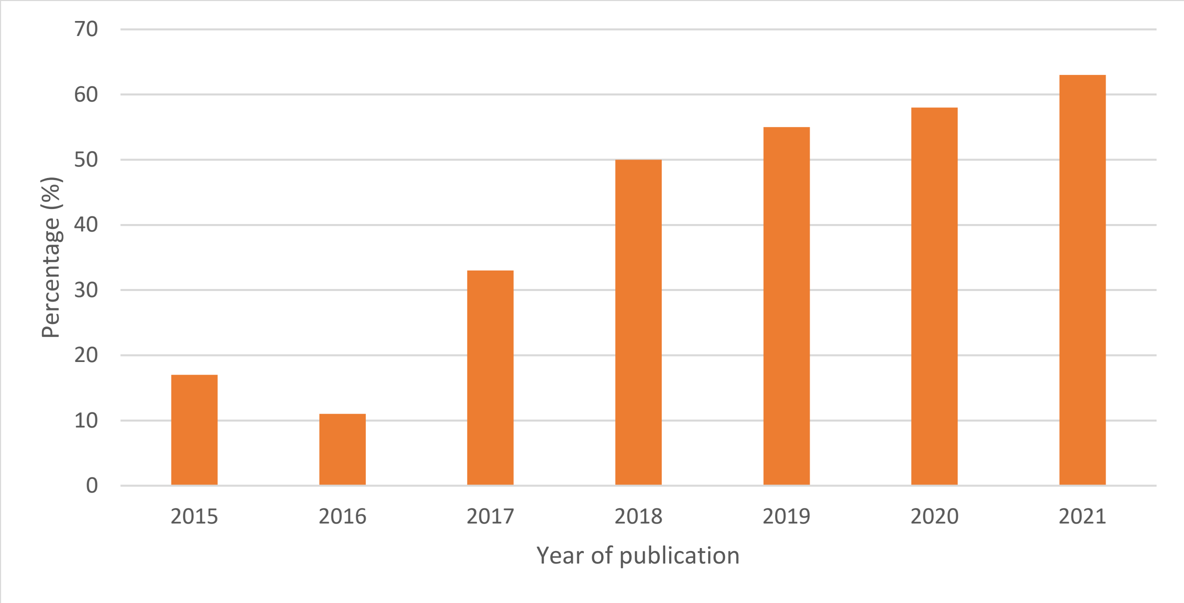

Figure 9. The percentage of articles published each year that mention welfare/wellbeing.

3. 3. Welfare indicators

A total of 112 unique welfare indicators were identified in this review. The number of welfare indicators allocated to each domain, their type (animal-based or resource-based) and sampling time (ante-mortem or post-mortem) are summarised in Table 7. A majority of the welfare indicators were animal-based (n = 66, 59%), with 59% (n = 39) of these sampled after the fish had died (post-mortem).

Table 7. The number of welfare indicators in each domain including the type of indicators and sampling time.

Domain	Number of indicators	Animal-based				Resource-based
		AM	PM	Both	Total	
Nutrition	26	2	15	4	21	5
Physical Environment	25	1	-	-	1	24
Health	56	8	24	7	39	17
Behavioural Interactions	5	5	-	-	5	-
Total	112	16	39	11	66	46

Note: Ante-mortem = AM and post-mortem = PM.

Of the 112 indicators, half (n = 56) were indicators of health/functional status, 26 (23%) were indicators of nutritional status, 25 (22%) related to the physical environment, and five (4%) related to behavioural interaction. The 56 indicators of health/functional status were identified from 55 different articles and included indicators such as mortality (n = 30), blood parameters (n = 20), and histopathology of tissues (n = 17) (Table 8). The 26 indicators of nutritional status were identified from 42 different articles and included indicators such as weight (n = 38), length (n = 19), growth rates (n = 16), diet additives (n = 16), and feed intake (n = 12) (Table 9). Under the physical environment domain, 25 indicators (22%) were identified from 53 different articles. The most common resource-based indicators assigned to the physical environment domain included water temperature (n = 50), salinity (n = 26), light regime (n = 26), and dissolved oxygen (n = 23) (Table 10). Only five indicators were assigned to the behavioural interaction domain, including schooling behaviour (n = 3), escape behaviour (n = 2), and exploratory behaviour (n = 2) (Table 11). Throughout the data extraction process, it was found that there was no explicit investigation of the affective experiences of fish. The term 'stress', however, was mentioned in 27 articles.

Table 8. Animal-based and resource-based welfare indicators assigned to Domain 3: Health. Type of indicator = Animal-based (AB) or Resource-based (RB); Sampling point = Antemortem (AM) or post-mortem (PM). For resource-based indicators sampling point is not relevant.

Welfare Indicator	Type of indictor	Sampling point	Number of studies	References
Mortality	AB	PM	30	Timmerhaus et al. (2021), Romero et al. (2021), Brown et al. (2021), Holborn et al. (2020), Purcell et al. (2020), Fraser et al. (2020), Meyer et al. (2019), Martín et al. (2019), Arriagada et al. (2019), Martinsen et al. (2018), Metochis et al. (2016), (Hauge et al., 2016), Larsen et al. (2015), Sun et al. (2015), Zalcman et al. (2021), Delphino et al. (2021), Wynne et al. (2020), Qian et al. (2020), Frisk et al. (2020), Bui et al. (2020), Wang et al. (2019), Ruyter et al. (2019), Long et al. (2019), Davie et al. (2019), Småge et al. (2018), Leblanc et al. (2018), Downes et al. (2018), Norambuena et al. (2016), Emery et al. (2016), Kousoulaki et al. (2015)
Blood parameters	AB	AM/PM	20	Timmerhaus et al. (2021), Purcell et al. (2020), Poirier et al. (2020), Fraser et al. (2020), Martín et al. (2019), Li et al. (2019), Llewellyn et al. (2017), Andrewartha et al. (2016), Metochis et al. (2016), (Hauge et al., 2016), Sun et al. (2015), Thörnqvist et al. (2015), Zanuzzo et al. (2020), Qian et al. (2020), Wang et al. (2019), Ruyter et al. (2019), Long et al. (2019), Småge et al. (2018), Espe et al. (2016), Kousoulaki et al. (2015)

Welfare Indicator	Type of indictor	Sampling point	Number of studies	References
Presence of pathogenic agent in tissues	AB	PM	20	Su et al. (2021), Romero et al. (2021), Brown et al. (2021), Purcell et al. (2020), Poirier et al. (2020), Holborn et al. (2020), Martín et al. (2019), Wessel et al. (2017), Metochis et al. (2016), Hauge et al. (2016), Delphino et al. (2021), Zanuzzo et al. (2020), Long et al. (2019), Chang et al. (2019), Småge et al. (2018), Leblanc et al. (2018), Downes et al. (2018), Downes et al. (2017), Vanderstichel et al. (2015), Fernandez-Senac et al. (2020)
Histopathology of tissues	AB	PM	17	Timmerhaus et al. (2021), Su et al. (2021), Brown et al. (2021), Purcell et al. (2020), Poirier et al. (2020), Li et al. (2019), Wessel et al. (2017), Remen et al. (2016), Hauge et al. (2016), Silva et al. (2015), Ruyter et al. (2019), Chang et al. (2019), Yossa et al. (2018), Småge et al. (2018), Downes et al. (2018), Downes et al. (2017), Kousoulaki et al. (2015)
Viral load of tissues	AB	PM	13	Su et al. (2021), Brown et al. (2021), Purcell et al. (2020), Holborn et al. (2020), Martín et al. (2019), Wessel et al. (2017), Hauge et al. (2016), Delphino et al. (2021), Chang et al. (2019), Leblanc et al. (2018), Downes et al. (2018), Downes et al. (2017), Fernandez-Senac et al. (2020)
Sea lice count on fish	AB	AM/PM	11	Contreras et al. (2020), Meyer et al. (2019), Arriagada et al. (2019), Llewellyn et al. (2017), Whittaker et al. (2021), Bui et al. (2020), Long et al. (2019), Jevne and Reitan (2019), Marín et al. (2018), Gautam et al. (2017), Samsing et al. (2016)
Presence of pathogenic agent in water	RB		10	Brown et al. (2021), Contreras et al. (2020), Arriagada et al. (2019), Martinsen et al. (2018), Wright et al. (2017), Llewellyn et al. (2017), Whittaker et al. (2021), Wynne et al. (2020), Long et al. (2019), Downes et al. (2017)

Welfare Indicator	Type of indictor	Sampling point	Number of studies	References
Infection treatment regime	RB		8	Martinsen et al. (2018), Martín et al. (2019), Zalcman et al. (2021), Bui et al. (2020), Marín et al. (2018), Jevne and Reitan (2019), Downes et al. (2018), Gautam et al. (2017)
Exposure to infected fish	RB		8	Su et al. (2021), Romero et al. (2021), Holborn et al. (2020), Wessel et al. (2017), Hauge et al. (2016), Småge et al. (2018), Leblanc et al. (2018), Fernandez-Senac et al. (2020)
Gill score	AB	PM	7	Martinsen et al. (2018), Wright et al. (2017), Bui et al. (2020), Chang et al. (2019), Downes et al. (2018), Downes et al. (2017), Fernandez-Senac et al. (2020)
Transcript levels/gene expression for immune related genes	AB	PM	7	Su et al. (2021), Romero et al. (2021), Li et al. (2019), Wessel et al. (2017), Zanuzzo et al. (2020), Wang et al. (2019), Kousoulaki et al. (2015)
Viral load of water	RB		7	Brown et al. (2021), Contreras et al. (2020), Martinsen et al. (2018), Wright et al. (2017), Llewellyn et al. (2017), Whittaker et al. (2021), Long et al. (2019)
Skin damage	AB	AM/PM	6	Timmerhaus et al. (2021), Llewellyn et al. (2017), Hauge et al. (2016), Bui et al. (2020), Long et al. (2019), Småge et al. (2018)
Vaccination status	RB		5	Su et al. (2021), Fraser et al. (2020), Contreras et al. (2020), Metochis et al. (2016), Småge et al. (2018)
Vertebral deformities	AB	AM/PM	4	Timmerhaus et al. (2021), Fraser et al. (2020), Bui et al. (2020), Davie et al. (2019)
Season	RB		4	Poirier et al. (2020), Jevne and Reitan (2019), Gautam et al. (2017), Vanderstichel et al. (2015)
Eye damage	AB	AM	3	Timmerhaus et al. (2021), Fraser et al. (2020), Bui et al. (2020)
Na^+K^+-ATPase Activity	AB	PM	3	Fraser et al. (2020), Frisk et al. (2020), Chang et al. (2019)
Sea lice skirt use	RB		3	Jónsdóttir et al. (2021), Bui et al. (2020), Jevne and Reitan (2019)

Welfare Indicator	Type of indictor	Sampling point	Number of studies	References
Snout damage	AB	AM/PM	3	Timmerhaus et al. (2021), Småge et al. (2018), Wynne et al. (2020)
Viral load of blood	AB	PM	3	Wessel et al. (2017), Hauge et al. (2016), Leblanc et al. (2018)
Cleaner fish density	RB		2	Bui et al. (2020), Jevne and Reitan (2019)
Emergence time	RB		2	Larsen et al. (2015), Thörnqvist et al. (2015)
Fin damage	AB	AM	2	Timmerhaus et al. (2021), Bui et al. (2020)
Jaw deformities	AB	AM	2	Timmerhaus et al. (2021), Bui et al. (2020)
Latitude	RB		2	Meyer et al. (2019), Zalcman et al. (2021)
Microbial composition of mucus	AB	AM	2	Llewellyn et al. (2017), Wynne et al. (2020)
Microbial composition of tissues	AB	PM	2	Brown et al. (2021), Poirier et al. (2020)
Opercula damage	AB	AM/PM	2	Timmerhaus et al. (2021), Bui et al. (2020)
Ploidy	AB	AM	2	Brown et al. (2021), Sun et al. (2015)
Presence of pathogenic agent in blood	AB	PM	2	Wessel et al. (2017), Hauge et al. (2016)
Sea-way distance from infected farm	RB		2	Meyer et al. (2019), Vanderstichel et al. (2015)
Sexual maturation state	AB	AM/PM	2	Fraser et al. (2020), Bui et al. (2020)
Transcript levels/gene expression for inflammation genes	AB	PM	2	Timmerhaus et al. (2021), Ruyter et al. (2019)
Transcript levels/gene expression for oxidative stress	AB	PM	2	Ruyter et al. (2019), Kousoulaki et al. (2015)
Transcript levels/gene expression for stress	AB	PM	2	Thörnqvist et al. (2015), Ruyter et al. (2019)
Biofilm microbiome	RB		1	Llewellyn et al. (2017)

Welfare Indicator	Type of indictor	Sampling point	Number of studies	References
Brain monoamine levels	AB	PM	1	Thörnqvist et al. (2015)
Cardiac pathology	AB	PM	1	Frisk et al. (2020)
Disease prevalence	AB	PM	1	Wright et al. (2017)
Fish temperature	RB		1	Poirier et al. (2020)
Infection behaviour	AB	AM	1	Llewellyn et al. (2017)
Liver enzyme activity	AB	PM	1	Ruyter et al. (2019)
Liver proteome	AB	PM	1	Nuez-Ortin et al. (2016)
Microbial composition of faeces	AB	PM	1	Neuman et al. (2018)
Microbial composition of marine aggregates	RB		1	Poirier et al. (2020)
Microbial composition of water	RB		1	Llewellyn et al. (2017)
Presence of pathogenic agent in faeces	AB	AM	1	Hauge et al. (2016)
Presence of pathogenic agent in mucus	AB	PM	1	Wynne et al. (2020)
Smolt index	AB	AM	1	Frisk et al. (2020)
Smolt production protocol	RB		1	Frisk et al. (2020)
Salmonid rickettsial syndrome necropsy	AB	PM	1	Martín et al. (2019)
Time since entering seawater	RB		1	Meyer et al. (2019)
Transcript levels/gene expression for detoxification genes	AB	PM	1	Li et al. (2019)

Welfare Indicator	Type of indictor	Sampling point	Number of studies	References
Transcript levels/gene expression for erythrocyte genes	AB	PM	1	Kousoulaki et al. (2015)
Transcript levels/gene expression for heart pathology genes	AB	PM	1	Frisk et al. (2020)
Total			55	

Table 9. Animal-based and resource-based welfare indicators assigned to Domain 1: Nutrition. Type of indicator = Animal-based (AB) or Resource-based (RB); Sampling point = Antemortem (AM) or post-mortem (PM). For resource-based indicators sampling point is not relevant.

Welfare indicator	Type of indicator	Sampling point	Number of studies	References
Weight	AB	AM/PM	38	Timmerhaus et al. (2021), Romero et al. (2021), Fraser et al. (2020), Holborn et al. (2020), Johannesen et al. (2020), Martinsen et al. (2018), Lerfall et al. (2016), Harvey et al. (2016), Fernandez-Senac et al. (2020), Norambuena et al. (2016), Belghit et al. (2018), Wessel et al. (2017), Llewellyn et al. (2017), Metochis et al. (2016), Yossa et al. (2018), Chang et al. (2019), Davie et al. (2019), Wang et al. (2019), (Qian et al., 2020), Zanuzzo et al. (2020), Hauge et al. (2016), Li et al. (2019), Nuez-Ortin et al. (2016), Espe et al. (2016), Neuman et al. (2018), Ruyter et al. (2019), Bui et al. (2020), Jónsdóttir et al. (2021), Aslam et al. (2019), Arriagada et al. (2019), Gu et al. (2015), Sun et al. (2015), Thörnqvist et al. (2015), Frisk et al. (2020), Martín et al. (2019), Wynne et al. (2020), Zalcman et al. (2021), Larsen et al. (2015)

Welfare indicator	Type of indicator	Sampling point	Number of studies	References
Length	AB	AM/PM	19	Romero et al. (2021), Fraser et al. (2020), Lerfall et al. (2016), Harvey et al. (2016), Fernandez-Senac et al. (2020), Norambuena et al. (2016), Belghit et al. (2018), Llewellyn et al. (2017), Metochis et al. (2016), Chang et al. (2019), Wang et al. (2019), Qian et al. (2020), Zanuzzo et al. (2020), Nuez-Ortin et al. (2016), Kousoulaki et al. (2015), Poirier et al. (2020), Bui et al. (2020), Frisk et al. (2020), Larsen et al. (2015)
Diet additives	RB		16	Nuez-Ortin et al. (2016), Ruyter et al. (2019), Lerfall et al. (2016), Emery et al. (2016), Kousoulaki et al. (2015), Belghit et al. (2018), Yossa et al. (2018), Metochis et al. (2016), Romero et al. (2021), Neuman et al. (2018), Norambuena et al. (2016), Li et al. (2019), Sun et al. (2015), Emery et al. (2016), Qian et al. (2020), Silva et al. (2015)

Welfare indicator	Type of indicator	Sampling point	Number of studies	References
Growth rates	AB	AM/PM	16	Timmerhaus et al. (2021), Romero et al. (2021), Fraser et al. (2020), Llewellyn et al. (2017), Metochis et al. (2016), Larsen et al. (2015), Sun et al. (2015), Qian et al. (2020), Frisk et al. (2020), Ruyter et al. (2019), Wang et al. (2019), Yossa et al. (2018), Belghit et al. (2018), Nuez-Ortin et al. (2016), Norambuena et al. (2016), Emery et al. (2016), Kousoulaki et al. (2015)
Viscero-somatic indices	AB	PM	13	Timmerhaus et al. (2021), Emery et al. (2016), Belghit et al. (2018), Li et al. (2019), Zanuzzo et al. (2020), Nuez-Ortin et al. (2016), Frisk et al. (2020), Ruyter et al. (2019), Espe et al. (2016), Norambuena et al. (2016), Yossa et al. (2018), Kousoulaki et al. (2015), Sun et al. (2015)
Feed intake	RB		12	Romero et al. (2021), Norambuena et al. (2016), Emery et al. (2016), Belghit et al. (2018), Metochis et al. (2016), Yossa et al. (2018), Qian et al. (2020), Li et al. (2019), Nuez-Ortin et al. (2016), Kousoulaki et al. (2015), Sun et al. (2015), Martín et al. (2019)

Welfare indicator	Type of indicator	Sampling point	Number of studies	References
Feed conversion ratio	AB	PM	11	Romero et al. (2021), Li et al. (2019), Metochis et al. (2016), Sun et al. (2015), Wang et al. (2019), Yossa et al. (2018), Belghit et al. (2018), Nuez-Ortin et al. (2016), Norambuena et al. (2016), Emery et al. (2016), Kousoulaki et al. (2015)
Condition factor	AB	PM	10	Timmerhaus et al. (2021), Fraser et al. (2020), Li et al. (2019), (Lerfall et al., 2016), Frisk et al. (2020), Bui et al. (2020), Belghit et al. (2018), Nuez-Ortin et al. (2016), Emery et al. (2016), Kousoulaki et al. (2015)
Weight gain	AB	PM	6	Metochis et al. (2016), Wang et al. (2019), Espe et al. (2016), Aslam et al. (2019), Sun et al. (2015), Norambuena et al. (2016)
Biomass	AB	AM/PM	5	Metochis et al. (2016), Jónsdóttir et al. (2021), Ruyter et al. (2019), Downes et al. (2017), Espe et al. (2016)
Emaciation	AB	AM	3	Timmerhaus et al. (2021), Fraser et al. (2020), Bui et al. (2020)

Welfare indicator	Type of indicator	Sampling point	Number of studies	References
Transcript levels/gene expression for lipid metabolism	AB	PM	3	Timmerhaus et al. (2021), Li et al. (2019), Ruyter et al. (2019)
PER/LER[1]	AB	PM	3	Belghit et al. (2018), Kousoulaki et al. (2015), Espe et al. (2016)
Transcript levels/gene expression for muscle growth rates	AB	PM	3	Timmerhaus et al. (2021), Kousoulaki et al. (2015), Espe et al. (2016)
Intestinal content	AB	PM	2	Li et al. (2019), Hauge et al. (2016)
Stomach content	AB	PM	2	Hauge et al. (2016), Martín et al. (2019)
Amount of feed offered	RB		1	Sun et al. (2015)
Faecal viscosity	AB	PM	1	Sun et al. (2015)
Body contour	AB	AM	1	Timmerhaus et al. (2021)
Liver nutrient concentrations	AB	PM	1	Ruyter et al. (2019)
Pellets at bottom of tank	RB		1	Zanuzzo et al. (2020)
Pellet size	RB		1	Larsen et al. (2015)

Welfare indicator	Type of indicator	Sampling point	Number of studies	References
Proteolytic enzyme activity	AB	PM	1	Espe et al. (2016)
Transcript levels/gene expression for metabolism genes	AB	PM	1	Timmerhaus et al. (2021)
Trypsin activity	AB	PM	1	Belghit et al. (2018)
Total			43	

Note: [1]PER/LER = protein/lipid efficiency ratio.

Table 10. Animal-based and resource-based welfare indicators assigned to Domain 2: Physical Environment. Type of indicator = Animal-based (AB) or Resource-based (RB); Sampling point = Antemortem (AM) or post-mortem (PM). For resource-based indicators sampling point is not relevant.

Welfare indicator	Type of indictor	Sampling point	Number of studies	References
Water temperature	RB		50	Timmerhaus et al. (2021), Romero et al. (2021), Brown et al. (2021), Purcell et al. (2020), Poirier et al. (2020), Fraser et al. (2020), (Contreras et al., 2020), Meyer et al. (2019), Martín et al. (2019), Aslam et al. (2019), Arriagada et al. (2019), Martinsen et al. (2018), Wright et al. (2017), Llewellyn et al. (2017), Andrewartha et al. (2016), Metochis et al. (2016), Hauge et al. (2016), Larsen et al. (2015), Sun et al. (2015), Silva et al. (2015), Thörnqvist et al. (2015), Zalcman et al. (2021), Whittaker et al. (2021), Zanuzzo et al. (2020), Wynne et al. (2020), Qian et al. (2020), Frisk et al. (2020), Bui et al. (2020), Wang et al. (2019), Ruyter et al. (2019), Long et al. (2019), Jevne and Reitan (2019), Davie et al. (2019), Chang et al. (2019), Yossa et al. (2018), Småge et al. (2018), Neuman et al. (2018), Marín et al. (2018), Leblanc et al. (2018), Downes et al. (2018), Gautam et al. (2017), Downes et al. (2017), Samsing et al. (2016), Nuez-Ortin et al. (2016), Norambuena et al. (2016), Harvey et al. (2016), Espe et al. (2016), Emery et al. (2016), Kousoulaki et al. (2015), Fernandez-Senac et al. (2020)

Welfare indicator	Type of indictor	Sampling point	Number of studies	References
Light regime	RB		26	Timmerhaus et al. (2021), Su et al. (2021), Romero et al. (2021), Fraser et al. (2020), Brown et al. (2021), Martinsen et al. (2018), Harvey et al. (2016), Norambuena et al. (2016), Whittaker et al. (2021), Leblanc et al. (2018), Småge et al. (2018), Wessel et al. (2017), Metochis et al. (2016), Yossa et al. (2018), Wang et al. (2019), Zanuzzo et al. (2020), Silva et al. (2015), Li et al. (2019), Nuez-Ortin et al. (2016), Espe et al. (2016), Ruyter et al. (2019), Bui et al. (2020), Sun et al. (2015), Thörnqvist et al. (2015), Frisk et al. (2020), Larsen et al. (2015)
Salinity	RB		26	(Timmerhaus et al., 2021), Romero et al. (2021), Brown et al. (2021), Wright et al. (2017), Martinsen et al. (2018), Marín et al. (2018), Fernandez-Senac et al. (2020), Samsing et al. (2016), Whittaker et al. (2021), Emery et al. (2016), Småge et al. (2018), Wessel et al. (2017), Llewellyn et al. (2017), Yossa et al. (2018), Chang et al. (2019), Wang et al. (2019), Silva et al. (2015), Long et al. (2019), Kousoulaki et al. (2015), Poirier et al. (2020), Neuman et al. (2018), Bui et al. (2020), Aslam et al. (2019), Arriagada et al. (2019), Sun et al. (2015), Martín et al. (2019)
Dissolved oxygen	RB		23	Timmerhaus et al. (2021), Fraser et al. (2020), Norambuena et al. (2016), Emery et al. (2016), Leblanc et al. (2018), Småge et al. (2018), Llewellyn et al. (2017), Metochis et al. (2016), Yossa et al. (2018), Chang et al. (2019), Wang et al. (2019), Qian et al. (2020), Zanuzzo et al. (2020), Silva et al. (2015), Hauge et al. (2016), Nuez-Ortin et al. (2016), Poirier et al. (2020), Neuman et al. (2018), Meyer et al. (2019)05, Aslam et al. (2019), Sun et al. (2015), Thörnqvist et al. (2015), Martín et al. (2019)

Welfare indicator	Type of indictor	Sampling point	Number of studies	References
Water velocity	RB		16	Timmerhaus et al. (2021), Romero et al. (2021), Johannesen et al. (2020), Norambuena et al. (2016), Samsing et al. (2016), Småge et al. (2018), Metochis et al. (2016), Silva et al. (2015), Hauge et al. (2016), Nuez-Ortin et al. (2016), Kousoulaki et al. (2015), Ruyter et al. (2019), Jónsdóttir et al. (2021), Aslam et al. (2019), Martín et al. (2019), Larsen et al. (2015)
Stocking density	RB		14	Fraser et al. (2020), Wright et al. (2017), Johannesen et al. (2020), Martinsen et al. (2018), Lerfall et al. (2016), Llewellyn et al. (2017), Yossa et al. (2018), Wang et al. (2019), Silva et al. (2015), Kousoulaki et al. (2015), Poirier et al. (2020), Larsen et al. (2015), Martín et al. (2019), Zalcman et al. (2021),
Water nitrogen oxide concentrations	RB		8	Timmerhaus et al. (2021), Metochis et al. (2016), Yossa et al. (2018), Qian et al. (2020), Zanuzzo et al. (2020), Nuez-Ortin et al. (2016), Aslam et al. (2019), Norambuena et al. (2016)
Water pH	RB		8	Timmerhaus et al. (2021), Norambuena et al. (2016), Metochis et al. (2016), Yossa et al. (2018), Qian et al. (2020), Nuez-Ortin et al. (2016), Aslam et al. (2019), Sun et al. (2015)
Water ammonia concentration	RB		6	Timmerhaus et al. (2021), Metochis et al. (2016), Yossa et al. (2018), Wang et al. (2019), Qian et al. (2020), Zanuzzo et al. (2020)
Depth distribution of fish in cage/tank	AB	AM	5	Timmerhaus et al. (2021), Johannesen et al. (2020), Wright et al. (2017), Bui et al. (2020), Samsing et al. (2016)
Total water ammonia concentration	RB		3	Aslam et al. (2019), Qian et al. (2020), Wang et al. (2019)
Water alkalinity	RB		3	Aslam et al. (2019), Qian et al. (2020), Yossa et al. (2018)

Welfare indicator	Type of indictor	Sampling point	Number of studies	References
Turbidity	RB		2	Poirier et al. (2020), Aslam et al. (2019)
Water ammonium concentration	RB		2	Yossa et al. (2018), Norambuena et al. (2016)
Water carbon dioxide	RB		2	Timmerhaus et al. (2021), Aslam et al. (2019)
Water volume	RB		2	Aslam et al. (2019), Thörnqvist et al. (2015)
Cumulative degree-days	RB		1	Zalcman et al. (2021)
Net cage characteristics	RB		1	Jónsdóttir et al. (2021)
Social rearing environment	RB		1	Larsen et al. (2015)
Total suspended solids	RB		1	Aslam et al. (2019)
Water conductivity	RB		1	Aslam et al. (2019)
Water elemental concentrations	RB		1	Aslam et al. (2019)
Water redox potential	RB		1	Aslam et al. (2019)
Wave height	RB		1	Johannesen et al. (2020)

Welfare indicator	Type of indictor	Sampling point	Number of studies	References
Wind speed	RB		1	Jevne and Reitan (2019)
Total			53	

Table 11. Animal-based and resource-based welfare indicators assigned to Domain 4: Behavioural interactions. Type of indicator = Animal-based (AB) or Resource-based (RB); Sampling point = Antemortem (AM) or post-mortem (PM). For resource-based indicators sampling point is not relevant.

Welfare Indicator	Type of indicator	Sampling point	Number of studies	References
Schooling behaviour	AB	AM	3	Timmerhaus et al. (2021), Johannesen et al. (2020), Bui et al. (2020)
Escape behaviour	AB	AM	2	Larsen et al. (2015), Thörnqvist et al. (2015)
Exploratory behaviour	AB	AM	2	Larsen et al. (2015), Thörnqvist et al. (2015)
Locomotion	AB	AM	1	Larsen et al. (2015)
Cleaner-client behaviour	AB	AM	1	Whittaker et al. (2021)
Total			6	

4. Discussion

Similar to other animal-based industries, aquaculture production systems and husbandry practices have the potential to influence the welfare of farmed salmon. There is a need to evaluate and monitor the welfare of individuals in these systems to ensure their welfare is protected and enhanced where possible. Therefore, the aim of this scoping review was to examine globally published literature to identify animal- and resource-based measurements/observations that could be used as indicators of the welfare state of commercially farmed salmon in New Zealand.

4. 1. Characterising the dataset

Most articles did not interpret their results in terms of fish welfare. Only forty-three percent of articles mentioned welfare at all - likely related to the way the search strategy was conducted. The search strategy focused on retrieving information relevant to the assessment of fish welfare as opposed to retrieving information explicitly on fish welfare. Although many of the articles included in this review did not set out to assess fish welfare, many papers reported work related to fish welfare and were thus included in the review. Of the papers that did put some emphasis on welfare, only half provided any discussion of what their findings meant in terms of fish welfare. Limited specific focus on welfare was also previously reported in a systematic review of on-animal sensor technology used in sheep research, which reported that only 6.1 % of the article dataset focused on monitoring welfare (Fogarty et al., 2018). In contrast, Rowe et al. (2019) reported a major focus on animal health and welfare in their review of precision livestock farming in the poultry sector. This is likely due to the combination of the terms health and welfare – results may have differed if the two terms were separated.

While welfare may not have been the explicit focus of many articles, of those that did mention welfare in their discussion, none provided a clear characterisation or description of their conceptualisation of animal welfare. Without a clear characterisation of animal welfare, the interpretation of results with regards to the impact on the animal's wellbeing can vary among people, potentially leading to conflicting real world actions (e.g., welfare impacts and regulation of gestation stalls for pigs (Fraser, 2003)). This indicates a need to collaborate with animal welfare scientists to provide guidance and a clear animal welfare framework from which to interpret findings.

The indicators identified focused mainly on welfare impacts in the health, nutrition, and physical environment domains. The focus on these domains suggests a biological functioning approach to the understanding and assessment of salmon welfare. This is consistent with expectation for several reasons. Firstly, a biological function focus may relate to the search strategy used, resulting in the retrieval of many papers focused on fish production rather than fish welfare explicitly. Secondly, it is reflective of the current state of thinking within the aquaculture industry which is focused on efficient production (Segner et al., 2019; Størkersen et al., 2021). Thirdly, indicators in the health, nutrition and physical environment domains reflect what is technically possible to measure in aquatic animals (Segner et al., 2019; Winckler, 2019).

Next, I discuss indicators in terms of their assignment to different domains within the Five Domains framework.

4. 2. Health domain

Half of the identified indicators were categorised as indicators of health status (Domain 3), with the majority being post-mortem, animal-based indicators (Table 8). The prevalence of post-mortem indicators may be attributed to several factors. Firstly, ease of handling. Similar to the use of anaesthetics to immobilise fish during husbandry procedures, sampling after death removes the challenges associated with handling a conscious, moving animal (Schroeder et al., 2021). Likewise, sampling after death prevents unnecessary stress on the animal itself. Handling of conscious fish during husbandry procedures is associated with negative impacts on fish welfare (Delfosse et al., 2021). Lastly, invasive sampling techniques may result in functional impairment or death of live fish. For example, sampling may require the removal of organs such as the liver and kidney, or result in severe tissue damage which could leave the animal functionally impaired (Noble et al., 2018). A feature, and limitation, of post-mortem indicators is that they can only provide evidence of a previous issue in the animal sampled. It is too late to mitigate any welfare issues for the individual. However, the indicator may point to a potential welfare problem within the wider population. This can be exemplified by the indicator 'mortality' below.

Mortality was the most common indicator mentioned among all studies. Increased rates of mortality point to a systematic welfare problem within a population. Mortality of farmed fish can occur as a result of a number of causes including severe infection (Dahle et al., 2020) and poor water quality (e.g., low oxygen and high temperature) (Fivelstad et al., 2003). There is potential for the animal to have suffered prior to death (Boulton et al., 2018; Ellis, Berrill, et al., 2012). However, mortality alone is unable to provide information on the likely suffering before death. It instead provides an indication of a potential problem within the farming unit. A necropsy of mortalities is required to determine cause of death and welfare assessments should be performed on individuals remaining in the population to identify potential issues. Notably, the absence of mortality or a low mortality rate is not an indicator of good welfare. Thus, mortality is referred to as a crude indicator of welfare state (Ellis, Berrill, et al., 2012).

Ante- and post-mortem indicators provide different information about fish welfare. For example, increased plasma cortisol measured antemortem may provide evidence of current mental experiences such as fear. Plasma cortisol is released in response to a real or perceived threat, initiating physiological and behavioural responses which prepare the animal to respond to perceived danger (Ellis, Yildiz, et al., 2012). Elevated cortisol levels can be measured in the blood within minutes of threat perception, allowing relatively rapid detection of 'stressed' fish and indication of a potential negative affective state (dependent on the stimulus and the animal's perception of it) at the time of sampling. In contrast, plasma cortisol measured post-mortem can only provide evidence of previous physiological stress and associated mental experience. Post-mortem measurement cannot provide evidence of current mental experience because the animal is no longer conscious and therefore unable to perceive (Mellor, 2019). Post-mortem measures can, at best, provide evidence of a previous mental experience and direct attention towards potential welfare problems in the wider population.

Plasma cortisol measures were the most common blood parameter among retrieved articles. There are, however, limitations to cortisol measurements sampled both ante- or post-mortem. Ante-mortem measurements alone provide limited information about the affective quality of an animal's experience. The affective quality associated with ante-mortem measurements of cortisol is context dependent. Elevated cortisol may result from the presence of a predator associated with fear or may result from playful interactions associated with excitement (Part et al., 2014). Ante-mortem measurements should be used alongside behavioural observations to understand specific welfare impacts on the animal (Ellis, Yildiz, et al., 2012). Contextual information should also be used when interpreting post-mortem cortisol measurements as elevated cortisol levels may be associated with the specific slaughter method (e.g., electrical stunning (Gräns et al., 2016)) as opposed to a separate welfare impact prior to slaughter. Caution should be taken when interpreting cortisol measures alone with regards to affective experiences.

Infection treatment regime as a resource-based indicator can reflect both negative and positive impacts on salmon welfare. Infection treatment regimens are implemented to alleviate and/or prevent infection of fish and the associated welfare impacts of infection such as inflammatory lesions associated with cardiomyopathy syndrome (CMS) (Su et al., 2021) and winter ulcer disease (de O. Roberti Filho et al., 2019). However, high vaccine doses and/or high numbers of repeated treatments have been shown to increase fish mortality rate (Meyer et al., 2019; Overton et al., 2019). In other production systems it is not expected that animals die as a result of a preventative treatment. Elevated mortalities suggests that farmed fish may be under high amounts of physiological stress such that an addition of another stressor can be lethal (Meyer et al., 2019). The mortality seen in fish may also be due to increased physiological work associated with mounting an immune response (Fraser et al., 2020; Martin et al., 2010) or due to repeated handling for treatment or vaccination (Overton et al., 2019). As the application of treatment is a resource-based indicator, there is a need to look directly at the animal to ascertain *if* there is a welfare impact and *what* that welfare impact may be.

4. 3. Nutrition domain

The most commonly used indicators of nutritional status (Domain 1) are morphometric indicators. Short-term and long-term measures of nutritional status are relevant to animal welfare and can be measured using different indicators. Morphometric indicators (e.g., bodyweight, length, growth rates, viscero-somatic indices, and condition factor) are better indicators of long-term nutritional status (Losada-Espinosa et al., 2018). Increases in morphometric indices are often used as measures of good productivity. However, very low or high morphometric indices can be indicative of an animal's increased susceptibility to other insults/welfare impacts such as infection and competition (Noble et al., 2018). For example, high growth rates and cardiac somatic index values may reflect good nutrition and growth of fish (Frisk et al., 2020; Timmerhaus et al., 2021). However, high values of these indicators in young salmon are also associated with cardiac deformities and CMS-related cardiac rupture as Atlantic salmon mature (Frisk et al., 2020). The reverse of this may also be true, whereby non-nutritional factors (i.e., factors not specific to nutrition) can influence morphometric parameters. For example, exposure to warm, hypoxic environmental conditions combined with saline injections has been shown to decrease spleen somatic index (Zanuzzo et al., 2020) and is associated with emaciation in Atlantic salmon (Fraser et al., 2020). It is not known what morphometric indicators mean in terms of affective experiences for fish at this time. Most morphometric indicators were sampled post-mortem. Once again, there is a potential issue of prolonged negative experience prior to death, and inability to make changes to correct potential issues.

4. 4. Physical environment domain

It is appropriate that indicators in this domain were mostly resource-based indicators of water quality. These features of the environment have significant impacts on fish welfare affecting the animal's respiration as well as osmotic and thermal regulation (Stien et al., 2020). Salmon operate within optimal ranges of gaseous and elemental concentrations, as well as temperature levels. Prolonged exposure to sub-optimal carbon dioxide and water pH levels is associated with elevated physiological stress responses, increased prevalence of gill pathologies and elevated cumulative mortality rates in Atlantic salmon (Fivelstad et al., 2003). As poikilotherms, the metabolism of salmon fluctuates with changes in water temperature. An acute rise in water temperature can result in increased oxygen consumption, and elevated activity levels as fish move to locate cooler water (Noble et al., 2018; Timmerhaus et al., 2021). The impacts of water quality parameters are described in the literature in terms of growth and survival. It is not yet known the affective experiences of these impacts on fish, but it is likely that fish may experience forms of respiratory and thermal discomfort, and potentially helplessness if they are unable to move into an area of water of optimal quality.

Assigning welfare indicators to Domain 2 can be difficult. This is because the impact of resource-based indicators in Domain 2 can be observed in the animal in Domain 3 (Health). For example, the effects of increased salinity levels (Domain 2) in the environment can result in increases in plasma chloride and cortisol levels and elevated $Na^{+}K^{+}$-ATPase activity (Domain 3) (Hvas et al., 2018). The indicators respectively assigned to either domain both provide evidence of the same welfare problem – osmotic imbalance and threat of dehydration. Therefore, factors of the animal's environment can potentially be assigned to Domain 3, while the animal-based indicators for the same problem may also be assigned to Domain 2. This is a challenge of operationalising the Five Domains Model whereby the welfare impact is double counted in separate domains.

4. 5. Behavioural interactions domain

This review found very few published indicators relevant to Domain 4. Indicators in Domain 4 are situation-related factors (i.e., internally generated factors which give rise to affects that motivate the animal to restore homeostasis and ensure survival), not survival-critical factors (i.e., externally generated factors relevant to the animal's perception of external circumstances) (Mellor et al., 2020). When welfare consideration is relatively new to an animal industry, invariably focus begins on survival-critical impacts (which affect production) before there is exploration of situation-related factors in Domain 4. In other words, there is always a focus on factors that influence productivity first before focus is shifted towards factors that influence an animal's behavioural interactions (Mellor & Beausoleil, 2020; Mellor et al., 2020). However, behavioural interactions have previously been shown to impact livestock productivity. For example, low stress handling of pigs (Hemsworth et al.,

2002) and dairy cattle (Hemsworth et al., 2000) can improve productivity. Similarly in Atlantic salmon, perimortem and pre-spawning handling stress can negatively impact fillet quality (Sigholt et al., 1997) and offspring survival (Eriksen et al., 2006). Relatively little emphasis was placed on behavioural interaction in the articles included in this review, despite clear evidence from other industries and research not identified in this review that human interaction with animals can directly influence productivity.

The limited number of studies addressing behavioural interactions may also relate to challenges associated with assessing fish behaviour in a commercial aquaculture setting. Firstly, the recognition of fish behaviours in an aquaculture setting is impeded by the relative inaccessibility of farmed fish. In terrestrial agricultural settings farmers are able to build relationships with individual animals, facilitating the recognition of abnormal behaviour or changes in behaviour patterns (Macaulay et al., 2021). However, fish farmers do not have the same level of access to a large number of individuals underwater in a cage or tank (Stien et al., 2020). The inability to form relationships with these animals may impair the farmers ability to recognise the expression of behaviour in the first instance, as well as the recognition of variations in the behaviour of individuals as behavioural responses to the same stimuli will not be uniform across all fish in a population (Macaulay et al., 2021). Secondly, observation and qualitative analysis of fish behaviour is subjective, requiring the observer to have knowledge of appropriate behaviours for a given lifecycle stage, production system and species (Noble et al., 2018). In comparison to terrestrial livestock, there is limited research available on the full repertoire of farmed fish behaviour (Macaulay et al., 2021). Thirdly, assessing absolute or quantitative changes in fish behaviour is laborious as it requires later analysis of collected data (Noble et al., 2018).

It is possible that indicators assigned to other domains are also relevant to Domain 4. Categorisation of welfare indicators was performed by the authors after data extraction. As noted above, infection treatment regime-related mortalities might be due to the negative impacts of repeated handling (a form of human-animal interaction). If the authors were performing a holistic welfare assessment using the Five Doman Model, this indicator could be placed in Domain 4. However, infection treatment regime was assigned to Domain 3 because the indicator more explicitly relates to the health of salmon through the prevention and mitigation of infection.

All behavioural observations in Domain 4 are ante-mortem, animal-based indicators. A benefit of focussing more attention on behavioural indicators in Domain 4 is the ability to infer potential mental experiences in the present moment. Behaviour has been described as the outcome of an animal's decision-making in relation to integrated stimuli from their environment (Budaev et al., 2019; Dawkins, 2004). In other words, changes in behaviour occur in response to the individual's perception of their external situation. For example, in the presence of a potential threat such as a fishing net, salmon exhibit escape behaviours such as an increase in tail beats (Larsen et al., 2015). In this situation the fish is likely to be experiencing fear and in response to the potential threat the fish actively attempts to move away from danger. At the time of observation, escape behaviour may provide direct evidence of current mental experiences of fear. Therefore, behavioural indices in Domain 4 may be used as output measures reflective of an animal's present perception of their external situation (Mellor et al., 2020).

4. 6. Study methodological considerations

Group-level indicators are more practical to measure in a farm context relative to individual-level indicators due to the large number of individual animals on farm. Over half of the article set reported indicators at a laboratory tank level, i.e., at a group-level. As mentioned above, one challenge with fish farming is accessibility to all individuals within a system, thus making it difficult to conduct individual assessments (Stien et al., 2020). Group-level indicators utilise a sub-sample of fish within a farming unit, forgoing the laborious challenge of assessing potentially thousands of animals. When using group-level indicators there is an assumption that the welfare of animals in the sub-sample is representative of the farming unit (Stien et al., 2020). However, due to individual variability within a population, it is possible that group-level evaluations may not apply uniformly across the farming unit i.e., individuals with poor welfare may not be included in the sub-sample assessed (Winckler, 2019). Although group-level observations can potentially limit the recognition of individual welfare compromise, they may trigger group-level intervention where the individual compromise is indirectly addressed (Stien et al., 2020; Winckler, 2019). Therefore, group-level indicators are advantageous on-farm due to their practicality at a commercial farm scale. However, assessors should be cognizant of the limitations of group-level indicators relating to individual variation within a population.

This review found a lack of explicit investigation of affective states in salmon. This is likely because more than half of the articles did not explicitly focus on animal welfare, but emphasised productivity. The term 'stress' featured in approximately one quarter of the articles. It is likely that the use of the term 'stress' was not in reference to an affective state. In scientific terms, 'stress' typically refers to physiological stress activation. There is likely some degree of unpleasantness associated with stress (Ellis, Yildiz, et al., 2012); however, it is not explicit that an affective state is being discussed. For example, two articles (Larsen et al., 2015; Thörnqvist et al., 2015) investigated different stress coping personalities with regards to stress responses during stressful interventions. Physiological and behavioural responses were referred to, but the explicit unpleasantness of these events was not discussed – although likely inherently implied (Ellis, Yildiz, et al., 2012). However, it is difficult to extrapolate reference to affective states without 'welfare' first being characterised. It should also be noted that in a welfare assessment context, assessors often look for specific pain and fear indicators in animals (Hemsworth et al., 2002). However, specific investigation of other affective experiences (particularly positive experiences such as joy) is rare, in part because of a lack of tools to identify specific indicators of such experiences. This is a likely explanation for not finding indicators of potential positive mental experiences in fish.

The articles reviewed demonstrated an increasing trend in salmon welfare-related research, with a notable increase in publication activity in 2021. This trend is also seen outside of the published scientific literature through specific fish welfare guidelines, assurance schemes, and funding activities. Codes of Practice for farmed salmonids exist in Canada (National Farm Animal Care Council, 2021) and some industries themselves have developed fish welfare principles (New Zealand Salmon Farmers Association, 2021; Scottish Salmon Producers Organisation, 2015). Fish welfare assurance schemes developed by the Royal Society for the Prevention of Animal Cruelty are also active in Australia (RSPCA, 2020) and the United Kingdom (RSPCA, 2021). There are also research funding opportunities aimed at improving fish welfare including scholarships funded by Universities Federation for Animal Welfare (UFAW, 2022). The increase in fish welfare research may be related to increased recognition of sentience in fish and increasing consumer demand for welfare assurance (Lara & Boyland, 2019).

Lifecycle stage needs to be considered when selecting welfare indicators for assessment. Indicators relevant to one lifecycle stage may not be relevant to another. For example, schooling behaviour is reported as a welfare indicator of social cohesion relevant to smolt and adult salmon, but not for fry and parr (Noble et al., 2018). Schooling behaviour is not present in fry and parr populations as these lifecycle stages are relatively territorial and aggressive towards conspecifics (Taylor, 1990). The behaviour only develops during smoltification and is expressed by older salmon. Therefore, schooling behaviour is an appropriate welfare indicator for smolt and adult salmon, but inappropriate for younger fish. Specificity of welfare indicators to lifecycle stages has been discussed in other animal-based industries, for example body condition score is a relevant indicator of long-term nutritional status in adult ewes, but not in lambs (Beausoleil & Mellor, 2017). This emphasises that contextual information must be considered when selecting indicators for welfare assessment.

4. 7. Potential limitations of study methodology

The large number of irrelevant articles retrieved (low percentage accuracy) may be attributed to the operators used in the final search string. Operators (e.g., AND and OR) were utilised in a way that did not limit the review to articles that explicitly discussed fish welfare but allowed for the retrieval of articles that discussed aspects that the investigators believed were related to fish welfare. For the purposes of this review, use of a welfare-strict search string (i.e., including only articles that contain the term 'welfare' in their titles, abstracts, and/or keywords) would have failed to retrieve all possibly relevant articles. This was demonstrated by the non-systematic search performed before formal article retrieval began (see section 2. 2. 3).

Human error can impact the outcomes of scoping reviews through subjective interpretation of criteria and reviewer fatigue. Decision-making can be influenced by a reviewer's specific pattern of thought and further affected through prolonged exposure to the review task (consequence of large data sets) (Belur et al., 2021). These challenges were minimised by employing two reviewers during the screening process, performing calibration screens, and holding on-going discussion between reviewers.

Date restriction and sub-sampling during the screening process may have impacted the final set of identified indicators. It is possible that articles published before 2015 contained relevant indicators that were not identified. Similarly, relevant indicators from articles not included in the sub-sample used for data extraction may not have been identified. However, in terms of welfare domains, the authors are confident that the sub-sample is representative of the overall dataset and that the date restriction did not significantly impact the review's outcome (see Appendix B).

Issues that did not emerge from analysis of the sub-sample that might have been expected in exploration of fish welfare include slaughter and predation. Slaughter processes which do not result in immediate loss of consciousness may significantly impact the animal's welfare through painful injuries and high stress associated with handling (Lines & Spence, 2012). Similarly, interactions with predators (e.g., birds and seals) may result in painful injuries and experiences of fear (Huntingford et al., 2006). It is important to be aware of indicators related to these events in order to identify and subsequently prevent or mitigate any associated welfare issues. The absence of any articles related to these issues in this review may be due to sampling bias as not all available indicators of welfare may have been present in the sub-sample. It could also be related to the specific search terms used as the search string did not include specific keywords related to slaughter or predation. Future research should include expert/industry consultation on potential welfare issues to identify any indicators missing from this review.

5. Conclusion

This scoping review identified a total of 112 potential animal- and resource-based indicators of farmed salmon welfare from 60 articles. There was a clear focus on the use of survival-critical indicators reflecting welfare impacts in Domains 1-3 (nutrition, health, and physical environment). A limited number of situation-related indicators (Domain 4) were identified. Of the identified indicators, a large proportion were classified as animal-based indicators sampled post-mortem. These indicators can only provide evidence of a previous experience in the animal assessed. Contextual information is necessary for appropriate application and interpretation of welfare indicators with regards to affective experiences. Similarly, a range of indicators across Domains 1-4 should be used in combination in order to perform a holistic assessment of farmed salmon welfare.

Chapter 3 – General discussion

As aquaculture industries increase production outputs to meet an increased global demand for aquatic-based protein, there has been a heightened interest in the welfare of farmed fish. There is an expectation of animal-based industries to evaluate their practices to maintain and enhance the welfare of their animals. The New Zealand aquaculture industry is at the start of this process with the aim of developing a Code of Welfare for farmed fish. In order to develop a robust and reliable Code of Welfare, several steps must be taken towards creating an effective welfare assessment tool to form the basis of the Code. Such steps include developing an understanding of the current and future New Zealand salmon farming industry, identifying potential areas within production systems where fish welfare may be influenced, and identifying potential indicators of farmed salmon welfare.

1. Overview of salmon production and potential welfare issues in Aotearoa New Zealand

Chapter one of this thesis provided an overview of the current and future production systems used to farm salmon in New Zealand and the potential impacts on salmon welfare within these systems. Freshwater and marine-based operations are used in New Zealand to farm King salmon for human consumption. Juvenile salmon are reared in freshwater hatcheries until they have reached an acceptable weight and/or stage of smoltification before being transferred into grow-out cages. Smolt are grown out in net cages, situated within either freshwater hydro-canals or coastal bays of the South Island, until they reach a harvest weight of ~4kg.

Prior to harvest, there are a various points within the production cycles where salmon welfare maybe influenced. The Five Domains Model was used as a guide to evaluate areas of potential welfare impacts within New Zealand salmon farming systems. In terms of nutritional impacts (Domain 1), management of feed withdrawal regimes and factors associated with underfeeding may negatively impacts salmon welfare. Water quality parameters are crucial for the maintenance of adequate environmental living conditions (Domain 2). Water quality parameters that may influence salmon welfare include water flow rate, temperature, oxygen saturation, carbon dioxide concentration, ammonia concentration, and salinity. Impacts on salmon health (Domain 3) may arise from the contraction of infectious bacterial diseases, physical injuries, and the development of spinal deformities. Lastly, in Domain 4, salmon welfare may be impacted through interactions with humans (e.g., handling stress during handling events), other non-human animals (e.g., aggressive interaction with conspecifics or presence of predators), and the environment (e.g., limited ability to exercise agency within barren and confined environments).

During harvest, stunning and slaughter methods may also impact salmon welfare. Methods that are currently utilised in a commercial slaughter setting include Aqui-S and carbon dioxide immersion, manual and automatic percussion, and electrical stunning. Brain spiking, also known as the iki-jime method, is used to euthanise broodstock prior to stripping. During stunning and slaughter, negative welfare impacts may arise when a stunning or slaughter method 1) does not result in immediate loss of consciousness and/or death of the fish, or 2) does not cause the fish to remain unconscious until death.

2. Identification of potential indicators of farmed salmon welfare

Following the evaluation of potential welfare impacts in New Zealand's salmon farming industry, indicators of fish welfare were identified to understand the tools that may be used to assess the welfare of fish on-farm. The aim of the scoping review presented in Chapter 2 was to identify a list of potential indicators of farmed salmon welfare for use in on-farm assessments in New Zealand. Examination of globally published literature related to measures of salmon welfare resulted in the identification of 112 unique welfare indicators from 60 papers. Identified indicators were assigned to a one of the first four physical/functional domains of the Five Domains Model. Indicators relevant to welfare impacts across all four physical/functional domains were identified. However, a majority of indicators (n = 107) reflected welfare impacts in the nutrition, physical environment, and health domains. Only five indicators reflected welfare impacts in Domain 4 (behavioural interactions). The proportion of indicators identified in the first three domains suggests a biological function understanding and assessment of salmon welfare within the examined published literature. It may also be reflective of what is currently technically possible to measure in aquatic animals as behavioural measures are difficult to measure in an aquaculture setting due to the sheer number of individuals farmed and the aquatic medium within which they are farmed (Segner et al., 2019; Stien et al., 2020). Identification of further indicators relevant to Domain 4 will be beneficial for the development of holistic welfare assessments.

Identified indicators were also analysed in terms of their temporal validity, i.e., their ability to demonstrate *when* a particular mental state may have been experienced by a fish in relation to the time of indicator observation. Of the identified indicators, the most common indicator type within the literature examined were animal-based indicators, sampled post-mortem. Indicator type and sampling time influence the conclusions which are able to be drawn from observation of particular indicators. Post-mortem animal-based indicators are unable to provide evidence of a present mental experience in the fish because the fish is no longer conscious and sensible to any sensory or affective experiences. However, post-mortem animal-based indicators are able to provide information of the potential risk of a welfare issue present within the remaining farming unit. Similarly, resource-based indicators cannot point to a present mental experience as these are considered indirect measures of welfare. As such, resource-based indicators can only ever provide evidence of a potential risk to the welfare of salmon. In contrast to post-mortem animal-based indicators and resource-based indicators, ante-mortem indicators can potentially provide evidence of present mental experience at the time of their observation. However, no one welfare indicator can provide definite evidence of a mental experience. There is a need to use multiple types of welfare indicators in welfare assessments to confidently conclude the experience of a particular mental state.

2. 2. Relevance of identified welfare indicators in a New Zealand context

Although a majority of the indicators identified originated from papers published in countries other than New Zealand (e.g., Norway and Canada), the indicators identified are still relevant to the assessment of salmon welfare in a New Zealand context. King salmon are not endemic to New Zealand and are not exposed to the majority of diseases affecting endemic Atlantic salmon populations in other countries (e.g., infectious pancreatic necrosis and bacterial kidney disease) (Lane et al., 2022). As such, many infection-related indicators identified in this review are not currently relevant in New Zealand. In the one study from New Zealand, no infection-specific welfare indicators were mentioned. However, indicators of enteric red mouth disease and vibriosis in Atlantic salmon are likely relevant to King salmon in New Zealand as these two diseases have been identified by industry experts in New Zealand (Chapter 1). Also, previous summer mortality events have been reported to have potential links to outbreaks of New Zealand rickettsia-like organism and *Tenacibaculum maritimum* (Brosnahan et al., 2019). Although disease outbreaks are not a major issue affecting the New Zealand industry currently, rising seawater temperatures are likely increase the risk of future disease outbreaks (Lane et al., 2022). Therefore, infection-specific indicators identified in this review will likely be of use in the future.

Similarly, the vast majority of research reviewed studied Atlantic salmon populations. This was expected as King salmon production accounts for less than 1% of global farmed salmon production, with the majority being Atlantic salmon (Araujo et al., 2021). Therefore, research efforts are more likely to focus on the productivity and welfare of Atlantic salmon. However, indicators of Atlantic salmon welfare are likely transferable to King salmon as the two species are similar in key regards according to industry experts (M. Preece, personal communication, May 18, 2021). However, this is in slight contradiction to a 2017 report which states "the animal husbandry, biology, physiology and other characteristics of King salmon as compared with Atlantic salmon differ" (Preece, 2017). It is advised that in order to apply indicators of Atlantic salmon welfare to King salmon, contextual information such as optimal physiological ranges of the species, behaviour, husbandry practices, and features of the animal's environment should be considered during welfare evaluation.

In conclusion, the 112 unique welfare indicators identified and the understanding of their collective value to the welfare of commercially farmed salmon in New Zealand presented in this review provides industry, government, and policy makers the necessary information to develop regulations and on-farm welfare assessments.

3. Future research

The next step towards the development of a Code of Welfare for farmed fish should evaluate the validity and practicality of the identified welfare indicators on farm. Scientific validity (or construct) denotes a real indication or provision of relevant information about the welfare state of an animal (Waran & Randle, 2017) and is considered the most important requirement for welfare indicators to have meaning (Wemelsfelder & Mullan, 2014). Previous research has evaluated the validity of indicators relevant to sheep and cattle welfare on-farm (Phythian et al., 2011) and in commercial abattoirs (Llonch et al., 2015; Losada-Espinosa et al., 2018). However, in these papers, only face and consensual validity was achieved. In order to demonstrate construct validity (true scientific validity), Beausoleil and Mellor (2017) propose a two-step framework requiring evidence of a relationship between the indicator (e.g., body condition score) and a physical impact on the animal (e.g., nutritional status) along with evidence of a relationship between the physical impact and mental experience of the animal (e.g., hunger). Experimental studies would be necessary to validate the relationship between the 112 welfare indicators and potential mental experiences in King salmon.

The practicality of an indicator is dependent on the context in which it is being utilised. In the case of farm animal welfare, practicality relates to the indicator's utility on farm, during transport and prior to slaughter (Beausoleil & Mellor, 2017; Llonch et al., 2015). Indicators are considered practical if they are not time consuming or costly to observe/measure, and do not significantly interfere with normal production routines (Beausoleil & Mellor, 2017; Llonch et al., 2015). On-farm application of each of the 112 welfare indicators would be required to evaluate the practicality of each individual welfare indicator.

The practicality and validity of indicators are also dependent on the assessment context in terms of species and life stage of the animal, and production system and cycle stage (Beausoleil & Mellor, 2017; Noble et al., 2018). For example, body condition scoring can be used as an accurate indicator of nutritional welfare factors for adult ewes, but is inaccurate for growing lambs (Beausoleil & Mellor, 2017; Llonch et al., 2015). Ongoing work needs to be done in order to make the acquired list of indicators meaningful and operational for use in a Code of Welfare and in on-farm welfare assessments.

References

Allen, C., & Bekoff, M. (2007). Animal consciousness. In M. Velmans & S. Schneider (Eds.), *The Blackwell Companion to Consciousness* (pp. 58-71). Blackwell Publishing.

Andrewartha, S. J., Munns, S. L., & Edwards, A. (2016). Calibration of the HemoCue point-of-care analyser for determining haemoglobin concentration in a lizard and a fish. *Conservation Physiology, 4*(1). https://doi.org/10.1093/conphys/cow006

Animal Welfare Act, Pub. L. No. 142, (1999). https://www.legislation.govt.nz/act/public/1999/0142/latest/DLM49664.html

Aquaculture New Zealand. (2020). *Aquaculture for New Zealand: A sector overview with facts and statistics for 2020*. https://drive.google.com/file/d/1yAlnzMbUPyuvbJxtD8OnaoLXydEl5Vic/view

Aqui-S™. (n.d.). *Aqui-S: Aquatic anaesthetic*. https://www.aqui-s.com/products/aqui-s-aquatic-anaesthetic

Araujo, B. C., Symonds, J. E., Glencross, B. D., Carter, C. G., Walker, S. P., & Miller, M. R. (2021). A review of the nutritional requirements of chinook salmon (*Oncorhynchus tshawytscha*). *New Zealand Journal of Marine and Freshwater Research*, 1-30. https://doi.org/https://doi.org/10.1080/00288330.2021.1985530

Arriagada, G., Hamilton-West, C., Nekouei, O., Foerster, C., Müller, A., Lara, M., & Gallardo-Escárate, C. (2019). Caligus rogercresseyi infestation is associated with Piscirickettsia salmonis-attributed mortalities in farmed salmonids in Chile. *Preventive Veterinary Medicine, 171*, Article 104771. https://doi.org/10.1016/j.prevetmed.2019.104771

Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. *Applied Animal Behaviour Science, 104*(3-4), 199-235.

Aslam, S. N., Navada, S., Bye, G. R., Mota, V. C., Terjesen, B. F., & Mikkelsen, Ø. (2019). Effect of CO2 on elemental concentrations in recirculating aquaculture system tanks. *Aquaculture, 511*, Article 734254. https://doi.org/10.1016/j.aquaculture.2019.734254

Barreto, M. O., Rey Planellas, S., Yang, Y., Phillips, C., & Descovich, K. (2022). Emerging indicators of fish welfare in aquaculture. *Reviews in Aquaculture, 14*(1), 343-361.

Beausoleil, N. J., & Mellor, D. J. (2017). Validating indicators of sheep welfare. In *Achieving Sustainable Production of Sheep* (pp. 349-370). Burleigh Dodds Science Publishing.

Belghit, I., Liland, N. S., Waagbø, R., Biancarosa, I., Pelusio, N., Li, Y., Krogdahl, Å., & Lock, E.-J. (2018). Potential of insect-based diets for Atlantic salmon (*Salmo salar*). *Aquaculture, 491*, 72-81. https://doi.org/10.1016/j.aquaculture.2018.03.016

Belur, J., Tompson, L., Thornton, A., & Simon, M. (2021). Interrater reliability in systematic review methodology: Exploring variation in coder decision-making. *Sociological methods & research, 50*(2), 837-865. https://doi.org/10.1177/0049124118799372

Birch, J., Burn, C., Schnell, A., Browning, H., & Crump, A. (2021). *Review of the evidence of sentience in cephalopod molluscs and decapod crustaceans*. LSE Consulting. https://www.lse.ac.uk/news/news-assets/pdfs/2021/sentience-in-cephalopod-molluscs-and-decapod-crustaceans-final-report-november-2021.pdf

Boulton, A., Kells, N., Beausoleil, N. J., Cogger, N., Johnson, C., Palmer, A., Laven, R., O'Connor, C., & Webster, J. (2018). *Bobby calf welfare across the supply chain - Final report for year 1* (2018/44). Ministry for Primary Industries. https://www.mpi.govt.nz/dmsdocument/30005-Bobby-Calf-Welfare-Across-the-Supply-Chain-Final-Report-Year-One-

Bowman, J., van Nuland, N., Hjelmstedt, P., Berg, C., & Gräns, A. (2020). Evaluation of the reliability of indicators of consciousness during CO2 stunning of Rainbow trout and the effects of temperature. *Aquaculture Research, 51*(12), 5194-5202.

Broom, D. (2014). *Sentience and animal welfare*. CABI.

Brosnahan, C. L., Munday, J. S., Ha, H. J., Preece, M., & Jones, J. B. (2019). New Zealand rickettsia-like organism (NZ-RLO) and *Tenacibaculum maritimum*: Distribution and phylogeny in farmed Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Fish Diseases, 42*(1), 85-95. https://doi.org/https://doi.org/10.1080/00288330.2020.1848887

Brown, C. (2015). Fish intelligence, sentience and ethics. *Animal cognition, 18*(1), 1-17.

Brown, R., Moore, L., Mani, A., Patel, S., & Salinas, I. (2021). Effects of ploidy and salmonid alphavirus infection on the skin and gill microbiome of Atlantic salmon (*Salmo salar*). *PLoS ONE, 16*(2). https://doi.org/10.1371/journal.pone.0243684

Budaev, S., Jørgensen, C., Mangel, M., Eliassen, S., & Giske, J. (2019). Decision-making from the animal perspective: Bridging ecology and subjective cognition. *Frontiers in Ecology and Evolution, 7*, 164. https://doi.org/https://doi.org/10.3389/fevo.2019.00164

Bui, S., Stien, L. H., Nilsson, J., Trengereid, H., & Oppedal, F. (2020). Efficiency and welfare impact of long-term simultaneous in situ management strategies for salmon louse reduction in commercial sea cages. *Aquaculture, 520*, Article 734934. https://doi.org/10.1016/j.aquaculture.2020.734934

Calabrese, S., Nilsen, T. O., Kolarevic, J., Ebbesson, L. O. E., Pedrosa, C., Fivelstad, S., Hosfeld, C., Stefansson, S. O., Terjesen, B. F., Takle, H., Martins, C. I. M., Sveier, H., Mathisen, F., Imsland, A. K., & Handeland, S. O. (2017). Stocking density limits for post-smolt Atlantic salmon (Salmo salar L.) emphasis on production performance and welfare [Article]. *Aquaculture, 468*, 363-370. https://doi.org/10.1016/j.aquaculture.2016.10.041

Carey, J. B., & McCormick, S. D. (1998). Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr. *Aquacultural, 168*(1-4), 237-253. https://doi.org/https://doi.org/10.1016/S0044-8486(98)00352-4

Casanovas, P., Walker, S. P., Johnston, H., Johnston, C., & Symonds, J. E. (2021). Comparative assessment of blood biochemistry and haematology normal ranges between Chinook salmon (*Oncorhynchus tshawytscha*) from seawater and freshwater farms [Article]. *Aquaculture, 537*, Article 736464. https://doi.org/10.1016/j.aquaculture.2021.736464

Chang, Y. C., Hamlin-Wright, H., Monaghan, S., Herath, T., Baily, J., del Pozo, J., Downes, J., Preston, A., Chalmers, L., Jayasuriya, N., Bron, J. E., Adams, A., & Fridman, S. (2019). Changes in distribution, morphology and ultrastructure of chloride cell in Atlantic salmon during an AGD infection. *Journal of Fish Diseases, 42*(10), 1433-1446. https://doi.org/10.1111/jfd.13073

Chu, Y., Wang, C. M., Park, J. C., & Lader, P. F. (2020). Review of cage and containment tank designs for offshore fish farming. *Aquaculture, 519*, Article 734928.

Contreras, M., Karlsen, M., Villar, M., Olsen, R. H., Leknes, L. M., Furevik, A., Yttredal, K. L., Tartor, H., Grove, S., Alberdi, P., Brudeseth, B., & de la Fuente, J. (2020). Vaccination with ectoparasite proteins involved in midgut function and blood digestion reduces salmon louse infestations. *Vaccines, 8*(1), 32. http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edb&AN=142496593&site=eds-live&scope=site&authtype=ip,sso&custid=s3027306

Cooke, S. J., & Sneddon, L. U. (2007). Animal welfare perspectives on recreational angling. *Applied Animal Behaviour Science, 104*(3-4), 176-198.

Dahle, O. M. V., Blindheim, S. H., Nylund, A., Karlsbakk, E., Breck, O., Glosvik, H., & Andersen, L. (2020). Atlantic salmon *Salmo salar* and ballan wrasse *Labrus bergylta* display different susceptibility to clonal strains of *Paramoeba perurans*. *Diseases of Aquatic Organisms, 140*, 55-72. https://doi.org/10.3354/dao03483

Davie, P. S., Walker, S. P., Perrott, M. R., Symonds, J. E., Preece, M., Lovett, B. A., & Munday, J. S. (2019). Vertebral fusions in farmed Chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand. *Journal of Fish Diseases, 42*(7), 965-974. https://doi.org/10.1111/jfd.13013

Dawkins, M. S. (2004). Using behaviour to assess animal welfare. *Animal Welfare, 13*(1), 3-7.

de O. Roberti Filho, F., Koch, J. F. A., Wallace, C., & Leal, M. C. (2019). Dietary β-1,3/1,6-glucans improve the effect of a multivalent vaccine in Atlantic salmon infected with *Moritella viscosa* or infectious salmon anemia virus [Article]. *Aquaculture International, 27*(6), 1825-1834. https://doi.org/10.1007/s10499-019-00436-9

DeGrazia, D. (2020). Sentience and consciousness as bases for attributing interests and moral status: Considering the evidence and speculating slightly beyond. In L. Johnson, A. Fenton, & A. Shriver (Eds.), *Neuroethics and nonhuman animals* (pp. 17-31). Springer Nature. https://doi.org/https://doi.org/10.1007/978-3-030-31011-0_2

Delfosse, C., Pageat, P., Lafont-Lecuelle, C., Asproni, P., Chabaud, C., Cozzi, A., & Bienboire-Frosini, C. (2021). Effect of handling and crowding on the susceptibility of Atlantic salmon (*Salmo salar L.*) to Lepeophtheirus salmonis (*Krøyer*) copepodids. *Journal of Fish Diseases, 44*(3), 327-336. https://doi.org/10.1111/jfd.13286

Delphino, M. K. V. C., Mardones, F. O., Neumann Heise, J., Gallardo, A., Jimenez, D., Peña, A., Rozas-Serri, M., & Gardner, I. A. (2021). Cost-effectiveness of longitudinal surveillance for *Piscirickettsia salmonis* using qPCR in Atlantic salmon farms (*Salmo salar*) in Chile. *Journal of Fish Diseases, 44*(3), 315-326. https://doi.org/10.1111/jfd.13285

Digfish Services. (2013). *Salmon - Chinook*. Retrieved January 25 from https://www.ikijime.com/fish/salmon-chinook/

Diggles, B. (2016). Development of resources to promote best practice in the humane dispatch of finfish caught by recreational fishers. *Fisheries management and ecology, 23*(3-4), 200-207.

Diggles, B., Hine, P., Handley, S., & Boustead, N. (2002). A handbook of diseases of importance to aquaculture in New Zealand. *NIWA Science and Technology Series*, 200, Article 49.

Downes, J. K., Rigby, M. L., Taylor, R. S., Maynard, B. T., MacCarthy, E., O'Connor, I., Marcos-Lopez, M., Rodger, H. D., Collins, E., Ruane, N. M., & Cook, M. T. (2017). Evaluation of non-destructive molecular diagnostics for the detection of *Neoparamoeba perurans*. *Frontiers in Marine Science, 4*, Article 61. https://doi.org/10.3389/fmars.2017.00061

Downes, J. K., Yatabe, T., Marcos-Lopez, M., Rodger, H. D., MacCarthy, E., O'Connor, I., Collins, E., & Ruane, N. M. (2018). Investigation of co-infections with pathogens associated with gill disease in Atlantic salmon during an amoebic gill disease outbreak [Article]. *Journal of Fish Diseases, 41*(8), 1217-1227. https://doi.org/10.1111/jfd.12814

Drach, A., Tsukrov, I., Decew, J., Robinson Swift, M., Celikkol, B., & Hurtado, C. F. (2013). Design and modeling of submersible fish cages with copper netting for open ocean aquaculture. MARINE V: proceedings of the V International Conference on Computational Methods in Marine Engineering, Germany.

Dunlop, R., & Laming, P. (2005). Mechanoreceptive and nociceptive responses in the central nervous system of Goldfish (*Carassius auratus*) and Trout (*Oncorhynchus mykiss*). *The journal of pain, 6*(9), 561-568.

Dunlop, R., Millsopp, S., & Laming, P. (2006). Avoidance learning in Goldfish (*Carassius auratus*) and Trout (*Oncorhynchus mykiss*) and implications for pain perception. *Applied Animal Behaviour Science, 97*(2-4), 255-271.

Eddy, F. (2005). Ammonia in estuaries and effects on fish. *Journal of Fish Biology, 67*(6), 1495-1513.

Ellis, T., Berrill, I., Lines, J., Turnbull, J. F., & Knowles, T. G. (2012). Mortality and fish welfare. *Fish Physiology and Biochemistry, 38*(1), 189-199.

Ellis, T., Yildiz, H. Y., López-Olmeda, J., Spedicato, M. T., Tort, L., Øverli, Ø., & Martins, C. I. (2012). Cortisol and finfish welfare. *Fish Physiology and Biochemistry, 38*(1), 163-188.

Emery, J. A., Smullen, R., Keast, R. S. J., & Turchini, G. M. (2016). Viability of tallow inclusion in Atlantic salmon diet, as assessed by an on-farm grow out trial. *Aquaculture, 451*, 289-297. https://doi.org/10.1016/j.aquaculture.2015.09.023

Eriksen, M., Bakken, M., Espmark, Å., Braastad, B., & Salte, R. (2006). Prespawning stress in farmed Atlantic salmon *Salmo salar*: Maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. *Journal of Fish Biology, 69*(1), 114-129.

Erikson, U. (2011). Assessment of different stunning methods and recovery of farmed Atlantic salmon (*Salmo salar*): Isoeugenol, nitrogen and three levels of carbon dioxide. *Animal Welfare-The UFAW Journal, 20*(3), 365.

Espe, M., Veiseth-Kent, E., Zerrahn, J. E., Rønnestad, I., & Aksnes, A. (2016). Juvenile Atlantic salmon decrease white trunk muscle IGF-1 expression and reduce muscle and plasma free sulphur amino acids when methionine availability is low while liver sulphur metabolites mostly is unaffected by treatment. *Aquaculture Nutrition, 22*(4), 801-812. https://doi.org/10.1111/anu.12294

Evans, D. (2011). Osmotic, ionic and nitrogenous-waste balance. In A. P. Farrell (Ed.), *Encyclopedia of Fish Physiology From Genome to Environment* (Vol. 2, pp. 1348-1353). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-374553-8.00210-0

Evans, D. H. (2010). A brief history of fish osmoregulation: The central role of the Mt. Desert Island Biological Laboratory. *Frontiers in Physiology*, 13.

Fast, M. D., Hosoya, S., Johnson, S. C., & Afonso, L. O. (2008). Cortisol response and immune-related effects of Atlantic salmon (*Salmo salar Linnaeus*) subjected to short-and long-term stress. *Fish & Shellfish Immunology, 24*(2), 194-204.

Feinberg, T. E., & Mallatt, J. M. (2016). *The ancient origins of consciousness: How the brain created experience*. MIT Press.

Fernandez-Senac, C., Fridman, S., Sokolowska, J., Monaghan, S. J., Garzon, T., Betancor, M., Paladini, G., Adams, A., & Bron, J. E. (2020). A comparison of the use of different swab materials for optimal diagnosis of amoebic gill disease (AGD) in Atlantic salmon (*Salmo salar L.*). *Journal of Fish Diseases, 43*(11), 1463-1472. https://doi.org/10.1111/jfd.13243

Fischer, J., & Appleby, J. (2017). *Intelligence report: NZ-RLO & T. maritimum 2015 response*. https://www.mpi.govt.nz/dmsdocument/18253-NZ-RLO-T.-maritimum-2015-Intelligence-Report

Fivelstad, S., Waagbø, R., Zeitz, S. F., Hosfeld, A. C. D., Olsen, A. B., & Stefansson, S. (2003). A major water quality problem in smolt farms: Combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (*Salmo salar L.*) smolts on physiology and growth. *Aquaculture, 215*(1-4), 339-357.

Fogarty, E. S., Swain, D. L., Cronin, G., & Trotter, M. (2018). Autonomous on-animal sensors in sheep research: A systematic review. *Computers and Electronics in Agriculture, 150*, 245-256.

Food and Agriculture Organisation of the United Nations (FAO). (2022). *Salmo salar*. Cultured Aquatic Species Information Programme. https://www.fao.org/fishery/en/culturedspecies/salmo_salar/en

Fraser, D. (2003). Assessing animal welfare at the farm and group level: The interplay of science and values. *Animal Welfare, 12*(4), 433-443.

Fraser, D. (2009). Assessing animal welfare: Different philosophies, different scientific approaches. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association, 28*(6), 507-518.

Fraser, T. W. K., Fjelldal, P. G., Sommerset, I., Softeland, T., Hostmark, O., Powell, M. D., Heen, V., & Hansen, T. J. (2020). No environmental effect on vaccine-induced reduced growth in Atlantic salmon (*Salmo salar*) smolts. *Aquaculture Environment Interactions, 12*, 327-338. https://doi.org/10.3354/aei00367

Frisk, M., Høyland, M., Zhang, L., Vindas, M. A., Øverli, Ø., & Johansen, I. B. (2020). Intensive smolt production is associated with deviating cardiac morphology in Atlantic salmon (*Salmo salar L.*). *Aquaculture, 529*. https://doi.org/10.1016/j.aquaculture.2020.735615

Gautam, R., Vanderstichel, R., Boerlage, A. S., Revie, C. W., & Hammell, K. L. (2017). Evaluating bath treatment effectiveness in the control of sea lice burdens on Atlantic salmon in New Brunswick, Canada [Article]. *Journal of Fish Diseases, 40*(7), 895-905. https://doi.org/10.1111/jfd.12569

Gräns, A., Niklasson, L., Sandblom, E., Sundell, K., Algers, B., Berg, C., Lundh, T., Axelsson, M., Sundh, H., & Kiessling, A. (2016). Stunning fish with CO2 or electricity: Contradictory results on behavioural and physiological stress responses. *Animal, 10*(2), 294-301.

Grimsbø, E., Nortvedt, R., Hjertaker, B. T., Hammer, E., & Roth, B. (2016). Optimal AC frequency range for electro-stunning of Atlantic salmon (Salmo salar). *Aquaculture, 451*, 283-288.

Gu, M., Gu, J. N., Penn, M., Bakke, A. M., Lein, I., & Krogdahl, Å. (2015). Effects of diet supplementation of soya-saponins, isoflavones and phytosterols on Atlantic salmon (Salmo salar, L) fry fed from start-feeding [Article]. *Aquaculture Nutrition, 21*(5), 604-613. https://doi.org/10.1111/anu.12187

Hampton, J. O., Hyndman, T. H., Laurence, M., Perry, A. L., Adams, P., & Collins, T. (2016). Animal welfare and the use of procedural documents: Limitations and refinement. *Wildlife Research, 43*(7), 599-603.

Hardy, R. W. (1995). Current issues in salmonid nutrition. In C. E. Lim & D. J. Sessa (Eds.), *Nutrition and uUtilization Technology in Aquaculture* (pp. 26-35). The American Oil Chemists Society Press.

Harvey, A. C., Glover, K. A., Taylor, M. I., Creer, S., & Carvalho, G. R. (2016). A common garden design reveals population-specific variability in potential impacts of hybridization between populations of farmed and wild Atlantic salmon, Salmo salar L [Article]. *Evolutionary Applications, 9*(3), 435-449. https://doi.org/10.1111/eva.12346

Harvey, A. M., Beausoleil, N. J., Ramp, D., & Mellor, D. J. (2020). A ten-stage protocol for assessing the welfare of individual non-captive wild animals: Free-roaming horses (*Equus ferus caballus*) as an example. *Animals, 10*(1), 148.

Hauge, H., Dahle, M., Moldal, T., Thoen, E., Gjevre, A. G., Weli, S., Alarcón, M., & Grove, S. (2016). Piscine orthoreovirus can infect and shed through the intestine in experimentally challenged Atlantic salmon (Salmo salar L.) [Article]. *Veterinary Research, 47*(1). https://doi.org/10.1186/s13567-016-0343-z

Hawkins, L., Magurran, A., & Armstrong, J. (2007). Innate abilities to distinguish between predator species and cue concentration in Atlantic salmon. *Animal Behaviour, 73*(6), 1051-1057.

Haworth, J. (2010). *Swimming upstream: How salmon farming developed in New Zealand*. Wily Publications.

Hayashi, M., Kita, J., & Ishimatsu, A. (2004). Acid-base responses to lethal aquatic hypercapnia in three marine fishes. *Marine biology, 144*(1), 153-160.

Hemsworth, P. H., Barnett, J. L., Hofmeyr, C., Coleman, G. J., Dowling, S., & Boyce, J. (2002). The effects of fear of humans and pre-slaughter handling on the meat quality of pigs. *Australian Journal of Agricultural Research, 53*(4), 493-501.

Hemsworth, P. H., Coleman, G. J., Barnett, J. L., & Borg, S. (2000). Relationships between human-animal interactions and productivity of commercial dairy cows. *Journal of Animal Science, 78*(11), 2821-2831.

Holborn, M. K., Ang, K. P., Elliott, J. A. K., Powell, F., & Boulding, E. G. (2020). Genome wide analysis of infectious salmon anemia resistance in commercial Saint John River Atlantic salmon [Article]. *Aquaculture, 514*. https://doi.org/10.1016/j.aquaculture.2019.734514

Huntingford, F., & Kadri, S. (2014). Defining, assessing and promoting the welfare of farmed fish. *Revue scientifique et technique (International Office of Epizootics), 33*(1), 233-244.

Huntingford, F. A., Adams, C., Braithwaite, V., Kadri, S., Pottinger, T., Sandøe, P., & Turnbull, J. (2006). Current issues in fish welfare. *Journal of Fish Biology, 68*(2), 332-372.

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

Hvas, M., Nilsen, T. O., & Oppedal, F. (2018). Oxygen uptake and osmotic balance of Atlantic salmon in relation to exercise and salinity acclimation. *Frontiers in Marine Science, 5*, 368.

Hvas, M., Stien, L. H., & Oppedal, F. (2020). The metabolic rate response to feed withdrawal in Atlantic salmon post-smolts [Article]. *Aquaculture, 529*, Article 735690. https://doi.org/10.1016/j.aquaculture.2020.735690

Iremonger, G. (2008). *Smoltification and growth retardation in New Zealand King salmon (Oncorhynchus tshawytscha) (Walbaum)* [Thesis]. University of Canterbury.

Iversen, M., Finstad, B., McKinley, R. S., & Eliassen, R. A. (2003). The efficacy of metomidate, clove oil, Aqui-S™ and Benzoak® as anaesthetics in Atlantic salmon (Salmo salar L.) smolts, and their potential stress-reducing capacity. *Aquaculture, 221*(1-4), 549-566.

Jevne, L. S., & Reitan, K. I. (2019). How are the salmon lice (Lepeophtheirus salmonis Krøyer, 1837) in Atlantic salmon farming affected by different control efforts: A case study of an intensive production area with coordinated production cycles and changing delousing practices in 2013–2018 [Article]. *Journal of Fish Diseases, 42*(11), 1573-1586. https://doi.org/10.1111/jfd.13080

Johannesen, Á., Patursson, Ø., Kristmundsson, J., Dam, S. P., & Klebert, P. (2020). How caged salmon respond to waves depends on time of day and currents [Article]. *PeerJ, 2020*(6), Article e9313. https://doi.org/10.7717/peerj.9313

Johansson, D., Ruohonen, K., Kiessling, A., Oppedal, F., Stiansen, J.-E., Kelly, M., & Juell, J.-E. (2006). Effect of environmental factors on swimming depth preferences of Atlantic salmon (*Salmo salar L.*) and temporal and spatial variations in oxygen levels in sea cages at a fjord site. *Aquaculture, 254*(1-4), 594-605.

Johnsson, J. I., Höjesjö, J., & Fleming, I. A. (2001). Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences, 58*(4), 788-794.

Jónsdóttir, K. E., Klebert, P., Volent, Z., & Alfredsen, J. A. (2021). Characteristic current flow through a stocked conical sea-cage with permeable lice shielding skirt [Article]. *Ocean Engineering, 223*, N.PAG-N.PAG. https://doi.org/10.1016/j.oceaneng.2021.108639

Kotrschal, A., & Kotrschal, K. (2020). Fish brains: Anatomy, functionality, and evolutionary relationships. In T. S. Kristiansen, A. Fernö, M. A. Pavlidis, & H. van de Vis (Eds.), *The Welfare of Fish* (1st ed., Vol. 20, pp. 129-148). Springer.

Kousoulaki, K., Ostbye, T. K. K., Krasnov, A., Torgersen, J. S., Morkore, T., & Sweetman, J. (2015, Jun). Metabolism, health and fillet nutritional quality in Atlantic salmon (Salmo salar) fed diets containing n-3-rich microalgae. *Journal of Nutritional Science, 4*, Article e24. https://doi.org/10.1017/jns.2015.14

Lambooij, E., Grimsbø, E., Van de Vis, J., Reimert, H., Nortvedt, R., & Roth, B. (2010). Percussion and electrical stunning of Atlantic salmon (Salmo salar) after dewatering and subsequent effect on brain and heart activities. *Aquaculture, 300*(1-4), 107-112.

Lane, H. S., Brosnahan, C. L., & Poulin, R. (2022). Aquatic disease in New Zealand: Synthesis and future directions. *New Zealand Journal of Marine and Freshwater Research, 56*(1), 1-42.

Lara, E., & Boyland, N. (2019). Fish welfare: an NGO's point of view. Derecho Animal. Forum of Animal Law Studies,

Larsen, M. H., Johnsson, J. I., Winberg, S., Wilson, A. D. M., Hammenstig, D., Thörnqvist, P.-O., Midwood, J. D., Aarestrup, K., & Höglund, E. (2015). Effects of emergence time and early social rearing environment on behaviour of Atlantic salmon: Consequences for juvenile fitness and smolt migration [Article]. *PLoS ONE, 10*(3), 1-17. https://doi.org/10.1371/journal.pone.0119127

Leblanc, F., Leadbeater, S., Laflamme, M., & Gagné, N. (2018). In vivo virulence and genomic comparison of infectious Salmon Anaemia Virus isolates from Atlantic Canada [Article]. *Journal of Fish Diseases, 41*(9), 1373-1384. https://doi.org/10.1111/jfd.12832

Lerfall, J., Bendiksen, E. T., Olsen, J. V., Morrice, D., & Østerlie, M. (2016). A comparative study of organic- versus conventional farmed Atlantic salmon. I. Pigment and lipid content and composition, and carotenoid stability in ice-stored fillets [Article]. *Aquaculture, 451*, 170-177. https://doi.org/10.1016/j.aquaculture.2015.09.013

Li, Y., Kortner, T. M., Chikwati, E. M., Munang'andu, H. M., Lock, E. J., & Krogdahl, Å. (2019). Gut health and vaccination response in pre-smolt Atlantic salmon (Salmo salar) fed black soldier fly (Hermetia illucens) larvae meal [Article]. *Fish and Shellfish Immunology, 86*, 1106-1113. https://doi.org/10.1016/j.fsi.2018.12.057

Lines, J., & Spence, J. (2012). Safeguarding the welfare of farmed fish at harvest. *Fish Physiology and Biochemistry, 38*(1), 153-162.

Llewellyn, M. S., Leadbeater, S., Garcia, C., Sylvain, F. E., Custodio, M., Ang, K. P., Powell, F., Carvalho, G. R., Creer, S., Elliot, J., & Derome, N. (2017). Parasitism perturbs the mucosal microbiome of Atlantic Salmon [Article]. *Scientific Reports, 7*, Article 43465. https://doi.org/10.1038/srep43465

Llonch, P., King, E. M., Clarke, K. A., Downes, J., & Green, L. E. (2015). A systematic review of animal based indicators of sheep welfare on farm, at market and during transport, and qualitative appraisal of their validity and feasibility for use in UK abattoirs. *The Veterinary Journal, 206*(3), 289-297.

Long, A., Garver, K. A., & Jones, S. R. M. (2019). Differential Effects of Adult Salmon Lice Lepeophtheirus salmonis on Physiological Responses of Sockeye Salmon and Atlantic Salmon [Article]. *Journal of Aquatic Animal Health, 31*(1), 75-87. https://doi.org/10.1002/aah.10053

Losada-Espinosa, N., Villarroel, M., María, G. A., & Miranda-de la Lama, G. C. (2018). Pre-slaughter cattle welfare indicators for use in commercial abattoirs with voluntary monitoring systems: A systematic review. *Meat Science, 138*, 34-48.

Macaulay, G., Bui, S., Oppedal, F., & Dempster, T. (2021). Challenges and benefits of applying fish behaviour to improve production and welfare in industrial aquaculture. *Reviews in Aquaculture, 13*(2), 934-948.

Madaro, A., Olsen, R. E., Kristiansen, T. S., Ebbesson, L. O., Nilsen, T. O., Flik, G., & Gorissen, M. (2015). Stress in Atlantic salmon: Response to unpredictable chronic stress. *The Journal of Experimental Biology, 218*(16), 2538-2550.

Marín, S. L., González, M. P., Madariaga, S. T., Mancilla, M., & Mancilla, J. (2018). Response of Caligus rogercresseyi (Boxshall & Bravo, 2000) to treatment with Hydrogen Peroxide: Recovery of parasites, fish infestation and egg viability under experimental conditions [Article]. *Journal of Fish Diseases, 41*(6), 861-873. https://doi.org/10.1111/jfd.12691

Martín, B. S., Fresno, M., Cornejo, J., Godoy, M., Ibarra, R., Vidal, R., Araneda, M., Anadón, A., & Lapierre, L. (2019). Optimization of florfenicol dose against Piscirickettsia salmonis in Salmo salar through PK/PD studies [Article]. *PLoS ONE, 14*(5), Article e0215174. https://doi.org/10.1371/journal.pone.0215174

Martin, S. A., Douglas, A., Houlihan, D. F., & Secombes, C. J. (2010). Starvation alters the liver transcriptome of the innate immune response in Atlantic salmon (Salmo salar). *BMC Genomics, 11*(1), 1-20.

Martinsen, K. H., Lillehammer, M., & Thorisdottir, A. (2018). Effect of hydrogen peroxide as treatment for amoebic gill disease in Atlantic salmon (Salmo salar L.) in different temperatures [Article]. *Aquaculture Research, 49*(5), 1733-1739. https://doi.org/10.1111/are.13627

McCormick, S. D., Moriyama, S., & Bjornsson, B. T. (2000). Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 278*(5), R1352-R1361.

Mellor, D. J. (2016). Updating animal welfare thinking: Moving beyond the "Five Freedoms" towards "a Life Worth Living". *Animals, 6*(3), 21.

Mellor, D. J. (2019). Welfare-aligned sentience: Enhanced capacities to experience, interact, anticipate, choose and survive. *Animals, 9*(7), 440.

Mellor, D. J., & Beausoleil, N. J. (2019). Moving beyond a problem-based focus on poor animal welfare toward creating opportunities to have positive welfare experiences. In F. D. McMillan (Ed.), *Mental Health and Well-being in Animals* (2nd ed., Vol. 50). CAB International.

Mellor, D. J., & Beausoleil, N. J. (2020). Moving beyond a problem-based focus on poor animal welfare toward creating opportunities to have positive welfare experiences. In F. D. McMillan (Ed.), *Mental Health and Well-being in Animals* (2 ed., pp. 50-66). CABI. https://doi.org/10.1079/9781786393401.0050

Mellor, D. J., Beausoleil, N. J., Littlewood, K. E., McLean, A. N., McGreevy, P. D., Jones, B., & Wilkins, C. (2020). The 2020 Five Domains Model: Including human–animal interactions in assessments of animal welfare. *Animals, 10*(10), 1870.

Metochis, C. P., Spanos, I., Auchinachie, N., Crampton, V. O., Bell, J. G., Adams, A., & Thompson, K. D. (2016, 12/01/December 2016). The effects of increasing dietary levels of soy protein concentrate (SPC) on the immune responses and disease resistance (furunculosis) of vaccinated and non-vaccinated Atlantic salmon (Salmo salar L.) parr [Article]. *Fish and Shellfish Immunology, 59*, 83-94. https://doi.org/10.1016/j.fsi.2016.10.016

Meyer, A., Burroughs, A., Sadler, R., Happold, J., Cowled, B., Mackenzie, C., Gallardo Lagno, A. L., & Cameron, A. (2019). Quantifying the effects of sea lice burden and lice bathing treatments on salmonid rickettsial septicaemia in commercial salmon and trout farms in Chile [Article]. *Aquaculture, 513*, Article 734411. https://doi.org/10.1016/j.aquaculture.2019.734411

Molony, V., & Kent, J. E. (1997). Assessment of acute pain in farm animals using behavioral and physiological measurements. *Journal of Animal Science, 75*(1), 266-272.

Mota, V. C., Nilsen, T. O., Gerwins, J., Gallo, M., Ytteborg, E., Baeverfjord, G., Kolarevic, J., Summerfelt, S. T., & Terjesen, B. F. (2019). The effects of carbon dioxide on growth performance, welfare, and health of Atlantic salmon post-smolt (Salmo salar) in recirculating aquaculture systems [Article]. *Aquaculture, 498*, 578-586. https://doi.org/10.1016/j.aquaculture.2018.08.075

Munday, J., Perrott, M., Symonds, J., Walker, S., Preece, M., & Davie, P. (2018). Prevalence of spinal abnormalities in Chinook salmon smolt and influence of early rearing temperature and growth rates. *Journal of Fish Diseases, 41*(7), 1111-1116.

Näslund, J., & Johnsson, J. I. (2016). Environmental enrichment for fish in captive environments: Effects of physical structures and substrates. *Fish and Fisheries, 17*(1), 1-30.

Näslund, J., Rosengren, M., Del Villar, D., Gansel, L., Norrgård, J. R., Persson, L., Winkowski, J. J., & Kvingedal, E. (2013). Hatchery tank enrichment affects cortisol levels and shelter-seeking in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences, 70*(4), 585-590.

National Animal Welfare Advisory Committe (NAWAC). (2018a). *Code of Welfare: Commercial Slaughter*. https://www.mpi.govt.nz/dmsdocument/46018-Code-of-Welfare-Commercial-slaughter

National Animal Welfare Advisory Committe (NAWAC). (2018b). *Code of Welfare: Transport*. https://www.mpi.govt.nz/dmsdocument/1407-Transport-within-New-Zealand-Animal-Welfare-Code-of-Welfare

National Farm Animal Care Council. (2021). *Code of practice for the care and handling of farmed salmonids*. Canadian Aquaculture Industry Alliance. https://www.nfacc.ca/pdfs/codes/farmed_salmonid_code_of_practice.pdf

Neuman, C., Hatje, E., Smullen, R., Bowman, J. P., & Katouli, M. (2018). The effect of fishmeal inclusion and prebiotic supplementation on the hindgut faecal microbiota of farmed Tasmanian Atlantic salmon (Salmo salar L.) [Article]. *Journal of Applied Microbiology, 125*(4), 952-963. https://doi.org/10.1111/jam.13930

New Zealand Government. (2018). *The New Zealand Government: Aquaculture strategy* (978-1-99-000833-7). https://drive.google.com/file/d/1gLidnTyUSu1my4URexhNOOvELL1Jbf-0/view

New Zealand King Salmon (NZKS). (2017). *New Zealand King Salmon operations report*. https://www.kingsalmon.co.nz/new-zealand-king-salmon-operations-report/

New Zealand Salmon Farmers Association. (2021). *A+ New Zealand farmed salmon welfare standards*. http://www.salmon.org.nz/2021/09/14/a-new-zealand-farmed-salmon-welfare-standards/

Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H., & Turnbull, J. F. (2018). *Welfare indicators for farmed Atlantic salmon: Tools for assessing fish welfare*. Nofima.

Norambuena, F., Rombenso, A., & Turchini, G. M. (2016). Towards the optimization of performance of Atlantic salmon reared at different water temperatures via the manipulation of dietary ARA/EPA ratio [Article]. *Aquaculture, 450*, 48-57. https://doi.org/10.1016/j.aquaculture.2015.06.044

Nordgreen, J., Horsberg, T. E., Ranheim, B., & Chen, A. C. (2007). Somatosensory evoked potentials in the telencephalon of Atlantic salmon (*Salmo salar*) following galvanic stimulation of the tail. *Journal of Comparative Physiology A, 193*(12), 1235-1242.

Nuez-Ortin, W. G., Carter, C. G., Wilson, R., Cooke, I., & Nichols, P. D. (2016, Aug). Preliminary validation of a high docosahexaenoic acid (DHA) and -linolenic Acid (ALA) dietary oil blend: Tissue fatty acid composition and liver proteome response in Atlantic salmon (Salmo salar) smolts. *PLoS ONE, 11*(8), Article e0161513. https://doi.org/10.1371/journal.pone.0161513

Oppedal, F., Vågseth, T., Dempster, T., Juell, J.-E., & Johansson, D. (2011). Fluctuating sea-cage environments modify the effects of stocking densities on production and welfare parameters of Atlantic salmon (Salmo salar L.). *Aquaculture, 315*(3-4), 361-368.

Overton, K., Dempster, T., Oppedal, F., Kristiansen, T. S., Gismervik, K., & Stien, L. H. (2019). Salmon lice treatments and salmon mortality in Norwegian aquaculture: A review. *Reviews in Aquaculture, 11*(4), 1398-1417.

Palmer, A. L. (2017). *Prevalence of potential animal-based indicators of poor welfare status in New Zealand bobby calves: a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Physiology at Massey University, Manawatū, New Zealand* Massey University].

Part, C., Kiddie, J., Hayes, W. A., Mills, D., Neville, R. F., Morton, D., & Collins, L. (2014). Physiological, physical and behavioural changes in dogs (Canis familiaris) when kennelled: Testing the validity of stress parameters. *Physiology & Behavior, 133*, 260-271.

Perrott, M. R., Symonds, J. E., Walker, S. P., Hely, F. S., Wybourne, B., Preece, M. A., & Davie, P. S. (2018, Apr). Spinal curvatures and onset of vertebral deformities in farmed Chinook salmon, Oncorhynchus tshawytscha (Walbaum, 1792) in New Zealand. *Journal of Applied Ichthyology, 34*(2), 501-511. https://doi.org/10.1111/jai.13663

Phythian, C., Michalopoulou, E., Jones, P., Winter, A., Clarkson, M., Stubbings, L., Grove-White, D., Cripps, P., & Duncan, J. (2011). Validating indicators of sheep welfare through a consensus of expert opinion. *Animal, 5*(6), 943-952.

Poirier, I., Benhaïm, D., Poizot, E., Gallon, R. K., Cauvin, E., Lemarchand, A., Bertrand, M., Lelièvre, C., Murat, A., Benoit, F., & Méar, Y. (2020). Marine aggregates in North Atlantic coast: Microbial characteristics and potential interactions with farmed Atlantic salmon (Salmo salar) [Article]. *Marine Environmental Research, 157*, Article 104864. https://doi.org/10.1016/j.marenvres.2019.104864

Poli, B. M., Parisi, G., Scappini, F., & Zampacavallo, G. (2005). Fish welfare and quality as affected by pre-slaughter and slaughter management. *Aquaculture International, 13*(1), 29-49.

Preece, M. (2017). *Statement of evidence of Mark Anthony Preece in support of the New Zealand King Salmon Co. Limited's submission*. Ministry for Primary Industries. https://www.mpi.govt.nz/dmsdocument/17503/direct

Purcell, M. K., Powers, R. L., Taksdal, T., McKenney, D., Conway, C. M., Bilott, D. G., Polinski, M., Garver, K., & Winton, J. (2020, Jul). Consequences of Piscine orthoreovirus genotype 1 (PRV-1) infections in Chinook salmon (Oncorhynchus tshawytscha), Coho salmon (O. kisutch) and Rainbow trout (O. mykiss). *Journal of Fish Diseases, 43*(7), 719-728. https://doi.org/10.1111/jfd.13182

Qian, C., Hart, B., & Colombo, S. M. (2020). Re-evaluating the dietary requirement of EPA and DHA for Atlantic salmon in freshwater [Article]. *Aquaculture, 518*, Article 734870. https://doi.org/10.1016/j.aquaculture.2019.734870

Remen, M., Sievers, M., Torgersen, T., & Oppedal, F. (2016, 11/01/November 2016). The oxygen threshold for maximal feed intake of Atlantic salmon post-smolts is highly temperature-dependent [Article]. *Aquaculture, 464*, 582-592. https://doi.org/10.1016/j.aquaculture.2016.07.037

Richman III, N. H., de Diaz, S. T., Nishioka, R. S., Prunet, P., & Bern, H. A. (1987). Osmoregulatory and endocrine relationships with chloride cell morphology and density during smoltification in coho salmon (Oncorhynchus kisutch). *Aquaculture, 60*(3-4), 265-285.

Robb, D., & Kestin, S. (2002). Methods used to kill fish: field observations and literature reviewed. *Animal Welfare, 11*(3), 269-282.

Robb, D., O'Callaghan, M., Lines, J., & Kestin, S. (2002). Electrical stunning of Rainbow trout (*Oncorhynchus mykiss*): Factors that affect stun duration. *Aquaculture, 205*(3-4), 359-371.

Robb, D., Wotton, S., McKinstry, J., Sørensen, N., Kestin, S., & Sørensen, N. (2000). Commercial slaughter methods used on Atlantic salmon: determination of the onset of brain failure by electroencephalography. *Veterinary Record, 147*(11), 298-303.

Robb, D. H., & Roth, B. (2003). Brain activity of Atlantic salmon (*Salmo salar*) following electrical stunning using various field strengths and pulse durations. *Aquaculture, 216*(1-4), 363-369.

Romero, A., Pérez, T., Santibáñez, N., Vega, M., & Miranda, P. (2021, 02/25/February 2021). Phytogenic feed additive (PFA) standardized in labdane diterpens have a protective effect in Salmo salar against Piscirickettsia salmonis [Short Communication]. *Aquaculture, 533*. https://doi.org/10.1016/j.aquaculture.2020.736170

Roth, B., Slinde, E., & Robb, D. H. (2007). Percussive stunning of Atlantic salmon (Salmo salar) and the relation between force and stunning. *Aquacultural Engineering, 36*(2), 192-197.

Rowe, E., Dawkins, M. S., & Gebhardt-Henrich, S. G. (2019). A systematic review of precision livestock farming in the poultry sector: Is technology focussed on improving bird welfare? *Animals, 9*(9), 614.

Royal Society for the Prevention of Cruelty Animals. (2021). *RSPCA welfare standards for farmed Atlantic salmon*. https://science.rspca.org.uk/documents/1494935/9042554/RSPCA+welfare+standards+for+farmed+Atlantic+salmon+%28PDF%29.pdf/60ae55ee-7e92-78f9-ab71-ffb08c846caa?t=1618493958793

Royal Society for the Prevention of Cruelty Animals (RSPCA). (2020). *RSPCA approved farming scheme standard: Farmed Atlantic salmon*. https://rspcaapproved.org.au/wp-content/uploads/2020/05/2020-05_FARMEDATLANTICSALMON_Standard.pdf

Ruyter, B., Sissener, N. H., Ostbye, T. K., Simon, C. J., Krasnov, A., Bou, M., Sanden, M., Nichols, P. D., Lutfi, E., & Berge, G. M. (2019). N-3 Canola oil effectively replaces fish oil as a new safe dietary source of DHA in feed for juvenile Atlantic salmon [Article]. *British Journal of Nutrition, 122*(12), 1329-1345. https://doi.org/10.1017/S0007114519002356

Samsing, F., Johnsen, I., Stien, L. H., Oppedal, F., Albretsen, J., Asplin, L., & Dempster, T. (2016). Predicting the effectiveness of depth-based technologies to prevent salmon lice infection using a dispersal model [Article]. *Preventive Veterinary Medicine, 129*, 48-57. https://doi.org/10.1016/j.prevetmed.2016.05.010

Schroeder, P., Lloyd, R., McKimm, R., Metselaar, M., Navarro, J., O'Farrell, M., Readman, G. D., Speilberg, L., & Mocho, J.-P. (2021). Anaesthesia of laboratory, aquaculture and ornamental fish: Proceedings of the first LASA-FVS Symposium. *Laboratory Animals, 55*(4), 317-328.

Scottish Salmon Producers Organisation. (2015). *Code of Good Practice for Scottish Finfish Aquaculture*. http://scottishsalmon.co.uk/cogp/

Segner, H., Reiser, S., Ruane, N., Rösch, R., Steinhagen, D., & Vehanen, T. (2019). Welfare of fishes in aquaculture.

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T., & Seland, A. (1997). Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (Salmo salar). *Journal of Food Science, 62*(4), 898-905.

Silva, P. F., McGurk, C., Knudsen, D. L., Adams, A., Thompson, K. D., & Bron, J. E. (2015, Aug). Histological evaluation of soya bean-induced enteritis in Atlantic salmon (Salmo salar L.): Quantitative image analysis vs. semi-quantitative visual scoring. *Aquaculture, 445*, 42-56. https://doi.org/10.1016/j.aquaculture.2015.04.002

Småge, S. B., Frisch, K., Vold, V., Duesund, H., Brevik, Ø. J., Olsen, R. H., Sjaatil, S. T., Klevan, A., Brudeseth, B., Watanabe, K., & Nylund, A. (2018). Induction of tenacibaculosis in Atlantic salmon smolts using Tenacibaculum finnmarkense and the evaluation of a whole cell inactivated vaccine [Article]. *Aquaculture, 495*, 858-864. https://doi.org/10.1016/j.aquaculture.2018.06.063

Sneddon, L. U. (2003a). The evidence for pain in fish: the use of morphine as an analgesic. *Applied Animal Behaviour Science, 83*(2), 153-162.

Sneddon, L. U. (2003b). Trigeminal somatosensory innervation of the head of a teleost fish with particular reference to nociception. *Brain research, 972*(1-2), 44-52.

Solstorm, F., Solstorm, D., Oppedal, F., Olsen, R. E., Stien, L. H., & Fernö, A. (2016). Not too slow, not too fast: Water currents affect group structure, aggression and welfare in postsmolt Atlantic salmon Salmo salar [Article]. *Aquaculture Environment Interactions, 8*, 339-347. https://doi.org/10.3354/aei00178

Stenton-Dozey, J. M., Heath, P., Ren, J. S., & Zamora, L. N. (2021). New Zealand aquaculture industry: Research, opportunities and constraints for integrative multitrophic farming. *New Zealand Journal of Marine and Freshwater Research, 55*(2), 265-285.

Stien, L. H., Bracke, M., Noble, C., & Kristiansen, T. S. (2020). Assessing fish welfare in aquaculture. In T. S. Kristiansen, A. Fernö, M. A. Pavlidis, & H. van de Vis (Eds.), *The Welfare of Fish* (1st ed., Vol. 20, pp. 303-321). Springer.

Stien, L. H., Bracke, M. B., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P. J., Vindas, M. A., & Øverli, Ø. (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*(1), 33-57.

Størkersen, K. V., Osmundsen, T. C., Stien, L. H., Medaas, C., Lien, M. E., Tørud, B., Kristiansen, T. S., & Gismervik, K. (2021). Fish protection during fish production. Organizational conditions for fish welfare. *Marine Policy, 129*, 104530.

Su, H., van Eerde, A., Steen, H. S., Heldal, I., Haugslien, S., Ørpetveit, I., Wüstner, S. C., Inami, M., Løvoll, M., Rimstad, E., & Clarke, J. L. (2021, 01/01). Establishment of a piscine myocarditis virus (PMCV) challenge model and testing of a plant-produced subunit vaccine candidate against cardiomyopathy syndrome (CMS) in Atlantic salmon Salmo salar [Article]. *Aquaculture*. https://doi.org/10.1016/j.aquaculture.2021.736806

Sun, G., Liu, Y., Li, Y., Li, X., & Wang, S. (2015). Evaluation of feed and feeding regime on growth performance, flesh quality and fecal viscosity of Atlantic salmon (Salmo salar L.) in recirculating aquaculture systems [Article]. *Journal of Ocean University of China, 14*(5), 849-857. https://doi.org/10.1007/s11802-015-2565-5

Sundh, H., Kvamme, B. O., Fridell, F., Olsen, R. E., Ellis, T., Taranger, G. L., & Sundell, K. (2010). Intestinal barrier function of Atlantic salmon (Salmo salar L.) post smolts is reduced by common sea cage environments and suggested as a possible physiological welfare indicator. *BMC physiology, 10*(1), 1-13.

Symonds, J., King, N., Camara, M., Ragg, N., Hilton, Z., Walker, S., Roberts, R., Malpot, E., Preece, M., & Amer, P. (2018). New Zealand aquaculture selective breeding: from theory to industry application for three flagship species. Proceedings of the World Congress on Genetics Applied to Livestock Production,

Symonds, J. E., Clarke, S. M., King, N., Walker, S. P., Blanchard, B., Sutherland, D., Roberts, R., Preece, M. A., Tate, M., & Buxton, P. (2019). Developing successful breeding programs for New Zealand aquaculture: a perspective on progress and future genomic opportunities. *Frontiers in Genetics, 10*, 27.

Taylor, E. B. (1990). Phenotypic correlates of life-history variation in juvenile Chinook salmon, Oncorhynchus tshawytscha. *The Journal of Animal Ecology*, 455-468.

Thörnqvist, P.-O., Höglund, E., & Winberg, S. (2015). Natural selection constrains personality and brain gene expression differences in Atlantic salmon (Salmo salar) [Article]. *Journal of Experimental Biology, 218*(7), 1077-1083. https://doi.org/10.1242/jeb.114314

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 [Article]. *Aquaculture, 532*, Article 736076. https://doi.org/10.1016/j.aquaculture.2020.736076

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.

Ueda, H. (2011). Physiological mechanism of homing migration in Pacific salmon from behavioral to molecular biological approaches. *General and Comparative Endocrinology, 170*(2), 222-232.

Universities Federation for Animal Welfare (UFAW). *UFAW SAWI Fund Animal Welfare Student Scholarships*. Retrieved February 3 from https://www.ufaw.org.uk/ufaw-sawi-fund-grants-and-awards/ufaw-sawi-fund-animal-welfare-student-scholarships

van de Vis, H., Kolarevic, J., Stein, L. H., Kristiansen, T. S., Gerritzen, M., van de Braak, K., Abbink, W., Sæther, B.-S., & Noble, C. (2020). Welfare of farmed fish in different production systems and operations. In T. S. Kristiansen, A. Fernö, M. A. Pavlidis, & H. van de Vis (Eds.), *The Welfare of Fish* (1st ed., Vol. 20, pp. 323-361). Springer https://doi.org/https://doi.org/10.1007/978-3-030-41675-1_14

van de Vis, J., Abbink, W., Lambooij, B., & Bracke, M. (2014). Stunning and killing of farmed fish: How to put it into pratice? In *Encyclopedia of Meat Sciences (Second Edition)* (pp. 421-426). Academic Press.

Vanderstichel, R., St-Hilaire, S., Ibarra, R., Lyngstad, T. M., Rees, E., & Medina, M. H. (2015). Space-time cluster analysis of the non-pathogenic infectious salmon anemia virus (HPR0 ISAV) in Chile, 2011-2012 [Article]. *Aquaculture, 437*, 120-126. https://doi.org/10.1016/j.aquaculture.2014.11.027

Wang, Y., Chi, L., Liu, Q., Xiao, Y., Ma, D., Xiao, Z., Xu, S., & Li, J. (2019). Effects of stocking density on the growth and immunity of Atlantic salmon salmo salar reared in recirculating aquaculture system (RAS) [Article]. *Journal of Oceanology and Limnology, 37*(1), 350-360. https://doi.org/10.1007/s00343-019-7350-7

Waran, N., & Randle, H. (2017). What we can measure, we can manage: The importance of using robust welfare indicators in Equitation Science. *Applied Animal Behaviour Science, 190*, 74-81.

Wells, N., & Rodriguez-Ferrere, M. (2018). *Wells on animal law* (2nd ed.). Thomson Reuters,.

Wemelsfelder, F., & Mullan, S. (2014). Applying ethological and health indicators to practical animal welfare assessment. *OIE Scientific and Technical Review, 33*(1), 111-120.

Wendelaar Bonga, S., & Lock, R. (1992). Toxicants and osmoregulation in fish.

Wessel, Ø., Braaen, S., Alarcon, M., Haatveit, H., Roos, N., Markussen, T., Tengs, T., Dahle, M. K., & Rimstad, E. (2017). Infection with purified Piscine orthoreovirus demonstrates a causal relationship with heart and skeletal muscle inflammation in Atlantic salmon [Article]. *PLoS ONE, 12*(8), Article e0183781. https://doi.org/10.1371/journal.pone.0183781

Whittaker, B. A., Maeda, S., & Boulding, E. G. (2021). Strike a pose: Does communication by a facultative cleaner fish, the cunner wrasse (Tautogolabrus adspersus), facilitate interaction with Atlantic salmon (Salmo salar)? [Article]. *Applied Animal Behaviour Science, 236*, Article 105275. https://doi.org/10.1016/j.applanim.2021.105275

Winckler, C. (2019). Assessing animal welfare at the farm level: Do we care sufficiently about the individual. *Animal Welfare, 28*(1), 77-82.

Wong, D., von Keyserlingk, M. A., Richards, J. G., & Weary, D. M. (2014). Conditioned place avoidance of Zebrafish (*Danio rerio*) to three chemicals used for euthanasia and anaesthesia. *PLoS ONE, 9*(2), e88030.

Woodruff, M. L. (2017). Consciousness in teleosts: There is something it feels like to be a fish. *Animal Sentience, 2*(13), 1. https://doi.org/10.51291/2377-7478.1198

Wright, D. W., Nowak, B., Oppedal, F., Bridle, A., & Dempster, T. (2017). Free-living Neoparamoeba perurans depth distribution is mostly uniform in salmon cages, but reshaped by stratification and potentially extreme fish crowding [Article]. *Aquaculture Environment Interactions, 9*, 269-279. https://doi.org/10.3354/AEI00233

Wynne, J. W., Thakur, K. K., Slinger, J., Samsing, F., Milligan, B., Powell, J. F. F., McKinnon, A., Nekouei, O., New, D., Richmond, Z., Gardner, I., & Siah, A. (2020). Microbiome profiling reveals a microbial dysbiosis during a natural outbreak of Tenacibaculosis (Yellow mouth) in Atlantic salmon [Article].

Frontiers in Microbiology, 11, Article 586387. https://doi.org/10.3389/fmicb.2020.586387

Yamauchi, K., Ban, M., Kasahara, N., Izumi, T., Kojima, H., & Harako, T. (1985). Physiological and behavioral changes occurring during smoltification in the masu salmon, Oncorhynchus masou. *Aquaculture, 45*(1-4), 227-235.

Yossa, R., Levesque, S., Groman, D. B., & Lora, J. H. (2018, 07 / 03 /). Preliminary evaluation of purified lignin and hemicellulose as prebiotics candidates for Atlantic Salmon, Salmo salar L [Article]. *Journal of Applied Aquaculture, 30*(3), 256-271. https://doi.org/10.1080/10454438.2018.1439795

Young, T., Walker, S. P., Alfaro, A. C., Fletcher, L. M., Murray, J. S., Lulijwa, R., & Symonds, J. (2019). Impact of acute handling stress, anaesthesia, and euthanasia on fish plasma biochemistry: implications for veterinary screening and metabolomic sampling [Original Paper]. *Fish Physiology and Biochemistry, 45*(4), 1485. https://doi.org/10.1007/s10695-019-00669-8

Zahl, I. H., Kiessling, A., Samuelsen, O. B., & Olsen, R. E. (2010). Anesthesia induces stress in Atlantic salmon (Salmo salar), Atlantic cod (Gadus morhua) and Atlantic halibut (Hippoglossus hippoglossus). *Fish Physiology and Biochemistry, 36*(3), 719-730.

Zahl, I. H., Samuelsen, O., & Kiessling, A. (2012). Anaesthesia of farmed fish: implications for welfare. *Fish Physiology and Biochemistry, 38*(1), 201-218.

Zalcman, E., Burroughs, A., Meyer, A., Hillman, A., Sadler, R., Madin, B., Mackenzie, C., Ward, M. P., Stevenson, M., Happold, J., Hutchison, J., Gallardo Lagno, A. L., Cameron, A., & Cowled, B. (2021). Sea lice infestation of salmonids in Chile between 2011 and 2017: Use of regulatory data to describe characteristics and identify risk factors [Article]. *Aquaculture, 530*, Article 735752. https://doi.org/10.1016/j.aquaculture.2020.735752

Zanuzzo, F. S., Beemelmanns, A., Hall, J. R., Rise, M. L., & Gamperl, A. K. (2020). The innate immune response of Atlantic salmon (Salmo salar) is not negatively affected by high temperature and moderate hypoxia [Article]. *Frontiers in Immunology, 11*, Article 1009. https://doi.org/10.3389/fimmu.2020.01009

Appendices

Appendix A: Data charting form.

Article Characteristics	
Citation details (author/s and article title):	[e.g., Taylor et al., Fish welfare indicators]
Country of origin	[e.g., New Zealand]
Aim of study:	[should be found in the introduction]
Year of publication:	[e.g., 2019]
Species of fish:	[King or Atlantic salmon]
Lifecycle stage:	[x years or months old/smolt, grow-out, adult etc]
Level of observation:	[farm-level, cage-level, aquaculture tank-level, laboratory tank-level, or individual-level]
Context of investigation:	[aquaculture/laboratory setting]
Type of study:	[experimental, observational, or mixed]
Did the article explicitly mention 'welfare'?	[yes or no]
If yes, is welfare characterised?	[yes or no]
What section of the article is the term welfare mentioned?	[introduction, methods, results, or discussion]
Welfare Indicator Characteristics	
Indicator/s investigated:	[e.g., plasma cortisol
Animal-based or resource-based:	[e.g., plasma cortisol is animal-based]
Sampled post-mortem or ante-mortem	[e.g., samples taken after euthanasia = post-mortem]
Related physical/functional state:	[e.g., hypoxia or disease state]
Affective experience (if mentioned):	[e.g., pain]

Appendix B: Number and percentage (%) of articles which referenced different aspects of welfare (nutrition, environment, health, behaviour, and mention of welfare) in their titles and abstracts.

Year published (Start of period to 2021; number of articles)	Nutrition	Environment	Health	Behaviour	'Welfare'
2015 (802)	377 (47%)	316 (39%)	506 (63%)	125 (16%)	137 (17%)
2014 (875)	409 (46%)	344 (39%)	548 (62%)	135 (15%)	149 (17%)
2013 (957)	455 (48%)	373 (39%)	593 (62%)	141 (15%)	157 (16%)
2012 (1029)	492 (48%)	396 (38%)	640 (62%)	154 (15%)	168 (16%)
2011 (1097)	524 (48%)	418 (38%)	677 (62%)	167 (15%)	175 (16%)
2010-2021 (1157)	556 (48%)	444 (38%)	707 (61%)	174 (15%)	179 (15%)

Appendix C: Full list of measurements and observations extracted from articles.

Over-arching welfare indicator	Related measurements and observations
Blood parameters	Amino acid and nitrogen metabolic balance, amylase activity, antibody titre, complement haemolytic activity, blood pH, erythroblasts, carbon dioxide partial pressure, fat content and fatty acid composition of erythrocytes, growth hormone, haematocrit, haemoglobin content, head-kidney-derived respiratory burst, immunoglobulin M levels, intracytoplasmic inclusion bodies, leucocytes, lipase activity, lysozyme activity, phagocytic activities, plasma concentration of florfenicol and florfenicol amine, plasma cortisol, plasma creatine kinase, plasma electrolytes (calcium, chloride, magnesium, potassium, sodium ion), plasma lactate, plasma osmolality, plasma glutamate pyruvate transaminase, plasma glutamate oxaloacetate transaminase, plasma thyroxine, plasma lysozyme concentration, protease activity, respiratory burst, serum glucose, serum protein, salmon plasma leptin hormone levels, smudge cells

Over-arching welfare indicator	Related measurements and observations
Cardiac pathology	Bulbus misalignment, cardiac rupture, irregularity of the compact myocardium, number of bulbar coronary collateral, relative ventricle mass, ventricular asymmetry, ventricular height : width ratio
Cleaner-client behaviour	Chase, contact, inspection, peck, pose, jolt
Diet additives	Agri-pro prebiotic, animal protein, arachidonic acid/eicosapentaenoic acid ratio, black soldier fly larvae meal, crude fat amount, crude protein amount, docosahexaenoic acid and alpha-linolenic acid from Camelina seeds, docosahexaenoic acid from canola oi, tallow, fish oil, poultry by-product oil, insect meal, canola oil, vegetable oil, hemicellulose, lignin, lupin meal, methionine concentration, fishmeal, spray-dried algae, soya bean meal, soya saponin concentration, soy protein concentration, vegetable protein, phytogenic feed additive
Escape behaviour	Tail beat
Exploratory behaviour	Approach novel object, latency to approach novel object, time spent with novel object
Eye damage	Cataracts
Fin damage	Active fin splitting, healed fin splitting

Over-arching welfare indicator	Related measurements and observations
Gill score	Healthy red colour, light scaring, macroscopic amoebic gill disease spots, mucus patch, necrosis streaking, white mucoid spots, plaques on the gill surface, percentage lesioned area
Growth rates	Specific growth rates, daily growth index, thermal growth coefficient
Histopathological changes in tissues	**Histopathology of gills:** Lamellar fusion, abundance of mucous cells, aneurysm, consolidation of inter-lamella space, epithelial lifting, epitheliocystis, formation of lacunae, intercellular oedema, thickening of basal region of lamella, clubbing, haemorrhage, hypertrophy, hyperplasia, hyperaemia, hydropic degeneration of epithelial cells, lamellar congestion, micro abscess, necrosis, thrombosis, number of lesions, gill inflammation, vesicle formation **Histopathology of gut:** Villi length, presence and size of supranuclear vacuoles, **Histopathology of the heart:** Leukocyte number, percentage area affected, myocardial inflammation, epicarditis, inflammatory lesions, lymphohistiocytic myocarditis
Infection behaviour	Jumping, rapid swimming, flashing

Over-arching welfare indicator	Related measurements and observations
Infection treatment regime	Hydrogen peroxide concentration, antibiotic concentration, number of cages treated, number of treatments, treatment delivery method (oral treatment, mechanical treatment by heat, medicinal hose treatment, non-chemical treatment, freshwater treatment, bath treatment)
Liver enzyme activity	Catalase activity, glutathione peroxidase activity, superoxide dismutase activity
Liver nutrient concentrations	Cholesterol concentrations, phytosterol concentrations, vitamin E concentrations, vitamin K concentrations
Locomotion	Time spent active, average speed, basal activity pattern
Microbial composition of tissue	Microbial composition of gills and skin
Na^+K^+-ATPase activity	Na^+K^+-ATPase gene expression in gills, NKA1a (freshwater ATPase), elongation-factor-1, beta actin, cofilin-2
Net cage characteristics	Conical shape, net solidity
Presence of pathogenic agents in tissues	Presence of pathogenic agents in brain, gills, heart, intestinal tract, kidney, liver, skeletal muscle, skin, spleen, and stomach
Sea lice count on fish	Net pen-level sea lice abundance

Over-arching welfare indicator	Related measurements and observations
Skin damage	Erosion of epidermis, raised scales, scale loss, skin lesions, skin pH, skin haemorrhage, subcutaneous oedema, ulcers
Smolt production protocol	Slow production, fast production
Snout damage	Mouth erosion, multifocal raised yellow plaques around palate and teeth, gross mouth lesions
Social rearing environment	Mono-culture, co-culture
Salmonid rickettsia syndrome necropsy	Bullae, congestive brain, congestive enteritis, haemorrhage in adipose tissue and pyloric caeca, hepatomegaly, lesions on liver, muscle caverns, presence of food in stomach, splenomegaly, ulcers, vesicles on skin
Vertebral deformities	Presence of fusion, reduced intervertebral space, irregular internal structure, lesions, aggravated fusions, one-sided compression, number of fused vertebra
Viral load in tissues	Viral load in brain, gills, heart, intestinal tract, kidney, liver, skeletal muscle, spleen, and stomach
Viscero-somatic indices	Cardiac somatic index, distal intestine somatic index, hepatosomatic index, mid intestine somatic index, proximal somatic index, pyloric caeca somatic index, spleen somatic index, visceral somatic index, whole gut somatic index

Over-arching welfare indicator	Related measurements and observations
Water nitrogen oxide concentration	Nitrate concentration, nitrite concentration

Appendix D: Accomplishments achieved during this Masters project.

- Completed first year of postgraduate coursework with an A+ (90-100%) average.
- Awarded one of six MPI Postgraduate Masters Scholarships in 2020 for research proposal of my Masters project.
- Awarded Massey Masterate Research Scholarship in 2021.
- Awarded high calibre UFAW Animal Welfare Student Scholarship in 2021 (also known as the Ruth Harrison Student Scholarship). This is the first scholarship of its kind to be awarded to a student in the Animal Welfare Science and Bioethics Centre.
- Formed a relationship with aquaculture industry representatives, NAWAC and the animal welfare directorate at MPI through working with an MPI mentor (Marie McAninch – former senior animal welfare advisor).
- Participated in November 2020 NAWAC meeting regarding fish farming and fish welfare.
- Invited to disseminate work on sentience in fish to NAWAC, members of the Animal Health and Welfare directorate at MPI, as well as members of Fisheries NZ.
- Keynote speaker at fish welfare symposium held by NAWAC and the National Animal Ethics Advisory Committee in 2021. I presented a talk on the current state of fish welfare and the development of a Code of Welfare for farmed fish.
- Attended scoping trip to salmon farms in the Marlborough Sounds with NAWAC in mid-May 2021.

- Organised and attended a scoping trip to salmon farms in Twizel to hold discussions with farm managers of Mount Cook Alpine Salmon and High Country Salmon in late-May 2021.
- Invited to review the SPCA Certified Standards for Chinook salmon. The first set of welfare standards of its kind for farmed salmon in New Zealand.
- Gained Certificate for Advanced Training: Fish Welfare Online Course from the Centre of Marine Sciences, University of the Algarve, Gambelas Campus, Portugal.
- Invited to perform animal welfare assessments at the 2021 Rural Games and provided a report on the state of welfare of the animals participating in the Rural Games.
- Provided tech support for the Australia and New Zealand College of Veterinary Scientists (ANZVCS) Animal Welfare Chapter conference 2021.
- Joined the Food and Fibre Youth Network and participated in the Network's October meeting. The discussions held throughout this meeting were submitted to the Primary Industry Committee regarding the future workforce of our primary industry in New Zealand.
- Guest lecturer for 300-level animal welfare course in 2021.
- I developed a welfare indicator characterisation matrix which allows for categorisation of indicators according to their temporal validity. The matrix has been introduced and presented to industry experts and researchers at international conferences and national symposiums. The matrix is currently being taught to 300-level animal science students at Massey and is a significant component of their final assessment for their course (117.332).
- Joined and presented welfare indicator matrix to the Philosophy of Human-Animal Interaction Lab group.

- Presented fish welfare research at ANZVCS Animal Welfare Chapter conference in June 2022.
- Presented at fish welfare research at an MPI Science Symposium (July 5th, 2022).
- Invited to present to the office of the Chief Science Advisor at MPI on fish welfare in Aotearoa (July 25th, 2022).
- Started new position (January 2022) at Massey University in the School of Veterinary Science as a Tutor in Animal Behaviour and Welfare.
- Involved as a co-supervisor for two MSc students within the AWSBC (2022-2023).
- Invited to, and joined, the KiwiCAM conference planning committee. An annual conference for students of cognition and memory.