COLOUR MATTERS: COHO SALMON (*ONCORHYNCHUS KISUTCH*) PREFER AND ARE LESS AGGRESSIVE IN DARKER COLOURED TANKS

by

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Abstract

Fish are capable of colour vision and certain colours have been shown to affect growth and survival, skin colour, stress response, and reproduction. Beyond these physiological consequences, colour has also been shown to affect aggression levels, which is a widespread problem in aquaculture. The compatibility of fish with tank colour has been largely neglected within the aquaculture industry. Common practice is to use light blue tanks but there is no scientific basis for this choice. Closed containment aquaculture systems provide a good model to investigate the effects of tank colour on fish. Though closed containment aquaculture systems provide the opportunity for full control of environmental conditions, little research to date has investigated which parameters within these systems promote fish welfare. The aim of this study was to assess preferences of coho salmon for tank colour and determine the effects of colour on aggression. Coho salmon (n=100) were randomly assigned to 10 tanks, each bisected to allow fish to choose between two colours. Using a Latin-square design, each tank was tested with each of the following colour choices: blue vs. white, light grey, dark grey, and black, as well as black vs. white, light grey, dark grey, and a mixed dark grey/black pattern. Fish showed a strong preference for black over all other tank background options ($p < 0.0001$) with the exception of pattern, which was still significant but slightly less strong ($p < 0.01$). Moreover, darker colours in the environment resulted in lower rates of aggressive behaviours compared to lighter colours ($p < 0.0001$). These results present the first evidence that darker tanks are preferred by and decrease overall tank aggression levels in salmonids.

Preface

All research and associated methods were approved by the University of British Columbia Animal Care Committee (Certificate Number: A13-0210).

Leigh Gaffney and Drs. M.A.G. von Keyserlingk, D. Weary, and J.G. Richards designed the experiment collaboratively. J.G. Richards supplied the coho salmon and lab space. The execution of the experiment and data collection was performed by Leigh Gaffney. Leigh Gaffney and B. Franks analyzed the data. Drs. M.A.G. von Keyserlingk, D. Weary, and J.G. Richards supervised data analysis, interpretation, and manuscript preparation.

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Chapter 1: Introduction

1.1 Overview

This thesis seeks to assess coho salmon preferences for tank colour and the effect of tank colour on fish aggression; potential determinants of the welfare of salmon in closed-containment aquaculture. This introductory chapter sets the stage by briefly reviewing the natural history of coho salmon and the use of salmonids in aquaculture, highlighting closed-containment systems. Secondly, animal welfare assessments and the capacity for fish to experience pain and fear will be discussed. Salmon aggression and the effects of tank colour on aggression levels, as well as other aspects of fish will be then be reviewed followed by a section on fish vision, measuring colour, and preference testing. Lastly, the overall objectives and hypothesis of this study will be stated.

1.2 Natural history

Coho salmon (*Oncorhynchus kisutch*) are one of the seven recognised species of Pacific salmon (Sandercock, 1991). Their natural range includes the far east of Russia around the Bering Sea, over to Alaska, and along the west coast of North America down to California (Hart, 1973). The life history of coho salmon begins as adult fish migrate from the sea into streams, during the fall months, where they deposit their eggs into gravel (Sandercock, 1991). As soon as they finish spawning, the male and female adult salmon die (Shapovalov and Taft, 1954). After the eggs incubate during winter in the gravel, free-swimming coho fry emerge in the spring. Fry tend to emerge at night and begin to school immediately (Sandercock, 1991). As they mature, individuals break off from the school and acquire their own territories for feeding (Shapovalov and Taft, 1954). Once territories have been established, juvenile coho begin to engage in intraspecific aggressive behaviours in the form of territory defense and hierarchy establishment (Shapovalov and Taft, 1954). Juvenile coho generally feed on other fry and on drifting invertebrates, such as ants, flies, and assorted larva (Shapovalov and Taft, 1954).

The juvenile coho's primary predators in the stream include fellow coho and other salmon species, as well as garter snakes and birds such as kingfishers and great blue herons (Shapovalov and Taft, 1954). After a year or more in the stream, sometime between March and April, the salmon begin their seaward migration downstream, undergoing a metamorphic process called smoltification (Sandercock, 1991). When they reach the ocean, coho spend approximately 18 months at sea feeding and growing before reaching sexual maturity (Shapovalov and Taft, 1954). Mature adults exhibit a "homing instinct" and travel back to their freshwater stream of origin to spawn (Sandercock, 1991).

1.3 Salmonids in aquaculture

Aquaculture, as defined by the Food and Agriculture Organization of the United Nations, is the farming of aquatic organisms, with human consumption being the most common reason for harvest (FAO, 2014). Over the past 20 years, aquaculture has expanded worldwide and this growth is expected to continue (Naylor et al., 2000). World demand for fish and fishery products is projected to increase by almost 45 million tonnes to 187 million tonnes by 2030 with aquaculture supplying the majority of this growth and accounting for over 50% of global fish production (FAO, 2014).

Although a variety of organisms such as molluscs, crustaceans, and aquatic plants, are reared in aquaculture, over half of all aquaculture production is finfish farming (FAO, 2014). Currently, Atlantic salmon (*Salmo salar*) are the most globally produced species of salmonid and are primarily farmed in Canada, Norway, Chile, Scotland, and Australia (FAO, 2014). Atlantic salmon became popular due to a number of factors, including market demand, high growth rates, low aggression and high disease resistance (Masser and Bridger, 2007; Canada House of Commons, 2013).

To date, the industry standard has been to raise salmonids in land-based recirculating aquaculture systems until smoltification. After this, the smolts are transferred to open ocean net-pens to continue their growth until they become market-sized adults. Ocean net-pen systems typically consist of 6-24 floating, mesh, cage-like structures, made of plastic, steel or aluminum, that are anchored to the ocean floor (Masser and Bridger, 2007). They are located in sheltered bays and fjords where they are protected from extreme currents and storms (Masser and Bridger, 2007). It is now well recognized, however, that this form of production has multiple negative impacts on the ocean environment e.g., organic waste release, fish escapes, and disease threats to natural populations (Black, 2001). Several alternative aquaculture methods that avoid these negative impacts have been proposed. The most feasible and fastest growing alternative is land-based, closed-containment aquaculture (Thorarensen and Farrell, 2011).

In British Columbia, Canada, there is a growing interest in culturing native, Pacific salmonids (e.g. coho salmon, *Oncorhynchus kisutch*), as an alternative to non-native Atlantic salmonids (Canada House of Commons, 2013). Coho salmon provide better opportunities for niche marketing, thus generating more profit with higher prices per kilogram of fish (Canada House of Commons, 2013). As a result, the industry is interested in investigating the culture of post-smolt coho salmon in closed containment aquaculture systems (Canada House of Commons, 2013).

1.3.1 Closed-containment recirculating aquaculture systems

Closed-containment recirculating aquaculture systems use large, circular, fiberglass tanks that are arranged close together, on land, in modules. In contrast to ocean net-pens, they physically separate fish from the external environment so there are no vectors for disease, pathogen or parasite transfer between wild and farmed salmonid populations (Canada House of Commons, 2013). Considering these systems are land-based, the modules must be located in proximity to a sufficient supply of either groundwater or seawater (Masser and Bridger, 2007) that is continuously pumped into the tanks and is constantly recirculated. Water quality is maintained through various means, including mechanical and biological filtration, ultraviolet (UV)-sterilization, heat exchange pumps, oxygen injection, and drainage of solid wastes through the tank bottoms. Through this constant treatment and recirculation of water within the tanks, these systems can reuse 98% of their input water (Canada House of Commons, 2013). Land-based closed-containment aquaculture systems also offer more control over tank conditions such as salinity, temperature, ammonia, CO_2, stocking density, and product quality (Thorarensen and Farrell, 2011). Surprisingly, little research to date has addressed the environmental parameters within closed containment systems that are needed to ensure high levels of fish welfare. Improved fish welfare has the potential to improve industry production quality and quantity as well as public perception and product acceptance (Broom, 1998; Southgate and Wall, 2001; FSBI, 2002). Thus, science-based recommendations on conditions to ensure good welfare in closed containment systems has the potential to greatly influence the long-term success of the industry.

1.4 Assessing animal welfare

Animal welfare scientists use multiple indicators to assess animal welfare, with most adopting the following three categories: 1) biological functioning and physical health, 2) emotional or affective states, and 3) the animals' ability to lead reasonably natural lives and to perform natural behaviours (Fraser et al., 1997). With the rapid expansion of fish aquaculture, the welfare of farmed fish has received increasing attention (Ashley, 2007). However, the majority of studies on fish welfare in aquaculture have focused on the biological functioning of fish, addressing issues such as growth rates, flesh quality, injury, disease, and reproductive problems that are bad for the fish and also for farm efficiency and quality (e.g. Schreck et al., 2001; Kristiansen et al., 2004; Poli et al., 2005). However, good animal welfare goes beyond just physical health. It also involves the ability of an animal to express natural behaviours and a lack of mental suffering from pain, fear, etc. The three categories of animal welfare do overlap and there are issues with measuring and focusing on a single category (Fraser et al., 1997; von Keyserlingk et al., 2009). Thus, the best solutions for improving the welfare of fish in closed containment systems should address all three categories of animal welfare. Unfortunately, scientific research addressing these aspects of fish welfare is lagging behind studies of biological functioning.

1.4.1 Affective states in fish

Some authors have argued that fish are unable to experience negative affective states (Rose, 2002); this argument is based on fish lacking a neocortex, the region of the brain linked to the emotional component of pain in mammals. However, there is a growing body of physiological, behavioural, and anatomical evidence indicating that fish are able to experience pain and fear (e.g. Braithwaite and Huntingford, 2004; Sneddon, 2002, Sneddon et al. 2003a,b; Dunlop and Laming, 2005). Research has confirmed the presence of nociceptors in the head and face of teleost fish, such as trout (Sneddon, 2002; Sneddon et al., 2003a,b). This indicates that fish have the needed neuroanatomy and neurophysiology to perceive and process information about stimuli, such as mechanical pressure or temperature that could cause pain in humans. Furthermore, studies conducted by Sneddon et al. (2003a,b) demonstrated that rainbow trout react to pain stimuli and display fear responses. Fish received injections in their lips with saline (control), bee venom, and acetic acid; in addition, some fish received morphine (an analgesic). The fish that received morphine spent less time rubbing their face against the tank enclosure and more time eating, indicating that these responses are pain specific. Fear responses were measured as the amount of time fish spent avoiding a novel object temporarily placed in its tank. The fish that received bee venom and acetic acid injections had a reduced fear response compared to fish that received saline and morphine injections. These results suggest that nociceptive stimulation preoccupies the fishes' attention and reduces the amount of attention directed at responding to the fear of the novel object. Studies have also shown that teleost fish produce complex behaviours and have the capacity for simple mental representations and, as such, they have the potential to experience pain (Rodriguez et al., 1994; Braithwaite, 1998; Odling-Smee and Braithwaite, 2003b; Laland et al., 2003; Kelley and Magurran, 2003). Considering this evidence, affective states should be considered when describing fish welfare in aquaculture.

1.5 Aggression in salmonids

As juvenile salmonids become territorial, they form social dominance hierarchies (Ellis et al., 2002; Ashley, 2007). Thus, increasing fish density generally increases social stress (Schreck et al., 1997; Ellis et al., 2002) and aggressive behaviours between conspecifics, such as charging, chasing, and biting (Abbott and Dill, 1985; Turnbull et al., 1998, 2005). These aggressive interactions are often the primary cause of fin and skin damage in fish (Abbott and Dill, 1985; Turnbull et al., 1998, 2005).

Fin damage refers to injury, or loss, of the tissue in the rayed fins of fish (Latremouille, 2003). A study conducted by Chervova (1997) demonstrated experimentally that fish fins are capable of nociception. Thus, injury to fin tissue is probably associated with pain. The fins of salmonids serve important functions for locomotion and intraspecific communication (Abbott and Dill, 1985; Pelis and McCormick, 2003); thus, fin damage has the potential to affect behaviour. In contrast, skin damage refers to injury of the epidermis of a fish, including scale loss, wounds and ulcers (Bouck and Smith, 1979). Skin damage can result in a loss of body water and changed ion balance, which produces an osmotic stress that can be life threatening (Bouck and Smith, 1979). Both fin and skin damage have the potential to cause inflammation and pain, and represent routes for pathogens leading to infection, illness, and reduced survival in salmon (Schneider and Nicholson, 1980; Ashley, 2007). In consideration of these factors, determining the mechanisms and factors associated with lowered intraspecific aggression has the potential to improve the welfare of fish.

1.6 The effect of colour on fish

1.6.1 The effect of colour on aggression

In a number of different fish species, certain colours have been shown to affect aggression levels. For example, black tank backgrounds decreased agonistic behaviour and social stress in pairs of Arctic charr (*Salvelinus.alpinus*; Höglund et al., 2002) and Nile tilapia (*Oreochromis niloticus*; Merighe et al., 2004).

Some teleost fish have the ability to adjust the colour of their skin in response to background colour (e.g. Baker et al., 1985; Fujimoto et al., 1991; Höglund et al., 2000). The two peptide hormones released from the pituitary, α -MSH (α-melanocyte-stimulating hormone) and MCH (melanin-concentrating hormone), are involved in the long-term hormonal control of colour changes in fish skin (Imanpoor and Abdollahi, 2011). When fish are placed on a white background, MCH is released and α -MSH is inhibited, causing a concentration of pigments in the dermal melanphores of the skin leading to an overall paling of the fish (Imanpoor and Abdollahi, 2011). In contrast, black backgrounds result in the activation of the MSH cells and the increased release of α -MSH into the blood stream, making fish skin darker in colour (Imanpoor and Abdollahi, 2011).

In salmonids and Arctic charr, lightening of the skin and eyes signals social dominance, while darkening signals social subordination (Keenleyside and Yamamoto, 1962; O'Connor et al*.,* 1999; Höglund et al*.,* 2000, 2002). Thus, a darker coloured fish may represent less of a threat and elicit less aggression than a conspecific displaying paler body coloration. Höglund et al. (2002), compared pairs of Arctic charr (*Salvelinus alpinus*), and showed that fish interacting on a white background displayed initial pale colouration and high levels of aggressive behaviour while fish interacting on a black background, displayed initial dark colouration and a lower frequency of aggressive interactions. While some of the fish on the white background became subordinate and took on a darker body colouration, the subordinate fish on the black background did not show any additional darkening. This result suggests that the use of dark tank colour has the potential to reduce aggressive behaviour and social stress in Arctic charr. To my knowledge, however, this has never been tested in any other fish species.

1.6.2 The effect of colour on stress and growth

In addition to aggression levels, a number of studies have demonstrated that various coloured tank lights impact fish stress responses and growth. An experiment conducted by Volpato and Barreto (2001), for example, exposed groups of Nile tilapia (*Oreochromis niloticus*) to green, blue, or white coloured environments by covering the light source with coloured cellophane (green or blue; no cellophane was used for white light). Certain fish were then confined to a small area of the tank with an opaque partition to induce a stress response. Confinement was shown to increase cortisol levels in fish held in green and white light, but no effect occurred when these fish were maintained under blue light. Blue light was thus shown to prevent an increase of stress-induced cortisol in Nile tilapia and was the recommended colour. In contrast, Karakatsouli et al. (2012) failed to conclude whether

European sea bass (*Dicentrarchus labrax*) were more or less stressed in blue or white environmental light conditions. In another study by Karakatsouli et al. (2010), the effect of white, red and blue lights on the growth of scaled and mirror common carp species (*Cyprinus carpio)* was compared. Results of this study showed that specific growth rate, weight gain, and feed efficiency were positively affected by red light and blue light at low and high stocking densities of scaled common carp, respectively. For mirror common carp, however, the coloured light sources did not induce many differences in growth performance. Conversely, red light, in comparison to blue, violet, red, green, and yellow light, limited weight gain for individually held Nile tilapia and increased weight heterogeneity for groups of Nile tilapia (*Oreochromis niloticus*; Luchiari and Freire, 2009). Lastly, blue light was shown to have negative growth effects for rainbow trout (*Oncorhynchus mykiss*), especially after 8 weeks under experimental conditions, while blue light seemed to be favourable in gilthead seabream (*Sparus aurata*) (Karakatsouli et al., 2007).

Tank substrate colour has also been shown to influence fish growth. For example, the presence of blue and red-brown substrates, in comparison with green or no substrate, was shown to enhance growth in gilthead seabream (*Sparus aurata*; Batzina and Karakatsouli, 2012).

Lastly, tank wall colour has been shown to affect the stress response and growth of a number of fish species. For example, Barcellos et al. (2009) revealed that jundiá (*Rhamdia quelen*), either in the blue or white tanks, presented similar amounts of whole-body cortisol (a glucocorticoid hormone released in response to stress) after exposure to an acute stressor (pursuit with a net for 60 seconds). However, when shelters that provided hiding places for the fish were added to the tanks, the fish kept in the blue tanks had the lowest cortisol concentrations compared to the fish kept in the white tanks after exposure to the acute stressor. Similarly, Nile tilapia (*Oreochromis niloticus*) and summer flounder (*Paralichthys dentatus*) were shown to have reduced cortisol levels when maintained in darker blue and red coloured tanks with the highest levels of cortisol detected in light blue tanks (McLean et al., 2008). The greatest overall weight increases for Nile tilapia and summer flounder were also observed for fish held in the red tanks (McLean et al., 2008). In contrast, Imanpoor and Abdollahi (2011) saw a higher final body mass and a lower stress response for juvenile Caspian kutum (*Rutilus kutum)* reared in yellow tanks, as opposed to red, blue, white or black tanks. Finally, for juvenile barramundi (*Lates calcarifer*), Ullmann et al. (2011) showed that mean fish weight in red and yellow tanks was significantly greater than that of green and blue tanks. Interestingly enough, Ullmann et al. (2011) also looked at fish preference for tank colour using square aquaria divided into four quadrants lined with red, yellow, green, or blue colour. Juvenile barramundi were found to have an inherent preference for the blue and green quadrants.

1.6.3 Tank colour in aquaculture

The majority of studies on the effects of colour on fish have focused on how they influence aggression levels, stress responses and growth. Salmonid compatibility with tank colour, however, has been largely neglected and to my knowledge no study to date has examined tank colour preference in any species of salmon. Although it is possible to have tanks fabricated in any colour, the most popular tank colour for fish in land-based closed containment aquaculture systems in North America is light blue. A total of four tank suppliers and manufacturers were surveyed via telephone interview to determine the most common tank color sold. Fabricators and suppliers included D&T Fiberglass Inc. (Sacramento, CA, USA), PR Aqua Supplies Ltd. (Nanaimo, BC, Canada), AgraMarine Holdings (Powell River, BC, Canada), and Marine Harvest (Canada, Campbell River, BC, Canada). It is unclear why light blue is the most popular colour choice but it likely took place without any considerations of the spectral sensitivities of fish (McLean et al., 2008).

1.7 Vision in fish

Salmon rely almost entirely on vision to detect movements and colour changes associated with prey, predators, and mates (Stradmeyer and Thorpe, 1987; Levine and MacNichol Jr, 1982). Furthermore, photic environments vary widely in marine and freshwater environments, ranging from almost full sunlight at the waters surface to very little light in the deep ocean (Levine and MacNichol Jr, 1982). The spectrum of light available ranges from a broad spectrum of ultraviolet (UV) to the far red at the surface and narrows to blue wavelengths of 460–480 nm in the deep (Levine and MacNichol Jr, 1982). This range in photic environment probably helped to drive the evolution of visual systems in fish.

The vertebrate retina contains both rod and cone cells (Fein and Szuts, 1982). The highly sensitive rod cells are responsible for vision at low light levels and provide monochromatic vision while, cone cells are colour sensitive and are active in bright light conditions (Bowmaker, 1995). The rod and cone cells of fish contain visual pigments composed of a protein (opsin) attached to a chromophore (the aldehyde of vitamin A, retinal) (Bowmaker, 1995). These visual pigments are sensitive to ultraviolet (UV) wavelengths, as well as short, medium, and long wavelengths (Temple et al., 2008). Thus, fish are capable of colour vision and the visual pigments of fish exhibit one of the greatest ranges among vertebrates.

The visual pigments of fish are maximally sensitive to a specific region of the spectrum and can be altered as fish develop and undergo ontogenetic or seasonal migrations to different environments with varying light conditions (Temple et al., 2006, 2008). Coho salmon, for example, shift their spectral sensitivity seasonally by changing their vitamin A1 and vitamin A2 chromophore ratio with A2 increasing during winter and decreasing in summer (Temple et al., 2006). Coho salmon also shift their spectral sensitivity ontogenetically by altering the opsin expression in their medium wavelength-sensitive and long wavelength-sensitive cones (Temple at al., 2008). Based on these findings, coho possess one of the most naturally flexible vertebrate visual pigment systems discovered to date (Temple et al., 2008).

1.8 Measuring colour

Although studies have looked at the effects of environmental light, substrate, and tank colour on fish, no study to date has provided detailed descriptions of the colours used in the experiments or justified the selection of those particular colours. For example, to look at the effects of tank colour on the growth rates and stress responses of juvenile Caspian kutum (*Rutilus kutum)*, Imanpoor and Abdollahi (2011) compared yellow, red, blue, white and black tanks. However, they did not justify their choice of tank colours or provide a detailed description of these colours. One possible way to describe tank colour is to use the HSV model, following Smith (1978), which describes colour in three dimensions: hue, saturation, and value.

Hue defines pure colour in terms of “green”, “red”, and “blue” or mixtures of two pure colours such as “red-blue” (=”purple”). Hue is measured as an angle on the colour wheel in degrees ranging from 0° to 360°. Every degree corresponds to a single color, for example, 0° is red, 45° is a shade of orange and 55° is a shade of yellow.

Saturation describes the intensity of a colour. More saturation equates to more pigment and a brighter more vivid colour. Saturation is measured in % and ranges from 0 to 100%. Thus, 0% represents a shade of grey between black and white and 100% represents an intense colour.

Value is essentially the relative lightness of a colour. Less value equates to a darker colour while more value represents a lighter colour. Value varies from 0% (black; the total absence of transmitted or reflected light) to 100% (white; the total transmission or reflection of light at all visible wavelengths).

A colour is fully specified by listing the three numbers for hue, saturation, and value in order. For example, black would be represented by 0°, 0%, 0%, white would be 0°, 0%, 100%, and dark grey would be 0°, 0%, 45%. The industry standard blue tanks used in the current study measured 200.4°, 22.8%, and 85%.

1.9 Preference testing

Preference tests allow animals to choose between environments or resources and are used to make inferences about animal motivation and welfare (e.g. Hughes and Black, 1973; Fraser, 1985; Tucker et al., 2003). This experimental paradigm has been successfully applied in many species helping determine, for example, the best environmental temperature for sows (Phillips et al., 2000), lighting type and intensity for poultry (Widowski et al., 1992; Davis et al., 1999), type of bedding and flooring (Tucker et al., 2003) or housing system (Legrand et al., 2009) for dairy cows, and nesting material for laboratory mice (Van de Weerd et al., 1997). The simplest experiment of this kind involves giving an animal the choice between two different resources or environmental conditions. The environment or resource that the animal chooses more frequently, consumes a larger amount of, or spends more time in, is taken to be preferred. This technique could prove to be useful for determining fish preferences for tank colours.

Preference tests have been subject to a number of criticisms (e.g. Dawkins, 1983; Duncan, 1992; Fraser and Matthews, 1997; Fraser, 2008). Firstly, preference tests must account for internal and external variables, such as temperature, age of the animal, and time of day, as they have the potential to influence the motivational state of animals (Fraser, 2008). Preference tests should also consider the past experiences of animals because animals may prefer environments they are used to (Fraser, 2008). This can be addressed by controlling the amount of exposure to a certain environment at an early age and the amount of exposure to each option within a preference test (Dawkins, 1983). Preference tests must also use options that an animal has the capacity to distinguish between (Fraser and Matthews, 1997). Lastly, animals do not always make choices that are best for their long-term welfare (Dawkins, 1983). Preference tests must be designed to account for these criticisms and interpreted with caution.

1.10 Objectives and hypotheses

Manufacturing the tanks used in land-based closed containment aquaculture systems in different colours is easy to do and may benefit fish welfare by reducing aggression. However, there appears to be no work investigating whether salmonid species show a preference for a particular tank colour and whether different colours elicit differences in coho salmon aggression.

The first aim of this thesis was to assess coho salmon preferences for tank colour using a preference test. The second aim was to determine the effects of tank colour on aggression (charging and chasing). As previous research had shown beneficial effects of dark tanks (O'Connor et al., 1999; Höglund et al., 2000, 2002; Merighe et al., 2004), we hypothesized that coho salmon would prefer darker tank backgrounds to lighter backgrounds and that darker tanks would reduce aggression.

Chapter 2: Colour matters: coho salmon (*Oncorhynchus kisutch)* prefer and are less aggressive in darker coloured tanks

2.1 Introduction

Fish are capable of colour vision (Cheng and Flamarique, 2004) and can be profoundly affected by the colour of their environment. Certain colours have been shown to affect growth and survival (e.g. Boeuf and Le Bail, 1999; Head and Malison, 2000; Ruchin, 2004; Giri et al., 2002), skin colour (Van der Salm et al., 2004; Imanpoor and Abdollahi, 2011), stress response (Head and Malison, 2000; Volpato and Barreto, 2001; Szisch et al., 2002), and reproduction (Naor et al., 2003; Boulcott et al., 2005). Beyond these physiological consequences, tank colour has also been shown to affect aggression levels (Höglund et al., 2002; Merighe et al., 2004). The effects of tank colour on aggression pose a major welfare concern as aggression is a prevalent side-effect of housing animals in captive groups (Ashley, 2007). As nearly all fish are socially housed, determining which colours are most effective at reducing aggression and the mechanisms involved is therefore central to advancing our understanding of fish welfare.

Previous research has suggested that black backgrounds may reduce aggressive behaviour and social stress over white tanks in pairs of Nile tilapia (*Oreochromis niloticus*; Merighe et al., 2004) and Arctic charr (*S. alpinus*; Höglund et al., 2000, 2002). Aggressive acts between fish in aquaculture systems, such as charging and chasing, are common and may lead to fin and skin damage (Abbott and Dill, 1985; Turnbull et al., 1998, 2005).

Damaged epithelial structures may also provide routes for infection, and reduce survival in fish (Schneider and Nicholson, 1980; Ashley, 2007).

Despite colour having a profound impact on fish behaviour and biological functioning, the compatibility of fish with tank colour has been largely neglected within the aquaculture industry. While it is possible to have tanks manufactured in any colour, in North America, the most popular colour of tank for fish aquaculture is light blue. The origin of this colour selection is unclear but likely took place without any considerations of fish preferences (McLean et al., 2008).

Atlantic salmon (*Salmo salar*) are the most produced species of fish in aquaculture (FAO, 2014) but there is a growing interest in farming other salmonids, including coho salmon, *Oncorhynchus kisutch* (Canada House of Commons, 2013). The standard practice has been to raise post-smolt salmonids in open ocean net-pens (Thorarensen and Farrell, 2011); however, there have been negative environmental impacts associated with this form of production e.g., organic waste release, fish escapes, and disease threats to natural populations (Black, 2001). Several alternative aquaculture methods have been proposed with the most feasible and fastest growing alternative being a land-based, closed containment aquaculture system (Thorarensen and Farrell, 2011). Closed containment aquaculture systems provide a good model to investigate the effects of tank colour on salmon. These systems provide the opportunity to control conditions such as salinity, temperature, ammonia, CO_2, stocking density, and tank colour (Thorarensen and Farrell, 2011; Canada House of Commons, 2013), yet surprisingly little is known regarding the optimal parameters for salmon growth and no work to date has addressed fish welfare under these conditions.

Preference tests allow animals to choose between environments or resources and are used to make inferences about animal motivation and welfare (e.g. Hughes and Black, 1973; Fraser, 1985; Tucker et al., 2003). The simplest experiment of this kind involves giving an animal the choice between two different resources or environmental conditions. The environment or resource that the animal chooses more frequently, consumes a larger amount of, or spends more time in, is taken to be preferred. This technique may be useful for determining fish preferences for tank colour.

Thus, the first aim of the current study was to assess preferences of coho salmon for tank colour using a preference test. Our second aim was to determine the effects of tank colour on aggression (charging and chasing). As previous research had shown beneficial effects of dark tanks (Höglund et al., 2000, 2002; Merighe et al., 2004), we predicted that coho salmon would prefer darker backgrounds and that access to these darker environments would reduce aggression.

2.2 Materials and methods

2.2.1 Ethics statement

This protocol was approved by the University of British Columbia Animal Care Committee (application number: A13-0210). All experimental procedures were performed in accordance with the Canadian Council on Animal Care guidelines on care and use of fish in research. For ethical reasons we used surplus fish from another experiment that were culled for reasons of low body weights.

2.2.2 Animals and housing

One hundred, 2-year-old coho salmon (mixed sex, mean ± s.d. weight 100 ± 5 g) were obtained from a collaborating research laboratory (InSEAS aquatic research facility, Department of Zoology, UBC, Vancouver, BC, Canada) and originally purchased from Target Marine Hatcheries Ltd. (Sechelt, BC, Canada). Although the mean weight of the fish used in this experiment is lower than what has been previously reported in the literature for coho salmon of similar age (e.g. Devlin et al., 1999; Vøllestad and Quinn, 2003) previous work indicates that the social dynamics and dominance hierarchies of fish are re-established when fish are re-grouped (e.g. Buchheim and Hixon, 1992; Huntingford and Garcia de Leaniz, 1997; Webster and Hixon, 2000; Whiteman and Côté, 2003) and thus body weight will not be considered further.

Fish were randomly assigned to groups of ten, each housed in a circular 600 L, 107 cm diameter (0.7m^3), light blue, fiber glass tanks (D&T Fiberglass Inc., Sacramento, CA, USA). The 10 tanks were serviced by a freshwater recirculating system providing mechanical and biological filtration, UV-sterilization, and oxygen injection (components were supplied by Integrated Aqua Systems, Inc., Escondido, CA, USA). During the experiment, mortality, water temperature, dissolved oxygen content, and pH were measured daily and ammonia-N was measured weekly. Water temperature was maintained between 8 and 12°C. The dissolved oxygen content of the water was kept between 70-90%, pH between 6.0 and 6.8 and ammonia-N was less than 0.5 ppt. The average water flow rate was 360 L/min. Tanks were cleaned once a week.

Fish were hand fed with a commercially available pelleted diet (Transfer Plus 3.0MM and 4.0MM, Skretting Canada, Vancouver, BC, Canada) once daily from Monday to Saturday at 1000h; no food was given on Sunday. All experimental populations were housed under a 10-h light; 14-h dark cycle (lights on and off at 0730h and 1700h, respectively). To ensure even light distribution in each tank and to avoid complications due to room lighting, a full spectrum daylight fluorescent lamp (CFL T2 23W Daylight Bulb, Globe Electric, Montreal, QC, Canada) was suspended 1 m above the center of each tank and light intensity was adjusted to 410 lx at water surface.

2.2.3 Experimental procedures

The interior sides and base of each of the 10 light blue experimental tanks were lined with laminated paper (InPrint Graphics & Copying Ltd., Vancouver, BC, Canada) varying in pigmentation: black (0°, 0%, 0%; hue, saturation, value, respectively), white (0°, 0%, 100%), light grey (0°, 0%, 85%), dark grey (0°, 0%, 45%), and a mixed dark grey/black sponge pattern. The industry standard, original blue tank background had a hue of 200.4°, a saturation of 22.8%, and a value of 85% (the same % value as the light grey laminated sheets). Sixteen 1.27cm x 0.32cm magnets (Rare-Earth Circular Magnets, Lee Valley Tools Ltd. and Veritas Tools Inc., Vancouver, BC, Canada) were used to hold the laminated pieces of paper in place in each tank. To habituate fish to each of the conditions, tanks were lined with each background option for a total of 3 days before preference testing began. Order of exposure was set by a replicated 5x5 Latin square.

Blue-trial preference tests of: blue versus white, blue versus light grey, blue versus dark grey, and blue versus black; as well as, black-trial preference tests of: black versus white, black versus light grey, black versus dark grey, and black versus dark grey/black sponge pattern, were conducted in each of the 10 tanks. All tanks were split into two to provide fish a choice of background colour. For example, in one treatment, black and white laminated sheets were used on the two sides of the tank. To avoid an effect of side on preferences, colour placement was alternated across the 10 tanks (e.g. white was on the left and black was on the right in tanks 1, 3, 5, 7, 9; black was on the left and white was on the right in tanks 2, 4, 6, 8, 10). The positions of the fish in each tank were video recorded from above using a Microsoft LifeCam Studio™ webcam (Redmon, WA, USA). Experimental trials began 15 minutes after the camera was placed above the tank to control for disturbances. The fish in each tank were recorded for a total of 10 min, starting at 1100h. Videos were scored using scan sampling at 30-second intervals to determine the number of fish on each side of the tank, yielding 20 observations/trial. The side of the tank that the fish spent most time on determined the 'preferred' side. If the fish straddled the two sides, the recorded position was that where the head was located. Videos were scored continuously for aggressive acts (charging, a rapid dart towards another fish with attempts to bite the other fish, and chasing, rapid pursuit of another fish) through out each 10-minute trial. The number of aggressive acts occurring on each side of the tank was recorded.

2.2.4 Statistical Methods

2.2.4.1 Tank colour preference

To determine side preferences in the blue- and black-trials, we ran one-sample t-tests to see whether the mean number of fish on the preferred side exceeded 5, indicating preference.

2.2.4.1 Aggressive acts

To model aggression, we applied multilevel models, which control for repeated testing of tanks and avoid pseudoreplication, with a Poisson error structure and a log link, which appropriately model the non-normality of count data (Gelman and Hill, 2006; Rabe-Hesketh and Skrondal, 2008; Snijders and Bosker, 2012). Generalized multilevel models assume asymptotic sampling distributions and therefore use z-statistics to test for the significance of fixed effects. For large sample sizes, such as the ones in the models presented here, the consequence of violating this assumption is minimal (Bolger and Laurenceau, 2013).

We modeled aggression on a given side of the tank using tank and trial as random effects and the following fixed effects. Firstly, to test the extent to which aggression on a given side of the tank was determined by how dark the background was, we created a darkness variable such that white was scored as 0 and black was scored as 1. Intermediate colours were assigned scores depending on their brightness value referred to in the methods. Secondly, we created indicator variables (0 or 1) for (i) blue and (ii) pattern backgrounds to account for effects of blue and pattern backgrounds beyond how dark they were. Thirdly, to test the extent to which aggression on a given side of the tank was determined by how dark the *opposite (non-preferred)* side of the tank was, we included the darkness score of the opposite side of the tank as an additional variable. Finally, as densities were highest on the preferred side and thus created a confound for modeling aggression on a given side of the tank, we controlled for average fish density (preference) on that side of the tank.

To model overall aggression within a trial, we specified tank as a random effect and predicted total aggression with (i) whether the test was a blue-trial or a black-trial and (ii) whether the comparison colour was white, light grey, or dark grey.

For all data processing, modeling, and plotting, we used R v. 3.1.0 (R Core Team, 2014) and the following packages: plyr (Wickham, 2011), reshape (Wickham, 2007), and lme4 (Bates et al., 2014).

2.3 Results

2.3.1 Tank colour preference

The fish showed a strong preference for darker backgrounds. Pair-wise comparisons for the blue-trials revealed that fish did not prefer blue over white (df = 9, t = 0.47, p > .6), but tended to prefer light grey over blue (df = 9, t = 2.22, p < .1), and preferred dark grey and black over blue (df = 9, t = 5.63, p < .001, and df = 9, t = 13.06, p < .0001, respectively; Fig. 2.1a). In the black-trials, the fish showed a strong preference for the black background over all other options (pair-wise comparisons: df's = 9; all t's > 11, p < .0001, except pattern: t = 3.66, p < .01; Fig. 2.1b). In fact, all 10 tanks in all trials showed a unanimous preference for the black background for all options other than dark patterned background.

2.3.2 Aggressive Acts

As dark colours were preferred, fish densities were highest on the darker side of the tank, so the effect of colour on aggression was tested controlling for density. After controlling for the effects of fish density, our results provide the first evidence that both background colours affected the total amount of aggression behaviour observed within a specific tank. Specifically, darker backgrounds not only decreased aggression on the focal-side, but also decreased aggression on the comparison-side as well (effect of focal side darkness: $z = 4.27$, $p < .0001$; effect of comparison-side-darkness: $z = 13.50$, $p < .0001$; Fig. 2.2). Thus, conditions on the preferred side also affected behaviour on the non-preferred side. For example, Figure 2.2 shows that when 4 of the 10 fish were on the blue side of the tank (fish density of 4), aggression on the blue side was much lower when it was paired with black than when it was paired with white. Collectively across all trials, this phenomenon resulted in lower overall rates of aggressive behaviours in the black trials compared to the blue trials ($z = 18.57$, $p < .0001$; Fig. 2.3).

2.4 Discussion

Our assessment of preference was based on a disproportionate number of fish occupying one side of the test tank. The fish in our study showed a clear preference for black backgrounds over all other colour options and for darker colours in general.

The preference for darker colours may be explained, in part, by the feeding habits of coho salmon. Salmon rely almost entirely on vision to detect their prey, which are mostly drifting, light-coloured invertebrates (Keenleyside, 1962; Stradmeyer and Thorpe, 1987). It is likely that these prey are easier to detect against a dark background (Naas et al*.,* 1996).

Additionally, bright environments, especially those without cover, may induce a stress response (Volpato et al., 2001). Fish tend to seek and hide in dark environments when anxious (Chaouloff et al., 1997; Steenbergen et al., 2011), likely as a form of antipredator behaviour (Fraser et al., 1993). Thus, fish preference for black tanks may also be related to protective, escape behaviours.

Salmonids switch from diurnal to nocturnal foraging when water temperatures drop below about 10 °C (Fraser and Metcalfe, 1997), such that fish take shelter in crevices during the day (Rimmer et al., 1983) and emerge to feed at night (Fraser et al., 1993; Fraser and Metcalfe, 1997). Water temperatures for this experiment ranged from 8-12°C; thus, it is possible that the preference for darker tanks is related to a change in foraging behaviour and might be even stronger at colder temperatures.

In our study, darker backgrounds were also shown to reduce fish aggression levels, regardless of where the aggression took place (on the darker side or on the lighter side). Salmonids are naturally territorial, and aggressive behaviours (charging and chasing) are known to increase with increasing density (Ashley, 2007). Aggression is thus of particular concern in closed containment aquaculture systems (Marchesan et al., 2005). Determining the factors associated with aggression may have great potential to improve the welfare of fish reared in these systems.

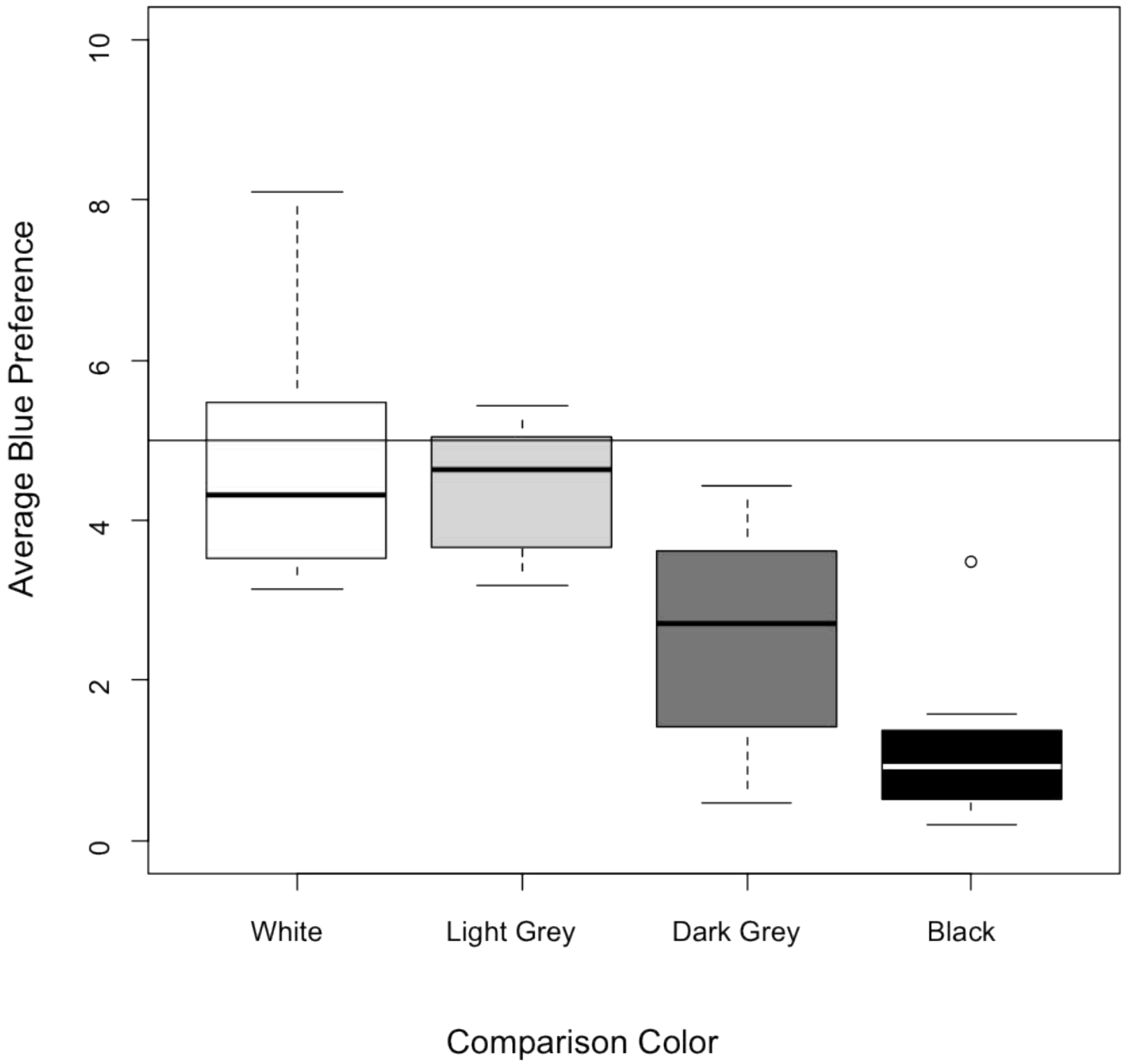
Salmonids, like other teleost fish, have the ability to adjust the colour of their skin in response to changes in background colour (Baker et al., 1981; Fujimoto et al., 1991; O'Connor et al*.,* 1999). In salmon, darkening of the skin and eyes signals social subordination, while lightening of the skin signals dominance (Keenleyside and Yamamoto, 1962; Abbott et al*.,* 1985; O'Connor et al*.,* 1999). Thus, a darker fish may represent less of a threat and elicit less aggression than a conspecific displaying paler body coloration. A study conducted by Höglund et al. (2002), comparing pairs of Arctic charr (*Salvelinus alpinus)*, showed that fish interacting on a white background displayed initial pale colouration and high levels of aggressive behaviour while fish interacting on a black background, displayed initial dark colouration and a lower frequency of aggressive interactions. While some of the fish on the white background became subordinate and took on a darker body colouration, the subordinate fish on the black background did not show any additional darkening. These findings may explain why darker backgrounds in our study not only decreased aggression on the focal side, but also decreased aggression on the comparison-side of the tank. Black backgrounds may have caused the fish within the tanks to take on darker body colourations, reducing overall aggression throughout the tank.

Preference tests allow animals to express their priorities, giving us insight into what is important to them (Fraser and Matthews, 1997); they are widely used in the welfare literature, for example, to assess flooring temperatures for sows (Phillips et al., 2000), lighting type and intensity for poultry (Widowski et al., 1992; Davis et al., 1999), bedding for dairy cows (Tucker et al., 2003), and nesting material for laboratory mice (Van de Weerd et al., 1997). However, the results of preference tests vary with context (Fraser, 2008) including the animal's previous experiences (Dawkins, 1977). Moreover, animals do not always make choices that are best for their long-term health and welfare (Duncan, 1978). Results from preference tests must therefore be interpreted with much care. For example, the data from this study could have led to conflicting interpretations. The fish preferred the darker sides, but were also observed to engage in more aggressive acts on those sides. However, the higher frequency of aggressive interactions observed on the black sides was explained entirely by the greater fish densities. Moreover, accounting for the higher densities revealed the reverse pattern: darker colours actually decreased aggression. Thus, once we controlled for differences in fish density, our results clearly showed that the presence of dark backgrounds decreased aggression on both the focal and comparison-side of the tanks causing an overall *decrease* in aggression and thus, improved fish welfare.

2.5 Conclusions

Coho salmon prefer dark tanks, and when these fish are housed with access to black backgrounds they show reduced agonistic behaviour. The growing interest in closed containment aquaculture means that there is increasing need to understand the effects of tank colour on fish welfare and behaviour.

(a)


Average Blue Preference
0
2
4
6
8
10
White
Light Grey
Dark Grey
Black
Comparison Color

(b)

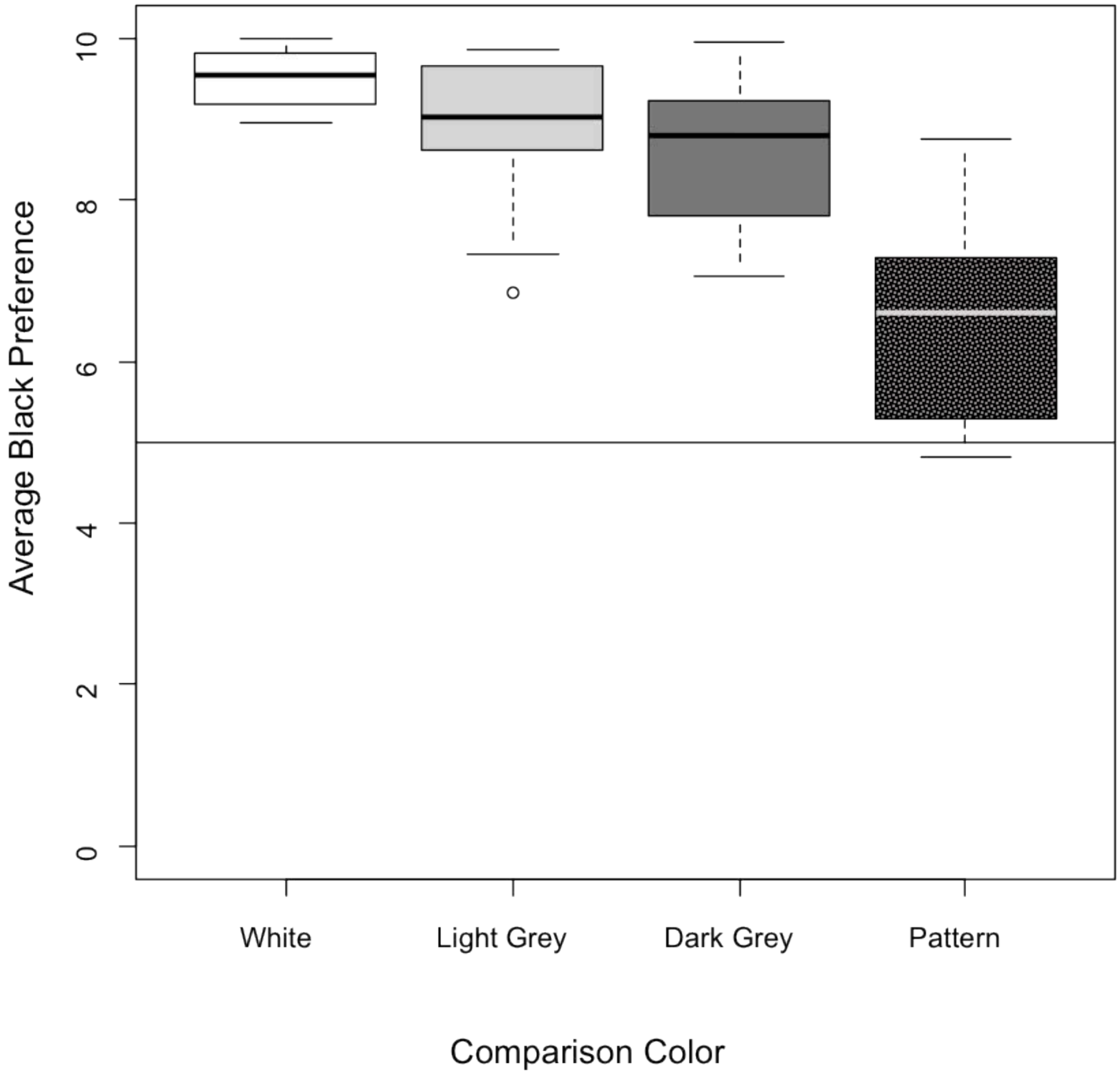


Figure 2.1: Box plots of average number of fish located on each side of the tank (per 10-minute trial) for (a) blue-comparison preference trials and (b) black-comparison preference trials. Each tank contained 10 fish.

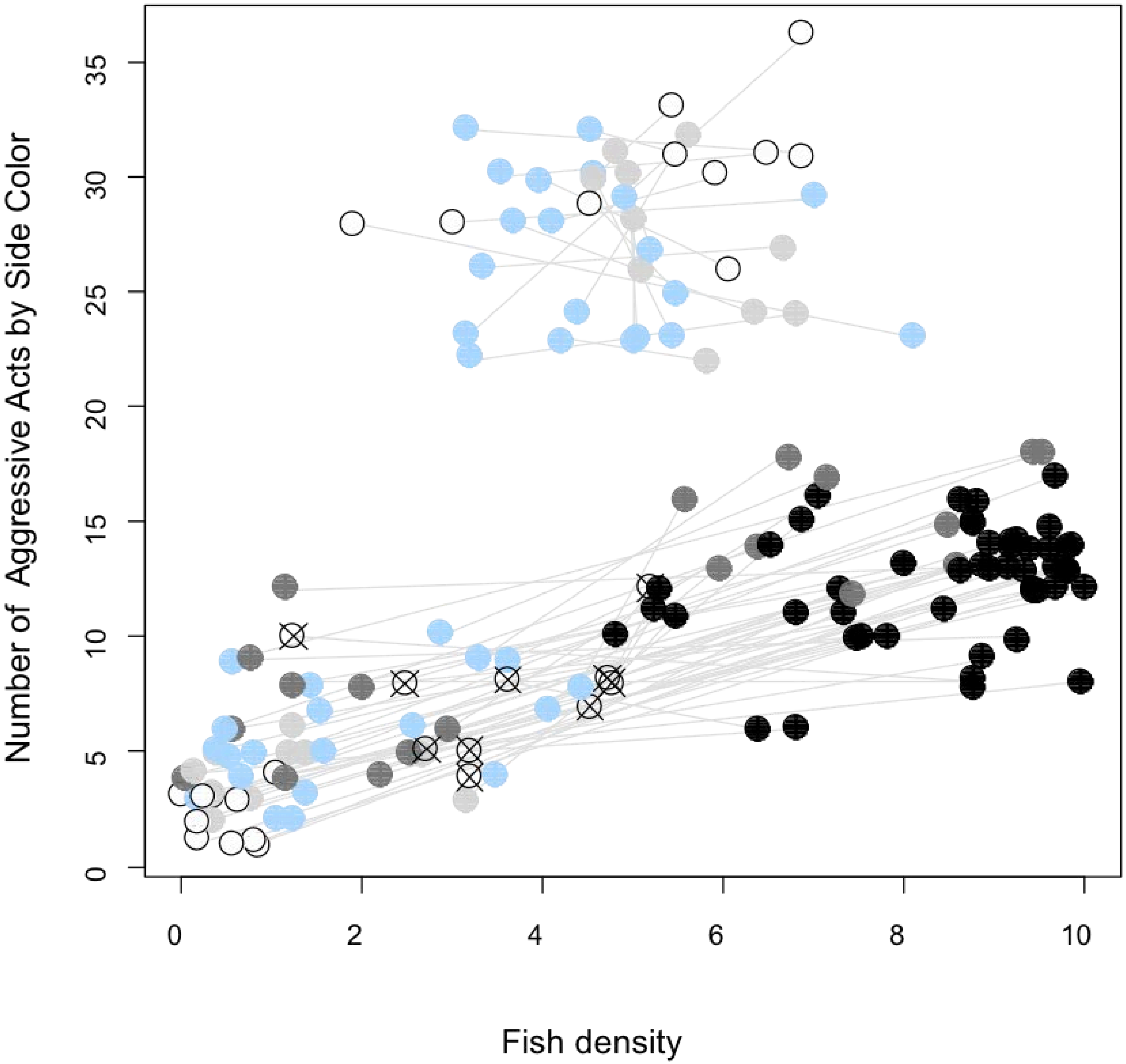


Figure 2.2: Total number of aggressive acts occurring on each side of the tank (per 10-minute trial) in relation to fish density (n = 10) for blue- and black-comparison trials. Fill of dots corresponds to tank side colour and dots with X represent the dark grey/black pattern sides. Grey lines connect the two sides of a tank in a single trial.

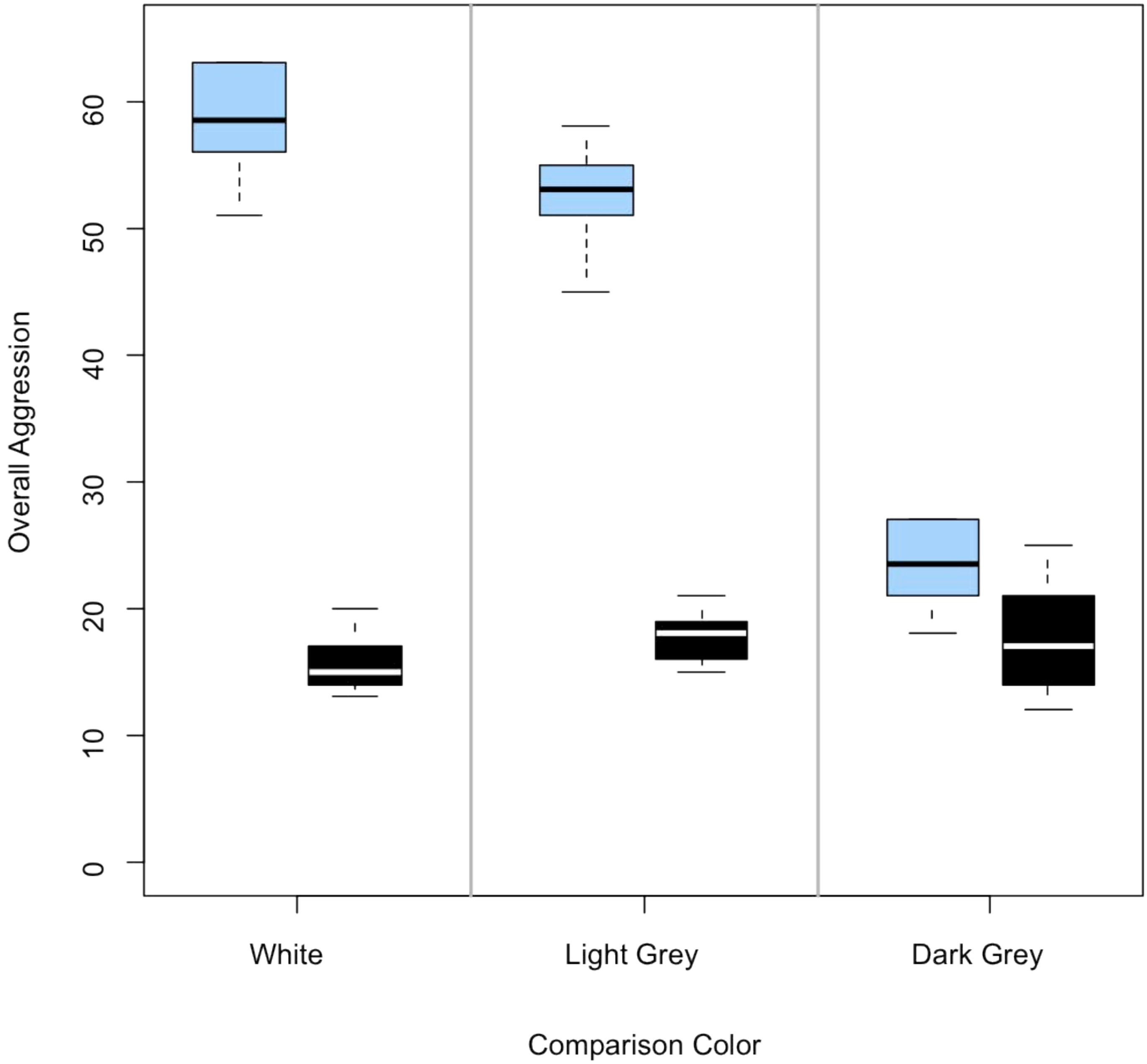


Figure 2.3: Total number of aggressive acts occurring on the blue and black sides of the tanks for white, light grey, and dark grey comparisons (n = 60; 6 trials, 10 tanks).

Chapter 3: General discussion and conclusions

3.1 Contributions and implications

Although the welfare of farmed fish is receiving increased attention in the literature (Ashley, 2007), the majority of studies on fish welfare have focused on measures of biological functioning (e.g. Schreck et al., 2001; Kristiansen et al., 2004; Poli et al., 2005). However, good animal welfare goes beyond just physical health; it also involves the ability of an animal to express natural behaviours and a lack of mental suffering from negative emotional states, such as pain and fear. Unfortunately, scientific research addressing these aspects of fish welfare is lagging behind studies of biological functioning.

Colour can affect the biological functioning of certain fish species, influencing growth rates, stress responses, and aggression. For example, the use of different coloured lights held above fish tanks and substrates within tanks have been shown to affect cortisol levels and growth rates of several species, including scaled common carp (*Cyprinus carpio;* Karakatsouli et al., 2010), Nile tilapia (*Oreochromis niloticus*; Volpato and Barreto, 2001; Luchiari and Pirhonen, 2009), rainbow trout (*Oncorhynchus mykiss*; Karakatsouli et al., 2007), and gilthead seabream (*Sparus aurata*; Karakatsouli et al., 2007; Batzina and Karakatsouli, 2012). Tank wall colour has been shown to affect stress responses and growth rates in jundiá (*Cachoeira Jundiá;* Barcellos et al., 2009), Nile tilapia (*Oreochromis niloticus*; McLean et al., 2008), summer flounder (*Paralichthys dentatus*; McLean et al., 2008), and juvenile Caspian kutum (*Rtilus frisii Kutum;* Ullmann et al., 2011). Tank wall colour has also been shown to affect fish aggression. For example, black tank backgrounds decreased aggressive behaviour and social stress in pairs of Arctic charr (*Salvelinus alpinus*;

Höglund et al., 2000) and Nile tilapia (*Oreochromis niloticus*; Merighe et al., 2004). No study to date, however, has looked at tank colour preference and the effects of tank colour on aggression in any species of salmon.

Salmonids are the most produced species of fish in aquaculture (FAO, 2014) and there is growing interest in cultivating post-smolt coho salmon in land-based, closed containment aquaculture systems (Canada House of Commons, 2013; Thorarensen and Farrell, 2011). Aggression is a major concern in closed containment aquaculture and is often the primary cause of fin and skin damage in fish (Ashley, 2007). Fin and skin damage have the potential to cause inflammation and pain, and may lead to infection, reduced survival in salmon, and a decrease in aquaculture production and product quality (Schneider and Nicholson, 1980; Ashley, 2007; Stien et al., 2013; Abbott and Dill, 1985; Turnbull et al., 1998, 2005). Closed containment aquaculture systems provide a good model to examine the effects of tank colour on salmon. The aims of the current study were to assess coho salmon preference for tank colour and determine the effect of tank colour on aggression levels. We predicted that darker tank backgrounds would be preferred by coho salmon and would reduce aggression.

The results from this study support our hypotheses. This thesis presents the first evidence that salmonids have a strong preference for darker tank backgrounds to lighter backgrounds and that darker backgrounds reduce aggression levels. This thesis shows that tank colour matters to fish and can alter social behaviour in the immediate vicinity, as well as in an adjacent area. Specifically, darker backgrounds not only decreased fish aggression on the focal-side, but also decreased aggression on the comparison-side. My work provides evidence that darker tanks improve the welfare of coho salmon living in closed containment aquaculture systems and contributes to the basic literature on fish welfare. Considering the growing interest in closed containment aquaculture, the results of this thesis have the potential to improve the welfare of countless numbers of fish.

3.2 Limitations and future research

Although outcomes of this study provide novel contributions to the literature on colour preference and how colours present in the immediate environment affect aggressive behaviour, there were some limitations that should be addressed in future research. One limitation was that the fish used in this study were kept under a relatively narrow range of environmental conditions. Preference tests depend on context, thus external and internal variables such as, temperature, age of the animal, or time of the year, must be considered (Fraser, 2008). Preferences may also fluctuate in response to changing variables; for example, pigs prefer to rest on straw in the morning when it is cold, but not in the evening when it is warmer (Steiger et al., 1979). The closed containment aquaculture system that I had access to supplied recirculating water to the ten, 0.7m^3 tanks used in the study and to ten larger, 5.6m^3 tanks, involved in a different study. Therefore, the water quality conditions of our tanks were interconnected. Another experiment investigated the effects of varying water salinity levels and temperatures on the growth of coho salmon and required that the salinity and temperature be maintained at 0ppt and between 8 and 12°C, respectively, throughout all tanks. In the case of wild coho salmon, the majority of their first year of life is spent living in freshwater streams, after which they migrate to the ocean, undergoing a metamorphic process called smoltification (Sandercock, 1991). Thus, the salinity of water ranges from 0ppt (freshwater) to 35ppt (saltwater) and temperature ranges from 0°C to 25°C during the lifecycle of a wild coho salmon (Sandercock, 1991). Considering the visual pigments of coho salmon are maximally sensitive to a specific region of the spectrum and can be altered as fish develop and undergo migration (Cheng and Flamarique, 2004; Temple et al., 2006, 2008), changes in water salinity or temperature may affect how coho salmon react to different tank colours. I suggest that future work look at how colour affects aggression levels and colour preferences in coho salmon held in differing water salinity and temperature levels. Closed containment aquaculture systems would serve as an ideal model for this investigation as they offer complete control over tank colour, water salinity, and water temperature.

Unfortunately the nature of the experimental paradigm used in the present study was only able to determine preference for specific tank colour and not motivation. In other words, to make a choice in my experiment, the fish simply swam to their preferred side of the tank; there was little indication of the importance of this choice to the individual fish. My prediction, however, is that fish were highly motivated to spend time on the darker side of the tank; for example, I observed fish trying to lift up the black laminated pieces of poster board held against the tank walls in order to hide underneath them. In some cases, fish pushed the magnets away from the edges of the poster board to swim underneath. Unfortunately, I was not able to reliably score these interactions, as they cannot be seen using video. In the future, it would be beneficial to assess the strength of coho salmon preference for darker tank backgrounds using a motivational test. A recent study by Galhardo et al. (2011) adapted a push-door to quantify motivation in a cichlid fish, the Mozambique tilapia (*Oreochromis mossambicus*). The males of this species have strong snouts and are thus suited to push. The fish were required to work the door (push/touch) at an ascending cost in order to have access to food and social partners. The measurements of motivation included latency to open the door, work attention and maximum price paid. From my own observations, I predict that coho salmon could also be trained to push objects. Thus, this study design could be used to assess the motivation of coho salmon to gain access to different coloured tank environments.

Another limitation was that it was difficult to identify the actions of individual fish on video. I was unable to determine whether some fish were initiating more aggressive acts than others or whether certain fish had stronger or weaker preferences for the various tank colours. It would thus be useful to attach tags to the caudal fins of fish to better track the movements of individuals. Höglund et al. (2002) used caudal fin tags successfully in an experiment with Arctic charr (*Salvelinus alpinus*) to differentiate between the aggressive acts of individuals interacting on white or black backgrounds. Similarly, the use of tags could help determine whether coho salmon show individual variation and whether this has an affect on aggression or colour preference in closed containment aquaculture systems.

Compared with previous research on the effect of tank colour on aggression in pairs Nile tilapia (*Oreochromis niloticus*; Merighe et al., 2004) and Arctic charr (*S. alpinus*; Höglund et al., 2002), I used relatively large groups of fish (10 fish per tank) and tank sizes ($0.7m^3$) for the behavioural observations of aggression in my experiment. The stocking density of my tanks, measured using the weight of fish per unit volume of the tanks (expressed as kg fish m^{-3}), was approximately 14 kg m^{-3} (compared to approximately 3 kg m^{-3} used in the other studies). However, the number of fish and the size of each tank in my study was considerably smaller than what is normally used in commercial salmonid aquaculture farms, where stocking densities in excess of 80 kg m^{-3} are common (Thorarensen and Farrell, 2011). Salmonids are naturally territorial, and aggressive behaviour is known to increase with increasing density (Ellis et al., 2002; Turnbull et al., 2005). Thus, I predict that tank colour would have an even greater impact on aggression levels in these larger, more densely stocked tanks. There is a considerable amount of evidence linking high stocking density with decreased fish welfare in rainbow trout (*O. mykiss*). For example, high density has been shown to cause decreased growth and reductions in food intake (Refstie, 1977; Holm et al., 1990; Boujard et al., 2002), increased fin erosion and skin damage (Ellis et al., 2002), a reduced immune capacity (Ellis et al., 2002), and differences in swimming behaviours (Anras and Lagardere, 2004). Given the known effects of higher stocking densities on fish aggression, I recommend that future work on the effect of tank colour on salmonid aggression be conducted at higher densities more similar to those found on commercial farms.

Although the results of my thesis show that black tanks are preferred and decrease overall tank aggression levels in coho living in closed containment systems, I was not able to assess growth in relation to tank colour. Fish growth rates and product quality are two of the most important issues for fish farmers (Thorarensen and Farrell, 2011). Therefore, testing the effect of tank colour on the growth and incidence of fin and skin damage in coho salmon reared in commercial scale closed containment aquaculture systems would be a logical next step. To test this, juvenile fish could be reared until adulthood in either blue (industry standard blue) or black, fully lined tanks. At the end of the rearing period, all fish would be slaughtered. Individuals would then be weighed and externally examined for amounts and severity of skin and fin damages. The tank colour associated with the highest fish weights (highest fish growth rates) and lowest amounts of fin and skin damages would represent the best tank colour for the aquaculture industry. Given my own findings, I predict that black tanks would result in less aggression overall, leading to lower amounts of fin and skin damage.

3.3 Conclusions

The results from my study show that black tanks are the preferred tank background colour for coho salmon. Black tanks were also associated with an overall lower number of aggressive acts than the industry standard, blue tanks. I recommend the adoption of black tanks for coho aquaculture in closed containment systems. The emerging interest for on-land, closed containment systems opens up an opportunity toward application of the suggested tank colour changes. It is encouraging that a simple change of fish rearing environment (such as the colour of tank used) may have multiple beneficial aspects for fish and producers. A small change in aquaculture procedures can reduce aggression levels in reared fish and hence improve fish welfare and likely also economic return.

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