CODE OF PRACTICE FOR THE CARE AND HANDLING OF FARMED SALMONIDS: REVIEW OF SCIENTIFIC RESEARCH ON PRIORITY ISSUES

September 2020

Farmed Salmonids Code of Practice Scientific Committee

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Excerpt from Scientific Committee Terms of Reference

Background

It is widely accepted that animal welfare codes, guidelines, standards or legislation should take advantage of the best available knowledge. This knowledge is often generated from the scientific literature.

In re-establishing a Code of Practice development process, NFACC recognized the need for a more formal means of integrating scientific input into the Code of Practice process. A Scientific Committee review of priority animal welfare issues for the species being addressed will provide valuable information to the Code Development Committee in developing or revising a Code of Practice. As the Scientific Committee report is publicly available, the transparency and credibility of the Code is enhanced.

For each Code of Practice being developed or revised, NFACC will identify a Scientific Committee. This committee will consist of a target number of 6 scientists familiar with research on the care and management of the animals under consideration. NFACC will request nominations from 1) Canadian Veterinary Medical Association, 2) Canadian Society of Animal Science, and 3) Canadian Chapter of the International Society for Applied Ethology. At least one representative from each of these professional scientific bodies will be named to the Scientific Committee. Other professional scientific organizations as appropriate may also serve on the Scientific Committee.

Purpose & Goals

The Scientific Committee will develop a report synthesizing the results of research relating to key animal welfare issues, as identified by the Scientific Committee and the Code Development Committee. The report will be used by the Code Development Committee in drafting a Code of Practice for the species in question.

The Scientific Committee report will not contain recommendations following from any research results. Its purpose is to present a compilation of the scientific findings without bias.

The full Terms of Reference for the Scientific Committee can be found within the NFACC Development Process for Codes of Practice for the Care and Handling of Farm Animals, available at www.nfacc.ca/code-development-process#appendixc.

CODE OF PRACTICE FOR THE CARE AND HANDLING OF FARMED SALMONIDS: REVIEW OF SCIENTIFIC RESEARCH ON PRIORITY ISSUES

Farmed Salmonids Code of Practice Scientific Committee Report

September 2020

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Conclusions:

1. **The definitions of "sentience" and "consciousness" vary widely across and within several disciplines, including neuroscience, psychology, and philosophy. Furthermore, the "problem of other minds" makes demonstrating conclusive evidence of sentience impossible. This renders a cogent and conclusive debate on the issue challenging.**

2. **Nociception, which does not require the emotive, subjective aspect of pain, triggers a wide range of physiological and behavioural responses in all vertebrates called the nocifensive response.**

3. **Arguments suggesting that fish cannot feel pain largely centre around the neurological requirements for sentience in humans and the absence of these in fish.**

4. **Arguments suggesting that fish can feel pain largely centre around the expression of pain-like responses to noxious stimuli and flexible behavioural responses suggesting the presence of conscious affective states.**

5. **Based on the scientific studies published to date and the enduring philosophical problem of other minds, we do not know if fish are sentient or not. However, we do know that fish consistently exhibit pain-like responses to noxious stimuli. Thus, given the possible moral consequences of assuming that they lack sentience, the Scientific Committee concludes that a prudent approach is most appropriate, wherein producers employ measures to mitigate nocifensive and maladaptive stress responses that could result in conscious pain or other negative affective states whenever possible.**

1.1   Introduction

There has been significant recent debate around whether fish are capable of sentience, which has crystallized around whether they can feel pain. This topic is highly complex, aside from also being quite controversial and polarizing. Much of the difficulty in summarizing the evidence for and against fish sentience stems from the interdisciplinary nature of the debate: the literature broadly spans different fields including neurophysiology, psychology, ethology, philosophy, and animal welfare science, each with unique approaches and field-specific language. In this chapter, we endeavour to 1) briefly and critically summarize the relevant arguments for and against fish sentience, primarily in the context of the debate around fish pain, and 2) describe and explain the Scientific Committee's position on the consideration of fish welfare.

However, before delving into the debate around fish specifically, it is worthwhile to review key terms and concepts. Sentience can be equated to "phenomenal consciousness" (Allen & Trestman, 2017), a type of consciousness originally distinguished by Block (1995) that refers to the qualitative character of an experience, or "what it is like" to be in a particular state (Nagel, 1974). In animal welfare science and ethics, the term "sentience" is usually narrowed to encompass specifically subjective or affective experiences (e.g., Singer, 1975; Varner, 2012).

Practically, this means that to be sentient is to feel—not just unconsciously or reflexively sense and/or respond to a stimulus. Feeling is generally thought to involve sensory input, intervening variables of emotion and motivation that help interpret the sensory information, and behavioural output (e.g., Brown & Farber, 1951; Berridge, 1996; Mason & Bateson, 2009; Cabanac et al., 2009). Without those intervening variables, sentience does not exist. For example, a human may sense heat from a stove on their hand (sensory input), feel pain (emotion) and wish to avoid it (motivation), and put the burnt hand in milk to soothe it (behavioural output). By contrast, a robot may be programmed to sense the temperature of a stove (sensory input) and, above a certain threshold, place its temperature sensor in milk (behavioural output), but the behavioural output is not the result of intervening emotion and/or motivation.

1.1.1   Other Minds

The struggle in probing these intervening emotional and motivational variables is that they are private and available only to the being experiencing them. This problem, often termed "the problem of other minds" has been summarized by Avramides (2001), who notes that most of our understanding about sentience, consciousness, and what a "mind" is comes from our own experience of having one. In essence, we cannot truly know whether anyone but ourselves is sentient. However, we routinely assume most other humans are sentient, primarily because there is anatomical homology between our own nervous systems (which presumably produce our sentience) and those of other humans, as well as our ability to communicate with one another about our private emotional experiences. Most non-human mammalian species with nervous systems similar to humans are also assumed to be capable of sentience, despite our inability to communicate with them. However, this assumption becomes more complex in lineages that do not share as many neurological features with humans, such as birds and fish (e.g., Bateson, 1991; cf. Nieuwenhuys, 2009), since assumptions based on anatomical homology are not as strongly supported and, like non-human mammals, they are unable to self-report experiences.

1.2   Relevance of Sentience to Welfare

The ability to experience pain, suffering, or any other objectionable, negative affective state (i.e., to be capable of sentience) is considered by many welfare scientists and ethicists to entitle an animal to have its welfare considered (e.g., Duncan, 1993). NFACC's current definition of "welfare" includes consideration of affective states, as well as health and biological functioning, and exhibition of both normal and important behaviours. This definition mirrors the "three circles of welfare" approach outlined by Fraser (2008), which posits that welfare is comprised of three overlapping concepts: 1) health and biological functioning, 2) affective states, and 3) natural living. Therefore, consideration of whether fish possess the capacity for conscious affective states (i.e., sentience) is an important discussion. An explanation of the Scientific Committee's position on the state of the literature and how to proceed with the consideration of fish welfare is necessary in order to place the remainder of this report in context.

1.3   The State of the Literature

1.3.1   Nociception

There is full agreement that fish possess the necessary neural components for nociception (a reflexive response to noxious stimuli) and can sense a variety of types of stimuli. They have

functioning nociceptors and they integrate sensory inputs from a variety of sensory organs. Fish nociceptors are similar to other vertebrates, with the single exception that there is no evidence for cold nociception in fishes (Ashley et al., 2007). Importantly, nociception is not pain perception, and hence is not evidence of sentience. Rather, it is an entirely unconscious process that identifies tissues that might be threatened or damaged and produces a nocifensive response.

1.3.2   Arguments against Fish Sentience

Those sceptical of the claim that fish can feel pain (arguments provided primarily by Rose et al., 2014, and Key, 2016) mainly focus on the neocortex, which they argue is necessary for human consciousness but is absent in teleost brains. Their argument—that without a neocortex (which facilitates the transmission of information through a network to produce consciousness), sentience is impossible—is necessarily anthropocentric. Since humans are capable of self-reporting experiences and are most convincingly assumed to be sentient (see Section 1.1.1: Other Minds), most research has assumed that the structures required to produce sentience in humans are the only structures capable of producing sentience.

This argument is complicated by our limited understanding of fish brain function. Briscoe and Ragsdale (2019) state that "evolutionary relationships of telencephalon-like structures from mammals to ray-finned fishes are unresolved. One illustrative case study is the question of whether ray-finned fishes possess a homolog of the hippocampus, a structure in the mammalian dorsal telencephalon (or pallium). Researchers have variously proposed that the entire fish pallium is homologous with the hippocampus, that fish do not have a hippocampus homolog, or that a fish hippocampus is found in the medial or the lateral pallium. This example captures in essence the chaos of conflicting telencephalon homologies proposed over the history of comparative neuroanatomy." There has been pioneering functional work done, wherein regions of the teleost brain have been lesioned and subjects have subsequently been tested in a variety of behavioural paradigms (reviewed in Broglio et al., 2005, and Demski, 2013). However, our understanding of the inter-relationships between regions and their functional repertoires remains limited relative to our understanding of mammalian neuroanatomy and function.

1.3.3   Arguments for Fish Sentience

The controversy concerning sentience and "feeling" or experiencing pain in fish is thoroughly reviewed in Braithwaite's (2010) book supporting the claim that fish can consciously feel pain. The majority of arguments that fish are sentient are supported by behavioural experiments, wherein researchers argue that fish may use a different set of neuroanatomical structures than mammals to produce conscious pain and show sufficient ability for complex cognition to support that they may have the capacity for consciousness (e.g., Chandroo et al., 2004; see *Section 1.3.4: Theoretical Considerations*). For example, rainbow trout respond to noxious stimuli (an injection of acetic acid in the lips) with a variety of altered behaviours, including increased opercular beat rate and anomalous rocking and rubbing of the mouth on the sides of the tank (Sneddon, 2003). The trout's attention to novel objects is also altered when subjected to noxious injections (Sneddon et al., 2003). Finally, treatment with morphine reduces these behavioural alterations and opercular beat rate (Sneddon, 2003). The authors and other researchers have inferred that these behavioural changes were evidence of conscious pain perception (i.e., sentience). Though these foundational studies have been criticized for methodological issues (e.g., doses of

analgesia; cf. Newby & Stevens, 2008) and for assuming that responses that are more complex than simple reflexes must involve consciousness when that is not actually the case (cf. Rose et al., 2014, and next paragraph), further work in this subject area has been conducted and pain-like responses have been observed in numerous fish species in a variety of experimental paradigms (reviewed in Schroeder, 2018, and Sneddon, 2019). These types of responses are usually taken as evidence of sentience in mammalian species (e.g., see Table 1 in Brown & Dorey, 2019); however, alternative explanations still exist for the responses exhibited by fish exposed to noxious stimuli (see *Section 1.1.1: Other Minds*). For example, the presence of opioid receptors and a response to the administration of opioids or anesthetics during exposure to a noxious stimulus both occur in lower organisms such as invertebrates (e.g., Harrison et al., 1994) and even plants (e.g., Baluška, 2016).

Other scientists have used learning paradigms to study the ability of fish to sense their environment (e.g., Popper, 1973), but the capacity to learn and commit relationships to memory does not necessarily imply consciousness. In fact, spinally transected rats can learn to lift their hindlimbs to avoid painful stimuli for increasingly long periods of time (Grau et al., 1998) and fully anesthetized rats can learn associations between tones and electric shocks (Edeline & Neuenschwander-El Massioui, 1988). Some have used more complex learning paradigms (e.g., trace classical conditioning) under the assumption that positive results would suggest the type of memory referred to as "declarative memory" and imply some conscious awareness (Nordgreen et al., 2010), but this assumption has been refuted by several authors (Eichenbaum, 1999; Price, 2015; Perruchet et al., 2016). Still others have suggested that tasks requiring flexible responses (e.g., route planning, motivational trade-offs) would more convincingly suggest sentience, since conscious affective states are suspected to mediate certain forms of flexible decision-making and types of goal-seeking that require innovation or planning (e.g., Mason, 2011; Droege & Braithwaite, 2015). Some species of fish exhibit these types of behaviours (reviewed in Bshary et al., 2002; Brown et al., 2006; Brown, 2015; and Droege & Braithwaite, 2015), though to our current knowledge no examples exist for salmonid species specifically.

1.3.4   *Theoretical Considerations*

We accept the hypothesis that the brain is the primary substrate for sentience in most vertebrate species. We also accept that sentience requires the transmission of nerve signals within complex nervous networks (e.g., LeDoux, 2019). However, there is growing support for the idea that human sentience and consciousness may be not be as tightly tied to the neocortex as Key (2016) and Rose et al. (2014) suggest. For example, some (Damasio & Damasio, 2016; Shriver, 2016) show that, in humans, sentience is not exclusively dependent on the neocortex, and Parvizi and Damasio (2001) hold that simple forms of consciousness can be produced by the brainstem. This latter claim is of particular interest, since the brains of teleost fish are largely dominated by highly developed regions of the brainstem (Nieuwenhuys et al., 1998), with many behaviours normally mediated by the mammalian forebrain instead being remarkably preserved when the fish forebrain is removed (e.g., Overmier & Hollis, 1983). Furthermore, other researchers who hold the view that fish do, or are likely to, feel pain argue that, given that fish and mammalian lineages diverged ~400 million years ago and sentience likely has adaptive benefits (cf. Cabanac, 1992; Dawkins, 1990, 1998; Rolls, 2014), convergent evolution may have produced something like mammalian sentience in other lineages using other brain regions or anatomical structures (i.e., other than those proposed to be necessary for sentience in humans; e.g., Velmans, 2012;

Allen, 2013) or, indeed, consciousness may pre-date the divergence entirely (though this is particularly difficult to scientifically support). Additionally, Birch (2018) and Varner (2017) suggest that there may be different degrees of sentience across different evolutionary lineages. Though these considerations may have a solid theoretical foundation and call some arguments against fish sentience into question, they still do not constitute empirical evidence for fish sentience.

1.4   What to Do?

1.4.1   The Conundrum

Fish sentience implies that they could experience a range of emotions that have implications for their welfare (see *Section 1.2: Relevance of Sentience to Welfare*). The conundrum is that due to the problem of other minds, our limited understanding of the teleost brain, and challenges in determining the neurological correlates of sentience, conclusive evidence of fish sentience is exceedingly difficult to demonstrate. Yet, the current lack of evidence for fish sentience does not negate the possibility of its existence and potential impact on welfare (absence of evidence is not evidence of absence). As of 2018, there were 149 418 tonnes of farmed finfish in production across Canada, with salmonids making up at least 132 572 of those tonnes (DFO, 2018). If there is a chance those fish are sentient, treating them as if they are not constitutes a considerable moral burden of suffering. So, what are we to do?

1.4.2   The Precautionary Principle

The precautionary principle is used by policy-makers to reduce risk, especially in cases that may be near a tipping point, when the scientific evidence to support the policy is uncertain or incomplete. It was initially endorsed in the United Nations World Charter for Nature and internationally implemented in the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer, and has since been widely used by policy-makers. Birch (2017) recently applied the principle to fish welfare with his Animal Sentience Precautionary Principle: "Where there are threats of serious, negative animal welfare outcomes, lack of full scientific certainty as to the sentience of the animals in question shall not be used as a reason for postponing cost-effective measures to prevent those outcomes." His proposed application of the principle has been criticized (e.g., Woodruff, 2017), but the premise is widely supported by scientists and philosophers studying animal welfare (e.g., Kaiser, 2005; Knutsson & Munthe, 2017; Jones, 2016). For example, Lund et al. (2007) conclude "that farmed fish should be given the benefit of the doubt and we should make efforts that their welfare needs are met as well as possible."

However, the precautionary principle has drawn considerable criticism, particularly when implemented as a "decision-rule," wherein it dictates which policies or actions are selected (Ahteensuu & Sandin, 2012). In response to a decision-rule precautionary approach to fish sentience and welfare, many have invoked Dawkins' (2017) "pragmatic approach" (e.g., Arlinghaus & Cooke, 2009; Browman et al., 2018; Chatigny, 2019), wherein she argues that "Animal welfare (well-being) can be defined objectively in terms of animal health and what animals want. Such a conscious-free definition is readily understandable by people with very different views about animals and yet is practical enough to point to what factual scientific information is needed in any given case." However, this definition of animal welfare is confusing (e.g., how do insentient animals have "wants"?) and incompatible with many more widely

accepted definitions (see *Section 1.2: Relevance of Sentience to Welfare*). Furthermore, there are obvious exceptions to this pragmatic approach; for example, slaughter, where an animal's health and wants are both compromised, yet welfare is still considered.

Therefore, we propose that a precautionary approach to fish sentience take a different form, wherein general conditions are placed on how actions and policies chosen (Ahteensuu & Sandin, 2012). In practice, the precautionary approach thus becomes a sort of lens through which we can view and re-evaluate policies and practices. Specific wording further supports this, in that the precautionary "approach" (vs. "principle") recognizes that 1) locally, there may be differences in producers' ability to apply the approach and 2) cost-effectiveness should be taken into account when applying the approach (Garcia, 1995). Moving forward with the precautionary approach, consideration of fish sentience and welfare then guides policy discussions and encourages that steps be taken to limit potentially negative affective experiences and promote potentially positive ones.

1.5   The Scientific Committee's Position

Based on the state of knowledge summarized here and the enduring problem of other minds, we do not know if fish are sentient or not. However, we do know that fish consistently exhibit pain-like responses to noxious stimuli. Given the possible moral consequences of assuming that they lack sentience, the Scientific Committee suggests a prudent, precautionary approach is most appropriate, wherein producers employ measures to mitigate nocifensive and maladaptive stress responses that could result in conscious pain or other negative affective states (if they exist for fish) whenever possible. As such, the chapters that follow report scientific evidence relevant to fish welfare, which involves an assumption of their ability to experience conscious negative and positive affective states.

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2   Stress Indicators

Conclusions

1. **Responses to internal and external stressors are normal adaptations that maintain physiological homeostasis and increase a fish's survival probability.**

2. **While physiological and behavioural responses to acute stressors can be beneficial, prolonged or chronic stress is maladaptive and can result in physiological responses and immunosuppressive changes that adversely affect salmonid welfare and increase susceptibility to diseases.**

3. **Measuring cortisol has been the primary means of assessing stress in experimental laboratory situations; however, it is a difficult assay and unlikely to be useful on a fish farm except in well-controlled experimental circumstances.**

4. **Monitoring swimming activity and feeding behaviour can be useful when assessing fish welfare during and after acute stress, such as crowding during grading or transport.**

2.1   Introduction

The stress response is a normal, highly conserved, adaptive response of all vertebrates, including fish, to biotic and abiotic stressors. It results in a variety of adaptive changes that restore the normal or optimal physiological state, a process called allostasis (Schreck & Tort, 2016). Farmed fish typically cannot escape from some stressors, resulting in a prolonged stress response that can threaten their health and welfare (Barton, 2002). Good welfare is reflected by the ability of the fish to cope with infectious and non-infectious stressors (e.g., Broom, 1996), so stressful husbandry conditions can result in impaired health and a decrease in coping ability and welfare. In this review, we briefly describe the salmonid stress response and review practical indicators of stress and their interpretations.

2.2   The Stress Response

In stressful situations, fish launch an endocrine (primary) stress response like that mounted by other vertebrates (see Figure 2.1; Donaldson, 1981; Pickering & Pottinger, 1989; Wendelaar Bonga, 1997). The autonomic nervous system results in catecholamine release into the circulating blood within seconds. Blood cortisol levels rise dramatically within minutes, through a hormonal cascade referred to as the hypothalamic-pituitary-interrenal (HPI) axis in fish (analogous to the HPA axis in mammals). Cortisol acts by either a direct effect on tissues or by up- or down-regulation of genes that mediate a mobilization of energy reserves to restore pre-stress conditions. The restoration of pre-stress conditions may be important in some acute stressful aquacultural circumstances that are discussed below. Cortisol is an essential component of the stress response in fish but also plays a significant role in osmoregulation, growth, and reproduction. Secondary responses to hormones include changes in metabolism, hydromineral balance, and cardiovascular, respiratory, and immune functions (see Figure 2.1). Tertiary responses are whole-animal changes in performance, such as in growth, disease resistance, and behaviour (see Figure 2.1; Schreck & Tort, 2016; Barton, 2002). Cortisol binds to receptors in the fish forebrain, suggesting a potential mechanism for the observation that stress interferes with

cognitive function (Schreck et al., 1997). Prolonged chronic or repeated stress is detrimental; for example, it is associated with decreased growth and/or leads to disease states. Mimicking prolonged stress with cortisol implants results in decreased growth in rainbow trout (Barton et al., 1987). Prolonged stress (high stocking density combined with poor water quality) results in a primary stress response (increase in plasma cortisol) that decreases with time and also results in a compromised barrier function of the skin, gill, and intestine that is associated with increased rate of movement of bacteria into the fish that lasts much longer than the increase in cortisol (Sundh et al., 2019). The mechanistic relationships between the primary response (catecholamines and cortisol), changes in barrier integrity, and changes in immune function are poorly understood. For example, Valenzuela et al. (2018) showed that chronic stress involves two different and nonoverlapping proteolytic pathways in fish muscle.

2.3   Categories of Stress Indicators

In salmonid aquaculture, measuring stress indicators is essential to estimate how health, performance, and welfare are being altered by rearing conditions and husbandry. It is generally recognized that reducing stressors, especially chronic stressors, results in increased survival and growth of all developmental stages, from egg to broodstock. Stress indicators for fishes have been reviewed by Sopinka et al. (2016), Thorarensen and Farrell (2011), and Noble et al. (2018). We have focused on the review by Sopinka et al. (2016), since they provide an intensive and extensive review of stress indicators in fishes, including the indicators, the samples required (e.g., blood, plasma, tissue), and each indicator's strengths and weaknesses at three levels of organization:

1. **Cellular and Molecular**: All indicators in this category involve extremely complex assays that are unlikely to be used on a fish farm. Depending on technological advances, it is possible that they may be used in future by inspectors who could analyze samples in a lab setting. They include reactive oxygen species, heat shock proteins, genes regulated by cortisol, and intracellular enzymes.

2. **Primary and Secondary Physiological**: Of all the factors in this category, blood cortisol concentrations are considered the gold standard in laboratory and research situations. It is not a useful on-farm metric because the fish must be rapidly caught and sampled without disturbance for an accurate measurement. Furthermore, both assays (an enzyme- or radio-immunoassay) are complex and the instruments required are expensive. At present, none of the other indicators in this category are likely to be useful on farm, but, similar to above, technological advances may make these more feasible in future.

3. **Whole Organism**: Indicators in this category are likely the most useful on a fish farm, particularly after transient stress: transportation, grading, and during or immediately before harvest. Prolonged stress effects could be monitored by observations of behaviour, especially during feeding; for example, a decrease in response to feed, month to month feed intake, biomass gain, and feed conversion. In addition, monitoring morphological changes such as colour change and fin erosion may be useful indicators. The incidence of daily mortalities is an important indicator of the overall health of a tank/cage of fish (Iversen et al., 2005), though ideally other indicators would be used before stress caused mortality. Examples chronic stressors include mycotoxins in feed, or high stocking density combined with poor water quality (Sundh et al., 2019).

2.4   Stressful Procedures

2.4.1   Stress During Transport

The transportation of salmonids between rearing sites (e.g., from freshwater tanks to marine net pens or freshwater raceways) or to slaughter could be stressful. Many disease outbreaks occur during the first months after transfer of smolts to sea sites (Iversen et al., 2005); however, some reports indicate that smolt transport is only "moderately stressful" (Nomura et al., 2009). Iversen el al. (2005) reported that loading was the most stressful event during transport (i.e., produced the largest cortisol response) and that multiple stressors (e.g., heavy seas during transport) increased mortalities in the subsequent 30-day period almost 20-fold to a total of 18%. Plasma cortisol was correlated with subsequent mortality whereas blood glucose and lactate were not. With current technology, there are few metrics that can be practically measured during the transport process, except for marked increases in swimming behaviour as the fish are crowded (Chandroo et al., 2005). However, in the days following transportation, changes in feeding behaviour and mortalities (discussed below) could be useful.

2.4.2   Stress During Grading

Fry are usually graded before grow-out. Grading can reduce size-related agonistic interactions (e.g., Adams et al., 2000; Lahti & Lower, 2000 Wipf & Barnes, 2011; Cvetkovikj et al., 2015), and allows for optimally sized feed pellets. Like transport, there are few metrics that could be applied during the grading process except for marked increases in swimming behaviour (Chandroo et al., 2005). However, changes in feeding behaviour and cumulative mortalities (discussed below) could be useful in the days following the grading of fish.

2.5   Stress Indicators

Stress after a stressful procedure mentioned above or effects of prolonged stress could be monitored by observations of indicators mentioned in the Whole Organism category (Sopinka et al., 2016). Some indicators may be detected during routine checks, during feeding, or with video cameras in the net-pens, but some may require a specific test. Indicators must be relevant to the fish, usable on the farm, reliable enough to make informed decisions on the farm, and suitable for use in the particular farming operation (Noble et al., 2018).

2.5.1   Morphological Changes

Two morphological changes that may indicate chronic stress are skin colour and fin erosion. Colouration in fish is due to cells in the scales called "chromatophores" that contain pigment or "iridophores" that produce iridescence through diffraction of light. Colour change is achieved by contraction (less colour or "paling") or dispersion of these cells. Chromatophores are regulated both by hormones and nerves and have alpha and/or beta-adrenergic receptors on their membranes, both of which may be stimulated by noradrenaline to produce contraction and dispersal. In general, catecholamines released during stress cause contraction of chromatophores such that fish appear pale (Thorsen, 2019).

Fin erosion can result from abrasion with rough surfaces, agonistic interactions, nutritional deficiency, and bacterial or sea-lice infection (Hamoutene et al., 2016), though it is sometimes unclear whether fin damage persists and is therefore stressful, and/or persists because a stress response to another stimulus suppresses immune function and wound healing (for example, see *Section 5: Biodensity*).

2.5.2   Behavioural Changes

Behaviour is probably one of the best day-to-day indicators of stress and welfare. It is non-invasive and gives an immediate indication of status but must be interpreted carefully since there is some risk that improperly collected data may result in a subjective interpretation. Many farms have video cameras and/or echo sounders installed to monitor behaviour (especially feeding and feed wastage) but these also can be used to monitor normal schooling behaviour and daily activity and swimming patterns (e.g., Juell & Fosseidengen, 2004).

2.5.2.1   Appetite and Behaviour During Feeding

Though specific mechanisms linking appetite suppression to the stress response have only been partially determined in fish, there is evidence that elements of the HPI axis mediate the appetite-suppressing effects of stress (Bernier & Peter, 2001). In practice, changes in exploratory behaviour, feed anticipatory activity, latency to feed, or amount of food required to achieve satiation all would indicate a stress response. In Atlantic salmon, the stress associated with changes in water quality (Espmark & Baeverfjord, 2009) or of vaccination (Sørum & Damsgård, 2004) or predation may result in reduced feed intake. A decreased anticipatory response to feeding can also be a sign of stress (Folkedal et al., 2012).

2.5.2.2   Other Behaviours

Unusual behaviours or a marked change in behaviour such as loss of equilibrium, slow swimming, or unusual vertical position (Fernö et al., 1995) of some fish or of most fish (Bui et al., 2013) may indicate acute stress. Unstructured swimming near the bottom of net-pen, tank, or container can also be an indicator of acute stress (Bui et al., 2013). Salmon usually swim continuously in a school in the same direction (polarized, but the direction of swimming varies between pens) during the day with few fish in the centre or near the edge of the pen (Sutterlin et al., 1979). Surface activity (e.g., jumps and/or rolls) over time should be measured and, for example, can be used to calculate jumps per fish in the pen over 5 minutes. The number of jumps and rolls increases markedly during the initial stages of sea-lice infestation (Samsing et al., 2015; Webster et al., 2007). Orientation relative to current flow towards high flow areas is common in stressed salmonids (Ross et al., 1995).

2.5.2.3   Reflex Behaviours

The righting reflex (return to normal upright position after being turned ventral side up) and tail-pinch reflex (attempt to escape after having the tail pinched) can be used as indicators of stress (Davis, 2010). These indicators are altered after imposition of a stressor (Davis, 2010) and the tests are simple, quick, inexpensive, and are easily used in field settings (e.g., Campbell et al., 2009; McLean et al., 2016). However, there are no data to link changes in these reflexes to

cortisol levels or other metrics of stress, and this indicator needs further validation, not least to determine how stressed a fish must be before reflexes become impaired.

2.5.2.4   *Ventilation Rate*

An increase in respiration rate (opercular movements) or gasping at the surface may indicate low oxygen in the water or a stress response to other variables. For example, it increases from 55 to 81 opercular beats/min when fish are crowded prior to slaughter (Erikson et al., 2016) and from 108 to 162 opercular beats/min in smolts during delousing (Vigen, 2008). Although it must be noted that many stressors used in the lab to initiate the stress response during research (e.g., crowding, netting, and lifting fish out of the water) also make the fish hypoxaemic.

2.6   Caveats

There are some caveats or uncertainties when interpreting the stress indicators outlined above, as discussed by Sopinka et al. (2016). Firstly, two fish can have the same level of elevated stress indicator at the same sampling time point (e.g., plasma cortisol) but one fish may be on a trajectory to death while the other on a trajectory to recovery. For example, in other animals, cortisol is elevated in response to positive and negative affective experiences, and this may also be the case for fish (cf. Ellis et al., 2012), necessitating an examination of the context in which cortisol is elevated, not just the cortisol level alone. Secondly, when exposed to an identical stressor, plasma cortisol, immune function, and behaviour can vary among species, including closely related species (e.g., pink salmon and sockeye salmon; Donaldson et al., 2014), between sexes of the same species (e.g., adult female Pacific salmon have higher stressor-induced plasma cortisol levels [Donaldson et al., 2014] and a greater likelihood of temperature-induced stress and mortality relative to males [Martins et al., 2012]), or even between individuals within the same species (e.g., coping styles can vary at the individual level: Vaz-Serrano et al., 2011). Thirdly, stress has carryover effects: that is, stress experienced by parents can be expressed by changes in performance of the next generation and the magnitude of the stress response may be heritable (e.g., Atlantic salmon and rainbow trout have been bred to express high or low plasma cortisol and behavioural responses to a standardized stressor [Sopinka et al., 2016]). Fourth, the magnitude of the stress response varies with life stage; salmonids are typically hyporesponsive in early development, but hyperresponsive during smolting. So, care must be taken to use indicators of stress that are either specific to the appropriate life stage or that vary in their interpretation with specific life stages in a known way.

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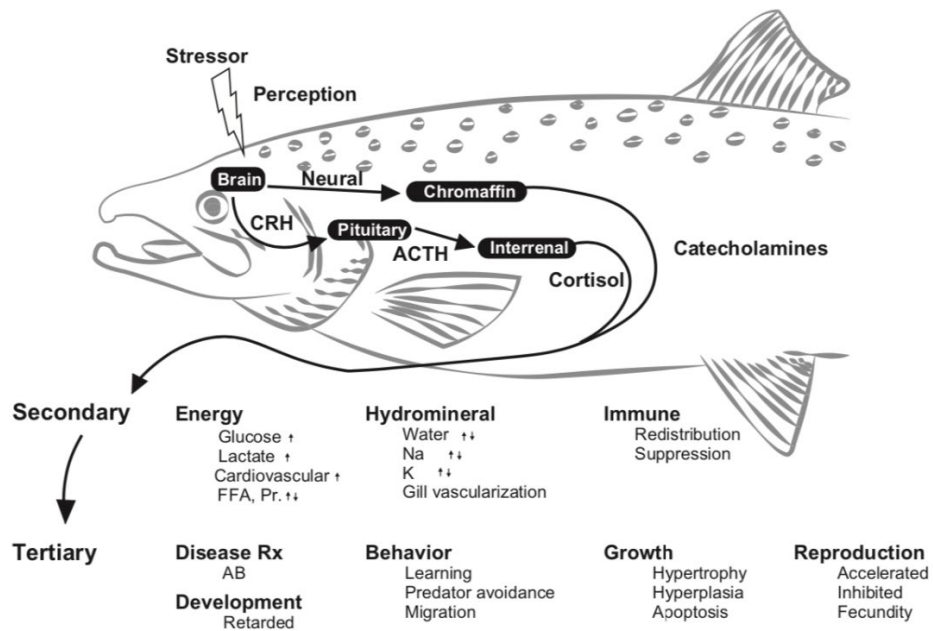
Figure 2.1 Primary, secondary, and tertiary responses of fish during distress.

After Schreck & Tort (2016). CRH: corticotropin releasing hormone; ACTH: adrenocorticotropic hormone; FFA: free fatty acids; P: *proteins*; AB: antibodies.

3   Sea Lice: Infection and Treatment

Co-authored by Mark Fast, PhD (University of Prince Edward Island)

Conclusions

1. **Sea lice infection can result in mild to severe skin ulceration that affects fish health, susceptibility to other pathogens, and the welfare of farmed fish.**

2. **The threshold number of lice per fish above which welfare is compromised varies by host life stage and size. Smaller fish (e.g., smolts) may experience mild stress at infection levels as low as 3 mobile lice/fish, but as the host increases in size the impact of the same infection level decreases.**

3. **There are a number of pharmaceutical agents used to control sea lice. Application of these agents may involve increased handling and stress for the fish being treated. Additionally, some agents are toxic at incorrect doses, so application must be done properly. Lice have also developed resistance to most chemotherapeutants and as such fish sometimes must be treated repeatedly. The effects of repeated treatment on fish are not well established in the literature.**

4. **Methods that reduce the risk of lice infection and do not necessitate crowding and handling (e.g., deep feeding, deepwater lighting) carry fewer welfare risks than those that involve handling, application of chemotherapeutants, and other potentially aversive factors.**

5. **Alternative/non-medicinal intervention strategies such as high water temperatures, low salinity, physical removal, and cage barriers need extensive research to determine their impact on fish welfare. Recent work has shown that exposure to the high water temperatures used during thermal treatment for ≤5 minutes are associated with panic reactions (and secondary issues) and that exposure for 72–140 seconds can cause thermal injury and mortality. Further work is required to determine the welfare impacts of other strategies.**

6. **Modelling approaches to more accurately depict farm network connectivity are needed, in conjunction with bioeconomic models to identify optimal treatment thresholds that protect wild stocks and cross-farm infection while minimizing costly treatments and selection for drug resistance.**

3.1   Introduction

Sea lice are common parasites of both wild and farmed marine fish. These parasitic infections can range in their impacts on the host and are affected by numerous environmental and management factors. The most commonly cultured species, Atlantic salmon (*Salmo salar* L.), is one of the more susceptible species to sea lice infection and is grown at stocking densities and in environments that can exacerbate infections. In most regions where salmon are farmed in sea cages (e.g., Norway, Chile, Scotland, Ireland, Canada), sea lice infections can transfer from wild fish populations and are magnified on farms to the point where significant management and

intervention strategies need to be employed to prevent welfare issues for farmed fish and spill-over infection pressure on wild salmon populations in the area.

Sea lice infections have proven difficult to manage and several chemical and non-chemical methods to control them have been used. All major salmon producing countries have established regulations requiring producers to regularly count and report numbers of sea lice on salmon in net pens and to treat them before numbers reach pre-determined levels. Unfortunately, lice have developed resistance to most of the chemical treatments used. Several new, non-chemical approaches to control infections show promise, including the use of altered temperature, salinity, lighting, "cleaner fish," physical removal, and mechanical barriers, some of which may modify fish behaviour to avoid infective sea lice stages. An integrated pest management (IPM) plan incorporating multiple different treatment/control modalities is likely the most promising approach for managing sea lice infections. This review describes important features of marine sea lice and how efforts to monitor and treat infections may affect optimal fish health and welfare.

3.2   Sea Lice Biology and Life Cycle

Sea lice is the common name referring to copepod ectoparasites within the family Caligidae. These parasites feed on the host's mucus, skin, and blood resulting in the range of impacts noted above. Multiple species of sea lice have been described in the North Atlantic and Pacific (Kabata, 1988; Jones & Johnson, 2015). *L. salmonis* susbsp. *Salmonis* (Atlantic) or *oncorhynchii* (Pacific) are of greatest concern in the Northern hemisphere, whereas in the South Pacific *C. rogercresseyi* is the species of major concern (Fast & Dalvin, *in press*). Other sea louse species found to infect farmed salmon include *C. elongatus* and *C. curtis* in the North Atlantic (Hogans & Trudeau, 1989; Hemmingsen et al., 2020) and *C. clemensii* and *Lepeophtheirus cuneifer* in the North Pacific (Jones & Johnson, 2015). In different locations, farmed or wild salmonids, whether resident or migratory, can act as sources of infection. These species of lice have a direct life cycle consisting of 8 stages, each separated by a moult (Gonzalez & Carvajal, 2003; Hamre et al., 2013). This life cycle consists of 3 larval planktonic stages, the last of which is parasitic and must find a host to infect before their energy reserves run out. Upon infection, the breakdown of stages can vary across Caligid copepod species (i.e. 2 chalimus and 2 pre-adult stages in *L. salmonis* vs. 4 chalimus and zero pre-adult stages in *C. rogercresseyi*) prior to maturation to adults. Following maturation and mating, adult females produce hundreds to thousands of free-swimming larvae capable of infecting other fish (Figure 3.1; Hamre et al., 2013).

Lice biology, infection, and development are highly dependent on water temperature and salinity. *Lepeophtheirus salmonis* and *C. rogercresseyi* naturally infect and develop on host salmon between 4–21°C and salinities >27 ppt. Below 5°C and/or below 27 ppt salinity, louse development (all planktonic and parasitic stages) and infection are severely compromised and at the higher range of temperatures (> 18°C), although development is much faster, adult survival and egg viability are compromised (Bricknell et al., 2006; Samsing et al., 2016; Montory et al., 2018; Hamre et al., 2019). For example at 10°C, the time from infection to first egg production in *L. salmonis* is approximately 47 days, whereas it would be 27 days at 15°C based on data from Hamre et al. (2019).

3.3   Impact of Sea Lice

High densities of the parasite on fish, in particular the mobile pre-adult and adult parasite life stages, can cause significant lesions, as they aggregate in large numbers around the head and posterior to the anal fin, feeding mainly on skin, mucus, and blood (Johnson & Fast, 2004; Wagner et al., 2008; Heggland, 2020). Osmoregulatory stress in juveniles and or stress associated with intensive blood feeding across all sizes of host fish can lead to chronic cortisol elevation and reductions in specific growth rate and overall growth (reviewed in Johnson & Fast, 2004; reviewed in Wagner et al., 2008; Fjelldal et al., 2019). Abolofia et al. (2017) investigated the total biomass lost per production cycle in Norway (through lice impacts on growth and mortality) and estimated that it varied from 3.6–16.6%, despite different control measures, particularly chemical lice treatment (application of parasiticides).

Additional indirect effects from lice infection can include pathogen vector transmission and/or increased host susceptibility to other infections. Numerous pathogens of salmon have either been identified from *L. salmonis/C. rogercresseyi* individuals or shown to cause greater pathology to salmon hosts during co-infection with sea lice. These salmon pathogens include infectious hematopoetic necrosis virus (Long et al., 2019), *Loma salmonae* (Mustafa et al., 2000), *Piscirickettsia salmonis* (Figueroa et al., 2017), and infectious salmon anemia virus (ISAv; Oelckers et al., 2014; Barker et al., 2019). Although there is a lack of evidence for sea lice acting as a true vector for the pathogens listed above (i.e. presence of pathogen within lice is not necessarily evidence of pathogen transmission by lice), sea lice are well-known immunomodulators of their hosts and may increase fish sensitivity to these pathogens (Fast, 2014).

3.4   Monitoring, Controlling, and Treating Sea Lice Infections

3.4.1   Monitoring Sea Lice Infections

Determining the numbers of sea lice infecting salmon is most often done by sampling salmon in a net pen and manually counting sea lice. However, cameras and optical underwater counting systems used in salmon net pens to monitor feeding, behaviour, etc., are gaining popularity and being adapted to count sea lice on individual fish (Gautam et al., 2017). This shift may be beneficial for salmon welfare, as it will limit acute stress associated with repeated handling for sea lice counting.

Thresholds at which intervention is required (usually expressed as numbers of sea lice of a particular sex or life stage per fish) are used by farming industry authorities across the Northern Hemisphere (for example, action including treatment or harvesting is required in British Columbia between April and June if *L. salmonis* levels are >3 mobile lice per fish). In some countries, such as Norway, thresholds have been set as low as 0.5 mature female lice per fish (0.2 during migration); however, in both cases these thresholds are not set due to welfare concerns for farmed fish but rather conservation purposes, in an effort to limit spillover infection of wild smolts passing by the net pens. Prior work has shown that post-smolts can exhibit signs of chronic/acute stress during infections with as low as 3 mobile lice per fish (Nolan et al., 1999) and susceptibility to ISAv may be increased at these infection levels (Barker et al., 2019). Stien et al. (2013) and Grimnes and Jakobsen (1996) suggest that sea lice infections become lethal around 0.12–0.15 lice per cm^2 of fish. But as noted by Johnson and Fast (2004) and Wagner et al.

(2008), the impacts of mobile lice are dependent on host species and size. So, although there is evidence of mild stress in post-smolts (~100–300 g) at these infection levels (3 mobile lice/fish), as fish grow the impact of the same infection level decreases. For Atlantic salmon in their second year at sea (≥1 kg), when treatments are most common, there is no direct evidence that they experience any physiological impacts of sea lice infection at this level, but further research on the sub-lethal impacts of sea lice infection (e.g., Brauner et al., 2012) is required at different life stages and for different species of salmonids. Thus, there is considerable uncertainty around these types of quantitative thresholds (e.g., Heuch et al., 2011) making it difficult to draw firm conclusions on when intervention is required. Ideally, treatment thresholds would protect migrating salmon, avoid costly cross-farm infections, and minimize selection for drug resistance. Recent modelling work has begun to set a framework for identifying optimal salmon lice treatment thresholds (Kragesteen et al., 2019). Results of this work demonstrate that too low a treatment threshold results in high rates of treatment, but in situations where equal connectivity (between farms) exists within a salmon farm network, low thresholds (0.1 gravid females/fish) can maximize salmon survival and weight (Kragesteen et al., 2019). While the authors point out estimating realistic connectivity is essential for obtaining useful model outputs, and this exercise may have oversimplified other essential inputs such as external infection pressure and treatment efficacy, these types of bioeconomic models will likely be important tools in the future for finely-tuning treatment thresholds by region and perhaps even farm network.

3.4.2   Chemical Sea Lice Treatment

A number of treatments have been used for the control of sea lice (Aaen et al., 2015; Grant, 2002; Hjeltnes et al., 2019), most of which were originally developed and used to control parasites in terrestrial livestock and humans. Lice-infected fish are typically treated by applying chemotherapeutants in tarpaulin-enclosed net pens (to contain the chemicals), as a bath in well-boats, or by including them in feed.

When used according to label instructions and/or as directed by a veterinarian, most anti-louse chemicals have few major adverse effects for fish, though they all have the potential to impact fish physiology due to direct interaction with target and non-target tissues. Incorrect dosages have caused mortality, which typically increases with increasing temperature (Overton et al., 2019). With regards to stress caused by these different anti-louse treatments, it is important to consider that the acute stress of treatment is likely less impactful to the fish than the chronic stress of the infection, as has been shown for avermectins (Whyte et al., 2019; though these products are no longer widely used). However, repeated administration of treatments due to lack of efficacy against juvenile lice may be required over a 2–3 week period, and the repeated exposure to some chemicals (e.g., for fish welfare and the development of resistance by sea lice) remains a major gap in our knowledge of anti-louse therapies.

In-feed treatments do not cause additional stress to salmon due to the method of application, but incorrect dosages can cause welfare issues. For example, overdosage or repeated treatment with ivermectin has been associated with behavioural changes, loss of equilibrium and skin discolouration, and neurotoxicity in some cases due to accumulation in the brain (Palmer et al., 1987; Johnson & Margolis, 1993; Whyte et al., 2019); however, this has not been described for the avermectin SLICE®.

Tarpaulin or well-boat associated bath treatments require increased fish handling and crowding. Calculating correct treatment dosages is also difficult since there is considerable error involved when estimating enclosed water volumes (Dobson & Tack, 1991). As well, tarpaulin/skirts have been shown to significantly reduce oxygen levels (<50%) in cases without supplemental oxygen (Stien et al., 2012), whereas the process of loading onto well-boats has been shown to increase acute stress (i.e. via changes in plasma cortisol, glucose, and lactate levels) which, in most cases, returns to baseline after short periods (i.e. transport lasting between 4–40 hours), and minor stress during unloading (Iversen et al., 2005). In some cases, the time-consuming nature of tarpaulin setup can also add to stress and poor water quality (via decreased water circulation, aeration, O_2 reductions etc.) and has led to restricted treatment times (i.e. 30 min maximum exposures) within some areas and/or times of year (Gautam et al., 2017. Organophosphates (e.g., azamethiphos, dichlorvos) can cause neurotoxicity through reductions in brain cholinesterase concentrations if fish are given insufficient recovery time between treatments (e.g., Raverty, 1987; Roth & Richards, 1992) and azamethiphos has been shown to cause chronic kidney injury in rainbow trout from iron deposition following repeated treatments (Barisic et al., 2019). Hydrogen peroxide baths can cause increased plasma cortisol and glucose levels, as well as minor alterations in plasma ion concentrations (sodium, chloride, and potassium), likely associated with changes in gill and skin morphology (Bowers et al., 2002; M. Fast pers. Obs.). However, according to Bowers et al. (2002), all of these hydrogen peroxide-related changes return to normal within 24 hours post bath treatment. Sublethal chronic toxicity of hydrogen peroxide has also recently been reported in Atlantic salmon, suggesting that these impacts are more significant in the first half of the day compared to administration of bath treatments later in the day (Vera & Migaud, 2016). It is also likely that pre-existing health issues (e.g., amoebic gill disease; AGD) or poor water quality may have a synergistic effect with some bath treatments, causing more severe negative effects (including high mortality; Noble et al., 2018) than would be expected from fish in good condition with proper water quality.

3.4.3   *Mechanical Sea Lice Barriers and Removal Methods*

Atlantic salmon can have their contact and infection with sea lice reduced by using deep net-pens and submerged lights, which provide light that attracts fish to deeper water (Juell et al., 2003; Stien et al., 2014; Frenzl et al., 2014; see *Section 7: Lighting*). Several mechanical methods for preventing contact with or for removing sea lice are also gaining attention (Overton et al., 2019). These barriers are typically used inside the sea cage and may produce a closed or semi-closed environment. They include "permaskirts" around the cages (Grøntvedt et al., 2018; Stien et al., 2018), "snorkel cages, tubes or nets" (Oppedal et al., 2017; Wright et al., 2017; Geitung et al., 2019), and deepwater feeding (Frenzl et al., 2014). Lice skirts can impede water flow and exchange, possibly leading to compromised water quality and increased stress and/or pathogen susceptibility. Small-scale studies of snorkels have not revealed any associated welfare issues, and recent commercial scale studies have not attempted to examine potential welfare impacts at the commercial scale (Noble et al., 2018; Geitung et al., 2019).

There are also mechanical delousing systems such as the "Hydrolicer" and "Optilicer" that require fish to be pumped through a high-pressure spray system that removes the lice. In some cases, these are used in conjunction with cameras on site that count and/or identify sea lice and laser delousers that aim concentrated photons to kill or injure lice once identified. As many of these are patented systems in development under different registered trade names, few

independent studies evaluating their efficacy, economic viability (Brakstad & Matanovic, 2017; Brakstad et al., 2019; Bui et al., 2020a), or impacts on fish welfare are currently available. Furthermore, the efficacy of these systems has not been investigated under the broad range of conditions found on farm (i.e. in high plankton and biofouling sites or low oxygen conditions requiring supplementation). The Hydrolicer and similar technologies require fish to be crowded prior to treatment (Overton et al., 2019), which may induce an acute stress response. A survey by Hjeltnes et al. (2018) shows that farmers commonly report scale loss and mortality, while gill bleeding and wounds were observed at least during developmental phase testing for mechanical treatments. Overton et al. (2019) report that uncompromised fish (defined as fish from groups with low mortality [<0.25%] the month before treatment) are most likely to emerge from mechanical delousing systems intact, but small, compromised fish (defined as fish from groups with high mortality [0.25–1%] the month before treatment, therefore possibly experiencing disease or other stressors) may experience increasing mortality if temperatures are too high during treatment (~10–16ºC; Overton et al., 2019).

As noted in *Section 3.2: Sea Lice Biology and Life Cycle*, seawater at >18ºC compromises adult sea lice survival, so exposing infected fish to considerably higher temperatures has been proposed and used as a sea lice treatment method. However, Poppe et al. (2018) suggested that the temperatures usually used during thermal de-lousing (28–34°C) are likely to be noxious for fish. Exposure to temperatures >28°C for ≤5 minutes initiates panic reactions and behaviours indicative of nociception (Nilsson et al., 2019), during which fish are likely to inflict serious self-damage. Gismervik et al. (2019) also showed that salmon exposed to water at 34–38°C for 72–140 seconds incur severe thermal injury to their gills, eyes, brains, and death within 2 min (Nilsson et al., 2019).

It is important to remember that mechanical treatments confer no long-lasting protection against sea lice infection, and therefore must be repeatedly performed to control infection levels, exposing fish to repeated acute stress and possible physical damage from handling and/or treatment. Noble et al. (2012) suggest that ISAv, winter ulcers, and piscirikettsiosis can all colonize sites of epidermal damage in marine environments, whereas *Saprolegnia* spp. Infections are most likely in freshwater. Noble et al. (2018) also report high mortality in fish with pre-existing health conditions (e.g., AGD) during mechanical/thermal treatments, as well as secondary effects like brain and eye hemorrhaging due to panic behaviour and traumatic injury during treatment. There is limited data on the average number of treatments required per production cycle, though a simulation by Kragesteen et al. (2019) estimates that, for 3 established Faroese farms in close proximity with a treatment threshold of 2 gravid lice/salmon, there are likely 3–4 treatments (each with 95% efficiency) performed per 500-day production cycle, though there may be far more treatments depending on the cage site and/or treatment threshold. Overton et al. (2019) report a dramatic shift from chemotherapeutants to non-medicinal techniques between 2012–2017, yet our understanding of how these newer treatments affect fish welfare remains limited.

3.4.4   *Biological Control Using Cleaner Fish*

A number of cleaner fish species have been used to effectively control sea lice and are gaining popularity as a biological alternative to chemical treatments and expensive technologies. For Atlantic salmon culture, the most effective species used in Europe have been the ballan wrasse

(*Labrus bergylta*) and now most common is the lumpfish (*Cyclopterus lumpus*; Cerbule, 2018. The natural distribution of most cleaner fish species overlaps with the natural distribution of marine salmonids (Pampoulie et al., 2014; Groner et al., 2016). However, due to temperature sensitivity in ballan wrasse, more recent work has shown lumpfish to be an effective coldwater alternative for efficient biological lice removal (Imsland et al., 2014, 2015, 2018). There are some concerns that stored cleaner fish may be infected with sea lice, so re-using cleaner fish in consecutive years may re-expose salmon and is therefore not recommended (Gonzalez & DeBoer, 2017). Furthermore, there is emerging evidence that cleaner fish may play a role in pathogen transfer between salmonids (e.g., *Tenacibaculum maritimum*; Småge et al., 2016, and others reviewed in Treasurer, 2002), though more research is required to determine if cleaner fish are terminal hosts or potential vectors for these pathogens.

The welfare of the cleaner fish themselves is also of considerable concern, though outside the scope of this review.

3.4.5   Other/Emerging Lice Control Approaches

A number of other techniques to control lice infection are being explored, including the use of low salinity and selective breeding, as well as IPM plans.

Observations that sea lice infection rates are lower on wild fish in low salinity (Jones & Hargreaves, 2007), that infested fish sometimes return to brackish and freshwater (Wells et al., 2007; Birkeland, 1996; Revie et al., 2003), and that fish returning to spawn in freshwater were identified as being fresh run by the presence of lice or late run by being free of lice (Costello, 2009; Connors et al., 2008) led to the suggestion that lower salinity can reduce louse infection (Gjelland et al., 2014). Similarly, farmed salmon also exhibit reduced lice numbers in low-salinity areas (Jones et al., 2007; Revie et al., 2003; Heuch, 1995). Elmoslemany et al. (2015) also observed a potential selectivity across copepod parasite species due to salinity tolerance, where very low prevalence of *C. clemensii* on salmon was found in Muchalat Inlet compared to *L. salmonis oncorhynchii*, whereas other farming regions contained a more mixed infection, and associated this with low salinity of the area. *Caligus elongatus* also shows significantly lower prevalence at low temperature and salinity (Heuch, 1995) and variability also exists within different family backgrounds of *L. salmonis salmonis*, with respect to thermal and haline sensitivity (Ljungfeldt et al., 2017). For these reasons, observations of louse behaviour suggest they may actively avoid waters with salinity of < 20$^{o}/_{oo}$ (Heuch, 1995; Crosbie et al., 2019). In general, when exposed to salinities below ~26$^{o}/_{oo}$ sea lice numbers on salmon are progressively reduced as salinity decreases, or if exposed to low salinity for longer periods, suggesting hyposalinity may be a useful treatment against lice in cultured settings (Sievers et al., 2019). At present, there is no evidence to suggest that the transfer of marine adults to freshwater causes any welfare issues, though few investigations have been conducted.

There have been initiatives to use selective breeding for sea lice resistance, to modulate the fish immune system through nutrition, and to develop sea lice vaccines. Gjerde et al. (2011) and Robledo et al. (2019) have shown that Atlantic salmon exhibit moderate heritabilities for the number of lice/fish in *L. salmonis* and *C. rogercresseyi*, respectively. Despite availability of SalmoBreed- and Aquagen-sourced fish with purportedly enhanced resistance to lice, and global interest in identifying these traits in individual broodstock programs, their efficacy has not yet

been demonstrated in scientific studies of industrial application. Minor advances have also been made since Raynard et al. (2002) in prototype lice vaccines (Carpio et al., 2011). However, these intervention strategies, along with anti-louse functional feeds, confer considerably less protection than the current control or treatment methods identified above.

There is new evidence that the simultaneous use of multiple sea lice control methods may show promise (e.g., cleaner fish, in-feed chemical treatment, and deepwater lighting; Bui et al., 2020b), though the welfare effects of different treatment combinations have yet to be fully investigated. IPM plans, which take into account sea lice biology, infection dynamics, and farm-level environmental factors to determine a suite of appropriate sea lice control measures, show considerable promise but require further research (e.g., Brooks, 2009).

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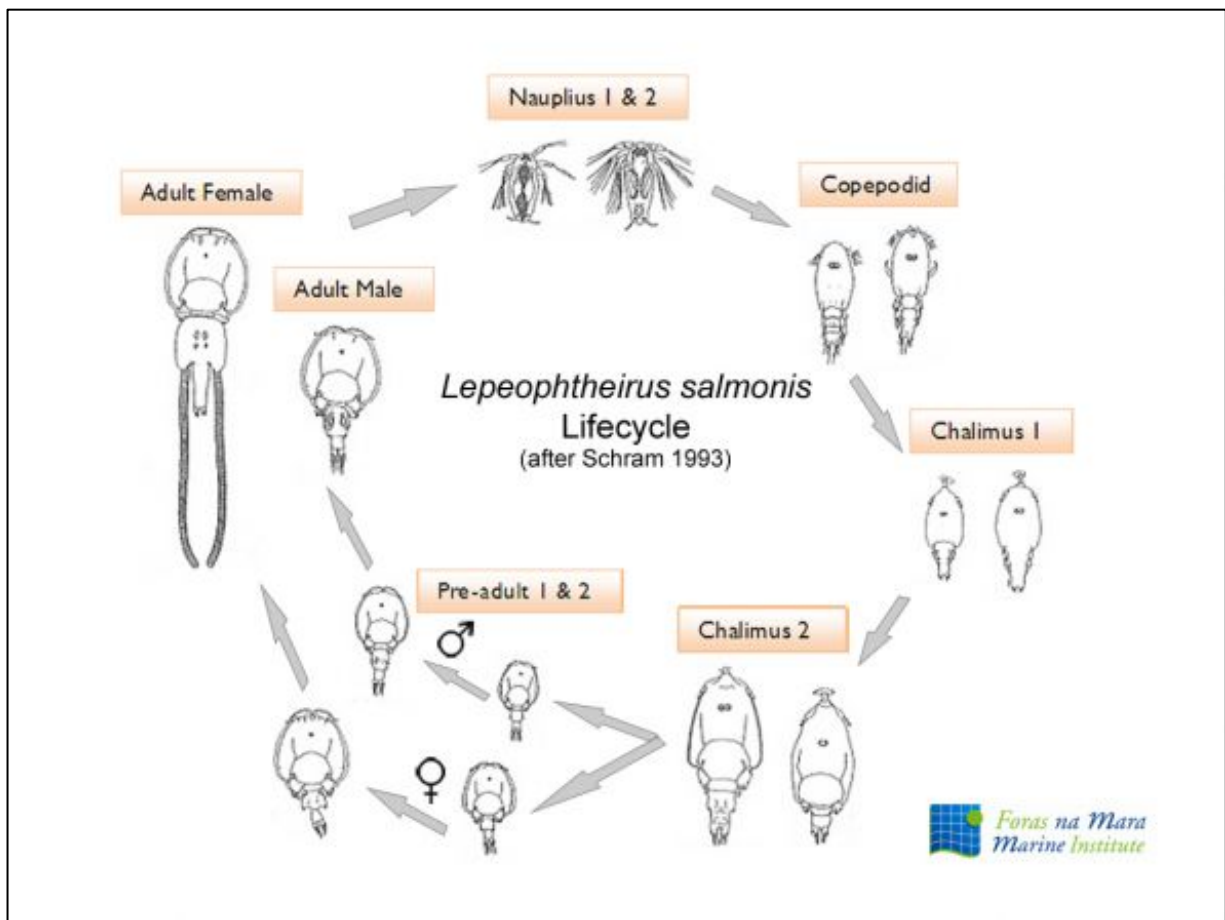
Figure 3.1. Life cycle of L. salmonis*, a common sea louse species infesting salmon.*

Three planktonic stages, nauplius I and II molt into the infective copepodid stage which attaches to the fish. The copepodid stage molts into sessile chalimus I and II stages, before moulting to the pre-adult stage that can move around on the surface of the fish and may enter the water column. The two pre-adult stages are followed by the final molt to the fully mature adult stage. From Marine Institute, https://www.marine.ie.

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4   Feed Deprivation

Conclusions

1. **Feed deprivation is either short-term, to empty the gut, or long-term, when fish rely on stored energy. The clearance/depletion rate is dependent largely on temperature and body size.**

2. **Short-term feed deprivation is beneficial to fish welfare prior to netting, handling, transportation, injection vaccination, slaughter, and acute phytoplankton blooms.**

3. **Long-term feed deprivation can be beneficial to fish welfare to reduce the risk of mortality due to chronic phytoplankton blooms and superchill in winter.**

4. **Broodstock close to the completion of sexual maturation exhibit a natural loss of appetite, resulting in feed deprivation beyond control of the farmer.**

5. **Welfare guidelines for the acceptable duration of short-term feed deprivation to empty the gut can be estimated reasonably well from the scientific literature, except for large Atlantic salmon harvested at < 5ºC.**

6. **Welfare guidelines for the acceptable duration of long-term feed deprivation may prove difficult to define but need to consider the transition from utilizing lipid reserves to breakdown of functional protein (Stage 2 to Stage 3 starvation).**

7. **Increases in aggression and dominance hierarchies resulting in fin damage may occur when feed is restricted (i.e., when fish are fed below satiation), even over very short periods.**

4.1   Introduction

This chapter reviews the primary scientific literature on the effects of feed deprivation on fish and attempts to identify pertinent welfare issues. Short-term feed deprivation of farmed salmonids, to empty their intestinal tracts, is standard practice preceding netting, transportation, and handling associated with vaccination and slaughter. An empty gut has both welfare and food safety benefits: lowered hypoxia risk by reducing the metabolism during feed digestion and uptake; reduced fouling of holding water with excreta, essential during transportation; and reduced risk of needle damage to internal organs from injection vaccination into peritoneal cavity. Herein, these practices are not further considered.

Long-term feed deprivation is practiced by farmers in four situations: 1) pre-slaughter, 2) during chronic harmful algae blooms, 3) during "superchill" events in sea-cages in Atlantic Canada, and 4) during sexual maturation in broodstock. These practices raise potential welfare concerns. Studies exploring feed deprivation to reduce the problem of early sexual maturation and improve disease resistance and meat quality are also included in this review.

4.2   Feed, Energy Reserves, Starvation Stress, and Behavioural Responses

Animals ingest feed for energy and other processes that require vitamins, minerals, etc.; some is used immediately, some is allocated to growth, and some is stored. Stored energy is vital to maintaining animal health when feed is scarce, a common occurrence among wild fish. The energy demand of an animal is often quantified indirectly by measuring oxygen consumption, which allows estimation of standard metabolic rate (minimum metabolic rate with an empty gut). Temperature and body size are the two dominant factors controlling metabolic rate. Warm-blooded farm animals have consistently high energy demands and therefore require regular meals. By contrast, the feed requirements of fish, most of which are ectotherms, are dependent on temperature, the principal controlling factor of metabolic rate (Fry, 1971). In Canada, the zone of thermal tolerance for farmed Atlantic salmon and rainbow trout is between 0.1 to 25°C in freshwater and 0 to 18°C in seawater. *Salvelinus* spp. in freshwater tolerate 0.1 to ~23°C. A 10ºC increase typically doubles the metabolic rate of many fish species (Clarke & Johnston, 1999). Body size and metabolic rate exhibit an inverse relationship in all animals, larger animals having a lower energy demand per unit body mass. Among fish, on a log-log scale, body mass and standard metabolic rate exhibit a straight-line relationship with a slope of 0.8 (Clarke & Johnston, 1999). Feed charts are derived from this relationship. For example, 5 g Atlantic salmon parr at 16ºC eat about 4% of their body weight per day (bw/d), compared with 1.7% bw/d for 70 g parr. By comparison, at 5ºC, the ration is much lower, about 1 and 0.25% bw/d respectively (Farmer et al., 1983). Feeding fish "to chart" is close to the maximum they will ingest and results in the highest somatic growth possible at a specific temperature. The maintenance ration (i.e., the amount of feed that results in neither gain or loss of body weight) is five- to seven-fold lower than the maximum ration, a measure of the capacity of fish to reduce their metabolic rate when feed is limited.

Appetite is dependent on stomach fullness. Salmonids will accept feed once their stomachs are about 85% full or less (Talbot et al., 1984). Gut evacuation rate is largely a function of both temperature and body size (see Figure 4.1), but temperature appears to be dominant (see Table 4.1). From the data in Table 4.1, most salmonids <1 kg complete gut evacuation within 15 degree days (ºD). However, in the only study on large 5 kg Atlantic salmon at 4ºC, typical of conditions at harvest in Canada, faeces were present in gut sections after 5–6 days, implying that 7 days of feed deprivation (28ºD) was needed to completely empty the gut (Waagbø et al., 2017).

The guts of farmed salmonids are mostly full most of the time, but among wild fish the incidence of fish with empty guts is high (Arrington et al., 2002). Consequently, fish in temperate regions have evolved strategies to survive long periods without feed (e.g., winter) by using stored energy. Salmonids are well-adapted to balancing periods of feed deprivation and reliance on stored energy with infrequent consumption of large high-quality prey items in the wild (Arrington et al., 2002). To survive winter, when both appetite and feed availability are low, the mobilization of stored lipids is vital to wild salmonids (Hutchings et al., 1999). Salmonids in freshwater in winter at <1ºC will ingest some feed, both in culture and wild (Cunjak & Power, 1987), but their appetite is very low, bordering on fasting, and weight gain is negligible.

Three stages of starvation have been identified in fish, similar to higher vertebrates (Soengas et al., 1996; Simpkins et al., 2003; Bar 2014):

Stage 1: Glycogen reserves in the liver are mobilized to maintain blood glucose levels, a critical fuel for the central nervous system. Both swimming activity and metabolic rate are reduced.

Stage 2: Lipids are mobilized, a process that involves the catabolism of triglycerides in adipose tissue and liberation of free fatty acids and glycerol into the blood circulation. Fatty acids are then processed in the liver into ketone bodies and circulated as energy sources for use by the brain. Protein catabolism begins, liberating amino acids, which are catabolized into keto acids and ammonia in the liver and used to produce glucose via gluconeogenesis.

Stage 3: Increased use of protein, which compromises vital tissue function and leads to death.

The timing of transition between each phase is species specific and strongly dependent on both body size and temperature (Bar, 2014).

Loss of body weight per day due to starvation among farm animals is rapid: chickens 5%, lactating goats 8%, and pigs 6% per day (McCue, 2010). By comparison, among 5 kg market sized Atlantic salmon at 4°C starved for 12 weeks, the rate of weight loss was much slower and decreased progressively from 0.6% per day at day 7, 0.23% per day at day 30, and 0.14% per day at day 86; overall, losing only 11% of their initial weight after 12 weeks of feed deprivation (Einen et al., 1998). However, fish welfare was not considered (Einen et al., 1998). Body weight change in fish is not always a reliable indicator of starvation, since losses of organic material can be masked by increases in water content. For example, the mean weight of Atlantic salmon (20–30 g at 14°C) starved for 7 to 14 days exhibited no change (Soengas et al., 1996). The salmon parr remained in Stage 2 for up to 6 weeks of feed deprivation and, after refeeding for 15 days, had fully recovered their body reserves (Soengas et al., 1996). Among starved rainbow trout (50 g; 7.5°C) whole body water content increased from 74 to 86% over 147 days and was highly correlated with a decrease in lipids (Simpkins et al., 2003). By comparison, rainbow trout (280 g at 6–8°C) starved for 29 days lost 18% of mean body weight compared with a 7% gain among fed controls (Pottinger et al., 2003).

Whole body lipid content is considered a useful measure of the physiological status of starved trout (Simpkins et al., 2003). Non-lethal estimates of fish body fat can be taken with an easy-to-use but expensive "fat-meter" scanner (CAD $8,000; distell.com). By comparison, plasma cortisol, an established indicator of stress in fish, appears to be an unreliable measure of starvation. Among rainbow trout starved for between 6 and 9 weeks in two studies, plasma cortisol remained basal (<10 ng/ml), similar to fed controls (Sumpter et al., 1991; Pottinger et al., 2003).

In another study of feral sea-run female Atlantic salmon, Cipriano et al. (2015) examined changes in metabolite profile during a 6–7 month-long spawning induced period of inappetence. During this period, the authors found no evidence for a shift to metabolic compounds that would indicate a pathological catabolic state, concluding instead that these fish may not have been stressed in an unnatural way and may be adapted, at this life stage, for such a state.

Starvation of Arctic charr for 18 weeks to explore the relationship between somatic growth and age at sexual maturity caught the attention of veterinarians and ethicists (Liu & Duston, 2016, with responses from O'Halloran, 2017, and Rollin, 2018). Whole body protein levels (16%) were unchanged throughout the deprivation period and fish were relying on their lipid reserves, which decreased from about 12 to 4% (Liu & Duston, 2016). The data indicate the fish were clearly deep into Stage 2 starvation but had not reached Stage 3.

Increased aggression among farmed salmonids is a serious potential consequence of food deprivation. Low frequency feeding (12 times/day) and automatic feeders where access to feed was limited have been shown to cause significant opercular and fin erosion in juvenile Atlantic salmon (Larsen et al., 2018). Rainbow trout (25 g, 8°C, 1.8 kg/m^3) offered reduced rations developed dominance hierarchies resulting in the greatest dorsal fin damage among subordinates (Moutou et al., 1998). Among Atlantic salmon parr (60 g, 10°C, 10 kg/m^3), food restriction (i.e., feeding below satiation) at short as 10 days resulted in significant increases in dorsal fin damage (Canon Jones et al., 2017). Such fin damage can produce secondary infections and is a significant welfare concern. Other risk factors influencing the incidence of aggression will include temperature, stocking density, species, strain, and even life stage.

It is possible that starvation stress increases the resistance of Atlantic salmon parr to cold water vibriosis (Damsgård et al., 2004), though this result has yet to be replicated in the species. Among non-salmonids, red sea bream (*Pagus major*) deprived of feed exhibited a significantly greater response from the liver than fed controls following exposure to *Edwardsiella tarda*, indicating that the phenomenon may be real, but the mechanism remains unknown (Mohapatra et al., 2017).

4.3   Aquaculture Practices that Extend Feed Deprivation Beyond Gut Evacuation

4.3.1   Deprivation Pre-Slaughter

The present review process failed to identify any clear advances in recent years defining acceptable limits of starvation in farmed salmonids. Therefore, a review of previously published welfare guidelines is included here. In 1996, the Farm Animal Welfare Council (UK) recommended that rainbow trout and Atlantic salmon could be deprived of feed for up to 48 and 72 hours, respectively, before slaughter for food hygiene reasons (FAWC, 1996). The rationale for these values was neither explained nor supported in the scientific literature at that time and ignored temperature. A review by Ashley (2007) urged that empirical studies be conducted on the effect of feed deprivation on stress physiology and behaviour. The European Food Safety Authority acknowledged that long periods of feed deprivation are commonly used prior to slaughter of Atlantic salmon and conceded that there was no published data on fish welfare (EFSA, 2008). EFSA (2009) stated a simple time duration of starving could not be specified, since its impact on welfare was dependent on temperature, body size, and lipid reserves, but did recommend that fasting beyond 50°D (e.g., 5 days at 10°C) should be avoided. FAWC (2014) recognized sudden feed withdrawal may increase aggression, and that deprivation periods in degree-days were more appropriate than absolute time. After 56°D of food deprivation (14 days at 4°C), large Atlantic salmon (5.6 kg) exhibited no negative effects on the general stress level, immunity, or health as judged by a suite of health markers, including gene expression, hormones, and metabolites (Waagbø et al., 2017). The same study reported the gut was empty in 7 days (28°D).

4.3.2   Chronic Harmful Algae Blooms

Algae blooms pose a serious threat to farmed salmonids in marine net-pens in British Columbia and pose an occasional but increasing threat in Atlantic Canada. Blooms can cause mass mortality due to either physical damage to gills, ichthyotoxins, or hypoxia. Globally, blooms have also caused massive losses of farmed salmon in Europe and Chile (e.g., Quinones et al., 2017) and rising ocean temperatures exacerbate the problem (Gobler et al., 2017). In BC, the daily decision to feed the fish is made only after the water column has been sampled with a plankton net and the species and abundance of algae determined (Haigh, 2017). The algae are typically concentrated relatively close to the surface where the photosynthesis rate is highest. Salmon normally remain in the safer, deeper water of the cage, but are attracted to the surface if feed is offered, with possible fatal consequences. The duration of food deprivation is often just a few hours, but in some instances can last for days to weeks (B. Milligan, pers. comm.).

4.3.3   Superchill Risk Management

"Superchill" is an industry term for the death of farmed salmonids from ice crystals forming in tissues when seawater water is at or below 0ºC and can be a serious problem for net-pens in certain coastal areas of Atlantic Canada (Saunders et al., 1975; Fletcher et al., 1988). Risk of superchill is greatest from late February to early March, when the seawater temperature can drop below the freezing point of cellular, plasma, and other body fluids (~-0.6 to -0.8ºC). The seawater can be below the freezing point of tissues and fish will remain alive in a super-cooled state (Scholander et al., 1957). Electrolytes in these tissues generally protect the fish from forming ice crystals, which disrupt cellular functions. However, when startled for any reason (e.g., by loud noises, feeding, human activity, encountering surface ice in net pens), sudden movements of the fish will result in rapid ice crystal formation—a process known as "seeding." If left undisturbed, the fish will often remain deep in the cage where the temperature may be slightly warmer than the surface. Therefore, farmers stay off the site and no feed is offered, a situation that can extend to 2 weeks or more in Newfoundland (A. Borchardt, Mowi, pers. comm.).

4.3.4   Sexual Maturation

Among all salmonids, the latter stages of sexual maturation are associated with a loss of appetite and a decrease in somatic tissue mass, while the gonads continue to grow. Among broodstock, this cessation of feeding has no negative effects on gamete quality but is associated with a decrease in disease resistance. By contrast, among fish intended for food production, sexual maturation is highly undesirable. For Atlantic salmon reared in Norway, Scotland, and Atlantic Canada, maturing fish start fasting and losing weight from June/July onwards, completing sexual maturation around November (Asknes et al., 1986; Stead et al., 1999; Duston & Saunders, 1999).

4.3.5   High Water Temperature

Atlantic salmon reared in sea-cages are increasingly at risk to high summer temperatures due to global climate change. The mass mortality in a Newfoundland fjord in September 2019 is the most worrying incident from Canada (CBC News, 2019). In Tasmania, Atlantic salmon were

exposed to a peak of 22.9°C seawater and >110 days above 18°C (Wade et al., 2019). Large fish (2.4 kg) were offered food daily, but their appetite decreased to become entirely absent for about two months, resulting in a loss of about 300 g of body weight. Smaller fish (1 kg) were more tolerant to high temperature: they stopped feeding for less than 1 week and their body weight remained stable. Oxygen was about 85% saturation (6.5 mg/L throughout) and mortality was not reported. From a welfare perspective, risky sea-cage sites need to be identified by modeling seawater temperatures, and supplemental oxygen needs to be available.

4.4   References

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Table 4.1 Gut evacuation rate (hours) of Atlantic salmon, brown trout and rainbow trout fed a variety of meals.

Data are sorted by temperature, from lowest to highest. With the exception of Waagbø et al. (2017), evacuation rates range from about 48 hours at 5–7°C to about 15 hours at 15–18°C, relatively independent of species and body size. Degree days (°D) for gut evacuation ranged from 9.1–15°D in nine studies, the exception being 28°D. Note gut evacuation rate should be one of several factors used to plan starve time, including careful consideration of context and potential welfare implications.

| Species	Body weight (g)	°C	Time (hours)	°D	Reference
Salmo salar	5600	4	168	28.0	Waagbø et al., 2017
Salmo trutta	90–300	5.2	42	9.1	Elliott, 1972
S. salar	695	7.1	48	14.2	Sveier et al., 1999
S. salar	150–200	9	30	11.3	Storebakken et al., 1999
S. trutta	90–300	9.8	27	11.0	Elliott, 1972
O. mykiss	142	10	28	11.7	Fauconneau et al., 1983
S. salar	900–1450	13.4	24	13.4	Aas et al., 2017
O. mykiss	91	15	24	15.0	Windell et al., 1969
S. trutta	90–300	15	15	9.4	Elliott, 1972
O. mykiss	140–145	18	14	10.5	Fauconneau et al., 1983
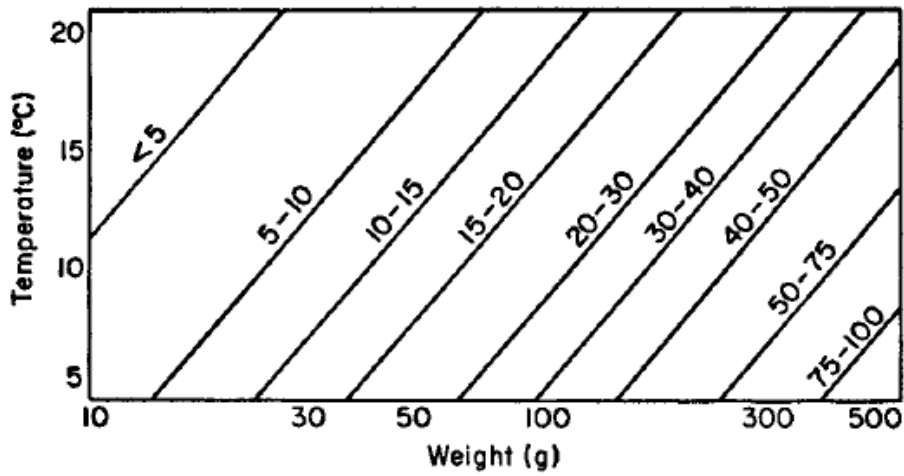
Figure 4.1 Gut evacuation time (hours) in rainbow trout relative to body weight (10–500 g) and temperature (5–20°C).

The diagonal lines represent the time in hours for the stomach to empty following a meal of 1–3% of body weight. Example: 100 g trout at 10°C evacuated stomach in about 20 hours. Figure from Grove et al. (1978).

5   Biodensity

Conclusions:

1. **Appropriate biodensities for rearing fish vary with life history stage, size, and species, as well as tank/net pen size and shape.**

2. **As long as water quality can be maintained, high biodensities may not have a significant impact on mortality rates but can produce an acute stress response to crowding that, if sustained, can be detrimental to fish health and welfare.**

3. **High biodensities can impair salmonid immune function, as it can exacerbate inflammatory responses and/or suppress innate and adaptive immune responses, leaving fish more susceptible to pathogens.**

4. **Generally, high biodensities can reduce growth rate, feeding efficiency, and fin quality for most salmonids.**

5. **Rates of aggression can increase with increasing biodensity until, above a certain point, aggressive behaviours are minimized. This relationship varies depending on species and body size variation within the group.**

6. **Studies that incorporate multiple welfare indicators into a single welfare index demonstrate that the relationship between welfare and biodensity is highly variable, complex, and confounded by a variety of other factors.**

7. **There are considerable differences between net pen and recirculating aquaculture systems in many respects, so these systems may require system-specific recommendations.**

5.1   Introduction

"Biodensity," used by some authors interchangeably with "stocking density," is defined as the fish biomass per unit volume of water and typically expressed in units of kg/m^3. Though this is a useful metric for comparison, it is important to recognize that "density" is a relative term and fish are rarely distributed evenly throughout a tank or net pen (i.e., with uniform nearest neighbour distances). Salmonids are social animals, who prefer the presence of, respond to, and learn from their conspecifics (e.g., Brown & Brown, 1996; Brown & Laland, 2002; Brown et al., 2003), so inappropriate biodensities can have considerable impacts on their welfare. In net pens, tanks, and recirculating aquaculture systems (RAS), biodensity also has implications for managing water quality, controlling pathogens, and mediating conspecific behavioural interactions.

Loading density is also a pertinent measure, especially for RAS and other land-based systems, and is typically expressed as fish biomass per unit water flow, i.e., kg fish per litre of water flow supplied to the rearing tank per minute (L/kg/min). To maximize return on financial investment, the goal of most RAS designs is to achieve the highest standards of product quality and fish welfare at the highest possible biodensity and lowest loading density (Martins et al., 2010). Increased fish biodensities and reduced water flow are design criteria to maximize productivity of RAS and may pose a threat to water quality and fish health and welfare. Establishing a causal

relationship between increased biodensity and fish welfare, *per se*, is difficult because associated deterioration in one or more water quality parameters is almost always a confounding factor.

Herein, we discuss the effects of changing the spatial relationship between conspecifics (i.e., altering biodensity), as opposed to the potential water quality perturbations that may be caused by different biodensities. For example, this chapter will not cover how increasing biodensity is related to oxygenation requirements or water ammonia concentrations, or the potential welfare impacts of compromised water quality (these topics are covered in *Section 6: Water Quality in RAS*). Instead, the objective of this chapter is to examine how biodensity in and of itself affects various welfare-relevant parameters in both sea-cage farming and RAS.

5.2   Tank and Net Size Considerations

Most of the data on optimal biodensities are derived from experiments conducted in small rearing tanks with relatively small fish. This approach might not be suitable for determining biodensities that avoid chronic stress and associated fin and opercula erosion in larger fish. Problems that may be evident in small experimental tanks might not be scalable to large production systems. The increased containment size may change the dynamics of different biodensities. For example, the ratio of a cylindrical tank's surface area to water volume decreases from 5:1 for a 1 m diameter tank 1 m deep (0.79 m^3 volume) to 0.9:1 for a 5 m diameter tank 2 m deep (39 m^3 volume). This may be a pertinent change when discussing welfare indicators such as fin erosion. Furthermore, net pens are flexible structures that are subject to a wide range of deformation, due to tidal and other hydraulic conditions and/or biofouling, which may result in inconsistent amounts of available living space (Turnbull et al., 2008).

5.3   Effects on Fish Health

5.3.1   *Mortality and Stress Response*

It appears that there is minimal impact of biodensity on salmonid mortality. Atlantic salmon parr (70 g) reared at a constant density of either 21, 43, 65, or 86 kg/m^3 for 14 weeks in 1 m^2 tanks (500 L) at 9.9°C (0.3 L/kg/min) all performed well and all reaching the smolt stage (100 g; Hosfeld et al., 2009). Atlantic salmon smolts (~80 g size) also showed no differences in mortality rates when stocked in 500 L tanks at either 20 or 100 kg/m^3 at 9.4–10°C (0.5 L/kg/min) for 57 days (Sveen et al., 2018). Post-smolts also showed similar mortality rates across biodensity treatments: Liu et al. (2017) found no differences in mortality of Atlantic salmon (~494 g) between biodensities of 9 to 53 kg/m^3 when raised in tanks of ~3 m^3 (2 m diameter and 1.3 m deep) tanks. When reared over 220 days, adult Atlantic salmon (~1400 g) at densities of 6 to 24 kg/m^3 also did not exhibit differences in mortality (Wang et al., 2019). In other species, a similar pattern has generally been found. Sevier et al. (2019) raised juvenile Arctic charr (~177 g) at densities ranging from 30 to 150 kg/m^3 in tanks of ~0.3 m^3 (dimensions: 1 × 1 × 0.27 m) and found no difference in mortality. Goebel et al. (2017) raised European whitefish fingerlings (~11 g) at densities from 3.75 to 15 kg/m^3 in 50 L tanks at 10.5°C and 14°C and found the same: no difference in mortality, regardless of temperature or density. For rainbow trout fingerlings (~0.21 g); however, mortality was reduced in lower density treatments (10 vs 12.5 kg/m^2) when reared in 2.7 m^3 raceways over 249 days (Timalsina et al., 2017). In most of the above studies, critical water quality factors like oxygen and ammonia were maintained within safe levels in an attempt to examine the density effects in the absence of water quality perturbations.

Despite the apparently minimal impact of biodensity itself on mortality of salmonids, higher or lower densities can elicit a stress response activation, depending on the species (see *Section 2: Stress Indicators*). In a seawater RAS (28–30 ppt) at 16.5ºC, Atlantic salmon post-smolts (~450 g) were stocked at 8, 15, and 30 kg/m^3 in large tanks (3000 L; 2 m diameter, 1.3 m deep). Over 10 weeks, the biodensities increased to 16, 31, and 61 kg/m^3 respectively (Liu et al., 2015). Mild, chronic stress was evident at the highest biodensity from week 3 onwards, as interpreted through elevated serum cortisol and alkaline phosphatase (which is involved in the initial stages of wound healing), though the stressor was not identified (Liu et al., 2015). Loading density was initially very good (>0.5 L/kg/min), but after 10 weeks at a biodensity of 61 kg/m^3, loading density was only 0.27 L/kg/min, indicating that water quality may have been compromised over the course of this study. In a trial of similar design, Liu et al. (2017) likewise found that after 9 weeks the salmon doubled in weight to 0.9 kg at final biodensities of 18, 37, 54 kg/m^3. Minor chronic stress was detected at the highest density (54 kg/m^3), including increased alkaline phosphatase and blood glucose (Liu et al., 2017). Post-smolts (~117 g) at the highest biodensity (70 kg/m^3) in 3.3 m^3 tanks showed an initial elevation in plasma cortisol and cortisol release rates that subsequently decreased over 57 days (Sundh et al., 2019). Calabrese et al. (2017) also found that post-smolts (~115 g) exhibited elevated cortisol, as well as elevated sodium levels, pCO_2, and decreased plasma pH when stocked at the highest density treatment (125 kg/m^3) in 500 L tanks for 8 weeks. This pattern was observed in adult Atlantic salmon (~1400 g) as well, with plasma cortisol increasing with increasing densities (from 6 to 24 kg/m^3) over 220 days (Wang et al., 2019). In contrast, Arctic charr (~177 g) showed an entirely opposite pattern to Atlantic salmon, with fish exhibiting elevated plasma cortisol (not correlated with feed efficiency or growth) at lower biodensities (30 vs 150 kg/m^3), especially early on when treatments were first applied (Sevier et al., 2019). After 80 days, juvenile rainbow trout at densities between 25 and 120 kg/m^3 in tanks of 1 m^3 (2 m diameter) did not exhibit any differences in plasma cortisol (Person-Le Ruyet et al., 2008). Rainbow trout (~43 g) in 300 L tanks (0.8 m diameter, 0.7 m deep) also had no differences in serum cortisol between biodensities (20 to 80 kg/m^3) after 60 days, but serum osmolality decreased at higher densities (80 kg/m^3), indicating impaired osmoregulatory capacity (Naderi et al., 2017a). Worthy of note is that blood cortisol levels may be unsuitable as an indicator of stress due to increased densities, and at the very least are difficult to interpret. Among both brown trout and rainbow trout, short-term increased densities produced only a transient increase in plasma cortisol levels, whereas there were significant decreases in the concentration of lymphocyte and neutrophil white blood cells (Pickering & Pottinger, 1987).

5.3.2   Immune Function and Disease

There is some evidence among salmonids that increasing biodensity, independent of water quality, has a detrimental impact on immune function, primarily the inflammatory response. Atlantic salmon smolts (~80 g) stocked at 100 kg/m^3 for 57 days showed (relative to those stocked at lower densities: 20 kg/m^3): delayed healing from a tissue-punch biopsy; altered wound contraction; upregulated immune genes (particularly genes associated with delayed wound healing); repressed genes for tissue repair; delayed epidermal repair; reduced numbers of mucous cells and amount of mucus (affecting a wound's ability to withstand secondary infection); and delayed mineralization of new scales (Sveen et al., 2018). Sundh et al. (2019) found similar effects for post-smolts (~177 g) at their highest density treatment (70 kg/m^3), with increased mRNA expression of pro-inflammatory cytokines and neutrophil infiltration into the gut (indicative of poor intestinal immune barrier). In adult Atlantic salmon (~1400 g), Wang et al.

(2019) found no difference in plasma lysozyme (an antimicrobial enzyme, particularly for Gram-positive bacteria) between biodensities (6 to 24 kg/m^3). In rainbow trout, juveniles (~43 g) exhibited elevated plasma lysozyme but decreased serum complement activity at higher biodensities after 60 days (20 to 80 kg/m^3; Naderi et al., 2017a). In contrast, juvenile rainbow trout (~65 g) reared at densities of 10, 40, and 80 kg/m^3 for 30 days showed not only similar decreases in serum complement and a decrease in serum IgM with increasing biodensity, but also a downregulated mRNA expression of pro-inflammatory immune genes. This may be a consequence of elevated cortisol at higher densities, which has been shown to suppress immune responses (Yarahmadi et al., 2016). Additionally, a sustained inflammatory response can be damaging to host tissues, repressing tissue healing and remodeling and possibly rendering fish more susceptible to pathogens. Furthermore, suppressed serum complement activity and reduced levels of serum IgM (measures of the innate and adaptive immune responses, respectively) may also indicate that salmonids are rendered more susceptible to pathogens at higher biodensities.

Several studies have suggested that elevated biodensity may increase horizontal transmission of pathogens and/or severity of disease, possibly due to increased conspecific interactions and/or the immune suppression described above (Mazur et al., 1993; LaPatra et al., 1996; Bebak-Williams et al., 2002: reviewed in Turnbull et al., 2008). However, the relationship between density, number of fish, pathogen transmission, and clinical disease is complex and may be true for some but not all pathogens. For example, Samsing et al. (2014) found that higher biodensities (68.5 kg/m^3 compared with 7.9 kg/m^3) resulted in lower total sea lice loads on Atlantic salmon post-smolts but did not affect lice infection success (% infection success = infection intensity × 100/infective dose). Furthermore, similar to what Frenette et al. (2020) found for cod, individual variation in disease susceptibility is likely to be high within a population.

5.3.3   Growth and Feeding

Growth may be compromised at different biodensities, possibly due to chronic stress-related somatic growth suppression or through a simple inability to access sufficient feed. Though studies linking growth and biodensity are potentially confounded by feed quantity and accessibility, reduced growth at high biodensities has been reported in several studies of salmonids. Atlantic salmon post-smolts (~115 g) stocked at >50 kg/m^3 exhibited a 42% reduction in specific growth rate (SGR) between 100 and 125 kg/m^3 and a linear reduction in feed efficiency as biodensity increased (Calabrese et al., 2017). A correlation between reduced feed intake and increased biodensity (between 50–125 kg/m^3) was also reported for these fish (Calabrese et al., 2017). So, not only did the fish eat less, but they also had poorer feed efficiency. Post-smolts (~494 g) in RAS stocked at 28–53 kg/m^3 weighed less after 66 days than fish stocked at either 18 or 19–36 kg/m^3, and SGR was highest at the lowest tested density (9–18 kg/m^3; Liu et al., 2017). For Atlantic salmon (~1413 g), high biodensities also impaired growth over 220 days in RAS, with fish in the highest density treatment (24 compared with 6 kg/m^3) exhibiting a lower SGR and reduced feed efficiency than those in the lowest density treatments (6 and 9 kg/m^3; Wang et al., 2019). However, Adams et al. (2007) did not find any significant differences in SGR of similarly sized Atlantic salmon (~980 g) between high (35 kg/m^3) and low (15 kg/m^3) densities after 51 days. Juvenile rainbow trout (~43 g) also show reduced growth, lower SGR, and lower feed intake after 60 days at high biodensities (80 compared with 20 kg/m^3; Naderi et al., 2017a, 2017b), though interestingly, these same fish showed improved feed efficiency at higher densities. Naderi et al. (2017b) suggest this may be because aggressive

interactions during feeding are less likely to occur at higher densities (see also *Section 5.4.2: Conspecific Interactions*). However, this result is not supported beyond densities of 80 kg/m^3, as another study of juvenile rainbow trout found that stocking at 120 kg/m^3 for 84 days produced poorer feed efficiency than fish held at either 25 or 74 kg/m^3 (Person-Le Ruyet et al., 2008). Fingerlings of some species may respond differently to high biodensities. For example, in European whitefish (~11 g) growth (SGR) and feed efficiency is impaired over 70 days with increasing biodensity (15 compared with 3.75 kg/m^3; Goebel et al., 2017) but rainbow trout (~180 g) showed no difference in final weight after 3 months at 10, 40, or 80 kg/m^3 (North et al., 2006). Young Arctic charr (~177 g), by comparison, exhibited impaired growth rates and feeding efficiency at very high (120 and 150 kg/m^3) but also at very low biodensities (30 kg/m^3) when compared with moderate densities (60 and 90 kg/m^3; Sevier et al., 2019). This pattern of impaired growth at low densities is further supported by a study of 2 year old Arctic charr (~55 g), who exhibited reduced growth and lower food intake at low densities (15 kg/m^3) compared with moderate and high densities (60 and 120 kg/m^3; Jørgensen et al., 1993). Thus, it appears that, for most salmonid species, higher biodensities (regardless of water quality perturbations) likely impair growth and feeding efficiency, though Arctic charr also experience impaired growth and feeding at very low densities with improved growth at elevated (but not extremely high) biodensities.

5.3.4   Fin Condition and Other Health Indices

Depending on the species, biodensity may compromise fin condition, possibly as a function of aggressive interactions, immune suppression and subsequent opportunistic infection, and/or a mismatch between density and tank parameters, like dimensions or material that can lead to abrasion trauma. Post-smolt Atlantic salmon (~115 g) held at densities ≥100 kg/m^3 exhibited increased pelvic fin damage (Calabrese et al., 2017). Another study found similar effects for post-smolts (~494 g) in RAS, with pectoral fin damage occurring at 53 kg/m^3 (compared with 36 kg/m^3; Liu et al., 2017). Juvenile Atlantic salmon (~113 g) exhibited dorsal fin erosion and splitting after 30 days in a high-density treatment (30 compared with 8 kg/m^3; Jones et al., 2011). After 84 days, juvenile rainbow trout fins were also less eroded at the lowest biodensity (25 compared with 120 kg/m^3) in a study by Person-Le Ruyet et al. (2008). After 3 months, North et al. (2006) found that rainbow trout (~180 g) fins were similarly smaller in the highest density treatment (80 kg/m^3). Ellis et al. (2002) reviewed seven earlier studies of rainbow trout fin condition, of which the majority also found an adverse effect of increasing biodensity. In contrast, Arctic charr (~177 g) stocked for 90 days at densities ranging from 30–150 kg/m^3 exhibited no caudal or dorsal fin damage, regardless of density (Sevier et al., 2019).

A variety of other health parameters have also been observed to be adversely affected by increasing biodensity, including cataracts, whose prevalence increased at higher densities for Atlantic salmon (Calabrese et al., 2017); intestinal barrier integrity for post-smolts (Sundh et al., 2019); and cholesterol for juvenile rainbow trout (Naderi et al., 2017b). However, serum total protein (a measure of nutritional status) did not seem to change with increasing density for juvenile rainbow trout in one study by Naderi et al. (2017a).

5.4   Effects on Fish Behaviour and Overall Welfare

5.4.1   Spatial Distribution

Spatial distribution of fish within a tank or net pen can be influenced by many factors, such as water flow (direction and current speed), lighting, temperature, feed dispersal, and/or the presence of shelter, and fish generally find safety in numbers when threatened (though this varies with life stage and species). However, when these factors are constant, spatial distribution may also be a useful indicator of how fish perceive, and react to, their conspecifics. If aggregating together, this may infer that they prefer the presence of conspecifics; if randomly distributed, they may not have a preference; and if evenly distributed, it is possible that they find their conspecifics somewhat aversive (Turnbull et al., 2008). Rainbow trout tend to distribute themselves evenly within tanks during culture from fingerling to market size (tank dimensions: $6.5 \times 1.5 \times 1.2$ m, density: ~14.75 kg/m^3; Heinen et al., 1996) but aggregate in net pens (net dimensions: $3.5 \times 3.5 \times 3.5$ m, density: ~0.35 kg/m^3; Phillips, 1985). Atlantic salmon (0.23–0.69 kg) often school in dense groups in net pens (Juell & Fosseidengen, 2004) and over a range of biodensities tend to occupy only between 45–85% of the available volume, depending on lighting conditions and water temperature, which highly influence their three-dimensional positioning in large enclosures (Bell, 2002: reviewed in Turnbull et al., 2008). Very little is known about Atlantic salmon social behaviour in the marine phase of their lives, so why they form these dense groups and whether this is normal or abnormal relative to their natural behaviour remains unknown.

5.4.2   Conspecific Interactions

Aggressive interactions in captive fish, beyond causing significant social stress, play a role in fin damage and body injuries, ability to access feed, disease susceptibility and horizontal transmission of pathogens, and sometimes even mortality. For salmonids, it is often assumed that housing fish at higher densities can help mitigate or eliminate aggression or the negative effects of a dominance hierarchy because the increased numbers of aggressive interactions required to maintain dominance become too costly or impossible to perform. However, the evidence is contradictory. Body size heterogeneity can be a useful indicator of a feeding hierarchy, as small fish often remain small due to limited access to feed. Liu et al. (2017) found that body sizes of post-smolt (~494 g) Atlantic salmon were homogeneous (i.e., coefficients of variation [CV] not significantly different between treatments) within low (9 kg/m^3; CV = 17.37) and high density treatments (53 kg/m^3; CV = 16.68), whereas body size was more variable at low densities (8.7 kg/m^3; CV = 14.33, compared with 44 kg/m^3; CV = 4.27) for Arctic charr fingerlings (~0.85 g; Brown et al., 1992). Actual rates of aggression tend to increase with increasing density for juvenile rainbow trout (Keeley, 2000; Cole & Noakes, 1980), whereas Arctic charr fingerlings (~0.85 g) are less aggressive at higher densities (44 kg/m^3; Brown et al., 1992). Adams et al. (2007) found that general rates of aggression (measured via pooled frequency of all aggressive actions: chasing, charging, displacement, and fin-nipping) between adult Atlantic salmon (~980 g) did not differ between density treatments (15, 25, and 35 kg/m^3), but during feeding these rates increased with decreasing density and levels of disturbance from husbandry staff. In contrast, Brown et al. (1992) found that at high densities (44 kg/m^3) juvenile Arctic charr (~0.85 g) exhibited significantly fewer aggressive interactions and spent more time shoaling over 12 weeks than at low densities (8.7 kg/m^3). It is also possible that the type of aggression changes with density: McNicol & Noakes (1984) observed that juvenile brook charr shift from overt aggression (i.e., nips and chasing) to aggressive displays with increasing density, though the densities used in their study are much lower than those used in culture and so their results may not hold in hatcheries or on-farm. Jones et al. (2011) observed the opposite pattern in juvenile

Atlantic salmon (~113 g), as they were significantly less aggressive at higher densities (30 compared with 8 kg/m^3) in terms of total number of aggressive behaviours observed, but exhibited an increase in biting frequency, which correlated with similarly increased levels of fin damage. From an ecological point of view, it can be theorized that, generally, as densities increase, the type of competition changes from "contest" behaviour (where aggressive individuals can monopolize food resources in a hierarchy) to "scramble" behaviour (where densities are so high that finite food resources cannot be monopolized and are instead distributed more equally among many scrambling individuals; Lomnicki 2009). Furthermore, beyond species, salmonid aggression may be influenced by a variety of environmental factors such as food composition and availability, particle distribution (through space and time), water flow rate, life stage, and temperature (Ellis et al., 2002), making it difficult to detect universal patterns of aggression as a function of density.

Interestingly, some studies have suggested that size heterogeneity may be a beneficial aggression management tool, depending on the species or life stage, and, in practice, fish farms tend to keep lots of fish with low size variability through breeding, sorting, and grading. Adams et al. (2000) suggest that the addition of a few large individuals may decrease rates of aggression; they studied 0+ Atlantic salmon parr and found that groups of small fish were highly aggressive, while groups with a few larger fish were far less aggressive. Wipf & Barnes (2011) similarly found that aggression amongst juvenile cutthroat trout increased as the density of small fish increased within a group. However, juvenile Arctic charr exhibit the opposite pattern: Lahti & Lower (2000) found that aggression was higher in groups of differently sized fish than groups of similar sizes. A study by Yue et al. (2006) investigated whether the presence of differently-sized rainbow trout induced a stress response or behavioural changes, finding no differences in medium-sized (~123 g) fish behaviour (measured as tank space use, locomotory behaviour, and aggression) in the presence of similar, smaller (~46 g), or larger (~314 g) conspecifics, though medium-sized fish experienced more aggression (chasing) when housed with larger fish. Plasma cortisol levels between medium-sized fish exposed to similar, smaller, and larger conspecifics were not significantly different, though they were all higher than baseline, indicating that exposure to new fish, regardless of size, may be stressful for rainbow trout. It has also been suggested that providing food such that it is unpredictable through time and space may serve to reduce food monopolisation and hierarchies in Atlantic salmon (Kadri et al., 1996). In fact, feeding regime, lighting (see *Section 7: Lighting*), and temperature may all be manipulated to control fish position within a container.

Non-aggressive interactions may also have welfare consequences at different densities. When feeding hierarchies can be established, they may be maintained by threats and displays that result in little to no physical damage (as in Nakano, 1994) but may keep subordinates in a state of chronic stress/poor welfare (Cubitt et al., 2008) or prevent them from accessing food (Huntingford et al., 1993). Though not extensively explored in recent literature, it has also been suggested that high densities may increase collisions both between fish and with tank/net pen walls, potentially increasing rates of injuries such as peduncle sores and snout abrasions (rainbow trout: Collins, 1972; Kils, 1989). Simultaneously, it has also been suggested that if densities are too low, the feeding response is impaired in rainbow trout (Purser & Hart, 1991; Winfree et al., 1998), as they are visual feeders who may benefit from being able to copy conspecific feeding behaviour (Phillips, 1985; Pitcher & Parrish, 1993).

5.4.3   Overall Welfare

Several studies have integrated multiple physiological and/or behavioural indicators to generate overall welfare scores (or indexes), for farmed salmonids. In a study of juvenile rainbow trout (~180 g) housed in freshwater flow-through tanks at densities of 10, 40, and 80 kg/m^3 over a 9-month period, North et al. (2006) analyzed various population effects (mortality, growth, size variation, feed efficiency), individual morphometrics (fish mass, fin condition, condition factor), and several physiological indices (haematocrit, plasma cortisol, lysozyme activity) using principal components analysis (PCA). They found a trend of higher plasma cortisol and lower lysozyme activity, as well as lower condition factors and higher size variation at low densities, indicating the presence of possible dominance hierarchies. PCA also identified a group of fish across all density treatments that had good fin condition but low condition factor. Finally, their analyses revealed that high densities did not produce a consistent effect on growth rate or physiological welfare indicators for rainbow trout. In a similar study of Atlantic salmon housed in 30 marine net pens at densities ranging from 9.7–34 kg/m^3, growth, fin condition, and plasma glucose and cortisol were combined using PCA (Turnbull et al., 2005). Welfare scores were consistent with farmer-reported estimations of welfare status. However, no trend between welfare and density emerged before densities reached 22 kg/m^3, after which increasing density was significantly related to decreasing welfare scores. Also of interest, there was no relationship found between welfare score and time since grading or sea lice treatment. Another study by Adams et al. (2007) used PCA to combine four measures of welfare (body condition, fin condition, plasma glucose, and cortisol) from Atlantic salmon (~980 g) housed in seawater tanks at biodensities of 15, 25, and 35 kg/m^3 over 51 days. Their analyses showed that high and low welfare scores were found at all biodensities, with the best mean welfare scores occurring in the moderate density treatment (25 kg/m^3). They also found an interaction between "disturbance" (a score based on how much husbandry activity and passing traffic the tanks experienced) and biodensity, suggesting that the link between biodensity and welfare is complex and possibly confounded by other factors.

5.5   Farming Situations Involving High Biodensities

Some industry practices involve temporary departures from normal biodensities, such as holding prior to grading, grading itself, and during transport. These practices elicit an acute stress response due to crowding that may temporarily compromise welfare (briefly reviewed in *Section 2: Stress Indicators*). There are few studies of how biodensity before or after crowding affects acute stress responses and recovery in salmonids. Cooke et al. (2000) implanted rainbow trout with telemetry devices and monitored swimming activity, finding that it increased with increasing biodensity (from 15 to 60 kg/m^3) but that diel patterns of activity (i.e., less at midday, more at night) were unaffected. They also found that oxygen consumption increased with increasing density, likely because of the increased activity. Chandroo et al. (2005) also used telemetry devices to track rainbow trout behaviour, finding that swimming activity and oxygen consumption both increased during truck transport, and returned to baseline during a 48-hour recuperation period. However, trout swimming performance (measured as critical speed and endurance) was compromised beyond this period relative to controls that were not transported. Though the telemetry devices used may constitute a stressor in and of themselves (e.g., Semple et al., 2018), these studies indicate that proper aeration of water during situations involving high biodensities because of crowding and transport is crucial.

5.6   References

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Section	Parameter	Species	General relationship with:		Exceptions/caveats
			Increasing biodensity	*Decreasing biodensity*	
5.3.1	Mortality	Atlantic salmon, Arctic charr, and European whitefish	No difference		Rainbow trout fingerlings have decreased mortality at low densities
	Stress response activation	Atlantic salmon	Increased		
		Rainbow trout	No difference		Impaired osmoregulation
		Arctic charr		Increased	
5.3.2	Immune function	Atlantic salmon and rainbow trout	Impaired		No studies of Arctic charr
5.3.3	Growth and feeding efficiency	Atlantic salmon, rainbow trout, European whitefish	Impaired		
		Arctic charr	Impaired when very high	Impaired when very low	
5.3.4	Fin damage	Atlantic salmon and rainbow trout	Increased		
		Arctic charr	No difference		
5.4.1	Spatial distribution	Atlantic salmon	N/A		Aggregate in net pens
		Rainbow trout	N/A		Evenly distributed through production cycle
5.4.2	Body size heterogeneity	Atlantic salmon	No difference		
		Arctic charr		More variable	
	Aggression	Atlantic salmon	Increased during feeding		
		Rainbow trout	Increased		
		Arctic charr		Increased	
	Type of aggression	Atlantic salmon	Increased biting		
		Brook charr	Displays of aggression	Overt aggression	
	Chance of collisions with conspecifics	Salmonids	Increased		
	Visually-mediated feeding response	Rainbow trout and other visually-feeding species	Impaired when very low		
5.4.3	Overall welfare score	Atlantic salmon	Decreased		Only above 22 kg/m3 in a study of net pens – no discernable effect in marine tanks
		Rainbow trout	Inconsistent welfare at all densities		In freshwater tanks

Table 5.1 Summary of general patterns in multiple welfare-relevant parameters for a number of salmonid species.

Specific references and deeper discussion given in text.

6   Water Quality Issues in Recirculation Aquaculture Systems (RAS)

Conclusions

1. **Temperature control can optimize growth rate but can raise welfare concerns around the completion of smolting and duration of the smolt window, and growout to 4 kg. For smolting, 10ºC is safe and >13ºC poses welfare risks if the fish are to be transferred to sea-cages. For growout to ≥4 kg in RAS, 14–17ºC appears to be safe.**

2. **80% oxygen saturation at the outlet drain is a useful guideline to avoid hypoxia. Spatial distribution of oxygen within large tanks is fairly uniform with appropriate water inlet manifolds and tank exchange rates of >0.3 L/kg/min.**

3. **Safe levels of carbon dioxide (CO_2) are uncertain, but 10 mg/L is suggested. 24 h cycles of CO_2 in RAS tanks may subject fish to episodic hypercapnia, but this is not documented in the scientific literature. Continuous monitoring of CO_2 is technically feasible but there are no published data. Nephrocalcinosis and cataracts sometimes result from chronic hypercapnia, but other pre-disposing factors are implicated though unidentified in the literature.**

4. **Suspended solids are a threat to water quality and salmon welfare in RAS due to variability in feed quality disrupting gut health and faecal caste integrity. Batch-to-batch variation in formulation of commercial feeds poses a threat to fish welfare in RAS. The goal of using fishmeal-free diets requires better knowledge of nutrient and binder interactions with the gut health and faecal caste integrity to manage suspended solid loading.**

5. **Safe levels for chronic ozone exposure in salmonids are currently uncertain.**

6.1   Introduction

Recirculation aquaculture systems (RAS) are established in the Atlantic salmon farming industry for production from egg to smolt. But record-high market prices for Atlantic salmon seem to make RAS production to harvest size economically viable, provided temperature and stocking densities are relatively high and both feed and electricity costs are minimized (Bjørndal & Tusvik, 2019; Song et al., 2019). However, growing salmon profitably at high densities in RAS will mean margins for error are narrow and welfare issues may arise quickly, requiring skilful management. Diploid Atlantic salmon were used in all the studies cited here. Triploids may become important in RAS production to address the significant problem of unwanted sexual maturation but would require specific guidelines beyond those for diploids. For example, the optimum temperature for triploid post-smolts is about 3°C lower than diploids (Sambraus et al., 2017). Summarized here are issues that have emerged since the excellent review by Thorarensen and Farrell (2011). This review is limited to Atlantic salmon because it currently dominates the industry and RAS research and development.

6.2   Temperature

Temperature control enables growth to be accelerated and, combined with photoperiod adjustment, allows the production of off-season smolts. Inappropriate manipulation of these two factors can disrupt smolting, resulting in high mortality following transfer to sea-cages. Regular sized juveniles (~0.1 kg) reared in freshwater are at most risk of mortality post-transfer. Fish transferred at 0.3–0.5 kg are likely well able to tolerate transfer to coastal seawater due to their large body size, but there is no information in the primary scientific literature. Among salmon reared to harvest in RAS, the concerns around smolting are much lower since temperatures/salinities can be controlled.

The highest safe freshwater temperature for completion of smolting is somewhere around 10 to 13°C, with interacting factors including photoperiod, genotype, and geography. Following 7 weeks under a short photoperiod (February-April), the transition to LL stimulated normal completion of smolting after 400 degree days (°D) in cold (4 increasing to 10.8°C between April–June) and medium (7–13.7°C) regimes. A warm regime (10.5–17.3°C), by comparison, advanced the timing of smolting but they were of poor quality (Sigholt et al., 1998). Rearing at 12ºC advanced smolting by about one month compared to 8ºC, but marine performance was not assessed (Handeland et al., 2013a). Similarly, another Norwegian stock reared at 12–13ºC through winter completed smolting about a month earlier than fish at ambient temperature, but following transfer to seawater their growth was relatively slow, indicating that smolting had been compromised (Solbakken et al., 1994). Among Saint John River (SJR) stock, the timing of smolting and degree of salinity tolerance was similar at constant 10 vs <5°C (Duston & Saunders, 1995). SJR stock reared at constant 16ºC from October onwards completed smolting in spring, but a lower temperature resulted in better smolt characteristics (Johnston & Saunders, 1981). In Tasmania, a flow-through system (4–9°C) better prepared smolts for transfer to the marine sites than the same cohort reared in a RAS at 14°C, but the role of temperature was open to speculation (van Rijn et al., 2020).

Following the completion of smolting, the amount of time allowable for safe transfer to seawater is temperature dependent. In traditional surface water-fed hatcheries, the SJR smolt window closed rapidly due to warming in May above 10°C, whereas at constant 10°C the window was about 6 weeks (Duston et al., 1991). In Norway, smolts held at either constant 6, 10, 12, or 14°C from early June exhibited similar high salinity tolerance for about 4 weeks through to early July (Stefansson et al., 1998).

Rearing post-smolts to 4–5 kg in RAS, in either fresh or saltwater, is the new frontier. At the Conservation Fund's Freshwater Institute (CFFI), Atlantic salmon were grown from 0.3 to 4 kg at 15–16°C with no welfare issues, as judged by growth, survival, and anecdotal observations of fish-to-fish interactions, food competition, and distribution throughout the tank (Davidson et al., 2016a). In seawater, optimum temperature for growth was 12.8°C at 70–150 g body size and 14°C at 150–300 g, whereas 18°C inhibited growth (Handeland et al., 2008). In sea-cages in a thermally stratified fjord in Norway, Atlantic salmon (1.3 kg) congregated between 16.3 and 17.5°C, suggesting this was their optimal temperature (Johansson et al., 2006). Similarly, 3.6–4.2 kg fish (genetic origins: River Philip, Nova Scotia) in sea-cages in Tasmania preferred 16.5–17.5°C but avoided >20.1°C (Stehfest et al., 2017).

6.3 Dissolved Oxygen (DO)

Hypoxia is a major threat to fish welfare. Moreover, the threat increases with warmer water since O_2 solubility decreases almost linearly with temperature, and O_2 demand increases exponentially. Consequently, oxygen injection and continuous monitoring of dissolved oxygen (DO) is essential in RAS. The inflow of water supersaturated with oxygen through correctly designed inlet manifolds can achieve uniform DO ~10 mg/L throughout 150 m^3 tanks stocked at 100 kg/m^3 (Davidson & Summerfelt, 2004). DO is typically reported at the outlet drain, where it is lowest. At CFFI, DO at the outlet drain was maintained at 100% saturation for growout of Atlantic salmon to 4 kg (10 mg/l at 15.2°C; Davidson et al., 2016a). In stocking density trials, DO >80% saturation at 10°C at the outlet drain was assumed not to be limiting (Sveen et al., 2016). Among post-smolts (0.3 kg) in seawater at 16°C subjected to hypoxia for 2 h per day for three weeks, 70% saturation (5.6 mg/l DO) was the estimated threshold for reduced growth and 60% saturation (4.9 mg/l) was suggested as a minimum acceptable threshold for fish welfare (Remen et al., 2012). Temperature and body size alter safe values: hypoxia tolerance thresholds for post-smolts in sea-cages (~0.5–0.6 kg) at 6, 12, 16, and 18°C were estimated at 30, 39, 47, and 55% saturation (2.9, 3.4, 3.8, and 4.2 mg/l; Remen et al., 2013). Saint John River stock Atlantic salmon (140 g) in 32 ppt at 12°C reared under 70% saturation (ca. 6.1 mg/L) for three weeks exhibited a 30% lower weight gain compared to fish reared at 100% saturation. During subsequent warming at 1°C/week up to 20°C, growth remained inferior at 70 vs. 100% saturation, with mortalities occurring after 1 month at 20°C/70% saturation, but the innate immune response to bacterial pathogens was not compromised (Gamperl et al., 2020; Zanuzzo et al., 2020).

Hyperoxia (>100% DO) is easy to achieve with O_2 injection but may also threaten fish welfare. DO up to 178% at 9°C for five weeks suppressed the breathing rate of pre-smolts (30 g) resulting in increased blood CO_2 levels, but had no negative effects on growth or survival (Hosfeld et al., 2010). The authors were careful to ensure the rearing water total gas partial pressures were normbaric and CO_2 concentration was low (<2 mg/L). If O_2 is injected improperly and the sum of the partial pressures of all the dissolved gases exceeds atmospheric pressure (hyperbaric hyperoxia) the risk of gas bubble disease is high (Bouck, 1980).

6.4 Carbon Dioxide

CO_2 excretion rate by fish is proportional to their O_2 demand, which in turn is largely driven by feed intake. In addition, the nitrifying bacteria in the biofilter produces 5.85 g of CO_2 per gram of ammonia consumed (Ebeling et al., 2006). Elevated CO_2 in RAS is a threat to fish welfare. Some marine teleosts are highly sensitive, with an 8 mg/L CO_2 concentration causing serious harm to cod (Moran et al., 2012). Salmonids are more tolerant, but recommended safe levels are edging downward from 20 mg/L to 10 mg/L (Thorarensen & Farrell, 2011). Growth of post-smolts over 12 weeks was lower at 19 mg/L CO_2, compared with 12 mg/L. At 5 mg/L CO_2, there was some indication of better performance compared with 12 mg/L, but there was considerable variation between replicates (Mota et al., 2019). Thorarensen and Farrell (2011) identified a critical data gap in the potential threat posed by temporal and spatial variation in CO_2 within RAS rearing tanks. Spatial variation in O_2 (and presumably CO_2) across the 6 m radius of a large tank stocked at 76 kg/m^3 was clearly evident but did not pose a threat to welfare, ranging from 8.6–10 mg/L (78 to 92% saturation; Gorle et al., 2018).

Temporal diel changes in CO_2 result from the daily cycle of photoperiod and feeding. CFFI rearing under continuous light (LD24:0) and feeding at regular intervals around the clock aim to minimize these fluctuations, although no diel data has been published (e.g., Davidson et al., 2016a). Under a simulated natural daylength cycle (Lat. 60°N), a clear diel cycle in CO_2 concentration was exhibited by post-smolts (114 g, 75 kg/m^3) in seawater (9.3°C) in November/December fed 2 to 4 meals per day (Kvamme et al., 2019). The tank water exchange rate was the key factor affecting the magnitude of the CO_2 spike, exceeding 20 mg/l during the afternoon at an exchange rate (loading density) of 0.2 L/kg/min. At 0.3 and 0.5 L/kg/min, by comparison, CO_2 concentration peaked at 11 and 7 mg/L respectively (Kvamme et al., 2019). Similarly, mean CO_2 concentration increased progressively as the water exchange rate was reduced from 0.5 to 0.2 L/kg/min among smolts reared at 75 kg/m^3, reaching 15.7 mg/L at the slowest exchange rate, 0.2 L/kg/min (Sveen et al., 2016). Moreover, a significant stress response in the skin was detected at ≤0.3 l/kg/min, with increased transcription of mucin genes (Sveen et al., 2016). During the freshwater RAS grow-out of Atlantic salmon (Cascade strain) in 150 m^3 tanks to >4 kg, the mean CO_2 concentration was 13–14 mg/L, with a final stocking density of 100 kg/m^3 and an exchange rate 0.33 l/kg/min (Davidson et al., 2016a). CO_2 concentration ranged from 2 to 24 mg/L which could have been stressful, but the fish "did not exhibit behavior indicative of compromised welfare" (anecdotally, fish were distributed evenly through the grow-out tank, docile, and did not compete aggressively for food or exhibit excessively agonistic interactions; Davidson et al., 2016a).

Because of its high solubility, stripping CO_2 from H_2O requires a degasser column with a water:forced air ratio of at least 1:10 (Summerfelt et al., 2000). Measuring CO_2, and hence assessing its effects, is complicated because it reacts with water and becomes part of the carbonate equilibrium (Colt et al., 2012). One option, allowing continuous monitoring, is an infrared sensor (Oxyguard: Moran et al., 2010). Titration with NaOH to an end-point pH of 8.3 to measure CO_2 concentration is confounded in a RAS if the water is discoloured due to humic acid derived from the feed/faeces. Seawater further complicates CO_2 measurement. To emphasize the complexity, Kvamme et al. (2019) calculated CO_2 by measuring total alkalinity in the inlet water, pH measurements at the tank outlet, temperature and salinity using an Excel spreadsheet developed by Raul H. Piedrahita (Professor Emeritus, University of California, Davis) based on methods from the US Department of Energy (1994).

Hypoxia, kidney damage, and cataracts are three symptoms and welfare consequences of hypercapnia. High CO_2 concentration in the water inhibits its excretion by diffusion from the blood at the gills. Exposing trout to an abrupt increase in CO_2 resulted in a rapid drop in blood pH, greatly reducing the binding of O_2 to haemoglobin, causing temporary hypoxaemia (Eddy et al., 1977). However, after a few hours, blood pH returned to normal, associated with a large rise in blood bicarbonate that remained chronically elevated. High HCO_3^- results in a high pH in the primary urine and deposits of calcium salts accumulate in kidney tubules called "nephrocalcinosis" (Eddy et al., 1979; Smart et al., 1979). The etiology of nephrocalcinosis is not fully understood, since its incidence varies greatly between studies (Mota et al., 2019). Also, the etiology of cataracts due hypercapnia is not known. Hypercapnia in RAS is preventable if the water exchange rate, degassing, and stocking density are correctly balanced with feeding strategies. The welfare effects of temporal fluctuations in CO_2 concentrations and compensation of blood pH in an overloaded RAS remains a critical gap in knowledge, as emphasized by Thorarensen and Farrell (2011). In conclusion, to prevent hypercapnia, a minimum exchange rate

of 0.3 L/kg/min seems important, and 10 mg/L of CO_2 a safe upper limit. Research incorporating continuous monitoring of CO_2 matched against welfare indicators would be valuable. In addition, the etiology of nephrocalcinosis and cataracts deserve attention.

6.5   Solids, Water Discolouration, and Ozone

Faecal solid waste is mostly settleable, but some fragments into suspended particles, which becomes dissolved organic carbon if not removed by mechanical filters and foam fractionators. The smaller the particle, the more difficult to manage and threatening to salmonid health in RAS. An intact faecal caste quickly sinks to the bottom of the tank, is drawn to the centre by centripetal force and efficiently removed at the centre drain by a steady outflow of 5–6 L of water/min per 1 m^2 of tank floor area (Davidson & Summerfelt, 2004). In the effluent pipes within the recirculation loop, the solids still pose a lethal threat if they accumulate due to either poor design or construction errors. Anaerobic decomposition of the sludge releases hydrogen sulphide (H_2S), which has resulted in several high mortality events in Norwegian RAS (Dunnette et al., 1985; Olsen, 2019). The potential threat is greater in seawater vs freshwater RAS: H_2S production was 20.5 vs 0.8 mg/g sludge respectively (Olsen, 2019). Nitrate, the end product of nitrification, neutralizes H_2S, and both should be monitored (Neori et al., 2007).

Feed composition greatly influences gut health and the physical viscoelastic characteristics of the faeces (Brinker et al., 2005; Gajardo et al., 2017). Fishmeal-free (FMF) diets are an important goal for the industry to satisfy the market demand for sustainable foods. Plant proteins, particularly soymeal, can include anti-nutritionals causing enteritis and diarrhea (Noble et al., 2005). The latter is a grave concern for RAS operators, but may go unnoticed in a flow-through system. An immediate effect of an increase in total suspended solids from 3 to 12 mg/L was doubling of oxygen demand within a large tank of smolts (Gorle et al., 2018).

RAS-reared salmon fed an experimental FMF diet high in mixed nut meal but no soy performed similar to controls fed a standard diet, with similar water quality and no welfare concerns, although gut health was not assessed (Davidson et al., 2016b). The feed industry is marketing diets for RAS, but not FMF. Skretting, for example, launched RecircReady diets in 2018, and in 2020 will release next generation feeds. A RAS operator complained that total suspended solids (TSS) can differ considerably depending on feed types, and even between batches of the same feed (pers. comm. to J. Duston). To develop better RAS diets, including FMF, molecular tools and microbial markers are being developed to assess the effect of novel dietary ingredients on Atlantic salmon gut health (Gajardo et al., 2017). RAS nutrition research is high priority both from a welfare and sustainability perspective.

Brown water is common in RAS due to humic acid compounds from the breakdown of food or faeces (Leonard et al., 2002). This discolouration limits light penetration which can disrupt smoltification (B. Milligan, pers. comm. to J. Duston). For optimal smoltification, a minimum light intensity of 43 lux was recommended (Handeland et al., 2013b; see *Section 7: Lighting*). Ozone can effectively clarify RAS water by oxidizing dissolved organics and microflocculation (Schroeder et al., 2011), and its use is of increasing importance in RAS management (Powell & Scolding, 2018).

Ozone provides both potential benefits and risks for RAS operators. Benefits include highly effective disinfection, flocculation of small particles, and reduction in off-flavour products such as geosmin (Gonçalves & Gagnon, 2011; Ji et al., 2020). As a powerful oxidizing agent, ozone can cause direct damage to fish tissues, especially the gills, but a greater threat specific to seawater RAS is the formation of highly toxic ozone-produced oxidants (OPOs), particularly free bromine and bromamines (Gonçalves & Gagnon, 2011). OPOs are more stable than ozone and can accumulate within seawater RAS if they are not removed by methods such as activated carbon filtration or UV radiation (Gonçalves & Gagnon, 2011). The threat of OPOs is compounded by fragmentary data on their toxicity in fish; the Scientific Committee found no data on effects of OPOs on Atlantic salmon. Juvenile turbot (*Psetta maxima* L.) exposed to OPO concentrations of 0.06, 0.10, and 0.15 mg/L for 21 days exhibited gill damage at 0.10–0.15 mg/L (Reiser et al., 2011). In a recent review, Powell and Scolding (2018) emphasized a need to define effective recommended doses and tolerance limits for ozone and OPOs in seawater RAS, cautioning these are likely to be specific to species, life-stage, and/or body size. Accurate and convenient measurement methods need to be applied consistently for both research and farm use. Buchan et al. (2005) compared five measurement methods and recommended a colorimetric test-kit (DPD; N,N-diethyl-p-phenylenediamine). Probes that measure oxidative redox potential (ORP; millivolts) are user-friendly but have a short lifespan and can be inaccurate (Buchan et al., 2005). The official APHA indigo colorimetric method was used by Gonçalves and Gagnon (2011).

6.6   Ammonia, Nitrite, and Nitrate

The high toxicity of unionized ammonia (NH_3) and nitrite (NO_2^-) are well established and the state of knowledge has not changed since a previous review (Thorarensen & Farrell, 2011). RAS operators understand how to manage biofilters and the importance of pH control. Nitrate (NO_3^-), the end-product of nitrification, is relatively non-toxic, but has attracted attention recently as a possible emerging threat in RAS because it can accumulate. Among turbot, nitrate >126 to 505 mg/l inhibited growth in dose-dependent manner (van Bussel et al., 2012). Among post-smolts, by comparison, nitrate maintained at 100 mg/L for 8 months in a freshwater RAS had no effect on growth or a number of health parameters (Davidson et al., 2017).

6.7   Microbial Ecosystem Management

Water quality in RAS is dependent on skilful management of the fish, an array of equipment, and the unseen contribution of a complex microbial community. Microbial biofiltration is essential for removal of ammonia and nitrite, and farmers are fully aware that stability of the biofilter is critical to RAS health. In addition, the high nutrient levels in RAS and the long retention times favour the accumulation of a wide range of microbes both in the water column and on surfaces, all of which may influence fish health (Pedersen et al., 2019). Stability is key to a healthy microbial ecosystem; basic changes in feed loading, for example, can be disruptive (Rojas-Tirado et al., 2018). Chemical therapeutants pose the most important threat. For example, formalin is used widely in the salmonid aquaculture industry to treat topical infections and can significantly reduce nitrification rate if the biofilter is not properly isolated (Pedersen et al., 2010). Residual formalin concentrations as low as 37 mg/L disrupted ammonia oxidizing bacteria and 10 mg/L could disrupt nitrite oxidizers, known to be more sensitive (Fredricks et al., 2018).

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7   Lighting

Conclusions

1. **Continuous, 24-hour light (LL) is used at certain stages of the lifecycle of both farmed trout and salmon but is particularly important in the Atlantic salmon industry. Evidence of the benefits of LL is considerable; evidence of negative effects is minor.**

2. **Eggs and alevins are typically incubated in stacks of trays or upwelling incubators in rooms with ceiling lights providing continuous white light, intensity >100 lux. Light intensity within each tray, however, is very low (<5 lux) to optimize survival. Exposure of each tray to bright light for a few minutes per week is necessary for visual health inspection and picking of dead eggs.**

3. **First feeding fry are reared under LL to maintain the fish in the water column, preventing the risk of nighttime suffocation due to fish settling on the tank floor and being drawn onto the outlet drain screen.**

4. **To stabilize the seasonal production cycle of Atlantic salmon smolts, daylength (photoperiod) regimes that include LL for several months are standard practice.**

5. **To prevent unwanted sexual maturation and associated health deterioration among large Atlantic salmon, sea-cages are illuminated with LL for several months during winter.**

6. **To reduce the risk of sea-lice infestation, submerged lights providing LL in deep sea cages reduce the risk of contact between the parasite and Atlantic salmon.**

7. **Egg production facilities produce up to three or more crops per year by maintaining broodstock in lightproof tanks under a simulated 12-month photoperiod cycle that is out of phase with the natural daylength cycle.**

7.1   Overview

Light has three components: colour, intensity, and duration (daylength or photoperiod), all of which can potentially influence animal welfare. In temperate regions, the life cycle of most organisms is entrained to the daily cycle of light intensity and the annual cycle of photoperiod, with temperature serving as a controlling factor for poikilotherms. Photoperiod manipulation is used widely in the Atlantic salmon farming industry to improve first-feeding, alter the timing of both smolting and sexual maturation, and reduce the incidence of unwanted sexual maturation. Regular full spectrum white light is usually used, with light-emitting diode (LED) technology enabling researchers to begin exploring the effects of colour. Light intensity needs to be sufficient for fish to see their food throughout the full depth of the rearing unit, but high intensity can be stressful. Photoperiod is the most powerful of the three components and can be used to manipulate the physiological state of salmonids at all life stages. The application of photoperiod manipulation to salmonid aquaculture was a popular research topic 15–20 years ago, as reflected in the older references cited here, and is now an established tool in the industry. Nevertheless, questions remain to be answered and better knowledge is needed of the appropriate lighting for

deep land-based tanks, the rate of transition between artificial and natural daylengths, and for reducing sea-lice infestation.

This review describes the uses of light manipulation in Atlantic salmon (*Salmo salar*) aquaculture in sequence from egg to adult, explaining the advantages and welfare concerns that are supported by empirical data. Perceived or potential welfare issues that may be a concern to the public are also included. Information on other species of farmed salmonids are included where evidence indicates differences from Atlantic salmon.

7.2   Egg Incubation and Alevin Stages

Wild salmonid eggs develop in darkness, buried in gravel redds (Crisp & Carling, 1989). Traditional hatchery practice followed suit: salmon eggs incubated in darkness resulted in better survival and bigger alevins than those incubated in the light (Bell & Hoar, 1950; Eisler, 1957). However, darkness makes egg management difficult. Eggs must be checked regularly for mortalities, which need to be picked out or treated with disinfectant. Dead eggs quickly become infected with *Saprolegnia* spp., which can quickly kill an entire batch of eggs (Thoen et al., 2011). Some hatcheries continue to operate in darkness, but most modern Atlantic salmon hatcheries operate on continuous 24-hour light, termed "LL," the eggs incubated in multi-deck Heath tray stacks, or a new version called Comphatch (Alvestad Marin AS, Norway). Each tray holds about 10,000 eggs, which are exposed to indirect light of low intensity (<5 lux). For visual health checks and picking, each tray is manually slid out, exposing the eggs to the room's ceiling light. Through to hatch and yolk-sac absorption, the alevins remain under LL either in Heath trays or in shallow tanks with biomats, either under or on top of which the fish congregate (Peterson & Martin-Robichaud, 1995). The aim of this hatchery practice is to provide stable conditions, maximize yolk-sac absorption, and minimize energetically wasteful movement among the alevins.

7.3   Swim-up, First Feeding Fry, and Early Parr Stages

Swim-up occurs when yolk sac absorption is almost complete, at about 750 degree days post-fertilization (for example, Saint John River stock; Peterson & Martin-Robichaud, 1995). At this stage, the fish are typically reared in shallow circular tanks. Continuous light is important in the early swim-up stages, ensuring the fish remain in the water column swimming actively. If the lights are switched off, the fry settle on the tank floor and are at risk of being drawn onto the tank's centre drain screen, and suffocating. Continuous light at first feeding keeps fish in the water column and allows for continuous feeding. As the fry grow and become better swimmers, the risk of suffocation decreases, and fish can be safely reared under a light-dark cycle, but most hatcheries continue with LL to maximize feeding and growth. Under natural conditions, wild Atlantic salmon fry experience a day length around the summer solstice of 17–24 hours, depending on latitude. A long photoperiod not only extends foraging activity for salmonids, which are visual feeders, but also triggers the secretion of growth hormone, stimulating appetite and growth (Björnsson et al., 1989; Peter & Marchant, 1995). Body weights of Atlantic salmon reared from first feeding for three months under LL (~500 lux at water surface) were 20% greater than fry reared under LD 16:8 or 12:12, with no welfare issues reported (Saunders & Henderson, 1988). Similarly, in Norway, growth rates were significantly higher among juvenile Atlantic salmon reared for four months under LL compared with 16 hours of light, 8 hours of

dark (LD 16:8), and plasma cortisol was similar between treatments, suggesting LL was not stressful (Stefansson et al., 1989). Deleterious effects of an unchanging photoperiod were evident when extended beyond three months, interfering with the completion of smolting (Stefansson et al., 1989; see *Section 7.4: Smoltification*).

7.4   Smoltification

The transformation from parr to smolt includes complex morphological, behavioural, and physiological changes (Hoar, 1976; Björnsson et al., 2011). Age at smolting depends on body size, ranging from about five years in cold, unproductive rivers to less than one year in intensive aquaculture with temperature control and high-quality feeds. Photoperiod is an important factor controlling the timing of smolting, serving to entrain an underlying circannual clock (Eriksson & Lundqvist, 1982; Duston & Saunders, 1990). The natural decrease in photoperiod in autumn is important for initiating smolting and the spring increase in photoperiod entrains the completion of the process, the most important change being the development of salinity tolerance. Failure to complete smolting results if fish are reared under LL through the autumn and spring (McCormick et al., 1987). Survival in 96 h salinity tolerance tests in May was 0% among fish reared on LL throughout the autumn and winter, compared with 100% survival among fish reared under a simulated natural daylength cycle (McCormick et al., 1987). Clearly, inappropriate use of LL causing disruption of smolting is a welfare issue, but with >30 years have elapsed since the discovery, the industry knows the timing and duration of LL treatment needs to be balanced with a short photoperiod.

Altering the timing of smolting to times of year other than the normal May window is an important strategy to optimize production efficiency. Production of off-season "underyearling" smolts in the fall/winter is common in British Columbia but is done less commonly in Atlantic Canada because the winter seawater temperature is too cold for smolt transfer. A typical photoperiod manipulation regime is LL from first feeding through to about 30–50 g, then vaccination and a short photoperiod LD 12:12 for 5 to 12 weeks, then a return to LL. The fish perceive the decrease in photoperiod as winter and the subsequent increase back to LL as a spring cue to complete smolting, with fish capable of tolerating transfer to sea-cages about six weeks after the return to LL. This accelerated production of smolts requires rapid growth at elevated temperature which can increase the incidence of spinal deformities (Fjelldal et al., 2006). Dietary phosphorus deficiency was subsequently causally linked to this welfare problem and remains a research priority (e.g., Sambraus et al., 2020). Completion of smolting is assessed by a variety of biochemical and physiological tests including salinity challenges and direct and indirect measures of gill Na^+K^+ATPase activity. Absolute daylength is not the critical factor, but rather the timing of the change in photoperiod from long to short and back to long again (Duston & Saunders, 1995). In Newfoundland, photoperiod manipulation is used to delay the timing of smolting to June to allow more time for the seawater to warm above 4°C (J. Nickerson, Cooke Aquaculture & A. Borchardt, Mowi, pers. comm.).

Daytime light intensity at the water surface is recommended to be greater than 20–40 lux for normal smolting; lower intensities are associated with poor growth and increased deformities (Handeland et al., 2013). In shallow rearing tanks the light intensity at the water surface is close to what the fish detects. In large tanks, typical of modern hatcheries, the light intensity at the bottom of the tank may be sub-optimal due to shading caused by the fish themselves (B.

Milligan, pers. obs.). The spectral composition of the lights had no effect on growth and smolting; however, the range of lights they tested was limited to only various hues of white and/or yellow (Stefansson & Hansen, 1989). Hatcheries are typically equipped with incandescent, fluorescent, and/or LED white light.

7.5   Sea-Cages (Marine Phase)

7.5.1   Preventing Sexual Maturation

To reduce the problem of sexual maturation prior to reaching harvest size, artificial lights on marine net-pens holding Atlantic salmon has been standard practice for ~20 years. Sexual maturation after one sea-winter, as grilse, results in a costly loss of growth and meat quality as energy is diverted into gonadal development (McClure et al., 2007). Moreover, sexual maturation can be a welfare threat, since the fish are more vulnerable to pathogenic disease due to a suppression of the immune system (Suzuki et al., 1997). Maturation among newly transferred smolts in sea cages is a relatively new problem in Norway that appears to be influenced by photoperiod and rearing temperature during the freshwater stage (Fjelldal et al., 2011). The early maturation problem is exacerbated by the industry moving to larger smolts to reduce rearing time in seawater and the associated risks of sea-lice infection. Age at maturity among salmonids is inversely related to body size (Hutchings & Jones, 1998). Male salmonids exhibit a much higher incidence of early maturation than females, hence the rainbow trout industry relies on all females or triploids (see *Section 8: Non-reproductive Fish, Induced Spawning, and Genetic Modification*). The Atlantic salmon industry in Canada remains mostly reliant on diploids. The heritability of age at maturity in Atlantic salmon is low, hence breeding programs have struggled to address the grilse problem (Gutierrez et al., 2015).

Maturation can be prevented by high intensity white light used continuously (i.e., LL) for 4–6 months during the fishes' first winter in seawater (Hansen et al., 1992; Taranger et al., 1998, 2010). The 24 h light disrupts the fishes' perception of time of year stopping sexual maturation (Taranger et al., 2010). The standard procedure to use LL follows the discovery by Hansen et al. (1992) that continuous light from October to June significantly reduced the incidence of maturation compared with controls reared under natural light in Norway. In land-based systems, the maturity rate among Atlantic salmon was significantly lower among fish reared under LL compared with a long photoperiod with a dark period of 4–6 hours (LL 7.5% vs LD 20:4: 25%, Berg et al., 1996; LL 27% vs LD 18:6: 41%, Good et al., 2016). No welfare concerns have been documented with use of LL. Bone development in Atlantic salmon can be inhibited by LL, but risk of spinal deformity is negligible provided dietary phosphorus is sufficient (Wargelius et al., 2009; Fjelldal et al., 2012). On the contrary, salmon reared on land under LL had a better condition factor and lower incidence of abnormal hearts compared with fish reared under LD20:4 (Berg et al., 1996).

7.5.2   Sea Lice Avoidance

Sea lice are a major threat to the health of Atlantic salmon in sea-cages. They are distributed relatively high in the water column. Consequently, the risk of Atlantic salmon coming into contact with infective copepodid stages and being infected can be reduced by using deep net-pens and submerged lights providing LL to attract the fish to deeper water (Juell et al., 2003;

Stien et al., 2014; Frenzl et al., 2014). This use of lights is a benefit to both fish health and welfare.

7.6   Photoperiod Manipulation of Timing of Spawning

The first reported use of lights to manipulate the timing of spawning was on brook trout (Hoover & Hubbard, 1937). Since then, the spawning of a wide range/array of species have been shown to respond to photoperiod, with salmonids receiving the most attention (Bromage et al., 2001). The most reliable regime for producing good quality gametes is a 12-month photoperiod cycle that is out-of-phase to the natural daylength cycle (e.g., a 6-month out-of-phase cycle: longest day December 21, shortest day June 21). Attempts to accelerate the maturation of Atlantic salmon and rainbow trout resulted in smaller eggs and poorer survival (Duston & Bromage, 1988; Taranger et al., 1998). The process of gonadal development in salmonids takes about 12 months to complete; hence, maintaining fish under a 12-month photoperiod cycle allows the normal chronology of maturation. Lightproof broodstock rearing facilities are land-based and typically have up to three spawning populations per year. To ensure good quality gametes all aspects of broodstock management must be of the highest welfare standards, since a host of potential stressors can disrupt the maturation process (Schreck, 2010).

7.7   References

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Lighting Conditions

Life Stage	Intensity	Duration (photoperiod)	References
Egg/Embryo	Low	• LL in the hatchery room, but eggs must be in Heath trays or Comphatchs with limited exposure to light	Bell & Hoar, 1950 Eisler, 1957
Hatched Alevin	Low	• LL in the hatchery room, but fry must be in Heath trays or under biomats with reduced exposure to light	Peterson & Martin-Robichaud, 1995
First Feeding		• LL in shallow circular tanks, to prevent fry from being suffocated on the tank's drain screen	
Parr		• LL or long photoperiod for no more than 3 months without changing	Saunders & Henderson, 1988 Stefansson et al., 1989
Smolt	>20–40 lux at water surface	• LL or long photoperiod (as in autumn) that changes to a short photoperiod (as in winter, e.g., LD 12:12) followed by a return to LL or long photoperiod (as in in spring)	Saunders et al., 1985 McCormick et al., 1987 Duston & Saunders, 1995 Handeland et al., 2013
Production Adult		• LL for first 4–6 months of first winter at sea to prevent maturation • Submerged lights to draw fish away from water's surface and prevent exposure to infective sea lice copepodids	Hansen et al., 1992 Berg et al., 1996 Taranger et al., 2010 Liu & Duston, 2019
Broodstock		• 12-month photoperiod regime to induce spawning without risk to egg survival • Where possible (i.e., a lightproof, land-based facility), may have different populations of fish on different phases of the photoperiod regime to maximize gamete production throughout the year	Taranger et al., 1998 Bromage et al., 2001

*Table 7.1 Summary of suitable intensity and duration of white light for Atlantic salmon (*Salmo salar*) at each stage of production.*

Abbreviations are LL (24 hour light) and LD (a ratio of Light:Dark, in hours).

8   Non-reproductive Fish, Induced Spawning, and Genetic Modification

Conclusions:

1. **Male broodstock often have wounds and associated secondary infections that can be reduced by supplementing freshwater with low concentrations of seawater (about 1% seawater, not to exceed 10% seawater).**

2. **As triploid fish mature through smoltification, rates of deformity may increase, and there is some evidence that they are less tolerant of poor water quality. At earlier life stages, triploids appear to perform as well as their diploid counterparts.**

3. **Inefficient triploid production may result in high rates of larval mortality and deformity.**

4. **There is evidence that triploids have different dietary requirements, and that some of the adverse effects reported may be mitigated nutritionally.**

5. **There are likely no serious welfare issues associated with production, including hormone use or use of monosex fish.**

8.1 Introduction

Production of sterile triploid salmonid fish can address the problem of potential breeding between farmed escapees and wild fish populations, and timing of reproduction is often controlled with hormones used to induce ovulation. Details of these procedures and potential associated welfare issues are discussed herein.

8.2 Broodstock

Some farms keep broodstock in land-based freshwater facilities for biosecurity reasons, with pit-tagged siblings in sea-cages used to identify better performing families, while others simply select them from the general population. Males and females are kept in the same tank for pheromonal communication. Photoperiod and temperature are often manipulated in order to advance or delay the time of spawning to have more than one spawning per year (welfare issues associated with photoperiod are addressed in *Section 7: Lighting*).

All potential broodstock are tagged and sampled for genotyping. Based on genotyping results, the broodstock are separated into those that will be used and those that will be culled. The tissue used for genotyping is sometimes a scale but is most often a fin clip taken from either the caudal fin or the adipose fin. The adipose fin develops differently in different fish groups, and there is good evidence that in salmonids it has a mechanosensory function. Elegant anatomical studies show that innervation and arrangement of collagen cables and layers are typical of mechanoreceptors (Buckland-Nicks, 2016). Removal of the adipose fin results in an 8% increase in caudal fin amplitude relative to unclipped fish during swimming but no change in tailbeat frequency, suggesting that it acts as a pre-caudal turbulence sensor (Reimchen & Temple, 2004). Electrophysiological studies show that it acts as a mechanoreceptor in catfish, with a response to small mechanical deformations. Based on the above, and on the observations that a fin clip wound in salmon heals quickly (closing within 6–12 hours even at low temperatures, epidermal normalization, and the absence of inflammation), Andrews et al. (2015) argue that adipose fin

clipping can be performed on salmonids without compromising fish welfare. However, Roques et al. (2010) claim that clipping the caudal fin of tilapia and carp, which lack an adipose fin, is a welfare issue because clipped fish showed increased swimming activity and more random movement (but the authors did not show any data and did not use anesthetic during fin clipping). In addition, there was change in location and secretion by gill mucus cells, and they claim this indicates stress-induced release of mucus (though there were no changes in other stress markers such as blood cortisol, glucose, lactate, or osmolality). There is other evidence that that the caudal fins of Cyprinidae species possess nociceptors, because carp respond to an electric shock to the tail that is attenuated with opioids (Chervova & Lapshin, 2011). The scientific committee therefore concludes that, though fin clipping may represent a noxious stimulus that induces a nocifensive response (possibly depending on the species), the associated welfare issues are likely only short-term and probably minimal, especially for clipping the adipose fin of salmonids.

During this pre-spawning period, fish are usually checked weekly for readiness using ultrasound, an effective and minimally invasive tool for monitoring reproductive readiness (e.g., Naeve et al., 2019), with stress only elicited by the handling required. They are also sexed using ultrasound and excess males are culled. The time of ovulation needs to be known because once the eggs are ovulated, they begin to lose their viability. Failure to strip the eggs at the appropriate time results in greatly reduced fertilization success. Milt can be collected a few hours before the expected time of ovulation; use of milt extenders is common (Nynca et al., 2016) and some producers now use cryomilt.

Before and during spawning, mature males show agonistic behaviour in the wild and probably in tanks, as farmed male broodstock often have wounds (Johnsson et al., 2001; Weir et al., 2005). Because of the wounds and the decrease in immune function associated with sexual maturity, there is an increase in fungal infections (*Saprolegnia sp.*) and in universal secondary infections that constitute a welfare hazard in broodstock. However, most opportunistic infections are related to poor husbandry and host condition (e.g., Wedemeyer et al. 1999, Beldomenico & Begon, 2010.). Freshwater is essential for sexual maturation, though supplementing with low concentrations of seawater (about 1% seawater, not to exceed 10% seawater) can reduce fungal infections and is not detrimental to sexual maturation (e.g., Long et al., 1977).

For all on-farm spawning, the fish are first sedated or anesthetized with tricaine methanesulfonate (TMS or MS-222), which is widely considered to be acceptable (see AVMA, 2020, for more on anesthesia). Eggs and milt can be removed manually, surgically, or in some cases through a process called "air-spawning." For iteroparous species (i.e., capable of multiple reproductive cycles; charr, trout, Atlantic salmon), gametes are sometimes removed manually. Manual stripping involves handling, which could cause stress. Furthermore, if improperly performed or if fish are not fully anesthetized, welfare may become compromised due to injury (e.g., from dropping or squeezing too hard) or infection from mishandling and disruption of the mucus layer. For semelparous species, it is essential that water quality in recovery baths is maintained. For semelparous species (i.e., have only one reproductive cycle prior to death; e.g., Pacific salmon), some farms use TMS plus percussion prior to surgical or manual stripping, whereas others add bleeding to the protocol. The disadvantage of using percussion or bleeding is that the gametes can become contaminated during the process, though gametes may also become contaminated if urine or feces are introduced through manual stripping. One small farm uses electro-immobilization for Chinook salmon, which does not alter embryo mortality or fry growth

in this species (Hudson et al., 2015). The fertilized eggs are usually disinfected with isotonic iodophor solution, rinsed, and water hardened in freshwater before being transferred to an incubator. Iodine disinfection of embryos is effective and does not appear to be toxic to Atlantic salmon embryos at low concentrations (50–75 mg/L; Chalupnicki et al., 2011).

Thus, the welfare issues associated with spawning and broodstock in salmonid farming are photoperiod manipulation (reviewed in *Section 7: Lighting*), the stress of handling and anesthesia associated with frequent handling to check the sex and assess readiness (indicators of stress are reviewed in *Section 2: Stress Indicators*).

8.3   Welfare Concerns Associated with Triploidy

Using triploidy has been proposed in aquaculture to produce sterile populations for aquaculture (considered desirable to minimize the potential impact of escapees on wild populations; Benfey, 2016). Triploids are usually produced using a shock treatment (heat/cold or pressure) of eggs shortly after fertilization, which interferes with the completion of meiosis. All cells of all triploids are larger than their diploid parents, as they contain an extra set of chromosomes. Females do not develop secondary sexual characteristics and have very reduced ovaries, whereas males develop typical secondary sexual characteristics and have large testes.

Inasmuch as the shock treatment is applied to eggs and not hatched fish, the procedure does not appear to introduce immediate welfare issues (Benfey, 2011), though inefficient triploid production methods may be associated with high rates of larval mortality and deformity in some species (e.g., Peruzzi & Chatain, 2000). Triploids also generally do not survive as well as diploids under conditions of chronic stress, such as high temperature combined with hypoxia, and/or changes in salinity (e.g., Myers & Hershberger, 1991; Blanc et al., 1992; Ojolick et al., 1995), though their response to more acute stressors appears to be similar to that of their diploid counterparts (Fraser et al., 2012a; Benfey & Devlin, 2018). Evidence suggests that this intolerance to high temperatures may be a result of a reduced ability to maintain proteostasis under stressful conditions; triploid fish have lower basal levels of heat shock proteins (HSPs) and therefore are not able to mount a heat shock response to acute high temperatures (Saranyan et al., 2017). At present, evidence from *in vivo* pathogen challenge trials suggests that ploidy does not affect disease susceptibility (e.g., Moore et al., 2017; Fraser et al., 2012a) or vaccination success (e.g., Yamamoto & Iida, 1995; Johnson et al., 2004), though triploids have increased numbers of irregular or deformed immune cells (e.g., Wlasow & Fopp-Bayat, 2011) and lower numbers of B-cells (e.g., Fraser et al., 2012b) in some species relative to diploids. There are some studies that report differences in disease susceptibility between ploidies (e.g., Myers & Hershberger, 1991; Ojolick et al., 1995; Cotter et al., 2002; Ching et al., 2010), but these are typically confounded by simultaneous heat stress, indicating that triploids may have compromised immune function while under additional temperature stress (Fraser et al., 2012a). It is possible that triploids have increased toxicological resistance relative to diploids; lower numbers of tumours after exposure to carcinogens (Thorgaard et al., 1999) and slower progression of lesion development post-exposure to aflatoxin B1 (a carcinogen; Arana et al., 2002). The effects of triploidy on growth and feed conversion efficiency are inconclusive (Fraser et al., 2012a; Benfey, 2016). When reared together with diploids, triploids may show reduced growth rates (e.g., Thorgaard et al., 1982; Galbreath et al., 1994) and increased fin erosion (e.g., Carter et al., 1994),

possibly due to the generally less aggressive demeanor often observed in triploids (Fraser et al., 2012a).

Though deformity rates among triploid fish in the early freshwater life stages are usually low (<2% of the population; Benfey, 2016), several studies show that triploid adult Atlantic salmon and rainbow trout have higher incidences of deformed jaws and other skeletal abnormalities (e.g., Sadler et al., 2001; Leclercq et al., 2011; Fraser et al., 2012a), cataracts (e.g., Leclercq et al., 2011), and abnormal gill filament development (Sadler et al., 2001) during and after smoltification. Triploids are generally more likely to be downgraded or discarded at harvest because of deformities (e.g., Fraser et al., 2013; Benfey, 2016) and so have not been adopted in Atlantic salmon farms in Canada (Benfey, 2016). There is recent evidence that deformities can be reduced with different diets because there are differences in nutritional requirements between ploidies (Vera et al., 2019), and more trials are being carried out at present in Norway and the European Union (EU). Smedley et al. (2018) recently reported that early phosphorus supplementation is crucial for skeletal development in triploids through to smoltification, though exact quantities of phosphorus required have yet to be determined. It has been argued that the differences at harvest between triploids and diploids need to be considered in selection trials to reduce or eliminate differences in downgrades and discards at harvest (Benfey, 2016). At present, all rainbow trout farms in New Brunswick must use either triploids or all-female populations depending on location (Province of New Brunswick, 2016).

8.4   Monosex

Similar to bovine, porcine, and ovine farming, sexual maturation results in a decrease in product quality and quantity, and measures are taken to mitigate these changes. In salmonids, males typically mature earlier (especially given the tendency for precocity among males), and maturation often produces increased aggression. In contrast, females tend to grow faster and bigger, and mature later (or sometimes do not mature at all before harvest). Thus, raising all females can be a profitable endeavour that also limits the rate of aggression among adult fish.

Diploid gynogenetic fish (all maternal inheritance) can be produced by blocking first extrusion of the second polar body or by blocking first cleavage during oocyte maturation. There is currently regulatory uncertainty about the use of hormones in sex manipulation, but salmonid production can be enhanced by culturing females only, a condition that manipulates the sex phenotype by exposing juvenile fish of the potential broodstock to 17-alpha-methyltestosterone either through immersion or incorporation in feed. All rainbow trout farms in New Brunswick must use either triploids or all-female populations depending on location (Province of New Brunswick, 2016). Some land-based salmon farms in Nova Scotia import all-female eggs from Iceland to reduce problems with early maturation in males. The scientific committee concludes that there are likely no serious welfare issues associated with production or use of monosex salmonids.

8.5   Hormone Use

Inasmuch as all salmonids in the wild dig nests in gravel (redds), and substrate is not available on the fish farm, all salmonids are strip-spawned for artificial gamete collection and fertilization. Efficiency requires control over timing and hormones can be of use in this regard, particularly to

induce ovulation. Although a number of different gonadotropin releasing hormone (GnRH) analogues are available, the only approved products for use in commercial aquaculture are Ovaplant (time release pellet implant; Syndel) and Ovaprim (injectable; Syndel), used in North America, and Gonazon™ (Intervet), used in Europe, which are efficacious in salmonids (Haffray et al., 2005; Svinger & Policar, 2013). Chorulon (chorionic gonadotropin; Merck) is approved in the USA. GnRHa is usually given in the form of two injections (10–100 μ/kg) spaced 3 days apart or a single application of a GnRHa-delivery system (10–50 μg/kg) given around two weeks before the onset of natural maturation of the broodstock. Both methods induce ovulation in 100% of the stock within two weeks after treatment. This type of treatment is not always used, but when females and males are not synchronized (e.g., if males and females are coming from different farms), they can be of particular use. The scientific committee had difficulty finding peer-reviewed literature pertaining to any welfare impacts due to action of the hormones themselves (though the doses used are close to physiological levels) or concerning the location of the pellet injection, but there is likely some stress associated with the application of said hormones due to the handling and anesthesia required prior to injection (indicators of stress are reviewed in *Section 2: Stress Indicators*) and possible infection at the site post-injection.

8.6   Genetic Modification

Genetic modification techniques are beginning to play a more significant role in salmonid production. Domestication results from selective breeding for specific phenotypes (e.g., rapid growth, disease tolerance) and may cause welfare issues through unintended negative consequences of the selected trait (e.g., increased somatic growth and skeletal problems, as in turkeys; Hocking, 2014) or through simultaneous unintended selection for other traits such as an increased stress response or aggression (e.g., Nicieza & Metcalfe, 1999). More recently, the introduction of novel genes into the salmonid genome through new technologies (e.g., AquAdvantage salmon; Smith et al., 2010) with the development of transgenic lines may also cause welfare issues (reviewed for animals generally by Ormandy et al., 2011, and for salmonids by Hallerman et al., 2007). It is possible that new genes for growth modulation may decouple proportional growth between tissues, causing a mismatch in the rate at which muscle tissues grows relative to bone or connective tissue, though there is no evidence of this in salmonids as yet. With the continuation of salmonid genetic modification and the emergence of gene editing (Gratacap et al., 2019), care should be taken to consider all possible welfare consequences, using information from other genetically modified species (e.g., mice; Wells et al., 2006) and with a thorough understanding of salmonid-specific welfare.

8.7   References

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9   Ice-Slurry Slaughter

Conclusions

1. **Rapid chilling of salmonids is not lethal if the ice-slurry is above -0.7°C, the freezing point of the blood. A brine ice-slurry can be below -0.7°C and therefore can be lethal. The effect will depend markedly on acclimation temperature prior to chilling.**

2. **There is no evidence that fish possess cold nociceptors, but studies are limited to trout and zebrafish.**

3. **Arctic charr have no behavioural responses or loss of reflexes to rapid chilling.**

4. **Physiological and/or behavioural responses to rapid chilling are likely not due to stimulation of nociceptors; rather, they are probably due to stimulation of temperature receptors involved in behavioural thermoregulation responses.**

5. **Rapid chilling takes 1-2 minutes for the brain to cool sufficiently to compromise neural and muscle function.**

6. **The cause of death during ice-slurry slaughter is currently unknown but is likely asphyxiation. It is possible that fish asphyxiate because of paralysis; cold temperatures block nerve conduction in other fish species, so chilling may arrest motor function, resulting in asphyxiation from a lack of gill irrigation. It is also possible that fish asphyxiate due to hypoxia; without supplemental oxygenation, oxygen levels in chilling tanks likely drop over time.**

7. **There is a lack of literature using validated experimental methods to test possible welfare issues with ice-slurry slaughter, limiting conclusions that can be made about the process and cause of death. Further research is required to determine if fish are insensible (i.e., incapable of perceiving and integrating sensory information) prior to death by asphyxiation.**

8. **Ice-slurry slaughter with CO_2 is generally considered inappropriate as a standard slaughter method as it induces considerable struggling and escape behaviours and an acute stress response.**

9.1   Introduction

In a recent review, Browning and Veit (2020) argue that "no slaughter can ever be completely humane." This is an ethical question, not easily or simply addressed with scientific evidence alone. For the purposes of this report, our definition of slaughter is the killing of animals for food. Humane slaughter is therefore slaughter that shows consideration for the animals' potential affective experience (see *Section 1: The Question of Sentience and Why It Matters* for discussion of this in fish) by inflicting minimal discomfort prior to death. Therefore, we can ask whether there is scientific evidence that fish are rendered insensible (that is, incapable of perceiving and integrating sensory information) as quickly as possible and with minimal distress by a given slaughter method.

Rapid chilling with ice slurries is the principal method of slaughter in the Ontario trout production industry. Typically, during ice-slurry slaughter, fish are transferred from holding nets or tanks into a non-oxygenated ice-water slurry where they remain until death. This chapter discusses the scientific literature pertaining to the rapid chilling of salmonids and uses this literature to estimate the possible relative contribution of different stressors during ice-slurry slaughter as it is currently practiced in industry in Canada, though there is no scientific literature directly examining fish welfare during the process in its entirety on the fish farm.

9.2   Is Live Chilling Lethal for Salmonids?

Salmonids are a cold-water fish, preferring temperatures between 4.5 and 15.5°C, depending on life stage and species (Sauter et al., 2001; McCullough, 2001). Like most poikilotherms, as body core temperature drops below optimal, most physiological metabolic functions become progressively slower (Crawshaw, 1979; Guderley & Blier, 1988). The temperature at which fish die when they are chilled depends on the salinity of the water, the temperature that the fish were acclimated to prior to chilling, and the species. Freshwater (FW) freezes at 0.0°C, seawater (SW) at -1.9°C, and blood plasma at -0.7°C. Thus, death due to freezing of the blood is impossible in FW ice-slurry, but possible in SW ice-slurry. Freezing point (FP) decreases with the addition of osmolytes (dissolved substances) by 1.86°C/osmole. The osmolality of salmonid plasma and tissue fluids is about 0.4 osmoles, which lowers FP to about 0.7°C. At temperatures below this, salmon body fluids will supercool or freeze.

Fish acclimate to their rearing temperature by undergoing adjustments in their biochemistry and cell membrane structure. The behavioural response to chilling and the lower lethal temperature both decrease as fish become adapted to lower temperatures. Consequently, their response to an abrupt decrease in temperature depends on their acclimation temperature. When Atlantic salmon are acclimated to 15°C, their lower lethal temperature is about 4°C, whereas in salmonids acclimated to 10°C subjected to an abrupt drop in temperature, death (quantified after 24 hours in the treatment) occurred between 0.5 and 3.0°C (Brett, 1952; Elliott & Elliot, 2010). For rainbow trout acclimated to 10°C, their tolerance (TLm; the temperature causing 50% mortality over 96 hours) was ~0.5°C. At temperatures below TLm, mortality usually occurred within the first 24 hours (Becker et al., 1977). Abrupt transfer of juvenile salmon (five Pacific species, ~5 g) from their acclimated temperatures to freshwater temperatures as low as 0.1°C resulted what the authors termed "suspended animation," from which some individuals transferred to 12°C afterwards recovered (Brett, 1952). A reliable indicator of cold death in Brett's study was the position of the opercula changing from closed to "fan-out." Similarly, Hovda & Linley (2000) claimed that pink salmon can be anesthetized in cold brine (-3 or -6°C) in less than a minute, and will recover in less than a minute if returned to temperature at the acclimation temperature (8.5°C). In another species, turbot (*Scophthalmus maximus*), recovery was possible after fish reared at ~13°C were submerged in seawater at -1.4°C for 90 minutes (Roth et al., 2009); however, differences in thermal tolerance exist between different species, so this result should be further investigated for salmonids specifically. The evidence therefore suggests that rapid chilling itself is not lethal for salmonids, but their resilience to chilling depends on multiple factors. This conclusion is concordant with that of the American Veterinary Medical Association (AVMA, 2016).

9.3   Effect of Rapid Chilling on Salmonid Brain, Nerve, and Muscle

9.3.1   Lack of Cold Nociceptors

Electrophysiological experiments using rainbow trout detected a variety of nociceptors that respond to very high temperatures, very high forces, low pH, and some chemicals that bind to nociceptive receptors in mammals. However, despite searching, they did not find the nociceptor that responds to cold that is present in other vertebrates (Ashley et al., 2006, 2007). Similarly, evidence from molecular biology shows that zebrafish lack a TRPM8 ortholog, and the two zebrafish TRPA1 paralogs are not thermosensitive (Chen et al., 2016). TRPM8 is also known as the cold and menthol receptor 1 (CMR1), and TRPM8 proteins are required for cold nociception in humans. In salmonid species with behavioural responses to chilling, the responses are therefore likely not a result of nociceptor stimulation. Rather, they are the response to stimulation of temperature receptors involved in behavioural thermoregulation: the fish seek warmer water.

9.3.2   Temperature Receptors in the Brain

The temperature-sensitive neurons involved in behavioural thermoregulation in fishes are located in the preoptic nucleus; cooling this area of the brain will result in the fish moving to warmer water, whereas warming it will result in the fish moving to cooler water, if there are a range of temperatures available (Crawshaw, 2011). During rapid chilling, the fish do not have a choice of temperatures but may exhibit increased swimming behaviour because they are actively seeking warmer water that is not available (e.g., Becker et al., 1977). This behaviour will persist until the low temperature reduces brain activity and/or muscle activity. When abruptly transferred to chilled water, it takes some time for the brain to cool (Stevens & Fry, 1970, 1974; also see *Section 9.3.3: Effect of Cooling on Brain Function*).

9.3.3   Effect of Cooling on Brain Function

There is very limited information on the effect of rapid chilling on fish brain function. Nerve conduction from catfish acclimated to 24°C was blocked at 3°C, and from fish acclimated to 10°C was blocked at 1°C (Prosser & Farhi, 1965). Reflexes tend to be blocked at slightly higher temperatures than that at which all nerve conduction is blocked (Prosser & Farhi, 1965). Visually evoked potentials (VEP, the response in the brain to a flash of light) have also been used to estimate brain function in rainbow trout (Kestin et al., 1991). When rainbow trout were restrained on their side on a bed of crushed ice at 2°C the VEP gradually decreased. Brain temperature was not measured, but given that the fish were in air (initiating a strong bradycardia reflex) the rate of cooling was probably much slower than it would be in water. Furthermore, when trout were removed from water they made "vigorous attempts to escape" restraint by twisting and turning in bouts lasting up to 20 seconds, in between which they appeared relatively calm. These experiments had no controls for restraining trout on their side in air independent of the chilling. Thus, their results are interesting but provide little insight into what likely happens during ice-slurry slaughter on farm. There is only one study using the VEP method during euthanasia in mammals (Cartner et al., 2007), but the significance of the results has been questioned (Hawkins et al., 2016). However, VEP recording is an interesting method and, using modern data logging techniques that could capture relevant data while fish remain in water (unlike current VEP recording techniques which require the fish to be removed from water), could provide valuable insight for future studies.

9.3.4   *Effect of Abrupt Cooling on Muscle Function*

Abrupt decreases in temperature result in an abrupt decrease in the ability of fish muscle to do effective work, largely because the rate of relaxation is very temperature sensitive and becomes very slow as the muscle is cooled (Luiker & Stevens, 1994; Rome & Swank, 1992). A 10°C decrease in temperature results in a 300% decrease in the rate of relaxation. Thus, when observing behaviour during rapid chilling, it is difficult to attribute a decrease in activity to a decrease in brain and/or nerve function or minimal behavioural stress response because of the concurrent considerable decrease in muscle function. Similarly, if we observe a decrease in respiratory movements (e.g., opercular movements) during rapid chilling, it is difficult to distinguish between an effect of reduced brain and/or nerve function from an effect of decreased muscle function.

9.4   **Stressors During Ice-Slurry Slaughter**

Drugs cannot be used during slaughter because of regulations concerning residues, so various alternate methods of salmonid slaughter exist across the industry. Freshwater trout production, particularly in Ontario, often uses ice-slurry slaughter (R. Moccia, pers. comm.). As noted previously, rapid chilling alone in freshwater is not necessarily lethal to salmonids; thus, it is possible that the cause of death during ice-slurry slaughter is actually asphyxiation due to the combined effect of decreasing oxygen levels in the water in addition to the cessation of gill irrigation (due to effect of low temperature on respiratory muscles and nerve conduction).

There are therefore three sources of stress during ice-slurry slaughter: 1) handling and crowding, 2) chilling, and 3) possibly low-level increases in CO_2 concentration and hypoxia. The magnitude of each of these stressors has been estimated separately. In Arctic charr, in the absence of handling plus crowding, rapid chilling had no effect on behaviour and a modest effect on cortisol (Seth et al., 2013). In Atlantic salmon, rapid chilling, in the absence of handling, resulted in no significant increases in blood glucose, lactate, cortisol, or sodium levels, whereas chilling with handling resulted in elevated stress indicators (Foss et al., 2012). An earlier study netting Atlantic salmon showed that live chilling (after netting) caused a modest increase in cortisol, glucose, lactate, and osmolality, whereas crowding caused larger increases in all variables and the crowding effect was not mitigated by live chilling (Skjervold et al., 2001). Low levels of CO_2 (50 mL CO_2/L water), like hypothermia, are suggested to have anesthetic-like effects (Kugino et al., 2016) through depression of the central nervous system activity leading to unconsciousness (see also *Section 9.5: Ice-Slurry Combined with CO₂*, discussing high concentrations of CO_2). Acute hypoxia, on the other hand, has been shown to induce a physiological stress response: for example, a drop from 90% to 40% oxygen saturation over 2 hours resulted in increased plasma cortisol, lactate, and chloride in Atlantic salmon (Remen et al., 2012). Thus, handling plus crowding may be the most significant stressor during ice-slurry slaughter, but is unavoidable. Chilling may be the smallest contributor to the total stress response prior to death during ice-slurry slaughter, though it is still a stressor in and of itself.

There is limited peer-reviewed literature that investigates fish welfare during the entire process of ice-slurry slaughter, as it is currently practiced in Canada. Studies of these practices would help confirm the actual cause of death during ice-slurry slaughter and would be of considerable value to our understanding of fish welfare during ice-slurry slaughter.

9.5   Ice-Slurry Combined with CO₂

Chilling with the addition of carbon dioxide (CO_2) has been historically used both for euthanasia and during the slaughter and processing of salmonids (Erikson et al., 2006). Though this practice is currently uncommon in industry, it may still be used by some producers and deserves brief review here.

Most species become unconscious after about 2–3 minutes from hypoxia and hypercapnia (Robb, 2001; Wall, 2001; Robb & Kestin, 2002) and will die if no oxygen is available. Fish respond to high concentrations of CO_2 with struggling behaviour and a stress response. This is possibly because carbolic acid is created when CO_2 is dissolved in water and, as mentioned previously, fish possess nociceptors that respond to low pH (Ashley et al., 2007). Seth et al. (2013) found that CO_2 had a large effect on both Arctic charr behaviour (anecdotally reported as "pronounced struggling and escape behavior") and cortisol indicating a considerable physiological and behavioural stress response. Importantly, the severe trauma caused by CO_2 was not mitigated by chilling down to 0.25°C (Seth et al., 2013). *Section 6: Water Quality Issues in RAS* also reviews important welfare consequences of abrupt increases in CO_2 concentration. Moreover, the stress due to CO_2 has been associated with down-grading of fillet quality (Merkin et al., 2018).

9.6   References

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Table 9.1. Lower lethal temperatures for salmonids.

Species	Acclimation Temperature (°C)				Incipient Lower Lethal Temperature (°C)	Reference
	2.6	**5**	**10**	**15**		
Onchorynchus nerka		0.0	3.1	4.1		Brett, 1952
O. tshawytscha			0.8	2.5		Brett, 1952
O. keta			0.5	4.7		Brett, 1952
O. kisutch	-0.1	0.2	1.7	3.5		Brett & Alderdice, 1958
Salmo salar					-0.8	Elliott & Elliott, 2010
S. trutta					-0.8	Elliott & Elliott, 2010
S. alpinus					-1.0	Elliott & Elliott, 2010

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