ASSESSING THE WELFARE OF ADULT ATLANTIC SALMON, *Salmo salar* DURING COMMERCIAL LIVE-HAUL TRANSPORT

by

STEPHEN TANG

B.Sc., Simon Fraser university, 2003

A thesis submitted in partial fulfillment of
the requirements for the degree of

Master of Science

in

The Faculty of Graduate Studies

(Zoology)

The University of British Columbia
Vancouver

February 2008

Abstract

I used physiological stress as in indicator of welfare of adult Atlantic salmon during transport onboard a commercial live-haul vessel, the *Sterling Carrier* under actual operational conditions. This state-of-the-art vessel incorporates both flow-thru (open-hold) and re-circulating (closed-hold) live-hold configurations to safely transport fish under diverse environmental conditions.

Measurements of bulk oxygen uptake rates (bulk $\dot{M}O_2$) for fish masses ranging from 20 to 40 tons during open-hold transports (n=89) revealed a slightly elevated bulk $\dot{M}O_2$ that was comparable to routine bulk $\dot{M}O_2$ measured in adult Atlantic salmon, *Salmo salar* held in large tanks and also to resting $\dot{M}O_2$ of individual Pacific salmonids measured in swim-respirometers. These results indicate a low level of stress, and suggest that open-hold live-haul transport aboard the *Sterling Carrier* does not compromise fish welfare.

While closed-hold transport protects fish from poor environments, water quality conditions progressively deteriorate as respiratory CO_2 accumulates in the water. I measured water CO_2 and pH changes during closed-hold transport experiments and used these data to model CO_2 and pH changes over a wide range of transport conditions. Model outputs demonstrated that the partial pressure of CO_2 (Pco_2) could accumulate to potentially deleterious levels (>10 torr) in 20-158 min depending on fish stress levels and loading densities. These data may be useful in estimating transport lengths possible under Pco_2 thresholds, which are presently lacking for live-haul transport.

The effects of 3-h and 24-h exposures to elevated water Pco_2 (hypercarbia) on blood pH and post-mortem flesh quality were also measured in adult Atlantic salmon. While elevated water Pco_2 disturbed blood pH as predicted, there were minimal effects on flesh quality based on

rigor mortis and flesh pH assessments, which were further reduced if fish were allowed to recover for 24 h after a hypercarbic exposure.

This study provides novel insights into a) current techniques of assessing fish welfare during live-haul transport, b) limitations associated with transporting fish under re-circulating conditions, and c) effects of elevated Pco_2 on flesh quality indicators in adult Atlantic salmon.

Table of Contents

List of Tables

List of Figures

Acknowledgements

This thesis would not have been possible without the guidance and support of many people. First and foremost, I must thank my supervisor, Dr. Tony Farrell for providing the extraordinary knowledge, constant motivation and inexhaustible patience required to see me across the finish line. The other members of my committee, Dr. Colin Brauner and Dr. Chris Wood deserve praise for their contributions as does Dr. Helgi Thorarensen for his pioneering work aboard the *Sterling Carrier*.

Many thanks to the members of the Farrell lab, past and present, for helping me to solve the never-ending mysteries of scientific research and writing. Special thanks to Miki, my partner in crime, Linda, without whom nothing would ever get done, and Erika, without whom nothing fun would ever get done. Thanks also to the Brauner lab for their support, especially Dan and Jodie for their CO_2 brains.

I also had enormous help from our industry partners, Marine Harvest and Batchelor Bay Management. Captain Jeff Turpin and the crew of the *Sterling Carrier* deserve special recognition for their knowledge and generosity that allowed this project to overcome many major obstacles.

I'd also like to thank my family for their support, especially May who's expertise in statistics was invaluable to the $\dot{M}O_2$ data analysis.

And finally, thank you Tasia, for your understanding and daily inspiration.

Co-authorship statement

All fieldwork, experiments and analysis were completed by me, Stephen Tang. The following co-authors are listed on the manuscripts: A.P. Farrell, C.J. Brauner, and H. Thorarensen. The co-authors and C.W. Wood were involved in the design, analysis of data and preparation of the literature review and manuscripts included in this thesis.

Chapter 1: Introduction and literature review

Fish Welfare

Welfare represents an animal's quality of life, which cannot be assessed by objective measures alone. A comprehensive assessment of welfare requires numerous subjective criteria, making any purely objective assessment impossible. However, by quantifying factors that contribute to welfare, we can generate objective evidence regarding the welfare of animals. During the live transport of fish in the aquaculture industry, conditions exist that can negatively affect the welfare of fish. The consequences of reduced welfare in agricultural animals can have implications for both economic and social aspects in industries exploiting animals for human consumption.

In this thesis I examine the welfare of adult Atlantic salmon, *Salmo salar* being live-hauled between sea-cages and processing plants in British Columbia, Canada. Fish welfare is assessed using objective measures of stress and their effects on the welfare of salmon during various conditions during transport are discussed.

Welfare in aquaculture

Global expansion in the production and consumption of aquaculture products (FAO, 2006) has resulted in increased social awareness regarding the welfare of cultured fish. Pressure to consider the welfare of fish is reinforced by public perception and scientific research suggesting that fish are sentient organisms (Chandroo et al., 2004; Huntingford et al., 2006). Fraser et al. (1997) proposes three qualifiers to good animal welfare: that animals should be able to lead natural lives; that they should live free of prolonged fear, stress and pain; and that they should not be forced to function beyond normal physiological or behavioral ranges. Standard

welfare requirements for fish raised in aquaculture environments vary widely among producers, but research to improve welfare conditions can result in widespread benefits especially when implemented during the emergent phase of an industry.

Current techniques for welfare assessment are primarily designed for use in mammalian or avian agriculture and are poorly suited for the assessment of fish welfare. Duncan and Fraser (1997) divided animal welfare assessments into three different approaches: feelings-based approaches that assess animal welfare through the psychological and emotional experiences of animals, nature-based approaches that assess animal welfare by comparing the captive lives of animals to the natural environments and their wild counterparts, and functioning-based approaches that assess animal welfare in terms of the physiological, biochemical and behavioral functioning of animals. A recent review of the subject advocated a 'feelings-based' approach to understanding the issue of fish welfare, arguing that in nature, physiological stress did not always constitute negative welfare and this approach would best represent the moral interest in the issue (Huntingford et al., 2006; Huntingford et al., 2007). Chandroo et al. (2004) reviewed anatomical, physiological and behavioral evidence for sentience in fish and concluded that fish do experience pain, fear and stress and that their capacity for suffering is more likely to exist than not. Other research has shown that fish can learn from negative experiences and perhaps exhibit fear in response to perceived stressors. Fish such as the pike, *Esox lucius* learned to avoid spinner baits after being hooked only a single time (Beukema, 1970), and avoidance conditioning to electric shocks has been documented in paradise fish, *Macropodus opercularis* (Topál and Csányi, 1999).

Nevertheless, the mental suffering and consciousness in fish are still considered poorly understood concepts, and fish welfare is better served by using assessments based on scientifically observable biochemical, physiological and behavioral indicators of health

(Arlinghaus et al., 2007). In this study, we determined that non-natural conditions during transport necessitated the use of a 'functioning-based' assessment of welfare based on the physiological responses of fish to the stressors during transport.

Under intensive fish culture conditions, the natural freedom of animals is necessarily restricted, and many procedures are known to induce significant stress in fish, altering various aspects of their biological functioning. Included are: water quality (Liao, 1971; Fivelstad et al., 1999a); stocking density (Jorgensen et al., 1993; Lefrancois et al., 2001; Ellis et al., 2002) crowding and handling (Barton et al., 1980; Barton et al., 1987; Barton, 2000) and transport (Barton et al., 1980; Carmichael, 1984, Barton, 2000). These have been shown to cause significant stress in fish. The welfare of intensively cultured fish represents a compromise between the practicality of aquaculture operations and the benefits of optimal welfare.

The stress response in fish

Fish respond to stress with numerous alterations to their physiology and behaviour. The physiological disturbances due to stress are some of the most comprehensively studied topics in fish physiology (McDonald and Milligan, 1997; Barton, 2002; Davis, 2006) and reviews of the subject are plentiful (see for example: Barton and Iwama, 1991; Bonga, 1997; McDonald and Milligan, 1997). The effects of acute stress on fish physiology can be described as primary, secondary or tertiary stress responses depending on whether they interact on hormone, system or organism levels.

The primary stress response involves the activation of adrenergic and endocrine signaling, releasing catecholamines (adrenaline/noradrenaline) and corticosteroids (cortisol) into the bloodstream via the hypothalamic-pituitary-interrenal (HPI) axis. Adrenaline and cortisol act in combination to initiate secondary responses on physiological processes, including increases in glycogenolysis, blood flow, ventilation rates, gill perfusion and spontaneous muscle activity

(Bonga, 1997; Barton, 2002). Tertiary stress responses are observed at the organism level and include changes in overall metabolic rates and performance indicators such as swimming performance (Wedemeyer and McLeay, 1981; McDonald and Milligan, 1997). The degree of the primary, secondary and tertiary responses to stress are typically proportional to the duration and magnitude of the stressor involved (Barton et al., 1980; Pickering and Pottinger, 1989), and primary and secondary responses may be cumulative with multiple stressors (Barton et al., 1986). These physiological and behavioral changes prepare fish for responding to non-specific stressors by increasing energy transport and mobilization and redistributing energy away from non-essential processes. One common aspect relating all levels of the stress response is the increase in metabolic activity associated with the increases in the hormone synthesis, oxygen and chemical distribution by blood, and rates of spontaneous activity. These metabolic costs are clearly manifested through increases in the oxygen consumption rate ($\dot{M}O_2$), ventilation rate and cardiac output during the stress response (Randall and Perry, 1992). The metabolic demand associated with stress cannot always be compensated through the effects of the stress response, and post-stress energy debt may occur following severe stress. As this energy debt is recovered, metabolic activity and oxygen consumption rates decline, but can remain elevated above the routine rate for extended periods of time (1-4 h; Brett, 1965; Scarabello et al., 1992; Lee et al., 2003a). Therefore acute stressors that are transient in nature can result in lasting physiological effects.

Under chronically stressed conditions, short-term adaptive responses become detrimental to the health and welfare of fish. Decreased growth rates (Barton et al., 1987), disease resistance (Pickering and Pottinger, 1989) and swimming performance (Pickering and Pottinger, 1989) have all been shown to occur with chronic stress. Captive fish are more likely to experience chronic stress than their wild counterparts, and the negative effects on growth rate and disease

resistance can be disastrous for commercial fish production. Multiple stressors can overwhelm compensatory mechanisms, especially if stressors have synergistic effects (when the effective result of two or more concurrent stressors is greater than the sum of the individual results).

In salmon aquaculture, the physiological cost of sub-lethal levels of stress amount to reduced welfare for fish and increased economic costs for operations through decreased growth rates, feed efficiency, disease resistance and potential impacts on product quality. Assessing the impacts of live-haul transport on fish welfare will aid in optimizing the process and potentially improve the overall welfare of cultured salmon.

Transport Welfare

Live-haul transport

In 2006, over 78,000 tonnes of salmon representing a value of over $400,000,000 CDN were raised and transported in British Columbia, yet few assessments of fish welfare during live-haul transport have been done. Live-haul transport has the potential to significantly impact fish welfare due to the multiple stressors involved. Crowding and handling, high loading densities and poor water quality can all cause stress and reduce fish welfare. The amount of stress fish experience during transport depends on many factors, including the layout of the transport vessel, water chemistry, and the species of fish (McDonald et al., 1993; Wedemeyer, 1996).

Advances in live-haul transport technology have resulted in the design of well-boats that allow continuous flushing of external water through the holds in a flow-through (open-hold) system. This allows the constant introduction of oxygenated water and continual removal of metabolic wastes from the hauling water, but connects live-wells to a dynamic external environment. Fish transported this way risk exposure to poor water quality in the environment (i.e. a large algal bloom or oil spill) which can impact fish health and welfare accordingly.

These well-boats can therefore also seal the valves connecting live-holds to the external water and transport fish in a more traditional re-circulating (closed-hold) system. Under these conditions, fish are protected from the external environment, but depletion of oxygen and build-up of metabolic excretory products such as CO_2 and ammonia in the hauling water can reduce water quality and welfare conditions for fish.

The benefits of increasing welfare by minimizing stress during transport are seen in increased survival rates both during and post-transport (Specker and Schreck, 1980; Schreck et al., 1989), reduction in the time required for resumption of normal behaviors (McDonald et al., 1993) and improved flesh quality characteristics (Berg et al., 1997; Erikson et al., 1997; Jittinandana et al., 2005).

Water quality

Regardless of the type of transport used, water quality has the greatest impact on fish welfare. In open-hold transport, external water flushed through the holds makes water quality dependent on external conditions, combined with the rate of flushing. In closed-hold transport the impacts of excretory products generated by fish metabolism determine water quality. Under both scenarios the greatest risk to welfare is the depletion of oxygen and buildup of CO_2 and ammonia. Low oxygen conditions (hypoxia) result in significant stress for fish (Perry and Reid, 1992), causing metabolic acidosis (Boutilier et al., 1988), impaired cardiac function (Steffensen, 1998), and impaired swimming performance (Farrell et al., 1998). Maintaining oxygen concentrations above hypoxic levels is the principal concern of live-haul operators, and aeration systems are an integral part of virtually every transport vehicle or vessel. Water quality and hence fish welfare can also be negatively impacted by both CO_2 and ammonia, topics which will be discussed in subsequent sections.

Loading density

Loading density affects fish directly through crowding (Mazeaud et al., 1977; Barton et al., 1980; Pickering and Pottinger, 1989; Pavlidis et al., 2003) and indirectly through density-dependent changes on water quality (Pickering and Stewart, 1984, Kebus et al., 1992). High loading densities may result in excessive contact between fish, increasing activity levels and causing physical damage. In addition, mucus and scale loss can severely reduce disease resistance and increase susceptibility to secondary infections. Social dominance behaviour between fish may be intensified under high densities and result in physical injuries and stress (Sloman et al., 2001).

The optimal transport density is difficult to predict, as the effects of transport variables such as water quality, the design of the transport vessel and the degree of interaction between fish may vary with loading density. Different species of fish show different levels of tolerance to high densities. Salmonids are typically more susceptible to transport stress due to their pelagic, predatory life-styles, yet numerous studies have documented successful high density transports of salmonid species. I observed the live-haul transport of adult Atlantic salmon, *Salmo salar*, at densities exceeding 150 kg m^{-3}, and Ostenfeld (1995) observed the 10.5 h truck transport of rainbow trout, *Oncorhynchus mykiss*, carried at densities of up to 174 kg m^{-3} without mortality. McDonald et al. (1993) measured transport stress in lake trout, *Salvelinus namaycush,* brook trout, *S. fontinalis* and splake, *S. fontinalis* x *S. namaycush* and reported that transport stress was not affected by density up to 170 kg m^{-3}. McDonald et al. (1993) further suggested that densities up to 500 kg m^{-3} were possible based on stress levels observed in controlled confinement experiments. In addition, matrinxã, *Brycon cephalus*, have been transported at densities up to 300 kg m^{-3} successfully (Carneiro and Urbinati, 2002), and Atlantic cod, *Gadus morhua*,

exposed to simulated transport conditions at densities up to 540 kg m^{-3} resulted in only low mortality rates (Staurnes et al., 1994).

Water temperature

Changes in temperature result in the alteration of many physiological processes in fish including: oxygen transport, acid/base regulation, osmotic regulation and metabolic rates (Crawshaw, 1979). The degree of physiological disturbance experienced is typically proportional to the magnitude and rate of temperature change experienced. Sudden fluctuations in temperature are acutely stressful and can have severe consequences. Pavlidis et al. (2003) observed mortality rates of over 40% in red porgy fry (*Pagrus pagrus*) during simulated transport at 19 °C after water at 14 °C was introduced over the course of an hour half-way through the transport. This was much higher than the 2% observed mortality in fish that were refreshed with 19°C water. Carmichael (1984) observed a 68.5% mortality rate in juvenile largemouth bass (*Micropterus salmoides*) that were transported in 15°C water and released into 28°C water. Fish released into 17°C water from the same transport suffered only 8% mortality.

Fish welfare during open-hold transport

Open-hold transport is characterized by the continuous flushing of live-holds with external water, by pumping or using valves to connect live-holds with the external environment. Open-hold transport is used extensively in the aquaculture industry, yet few studies to date have examined its effects on the welfare of salmon.

Erikson et al. (1997) measured the effects of loading, hauling, and slaughter stress on flesh quality characteristics of adult Atlantic salmon during a 4 h transport aboard a 90 m^3 well-boat. White muscle phosphocreatine (PCr) levels were depleted prior to transport, but recovered to values comparable to rested fish post-transport, which indicated that significant stress

experienced during the loading processes were not exacerbated during the transport and fish were able to at least partially recover during the 4 h they were aboard the well-boat.

Farrell (2006) examined live-haul welfare of over 60,000 kg of adult Atlantic salmon by measuring $\dot{M}O_2$ in a population of fish (bulk $\dot{M}O_2$), during a single 11 h transport aboard the *Sterling Carrier*, a 650 m^3 live-haul vessel. Very high rates of bulk $\dot{M}O_2$ were observed during the initial few minutes of transport, indicating fish were seriously stressed. However, bulk $\dot{M}O_2$ decreased during the course of transport, and by 4 h had reached rates comparable to routine $\dot{M}O_2$ of individual salmon, again indicating low stress levels and significant recovery during transport.

Since these studies were limited to single transports between sea-cages and processing plants with near optimal weather conditions and normal loading densities, the results may only represent the best-case scenario for open-hold transport, as loading and environmental conditions vary depending on the location and season. Therefore my thesis expanded on the current knowledge by examining many trips and a wide range of transport conditions occurring on the west coast of British Columbia.

Measuring oxygen uptake rate as an indicator of welfare

Increases in $\dot{M}O_2$ reflect changes in metabolism in response to stress (Barton and Iwama, 1991). During stress, increased energy requirements are supplied through mobilization of energy stores (via glycogenolysis) and increases in mitochondrial oxygen consumption rates (via Krebs cycle). With severe stress in salmonids, (i.e. exhaustive exercise) $\dot{M}O_2$ can increase up to 10x the resting rate (Brett, 1965; Farrell et al., 2003; Lee et al., 2003a) and result in oxygen debts that continue to elevate $\dot{M}O_2$ for 1-4 h post-stress (Brett, 1965; Lee et al., 2003a; Scarabello et al., 1992). Thus, the magnitude of stress fish experience is reflected in both the increase of $\dot{M}O_2$ and the time required for $\dot{M}O_2$ to return to pre-transport levels.

Traditional methods of measuring stress require bio-samples for analysis of primary stress hormones such as adrenaline and cortisol concentrations, or secondary stress responses such as changes in plasma metabolite concentrations. In contrast, our method of measuring bulk $\dot{M}O_2$ is a non-lethal, non-invasive method of assessing stress levels during transport.

Bergheim et al. (1991) measured bulk $\dot{M}O_2$ in up to 3,500 kg of adult Atlantic salmon in 50 m^3 salt-water culture tanks using the differences in dissolved oxygen concentrations of inflow and outflow water and found that bulk $\dot{M}O_2$ varied between 1.5-4.5 mg O_2 min^{-1} kg^{-1} over a period of 9 months. Forsberg (1997) also measured bulk $\dot{M}O_2$ under similar conditions, while varying the amount of feed supplied to fish and found that the range of bulk $\dot{M}O_2$ observed (0.89-2.15 mg O_2 min^{-1} kg^{-1}) was positively correlated to the amount of feed supplied (0%-0.75% BW day^{-1}).

During simulated live-haul transport, Staurnes et al. (1994) found that bulk $\dot{M}O_2$ in 183 kg of adult Atlantic cod, *Gadus morhua* was 2.0 ± 0.1 mg O_2 min^{-1} kg^{-1}. These rates were only slightly elevated compared to $\dot{M}O_2$ of individual resting cod, but a mortality rate of 2.4% was still observed.

Farrell (2006) measured bulk $\dot{M}O_2$ in over 30,000 kg of adult Atlantic salmon in the live-holds of a commercial well-boat and observed bulk $\dot{M}O_2$ rates in excess of 8 mg O_2 min^{-1} kg^{-1} during the first few minutes and between 1.93 and 5.78 mg O_2 min^{-1} kg^{-1} for the duration of the voyage. These findings suggest that stress during the first few minutes was significant, but that residual stress from loading procedures was also being measured. Significant recovery from loading stressors was thus observed during the transport and overall stress was low.

Other studies investigating transport stress also observe the greatest degree of stress immediately following loading processes, followed by the recovery of stress indices during and

after transport (Barton et al., 1980; Iversen et al., 2005; Iversen et al., 1998; McDonald et al., 1993; Specker and Schreck, 1980).

$\dot{M}O_2$ and other metabolic processes are temperature dependent in poikilothermic fish, as enzyme mediated processes and biochemical reaction rates change with temperature. Large and sudden temperature changes will stimulate a stress response, increasing $\dot{M}O_2$, but small or gradual changes can result in new steady-state metabolic rates and $\dot{M}O_2$ changes that are not the result of stress. The temperature coefficient (Q) explains the dependence of a process on temperature, which is typically reported as the slope of the rate change over a range of 10°C (Q_{10}). The Q_{10} values reported for routine $\dot{M}O_2$ in fish range from 1.4 to 2.4 (Clarke and Johnston, 1999), and Q_{10} values for resting and maximal $\dot{M}O_2$ in salmonids have been measured to be around 2.0 or more (Lee et al., 2003b; MacNutt et al., 2006).

Fish welfare during closed-hold transport

The majority of studies investigating transport stress use closed-hold conditions and cover numerous species, sizes and ages of salmonids (Barton et al., 1980; Specker and Schreck, 1980; Barton and Peter, 1982; Nikinmaa et al., 1983; Maule et al., 1988; McDonald et al., 1993; Ostenfeld et al., 1995; Iversen et al., 1998; Barton, 2000; Iversen et al., 2005). During closed-hold transport, fish are again exposed to crowding, handling and confinement stressors during loading and transport (Specker and Schreck, 1980), plus interactions between fish metabolism and water chemistry. Oxygen depletion and the buildup of CO_2 and ammonia result in the progressive deterioration of water quality. Closed-hold transports routinely utilize some form of aeration/oxygenation to offset oxygen depletion, but elevations in the partial pressure of CO_2 (Pco_2) and ammonia concentrations are more difficult to resolve, and can reduce water quality to dangerous levels.

Effects of carbon dioxide on water quality

The accumulation of respiratory CO_2 during closed-hold transport results in the elevation of Pco_2 in the water (hypercarbia) and affects fish physiology and water quality. CO_2 is highly soluble in water and reacts to form carbonic acid, H_2CO_3, a weak acid that dissociates into bicarbonate, HCO_3^- and carbonate, CO_3^{2-} ions. The relative concentrations of the different CO_2 species in water are dependent on the water pH, temperature and salinity.

Fish integuments are relatively impermeable to bicarbonate and carbonate ions, but dissolved CO_2 gas can easily pass between the blood and water across the gills of fish. The Pco_2 of ocean surface water is typically very low, in the range of 0.15-0.30 mmHg. The high solubility of CO_2 in water limits the Pco_2 in fish blood to 1-2 mmHg above environmental levels (Heisler, 1986) despite the continuous production of CO_2 in tissues. Because O_2 solubility in water is low, fish utilize a high ventilation rate to ensure sufficient O_2 uptake at the gills which also helps to remove CO_2 from the blood. When fish are exposed to hypercarbic water, the Pco_2 pressure gradient at the gills is reversed, and CO_2 passively diffuses into the blood, causing the elevation of Pco_2 in the blood (hypercapnia).

The major consequence of hypercapnia is plasma acidosis. Carbonic acid is formed from CO_2 in the blood and saturates blood buffering systems reducing pH of plasma. The hypercapnic acidosis is typically corrected within 1-3 days of exposure to moderate levels of hypercarbia (Eddy et al., 1977; Hyde and Perry, 1989) but may not be fully restored under high levels. Blood pH is restored through the accumulation of HCO_3^- via branchial HCO_3^-/Cl^- exchangers (Toews et al., 1983; Perry et al., 1987; Goss and Perry, 1993), which increases plasma buffering capacity and plasma pH. The rate of pH recovery is dependent on the rate of HCO_3^- uptake, which can be limited by concentrations of bicarbonate and calcium ions in the water (Larsen and Jensen, 1997). In salmonids, hypercapnia induces a number of physiological and behavioral responses

including; the reduction in plasma pH (Eddy et al., 1977; Thomas, 1982), an increase in ventilation rate and volume (Janssen and Randall, 1975; Thomas et al., 1983; Fivelstad et al., 1999b), an increase in ionic flux rates, especially the HCO_3^- and Cl^- transport at the gills (Goss and Perry, 1993; Larsen and Jensen, 1997), a reduction in blood oxygen content and carrying capacity (hypoxemia) (Eddy and Morgan, 1969; Eddy, 1976;) and at higher levels, narcosis, anesthesia and eventually death (Bernier and Randall, 1998).

The build-up of CO_2 also acidifies hauling water, and exponentially increases the Pco_2 levels by changing the proportion of the total CO_2 existing as dissolved gas in the water. The resulting acidic pH is highly stressful and potentially lethal for fish, though lethal Pco_2 tensions will occur before lethal pH is reached in sea-water. Nevertheless, sub-lethal exposures to acid pH have significant negative effects on fish physiology. Ye and Randall (1991) found that the maximum aerobic swimming speed in rainbow trout was reduced by up to 45% at pH less than 6. However, the effects of reducing water pH with hypercarbia are not the same as acidifying the water with a mineral acid (any acid derived from inorganic minerals, i.e. HCl or H_2SO_4). Kikkawa et al. (2004) compared mortality rates in larval red sea bream (*Pagrus major*) exposed to water acidified with either CO_2 or HCl and found 95% survival in water acidified to a pH of 5.9 with HCl and 0% survival in water acidified to the same pH with CO_2. At a pH of 6.2, mortality in HCl was less than 2% as compared to 60% in CO_2. The primary reason for these differences is the high permeability of fish gills to CO_2 and low permeability to H^+ ions, such that the plasma acidosis is induced at a much faster rate during hypercarbia than the acidosis induced by elevated H^+ concentrations in the water. Fish exposed to acidified water at normal Pco_2 tensions generally require days of exposure before plasma pH decreases to a constant level (Neville, 1979; McDonald and Wood, 1981) as compared to minutes during hypercarbia (Eddy et al., 1977; McKenzie et al., 2002).

Hypercapnic plasma acidosis causes hypoxemia through Bohr and Root effects. Hypoxemia severely limits the aerobic capacity of fish and has been shown to be the primary cause of catecholamine elevation during hypercapnia (Perry et al., 1989). Basu (1959) found that brook trout (a.k.a. speckled trout) exhibited a nearly 4-fold reduction in $\dot{M}O_2$ during moderate hypercarbia. Moreover, the minimum dissolved water oxygen concentration required to sustain 50% of the maximum aerobic scope increased from ~6 mg O_2 l^{-1} to greater than 11 mg O_2 l^{-1}. Consequently, many of the physiological and behavioral changes observed during hypercarbia are in response to the induced hypoxemia rather than elevated CO_2 *per se*.

Effects of ammonia on water quality

Ammonia is another product of metabolism in fish that accumulates in re-circulating water, existing in equilibrium between ionized (NH_4^+) and unionized (NH_3) forms. Gill membranes are much more permeable to NH_3 than NH_4^+, and thus elevated levels of NH_3 in water are much more toxic than similar levels of NH_4^+. Lethal concentrations of NH_3 for rainbow trout in seawater (15°C at pH 8.0) are approximately 0.45 mg l^{-1}, while recommended safe concentrations for intensive rearing purposes are less than 0.0125 mg l^{-1} under similar conditions (Smart, 1981). Fish exposed to elevated water ammonia levels exhibit hyperactivity, increased ventilation rates, coughing and eventually convulsions and mortality (Meade, 1985; Smart, 1978).

The NH_3 / NH_4^+ equilibrium (and thus toxicity) of ammonia is highly dependent upon water pH (Smart, 1978; Thurston et al., 1981b). Because the pK of the NH_3 / NH_4^+ reaction is 9.8 (at 10ºC) ammonia predominantly exists as NH_4^+ at the normal pH of seawater (pH=7.5-8). During closed-hold transport conditions, hypercarbia will acidify the hauling water, increasing the proportion of NH_4^+ and reducing ammonia toxicity. However, if water pH is being buffered or ammonia levels reach extremely high concentrations, or such changes occur in conjunction

with other water quality stressors such as hypoxia, ammonia toxicity can reduce welfare (Thurston et al., 1981a).

Flesh Quality

Effects of live-haul transport on flesh quality

The goal of salmon aquaculture is to produce a meat product, the quality of which is measured from numerous subjective and objective characteristics found in the flesh. Some flesh characteristics are set at the point of death, while others continue to change post-mortem. In addition, some characteristics can be significantly affected by processes occurring before, during and after death.

Strong correlations have been found between exposure of fish to ante-mortem stress, and changes in post-mortem flesh quality have been shown to be the result of changes in the biochemical properties of fish muscle at the time of death (Thomas et al., 1999). In particular, severe crowding and handling cause significant changes in muscle biochemistry, which can be observed in post-mortem muscle. These effects include: reduction of intracellular and extracellular pH (Kieffer et al., 1994; Jerrett and Holland, 1998; Kristoffersen et al., 2006), reduction of intracellular glycogen concentrations (Milligan and Wood, 1986; Booth et al., 1995; Skjervold et al., 2001a), and the reduction of PCr and adenosine triphosphate (ATP) concentrations (Berg et al., 1997; Erikson et al., 1997; Sigholt et al., 1997; Erikson et al., 1999; Thomas et al., 1999).

These biochemical conditions are in turn manifested at a macroscopic level, affecting overall flesh quality through changes in the timing and strength of *rigor mortis* (the characteristic stiffening of muscles post-mortem) (Lowe et al., 1993; Berg et al., 1997; Jerrett and Holland,

1998; Thomas et al., 1999; Kristoffersen et al., 2006; Roth et al., 2006a;), flesh texture (Roth et al., 2006a; Sigholt et al., 1997) and flesh color (Robb et al., 2000).

While the physical handling and crowding stressors imposed on adult Atlantic salmon during live-haul processes can significantly impact flesh quality, the effects of poor water quality conditions experienced during live-haul transport have not been studied in detail. Elevated CO_2 can cause significant stress during transport (see Chapter 3), affecting ante-mortem physiology and biochemistry and potentially affecting post-mortem flesh quality.

Our assessment of the impacts of live-haul transport on flesh quality requires the use of quantitative flesh quality indicators. *Rigor mortis* and white muscle pH are affected by the physiological and biochemical conditions at the time of death. Both evolve post-mortem, permitting the analysis of the absolute values and the rate at which they change over time. Furthermore, both indicators are commonly used in the industry and are suitable for use in either field or laboratory settings.

Rigor mortis

The progression of post-mortem *rigor mortis* represents a well-established flesh quality indicator that directly affects flesh quality through changes in flesh texture (Iwamoto et al., 1987). Processing of fish (gutting, filleting, de-boning) should take place during the pre-rigor period, typically within 24 h of slaughter, to minimize the occurrence of muscle gaping (separation of muscle sections), which significantly reduces flesh quality (Skjervold et al., 2001b; Skjervold et al., 2001c).

The typical methods used to assess rigor progression in fish measure the degree of stiffness of fish muscles or whole fish. Some of the methods are: the modified Cutting's method which measures the distance or degree of tail droop (Bito et al., 1983), mechanical methods that use pressure transducers or low-frequency vibrations to measure muscle stiffness as deflection

resistance or vibration propagation (Berg et al., 1997; Veland and Torrissen, 1999) and tactile assessments of muscle stiffness by trained professionals (Berg et al., 1997).

Rigor mortis results from a lack of intracellular ATP within muscles, which prevents the release of the myosin-actin complexes. Rigor does not occur immediately, as it takes time for ATP in the muscle to be consumed. Additionally, ATP is replenished from intracellular PCr and during anaerobic glycolysis. Initially, PCr is utilized until concentrations drop to levels comparable to normal ATP concentrations (of ~ 10 μmol) after which ATP is supplied by anaerobic glycolysis (Watabe et al., 1991). When glycolysis substrates are also depleted, myosin/actin complexes are unable to release, and the onset of rigor is observed. Rigor progresses through the whole animal until all muscles have reached the rigor state. This peak in rigor is followed by muscle softening during the resolution of *rigor mortis*, a process that does not involve release of myosin/actin complexes but rather the breakdown of connective tissue and fiber-to-fiber attachments in muscle (Ando et al., 1993; Taylor et al., 2002).

Therefore stressors such as exercise and physical handling that reduce energy stores in fish muscles also reduce the time required for fish to enter *rigor mortis* (Berg et al., 1997; Cappeln and Jessen, 2002).

Sigholt et al. (1997) demonstrated that significant decreases in muscle PCr and ATP occurred when adult Atlantic salmon were exposed to a 10-min handling stress before slaughter, resulting in a shorter pre-rigor period. Peak rigor occurred 6X faster, i.e. within 2 h in the stressed fish as opposed to 12 h in unstressed fish. Furthermore, flesh quality was reduced because of a softer texture and a lower breaking strength.

Berg et al. (1997) found that crowding and handling stress during commercial slaughtering processes also accelerated the onset of *rigor mortis* by 2-3X. The maximum rigor tension was also significantly lower in fish sampled before entering the processing line.

Differences in rigor strength were hypothesized to be the result of incomplete rigor as only parts of the muscle were entering rigor at the same time. This theory was supported by significantly higher ATP concentrations measured in non-stressed fish in peak rigor compared to almost no ATP in stressed fish in peak rigor.

White muscle pH

White muscle pH also represents an industry standard in assessing flesh quality that affects post-mortem enzymatic rates and protein denaturation rates. In conjunction with *rigor mortis*, muscle pH plays an important role in determining texture, shelf life and freshness indexes. Lactic acid is accumulated in post-mortem muscle during anaerobic glycolysis, which relies on glycogen (and glycogenolysis) to re-synthesize ATP. Therefore muscle pH decreases until intracellular glycogen has been exhausted by glycolysis. Rigor progression and muscle pH are correlated, as while ATP is being synthesized, lactic acid accumulates and pH declines but rigor is delayed. When ATP synthesis stops, lactic acid production ceases, pH stabilizes and *rigor mortis* begins.

The post-mortem white muscle pH is initially dependent on ante-mortem concentrations of intracellular lactic acid, while the final muscle pH is dependent on the amount of lactic acid produced post-mortem, which is determined by ante-mortem intracellular glycogen concentrations (Kobayashi et al., 1999).

Thomas et al. (1999) found that the exercise and crowding associated with transport and slaughter of adult Atlantic salmon significantly increased muscle lactate and reduced muscle pH immediately at the time of death. Unstressed fish had higher muscle pH immediately after death, but after 72 h of ice-storage all fish had similar muscle pH.

Jerrett and Holland (1998) found that ‘exercising’ chinook salmon, *O. tshawytscha* using post-mortem electrical stimulation resulted in significant reductions in muscle pH. The pH of

'rested' fish muscle at the time of death was 7.35 ± 0.08, while 'partially-exercised' (180 pulses of electricity) and 'exhausted' (360 pulses) were 7.02 ± 0.08 and 6.58 ± 0.08, respectively. Rigor was observed to occur at a pH of ~6.6 and the final muscle pH was ~6.2, regardless of the amount of 'exercise' used for each group. Robb et al. (2000) found similar results in rainbow trout 'exercised' using 2 min of electrical stimulation. However, the 'rested' fish were anaesthetized prior to sampling, yielding a much higher initial muscle pH of 7.8 ± 0.31. Exercised fish were comparable to the 'exhausted' fish of Jarrett and Holland (1998) with muscle pH of 6.7 ± 0.03 at the time of death.

Effects of hypercarbia on flesh quality

Strenuous exercise or handling induces a mixed metabolic and respiratory acidosis that reduces muscle pH and can affect flesh quality. Depending on the source of acidosis, the effects on post-mortem muscle may differ. A metabolic acidosis caused by lactic acid accumulation has lasting effects on muscle pH long after activity has ceased (Milligan and Wood, 1986), while the acidosis occurring during a respiratory acidosis is transient and only exists while internal or external elevations in CO_2 are present.

Fish exposed to hypercarbia during closed-hold, live-haul transport primarily experience a respiratory acidosis. Short-term (1-24 h) exposure to low or moderate hypercarbia will still result in significant acidosis, and partial or complete compensation of the respiratory acidosis will occur during this time (Eddy et al., 1977). Furthermore, the bicarbonate accumulation during hypercarbia may cause plasma alkalosis when atmospherically equilibrated Pco_2 tensions in the water (normocarbia) are restored (Eddy et al., 1977).

These physiological and biochemical changes may be sustained in post-mortem muscle, affecting flesh quality. It is unknown how these will change post-mortem, or their effects on flesh quality indicators such as *rigor mortis* and muscle pH.

Reductions in flesh quality with exposure to hypercarbia have been observed with the use of CO_2 stunning tanks, which involve very high Pco_2 (>100 mmHg) and very short exposure periods (5-10 min). Accelerated onset of *rigor mortis*, lower flesh pH and softer flesh texture were observed in response but were attributed to the vigorous physical reactions evoked by pumping fish into the hypoxic/anoxic CO_2 tanks rather than CO_2 exposure *per se* (Marx et al., 1997; Kiessling et al., 2004; Jittinandana et al., 2005; Erikson et al., 2006; Roth et al., 2006b). Danley et al. (2005) observed reduced growth rates in *O. mykiss* reared in low to moderate elevations of CO_2 (22-57 mg l^{-1}) but no significant differences in flesh quality were found after 96 days of exposure to hypercarbia.

Summary

This thesis was undertaken to address the current lack of knowledge regarding the welfare of adult salmon during commercial live-haul transport in BC's aquaculture industry. Fish welfare during open-hold live-haul has not been thoroughly studied despite its extensive use. While studies have observed fish exposed to elevated Pco_2 tensions during transport and the consequences of hypercapnia are well-established in salmonids, no study to date has examined this particular problem in the large masses and high densities commonly used in live-haul transport today. Furthermore, by investigating the effects that hypercarbic conditions created during closed-hold transport on flesh quality we hope to identify potential economic implications for poor welfare during live-haul transport. To the best of my knowledge, the effects of short-term exposure to sub-lethal CO_2 levels on flesh quality in Atlantic salmon have not been investigated. Another concern is the physiological and biochemical disturbances caused by returning fish acclimated to hypercarbic water to normocarbic water (Eddy et al., 1977). As these are potential risks for transported fish, studying these effects is an important aspect of assessing the welfare of fish during live-haul transport.

Research Objectives and Hypotheses

1. To provide a comprehensive assessment of the factors affecting fish welfare during open-hold live-haul of adult Atlantic salmon aboard the *Sterling Carrier*. It is hypothesized that fish welfare during open-hold transport will primarily be determined by water quality, which will be dictated by environmental conditions at the time of transport.

2. To provide a comprehensive assessment of the factors affecting fish welfare during closed-hold live-haul transport of adult Atlantic salmon aboard the *Sterling Carrier*. It is hypothesized that fish welfare will change with water quality and primarily be dictated by the Pco_2 in the hauling water.

3. To quantify the effects of hypercarbic exposure on the flesh quality of adult Atlantic salmon. It is hypothesized that hypercarbia will have negative effects on flesh quality.

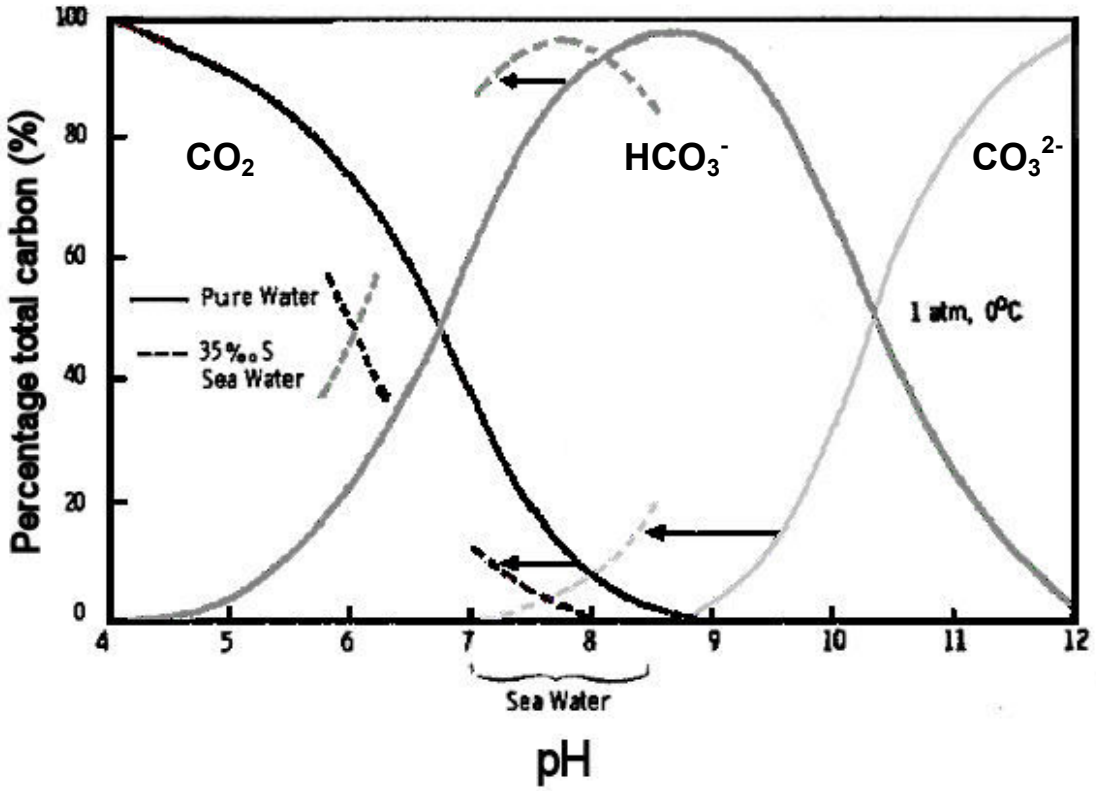

Figure 1.1: Distribution of carbon dioxide species in water. Solid lines indicate freshwater species, broken lines indicate shift in equilibrium in seawater. In seawater, the majority of CO_2 exists as a dissolved gas phase below pH 6.5, bicarbonate between pH 7-9, and carbonate above pH 9.

References

Ando, M., Toyohara, H., Shimizu, Y. and Sakaguchi, M. (1993). Postmortem tenderization of fish muscle due to weakening of pericellular connective-tissue. Nippon Suisan Gakk. 59, 1073-1076.

Arlinghaus, R., Cooke, S. J., Schwab, A. and Cowx, I. G. (2007). Fish welfare: a challenge to the feelings-based approach, with implications for recreational fishing. Fish Fish. 8, 57-71.

Barton, B. A. (2000). Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. N. Am. J. Aquacult. 62, 12-18.

Barton, B. A. (2002). Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517-525.

Barton, B. A. and Iwama, G. K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. of Fish Dis. 1, 3-26.

Barton, B. A. and Peter, R. E. (1982). Plasma-cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anesthesia, and cold shock. J. Fish Biol. 20, 39-51.

Barton, B. A., Peter, R. E. and Paulencu, C. R. (1980). Plasma-cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Can. J. Fish. Aquat. Sci. 37, 805-811.

Barton, B. A., Schreck, C. B. and Barton, L. D. (1987). Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis. Aquat. Organ. 2, 173-185.

Barton, B. A., Schreck, C. B. and Sigismondi, L. A. (1986). Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Trans. Am. Fish. Soc. 115, 245-251.

Berg, T., Erikson, U. and Nordtvedt, T. S. (1997). *Rigor mortis* assessment of Atlantic salmon (*Salmo salar*) and effects of stress. J. Food Sci. 62, 439-446.

Bergheim, A., Seymour, E. A., Sanni, S., Tyvold, T. and Fivelstad, S. (1991). Measurements of oxygen-consumption and ammonia excretion of Atlantic salmon (*Salmo salar* L.) in commercial-scale, single-pass fresh-water and seawater land-based culture systems. Aquacult. Eng. 10, 251-267.

Bernier, N. J. and Randall, D. J. (1998). Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment. J. Fish Biol. 52, 621-637.

Beukema, J. J. (1970). Acquired hook-avoidance in the pike *Esox lucius* L. fished with artificial and natural baits. J. Fish Biol. 2, 155-160.

Bito, M., Yamada, K., Mikumo, Y. and Amano, K. (1983). Difference in the mode of *rigor mortis* among some varieties of fish by modified Cutting's method. Bull. Tokai Reg. Fish. Res. Lab. 109, 89-96.

Bonga, S. E. W. (1997). The stress response in fish. Physiol. Rev. 77, 591-625.

Booth, R. K., Kieffer, J. D., Davidson, K., Bielak, A. T. and Tufts, B. L. (1995). Effects of late-season catch and release angling on anaerobic metabolism, acid-base status, survival, and gamete viability in wild Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 52, 283-290.

Boutilier, R. G., Dobson, G., Hoeger, U. and Randall, D. J. (1988). Acute exposure to graded-levels of hypoxia in rainbow trout (*Salmo gairdneri*) - metabolic and respiratory adaptations. Resp. Physiol. 71, 69-82.

Brett, J. R. (1965). Relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 22, 1491-1501.

Cappeln, G. and Jessen, F. (2002). ATP, IMP, and glycogen in cod muscle at onset and during development of *rigor mortis* depend on the sampling location. J. Food Sci. 67, 991-995.

Carmichael, G. J. (1984). Long-distance truck transport of intensively reared largemouth bass. Prog. Fish Cult. 46, 111-115.

Carneiro, P. C. F. and Urbinati, E. C. (2002). Transport stress in matrinxa, *Brycon cephalus* (Teleostei: Characidae), at different densities. Aquacult. Int. 10, 221-229.

Chandroo, K. P., Duncan, I. J. H. and Moccia, R. D. (2004). Can fish suffer? Perspectives on sentience, pain, fear and stress. Appl. Anim. Behav. Sci. 86, 225-250.

Clarke, A. and Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. J. Anim. Ecol. 68, 893-905.

Crawshaw, L. I. (1979). Responses to rapid temperature-change in vertebrate ectotherms. Am. Zool. 19, 225-237.

Danley, M. L., Kenney, P. B., Mazik, P. M., Kiser, R. and Hankins, J. A. (2005). Effects of carbon dioxide exposure on intensively cultured rainbow trout, *Oncorhynchus mykiss*: Physiological responses and fillet attributes. J. World Aquacult. Soc. 36, 249-261.

Duncan, I. J. H. and Fraser, D. (1997). Understanding animal welfare. In *Anim. Welf.*, (eds. M. C. Appleby and B. O. Hughes), pp. 19-32. Wallingford, UK: CAB International.

Eddy, F. B. (1976). Acid-base-balance in rainbow trout (*Salmo gairdneri*) subjected to acid stresses. J. Exp. Biol. 64, 159-171.

Eddy, F. B., Lomholt, J. P., Weber, R. E. and Johansen, K. (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high CO_2 tension. J. Exp. Biol. 67, 37-47.

Eddy, F. B. and Morgan, R. I. G. (1969). Some effects of carbon dioxide on blood of rainbow trout *Salmo gardneri* Richardson. J. Fish Biol. 1, 361-372.

Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M. and Gadd, D. (2002). The relationships between stocking density and welfare in farmed rainbow trout. J. Fish Biol. 61, 493-531.

Erikson, U., Hultmann, L. and Steen, J. E. (2006). Live chilling of Atlantic salmon (*Salmo salar*) combined with mild carbon dioxide anaesthesia I. Establishing a method for large-scale processing of farmed fish. Aquaculture 252, 183-198.

Erikson, U., Sigholt, T., Rustad, T., Einarsdottir, I. E. and Jorgensen, L. (1999). Contribution of bleeding to total handling stress during slaughter of Atlantic salmon. Aquacult. Int. 7, 101-115.

Erikson, U., Sigholt, T. and Seland, A. (1997). Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 149, 243-252.

FAO. (2006). The state of world fisheries and aquaculture. Rome: FAO.

Farrell, A. P. (2006). Bulk oxygen uptake measured with over 60,000 kg of adult salmon during live-haul transportation at sea. Aquaculture 254, 646-652.

Farrell, A. P., Gamperl, A. K. and Birtwell, I. K. (1998). Prolonged swimming, recovery and repeat swimming performance of mature sockeye salmon, *Oncorhynchus nerka* exposed to moderate hypoxia and pentachlorophenol. J. Exp. Biol. 201, 2183-2193.

Farrell, A. P., Lee, C. G., Tierney, K., Hodaly, A., Clutterham, S., Healey, M., Hinch, S. and Lotto, A. (2003). Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish Biol. 62, 64-84.

Fivelstad, S., Bergheim, A., Kloften, H., Haugen, R., Lohne, T. and Olsen, A. B. (1999a). Water flow requirements in the intensive production of Atlantic salmon (*Salmo salar* L.) fry: growth and oxygen consumption. Aquacult. Eng. 20, 1-15.

Fivelstad, S., Olsen, A. B., Kloften, H., Ski, H. and Stefansson, S. (1999b). Effects of carbon dioxide on Atlantic salmon (*Salmo salar* L.) smolts at constant pH in bicarbonate rich freshwater. Aquaculture 178, 171-187.

Forsberg, O. I. (1997). The impact of varying feeding regimes on oxygen consumption and excretion of carbon dioxide and nitrogen in post-smolt Atlantic salmon *Salmo salar* L. Aquac. Res. 28, 29-41.

Fraser, D., Weary, D. M., Pajor, E. A. and Milligan, B. N. (1997). A scientific conception of animal welfare that reflects ethical concerns. Anim. Welf. 6, 187-205.

Goss, G. G. and Perry, S. F. (1993). Physiological and morphological regulation of acid-base status during hypercapnia in rainbow trout (*Oncorhynchus mykiss*). Can. J. Zool. 71, 1673-1680.

Heisler, N. (1986). Acid-base regulation in animals. Amsterdam: Elsevier Science Publishers B.V.

Huntingford, F., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandoe, P. and Turnbull, J. F. (2007). The implications of a feelings-based approach to fish welfare: a reply to Arlinghaus *et al.* Fish Fish. 8, 277-280.

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandoe, P. and Turnbull, J. F. (2006). Current issues in fish welfare. J. Fish Biol. 68, 332-372.

Hyde, D. A. and Perry, S. F. (1989). Differential approaches to blood acid-base regulation during exposure to prolonged hypercapnia in 2 fresh-water teleosts - the rainbow trout (*Salmo gairdneri*) and the American eel (*Anguilla rostrata*). Physiol. Zool. 62, 1164-1186.

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

Iversen, M., Finstad, B. and Nilssen, K. J. (1998). Recovery from loading and transport stress in Atlantic salmon (*Salmo salar* L.) smolts. Aquaculture 168, 387-394.

Iwamoto, M., Yamanaka, H., Watabe, S. and Hashimoto, K. (1987). Effect of storage-temperature on rigor-mortis and ATP degradation in plaice *Paralichthys olivaceus m*uscle. J. Food Sci. 52, 1514-1517.

Janssen, R. G. and Randall, D. J. (1975). Effects of changes in pH and pCO_2 in blood and water on breathing in rainbow trout, *Salmo gairdneri*. Resp. Physiol. 25, 235-245.

Jerrett, A. R. and Holland, A. J. (1998). Rigor tension development in excised "Rested", "Partially exercised" and "Exhausted" Chinook salmon white muscle. J. Food Sci. 63, 48-52.

Jittinandana, S., Kenney, P. B., Mazik, P. M., Danley, M., Nelson, C. D., Kiser, R. A. and Hankins, J. A. (2005). Transport and stunning affect quality of arctic char fillets. J. Muscle Foods 16, 274-288.

Jorgensen, E. H., Christiansen, J. S. and Jobling, M. (1993). Effects of stocking density on food-intake, growth-performance and oxygen-consumption in Arctic charr (*Salvelinus alpinus*). Aquaculture 110, 191-204.

Kebus, M. J., Collins, M. T., Brownfield, M. S., Amundson, C. H., Kayes, T. B. and Malison, J. A. (1992). Effects of rearing density on the stress response and growth of rainbow trout. J. Aquat. Anim. Health 4, 1-6.

Kieffer, J. D., Currie, S. and Tufts, B. L. (1994). Effects Of Environmental-Temperature On The Metabolic And Acid-Base Responses Of Rainbow-Trout To Exhaustive Exercise. J. Exp. Biol. 194, 299-317.

Kiessling, A., Espe, M., Ruohonen, K. and Morkore, T. (2004). Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO_2 anaesthesia. Aquaculture 236, 645-657.

Kikkawa, T., Kita, J. and Ishimatsu, A. (2004). Comparison of the lethal effect of CO_2 and acidification on red sea bream (*Pagrus major*) during the early developmental stages. Mar. Pollut. Bull. 48, 108-110.

Kobayashi, M., Takatori, T., Nakajima, M., Saka, K., Iwase, H., Nagao, M., Niijima, H. and Matsuda, Y. (1999). Does the sequence of onset of *rigor mortis* depend on the proportion of muscle fibre types and on intra-muscular glycogen content? Int. J. Legal Med. 112, 167-171.

Kristoffersen, S., Tobiassen, T., Steinsund, V. and Olsen, R. L. (2006). Slaughter stress, postmortem muscle pH and rigor development in farmed Atlantic cod (*Gadus morhua* L.). Int. J. Food Sci. Tech. 41, 861-864.

Larsen, B. K. and Jensen, F. B. (1997). Influence of ionic composition on acid-base regulation in rainbow trout (*Oncorhynchus mykiss*) exposed to environmental hypercapnia. Fish Physiol. Biochem. 16, 157-170.

Lee, C. G., Farrell, A. P., Lotto, A., Hinch, S. G. and Healey, M. C. (2003a). Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. J. Exp. Biol. 206, 3253-3260.

Lee, C. G., Farrell, A. P., Lotto, A., MacNutt, M. J., Hinch, S. G. and Healey, M. C. (2003b). The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch)* salmon stocks. J. Exp. Biol. 206, 3239-3251.

Lefrancois, C., Claireaux, G., Mercier, C. and Aubin, J. (2001). Effect of density on the routine metabolic expenditure of farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture 195, 269-277.

Liao, P. B. (1971). Water requirements of salmonids. Prog. Fish Cult. 33, 210-215.

Lowe, T. E., Ryder, J. M., Carragher, J. F. and Wells, R. M. G. (1993). Flesh quality in snapper, *Pagrus auratus*, affected by capture stress. J. Food Sci. 58, 770-773.

MacNutt, M. J., Hinch, S. G., Lee, C. G., Phibbs, J. R., Lotto, A. G., Healey, M. C. and Farrell, A. P. (2006). Temperature effects on swimming performance, energetics, and aerobic capacities of mature adult pink salmon (*Oncorhynchus gorbuscha*) compared with those of sockeye salmon (*Oncorhynchus nerka*). Can. J. Zool. 84, 88-97.

Marx, H., Brunner, B., Weinzierl, W., Hoffmann, R. and Stolle, A. (1997). Methods of stunning freshwater fish: Impact on meat quality and aspects of animal welfare. Z. Lebensm. Unters. F. A. 204, 282-286.

Maule, A. G., Schreck, C. B., Bradford, C. S. and Barton, B. A. (1988). Physiological-effects of collecting and transporting emigrating juvenile chinook salmon past dams on the Columbia river. Trans. Am. Fish. Soc. 117, 245-261.

Mazeaud, M. M., Mazeaud, F. and Donaldson, E. M. (1977). Primary and secondary effects of stress in fish - some new data with a general review. Trans. Am. Fish. Soc. 106, 201-212.

McDonald, D. G., Goldstein, M. D. and Mitton, C. (1993). Responses of hatchery-reared brook trout, lake trout, and splake to transport stress. Trans. Am. Fish. Soc. 122, 1127-1138.

McDonald, D. G. and Wood, C. M. (1981). Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. J. Exp. Biol. 93, 101-118.

McDonald, G. and Milligan, L. (1997). Ionic, osmotic and acid-base regulation in stress. In *Fish stress and health in aquaculture*, (eds. G. K. Iwama A. D. Pickering J. P. Sumpter and C. B. Schreck), pp. 119-144. Cambridge, UK: Cambridge University Press.

McKenzie, D. J., Taylor, E. W., Dalla Valle, A. Z. and Steffensen, J. F. (2002). Tolerance of acute hypercapnic acidosis by the European eel (*Anguilla anguilla*). J. Comp. Physiol. B 172, 339-346.

Meade, J. W. (1985). Allowable ammonia for fish culture. Prog. Fish Cult. 47, 135-145.

Milligan, C. L. and Wood, C. M. (1986). Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. J. Exp. Biol. 123, 123-144.

Neville, C. M. (1979). Sublethal effects of environmental acidification on rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36, 84-87.

Nikinmaa, M., Soivio, A., Nakari, T. and Lindgren, S. (1983). Hauling stress in brown trout (*Salmo trutta*) - physiological-responses to transport in fresh-water or salt-water, and recovery in natural brackish water. Aquaculture 34, 93-99.

Ostenfeld, T., Thomsen, S., Ingolfdottir, S., Ronsholdt, B. and McLean, E. (1995). Evaluation of the effect of live haulage on metabolites and fillet texture of rainbow trout (*Oncorhynchus mykiss*). Water Sci. Technol. 31, 233-237.

Pavlidis, M., Angellotti, L., Papandroulakis, N. and Divanach, P. (2003). Evaluation of transportation procedures on water quality and fry performance in red porgy (*Pagrus pagrus*) fry. Aquaculture 218, 187-202.

Perry, S. F., Kinkead, R., Gallaugher, P. and Randall, D. J. (1989). Evidence that hypoxemia promotes catecholamine release during hypercapnic acidosis in rainbow trout (*Salmo gairdneri*). Resp. Physiol. 77, 351-363.

Perry, S. F., Malone, S. and Ewing, D. (1987). Hypercapnic acidosis in the rainbow trout (*Salmo gairdneri*). 1. Branchial ionic fluxes and blood acid-base status. Can. J. Zool. 65, 888-895.

Perry, S. F. and Reid, S. D. (1992). Relationship between blood O_2 content and catecholamine levels during hypoxia in rainbow trout and American eel. Am. J. Physiol. 263, R240-R249.

Pickering, A. D. and Pottinger, T. G. (1989). Stress responses and disease resistance in salmonid fish - effects of chronic elevation of plasma-cortisol. Fish Physiol. Biochem. 7, 253-258.

Pickering, A. D. and Stewart, A. (1984). Acclimation of the interrenal tissue of the brown trout, *Salmo trutta*-L, to chronic crowding stress. J. Fish Biol. 24, 731-740.

Randall, D. J. and Perry, S. F. (1992). Catecholamines. In *Fish Physiol.*, vol. XIIB, (eds. W. S. Hoar D. J. Randall and A. P. Farrell), pp. 255-300. San Diego, CA: Academic Press.

Robb, D. H. F., Kestin, S. C. and Warriss, P. D. (2000). Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. Aquaculture 182, 261-269.

Roth, B., Slinde, E. and Arildsen, J. (2006a). Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on *rigor mortis* and the physical properties of flesh. Aquaculture 257, 504-510.

Roth, B., Slinde, E. and Robb, D. H. F. (2006b). Field evaluation of live chilling with CO_2 on stunning Atlantic salmon (*Salmo salar*) and the subsequent effect on quality. Aquac. Res. 37, 799-804.

Scarabello, M., Heigenhauser, G. J. F. and Wood, C. M. (1992). Gas-exchange, metabolite status and excess postexercise oxygen-consumption after repetitive bouts of exhaustive exercise in juvenile rainbow trout. J. Exp. Biol. 167, 155-169.

Schreck, C. B., Solazzi, M. F., Johnson, S. L. and Nickelson, T. E. (1989). Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch*. Aquaculture 82, 15-20.

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

Skjervold, P. O., Fjaera, S. O., Ostby, P. B. and Einen, O. (2001a). Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 192, 265-280.

Skjervold, P. O., Fjaera, S. O., Ostby, P. B., Isaksson, T., Einen, O. and Taylor, R. (2001b). Properties of salmon flesh from different locations on pre- and post-rigor fillets. Aquaculture 201, 91-106.

Skjervold, P. O., Rora, A. M. B., Fjaera, S. O., Vegusdal, A., Vorre, A. and Einen, O. (2001c). Effects of pre-, in-, or post-rigor filleting of live chilled Atlantic salmon. Aquaculture 194, 315-326.

Sloman, K. A., Metcalfe, N. B., Taylor, A. C. and Gilmour, K. M. (2001). Plasma cortisol concentrations before and after social stress in rainbow trout and brown trout. Physiol. Biochem. Zool. 74, 383-389.

Smart, G. R. (1978). Investigations of toxic mechanisms of ammonia to fish gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. J. Fish Biol. 12, 93-104.

Smart, G. R. (1981). Aspects of water quality producing stress in intensive fish culture. In *Stress and fish*, (ed. A. D. Pickering), pp. 277-292. New York, NY: Academic Press.

Specker, J. L. and Schreck, C. B. (1980). Stress responses to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts. Can. J. Fish. Aquat. Sci. 37, 765-769.

Staurnes, M., Sigholt, T., Pedersen, H. P. and Rustad, T. (1994). Physiological-effects of simulated high-density transport of Atlantic cod (*Gadus morhua*). Aquaculture 119, 381-391.

Steffensen, J., Farrell, A. (1998). Swimming performance, venous oxygen tension and cardiac performance of coronary-ligated rainbow trout, *Oncorynchus mykiss*, exposed to progressive hypoxia. Comp. Biochem. Physiol. A Comp. Physiol. 119A, 585-592.

Taylor, R. G., Fjaera, S. O. and Skjervold, P. O. (2002). Salmon fillet texture is determined by myofiber-myofiber and myofiber-myocommata attachment. J. Food Sci. 67, 2067-2071.

Thomas, P. M., Pankhurst, N. W. and Bremner, H. A. (1999). The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. J. Fish Biol. 54, 1177-1196.

Thomas, S., Fievet, B., Barthelemy, L. and Peyraud, C. (1983). Comparison of the effects of exogenous and endogenous hypercapnia on ventilation and oxygen-uptake in the rainbow trout (*Salmo gairdneri* R.). J. Comp. Physiol. 151, 185-190.

Thomas, S., Le Ruz, H. (1982). A continuous study of rapid changes in blood acid-base status of trout during variations of water pCO_2. J. Comp. Physiol. B. 148, 123-130.

Thurston, R. V., Phillips, G. R., Russo, R. C. and Hinkins, S. M. (1981a). Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. Can. J. Fish. Aquat. Sci. 38, 983-988.

Thurston, R. V., Russo, R. C. and Vinogradov, G. A. (1981b). Ammonia toxicity to fishes - effect of pH on the toxicity of the un-ionized ammonia species. Environ. Sci. Technol. 15, 837-840.

Toews, D. P., Holeton, G. F. and Heisler, N. (1983). Regulation of the acid-base status during environmental hypercapnia in the marine teleost fish *Conger conger*. J. Exp. Biol. 107, 9-20.

Topál, J. and Csányi, V. (1999). Interactive learning in the paradise fish (*Macropodus opercularis*): an ethological interpretation of the second-order conditioning paradigm. Anim. Cogn. 2, 197-206.

Veland, J. O. and Torrissen, O. J. (1999). The texture of Atlantic salmon (*Salmo salar*) muscle as measured instrumentally using TPA and Warner-Brazler shear test. J. Sci. Food Agric. 79, 1737-1746.

Watabe, S., Kamal, M. and Hashimoto, K. (1991). Postmortem changes in ATP, creatine-phosphate, and lactate in sardine muscle. J. Food Sci. 56, 151-153.

Wedemeyer, G. A. (1996). Physiology of fish in intensive culture systems. New York: Chapman & Hall.

Wedemeyer, G. A. and McLeay, D. J. (1981). Methods for determining the tolerance of fishes to environmental stressors. In *Stress and Fish*, (ed. A. D. Pickering), pp. 247-276. New York, New York: Academic Press Inc.

Ye, X. M. and Randall, D. J. (1991). The effect of water pH on swimming performance in rainbow trout (*Salmo gairdneri*, Richardson). Fish Physiol. Biochem. 9, 15-21.

Chapter 2: Using bulk oxygen uptake to assess the welfare of adult Atlantic salmon, *Salmo salar* during commercial live-haul transport[1]

Introduction

Recent advances in live-haul technology have resulted in specialized well-boats equipped with large capacity live-holds that are continually flushed (open-hold) via valves mounted in the hull that use the forward momentum of the vessel to force water through the holds. During fish metabolism, oxygen is consumed while CO_2 and ammonia are excreted, adversely affecting water quality conditions. In traditional re-circulated (closed-hold) type transport, depletion of oxygen and accumulation of CO_2 and ammonia can reach levels that are highly stressful or even lethal to fish over time. Open-hold live-haul allows the bulk transport of large masses of fish while maintaining water quality by continually refreshing holds with oxygenated water and preventing the buildup of metabolic wastes. However, water quality is dependent on external conditions, which can vary significantly in ocean surface waters. While water quality is the main factor affecting fish welfare during transport, other factors such as handling and crowding during loading procedures and high loading densities also contribute significantly to stress during transport (Barton et al., 1980; Specker and Schreck, 1980; Barton and Peter, 1982; Nikinmaa et al., 1983; Maule et al., 1988; McDonald et al., 1993; Erikson et al., 1997; Iversen et al., 1998; Barton, 2000; Iversen et al., 2005).

The *Sterling Carrier* is a state-of-the-art dedicated live-haul vessel operating in the aquaculture industry of British Columbia, Canada. The 40 m vessel is capable of carrying over 100 000 kg of adult Atlantic salmon in a single trip at densities reaching 200 kg m^{-3}. While live-

[1] A version of this chapter will be submitted for publication. Tang, S., Brauner, C.J., Farrell, A.P., 2008. Using bulk oxygen uptake to assess the welfare of adult Atlantic salmon, *Salmo salar* during commercial live-haul transport.

haul transports under these conditions are cost-effective, they do not necessarily represent optimal conditions for fish welfare.

Open-hold live-haul transport has been used for years in both Canada and Norway, yet few assessments have been done. Studies have shown that compared to loading/unloading and post-transport slaughter processes, the transport phase may be the least stressful part of the process (Erikson et al., 1997; Iversen et al., 2005).

Typically fish are processed within 24 h of being unloaded at the processing facility, and transport is one of the final steps influencing the final product quality. The benefits of maintaining good welfare by minimizing stress during transport are seen in increased survival rates during and post-transport (Specker and Schreck, 1980; Schreck et al., 1989), reduction in the time required for resumption of normal behaviors (McDonald et al., 1993) and improved flesh quality (Berg et al., 1997; Erikson et al., 1997; Jittinandana et al., 2005).

Farrell (2006) first measured the bulk oxygen consumption rate (bulk $\dot{M}O_2$) in a large mass of salmon during transport by using the live-holds aboard the *Sterling Carrier* as large-scale respirometers. Changes in $\dot{M}O_2$ with the elevation of metabolic rate during stress are well-established, and measuring $\dot{M}O_2$ provides a number of advantages over traditional methods of quantifying stress. Measuring bulk $\dot{M}O_2$ allows the observation of stress levels in a fish population during actual live-haul transport, under actual conditions and without disturbing fish and potentially biasing results.

In a preliminary study, Farrell (2006) reported bulk $\dot{M}O_2$ greater than 8 mg O_2 min^{-1} kg^{-1} at the onset of transport, however bulk $\dot{M}O_2$ decreased to below 4 mg O_2 min^{-1} kg^{-1} within 20 min. After 4 h of transport, bulk $\dot{M}O_2$ remained at a steady rate of 3.1 mg O_2 min^{-1} kg^{-1}. In comparison with $\dot{M}O_2$ measured from individual salmon in swim-respirometers (Farrell et al., 2003; Lee et al., 2003b; MacNutt et al., 2006) this would indicate that fish were severely stressed

immediately post-loading but recovered to levels comparable to routine $\dot{M}O_2$ in a short period of time. Farrell (2006) acknowledged that rapidly fluctuating water conditions during the initial phase of transport could have resulted in overestimation of $\dot{M}O_2$ during this time. In addition the flow rate through the holds may have been overestimated, affecting dilution rate and wash-out time estimates. The objective of the current study was to address issues concerning the measurement of flow rate through the holds and to investigate the effects of loading density and water temperature on fish welfare by measuring bulk $\dot{M}O_2$ from a large number of transports occurring throughout the season.

Materials and Methods

Trip records from 45 individual transports occurring between May-September 2003 were analyzed. All transports originated from sea-cages located in Quatsino Sound, BC and finished at the Englewood processing plant at Beaver Cove, BC. Trips lasted a minimum of 10 h. Data for input and output oxygen concentrations, temperature, total fish mass and average fish weight were collected from a PT4 control unit (Point Four Systems, Richmond, BC, Canada) and compared to the ship's logs to determine the approximate departure and arrival times of each transport. Transported fish had not been fed for at least 7 days prior to transport.

Data treatment

Bulk $\dot{M}O_2$ data from each trip was visually assessed for evidence of prolonged oxygen injection beyond the first hour of the transport. The first 59 minutes was removed from the data set to account for washout time and residual oxygen that resulted from routine oxygen injection during loading and post-departure from the sea-cages. The total duration of the 45 transports ranged from 11-15 h, but for consistency only the first 10 h of transport time were analyzed for any trip. Trips showing signs of oxygen injection past the 1 h mark were discarded. Also, each

trip was assessed for erroneous bulk $\dot{M}O_2$ data resulting from sharp rises and falls in inflow oxygen content. Any bulk $\dot{M}O_2$ values less than 0 mg O_2 min^{-1} kg^{-1}or greater than 15 mg O_2 min^{-1} kg^{-1} were removed from the data set. If the proportion of discarded data points exceeded 25% during a single trip, the trip was not used. The data set used for the present analysis contained 89 data sets from 44 starboard and 45 port samples, collected from 45 individual trips.

Calibration of the vessel for respirometry

A detailed description of the *Sterling Carrier* and its equipment are documented in Farrell (2006). Briefly, the *Sterling Carrier* is a 40 m vessel with two 325 m^3 live-holds that are flooded via a series of 14" hydraulic valves (four forward and three aft valves per hold), producing a directional water flow from forward to aft while the vessel is in motion. Water can also be re-circulated in each hold using pumps, capable of re-circulating water at 20 m^3 min^{-1}. To maintain dissolved oxygen concentrations, pure O_2 gas is injected through diffusers directly into the re-circulating water before it re-enters the holds through perforated PVC diffusing columns that run lengthwise along the bottom. Oxygen electrodes (Oxyguard MKIII, Point Four Systems, Richmond, BC) are positioned in screened compartments at forward and aft ends of each hold to measure inflow and outflow water oxygen concentrations. The oxygen probes use internal temperature correction, but a temperature electrode (#10TRR001, Point Four Systems, Richmond, BC) is also positioned in the forward compartment of each hold to measure water temperature. Oxygen and temperature probes were air-calibrated to an accuracy of ± 0.1 mg O_2 l^{-1} and ± 0.1˚C, respectively.

As fish are pumped from the pens, the number of fish and total fish mass are measured using a video counting system (Wingfish, Wingan A/S, Norway). The counting system uses a glass chamber with two video cameras mounted at 90˚ angles that register images from approximately every 10^{th} fish passing through the chamber. Surface area is calculated from the

combined images and mass is calculated from calibrated surface area/mass algorithms. The fish number and mass measured during loading were within 1% of the estimated whole fish masses derived from processed fish weights at the processing plant, where each fish is measured individually. Fish mass per hold varied between 20,150 and 48,425 kg, representing hold densities of between 63 and 150 kg m^{-3}.

The flow rate through each hold, V_W, was measured in the upper rectangular section of the starboard hold, known as the combing. We measured the surface area of the combing (16.58 m^2) and marked the vertical wall with 20 cm increments starting 10 cm from the bottom of the combing. With the hull-valves closed, the water level in both holds was lowered to below the combing using the onboard pumps. Then all forward valves were opened simultaneously, and the time to fill each marked section of the combing was measured (each section had a volume of 3.32 m^3). The water level remains relatively steady at ~30 cm up the combing with both forward and aft hull-valves open under normal transport speed (~9 knots) but reached up to 50 cm with only the forward valves opened, allowing us to measure instantaneous flow rates at various water levels in the holds. The instantaneous flow rate at the normal transport water level was 51.2 m^3 min^{-1} (Fig. 2.1). The standard errors for the calculated flow rates were between 1.37 to 2.34 m^3 min^{-1}. The flow rate measurements were done while sailing in flat conditions during slack tide

A flow rate of 51.2 m^3 min^{-1} generates a dilution rate ($D=V_W / V_R$) of 0.20 in a hold with a fish mass of 75,000 kg, which represents a 50% washout every 3.45 min and a 95% washout every 15 min (Steffensen, 1989). The average residence time of a molecule of water ($1/D$) is 4.75 min, which represents the time required for a water molecule to travel through the system and the time correction we needed to apply between the inflow and outflow oxygen measurements to calculate the oxygen removal by the fish. For our oxygen measurements which were taken at 3-min intervals, a lag-time of either 3 or 6 min can be incorporated into the bulk $\dot{M}O_2$ calculations.

A shorter lag time will result in the overestimation of bulk $\dot{M}O_2$ during periods of decreasing oxygen or increasing bulk $\dot{M}O_2$ but a longer lag-time will underestimate $\dot{M}O_2$ during the same periods. Therefore, the 3-min lag-time underestimates bulk $\dot{M}O_2$ during periods of increasing stress (i.e. decreasing oxygen concentrations or increasing rates of oxygen uptake) and provides a larger margin of error for welfare purposes.

Bulk $\dot{M}O_2$ was calculated from each hold using the equation:

$$\dot{M}O_2 = \frac{V_w \times \left(C_wO_{2in} - C_wO_{2out}\right)}{bw} \quad \text{(Eqn. 2.1)}$$

V_w = water flow through respirometer (l min^{-1})

C_wO_{2in} = inflow water oxygen concentration in forward compartment (mg O_2 l^{-1})

C_wO_{2out} = outflow water oxygen concentration in aft compartment (mg O_2 l^{-1})

bw = fish mass in respirometer (kg)

Measurement error

There is a certain amount of error associated with bulk $\dot{M}O_2$ measurement, as control of conditions and accuracy of equipment is never perfect. This error can be significant, especially under field conditions using non-specialized equipment. The total rate of error in our $\dot{M}O_2$ measurements was less than 10% with error associated with; the accuracy of the oxygen probes (<2%), fish mass estimates from the counting system (<3%) and variability in flow rate (<5%). Generally, this represents an acceptable error for measuring bulk $\dot{M}O_2$ during live-haul.

Routine metabolic oxygen consumption

Routine oxygen consumption was measured in individual adult Atlantic salmon (N=6, mass=1.7 ± 0.5 kg, fork length= 53 ± 4 cm) raised in outdoor sea-water tanks at the Centre for

Aquaculture and Environmental Research (CAER) laboratory in West Vancouver, BC, Canada. Fish were fed 3-times a week on commercial trout diets (Skretting, Vancouver, BC, Canada) and were starved for at least 72 h before $\dot{M}O_2$ was measured. In the morning a fish was netted from the tanks and lightly anaesthetized using 1:20000 MS-222 (Sigma-Aldrich, Oakville, Ontario, Canada). The fish was quickly transferred into a 127 l swim-respirometer, the swim chamber was covered with a black plastic bag to minimize light exposure, and the fish was allowed to recover for 24 h at a water velocity of approximately 0.3 body lengths sec^{-1}. The respirometer received water at ambient temperature during recovery and flushing periods (water temperature = 12.1 ± 0.2 °C). At this velocity fish rarely swam and mostly rested on the bottom of the swim chamber. During $\dot{M}O_2$ measurement, incoming water flow was stopped and water was re-circulated within the respirometer. A pump sampled water from the respirometer and circulated it past a thermostatted oxygen electrode (Oxyguard Handy Mark II, Point Four Systems, Richmond, BC, Canada) connected to a PC through a data logger. Dissolved oxygen concentrations were recorded using LabView 6.1 (National Instruments, Austin, TX, USA). Resting oxygen consumption was measured in triplicate for each fish for up to 30 min, or until oxygen concentration in the respirometer had dropped 0.5 mg l^{-1}, at the same water velocity of ~0.3 BL s^{-1}. Between trials, the respirometer was flushed with fresh sea water until oxygen concentrations returned to starting levels. Oxygen concentration did not drop below 7.0 mg l^{-1} during any trial.

Statistical analysis

The change in mean $\dot{M}O_2$ values over time was analyzed using repeated measures ANOVA (SigmaStat 3.01, Systat Software Inc., San Jose, CA, USA). $\dot{M}O_2$ values are presented as mean ± 95% confidence intervals.

A mixed effects regression model was created in SAS v.9.01 (SAS Institute Inc., Cary, NC, USA) to isolate the effects of density and temperature on bulk $\dot{M}O_2$. The independent

variables included were; time (h), density (kg m^{-3}), and temperature (°C). Bulk $\dot{M}O_2$ was reported as the dependent variable. Time was log-transformed and density rounded to the nearest 5 kg m^{-3}. The model adjusts for the effects of both temperature and density on bulk $\dot{M}O_2$, as well as adjusting for the correlation between the individual holds on each trip. At a significance level of $p < 0.05$, no significant interactions were found between density and temperature, density and time, temperature and time or density, temperature and time. Therefore, only direct effects are reported.

3-D plots of loading density vs. time, and water temperature vs. time interactions on bulk $\dot{M}O_2$ were created using the Loess smoothing function, a local smoothing function employing tri-cube weighting and linear regression in SigmaPlot 10.0 (Systat Software Inc., San Jose, CA, USA).

Results

A significant decrease in mean bulk $\dot{M}O_2$ was observed over 10 h of transport. Mean bulk $\dot{M}O_2$ decreased progressively from 2.98 ± 0.13 mg O_2 min^{-1} kg^{-1} after 1 h of transport to 2.30 ± 0.07 mg O_2 min^{-1} kg^{-1} after 6.5 h of transport. After 6.5 h, mean bulk $\dot{M}O_2$ did not change significantly from the 2.03 ± 0.14 mg O_2 min^{-1} kg^{-1} measured after 10 h of transport (Fig. 2.2).

Individual bulk $\dot{M}O_2$ measured after 1 h reached up to 4.58 mg O_2 min^{-1} kg^{-1} and 3.60 mg O_2 min^{-1} kg^{-1} after 10 h, but was as low as 1.39 mg O_2 min^{-1} kg^{-1} and 0.42 mg O_2 min^{-1} kg^{-1} after 1 h and 10 h of transport, respectively.

The analysis of bulk $\dot{M}O_2$ data using a mixed effects regression model revealed significant direct effects of both time and temperature on bulk $\dot{M}O_2$. The mean water temperature at 6.5 h was 9.9 ± 0.1 ºC. Temperature showed a significant effect on $\dot{M}O_2$, with a positive slope of 0.24 mg O_2 min^{-1} kg^{-1} $ºC^{-1}$ around the mean temperature and across the range of temperatures

observed (7.8-15.0 ºC) (Table 2.1). Bulk $\dot{M}O_2$ was not significantly affected by loading density over the range tested (67-150 kg m^{-3}).

Routine $\dot{M}O_2$

The routine $\dot{M}O_2$ measured for individual adult Atlantic salmon measured in the swim respirometer was 1.32 ± 0.13 mg O_2 min^{-1} kg^{-1}. In comparison to the routine $\dot{M}O_2$ measured in a 2 kg fish, routine $\dot{M}O_2$ in the 5 kg Atlantic salmon typically hauled for processing will be 15-20% lower, depending on the allometric scaling coefficient used.

Discussion

In general, bulk $\dot{M}O_2$ was low; suggesting that the stress level of fish during live-haul was similarly low. The decrease in bulk $\dot{M}O_2$ over time suggests that recovery from loading stressors occurred between 1 h and 6.5 h of transport (Fig. 2.2). Recovery during transport was also indicated by the statistical model, which showed that mean bulk $\dot{M}O_2$ was negatively correlated with time. The log-linear relationship between time and bulk $\dot{M}O_2$ provided a better fit than a linear relationship in the statistical model, giving an exponential rate of bulk $\dot{M}O_2$ recovery during transport, coinciding with the exponential rates of $\dot{M}O_2$ recovery observed following exhaustive stress in other salmonid species (Brett, 1965; Lee et al., 2003a).

Transported fish are exposed to changes in ambient water temperature during open-hold transport, which can vary by as much as 8 °C during a single trip, and also vary seasonally. Large and small fluctuations in temperature affect fish metabolic processes, changing $\dot{M}O_2$. The regression model showed a positive correlation between $\dot{M}O_2$ and water temperature. During the first 6.5 h of transport (while bulk $\dot{M}O_2$ was still declining) the temperature quotient (Q_{10}) of bulk $\dot{M}O_2$ was 1.9 (from 8-14 °C) and from 6.5 h onwards (after which bulk $\dot{M}O_2$ had stabilized) Q_{10} was 2.0 (from 8-12 °C). These values are comparable to the range of Q_{10} of 1.4-2.4 for routine $\dot{M}O_2$ found by Clarke and Johnston (1999) from 69 different species of fish across a

temperature range of 40 °C and also the Q_{10} of ~2.3 for routine $\dot{M}O_2$ in adult sockeye salmon (from 10-20°C) as measured by Lee et al. (2003b).

A 3-D surface plot of water temperature, time and bulk $\dot{M}O_2$ (Fig. 2.3A) also shows a positive correlation between temperature and the bulk $\dot{M}O_2$ of salmon during transport, indicating that bulk $\dot{M}O_2$ was highest in the upper temperature range, and during the beginning of the transport period.

According to the regression model, changing the loading density from 63 to 150 kg m^{-3} did not significantly affect bulk $\dot{M}O_2$, which suggests that the high densities involved in live-haul do not acutely stress adult Atlantic salmon. Non-significant increases in bulk $\dot{M}O_2$ were observed at the extremes of the density range when density was analyzed categorically (data not shown), and the 3-D surface plot of loading density, time and bulk $\dot{M}O_2$ (Fig. 2.3B) shows increases in bulk $\dot{M}O_2$ at higher loading densities, and again at the beginning of the transport period. With very high loading densities, stress due to excessive crowding could result in the elevation of bulk $\dot{M}O_2$, but with very low loading densities, the increased space may encourage greater activity which will also elevate bulk $\dot{M}O_2$. The density at which a minimum bulk $\dot{M}O_2$ is observed may indicate an optimal transport density, however the benefits of lower bulk $\dot{M}O_2$ during transport must be assessed relative to the cost-effectiveness of using higher loading densities.

$\dot{M}O_2$ comparisons

Bulk $\dot{M}O_2$ during transport was comparable to routine and standard $\dot{M}O_2$ of various salmonid species but elevated over the routine $\dot{M}O_2$ measured for adult Atlantic salmon (Table 2.2). Increased $\dot{M}O_2$ due to swimming activity must be taken into account when comparing the routine $\dot{M}O_2$ measured from mostly non-swimming salmon in respirometers and the bulk $\dot{M}O_2$ of salmon swimming against a current (up to 0.5 BL s^{-1}) in the holds of the live-haul vessel. Bulk $\dot{M}O_2$ during transport was similar to bulk $\dot{M}O_2$ in adult Atlantic salmon measured in large salt-

water tanks by Bergheim et al. (1991) and Bergheim et al. (1993), which ideally represents the typical daily range of $\dot{M}O_2$ of salmon in intensive culture conditions (Table 2.2). However, the bulk $\dot{M}O_2$ studies conducted in the salt-water tanks measured fish being fed to satiation, which is known to significantly increase $\dot{M}O_2$ (Brett and Groves, 1979). Forsberg (1997) observed that $\dot{M}O_2$ in adult Atlantic salmon increased from 0.95 ± 0.06 mg O_2 min^{-1} kg^{-1} to 2.06 ± 0.09 mg O_2 min^{-1} kg^{-1} in fish fasted for 10 days or fed to satiation, respectively. Therefore the elevation of bulk $\dot{M}O_2$ during transport likely does not stress fish beyond levels experienced during normal rearing conditions. In comparison to the scope of $\dot{M}O_2$ measured in individual Pacific salmon using swim-respirometers, $\dot{M}O_2$ during transport was comparable to routine rates, again suggesting fish are not seriously stressed.

When routine $\dot{M}O_2$ is elevated, the aerobic scope of an animal is reduced (defined as the difference between maximum and minimum aerobic rates). A reduction in aerobic scope reduces energy available for other biological processes, which can have serious consequences when high levels of aerobic exertion are required, i.e. during foraging or ascending rapids. For cultured fish in controlled environments, these reductions in aerobic scope are less important, but may have implications in growth rates and feed efficiencies. Nevertheless, many species of salmonids have demonstrated very high aerobic swimming performance during experiments involving significantly reduced aerobic scope (Farrell et al., 2003).

Comparison to previous studies

One objective of this study was to expand on the initial findings of Farrell (2006) and provide a more comprehensive assessment of transport welfare using bulk $\dot{M}O_2$. Farrell (2006) had previously calculated the flow rate of water into the holds by measuring the time required to fill the empty holds while the vessel was in motion. Further investigation indicated that this had resulted in overestimation of the flow rate during the initial filling stages, when water is enters

through both forward and aft hull-valves, being driven by gravity. When the holds are full, water enters only through the forward hull valves, being driven by the forward motion of the vessel. When the flow rate of just the forward hull-valves was measured with the holds nearly full, the flow rate was only 59% of the previous calculations. Recalculating the bulk $\dot{M}O_2$ values measured by Farrell (2006) yields values in agreement with bulk $\dot{M}O_2$ values measured in the current study (Table 2.3).

Loading stress and recovery during transport

The highest stress levels were measured immediately after 1 h of transport, likely as a result of residual stress due to the loading procedures. This observation agrees with the findings of Farrell (2006) and others (Specker and Schreck, 1980; Barton and Peter, 1982; McDonald et al., 1993; Barton, 2000) that suggest that loading stressors are the greatest source of stress during fish transport. Even with considerable variation in conditions occurring during live-haul transport, recovery from initial loading stressors was seen. The true maximum rate of bulk $\dot{M}O_2$ most likely occurred within the 60 min of loading, but before measurements began. The amount of stress fish experienced during loading will largely be determined by the length of time spent loading fish, which can vary considerably depending on environmental conditions, equipment and operators. However, once fish are loaded and transport commences, decreasing bulk $\dot{M}O_2$ suggests that conditions are conducive for full recovery from even severe loading stressors.

Recovery of bulk $\dot{M}O_2$ during transport was complete by 6.5 h, which is longer than recovery times for $\dot{M}O_2$ of 3.2 h in juvenile sockeye (Brett, 1964) and 1.2 h in adult sockeye (Lee et al., 2003a) swum to exhaustion. This indicates that fish are likely still experiencing some degree of stress as they acclimate to their new environment and in response to fluctuating environmental conditions.

Conclusions

$\dot{M}O_2$ is useful in providing a general overview of stress levels in an animal. When measured in populations of fish, the bulk $\dot{M}O_2$ represents the average rate of $\dot{M}O_2$ of the individuals. The mean bulk $\dot{M}O_2$ measured during transport was comparable to routine rates of both the routine $\dot{M}O_2$ of individual salmon and standard rates of $\dot{M}O_2$ in fish during intensive culture. These low rates of oxygen uptake suggest that fish are experiencing relatively low stress levels in live-holds during transport and are not forced to function outside of their physiological means. While bulk $\dot{M}O_2$ may not be a comprehensive measure of stress or welfare, it can indicate the cumulative response of transported fish to the multiple stressors encountered during live-haul, including high loading densities and changes in water temperature. We conclude that bulk $\dot{M}O_2$ indicated adult Atlantic salmon experienced conditions that promoted good fish welfare during live-haul transport aboard the *Sterling Carrier*, as the measured values were low compared to the range of $\dot{M}O_2$ that adult salmonids are capable of achieving. Furthermore, we suggest that the benefits of quantitatively monitoring fish stress levels during transport without causing additional disturbances or using lethal sampling is itself beneficial for fish welfare.

Summary

1) Adult Atlantic salmon welfare was acceptable during transport as evidenced by low rates of bulk oxygen uptake (bulk $\dot{M}O_2$) throughout 10 h of live-haul transport.

2) Temperature affected bulk $\dot{M}O_2$ in a manner that agreed with observed temperature coefficients for $\dot{M}O_2$ in adult salmon.

3) Loading density did not significantly affect bulk $\dot{M}O_2$ over the range of 63-150 kg m^{-3}.

Table 2.1: Mixed effects linear regression of time, temperature and loading density on bulk $\dot{M}O_2$ during transport. Time and temperature significantly affected bulk $\dot{M}O_2$. Loading density did not significantly affect bulk $\dot{M}O_2$ over the range of densities tested.

Independent variable	Slope (ß – coefficient)	Standard Error	*p*
Time (log-h)	-0.14	0.03	<.0001
Temperature (°C)	0.24	0.01	<.0001
Loading density (kg m^{-3})	-0.01	0.01	0.6821

Table 2.2: Comparison of $\dot{M}O_2$ from various species of adult *Oncorhynchus* and *Salmo* species of salmon under various conditions. Transport $\dot{M}O_2$, routine $\dot{M}O_2$ and maximum $\dot{M}O_2$ rates measured in a) bulk or b) individually for various species of adult salmon.

Salmonid species	Activity level	$\dot{M}O_2$ (mg O_2 min^{-1} kg^{-1})	Temp (°C)	Source
Atlantic *Salmo salar* *(1.5-5.5 kg)*	Transport start[a]	2.98 ± 0.13	7.8-15.0	Current study
	Transport end[a]	2.00 ± 0.06		
	Routine[a]	1.32 ± 0.13	12.1 ± 0.2	
	Routine[a]	1.5-4.5	5.5-10.3	Bergheim et al. (1991)
	Routine[a]	1.7-3.5	7.2-9.1	Bergheim et al. (1993)
	Routine[a]	0.89-2.15	8.5	Forsberg (1997)
sockeye *Oncorhynchus nerka* *(1.9-3.3 kg)*	Routine[b]	2.99 ± 0.23	16.3 ± 0.3	Farrell et al. (2003)
	Maximum[b]	12.28 ± 0.75		
pink *O. gorbuscha* *(1.3-1.9 kg)*	Routine[b]	4.25 ± 0.69	11.8 ± 0.2	
	Maximum[b]	12.63 ± 0.44		
coho *O. kisutch* *(2.1-2.5 kg)*	Routine[b]	2.23 ± 0.09	5-12	Lee et al. (2003b)
	Maximum[b]	8.77 ± 0.		
chinook *O. tshawytscha* *(3.7 to 6.4 kg)*	Routine[b]	1.99 ± 0.15	8-17	Geist et al. (2003)
	Maximum[b]	10.94 ± 0.52		

Table 2.3: Comparison of previous bulk $\dot{M}O_2$ transport results to current results. Bulk $\dot{M}O_2$ values measured by Farrell (2006) re-calculated using a flow-rate of 51.2 m^3 min^{-1} are comparable to the mean bulk $\dot{M}O_2$ measured during the current study.

Time of transport	Bulk $\dot{M}O_2$ (Farrell, 2006) (mg O_2 min^{-1} kg^{-1})	Recalculated $\dot{M}O_2$ (mg O_2 min^{-1} kg^{-1})	Current study (mean ± 95% CI)
20 min	>8	5.3	N/A
1 h	4.5	2.7	2.98 ± 0.13
4 h	3.1	1.8	2.42 ± 0.20
10 h	3.1	1.8	2.03 ± 0.14

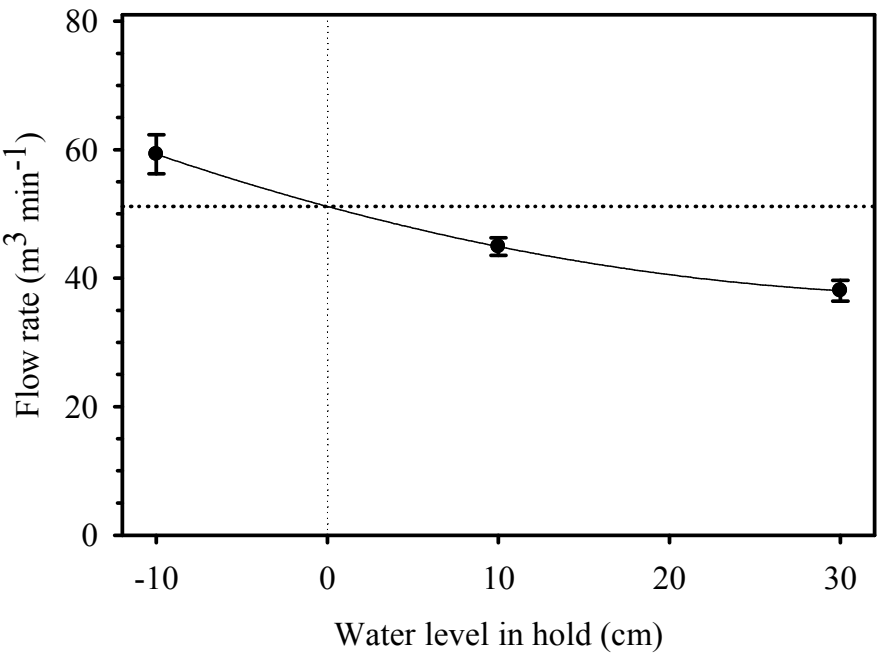

Figure 2.1: Calibration of flow-rate through live-holds. Flow-rate was measured by filling the top section of the hold with only the forward hull-valves opened. The dotted lines indicate the water level in the combing (0 cm) which corresponds to the flow rate (51.2 m^3 min^{-1}) that occurs during normal transport speeds with both forward and aft hull-valves open.

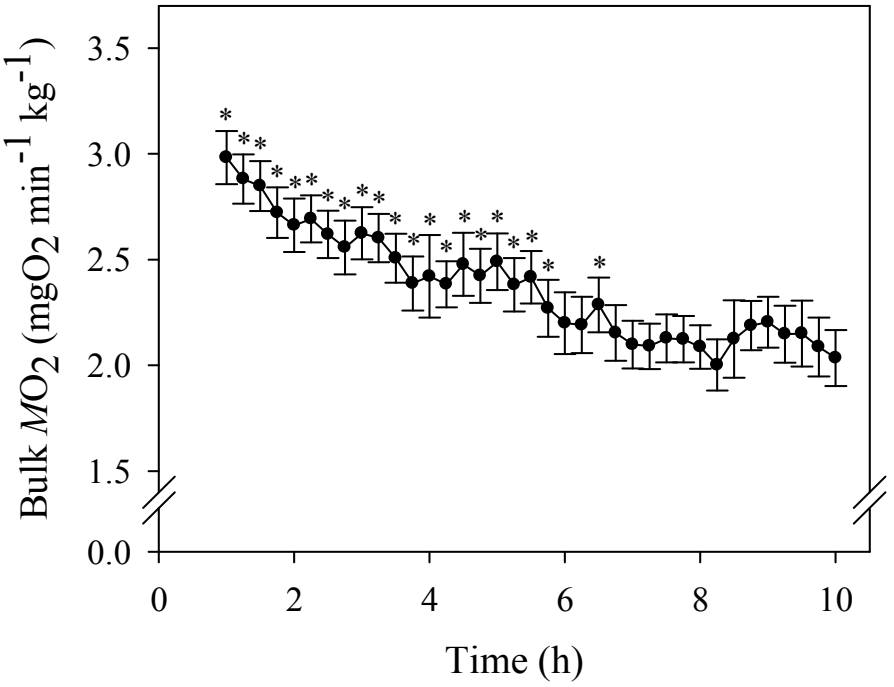

Figure 2.2: Bulk $\dot{M}O_2$ during open-hold live-haul transport. Mean bulk $\dot{M}O_2$ measured from port and starboard holds during open-hold live-haul transports occurring between May to September 2003. Values significantly different from the final $\dot{M}O_2$ at 10 h are indicated by an '*' (RMANOVA, $p < 0.05$). Values are mean ± 95% C.I., N=89.

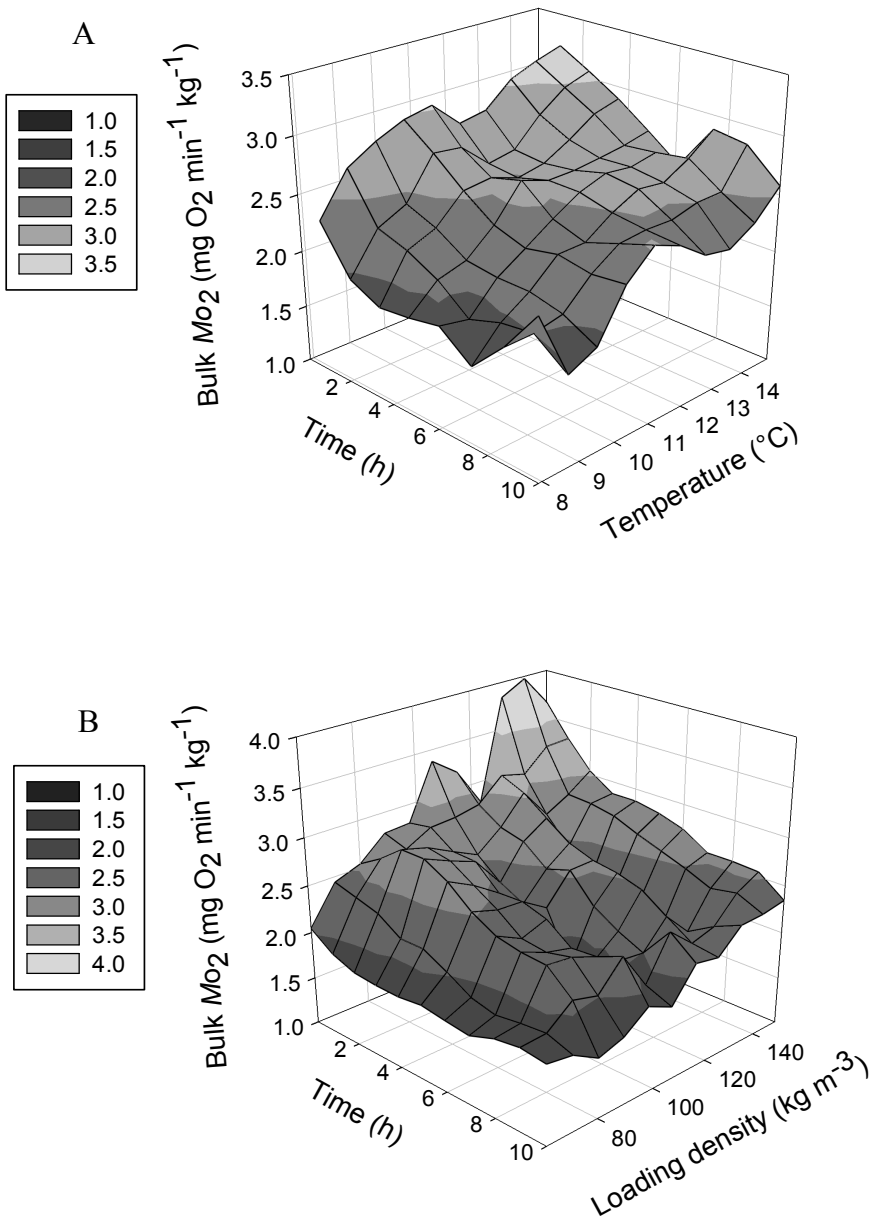

Figure 2.3: 3-D surface plots of water temperature, time, loading density and bulk $\dot{M}O_2$. 3-D surfaces were generated using Loess smoothing to approximate the effects of A) water temperature and time on bulk $\dot{M}O_2$ and B) loading density and time on bulk $\dot{M}O_2$. Surfaces are generated from combined port and starboard bulk $\dot{M}O_2$ data from all trips (N=89).

References

Barton, B. A. (2000). Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. N. Am. J. Aquacult. 62, 12-18.

Barton, B. A. and Peter, R. E. (1982). Plasma-cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anesthesia, and cold shock. J. Fish Biol. 20, 39-51.

Barton, B. A., Peter, R. E. and Paulencu, C. R. (1980). Plasma-cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Can. J. Fish. Aquat. Sci. 37, 805-811.

Berg, T., Erikson, U. and Nordtvedt, T. S. (1997). *Rigor mortis* assessment of Atlantic salmon (*Salmo salar*) and effects of stress. J. Food Sci. 62, 439-446.

Bergheim, A., Forsberg, O. I. and Sanni, S. (1993 Biological basis for landbased farming of Atlantic salmon: Oxygen consumption. In *Fish Farming Technology*, (eds. H. Reinertsen L. A. Dahle L. Jorgensen and K. Tvinnereim), pp. 289–295. Rotterdam: A.A. Balkema.

Bergheim, A., Seymour, E. A., Sanni, S., Tyvold, T. and Fivelstad, S. (1991). Measurements of oxygen-consumption and ammonia excretion of Atlantic salmon (*Salmo salar* L.) in commercial-scale, single-pass fresh-water and seawater landbased culture systems. Aquac. Eng. 10, 251-267.

Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21, 1183-1226.

Brett, J. R. (1965). Relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 22, 1491-1501.

Brett, J. R. and Groves, T. D. D. (1979). Physiological energetics. In *Fish Physiol.*, vol. VIII, (eds. W. S. Hoar D. J. Randall and J. R. Brett), pp. 279-352. Orlando, Florida: Academic Press.

Clarke, A. and Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. J. Anim. Ecol. 68, 893-905.

Erikson, U., Sigholt, T. and Seland, A. (1997). Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 149, 243-252.

Farrell, A. P. (2006). Bulk oxygen uptake measured with over 60,000 kg of adult salmon during live-haul transportation at sea. Aquaculture 254, 646-652.

Farrell, A. P., Lee, C. G., Tierney, K., Hodaly, A., Clutterham, S., Healey, M., Hinch, S. and Lotto, A. (2003). Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish Biol. 62, 64-84.

Forsberg, O. I. (1997). The impact of varying feeding regimes on oxygen consumption and excretion of carbon dioxide and nitrogen in post-smolt Atlantic salmon *Salmo salar* L. Aquac. Res. 28, 29-41.

Geist, D. R., Brown, R. S., Cullinan, V. I., Mesa, M. G., Vanderkooi, S. P. and McKinstry, C. A. (2003). Relationships between metabolic rate, muscle electromyograms and swim performance of adult chinook salmon. J. Fish Biol. 63, 970-989.

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

Iversen, M., Finstad, B. and Nilssen, K. J. (1998). Recovery from loading and transport stress in Atlantic salmon (*Salmo salar* L.) smolts. Aquaculture 168, 387-394.

Jittinandana, S., Kenney, P. B., Mazik, P. M., Danley, M., Nelson, C. D., Kiser, R. A. and Hankins, J. A. (2005). Transport and stunning affect quality of arctic char fillets. J. Muscle Foods 16, 274-288.

Lee, C. G., Farrell, A. P., Lotto, A., Hinch, S. G. and Healey, M. C. (2003a). Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. J. Exp. Biol. 206, 3253-3260.

Lee, C. G., Farrell, A. P., Lotto, A., MacNutt, M. J., Hinch, S. G. and Healey, M. C. (2003b). The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch)* salmon stocks. J. Exp. Biol. 206, 3239-3251.

MacNutt, M. J., Hinch, S. G., Lee, C. G., Phibbs, J. R., Lotto, A. G., Healey, M. C. and Farrell, A. P. (2006). Temperature effects on swimming performance, energetics, and aerobic capacities of mature adult pink salmon (*Oncorhynchus gorbuscha*) compared with those of sockeye salmon (*Oncorhynchus nerka*). Can. J. Zool. 84, 88-97.

Maule, A. G., Schreck, C. B., Bradford, C. S. and Barton, B. A. (1988). Physiological-effects of collecting and transporting emigrating juvenile chinook salmon past dams on the Columbia river. Trans. Am. Fish. Soc. 117, 245-261.

McDonald, D. G., Goldstein, M. D. and Mitton, C. (1993). Responses of hatchery-reared brook trout, lake trout, and splake to transport stress. Trans. Am. Fish. Soc. 122, 1127-1138.

Nikinmaa, M., Soivio, A., Nakari, T. and Lindgren, S. (1983). Hauling stress in brown trout (*Salmo trutta*) - physiological-responses to transport in fresh-water or salt-water, and recovery in natural brackish water. Aquaculture 34, 93-99.

Schreck, C. B., Solazzi, M. F., Johnson, S. L. and Nickelson, T. E. (1989). Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch*. Aquaculture 82, 15-20.

Specker, J. L. and Schreck, C. B. (1980). Stress responses to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts. Can. J. Fish. Aquat. Sci. 37, 765-769.

Steffensen, J. F. (1989). Some errors in respirometry of aquatic breathers - how to avoid and correct for them. Fish Physiol. Biochem. 6, 49-59.

Chapter 3: Modeling hypercarbia during high-density re-circulating transport of adult Atlantic salmon, *Salmo salar* based on observations aboard a commercial live-haul vessel[2]

Introduction

Global expansion in the production and consumption of aquaculture products (FAO, 2006) has increased social awareness regarding the welfare of the animals being cultivated. It is widely accepted that intensive fish culture imposes unavoidable stressors on fish and thus industries should maximize welfare conditions to enhance the quality of life of animals being raised and potentially the quality of product being produced as a result. To ensure the highest quality flesh products, the aquaculture industry, like many other livestock operations, live-haul their animals to dedicated processing plants. Currently, the salmon aquaculture industry in British Columbia, live-hauls over 55,000 tonnes of adult Atlantic salmon per year from sea-cages scattered along the coast to central processing plants.

Efficient, cost-effective transport requires large numbers of fish to be hauled at high densities and if transport conditions are poor, thousands of fish are put at significant risk. As such, live transport presents a serious risk for fish welfare. High densities, handling and poor water quality can all cause significant stress during transport. Ambient water quality can also become an issue during open live-haul in the event of an algal bloom or oil-spill as holds are flushed with ambient water. Alternately, water can be re-circulated within the ship and transport can occur in a re-circulating (closed-hold) system to prevent exposure of fish to dangerous ambient water conditions. Whenever water is re-circulated the rate of oxygen depletion by large masses of fish from relatively small volumes of water necessitates supplemental oxygenation,

[2] A version of this chapter will be submitted for publication. Tang, S., Thorarensen, H., Brauner, C.J., Farrell, A.P., 2008. Modeling hypercarbia during high-density re-circulating transport of adult Atlantic salmon, *Salmo salar* based on observations aboard a commercial live-haul vessel.

however, even if oxygen requirements are met, toxic excretory products such as CO_2 and NH_3 accumulate, resulting in a rapid and progressive deterioration of water quality.

Despite such concerns, and numerous studies documenting significant stress during closed-hold transport in salmonids (Barton et al., 1980; Specker and Schreck, 1980; Barton and Peter, 1982; Nikinmaa et al., 1983; Maule et al., 1988; McDonald et al., 1993; Erikson et al., 1997; Iversen et al., 1998; Barton, 2000; Iversen et al., 2005), few studies report changes in CO_2 buildup. To the best of our knowledge, the only study to report a significant accumulation of CO_2 during transport actually found that CO_2 did not significantly affect stress, but that stress levels decreased despite increasing CO_2 levels (Barton and Peter, 1982). This contrasts with general knowledge showing that elevated levels of CO_2 activate the primary stress response in fish (Perry et al., 1989) and cause significant disturbances in salmonid physiology including: a near immediate reduction in plasma pH (Eddy et al., 1977; Thomas, 1982); increased ventilation rate and volume (Janssen and Randall, 1975; Thomas et al., 1983; Fivelstad et al., 1999) reduced blood oxygen content and carrying capacity through Bohr and Root effects (Eddy and Morgan, 1969; Eddy et al., 1977; Wedemeyer, 1996); narcosis, anesthesia and eventually death at higher levels (Bernier and Randall, 1998).

The partial pressure of CO_2 (Pco_2) of surface waters in the ocean is usually very low, in the range of 0.15-0.30 mmHg. The Pco_2 of a fish's blood is only 1-2 mmHg above that of its environment (Heisler, 1986) despite continuous CO_2 production by tissues. Due to the high ventilation volume required for O_2 uptake and the high capacitance of water for CO_2, the gills can be considered hyperventilated relative to CO_2, which maximizes CO_2 removal from the blood. The permeability of fish gills to CO_2 is also high, and elevations in external Pco_2 (hypercarbia) are rapidly equilibrated internally, elevating Pco_2 of the blood (hypercapnia). The low Pco_2 of surface waters (0.15-0.30 mmHg) and fish blood mean that fish are highly

susceptible to even small elevations in environmental Pco_2, and the log-linear relationship between Pco_2 and pH results in large changes in pH with small increases in Pco_2 at the low Pco_2 tensions that typically exist in natural waters. Once inside the fish, CO_2 is rapidly converted into carbonic acid (via carbonic anhydrase), and the resulting acid load is quickly equilibrated throughout the body, resulting in a hypercapnic acidosis proportional to the degree of hypercarbia experienced.

Therefore, hypercapnia is capable of causing stress and negatively impacting fish welfare. Indeed, studies investigating re-circulating or closed conditions report significant stress during transport through elevated plasma cortisol concentrations (Barton et al., 1980; Specker and Schreck, 1980; Barton and Peter, 1982; Barton, 2000) plasma glucose and lactate concentrations (Mazeaud et al., 1977; Nikinmaa et al., 1983; Carneiro and Urbinati, 2002) or mortality rates (Specker and Schreck, 1980; Carmichael, 1984; Pavlidis et al., 2003).

Despite this knowledge, and the expectation that CO_2 could accumulate to potentially dangerous levels, there is a lack of knowledge regarding the rate of CO_2 accumulation during live haul transport. In fact, non-lethal thresholds for CO_2 exposure during transport of adult Atlantic salmon have not been described. Currently suggested CO_2 thresholds for Atlantic salmon of 12-30 mg/L (~ 5-12 mmHg Pco_2) are intended for rearing conditions, when CO_2 impacts on growth rate, feed conversion rate, and immune status must be considered (Smart et al., 1979; Wedemeyer, 1996; Fivelstad et al., 1998). Wedemeyer (1996) suggests that fish can likely tolerate levels of up to 40 mg l^{-1} under transport conditions due to the limited exposure periods involved. In addition, CO_2 thresholds measured using mg l^{-1} units cannot be applied universally, since concentrations of CO_2 do not indicate the Pco_2 in solution, which is the biologically relevant measure of CO_2 toxicity for fish.

With the help of the *Sterling Carrier*, a state-of-the-art live-haul vessel operating on the west coast of British Columbia, Canada, we observed fish under closed-hold transport and measured initial rates of CO_2 accumulation under commercial live-haul conditions. These experiments were limited in duration for the safety of the transported fish, but the collected data were used to create a model of CO_2 accumulation under various transport scenarios. In addition, by using measured bulk oxygen consumption rates (bulk $\dot{M}O_2$) for adult Atlantic salmon during live-haul transport (Chapter 2), CO_2 accumulation rates in the hauling water were calculated for typical respiratory exchange ratio (RER, the molar ratio of CO_2 excreted and O_2 consumed). RER typically varies between 0.7-1.3, increasing with activity level and in relation to the tissue level O_2 consumption and CO_2 production ratio, or respiratory quotient (RQ).

For the purposes of this study, an arbitrary Pco_2 threshold was set at 10 mmHg, which represents a significant elevation over environmental levels (~50x normal) but one that is typically non-lethal. By modeling different transport scenarios from the data collected during actual transports, we assessed the risk to fish welfare during high density live-haul transport of adult Atlantic salmon up to a 10 mmHg threshold of CO_2 exposure, though the models we developed can be extrapolated to levels beyond this threshold.

Materials and Methods

Transport vessel

A detailed description of the *Sterling Carrier* and its equipment is documented in Farrell (2006). Briefly, the *Sterling Carrier* is a 40 m vessel with two 325 m^3 live-holds that are flooded via a series of 14" hydraulic valves (four forward and three aft valves per hold), producing a directional water flow rate of ~50 m^3 min^{-1} from forward to aft while the vessel is traveling at 9 knots (routine transport speed for the vessel). If the hull valves are closed, water in each hold is

re-circulated through separate loops using pumps, capable of re-circulating water at 20 m^3 min^{-1} To maintain dissolved oxygen concentrations, pure O_2 gas is injected directly into the re-circulating water through spargers prior to re-entering the holds through a pair of perforated PVC diffusing columns that run lengthwise along the bottom of each hold

Closed-hold transport protocol

Six experiments were conducted (July 9-13, 2006 and November 13-16, 2006) during routine commercial transports around Vancouver Island, BC. Each experiment started at least 3.5 h into each trip to allow recovery from loading stressors (see Chapter 2), and experiments were scheduled to end at least 2 h before arrival at the processing plant to allow fish to recover before offloading. Only a single experiment was conducted per trip to ensure true replication. Mean fish density (± SEM) during the trips was 135 ± 4 kg m^{-3}, mean fish mass was 5.7 ± 0.2 kg, mean water temperature was 10.6 ± 1.2 ºC and mean salinity was 31 ± 1 ppt.

Visual observation of fish was possible from the hatches on deck and via closed-circuit cameras located in the holds. Fish behaviour was monitored during all experiments for signs of distress by both scientists on deck and the captain, on the bridge.

Water monitoring and sampling

Hull valves were closed in the starboard hold for 30 min for each re-circulation experiment. During this time, water parameters were recorded and water samples taken every 5 min during re-circulation and then for 15 min following re-establishment of flow-through conditions when valves were re-opened and the holds were flushed.

A water sampling circuit was set up using a submersible pump (5 l min^{-1}) sampling water from the starboard hold at a depth of 2 m. The sampling chamber was a 1 L Erlenmeyer flask into which the pH probe (Symphony 14002-764, VWR Scientific) from a handheld pH meter (Symphony SP301, VWR) was inserted. A 3-way stop-cock allowed water to be sampled from

the circuit into 250 ml BOD bottles. Water samples were drawn and 2.5 ml of saturated $HgCl_2$ was added (to prevent microbial respiration) before the bottles were sealed and all samples were analyzed within 7 days. Water leaving the circuit was returned to the hold below the surface.

Dissolved oxygen concentration was maintained above 7.0 mg l^{-1} during the experiments by injecting oxygen from onboard oxygenation systems and monitored by the onboard oxygen sensors mounted inside the holds (Oxyguard III, Point Four Systems, BC) and a handheld meter (Oxyguard model). Water pH and temperature were measured using an epoxy combination pH/temperature electrode (Symphony 14002-764, VWR Scientific) and handheld pH meter (Symphony SP301, VWR Scientific). This occurred both during the onboard water sampling and during analysis of total CO_2.

Water CO_2 measurements

Total CO_2 (TCO_2 – representing the concentration of all species of CO_2 in water) was analyzed at the University of British Columbia from water samples on a gas chromatograph (Carle Model III, Fullerton, California, USA) connected to a chart recorder (Soltec 1241, San Fernando, California, USA) as described in Brauner et al. (2000). A 2 ml water sample, a 4 ml of 1 M HCl saturated with N_2 gas and 6 ml of N_2 gas were drawn into a 10 ml syringe and mixed on a rotary mixer for 4 min. As the water was acidified, the CO_2 expelled into the N_2 gas phase was forced through a dehydrating filter directly into the sampling loop of the gas chromatograph.

TCO_2 was calculated from the area under the peak against standard curves measured from fresh bicarbonate standards made daily ($NaHCO_3$, Sigma-Aldrich, Oakville, Ontario, Canada). Pco_2 was calculated from TCO_2 and pH using the Henderson-Hasselbalch equation solved for Pco_2 (Eqn. 3.1; Cameron, 1972).

Henderson-Hasselbalch equation solved for Pco_2:

$$Pco_2 = \frac{TCO_2}{\alpha 10^{pH-pK^1}+1} \quad \text{(Eqn. 3.1)}$$

Pco_2 = partial pressure of CO_2 (mmHg)

TCO_2 = total CO_2 in solution (mmol l^{-1})

α = solubility constant of CO_2 (mmol l^{-1} $mmHg^{-1}$)

pH = pH of solution

pK^1 = first dissociation constant of CO_2

CO_2 accumulation model

A model was created to examine the effects of various re-circulating transport conditions on water quality. The user can input the transport water parameters, including: temperature, salinity, initial water pH, background TCO_2/Pco_2; the transport fish density; and the fish physiological parameters including: RER and $\dot{M}O_2$ (as a specific value or as percentage of the maximum $\dot{M}O_2$).

The model predicted changes in water Pco_2 due to fish respiration over time according to the following equation:

$$Pco_2 = \frac{TCO_{2ac} + TCO_{2bg}}{\alpha 10^{pH-pK^1}+1} \quad \text{(Eqn. 3.2)}$$

TCO_{2ac} = moles of accumulated total CO_2 from respiration (mmol l^{-1})

TCO_{2bg} = moles of background total CO_2 (mmol l^{-1})

The amount of CO_2 created through respiration is calculated from the equation:

$$TCO_{2ac} = \frac{\left(\dot{M}O_2 \times RER \times mass\right) \times t}{V_r} \quad \text{(Eqn. 3.3)}$$

$\dot{M}O_2$ = oxygen consumption rate (input as mg O_2 min^{-1} kg^{-1}, converted to mmol O_2 min^{-1} kg^{-1})

RER = respiratory exchange ratio (moles CO_2 excreted per mole O_2 consumed, 1.0 was used)

mass = total fish mass per hold (kg)

t = time (min)

V_r = water volume of hold (l)

The solubility (α) and 1st dissociation constant of CO_2 (pK^1) are derived from tables presented in Boutilier et al. (1984) and Mehrbach et al. (1973) (Table 3.1). The accumulation of TCO_2 in the system drives both an increase in Pco_2 and decrease in pH. As water Pco_2 is also highly dependent on pH, we needed to be able to predict changes in pH accurately with changes in TCO_2. Unfortunately, the relationship between TCO_2 and water pH derived from the collected water samples was poor, making pH difficult to predict from TCO_2. Since water pH and Pco_2 are tightly correlated, we generated a pH/Pco_2 curve (Fig 3.2) from the collected water samples and predicted changes in water pH according to Pco_2. However, to accurately predict the water Pco_2 (Eqn. 3.2) at a given point in time requires the water pH for the same time, but this was not possible when water pH was being derived from water Pco_2. Instead, water Pco_2 was calculated in 1 min increments using the water pH from the previous time increment, with the assumption that the change in water pH between increments would be small. This overestimation of pH results in an underestimation of Pco_2 proportional to the length of time between each calculation (the amount of TCO_2 accumulated). The amount of error accumulates with each progressive calculation, reaching ~10% after 1000 calculations (1000 min) but error was less than 2% at the

longest length of time modeled in this study, and occurred under the least stressful conditions examined.

Results

Closed-hold transport of adult Atlantic salmon

Water $P\text{co}_2$ increased at a nearly linear rate and water pH decreased non-linearly during the 30-min recirculation periods (Fig. 3.1). During this time, water $P\text{co}_2$ increased from 0.51 ± 0.04 mmHg to 2.49 ± 0.38 mmHg and water pH decreased 0.4 units from 7.76 ± 0.11 to 7.34 ± 0.09. After 15 min of flushing, $P\text{co}_2$ recovered to 0.78 ± 0.10 mmHg and pH recovered to 7.82 ± 0.09, neither of which were significantly different from the initial ambient water conditions ($p>0.05$).

During the later stages of re-circulation, fish were observed to increase activity and flush to a brighter blue color, suggesting that the fish were responding to the changing water quality conditions (i.e. $P\text{co}_2$ = 2.5 mmHg and pH = 7.3).

CO_2 accumulation model

Modeling CO_2 accumulation across a range of transport densities used for live-haul in British Columbia reveals that the time to reach the 10 mmHg $P\text{co}_2$ threshold at a routine metabolic rate is reduced as density increases (Fig. 3.3A). At the lowest density the 10 mmHg $P\text{co}_2$ threshold is reached in 150 min, and this decreases with fish density, such that at a density of 170 kg m^{-3} the same threshold is reached after only 56 min.

Elevating $\dot{M}O_2$ reduces the time required to reach the 10 mmHg $P\text{co}_2$ threshold (Fig.3.3B). At a maximal rate of $\dot{M}O_2$, the time required to reach the threshold under low density is reduced to 48 min and likewise at high density the time required to reach the 10 mmHg $P\text{co}_2$ threshold is reduced to only 19 min. Water pH decreases as $P\text{co}_2$ increases and under the

modeled conditions pH reached 6.60 ± 0.01 at 10 mmHg $P\mathrm{co}_2$. Changes in fish density or their $\dot{M}\mathrm{O}_2$ have no effect on the final pH, but will affect the rate of decrease (Fig. 3.3C,D).

Both the time to reach the 10 mmHg $P\mathrm{co}_2$ threshold and the pH at the $P\mathrm{co}_2$ threshold were affected by changes in ambient water temperature and salinity. Salinity within the range of the model (25-35 ppt) had little effect on the rate of CO_2 accumulation or final pH, temperature also had little effect on the final pH but significant effects on the rate of CO_2 accumulation over the range of the model (0-25 °C) (Table 3.2).

Discussion

During re-circulated transport, fish welfare is at risk as water quality deteriorates with the continuous accumulation of CO_2. This study represents the first time that welfare has been assessed and modeled for closed-hold conditions aboard a live-haul vessel carrying adult Atlantic salmon at commercial densities. We found that water $P\mathrm{co}_2$ increased in a nearly linear fashion, with a concomitant drop in water pH during 30 min of re-circulation. We also observed that re-opening the hull-valves (to permit flushing of the holds with ambient water) rapidly reduced $P\mathrm{co}_2$ levels and completely restored water quality within 15 min. During closed-hold experiments, the water pH was continuously monitored, and any experiment was terminated if water pH fell below pH 7.0, indicating a $P\mathrm{co}_2$ of ~6.5 mmHg based on local water conditions. After 30 min of re-circulation, $P\mathrm{co}_2$ was still well below the recommended CO_2 threshold of 10 mmHg. Other salmonids have been exposed to $P\mathrm{co}_2$ levels ranging from 15 mmHg (Eddy et al., 1977) to 78 mmHg (Bernier and Randall, 1998) without mortality and with full recovery following return to control conditions. Water pH was also substantially higher than the sub-lethal pH limits of 4 – 4.5 assessed by Butler et al. (1992) and still within the recommended pH range

of 6.5-8.5 (Wedemeyer, 1996) and thus Pco_2 and pH levels fish experienced were not expected to present a serious threat to fish welfare during closed-hold experiments.

The 10 mmHg water Pco_2 threshold was based on the findings of Fivelstad et al. (1998) who assessed safe levels of CO_2 exposure during rearing of post-smolt Atlantic salmon. Their results showed that exposure to Pco_2 levels up to 12 mmHg did not produce any significant decreases in growth factors or increases in mortality rate. However, their observed mortality rate of 1.1% at 12 mmHg (i.e. one fish died after 43 days of exposure in one of the two replicate groups) would be unacceptable during typical transports, where mortalities rarely reach 1%. The mortality rate on the *Sterling Carrier* was only 0.21% of 1.44 million fish in 116 trips during the period of Oct. 2005-Mar. 2006.

Monitoring Pco_2 during closed-hold live-haul presents its own challenges. Accurate methods of measuring Pco_2 are complicated, time-consuming and expensive and are not practical in a commercial live-haul setting. Standard titration methods are equally time consuming and can be extremely inaccurate and are poorly suited for monitoring progressively increasing Pco_2 levels. Since Pco_2 is largely determined by water pH, changes in Pco_2 are almost immediately reflected by changes in water pH. Measurement of pH is fast and accurate and could be used to continuously estimate Pco_2 levels in hauling water during a closed-hold event. Under the water conditions presented, it is evident that once pH dropped below 6.6, the 10 mmHg threshold was exceeded (Fig. 3.3). Due to shifts in the CO_2 equilibrium with decreases in the water pH, Pco_2 increases at an exponential rate, therefore increasing the Pco_2 threshold by 2X will not yield 2X longer transport times. The concern for fish welfare required that we model closed-hold conditions beyond 30 min as the risk to fish welfare under actual commercial conditions was considered too great.

While hypercarbia is associated with the activation of primary stress responses in fish, it is not the hypercapnia *per se* but the reduction in blood oxygen content due to Bohr and Root shifts of hemoglobin saturation and binding properties that are primarily responsible for increases in plasma catecholamine levels in trout (Perry et al., 1989). It is has been suggested that the stress associated with elevated levels of CO_2 is not acutely stressful if blood oxygen content can be maintained above hypoxic levels, possible in mixed hypercapnic and hyperoxic conditions (Dejours, 1975). However, hyperoxia also results in hypercapnia and respiratory acidosis due to reductions in ventilation rates (Wood and Jackson, 1980; Wood and Lemoigne, 1991). Nevertheless, Barton and Peter (1982) found that during 8 h of re-circulating transport in hyperoxic water (oxygen saturation of between 106-144%) CO_2 levels progressively increased from 6 mg l^{-1} to 48 mg l^{-1} but rainbow trout cortisol levels began to recover after 4 h of transport and had fully recovered by 8 h. However, when oxygen is below saturation levels, hypercarbia will exacerbate hypoxic conditions making the monitoring of O_2 levels equally important as CO_2 levels during closed-hold transport.

Using model outputs generated from the conditions observed during the actual closed-hold experiments, we compared the actual Pco_2 accumulation with rates modeled using different rates of $\dot{M}O_2$. The measured Pco_2 accumulation rates closely matched the modeled rates with an $\dot{M}O_2$ of 2.5 mg O_2 min^{-1} kg^{-1} circulation (Fig 3.4). The rate of the Pco_2 increase also suggests that $\dot{M}O_2$ remained relatively constant during the first 30 min of re-circulation.

Prolonged exposures to high CO_2 levels can also directly affect oxygen consumption rates. Elevating Pco_2 was found to depress $\dot{M}O_2$ at higher tensions (Basu, 1959), even though comparable reductions in water pH have been shown to elevate $\dot{M}O_2$ by as much as 40% (Butler et al., 1992). The anesthetic properties of CO_2 on fish were first described by Fish (1943) though relatively high tensions (> 30 mmHg) are required for anesthetic action (Bernier and Randall,

1998). It is thought that CO_2 narcosis may occur during severe hypercapnic acidosis by reducing brain pH (Yoshikawa et al., 1994; Yoshikawa et al., 1991). With Pco_2 tensions below 10 mmHg, the anesthetic effect is minimal, and the gradual accumulation during transport is much less likely to induce narcosis than abrupt changes.

The initial introduction of fish to hypercapnic conditions can trigger the stress response, resulting in a transient elevation of $\dot{M}O_2$ (Thomas et al., 1983). CO_2 stunning tanks are still used in some countries (including Canada) to stun fish prior to slaughter, though it is considered a less than optimal method (Poli et al., 2005) due to observations of violent struggling when fish enter the CO_2 tanks (Erikson et al., 2006; Roth et al., 2006). Kikkawa et al.(2006) found that up to 85% of juvenile Japanese sillago (*Sillago japonica*) were able to survive step-wise increases in Pco_2 to over 50 mmHg over a period of 18 h, but an immediate introduction to the final Pco_2 tensions resulted in 100% mortality within 15 min. Moreover, the sudden return to low Pco_2 conditions also caused significant mortality in fish returned to normocapnic water from Pco_2 tensions above 50 mmHg.

For the modeling experiments we used $\dot{M}O_2$ values that represented the total aerobic scope for adult Atlantic salmon during transport based on previous bulk $\dot{M}O_2$ measurements (Chapter 2). These were applied to a range of loading densities that routinely occur during live-haul aboard the *Sterling Carrier*. By using this range of $\dot{M}O_2$ values, we were able to simulate the effects of transporting fish under a large range of 'stress' levels. Highly stressed fish that are consuming large amounts of oxygen are also producing proportional amounts of CO_2, effectively increasing the rate of CO_2 accumulation in the holds and decreasing the amount of time required to reach a specific Pco_2 threshold. Poor welfare conditions preceding or during closed-hold transport that result in the elevation of $\dot{M}O_2$ can have serious consequences for the health of transported fish. The routine $\dot{M}O_2$ (2.5 mg O_2 min^{-1} kg^{-1}) modeled during transport was based on

the bulk $\dot{M}O_2$ measurements made by Farrell (2006) and the average bulk $\dot{M}O_2$ from 45 transports of 10 h or longer (Chapter 2). The maximum was based on the bulk $\dot{M}O_2$ of greater than 8 mg O_2 min^{-1} kg^{-1} measured by Farrell (2006) during the first few minutes of transport. While we suggest that these high bulk $\dot{M}O_2$ values were likely overestimated due to fluctuating O_2 conditions at the onset of transport (Chapter 2), $\dot{M}O_2$ of greater than 8 mg O_2 min^{-1} kg^{-1} has been measured in numerous other species of salmon. For example: 12 mg O_2 min^{-1} kg^{-1} in adult sockeye salmon (Farrell et al., 2003); 13 mg O_2 min^{-1} kg^{-1} in adult pink salmon (Farrell et al., 2003); and 10 mg O_2 min^{-1} kg^{-1} in adult coho salmon (Lee et al., 2003) (Chapter 2, Table 2.2). Adult Atlantic salmon are likely capable of a maximal $\dot{M}O_2$ of at least 8 mg O_2 min^{-1} kg^{-1}, justifying its use in the modeling experiments.

The effects of water temperature and/or salinity on the pH/Pco$_2$ relationship were minimal (Table 3.2). Increasing water temperature did increase the rate of Pco$_2$ accumulation due to changes in the solubility of CO_2. However, a greater concern is the temperature dependence of $\dot{M}O_2$. Increasing the water temperature will result in the increase of $\dot{M}O_2$, increasing CO_2 production and reducing the time required to reach the Pco$_2$ threshold. We found that $\dot{M}O_2$ can increase as much as 2.4 times over 10 °C (Chapter 2, Table 2.1). Since the water Pco$_2$ accumulation was modeled at 10 °C, increasing the water temperature by 10 °C would potentially increase the rate of CO_2 accumulation by over 2X, reducing the transport times accordingly.

In many re-circulating systems, the accumulation of metabolically derived ammonia can also be dangerous as unionized ammonia (NH_3) is highly toxic to fish. Elevated levels of ammonia can also affect $\dot{M}O_2$ in fish due to the activation of the stress response, increased activity levels or increased metabolic costs associated with ion regulation. Smart (1978) found that rainbow trout exposed to lethal concentrations of ammonia (273 mg l^{-1} NH_3-N in freshwater

@ 15 °C, pH 6.9) Shingles et al. (2001) also observed significant elevation of routine $\dot{M}O_2$ exposed to a sub-lethal concentration of ammonia (4.9 ± 0.3 mg l^{-1} NH_3-N in freshwater @ 16 ± 0.1 °C, pH 8.39 ± 0.02), but significant reductions in active $\dot{M}O_2$ when fish were exercised in a swim-respirometer. However, during transport the effects of NH_3 on $\dot{M}O_2$ will be negated by the narcotic effects of CO_2, which is excreted at 10X the rate of ammonia during respiration (Randall and Wright, 1989), and the toxic effects of CO_2 will be manifested much earlier than those of ammonia. In addition, the concomitant decrease in water pH with increasing CO_2 will also reduce ammonia toxicity by reducing the proportion of ammonia existing in the un-ionized form (Thurston et al., 1981). Finally, the starvation of fish prior to transport will minimize ammonia production and excretion during transport. Our preliminary re-circulation study conducted during a commercial transport on Dec. 4, 2004 (density = 115 kg m^{-3}, average fish mass = 5.9 kg), found that total ammonia concentration did not exceed 0.36 mg NH_3-N l^{-1} during a 90 minute re-circulation experiment, which was well below the acute lethal concentration of 22 mg NH_3-N l^{-1} and recommended safe concentration for intensive rearing purposes of 3.4 mg NH_3-N l^{-1} for rainbow trout (in sea water @ 15°C, pH 8.0; Smart, 1981).

We conclude that closed-hold transport for limited periods of time can be accomplished at the typical densities and masses involved in commercial live-haul of adult Atlantic without seriously compromising fish welfare. The ability to measure CO_2 levels is crucial to this process, as factors such as density and stress levels can significantly affect the rate of CO_2 accumulation. By measuring water pH as a surrogate for CO_2, water quality, and the risk to fish welfare can be accurately and continuously monitored during closed-hold transport conditions as water quality conditions during such transports will quickly deteriorate beyond typical conditions for salmonids. However, due to the log-linear relationship of pH and Pco_2, as pH drops towards the pK of the CO_2/HCO_3^- reaction (~ 5.9 in sea water @ 10 °C, 30 ppt, from Mehrbach et al. 1977)

much larger increases in Pco_2 are required to produce relatively small decreases in water pH, reducing the effectiveness of using water pH as a monitoring solution in high Pco_2/low pH conditions. Up to a Pco_2 of 10 mmHg, changes in water pH would still be large enough to accurately monitor incremental changes in Pco_2. Establishing accurate relationships between pH and CO_2 across the range of water conditions routinely encountered during transport and calculating pH thresholds for specific Pco_2 levels would enable live-haul operators to avoid exposing fish to CO_2 levels above 10 mmHg during closed-hold transport in order to protect fish welfare.

Summary

1) CO_2 levels increased from 0.51 ± 0.04 mmHg to 2.49 ± 0.38 mmHg during 30 minutes of closed-hold transport at 10 °C.
2) Modeling CO_2 accumulation up to a 10 mmHg threshold revealed that transport times could last up to 150 min, or less than 20 min depending on loading density and stress levels of fish.
3) Measuring the decrease in water pH would provide an accurate and simple method of monitoring the increase in Pco_2 during closed-hold transport up to a Pco_2 of ~ 10 mmHg.

Table 3.1: Constants used in CO_2 accumulation model.

Constant used in model	Value	Source
CO_2 solubility (α)	temperature 0-20°C salinity 25-35 ppt	Boutilier et al. (1984)
CO_2 dissociation (pK)		Mehrbach et al. (1977)
Hold volume (V_W) =	325 m^3	
Scaling coefficient (M^b)	0.79	Clarke and Johnston (1999)
Molar weight O_2	16.00 g mol^{-1}	
Molar weight CO_2	44.01 g mol^{-1}	

Table 3.2: Effect of water temperature and salinity on time required to reach Pco_2 threshold. Increasing temperature or decreasing salinity reduces the amount of time required to reach the Pco_2 threshold. Values modeled at a medium transport density (120 kg m^{-3}) at the routine $\dot{M}O_2$ (2.5 mg O_2 min^{-1} kg^{-1}) with a starting pH of 7.8.

Temperature (°C)	Salinity (ppt)	Time required to reach 10 mmHg Pco_2 threshold (min)	Final pH at 10 mmHg Pco_2
5	30	108	6.61
10	30	83	6.61
20	30	40	6.67
10	25	97	6.62
10	35	71	6.60

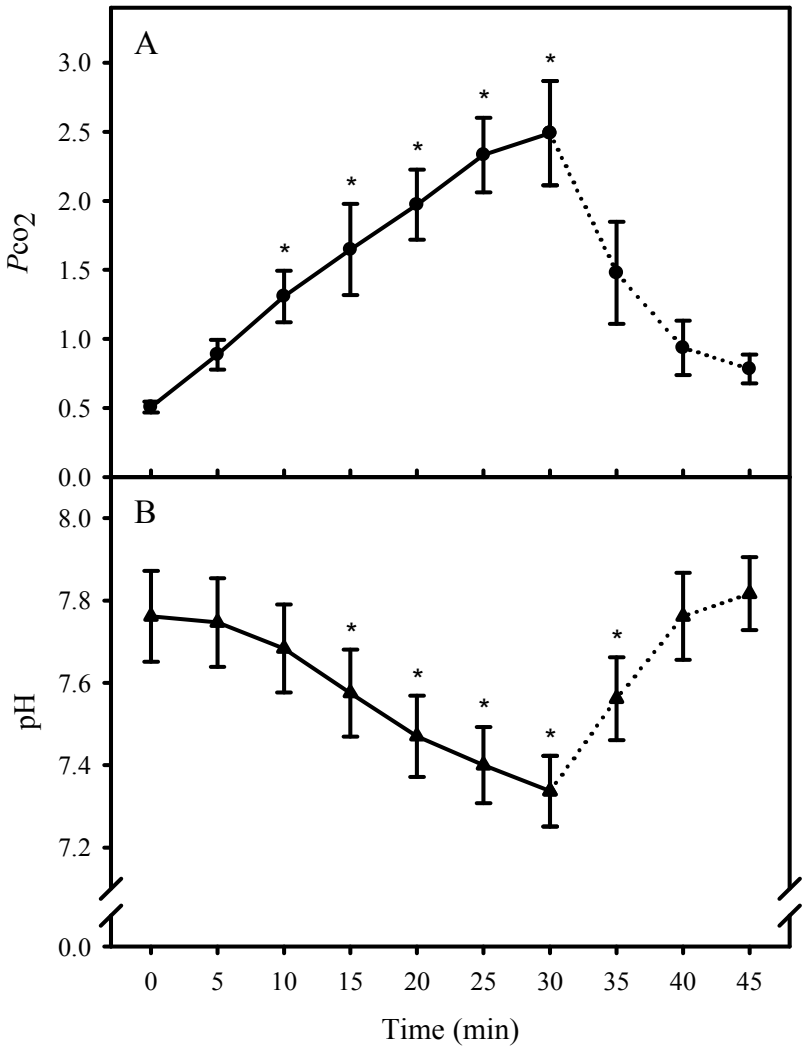

Figure 3.1: Changes in water Pco_2 and water pH during 30 min re-circulating transport. Measured changes in A) water Pco_2 calculated from total CO_2 in water samples (n=3) and B) water pH (n = 6) during 30 min re-circulating transport of 5.7 ± 0.2 kg Atlantic salmon at 135 ± 4 kg/m^3 (10.6 ± 1.2 ºC). Broken line represents first 15 min of recovery following resumption of open circulation. * indicates values significantly different from starting conditions (paired t-test, $p<0.05$).

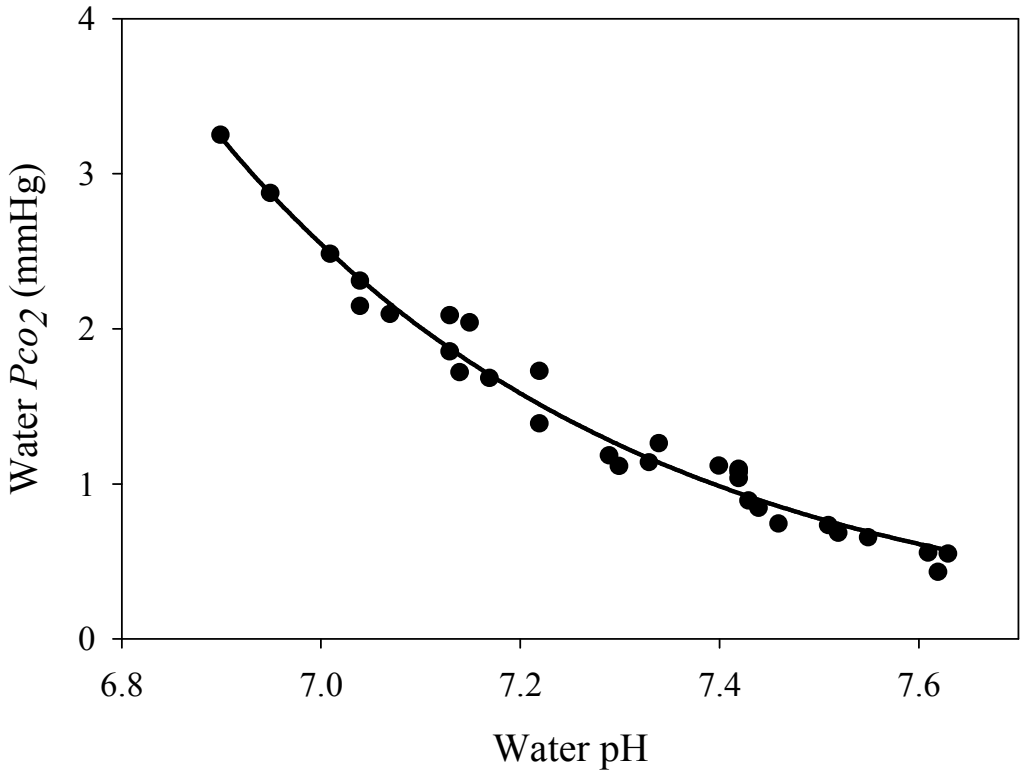

Figure 3.2: Relationship between water Pco_2 and water pH in closed-hold water samples. Water Pco_2 was calculated from total CO_2 and water pH measured from water samples taken during 30 min closed-hold experiments aboard the *Sterling Carrier*.

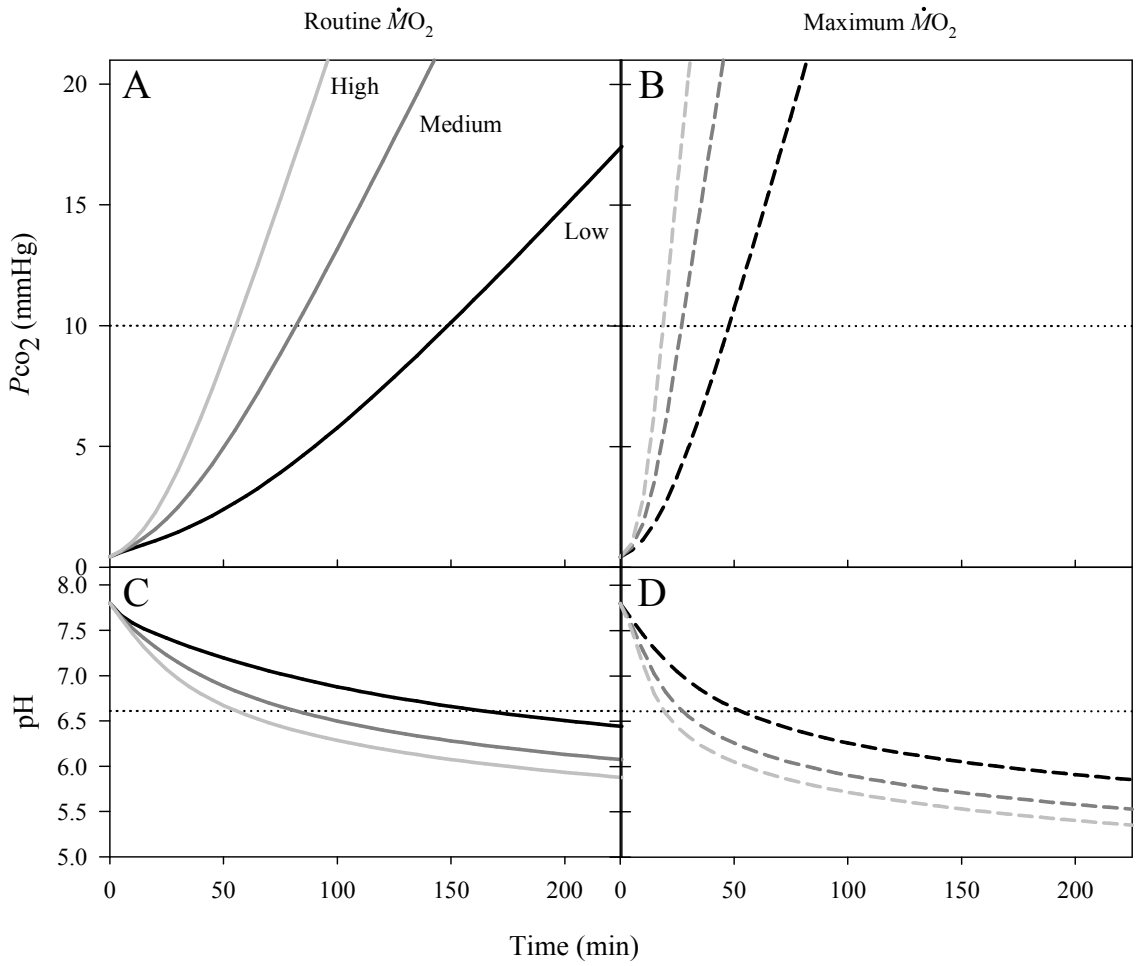

Figure 3.3: Modeled changes in water quality during various closed-hold transport scenarios. Simulated Pco_2 accumulation at routine $\dot{M}O_2$ (2.5 mg O_2 min^{-1} kg^{-1}, solid lines) and maximum $\dot{M}O_2$ (8.0 mg O_2 min^{-1} kg^{-1}, dashed lines) across a range of loading densities; low density (70 kg m^{-3}), medium density (120 kg m^{-3}) and high density (170 kg m^{-3}). (A) Under low stress conditions, low loading density allows a recirculation time of up to 150 min while at a high density this time is reduced to 56 min. (B) Under high stress conditions, low loading density levels allow recirculation time of 48 min while high loading densities further reduce the time to reach the 10 mmHg CO_2 threshold to 19 min. The associated water pH changes at routine $\dot{M}O_2$ (C) and maximum $\dot{M}O_2$ (D) at low, medium and high loading densities show that water pH decreases with increasing water Pco_2. The arbitrary threshold Pco_2 of 10 mmHg is reached at a water pH of 6.6 (dotted lines) under all scenarios. All trips were modeled using the same water conditions: temperature = 10 ºC, salinity = 30 ppt, starting water pH = 7.8.

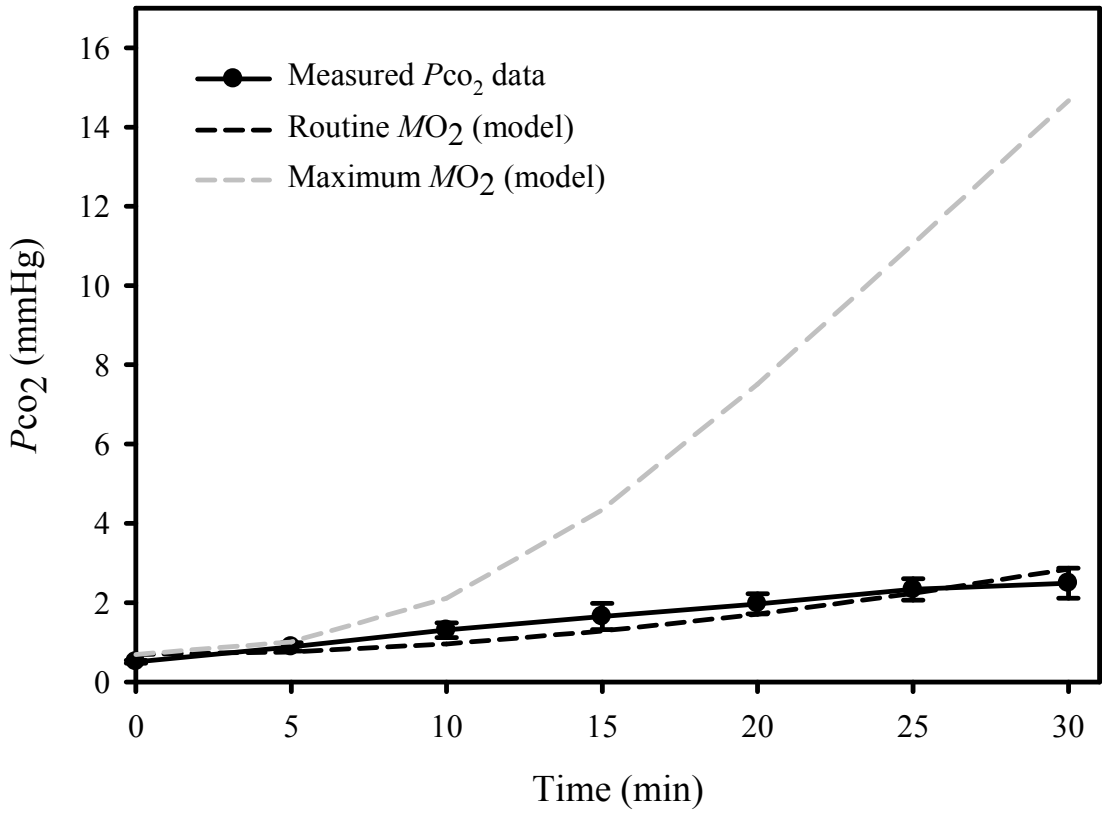

Figure 3.4: Comparison of actual $P\text{co}_2$ accumulation to modeled $P\text{co}_2$ accumulation. Actual $P\text{co}_2$ accumulation measured during closed-hold transports (n=3) was compared to modeled $P\text{co}_2$ accumulation modeled using transport $\dot{M}\text{O}_2$ (2.5 mg O_2 min^{-1} kg^{-1}) and maximum $\dot{M}\text{O}_2$ (8.0 mg O_2 min^{-1} kg^{-1}). Mean loading density (137 ± 6 kg m^{-3}), temperature (8.4 ± 0.3 ºC) and salinity (29 ± 2 ppt) and starting water pH (7.6 ± 0.1) were replicated in the modeled data. The $P\text{co}_2$ accumulation during actual closed-hold transport suggests that the rate of $\dot{M}\text{O}_2$ was similar to the transport $\dot{M}\text{O}_2$ measured in Chapter 2.

References

Barton, B. A. (2000). Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. N. Am. J. Aquacult. 62, 12-18.

Barton, B. A. and Peter, R. E. (1982). Plasma-cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anesthesia, and cold shock. J. Fish Biol. 20, 39-51.

Barton, B. A., Peter, R. E. and Paulencu, C. R. (1980). Plasma-cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Can. J. Fish. Aquat. Sci. 37, 805-811.

Basu, S. P. (1959). Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. J. Fish. Res. Board Can. 16, 175-212.

Bernier, N. J. and Randall, D. J. (1998). Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment. J. Fish Biol. 52, 621-637.

Boutilier, R. G., Heming, T. A. and Iwama, G. K. (1984). Appendix: Physicochemical parameters for use in fish respiratory physiology. Orlando, Florida: Academic Press.

Brauner, C. J., Thorarensen, H., Gallaugher, P., Farrell, A. P. and Randall, D. J. (2000). CO_2 transport and excretion in rainbow trout (*Oncorhynchus mykiss*) during graded sustained exercise. Resp. Physiol. 119, 69-82.

Butler, P. J., Day, N. and Namba, K. (1992). Interactive effects of seasonal temperature and low pH on resting oxygen uptake and swimming performance of adult brown trout *Salmo trutta*. J Exp Biol 165, 195-212.

Cameron, J. N. (1972). Principles of pysiological masurement. Orlando, Florida: Academic Press Inc.

Carmichael, G. J. (1984). Long-distance truck transport of intensively reared largemouth bass. Prog. Fish Cult. 46, 111-115.

Carneiro, P. C. F. and Urbinati, E. C. (2002). Transport stress in matrinxa, *Brycon cephalus* (Teleostei: Characidae), at different densities. Aquacult. Int. 10, 221-229.

Clarke, A. and Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. J. Anim. Ecol. 68, 893-905.

Dejours, P. (1975). Principles of comparative respiratory physiology. Amsterdam: North-Holland Publishing Company.

Eddy, F. B., Lomholt, J. P., Weber, R. E. and Johansen, K. (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high CO_2 tension. J. Exp. Biol. 67, 37-47.

Eddy, F. B. and Morgan, R. I. G. (1969). Some effects of carbon dioxide on blood of rainbow trout *Salmo gardneri* Richardson. J. Fish Biol. 1, 361-372.

Erikson, U., Hultmann, L. and Steen, J. E. (2006). Live chilling of Atlantic salmon (*Salmo salar*) combined with mild carbon dioxide anaesthesia I. Establishing a method for large-scale processing of farmed fish. Aquaculture 252, 183-198.

Erikson, U., Sigholt, T. and Seland, A. (1997). Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 149, 243-252.

FAO. (2006). The state of world fisheries and aquaculture. Rome: FAO.

Farrell, A. P. (2006). Bulk oxygen uptake measured with over 60,000 kg of adult salmon during live-haul transportation at sea. Aquaculture 254, 646-652.

Farrell, A. P., Lee, C. G., Tierney, K., Hodaly, A., Clutterham, S., Healey, M., Hinch, S. and Lotto, A. (2003). Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish Biol. 62, 64-84.

Fish, F. F. (1943). The anesthesia of fish by high carbon dioxide concentrations. Trans. Am. Fish. Soc., 25-29.

Fivelstad, S., Haavik, H., Lovik, G. and Olsen, A. B. (1998). Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.): ion regulation and growth. Aquaculture 160, 305-316.

Fivelstad, S., Olsen, A. B., Kloften, H., Ski, H. and Stefansson, S. (1999). Effects of carbon dioxide on Atlantic salmon (*Salmo salar* L.) smolts at constant pH in bicarbonate rich freshwater. Aquaculture 178, 171-187.

Heisler, N. (1986). Acid-base regulation in animals. Amsterdam: Elsevier Science Publishers B.V.

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

Iversen, M., Finstad, B. and Nilssen, K. J. (1998). Recovery from loading and transport stress in Atlantic salmon (*Salmo salar* L.) smolts. Aquaculture 168, 387-394.

Janssen, R. G. and Randall, D. J. (1975). Effects of changes in pH and pCO_2 in blood and water on breathing in rainbow trout, *Salmo gairdneri*. Resp. Physiol. 25, 235-245.

Kikkawa, T., Sato, T., Kita, J. and Ishimatsu, A. (2006). Acute toxicity of temporally varying seawater CO_2 conditions on juveniles of Japanese sillago (*Sillago japonica*). Mar. Pollut. Bull. 52, 621-625.

Lee, C. G., Farrell, A. P., Lotto, A., MacNutt, M. J., Hinch, S. G. and Healey, M. C. (2003). The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch)* salmon stocks. J. Exp. Biol. 206, 3239-3251.

Maule, A. G., Schreck, C. B., Bradford, C. S. and Barton, B. A. (1988). Physiological-effects of collecting and transporting emigrating juvenile chinook salmon past dams on the Columbia river. Trans. Am. Fish. Soc. 117, 245-261.

Mazeaud, M. M., Mazeaud, F. and Donaldson, E. M. (1977). Primary and secondary effects of stress in fish - some new data with a general review. Trans. Am. Fish. Soc. 106, 201-212.

McDonald, D. G., Goldstein, M. D. and Mitton, C. (1993). Responses of hatchery-reared brook trout, lake trout, and splake to transport stress. Trans. Am. Fish. Soc. 122, 1127-1138.

Mehrbach, C., Culberso.Ch, Hawley, J. E. and Pytkowic.Rm. (1973). Measurement of apparent dissociation-constants of carbonic-acid in seawater at atmospheric-pressure. Limnol. Oceanogr. 18, 897-907.

Nikinmaa, M., Soivio, A., Nakari, T. and Lindgren, S. (1983). Hauling stress in brown trout (*Salmo trutta*) - physiological-responses to transport in fresh-water or salt-water, and recovery in natural brackish water. Aquaculture 34, 93-99.

Pavlidis, M., Angellotti, L., Papandroulakis, N. and Divanach, P. (2003). Evaluation of transportation procedures on water quality and fry performance in red porgy (*Pagrus pagrus*) fry. Aquaculture 218, 187-202.

Perry, S. F., Kinkead, R., Gallaugher, P. and Randall, D. J. (1989). Evidence that hypoxemia promotes catecholamine release during hypercapnic acidosis in rainbow trout (*Salmo gairdneri*). Resp. Physiol. 77, 351-363.

Poli, B. M., Parisi, G., Scappini, F. and Zampacavallo, G. (2005). Fish welfare and quality as affected by pre-slaughter and slaughter management. Aquacult. Int. 13, 29-49.

Randall, D. J. and Wright, P. A. (1989). The interaction between carbon-dioxide and ammonia excretion and water pH in fish. Can. J. Zool. 67, 2936-2942.

Roth, B., Slinde, E. and Robb, D. H. F. (2006). Field evaluation of live chilling with CO_2 on stunning Atlantic salmon (*Salmo salar*) and the subsequent effect on quality. Aquac. Res. 37, 799-804.

Shingles, A., McKenzie, D. J., Taylor, E. W., Moretti, A., Butler, P. J. and Ceradini, S. (2001). Effects of sublethal ammonia exposure on swimming performance in rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 204, 2691-2698.

Smart, G. R. (1978). Investigations of toxic mechanisms of ammonia to fish gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. J. Fish Biol. 12, 93-104.

Smart, G. R. (1981). Aspects of water quality producing stress in intensive fish culture. In *Stress and fish*, (ed. A. D. Pickering), pp. 277-292. New York, NY: Academic Press.

Smart, G. R., Knox, D., Harrison, J. G., Ralph, J. A., Richards, R. H. and Cowey, C. B. (1979). Nephrocalcinosis in rainbow trout *Salmo gairdneri* Richardson - the effect of exposure to elevated CO_2 concentrations. J. Fish Dis. 2, 279-289.

Specker, J. L. and Schreck, C. B. (1980). Stress responses to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts. Can. J. Fish. Aquat. Sci. 37, 765-769.

Thomas, S., Fievet, B., Barthelemy, L. and Peyraud, C. (1983). Comparison of the effects of exogenous and endogenous hypercapnia on ventilation and oxygen-uptake in the rainbow trout (*Salmo gairdneri* R.). J. Comp. Physiol. 151, 185-190.

Thomas, S., Le Ruz, H. (1982). A continuous study of rapid changes in blood acid-base status of trout during variations of water pCO_2. J. Comp. Physiol. B. 148, 123-130.

Thurston, R. V., Russo, R. C. and Vinogradov, G. A. (1981). Ammonia toxicity to fishes - effect of pH on the toxicity of the un-ionized ammonia species. Environ. Sci. Technol. 15, 837-840.

Wedemeyer, G. A. (1996). Physiology of fish in intensive culture systems. New York: Chapman & Hall.

Wood, C. M. and Jackson, E. B. (1980). Blood acid-base regulation during environmental hyperoxia in the rainbow trout (*Salmo gairdneri*). Resp. Physiol. 42, 351-372.

Wood, C. M. and Lemoigne, J. (1991). Intracellular acid-base responses to environmental hyperoxia and normoxic recovery in rainbow trout. Resp. Physiol. 86, 91-113.

Yoshikawa, H., Kawai, F. and Kanamori, M. (1994). The relationship between the EEG and brain pH in carp, *Cyprinus carpio*, subjected to environmental hypercapnia at an anesthetic level. Comp. Biochem. Physiol. A. 107, 307-312.

Yoshikawa, H., Yokoyama, Y., Ueno, S. and Mitsuda, H. (1991). Electroencephalographic spectral-analysis in carp, *Cyprinus carpio*, anesthetized with high-concentrations of carbon-dioxide. Comp. Biochem. Physiol. A. 98, 437-444.

Chapter 4: Impacts of sub-lethal CO_2 exposure on the flesh quality of adult Atlantic salmon, *Salmo salar*[3]

Introduction

The goal of salmon aquaculture is to produce a meat product that has a value defined by numerous subjective and objective criteria including taste, texture, odor, appearance and nutritional content. As a meat product, the biochemical composition of the ante-mortem muscle ultimately determines the quality of the post-mortem flesh product.

The correlation between ante-mortem stress and the biochemical properties of post-mortem flesh have been found to directly or indirectly affect flesh quality (Thomas et al., 1999). Major changes in white muscle biochemistry occur with the recruitment of anaerobic respiration in response to physical stress in fish. The generated metabolic acidosis results in the reduction of intracellular pH and extracellular pH (Jerrett and Holland, 1998; Kieffer et al., 1994; Kristoffersen et al., 2006), reduction of intracellular glycogen concentrations (Booth et al., 1995; Milligan and Wood, 1986; Skjervold et al., 2001) and the reduction of PCr and ATP concentrations (Berg et al., 1997; Erikson et al., 1999; Erikson et al., 1997; Sigholt et al., 1997; Thomas et al., 1999).

These biochemical conditions are manifested in the flesh quality through changes in the timing and strength of muscle contractions during *rigor mortis* (Berg et al., 1997; Jerrett and Holland, 1998; Kristoffersen et al., 2006; Lowe et al., 1993; Roth et al., 2006; Thomas et al., 1999), flesh pH (Jerrett and Holland, 1998; Thomas et al., 1999) flesh texture (Roth et al., 2006; Sigholt et al., 1997) and flesh color (Robb et al., 2000).

[3] A version of this chapter will be submitted for publication. Tang, S., Farrell, A.P., 2008. Impacts of sub-lethal CO_2 exposure on flesh quality of adult Atlantic salmon, *Salmo salar*.

The live-haul transport of fish can be the source of significant physical stress, especially during loading and unloading stages (Barton et al., 1980; McDonald et al., 1993) and has predictable results on flesh quality. Water quality is probably the greatest concern during live-haul transport and especially during re-circulated (closed-hold) transport of fish. Water quality is a vital factor in the health and welfare of fish, and poor water quality results in stress causing considerable physiological and biochemical disturbances. The accumulation of respiratory CO_2 results in elevated water CO_2 levels (hypercarbia), affecting both fish physiology and water quality. During hypercarbia, the CO_2 partial pressure gradient between the fish and water is reversed, and external CO_2 enters the blood across the gills, causing an elevation in the CO_2 levels in the blood (hypercapnia). The uncatalyzed formation of carbonic acid in the blood saturates physiological buffering systems, reducing the pH of the plasma.

The respiratory acidosis associated with hypercarbia involves the reduction of plasma pH (Eddy et al., 1977; Thomas, 1982), increases in ionic flux rates of HCO_3^--, Na^+ and Cl^- at the gills (Goss and Perry, 1993; Larsen and Jensen, 1997), reduction in blood oxygen content and carrying capacity (Eddy, 1976; Eddy and Morgan, 1969) and at higher levels, narcosis, anesthesia and eventually death (Bernier and Randall, 1998).

During persistent hypercarbia, the plasma acidosis is compensated within 1-3 days at low to moderate levels of hypercarbia (Eddy et al., 1977; Hyde and Perry, 1989) by the accumulation of bicarbonate via branchial HCO_3^-/Cl^- exchangers (Goss and Perry, 1993; Perry et al., 1987; Toews et al., 1983). The increased bicarbonate concentration increases plasma buffering capacity, restoring plasma pH. This acidotic state and increased buffering capacity of the blood can be transmitted to the post-mortem muscle biochemistry and potentially affect flesh quality.

To the best of my knowledge, the effects of short-term exposure to sub-lethal CO_2 levels on flesh quality in Atlantic salmon have not been investigated. As these risks exist for

transported fish, studying these effects is an important aspect of assessing the welfare of fish during live-haul transport. By assessing flesh quality of adult Atlantic salmon exposed to hypercapnic conditions we can identify potential risks to flesh quality during the live-haul transport of salmon. There is also a lack of information available regarding CO_2 exposure thresholds for live-haul processes and the results of this study can be used to recommend CO_2 limits for optimizing fish welfare and flesh quality in the aquaculture industry.

Materials and Methods

Experimental animals

Experiments were conducted on adult Atlantic salmon, *Salmo salar*, raised in outdoor salt-water tanks at CAER and fed commercial pellet diets (Skretting, Vancouver, BC, Canada). A total of 40 fish were used, mean weight 1.60 kg (SD 0.31, SE 0.05), mean length 54 cm (SD 4.21, SE 0.72).

Surgery protocol

Fish were removed from the tanks, anesthetized with 1:10000 MS-222 and maintained under 1:20000 MS-222 during which the dorsal aorta was cannulated using PE50 tubing as described in Soivio (1975). Each fish was weighed and fork length measured before being placed in an 89 l isolation chamber supplied with sea-water at 10 l min^{-1} to recover for at least 24 h. During the recovery period, the cannula was flushed every 12 h with 1:250 IU (Li)heparin in Cortland saline (Wolf, 1963).

CO_2 exposure

Fish were exposed to hypercarbia at 10 mmHg Pco_2 by passing water through a 1.8 m (115 l) gas-exchange column before it entered the isolation chambers. The gas-exchange column was filled with plastic bio-balls and connected to a commercial gas mixer (Tescom Corp, Solon,

OH, USA) that provided CO_2 gas mixtures between CO_2 and compressed air (Praxair, Vancouver, BC, Canada). The gas mixer was calibrated to 10 mmHg by comparing the pH of calibrated gas mixtures equilibrated in sea-water (0.51%, 1% and 2% CO_2 balance air) to the pH in the isolation chambers. pH was measured using handheld pH meter with combination pH electrode (SP301 meter, 14002-764 electrode, VWR Scientific, Mississauga, Ontario, Canada).

Experimental treatments

Two CO_2 exposure durations (3 h and 24 h) and two recovery protocols (no recovery and 24-h recovery) were tested in a 2-way block design. Fish designated as controls were cannulated and placed in isolation chambers for the duration of each treatment but not exposed to CO_2.

Blood sampling

A 500 µl blood sample was drawn from the cannula at various times during the CO_2 exposure (0,1,2,3,6,12 and 24 h) and recovery periods (1,3,6,12 and 24 h). Blood pH was measured from 200 µl of whole blood with a combination pH electrode (GK2401C, Radiometer, France) connected to a PHM73 pH/blood gas monitor (Radiometer, Copenhagen). Total CO_2 (TCO_2) content in whole blood was measured from 25 µl blood injected into a Cameron chamber filled with de-carbonated 0.01N HCl according to the procedures of Cameron (1971). Pco_2 in the chamber was measured with a Pco_2 electrode (MODEL, Radiometer, Copenhagen). TCO_2 measurements and pH measurements were used to calculate the Pco_2 of blood samples using the Hendersen-Hasselbalch equation. Hemoglobin was measured using the Drabkin spectrophotometric assay and hematocrit was also measured. The remaining sample was centrifuged for 8 min at 3000 g and plasma and red blood cells were separated and frozen in liquid nitrogen for later analysis.

Sampling protocol

At the end of each treatment, the fish was stunned with a sharp blow to the head then ex-sanguinated by cutting all the gill arches on one side. The body was bled for approximately 5 min, transferred to an ice-filled cooler. Initial pH and *rigor mortis* measurements were taken within 15 min of the time of death, and the coolers were stored in a cold-room between post-mortem tests.

Rigor mortis measurement

Rigor mortis was measured as the Rigor Index (RI) by the modified Cutting's method (Bito et al., 1983). Measurements were made at 0, 24, 48 and 72 h post-mortem in the 3-h CO_2 and 3-h CO_2 + recovery treatments, and 0, 6, 12, 24, 48 and 72 h post-mortem during 24-h CO_2 and 24-h CO_2 + recovery treatments. The fish was placed on its side on a horizontally level board so that approximately half of its posterior length extended over the edge of the board. The angle between the edge of the board and the tip of the caudal penduncle was measured to the nearest degree. Measurements were made for each side and the average of the two sides reported as RI. If the fish displayed less than 0 degrees of tail drop on either side (as often occurred when fish become bowed under full rigor) both sides were considered to be in full rigor and a RI value of 100% was assigned for both sides. This was necessary to prevent underestimating RI in bowed fish.

Extracellular pH

Extracellular pH (pH_e) of the post-mortem muscle was measured using an ISFET stainless steel pH piercing tip micro-probe (PH37-SS, IQ Scientific, Carlsbad, CA, USA). The probe was connected to a handheld pH meter (IQ150, IQ Scientific, Carlsbad, CA, USA) and calibrated with precision pH solutions to an accuracy of ± 0.01 pH units. A small incision was

made in the skin and the probe was inserted into the white muscle in the shoulder muscle anterior to the dorsal fin.

Intracellular pH

Intracellular pH (pH_i) of the post-mortem muscle was measured from white muscle tissue samples excised and freeze-clamped following each rigor measurement. Approximately 1 cm^3 of white muscle was excised from a section anterior to the dorsal fin in the area that pH_e was measured. Each sample was freeze-clamped and stored at -80 ºC until analysis. White muscle pH_i was measured according to procedures outlined in Portner (1990). Briefly, ~200 mg of tissue was ground under liquid nitrogen and added to a pH neutral metabolic inhibitor to prevent H^+ generation. The extract is mixed thoroughly, centrifuged and the pH of the supernatant measured using a BMS Mk. 2 thermostatted glass capillary pH system (Radiometer, Copenhagen,) connected to a PHM71 pH meter (Radiometer, Copenhagen).

Statistical analysis

Mean values were compared using RMANOVA with Holm-Sidak multiple comparisons test in SigmaStat 3.0 (Systat Software Inc., San Jose, CA, USA).

Results

Fish exposed to 10 mmHg CO_2 experienced a significant respiratory acidosis. Arterial blood pH dropped from 7.76 ± 0.01 to 7.53 ± 0.02 during the first hour of hypercapnia. Blood pH had fully recovered by 12 h in the 24-h exposure group but remained significantly acidic in the 3-h exposure group. Following re-introduction to normocapnic water, both groups experienced a significant blood alkalosis (Fig 4.1). The alkalosis was fully compensated within 12 h in the 24-h exposure group, but required less than 6 h to recover in the 3-h exposure group as pH had recovered from the recovery alkalosis by this time.

Hematocrit and hemoglobin concentrations did not change significantly during the 3-h or 24-h hypercapnic treatments. The hemotocrit decreased significantly after 12 h of recovery in the 24-h hypercapnia + recovery treatment. This was likely due to hemodilution as 12 blood samples were taken from this group of fish.

The pH recovery was primarily accomplished through accumulation of HCO_3^- in the blood, which reached 17.9 ± 1.3 mmol l^{-1} after 3 h and 23.1 ± 3.2 mmol l^{-1} after 12 h (Fig. 4.2). During the 1st h, HCO_3^- increased along the slope of the buffer line (generated from rainbow trout blood; Fig 4.2). Blood pH had fully recovered within 12 h after which HCO_3^- accumulation ceased. HCO_3^- concentrations were rapidly reduced when fish were returned to normocapnic water, and in the 24-h exposure group HCO_3^- returned to pre-stress levels within 6 h of recovery.

Post-mortem effects of 10 mmHg CO_2 exposure on rigor progression in fish were minimal. All fish experienced the onset of rigor within 6 h post-mortem (Fig. 4.3, 4.4). In control and recovery treatments, peak rigor was measured at 24 h, 3-h CO_2 exposed fish did not show a definitive peak in rigor, but the greatest rigor was measured between 24-48 h. At 72 h the RI in the 3-h CO_2 fish was significantly higher compared to controls, but not significantly different from 3 h + recovery fish. Peak rigor in the 24-h CO_2 treatment was advanced by approximately 12 h compared to recovery and control groups. Both 24-h CO_2 and 24-h CO_2 + recovery showed significantly lower RI after 48 h post-mortem, but no differences in the RI were observed at 72 h post-mortem.

Both intracellular and extracellular muscle pH decreased over time in all treatments. Fish exposed to 3-h hypercapnia showed a significantly lower initial extracellular pH, but pH was not significantly different from control and recovery treatments by 24 h and no significant changes were observed in pH_i or pH_e after 72 h ice-storage. Fish exposed in the 24-h hypercapnia treatment showed a lower final pH 72 h post-mortem relative to control and 24-h + recovery

treatments. No significant differences were observed in intracellular pH between 24-h hypercapnia, 24-h hypercapnia + recovery and control treatments. Significant decreases in extracellular pH at 48 h postmortem in both 3-h and 24-h recovery treatments are likely artifacts, as the increase in mean pH in both groups at 72 h suggest the previous measurements are too low. One possible explanation for this could be that water loss of fish muscle, which would concentrate extracellular fluid and result in lower pH.

Discussion

Fish exposed to 10 mmHg CO_2 experienced large physiological disturbances but relatively minor changes in post-mortem flesh quality indices. The respiratory acidosis that resulted from 10 mmHg hypercapnia was fully compensated by 12 h. The accumulation of bicarbonate was the primary method of pH compensation during hypercapnia, increasing over 15 mmol l^{-1} in 12 h. By 3 h bicarbonate concentrations had already increased by over 10 mmol l^{-1}. The rates of bicarbonate accumulation and pH recovery of adult Atlantic salmon during hypercapnia are comparable to rainbow trout exposed to similar levels of hypercarbia in fresh water containing high concentrations of HCO_3^-, Ca^{2+} and Cl^- (Larsen and Jensen, 1997). Therefore, the rate at which HCO_3^-/Cl^- exchange mechanisms operate, and pH regulation in seawater and 'hard' freshwater do not appear to be limited by ion availability.

The elevation of HCO_3^- associated with compensation of the respiratory acidosis also resulted in a plasma alkalosis in fish when they were returned to normocapnic water after either 3 h or 24 h of hypercapnia (Fig. 4.1). Clearance of excess HCO_3^- from the blood returned the blood pH to control levels within 6 h of the recovery period.

Fish exposed to the short and long hypercapnia treatments would have possessed significantly different biochemical conditions than control and 24-h recovery treatments when

entering the post-mortem state. The 3-h hypercapnic fish still showed significant blood acidosis, with significant elevations in blood HCO_3^-. The 24-h hypercapnic fish did not exhibit blood acidosis, but had accumulated even greater blood HCO_3^-. Control and 24-h recovery fish would not be acidotic and would have low HCO_3^-. However, the effects of these differences in biochemical conditions on the measured flesh quality indicators were small.

The greatest changes were in the timing and strength of the *rigor mortis*. 3-h hypercapnic fish showed a lower overall rigor tension that was sustained, resulting in significantly greater RI at the end of 72 h post-mortem storage compared to control fish. However, *rigor mortis* was not assessed during the first 24 h in this group, as we were concerned with minimizing the effects of repeated handling on *rigor mortis* development (Berg et al., 1997). The lack of data resolution outweighed concerns over the possible acceleration of *rigor mortis* with handling, and thereafter rigor was measured four times during the first 24 h. The onset of *rigor* was advanced by 12 h in 24-h hypercapnia fish, within the time-frame expected for un-stressed fish (Jerrett and Holland, 1998; Sigholt et al., 1997) but approaching times indicative of stress experienced during 'routine' handling during slaughter processes (Berg et al., 1997). The strength of the rigor experienced by the 24-h hypercapnia fish was also significantly greater at 12 h compared to both recovery and control groups (Fig. 4.4), but reductions in the processing ability in these fish were likely small, as all groups were experiencing significant rigor by this point and

Muscle pH_i and pH_e were only slightly different than control conditions, and initial values were within the range (7.2-7.8) expected in un-stressed fish and well above pH_e measured in stressed and exhausted fish (6.6-7.0) (Erikson et al., 1997; Jerrett and Holland, 1998; Robb et al., 2000). Final pH_e in the 24-h hypercapnia group was significantly lower than the control group which suggests that lactate production during anaerobic glycolysis continued post-mortem and that intracellular glycogen concentrations were not depleted during hypercapnia. The

reduction in the pre-rigor period may be the result of changes in ATP/PCr concentrations or lower rates of ATP synthesis that resulted in muscles entering rigor before ATP substrates were fully depleted.

The difference between pH_e and blood pH at the time of death were quite large, even in the 3-h hypercapnia group which were still experiencing significant blood acidosis at the time of death. However, pH_e was much closer to blood pH of samples taken after the stunning but before exsanguinations using a caudal puncture. In the 3-h hypercapnia treatment, caudal blood pH of the caudal sample was 7.45±0.05, 24-h hypercapnia treatment caudal blood pH was 7.57±0.06, 3-h + recovery caudal blood pH was 7.34±0.03, 24-h + recovery treatment caudal blood pH was 7.35±0.05 and the control treatment caudal blood pH was 7.40±0.03. The lower blood pH is probably associated with struggling and muscle contractions experienced during the stunning procedures. The amount of struggling varied considerably among individual fish. However, fish sampled during hypercarbia may have been partially anaesthetized, struggled less and therefore had a higher initial post-mortem blood pH.

Compared to fish exercised to or stimulated exhaustively, the changes observed in flesh quality in response to exposure to 10 mmHg Pco_2 were minimal. Only small changes were observed between experimental and control groups, and these were typically reconciled after 24 h recovery in normocapnic water. The acceleration of rigor onset in the 24-h hypercapnia group indicates that the effects of hypercapnia on flesh quality are not negligible, but are still acceptable from industrial standards. Under the conditions examined we conclude that short-term exposures of fish to hypercarbia up to 10 mmHg that may occur during closed-hold live-haul transport do not pose a significant risk to either fish welfare (see Chapter 3) or flesh quality. Further investigation into higher Pco_2 levels may result in more significant reductions in flesh quality.

Summary

1) Exposure to short (3-h) or long (24-h) periods of elevated partial pressures of CO_2 (10 mmHg Pco_2) resulted in significant disturbances in blood pH and [HCO_3^-]. Allowing 24 h of recovery in low-CO_2 water returned these parameters to normal.
2) Flesh quality as measured by intracellular pH, extracellular pH and *rigor mortis* strength and progression were not significantly affected by exposure to a 10 mmHg water Pco_2.

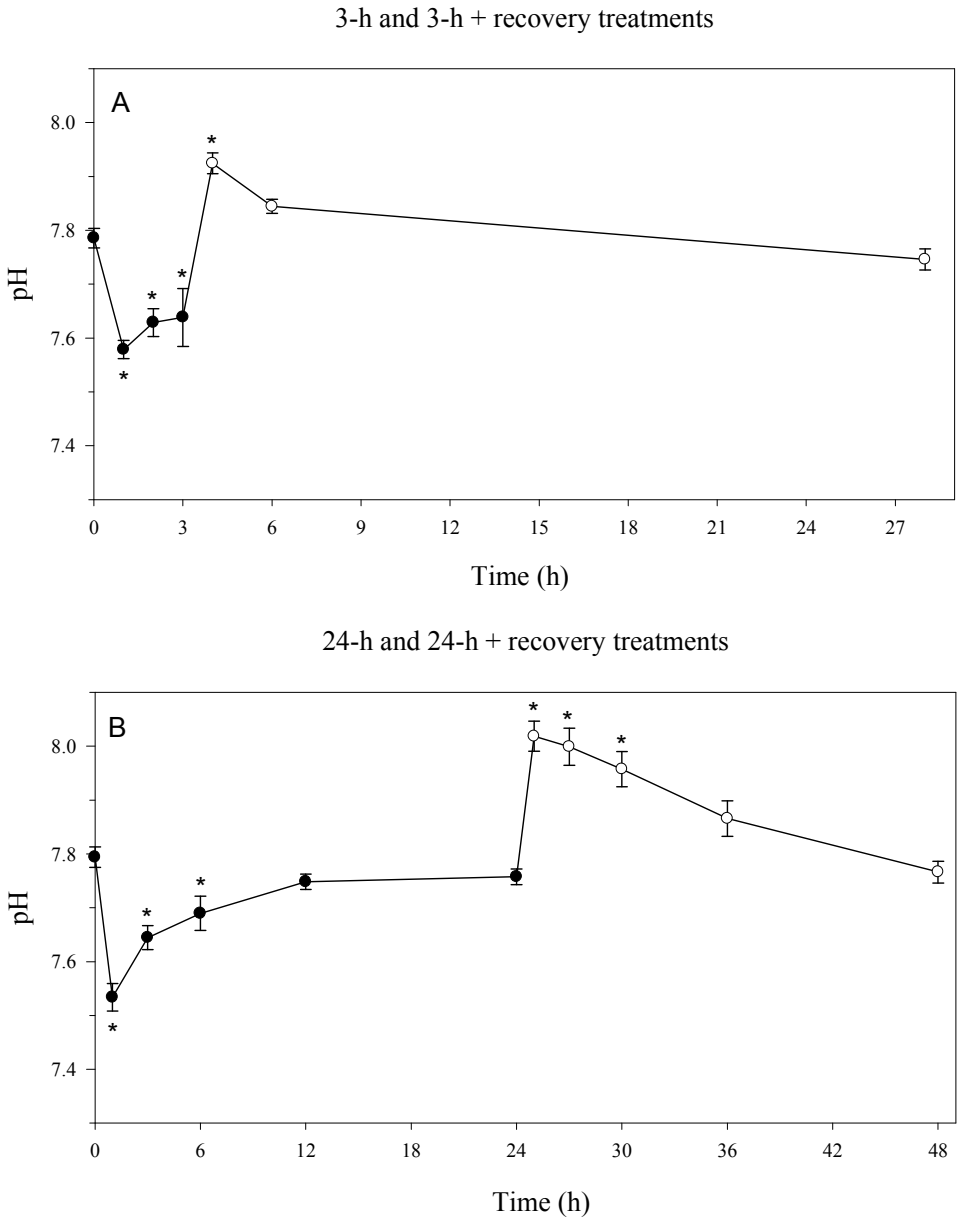

Figure 4.1: Changes in whole blood pH during hypercarbia and recovery treatments. Blood pH during hypercarbia (●) and recovery (○) during 3-h (A) and 24-h (B) exposures to 10 mmHg Pco_2. Fish experienced significant hypercapnia with compensation during moderate hypercarbia. Significant alkalosis and a subsequent recovery is also observed following the return to low Pco_2 conditions. Values significantly different from pre-exposure values are indicated with an '*'.

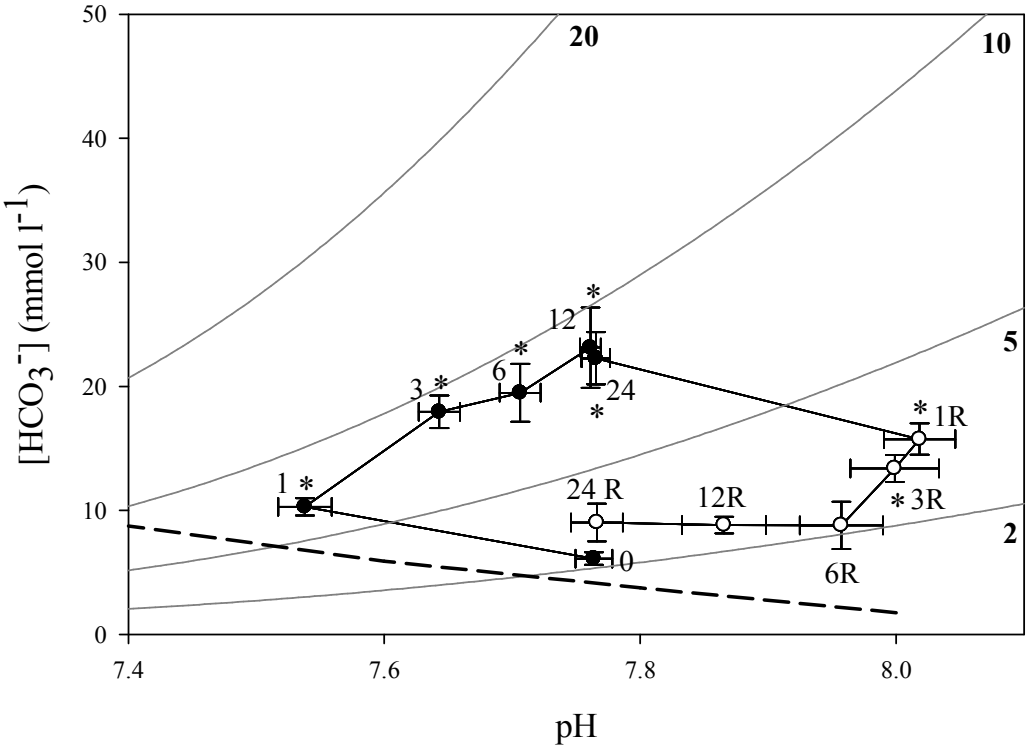

Figure 4.2: Davenport diagram of changes in whole blood bicarbonate concentration and pH during exposure to hypercarbia. Fish were exposed to 10 mmHg Pco_2, the sampling times during the 24 h of hypercarbia (●) and 24 h of recovery (○) are labeled. Isopleths represent Pco_2 tensions (mmHg). HCO_3^- values significantly different than pre-exposure levels are indicated by an '*'. The dashed line indicates the bicarbonate buffer line of whole blood (in rainbow trout, *S. gairdneri*; Wood et al., 1982). Fish accumulated bicarbonate, recovering blood pH during exposure to hypercarbia. Return to low CO_2 conditions resulted in a significant alkalosis, and blood pH was recovered by excreting bicarbonate.

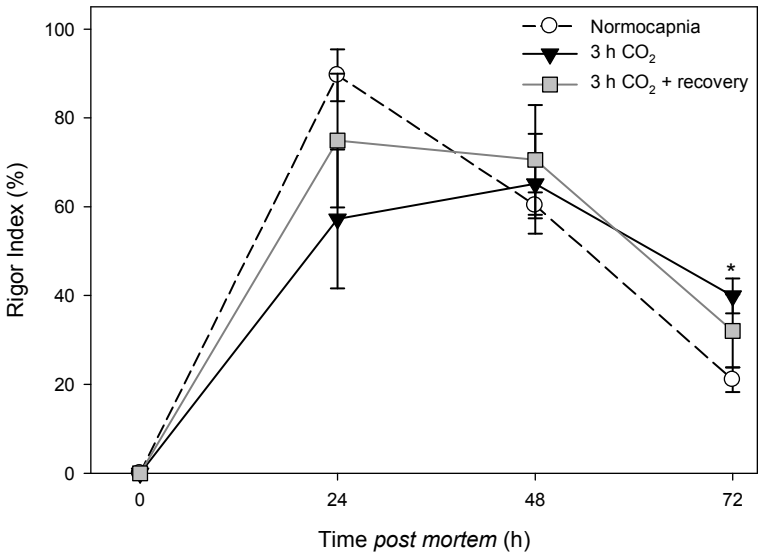

Figure 4.3: *Rigor mortis* progression of Atlantic salmon exposed to 10 mmHg Pco_2 for 3 h. Atlantic salmon were exposed to 10 mmHg Pco_2 for 3 h (n=7), 10 mmHg Pco_2 for 3 h followed by 24 h recovery period (n=5) , and control treatment (n=5). Rigor index (RI) is measured as percent change from time 0. Means ± SEM, any values significantly different from control values are indicated with an * (t-test, $p<0.05$). Recovery treatment values significantly different from hypercapnia values are indicated with an † ($p<0.05$).

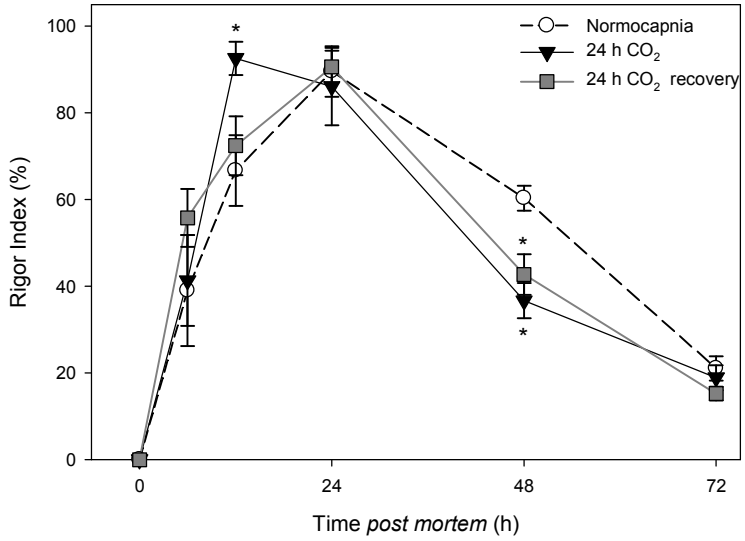

Figure 4.4: *Rigor mortis* progression of Atlantic salmon exposed to 10 mmHg Pco_2 for 24 h. Atlantic salmon were exposed to 10 mmHg Pco_2 for 24 h (n=7), 10 mmHg Pco_2 for 24 h followed by 24 h recovery period (n=5) , and control treatment (n=5). Rigor index (RI) is measured as percent change from time 0. Means ± SEM, any values significantly different from control values are indicated with an * (t-test, $p<0.05$). Recovery treatment values significantly different from hypercapnia values are indicated with an † ($p<0.05$).

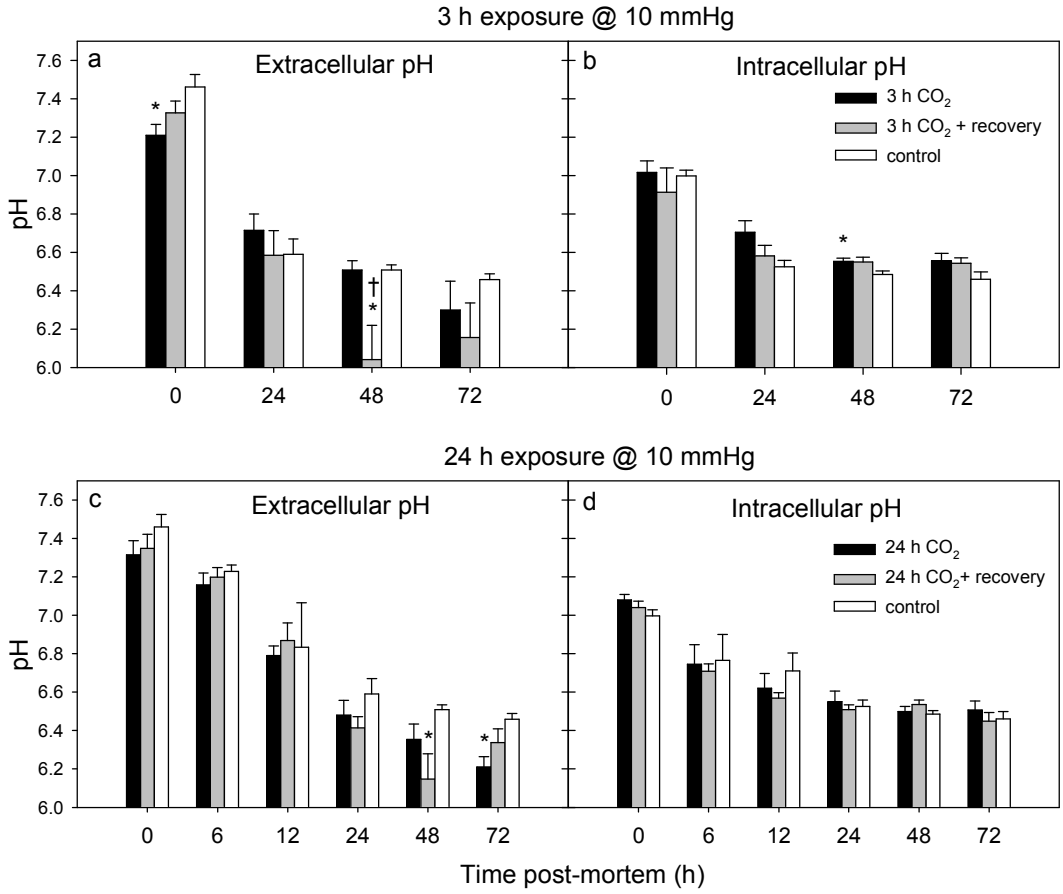

Figure 4.5: Flesh pH and white muscle intracellular pH of Atlantic salmon exposed to 10 mmHg Pco_2. Post-mortem flesh pH measured via muscle puncture of adult Atlantic salmon exposed to 10 mmHg Pco_2 for 3 h (a) without recovery (n= 6) vs. with 24 h recovery (n = 5) vs. controls (n = 5), or exposed to 10 mmHg Pco_2 for 24 h (c) without recovery (n= 5) vs. with 24-h recovery (n = 6) vs. controls (n = 5). Post-mortem intracellular white muscle pH measured from tissue samples in fish exposed to 10 mmHg Pco_2 for 3 h (b) without recovery (n= 7) vs. with 24-h recovery (n = 5) *vs.* controls (n = 4)or exposed to 10 mmHg Pco_2 for 24 h (d) without recovery (n= 6) vs. with 24 h recovery (n = 6) vs. controls (n = 4). Means ± SEM, values significantly different from control values are indicated with an * (t-test, $p<0.05$), recovery treatment values significantly different from hypercapnia values are indicated with an † (t-test, $p<0.05$).

References

Barton, B. A., Peter, R. E. and Paulencu, C. R. (1980). Plasma-cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Can. J. Fish. Aquat. Sci. 37, 805-811.

Berg, T., Erikson, U. and Nordtvedt, T. S. (1997). *Rigor mortis* assessment of Atlantic salmon (*Salmo salar*) and effects of stress. J. Food Sci. 62, 439-446.

Bernier, N. J. and Randall, D. J. (1998). Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment. J. Fish Biol. 52, 621-637.

Bito, M., Yamada, K., Mikumo, Y. and Amano, K. (1983). Difference in the mode of *rigor mortis* among some varieties of fish by modified Cutting's method. Bull. Tokai Reg. Fish. Res. Lab. 109, 89-96.

Booth, R. K., Kieffer, J. D., Davidson, K., Bielak, A. T. and Tufts, B. L. (1995). Effects of late-season catch and release angling on anaerobic metabolism, acid-base status, survival, and gamete viability in wild Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 52, 283-290.

Cameron, J. N. (1971). Rapid method for determination of total carbon dioxide in small blood samples. J. Appl. Physiol. 31, 632-634.

Eddy, F. B. (1976). Acid-base-balance in rainbow trout (*Salmo gairdneri*) subjected to acid stresses. J. Exp. Biol. 64, 159-171.

Eddy, F. B., Lomholt, J. P., Weber, R. E. and Johansen, K. (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high CO_2 tension. J. Exp. Biol. 67, 37-47.

Eddy, F. B. and Morgan, R. I. G. (1969). Some effects of carbon dioxide on blood of rainbow trout *Salmo gardneri* Richardson. J. Fish Biol. 1, 361-372.

Erikson, U., Sigholt, T., Rustad, T., Einarsdottir, I. E. and Jorgensen, L. (1999). Contribution of bleeding to total handling stress during slaughter of Atlantic salmon. Aquacult. Int. 7, 101-115.

Erikson, U., Sigholt, T. and Seland, A. (1997). Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 149, 243-252.

Goss, G. G. and Perry, S. F. (1993). Physiological and morphological regulation of acid-base status during hypercapnia in rainbow trout (*Oncorhynchus mykiss*). Can. J. Zool.71, 1673-1680.

Hyde, D. A. and Perry, S. F. (1989). Differential approaches to blood acid-base regulation during exposure to prolonged hypercapnia in 2 fresh-water teleosts - the rainbow trout (*Salmo gairdneri*) and the American eel (*Anguilla rostrata*). Physiol. Zool. 62, 1164-1186.

Jerrett, A. R. and Holland, A. J. (1998). Rigor tension development in excised "rested", "partially exercised" and "exhausted" chinook salmon white muscle. J. Food Sci. 63, 48-52.

Kieffer, J. D., Currie, S. and Tufts, B. L. (1994). Effects of environmental-temperature on the metabolic and acid-base responses of rainbow-trout to exhaustive exercise. J. Exp. Biol. 194, 299-317.

Kristoffersen, S., Tobiassen, T., Steinsund, V. and Olsen, R. L. (2006). Slaughter stress, postmortem muscle pH and rigor development in farmed Atlantic cod (*Gadus morhua* L.). Int. J. Food Sci. Tech. 41, 861-864.

Larsen, B. K. and Jensen, F. B. (1997). Influence of ionic composition on acid-base regulation in rainbow trout (*Oncorhynchus mykiss*) exposed to environmental hypercapnia. Fish Physiol. Biochem. 16, 157-170.

Lowe, T. E., Ryder, J. M., Carragher, J. F. and Wells, R. M. G. (1993). Flesh quality in snapper, *Pagrus auratus*, affected by capture stress. J. Food Sci. 58, 770-773.

McDonald, D. G., Goldstein, M. D. and Mitton, C. (1993). Responses of hatchery-reared brook trout, lake trout, and splake to transport stress. Trans. Am. Fish. Soc. 122, 1127-1138.

Milligan, C. L. and Wood, C. M. (1986). Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. J. Exp. Biol. 123, 123-144.

Perry, S. F., Malone, S. and Ewing, D. (1987). Hypercapnic acidosis in the rainbow trout (*Salmo gairdneri*). 1. Branchial ionic fluxes and blood acid-base status. Can. J. Zool.65, 888-895.

Portner, H. O., Boutilier, R. G., Tang, Y. and Toews, D. P. (1990). Determination of intracellular pH and pCO_2 after metabolic inhibition by fluoride and nitrilotriacetic acid. Resp. Physiol. 81, 255-274.

Robb, D. H. F., Kestin, S. C. and Warriss, P. D. (2000). Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. Aquaculture 182, 261-269.

Roth, B., Slinde, E. and Arildsen, J. (2006). Pre- or post-mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on *rigor mortis* and the physical properties of flesh. Aquaculture 257, 504-510.

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

Skjervold, P. O., Fjaera, S. O., Ostby, P. B. and Einen, O. (2001). Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 192, 265-280.

Soivio, A., Nynolm, K. and Westman, K. (1975). Technique for repeated sampling of blood of individual resting fish. J. Exp. Biol. 63, 207-217.

Thomas, P. M., Pankhurst, N. W. and Bremner, H. A. (1999). The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. J. Fish Biol. 54, 1177-1196.

Thomas, S., Le Ruz, H. (1982). A continuous study of rapid changes in blood acid-base status of trout during variations of water pCO_2. J. Comp. Physiol. B. 148, 123-130.

Toews, D. P., Holeton, G. F. and Heisler, N. (1983). Regulation of the acid-base status during environmental hypercapnia in the marine teleost fish *Conger conger*. J. Exp. Biol. 107, 9-20.

Wood, C. M., McDonald, D. G. and McMahon, B. R. (1982). The influence of experimental anemia on blood acid-base regulation *in vivo* and *in vitro* in the starry flounder (*Platichthys stellatus*) and the rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 96, 221-237.

Chapter 5: Major findings and conclusions

My thesis examined the welfare of adult Atlantic salmon during live-haul transport aboard a commercial well-boat, the *Sterling Carrier*. Three objectives were proposed to provide a comprehensive assessment of welfare during live-haul: 1) to quantitatively assess fish welfare during open-hold (flow-through) transport, 2) to quantitatively assess fish welfare during closed-hold (re-circulating) transport, and 3) to quantitatively assess the potential impacts of exposure to elevated levels of CO_2 on flesh quality.

Objective 1

By measuring the bulk oxygen uptake rate (bulk $\dot{M}O_2$) of salmon, we were able to indirectly assess stress and hence welfare during transport in a continuous and non-lethal manner. Low mean bulk $\dot{M}O_2$ measurements suggested that stress levels were relatively low. Indeed, bulk $\dot{M}O_2$ during open-hold transport was comparable to the bulk $\dot{M}O_2$ measured in land-based seawater rearing tanks (Bergheim et al., 1991; Bergheim et al., 1993; Forsberg, 1997). Bulk $\dot{M}O_2$ during transport was elevated relative to the routine $\dot{M}O_2$ of individual adult Atlantic salmon, but comparable to the routine $\dot{M}O_2$ of individual Pacific salmonid species (Farrell et al., 2003; Geist et al., 2003; Lee et al., 2003). The highest bulk $\dot{M}O_2$ occurred at the beginning of the transport procedure, but decreased steadily during transport. By 6.5 h it had reached a steady rate which persisted for the duration of the trip. These findings are in agreement with the results of other studies showing that loading procedures prior to transport caused the greatest amount of stress during transport (Specker and Schreck, 1980; Barton and Peter, 1982; McDonald et al., 1993; Barton, 2000) and that significant recovery from loading stressors can occur during transport (Barton and Peter, 1982; Erikson et al., 1997). Bulk $\dot{M}O_2$ during transport was not

affected by loading density throughout the range observed (62-150 kg m^{-3}), but was positively correlated with water temperature (0.24 mg O_2 min^{-1} kg^{-1} $^oC^{-1}$).

Objective 2

During closed-hold live-haul transport the greatest threat to fish welfare is the accumulation of respiratory CO_2 in the hauling water. In a fixed volume of water, CO_2 production is proportional to fish density and rate of oxygen consumption, and can quickly reach levels that are dangerous to fish health. Data collected from actual closed-hold live-haul transport experiments was used to construct a model capable of predicting the time required to reach an arbitrary Pco_2 threshold (10 mmHg) under various loading densities, fish stress levels and water conditions. We found that closed-hold transport of up to 150 min can occur without exceeding a Pco_2 of 10 mmHg under typical loading densities and low stress levels, but higher fish densities and/or stress that elevates $\dot{M}O_2$ can reduce the time to reach 10 mmHg to less than 20 min. The inability to reliably monitor Pco_2 levels during closed-hold transport represents a serious disadvantage when using closed-hold transport, considering the consequences of exposing fish to high CO_2, particularly the rapid plasma acidosis (Eddy et al., 1977; Thomas, 1982), which can result in respiratory distress (Eddy and Morgan, 1969; Eddy, 1976), and death (Bernier and Randall, 1998). Finally, we found that increases in Pco_2 were closely matched by decreases in water pH, and the relationship was relatively insensitive to changes in water chemistry. Measuring water pH during closed-hold transport may provide an accurate indication of Pco_2 levels, and would be easily implemented into most live-haul systems.

Objective 3

Exposing fish to elevated Pco_2 (10 mmHg) for 3 h or 24 h had significant impacts on fish physiology, but relatively small effects on flesh quality. Fish experienced a significant acidosis during CO_2 exposure, with an initial drop in blood pH of up to 0.4 units. Blood pH compensation occurred with accumulation of plasma HCO_3^-, and had returned to normal within 12 h. When returned to normocapnic water, the hypercapnic fish experienced a significant plasma alkalosis, which also lasted up to 12 h before blood pH had returned to normal. No changes in the progression of *rigor mortis* were observed in fish exposed to hypercarbia for 3 h, but 6 and 12 h samples were not taken. Onset of *rigor mortis* was advanced by approximately 12 h fish exposed to hypercarbia for 24 h. The rigor progression in fish allowed a 24-h recovery period was not significantly different from control fish. Neither intracellular nor extracellular pH showed large differences between hypercapnic, recovery or control groups. Significant decreases were observed immediately after death in 3-h hypercapnic fish and after 72 h in 24-h hypercapnic fish, but these were small compared to differences in muscle pH that have been observed with moderate handling and stress prior to slaughter (Erikson et al., 1997; Jerrett and Holland, 1998; Robb et al., 2000). The differences in both *rigor mortis*, intracellular and extracellular muscle pH would likely result in insignificant changes in flesh processing ability and quality compared to changes observed in highly stressed fish or even fish exposed to some routine slaughter techniques. However, the findings show that significant negative effects were detected in response to hypercapnia and much higher Pco_2 tensions could result in serious reductions in flesh quality.

Recommendations

During normal live-haul, open-hold transport maintained fish welfare and represented an excellent method of live-hauling adult Atlantic salmon and should be used whenever conditions permit. If closed-hold transport is necessary, monitoring changes in water pH, which indicate changes in CO_2 levels in the water is critical to prevent undue stress and reductions in flesh quality. Current recommendations for CO_2 exposure intended for culturing of post-smolt Atlantic salmon (Fivelstad et al., 1998) have limited application for transport procedures and more research into the effects of short-term CO_2 exposure on physiology and flesh quality are necessary to determine safe levels for transport.

References

Barton, B. A. (2000). Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. N. Am. J. Aquacult. 62, 12-18.

Barton, B. A. and Peter, R. E. (1982). Plasma-cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anesthesia, and cold shock. J. Fish Biol. 20, 39-51.

Bergheim, A., Forsberg, O. I. and Sanni, S. (1993). Biological basis for landbased farming of Atlantic salmon: Oxygen consumption. In *Fish Farming Technology*, (eds. H. Reinertsen L. A. Dahle L. Jorgensen and K. Tvinnereim), pp. 289–295. Rotterdam: A.A. Balkema.

Bergheim, A., Seymour, E. A., Sanni, S., Tyvold, T. and Fivelstad, S. (1991). Measurements of oxygen-consumption and ammonia excretion of Atlantic salmon (*Salmo salar* L.) in commercial-scale, single-pass fresh-water and seawater land-based culture systems. Aquacult. Eng. 10, 251-267.

Bernier, N. J. and Randall, D. J. (1998). Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment. J. Fish Biol. 52, 621-637.

Eddy, F. B. (1976). Acid-base-balance in rainbow trout (*Salmo gairdneri*) subjected to acid stresses. J. Exp. Biol. 64, 159-171.

Eddy, F. B., Lomholt, J. P., Weber, R. E. and Johansen, K. (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high CO_2 tension. J. Exp. Biol. 67, 37-47.

Eddy, F. B. and Morgan, R. I. G. (1969). Some effects of carbon dioxide on blood of rainbow trout *Salmo gardneri* Richardson. J. Fish Biol. 1, 361-372.

Erikson, U., Sigholt, T. and Seland, A. (1997). Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 149, 243-252.

Farrell, A. P., Lee, C. G., Tierney, K., Hodaly, A., Clutterham, S., Healey, M., Hinch, S. and Lotto, A. (2003). Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish Biol. 62, 64-84.

Fivelstad, S., Haavik, H., Lovik, G. and Olsen, A. B. (1998). Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.): ion regulation and growth. Aquaculture 160, 305-316.

Forsberg, O. I. (1997). The impact of varying feeding regimes on oxygen consumption and excretion of carbon dioxide and nitrogen in post-smolt Atlantic salmon *Salmo salar* L. Aquac. Res. 28, 29-41.

Geist, D. R., Brown, R. S., Cullinan, V. I., Mesa, M. G., Vanderkooi, S. P. and McKinstry, C. A. (2003). Relationships between metabolic rate, muscle electromyograms and swim performance of adult chinook salmon. J. Fish Biol. 63, 970-989.

Jerrett, A. R. and Holland, A. J. (1998). Rigor tension development in excised "Rested", "Partially exercised" and "Exhausted" Chinook salmon white muscle. J. Food Sci. 63, 48-52.

Lee, C. G., Farrell, A. P., Lotto, A., MacNutt, M. J., Hinch, S. G. and Healey, M. C. (2003). The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch)* salmon stocks. J. Exp. Biol. 206, 3239-3251.

McDonald, D. G., Goldstein, M. D. and Mitton, C. (1993). Responses of hatchery-reared brook trout, lake trout, and splake to transport stress. Trans. Am. Fish. Soc. 122, 1127-1138.

Robb, D. H. F., Kestin, S. C. and Warriss, P. D. (2000). Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. Aquaculture 182, 261-269.

Specker, J. L. and Schreck, C. B. (1980). Stress responses to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts. Can. J. Fish. Aquat. Sci. 37, 765-769.

Thomas, S. H. L. R. (1982). A continuous study of rapid changes in blood acid-base status of trout during variations of water pCO_2. J. Comp. Physiol. B. 148, 123-130.