THE STRESS OF MOVING OUT: PHYSIOLOGICAL AND
BEHAVIOURAL EFFECTS OF COMMERCIAL TRANSPORT ON
ATLANTIC SALMON (Salmo salar) SMOLTS

by

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ABSTRACT

Despite the controversy over environmental sustainability, salmon aquaculture in British Columbia is economically important for many coastal communities and is reported as being the largest agricultural export product for the province. This thesis examined the welfare status of commercially produced Atlantic salmon smolts during transport from freshwater farms to the saltwater net pens using physiology and behaviour to assess transport stress. Smolts were transported first by truck from the freshwater farm to the dock, and then in the flow-through cargo holds of a live-haul vessel to the saltwater net pens. Fish and water were sampled before and after truck transport, and several times aboard the vessel. Assessment of stress was based on measurement of plasma cortisol, glucose, lactate, potassium, sodium and chloride concentrations, as well as behavioural observations made on underwater video footage. Seven transports of fish originating from two different hatcheries were sampled; one was a land-based tank hatchery that required a 30-min drive to the dock, and the other a lake net pen facility that was 90 min to the dock. Analysis of plasma constituents supported previous studies that recovery from the stress accumulated during loading and truck transport can be quite rapid in a live-haul vessel. Underwater video footage, recorded at the freshwater farms and in the cargo holds of the Sterling Carrier, also suggested recovery onboard in that for the most part, behaviour onboard was similar to behaviour at the freshwater farms. There were some significant differences between fish from the two types of hatcheries, particularly in the original hatchery conditions and in their behavioural responses to transport conditions; however, post-transport growth and mortality rates reported by the saltwater farms showed no significant difference. Although fish were subjected to moderately stressful conditions during part of the process, smolt transport as currently carried out by our industry partners reflects good husbandry practices and fish welfare.
# Table of Contents

**Abstract** .............................................................................................................................. ii
**List of Tables** .......................................................................................................................... v
**List of Figures** ........................................................................................................................ v
**Acknowledgements** .............................................................................................................. vii
**Co-Authorship Statement** ..................................................................................................... viii

**Chapter One: Introduction & Literature Review** ............................................................... 1

1.1 Introduction ....................................................................................................................... 1
1.2 Transportation as a stressor ............................................................................................. 3
1.3 Stress physiology of salmonid smolts .......................................................................... 5
1.4 Stress and fish behaviour ............................................................................................... 10
1.5 Long-term welfare assessment ..................................................................................... 12
1.6 Research objectives and hypotheses ........................................................................... 12
**References** .......................................................................................................................... 20

**Chapter 2: Physiological Responses of Atlantic Salmon (Salmo salar) Smolts**
DURING COMMERCIAL TRANSPORT ON THE WEST COAST OF CANADA ........................................ 31
2.1 Introduction ..................................................................................................................... 31
2.2 Materials & Methods ..................................................................................................... 33
2.2.1 Experimental animals .............................................................................................. 33
2.2.2 Transport process ..................................................................................................... 34
2.2.3 Sampling procedures ............................................................................................... 36
2.2.3.1 Water sampling .................................................................................................. 36
2.2.3.2 Fish sampling ..................................................................................................... 36
2.2.3.3 Fish tissue handling ........................................................................................... 38
2.2.4 Analytical procedures ............................................................................................... 38
2.2.4.1 Water quality ................................................................................................... 38
2.2.4.2 Plasma chemistry .............................................................................................. 39
2.2.5 Growth and mortality ............................................................................................... 39
2.2.6 Statistical analysis .................................................................................................... 40
2.3 Results ............................................................................................................................. 40
2.3.1 Water quality ........................................................................................................... 40
2.3.2 Plasma chemistry .................................................................................................... 41
2.3.2.1 Routine status prior to transport .................................................................... 41
2.3.2.2 Plasma chemistry after truck transport ........................................................... 41
2.3.2.3 Plasma chemistry aboard the Sterling Carrier ............................................... 42
2.3.3 Growth and survival data from farms .................................................................... 43
2.4 Discussion ......................................................................................................................... 43
2.4.1 Water quality ........................................................................................................... 44
2.4.2 Routine status at the freshwater farms ................................................................... 45
2.4.3 Status after truck transport .................................................................................... 47
2.4.4 Status aboard the Sterling Carrier ......................................................................... 48
2.4.5 Post-transport growth and survival ....................................................................... 50
2.5 Conclusions ..................................................................................................................... 51
**References** .......................................................................................................................... 62
CHAPTER 3: QUANTIFYING BEHAVIOURS OF ATLANTIC SALMON (Salmo salar) SMOLTS DURING COMMERCIAL LIVE-HAUL TRANSPORT, AND CORRELATIONS WITH PHYSIOLOGICAL MEASURES..68
3.1 Introduction ..............................................................................................................68
3.2 Materials & Methods ...............................................................................................70
3.2.1 Experimental animals and transport procedures ...............................................70
3.2.2 Video recording and analysis ..............................................................................70
3.2.3 Behavioural analyses ...........................................................................................71
3.2.3.1 Fish orientation .................................................................................................72
3.2.3.2 Observed fish density .......................................................................................72
3.2.3.3 Swimming effort ................................................................................................72
3.2.3.4 Darterg and flashing behaviours .................................................................73
3.2.4 Statistical analysis ................................................................................................73
3.3 Results .......................................................................................................................73
3.3.1 Behaviour at the freshwater farms ......................................................................73
3.3.2 Behaviour on board the Sterling Carrier ............................................................74
3.4 Discussion .................................................................................................................76
3.4.1 Social density ........................................................................................................76
3.4.2 Schooling behaviour ............................................................................................76
3.4.3 Erratic behaviour ..................................................................................................78
3.5 Conclusions ..............................................................................................................79

CHAPTER 4: SUMMARY AND CONCLUSIONS.....................................................................90
Objective 1 ........................................................................................................................90
Objective 2 ........................................................................................................................91
Objective 3 ........................................................................................................................93
Conclusions and industry recommendations .................................................................94
Future studies ....................................................................................................................95
References ........................................................................................................................96
LIST OF TABLES

**Table 1.1**  Literature values for plasma cortisol concentrations in salmonids ................................ 14

**Table 1.2**  Literature values for plasma glucose concentrations in salmonids ................................. 15

**Table 1.3**  Literature values for plasma lactate concentrations in salmonids .................................... 16

**Table 1.4**  Literature values for plasma potassium concentrations in salmonids ............................... 17

**Table 1.5**  Literature values for plasma sodium concentrations in salmonids .................................... 18

**Table 1.6**  Literature values for plasma chloride concentrations in salmonids ................................... 19

**Table 2.1**  A comparison of the two freshwater facilities ........................................................................ 52

**Table 2.2**  Smolt transports using the *Sterling Carrier*, spring 2006 .................................................. 53

**Table 2.3**  Summary of plasma measurements taken aboard the *Sterling Carrier*, by hold ........... 54
LIST OF FIGURES

Figure 2.1  Map of study area showing locations of freshwater and saltwater facilities .......... 55
Figure 2.2  Diagram illustrating shape and dimensions of the *Sterling Carrier*’s holds .............. 56
Figure 2.3  A schematic of a typical smolt transport aboard the *Sterling Carrier* ..................... 57
Figure 2.4  pH, dissolved CO$_2$ and NH$_3$-N in rearing or transport water .................................. 58
Figure 2.5  Plasma cortisol, glucose and lactate concentrations in smolts ................................. 59
Figure 2.6  Plasma chloride, sodium and potassium concentrations in smolts ............................ 60
Figure 2.7  Mortality and growth rates of smolts, per pen, for 30 days post-transport ............... 61
Figure 3.1  Diagram of one of the *Sterling Carrier*’s cargo holds, indicating the flow of water and
position of the cameras ....................................................................................................................... 81
Figure 3.2  A screen capture of ImageJ, showing an example of an observed fish density
measurement ........................................................................................................................................ 82
Figure 3.3  Box plot of observed density and a line plot of actual rearing/loading density for
Dalrymple smolts and Georgie Lake smolts .................................................................................... 83
Figure 3.4  Box plot of observed density and a line plot of actual rearing/loading density for
Dalrymple smolts and Georgie Lake smolts .................................................................................... 84
Figure 3.5  Fish alignment, or schooling, and erratic behaviour at the freshwater farm and
aboard the *Sterling Carrier* ..................................................................................................................... 85
Figure 3.6  Tailbeat frequency at the freshwater farm and aboard the *Sterling Carrier* .......... 86
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CHAPTER ONE: INTRODUCTION & LITERATURE REVIEW

1.1 Introduction

While British Columbia’s wild salmon fishery continues to decline (DFO 2007), salmon aquaculture has steadily grown during the past 25 years: aquaculture products are now reported to be the largest agricultural export product for the province, worth almost $314 million CAD in 2005 (BCMAL 2006). Since salmon are one of a few high-end fishes that are very high in nutrients and low in contaminants (Dewailly et al., 2007; Ikonomou et al., 2007), demand will likely continue to grow. Aquaculture and its supporting services are already economically important for many rural, coastal BC communities, and with the recent approval of additional farm sites on the central coast (BCMAL 2007a), and approvals for pilot projects on closed-containment systems on Vancouver Island (BCMAL 2007b), salmon farming is undoubtedly a growth industry. Thus, sustainable practices are essential.

In just 40 years (Gjedrem et al., 1991; Tilseth et al., 1991), salmon culture practices have become very intense and highly commercialized. There is much concern in BC over the effects of open-cage aquaculture on native salmon species and the environment; however, little research has been directed to the welfare of the farmed fish. In intensive livestock production operations, animal welfare is becoming a paramount concern, not only for ethical reasons but also for product quality and profitability. It has been demonstrated repeatedly in various domesticated species that certain management practices such as excessive handling, confinement and crowding can lower an animal’s tolerance to disease, retard growth and affect meat quality (Mitchell et al., 1988; Van Weerd and Komen, 1998; Warriss, 1998). Although consumers may appear less concerned for fish compared to birds and mammals (Hastein et al., 2005; Lund et al., 2007), many government, non-government, and animal advocate organizations do exist for fish and aquatic invertebrates. For example, the World Organization for Animal Health (OIE) publishes an annual Aquatic
Animal Health Code (OIE, 2006a) and Manual of Diagnostic Tests for Aquatic Animals (OIE, 2006b). The Standing Committee of the European Convention for the protection of animals kept for farming purposes has recently adopted a recommendation concerning farmed fish (COE, 2006b), and in the UK, the Farm Animal Welfare Council (FAWC) has submitted a report and recommendations concerning the welfare of farmed fish to the UK government (FAWC, 1996). Also in the UK, the Royal Society for the Prevention of Cruelty to Animals (RSPCA) has a food labelling program called Freedom Foods, which requires member producers to adhere to strict animal welfare standards developed by the RSPCA, veterinarians and industry. Freedom Foods currently has 45 members from the salmon aquaculture industry, including a live-haul vessel similar to the one in this study (Scottish Sea Farms, 2005; RSCPA, 2007). There are currently no specific welfare guidelines for farmed fish in BC, but producers are aware that good welfare means high-quality products, which should translate to good economic sense.

So what exactly is animal welfare, and how do we assess it? Whereas animal rights or animal liberation advocates believe that humans do not have the right to use animals as resources (Regan, 1985; Singer, 1990), animal welfare simply represents a concern for the well-being of animals, particularly of those under human care (Fraser et al., 1997). Most animal welfare advocacy groups today have as its mandate the “Five Freedoms”, originally put forth by FAWC (1979). These five freedoms are: (1) freedom from hunger and thirst, (2) freedom from discomfort, (3) freedom from pain, injury or disease, (4) freedom to express normal behaviour, and (5) freedom from fear and distress. These freedoms are widely used in assessing the welfare state of animals, and in putting together guidelines or standards for their handling and care, such as those put forth by the BCSPCA or Canadian Council on Animal Care.

Assessment of welfare for animals is difficult because humans can never know exactly what an animal is feeling or thinking, or if it does at all. Objective assessments are usually based on resource-based criteria such as type of feed or housing provided, or animal-based criteria such
as physiological indicators of stress, behavioural responses to stress, and longer-term effects such as disease resistance, growth rate and longevity (Whay et al., 1993; Fraser, 1995). In the case of fish, there is a fundamental debate over whether they feel pain and distress (Chandroo et al., 2004; Ashley, 2007; Braithwaite, 2007). Nevertheless, because stress in fish often has direct consequences in growth and mortality, there are an exhaustive number of studies on the physiology of stress in fish (Mazeaud et al., 1977; Barton and Iwama, 1991; Wendelaar Bonga, 1997; Barton, 2002; Portz et al., 2006). Studies looking at the behaviour, or behaviour combined with physiology, of stress in fish have only recently been undertaken and are limited in number (Schreck et al., 1997; Huntingford, 2004; Gilmour et al., 2005; Ashley, 2007).

In this study, I was given the opportunity to study the welfare status of Atlantic salmon (Salmo salar) smolts during commercial transport. Because our industry partner, the live-haul vessel Sterling Carrier, had video cameras set up to monitor the fish within its cargo holds, a behavioural study was planned. However, due to the lack of scientific studies on collective smolt behaviour, an investigation of known parameters—namely the physiological measures of stress—against which to compare the behavioural observations was necessary. In effect, I would need to address the FAWC’s freedoms 2 and 5 (freedom from discomfort, and fear and distress) using freedom 4 (freedom to express normal behaviours). A review of the existing literature on transportation stress, salmonid smolt physiology and behaviour follows.

1.2 Transportation as a stressor

Domestic animals are subject to daily interaction with humans, be it something as simple and innocuous as feeding and visual inspections, or management practices such as vaccinations, transportation and slaughter. For terrestrial animals destined for human consumption, provincial health and safety regulations require that slaughter be performed at federally or provincially licensed facilities, making transportation of livestock unavoidable (BCMoH, 2004). In the case of
farmed salmon, an anadromous life history necessitates a move from fresh to saltwater for juveniles.

Transport is expensive, so producers must move as many animals as possible at a time. Handling, crowding and novel environments all impact the animals; so much so that there exist specific guidelines and regulations for the welfare of animals during transport in many countries (CARC, 2001; COE, 2006a; IATA 2006).

Fish must be transported in water, which adds a significant challenge. Not only is water medium required by fish for survival, it is extremely heavy, and must be contained to prevent spillage when transporting over land or by air. Although oxygen is supplied during commercial-scale transports, waste products still accumulate; and studies have shown that animals under stress will consume more oxygen and produce more waste (Sanni and Forsberg, 1996; Wedemeyer, 1997). The waste products of greatest concern during fish transport are carbon dioxide and ammonia (Smart, 1981; Wedemeyer, 1997).

Dissolved carbon dioxide (CO\textsubscript{2}) can dissociate and react with water to form bicarbonate and carbonate. When CO\textsubscript{2} levels increase, equilibrium shifts to the right and excessive amounts of carbonate accumulate; protons are released and the water becomes more acidic (Cameron, 1986):

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^- \leftrightarrow 2\text{H}^+ + \text{CO}_3^{2-}
\]

Increase in ambient CO\textsubscript{2} will lead to increased blood CO\textsubscript{2} and acidosis, compromising the O\textsubscript{2} carrying capacity of hemoglobin (Wedemeyer, 1997). The change in ambient pH will likewise impair ion regulation at the gills and disrupt acid-base balance (Wood and McDonald, 1982). Wedemeyer (1996; 1997) recommends that dissolved CO\textsubscript{2} be less than 10 mg l\textsuperscript{-1} in rearing tanks and pens, and that it not exceed 30 mg l\textsuperscript{-1} during transport. Chronically elevated CO\textsubscript{2} has been associated with nephrocalcinosis and reduced growth (Fivelstad et al., 1999; Fivelstad et al., 2003). However, for the relatively short-term exposure during live-haul, it is the abrupt change in water quality that is of greatest concern.
Mammals convert the products of protein metabolism from toxic ammonia into non-toxic urea before excretion. Teleosts secrete ammonia without conversion and, in the wild, the ammonia would be diluted immediately into insignificant concentrations (Brockway, 1950). Ammonia exists in ionized and unionized forms in water, and the equilibrium shifts to the left as pH increases:

\[
\text{NH}_3 + \text{H}_2\text{O} \leftrightarrow \text{NH}_4^+ + \text{OH}^-
\]

However, in intensive culture, where fish densities are high and food is provided liberally, the risk of ammonia accumulation is great. Excessive concentrations of ammonia can interfere with excretion at, or even passively diffuse into, the gills. Elevated levels of unionized NH\(_3\) are toxic to fish and have been associated with respiratory and osmoregulatory interference, as well as neurological failure and death (Smart, 1981; Meade, 1985; Randall and Tsui, 2002). As the ammonia equilibrium equation suggests, low pH (such as that caused by elevated CO\(_2\)) drives the equilibrium to the right, reducing the concentration of the toxic components (Wedemeyer, 1996). To be safe, freshwater aquaculturists should maintain NH\(_3\) concentrations in the rearing water below 0.02 mg l\(^{-1}\) (Smart, 1981; Wedemeyer, 1997).

### 1.3 Stress physiology of salmonid smolts

The stress response of fish is similar to that of mammals (Schreck, 1981; Wendelaar Bonga, 1997). When confronted by a stressor, the fish responds by secreting catecholamines, the so-called “fight or flight” hormones, and corticosteroids such as cortisol, the “stress” hormone. These primary humoral responses are almost immediate, and are detected in the blood within minutes. These in turn trigger secondary stress responses, which include changes in metabolic activity, osmoregulation, immunosuppression, and at the cellular level, upregulation of heat shock proteins (Mazcaud et al., 1977; Barton and Iwama, 1991). Responses at the whole-animal level such as changes in growth rate or behaviour are considered tertiary stress responses.

Stress assessment using catecholamines or cortisol is difficult under any conditions because
the process of capturing the animal for sampling can elicit these primary responses (Hunn and Greer, 1991; Waring et al., 1992), and this is further complicated in smolts because the physiological changes associated with smoltification include an elevation in plasma cortisol levels (Carey and McCormick, 1998; Shrimpton et al., 2000; Jørgensen et al., 2007). Cannulation generally yields the lowest resting plasma hormone values (Smith and Bell, 1964; Soivio et al., 1975; Karlsson et al., 2006), but this is difficult to do for small fish, or while in the field. A non-invasive method of measuring cortisol release into water is emerging, but these studies are still preliminary, requiring physiological sampling for comparison and validation (Lower et al., 2005; Ellis et al., 2007; Sebire et al., 2007).

Like their Pacific cousins, Atlantic salmon hatch in freshwater and migrate downstream to the sea. In the wild, they may spend anywhere from 2 to 6 years in freshwater, but domesticated Atlantic salmon are transferred to seawater at 0, 1 or 2 years of age (Clarke et al., 1996). The physical, behavioural and physiological changes that occur as juvenile salmon prepare to move to saltwater is known as smoltification, and is thought to be associated with fish size and change in day length (Shrimpton et al., 2000; Sundell et al., 2003). Smoltification may or may not be artificially induced in commercially raised 0 and 1 year smolts (Clarke et al., 1996).

Countless studies on physiological changes related to stress and to smoltification exist, and some of the relevant results have been summarized in Tables 1.1 - 1.6. The smolts used in the work described in this thesis were commercially reared, and thus subjected to daily disturbances such as feeding and cleaning. As I had no access to them prior to the study, I will compare my findings to literature values in order to assess their physiological status, and use my pre-transport values as a reference for recovery.

Resting plasma cortisol concentrations for parr (juvenile, unsmolted salmonids) and adults should be less than 5 ng ml$^{-1}$ (Barton and Iwama, 1991), whereas in smolts, resting values of 20 - 150 ng ml$^{-1}$ have been reported (Langhorne and Simpson, 1980; Virtanen and Forsman, 1987;
Shrimpton et al., 2000; Sundell et al. 2003; see Table 1.1). Poole et al. (2000; 2003) held and terminally sampled fish over time to develop a response curve to capture stress, which enabled them to extrapolate basal cortisol levels, at 30 - 40 ng ml\(^{-1}\). Increases in plasma cortisol concentration are evident within minutes of a stress event, but the timing and magnitude of the peak value depends on the type and severity of the stressor. For a single stress event, peak cortisol values occur in 20 - 60 min, and have been reported to be as high as 750 ng ml\(^{-1}\) for salmon smolts (Virtanen and Forsman, 1987; see Table 1.1). In most cases, recovery of plasma cortisol from a single acute stressor begins within hours, and returns to pre-stress levels in 12 - 48 h (Davis and Parker, 1982; Einarsdottir and Nilssen, 1996; Wendelaar Bonga, 1997). Since the animals in this study had been tested by Marine Harvest Canada for gill ATPase activity and deemed to be smolts, and because they were subject to human disturbance daily, I predicted a slight elevation in plasma cortisol before the transport event. Based on the routine values found in the literature for non-cannulated fish, I will accept any results below 50 ng ml\(^{-1}\) as routine, or “resting,” values, and those over 150 ng ml\(^{-1}\) as “severely stressed.”

Plasma glucose becomes elevated as a secondary response to stress; cortisol mediates the mobilization of glycogen in the liver, releasing glucose into the bloodstream and preparing the animal for action (Mazeaud and Mazeaud, 1981; Mommsen et al., 1999). Plasma glucose is often measured in stress studies because it can be measured easily using blood glucose meters designed for humans (Wedemeyer et al., 1990; Morgan and Iwama, 1997) and its slower response time renders it less sensitive to sampling stress. Blood glucose levels typically peak 1 - 3 h after a stress event, and take 48 h or more to recover to pre-stress levels (Waring et al., 1992; Carey and McCormick, 1998; Iversen et al., 1998; Sandodden et al., 2001; see Table 1.2). Resting values in Atlantic salmon parr and adults range from 3 - 4 mM, and in smolts, whose basal plasma cortisol is elevated, glucose can be slightly elevated to 6 - 7 mM. Peak glucose levels following stress in salmon smolts have been reported to reach up to 11 mM (Virtanen and Forsman, 1987; see Table
1.2). Thus, for my study I will assume routine plasma glucose concentrations to be below 4 mM, and severely stressed values to be over 10 mM.

Lactate is produced in and released into the blood by white muscle cells following strenuous exercise, and its concentration in the plasma increases with the intensity of physical activity (Wedemeyer et al., 1990; Milligan, 1996). Plasma lactate concentrations are therefore often measured to assess exercise and handling stress. Lactate production is not particularly affected by smoltification, and in a well-rested, unstressed fish its plasma concentration should be < 1.0 mM (Waring et al., 1992; Brauner et al., 2000; Sadler et al., 2000). Unless fish have been cannulated and allowed to recover, such low measurements are difficult to obtain as handling causes lactate to be released almost immediately into the blood (values given in Table 1.3 are for uncannulated fish, unless otherwise noted). Lactate typically peaks after 2 - 4 h, and has been reported to clear within 3 h (Waring et al. 1992) or up to 14 h (Turner et al., 1983) depending on the level of stress. Exhaustive exercise in rainbow trout has yielded maximum lactate values of up to 20 mM (Milligan, 1996). Given this information, I will assume resting plasma lactate concentration for Atlantic salmon smolts to be below 1 mM, however I do expect some elevation due to sampling stress. Plasma lactate values in fish that had struggled excessively may reach up to 20 mM.

Plasma potassium is another parameter used to assess exercise and handling stress. A higher potassium concentration is actively maintained inside animal cells, and so as muscle cells contract repeatedly, potassium ions are lost to the interstitial space and blood (Turner et al., 1983; McDonald and Milligan, 1992). As listed in Table 1.4, plasma potassium concentration in resting juvenile salmon is in the order of 2.3 - 2.7 mM (Waring et al., 1992; Quigley et al., 2006), and after exercise may rise to 3.3 - 3.6 mM (Waring et al., 1992), but values as high as 4.7 mM have been reported after exhaustive exercise in rainbow trout (Turner et al., 1983). When forced to exercise, plasma potassium values will rise steeply until peak values are reached at 1 - 2 h post-exercise, depending on the nature and duration of the exercise (Wood et al., 1983; Waring et al., 1992).
Significant recovery can occur in as little as 30 min post-exercise (Farrell et al., 2000), but recovery to pre-exercise values can take 12 h or more (Wood et al., 1983; Waring et al., 1992). For this study I will set routine plasma potassium concentrations at 2.5 mM, and the maximum stress response at 5.0 mM.

Commercially produced Atlantic salmon are reared for about 1 year in freshwater, where they develop an osmotic and ionic balance with their particular farm conditions, before transfer to the sea cages. Routine levels of plasma sodium for juvenile Atlantic salmon in freshwater can range between 135 ~ 170 mM (Table 1.5), and plasma chloride between 110 and 146 mM (Table 1.6). Because both are influenced by diet, water quality and developmental stage, there is a wide range reported in literature (Blackburn and Clarke, 1987; McDonald and Milligan, 1997). When stressed, the osmoregulatory homeostasis of freshwater fish is temporarily disturbed, so that ion loss occurs across the gills and in the urine, while excess water enters across the gills (Mazeaud et al., 1977; McDonald and Milligan, 1997). As a result, plasma sodium and chloride concentrations tend to decrease under stress in freshwater: sodium by ~5 mM and chloride by 15 - 20 mM (Virtanen and Forsman, 1987; Carey and McCormick, 1998; Iversen et al. 1998).

Before moving to seawater, the readiness of prospective smolts can be tested with a saltwater challenge test, an abrupt transfer to seawater to test osmoregulatory ability (Blackburn and Clarke, 1987). The higher concentration of ions in seawater results in fish losing water and gaining sodium and chloride ions, but if fish have smoltified sufficiently, a readjustment of plasma ion concentrations to a new, slightly higher steady state takes place in 8 - 14 days (Eddy, 1981; Nonnotte and Boeuf, 1995; Singer et al., 2002). This new ionoregulatory status is achieved by drinking sea water and developing more gill chloride cells to pump out excess sodium and chloride ions. In a successful seawater challenge test, smolts should have plasma sodium concentrations of 160 - 165 mM and chloride concentrations of 140 - 150 mM at 24 h post-transfer (Clarke et al., 1996). Fish that are unable to osmo- and ionoregulate will continue to gain ions and lose water,
and eventually succumb to the chemical imbalances. As mentioned previously, the study described in this thesis was conducted under commercial conditions; thus it was not practicable to sample the transported smolts at 24 h after transfer to seawater. At the last sampling point for my experimental protocol, there was a mixed population of smolts that had been in seawater for 4 - 9.5 h. Thus, I expect mean plasma sodium and chloride concentrations to be at the lower end of the ranges given above, at about 160 mM for sodium and 140 mM for chloride.

1.4 Stress and fish behaviour

Fish were kept in ponds for food and for ornamental purposes in ancient China and in Imperial Rome (Wedemeyer, 1997). Back then, the health and welfare of fish could only be determined by visual inspection of physical appearance and behaviour. Although we still use visual inspection to assess the health of fish, the behaviour of cultured fish was only documented in a subjective, qualitative manner, and only recently have behavioural studies emerged (Dawkins, 2004; Huntingford, 2004; Scott and Sloman, 2004; Chandroo et al. 2005).

Smoltification in salmonids involves physical and behavioural changes as well as physiological changes. Atlantic salmon parr are competitive and will defend their territories at the bottoms of rearing tanks, but as they begin to smolt they will take up more of the water column and begin to school (Folmar and Dickhoff, 1980; Bakshtanskiy et al., 1988; McCormick et al., 1998b).

The majority of information on schooling behaviour is limited to fish of economic importance such as anchovy, herring and cod (Graves, 1977; Domenici et al., 2002; Herbert and Steffensen, 2006) or smaller fish that can be easily managed in a lab (Symons, 1971; Svendsen et al., 2003). Very few schooling studies have been done on wild or cultured salmonids, but studies on individual behaviour such as swimming (Brett, 1964; Hinch and Rand, 2000; Enders et al., 2003) and aggression (Sloman et al., 2001; Øverli et al., 2004) or ecological studies on feeding, breeding

The definition of a school ranges from an aggregation of fish that choose to stay close to each other (Keenleyside, 1955) to a group of fish that is oriented in the same direction, maintain approximately the same distance from each other, and move at the same speed (Breder and Halpern, 1946). Concepts used to describe schools include volume of the school, the number and size of fish in the school, density and nearest neighbour distance (NND; Symons, 1970; Serebrov, 1976; Pitcher and Partridge, 1979). NND is the distance between two adjacent fish in a school, and is determined by the difference between the repulsive and attractive forces between fish (Breder, 1954). NND is conventionally given using the dimension of body length (bl), and measurements for various species reveal values <1.0 bl. Pitcher and Partridge (1979) determined that the NND ranged from 0.64 bl for cod, 0.82 bl for herring and up to 0.9 bl in saithe, and proposed that 0.9 bl is a reasonable estimate of NND for a generic school. More recently, nearest neighbour ratios and area enclosing a given group of fish were used to quantify school cohesion in laboratory experiments using juvenile chum salmon (Ryer and Olla, 1996).

Compared to parr, Atlantic salmon smolts are “less athletic” and prefer to swim at lower speeds (Kutty and Saunders, 1973; Virtanen and Forsman, 1987). During swimming tests, smolts were found to swim at about 2.0 - 5.5 bl s\(^{-1}\) while parr swam at 7.0 - 7.3 bl s\(^{-1}\) (reviewed by Folmar and Dickhoff, 1980). Ventilation rate in resting Atlantic salmon smolts is reported to be about 39 - 52 min\(^{-1}\) (Poleo and Muniz, 1993). A stressed fish may dart, flash, or otherwise exhibit struggling or abnormal swimming behaviour (van Raaij et al., 1996). Ryer and Olla (1996) measured “chases” during feeding to quantify agonistic behaviour among juvenile chum salmon; such measurements of individual behaviours are easy to use in dominance-subordination studies (Øverli et al., 1999; Sloman et al., 2001), preference studies (Bremset, 2000; Olsen et al, 2004), or other studies using a small number of fish.
1.5 Long-term welfare assessment

Delayed mortality is a major concern of fish transportation, be it a large-scale commercial operation or bringing home a goldfish from the pet store (Barton 2000; Lim et al., 2003). Starving, crowding, handling, closed containment, sudden changes in temperature and water quality, and novel environment are all factors that could weaken a fish’s immune system and leave it susceptible to opportunistic infections (Barton and Iwama, 1991; Wedemeyer, 1996). Thus, it is important to monitor post-transport mortality to identify potential problems that can be traced back to the transport date and source farm as necessary. Ideally, mortality should be 0, but for commercially transported smolts in sea cages, less than 2% over 30 days is considered an acceptable mortality rate (as reported by Iversen et al., 2005). To put this in perspective, mortality rates for farmed Atlantic salmon from seacage transfer to slaughter in New Brunswick, Canada is reported to be 5% (C. Graham, personal communication, New Brunswick Salmon Growers Association).

Growth rate is another important factor to be considered, as it can be negatively affected by stress and illness (Wendelaar Bonga, 1997; Van Weerd and Komen, 1998; McCormick et al., 1998a; Gregory and Wood, 1999). In most growth studies, specific growth rate (SGR) is used, which averages growth over the period of study (Rowe and Thorpe, 1990):

\[
SGR = 100 \times \frac{\log M_n - \log M_1}{(D_n - D_1)}
\]

Where \( M \) is fish mass at day 1 and day \( n \), and \( D_n - D_1 \) is number of days between days 1 and \( n \).

For Atlantic salmon post smolts in seawater, SGR is reported to be between 0.9 - 1.1 % mass day\(^{-1}\) (Fivelstad et al., 1998; Fivelstad et al., 1999; Fivelstad et al., 2003).

1.6 Research objectives and hypotheses

Our research partner, Batchelor Bay Management, operated the Sterling Carrier, a state-of-the-art live-haul vessel that anecdotally reported very low mortality rates during transport of adult and juvenile Atlantic salmon. My objective was to confirm that live smolts transported by
the Sterling Carrier were receiving the best care in terms of welfare, by:

1. Quantifying the primary, secondary and tertiary stress responses during the commercial transport process using plasma cortisol, glucose, lactate, chloride, sodium and potassium concentrations, as well as post-transport growth and mortality rates. The results will be used to quantify the level of stress during the transport process (trucking and ocean shipping), and to identify which procedures were most stressful to the smolts. The hypothesis is that the initial loading and truck transport using closed containment will be the most stressful part of the trip.

2. Plasma cortisol, glucose, lactate, chloride, sodium and potassium concentrations will be compared against those found in literature, and useful measures of stress in smolts during transport will be identified. Elevated cortisol and glucose are predicted to identify the direct stress effects of transport, whereas elevated lactate and potassium should indicate whether smolts were physically struggling. Elevated sodium and chloride will be compared to expected values for a seawater challenge test, to confirm that the smolts were physiologically ready to enter seawater.

3. Behavioural observations will be made using existing equipment (the cargo holds of the Sterling Carrier are equipped with a video monitoring system within its cargo holds). Simple, easy-to-use behavioural criteria that could later be adapted for use by staff and crew will be quantified, and compared to physiological measurements. It is hypothesized that behaviour, a tertiary stress response, will reflect degree of recovery to some extent.
Table 1. Literature values for plasma cortisol concentrations in salmonids. Values given are sample means. Parr, smolts and trout are in freshwater (fw) unless otherwise stated.

* Time to recovery indicates the time it takes for values to return to pre-treatment values.

<table>
<thead>
<tr>
<th>Routine [cortisol], ng ml(^{-1})</th>
<th>Maximally stressed [cortisol], ng ml(^{-1})</th>
<th>Time to max stress</th>
<th>Time to recovery*</th>
<th>Stressor</th>
<th>Study animal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9 ~ 8.3</td>
<td>132.7 ~ 193.9</td>
<td>20 min</td>
<td>24 h</td>
<td>Water level reduction</td>
<td>Atlantic post-smolts</td>
<td>Einarsdottir &amp; Nilssen 1996</td>
</tr>
<tr>
<td>&lt; 2.0</td>
<td>20 ~ 40</td>
<td></td>
<td></td>
<td>Natural smolification</td>
<td>Atlantic smolts</td>
<td>Shrimpton et al. 2000</td>
</tr>
<tr>
<td>2.5</td>
<td>25</td>
<td></td>
<td></td>
<td>Induced smolification (photoperiod)</td>
<td>Atlantic smolts</td>
<td>Sundell et al. 2003</td>
</tr>
<tr>
<td>9.4</td>
<td>Nutrition study</td>
<td></td>
<td></td>
<td>Atlantic adults</td>
<td>Waagba et al. 1994</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>243</td>
<td>3 h</td>
<td>8 h</td>
<td>Acute handling + 3 h confinement</td>
<td>Atlantic smolts</td>
<td>Carey &amp; McCormick 1998</td>
</tr>
<tr>
<td>10</td>
<td>160</td>
<td>2 h</td>
<td>24 h</td>
<td>Handling &amp; 2 h transport</td>
<td>Coho smolts</td>
<td>Specker &amp; Schreck, 1980</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>20 min</td>
<td></td>
<td>Dip-netting</td>
<td>Chinook parr</td>
<td>Strange et al. 1977</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td></td>
<td></td>
<td>Natural smolification</td>
<td>Atlantic smolts</td>
<td>Sundell et al. 2003</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td></td>
<td></td>
<td>Natural smolification</td>
<td>Atlantic smolts</td>
<td>McCormick et al. 2002</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>98</td>
<td></td>
<td></td>
<td>Natural smolification</td>
<td>Atlantic smolts</td>
<td>Langhorne &amp; Simpson, 1980</td>
</tr>
<tr>
<td>10 ~ 100</td>
<td>500</td>
<td>24 h</td>
<td>&gt; 48 h</td>
<td>Dip-netting &amp; confinement</td>
<td>Chinook parr</td>
<td>Strange et al. 1977</td>
</tr>
<tr>
<td>10.1 ~ 21.0</td>
<td>77.6 ~ 182.3</td>
<td>1 h</td>
<td></td>
<td>Loading &amp; truck transport (0.5 ~ 4.5 h)</td>
<td>Atlantic smolts</td>
<td>Iversen et al. 1998</td>
</tr>
<tr>
<td>16.3</td>
<td>~165</td>
<td>1 h</td>
<td>48 h</td>
<td>Netting confinement (9 min)</td>
<td>Atlantic adults (cannulated)</td>
<td>Waring et al. 1992</td>
</tr>
<tr>
<td>18</td>
<td>196</td>
<td>2 h</td>
<td>&gt; 48 h</td>
<td>Loading &amp; transport (2 h, fw)</td>
<td>Atlantic parr</td>
<td>Sanddoden et al. 2001</td>
</tr>
<tr>
<td>19 ~ 34</td>
<td>70</td>
<td>24 h</td>
<td>&gt; 24 h</td>
<td>Angling ~5 min</td>
<td>Rainbow trout (spawning, wild)</td>
<td>Pankhurst &amp; Dedual 1994</td>
</tr>
<tr>
<td>19.6 ~ 26.1</td>
<td>118.5 ~ 174.7</td>
<td>Post load (? h)</td>
<td>4 ~ 40 h</td>
<td>Loading &amp; well boat transport</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Iversen et al. 2005</td>
</tr>
<tr>
<td>23.5</td>
<td>47.6</td>
<td>30 min</td>
<td>&gt; 12 h</td>
<td>Netting confinement (30 min)</td>
<td>Atlantic smolts</td>
<td>Davis &amp; Parker 1982</td>
</tr>
<tr>
<td>24.6</td>
<td>75.5</td>
<td>2.5 h</td>
<td></td>
<td>Confinement (2.5 h)</td>
<td>Atlantic smolts</td>
<td>Sadler et al. 2000</td>
</tr>
<tr>
<td>25</td>
<td>165</td>
<td>2 h</td>
<td>24 h</td>
<td>Handling &amp; 2 h transport</td>
<td>Coho smolts (fw-sw)</td>
<td>Specker &amp; Schreck, 1980</td>
</tr>
<tr>
<td>25</td>
<td>125</td>
<td>2 h</td>
<td>4 h</td>
<td>Netting, confinement (2 min), tank transfer</td>
<td>Brown trout</td>
<td>Pickering et al., 1982</td>
</tr>
<tr>
<td>40.48 ~ 89.84</td>
<td></td>
<td></td>
<td></td>
<td>Natural smolification</td>
<td>Atlantic smolts (wild)</td>
<td>Poole et al. 2003</td>
</tr>
<tr>
<td>35.76 ~ 47.63</td>
<td></td>
<td></td>
<td></td>
<td>Natural smolification</td>
<td>Atlantic smolts (wild)</td>
<td>Poole et al. 2003</td>
</tr>
<tr>
<td>(60)</td>
<td>111</td>
<td>1 h</td>
<td>24 h</td>
<td>Angling 15 min</td>
<td>Rainbow trout (spawning, wild)</td>
<td>Pankhurst &amp; Dedual 1994</td>
</tr>
<tr>
<td>75 ~ 130</td>
<td>250 ~ 350</td>
<td>3 h</td>
<td>12 h</td>
<td>Netting &amp; transfer from sea-cage to tank</td>
<td>Atlantic adults</td>
<td>Hemre &amp; Krogdahl 1996</td>
</tr>
<tr>
<td>78</td>
<td>735</td>
<td>4 h</td>
<td>3.5 h</td>
<td>Hypoxia (90 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>van Raaij et al., 1996</td>
</tr>
<tr>
<td>118.2 ~ 134.3</td>
<td>104.2 ~ 249.5</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Brauner et al. 2000</td>
</tr>
<tr>
<td>150</td>
<td>750</td>
<td>8 h</td>
<td></td>
<td>Continuous swimming (8 h)</td>
<td>Atlantic smolts</td>
<td>Virtanen &amp; Forsman, 1987</td>
</tr>
</tbody>
</table>
Table 1.2  Literature values for plasma glucose concentrations in salmonids. Values given are sample means. Parr, smolts and trout are in freshwater (fw) unless otherwise stated.
* Time to recovery indicates the time it takes for values to return to pre-treatment values.

<table>
<thead>
<tr>
<th>Routine [glucose], mM</th>
<th>Maximally stressed [glucose], mM</th>
<th>Time to max stress</th>
<th>Time to recovery*</th>
<th>Stressor</th>
<th>Study animal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.93</td>
<td>4.1</td>
<td>3.75 h</td>
<td>0.75 h</td>
<td>Hypoxia (90 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>van Raaij et al., 1996</td>
</tr>
<tr>
<td>3.3</td>
<td>4.7 ± 1.2</td>
<td>2.5 h</td>
<td></td>
<td>Confinement (2.5 h)</td>
<td>Atlantic smolts</td>
<td>Sadler et al. 2000</td>
</tr>
<tr>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
<td>Nutrition study</td>
<td>Atlantic parr</td>
<td>Waagbø et al. 1994</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>1 h</td>
<td>&gt; 48 h</td>
<td>Loading &amp; truck transport (fw-fw, .5 ~ 4.5 h)</td>
<td>Atlantic smolts</td>
<td>Iversen et al. 1998</td>
</tr>
<tr>
<td>4.1</td>
<td>6.1</td>
<td>3</td>
<td>48 h</td>
<td>Netting confinement (9 min)</td>
<td>Atlantic adults (cannulated)</td>
<td>Waring et al. 1992</td>
</tr>
<tr>
<td>4.6</td>
<td>7.7</td>
<td>8 h</td>
<td>48 h</td>
<td>Acute handling + 3 h confinement</td>
<td>Atlantic smolts</td>
<td>Carey &amp; McCormick 1998</td>
</tr>
<tr>
<td>6.8</td>
<td>11</td>
<td>8 h</td>
<td></td>
<td>Continuous swimming (8 h)</td>
<td>Atlantic smolts</td>
<td>Virtanen &amp; Forsman, 1987</td>
</tr>
<tr>
<td>7</td>
<td>10.5</td>
<td>12 h</td>
<td>48 h</td>
<td>Netting &amp; transfer from sea-cage to tank</td>
<td>Atlantic adults</td>
<td>Hemre &amp; Krogdahl 1996</td>
</tr>
<tr>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
<td>Nutrition study</td>
<td>Atlantic adults</td>
<td>Waagbø et al. 1994</td>
</tr>
<tr>
<td>3 ~ 4</td>
<td>8.5</td>
<td>12 h</td>
<td>&gt;&gt; 48 h</td>
<td>Loading &amp; transport (2 h, fw)</td>
<td>Atlantic parr</td>
<td>Sandodden et al. 2001</td>
</tr>
<tr>
<td>3.8 ~ 5.7</td>
<td>Post load (? h)</td>
<td></td>
<td></td>
<td>Loading &amp; well boat transport</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Iversen et al. 2005</td>
</tr>
<tr>
<td>4.0 ~ 6.1</td>
<td>Natural smoltification</td>
<td></td>
<td></td>
<td>Atlantic smolts (wild)</td>
<td>Poole et al. 2003</td>
<td></td>
</tr>
<tr>
<td>4.4 ~ 6.1</td>
<td>7.7</td>
<td>4 h</td>
<td>8 h</td>
<td>Netting, confinement (2 min), tank transfer</td>
<td>Brown trout</td>
<td>Pickering et al., 1982</td>
</tr>
<tr>
<td>5.0 ~ 6.5</td>
<td>Natural smoltification</td>
<td></td>
<td></td>
<td>Atlantic smolts (wild)</td>
<td>Poole et al. 2003</td>
<td></td>
</tr>
<tr>
<td>8.3 ~ 12.7</td>
<td>6.3 ~ 9.4</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Brauner et al. 2000</td>
</tr>
<tr>
<td>2.7 ~ 4.3</td>
<td>water level reduction</td>
<td></td>
<td></td>
<td>Atlantic post-smolts</td>
<td>Einarsdottir &amp; Nilssen 1996</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.3. Literature values for plasma lactate concentrations in salmonids. Values given are means. Parr, smolts and trout are in freshwater (fw) unless otherwise stated.

* Time to recovery indicates the time it takes for values to return to pre-treatment values.

<table>
<thead>
<tr>
<th>Routine [lactate], mM</th>
<th>Maximally stressed [lactate], mM</th>
<th>Time to max stress</th>
<th>Time to recovery*</th>
<th>Stressor</th>
<th>Study animal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6.4</td>
<td>6 h</td>
<td>12 h</td>
<td>Hypoxia (90 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>van Raaij et al., 1996</td>
</tr>
<tr>
<td>~ 0.5</td>
<td>~ 13</td>
<td>2 h</td>
<td>&gt; 12 h</td>
<td>Swimming to exhaustion (6 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>Wood et al., 1983</td>
</tr>
<tr>
<td>0.7</td>
<td>0.7</td>
<td>48 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Maxime 2002</td>
</tr>
<tr>
<td>0.8 ~ 2.1</td>
<td>1.3 ~ 1.5</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Brauner et al. 2000</td>
</tr>
<tr>
<td>0.9</td>
<td>3.7</td>
<td>2.5 h</td>
<td></td>
<td>Confinement (2.5 h)</td>
<td>Atlantic smolts</td>
<td>Sadler et al. 2000</td>
</tr>
<tr>
<td>1</td>
<td>2.8 minutes</td>
<td>3 h</td>
<td></td>
<td>Netting confinement (9 min)</td>
<td>Atlantic adults (cannulated)</td>
<td>Waring et al. 1992</td>
</tr>
<tr>
<td>1</td>
<td>16.8</td>
<td>2 - 4 h</td>
<td></td>
<td>Swimming to exhaustion (10 min)</td>
<td>Coho post-smolts (cannulated)</td>
<td>Milligan &amp; McDonald, 1988</td>
</tr>
<tr>
<td>1</td>
<td>3.5</td>
<td>1 h</td>
<td>&gt; 48 h</td>
<td>Loading &amp; truck transport (fw-fw, .5 ~ 4.5 h)</td>
<td>Atlantic smolts</td>
<td>Iversen et al. 1998</td>
</tr>
<tr>
<td>1.1</td>
<td>6.1</td>
<td>2 h</td>
<td>4 h</td>
<td>Netting, confinement (2 min), tank transfer</td>
<td>Brown trout</td>
<td>Pickering et al., 1982</td>
</tr>
<tr>
<td>1.2 ~ 5.1</td>
<td>Post load (? h)</td>
<td></td>
<td></td>
<td>Loading &amp; well boat transport</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Iversen et al. 2005</td>
</tr>
<tr>
<td>1.4</td>
<td>4.8</td>
<td>1 h</td>
<td>24 h</td>
<td>Angling &lt;5 min</td>
<td>Rainbow trout (spawning, wild)</td>
<td>Pankhurst &amp; Dedual 1994</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>3 h</td>
<td>&lt; 8 h</td>
<td>Acute handling + 3 h confinement</td>
<td>Atlantic smolts</td>
<td>Carey &amp; McCormick 1998</td>
</tr>
<tr>
<td>(3.8)</td>
<td>7.2</td>
<td>1 h</td>
<td>24 h</td>
<td>Angling 15 min</td>
<td>Rainbow trout (spawning, wild)</td>
<td>Pankhurst &amp; Dedual 1994</td>
</tr>
</tbody>
</table>
Table 1.4. Literature values for plasma potassium concentrations in salmonids. Values given are means. Parr, smolts and trout are in freshwater (fw) unless otherwise stated. 
* Time to recovery indicates the time it takes for values to return to pre-treatment values.

<table>
<thead>
<tr>
<th>Routine [potassium], mM</th>
<th>Maximally stressed [potassium], mM</th>
<th>Time to max stress</th>
<th>Time to recovery*</th>
<th>Stressor</th>
<th>Study animal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3 ~ 2.5</td>
<td>2.73 ~ 2.8</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Quigley et al. 2006</td>
</tr>
<tr>
<td>2.3 ~ 2.4</td>
<td>2.7 ~ 2.8</td>
<td>48 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Maxime 2002</td>
</tr>
<tr>
<td>~ 2.5</td>
<td>~ 5</td>
<td>2 h</td>
<td>&gt; 12 h</td>
<td>Swimming to exhaustion (6 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>Wood et al., 1983</td>
</tr>
<tr>
<td>2.7</td>
<td>3.3 ~ 3.6</td>
<td>minutes</td>
<td>8 h</td>
<td>Netting confinement (9 min)</td>
<td>Atlantic adults (cannulated)</td>
<td>Waring et al. 1992</td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
<td></td>
<td></td>
<td>Hypoxia (90 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>van Raaij et al., 1996</td>
</tr>
<tr>
<td>3.1</td>
<td>1.7</td>
<td>3 h</td>
<td>24 h</td>
<td>Acute handling + 3 h confinement</td>
<td>Atlantic smolts</td>
<td>Carey &amp; McCormick 1998</td>
</tr>
<tr>
<td>4.5</td>
<td>4.0 ~ 4.7</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Brauner et al. 2000</td>
</tr>
</tbody>
</table>
Table 1.5. Literature values for plasma sodium concentrations in salmonids. Values given are means. Parr, smolts and trout are in freshwater (fw) unless otherwise stated.

* Time to recovery indicates the time it takes for values to return to pre-treatment values.

<table>
<thead>
<tr>
<th>Routine sodium, mM</th>
<th>Maximally stressed sodium, mM</th>
<th>Time to max stress</th>
<th>Time to recovery*</th>
<th>Stressor</th>
<th>Study animal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>135 ~ 142</td>
<td>165</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Brauner et al. 2000</td>
</tr>
<tr>
<td>135 ~ 145</td>
<td>~ 165</td>
<td>1 h</td>
<td>8 h</td>
<td>Swimming to exhaustion (6 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>Wood et al., 1983</td>
</tr>
<tr>
<td>137 ± 4</td>
<td>NS</td>
<td></td>
<td></td>
<td>Hypoxia (90 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>van Raaij et al., 1996</td>
</tr>
<tr>
<td>138.9 ~ 145.4</td>
<td></td>
<td></td>
<td></td>
<td>Natural smoltification</td>
<td>Atlantic smolts</td>
<td>Poole et al. 2003</td>
</tr>
<tr>
<td>143.3 ~ 148.7</td>
<td>153</td>
<td>8 h</td>
<td>14 d</td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Singer et al. 2002</td>
</tr>
<tr>
<td>147.3 ~ 156.2</td>
<td></td>
<td></td>
<td></td>
<td>Natural smoltification</td>
<td>Atlantic smolts (wild)</td>
<td>Poole et al. 2003</td>
</tr>
<tr>
<td>150</td>
<td>170</td>
<td>48 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Maxime 2002</td>
</tr>
<tr>
<td>153.5</td>
<td>167.1</td>
<td>48 h</td>
<td>14 d</td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Nonnotte &amp; Boeuf 1995</td>
</tr>
<tr>
<td>154 ~ 162</td>
<td>162 ~ 168</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Chinook, coho smolts (fw-sw)</td>
<td>Blackburn &amp; Clarke, 1987</td>
</tr>
<tr>
<td>155</td>
<td>180</td>
<td>3 h</td>
<td>24 h</td>
<td>Netting confinement (9 min)</td>
<td>Atlantic adults (cannulated)</td>
<td>Waring et al. 1992</td>
</tr>
<tr>
<td>155 ~ 165</td>
<td>176 ~ 211</td>
<td>24 h</td>
<td></td>
<td>Loading &amp; truck transport + SW challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Iversen et al. 1998</td>
</tr>
<tr>
<td>157</td>
<td>152</td>
<td>8 h</td>
<td>48 h</td>
<td>Acute handling + 3 h confinement</td>
<td>Atlantic smolts</td>
<td>Carey &amp; McCormick 1998</td>
</tr>
<tr>
<td>157</td>
<td>175</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Smith et al. 1991</td>
</tr>
<tr>
<td>159 ~ 166</td>
<td>167 ~ 171</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Quigley et al. 2006</td>
</tr>
</tbody>
</table>
Table 1.6. Literature values for plasma chloride concentrations in salmonids. Values given are means. Parr, smolts and trout are in freshwater (fw) unless otherwise stated.
* Time to recovery indicates the time it takes for values to return to pre-treatment values.

<table>
<thead>
<tr>
<th>Routine [chloride], mM</th>
<th>Maximally stressed [chloride], mM</th>
<th>Time to max stress</th>
<th>Time to recovery*</th>
<th>Stressor</th>
<th>Study animal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>?</td>
<td>138 ~ 150</td>
<td></td>
<td></td>
<td>water level reduction</td>
<td>Atlantic post-smolts</td>
<td>Einarsdottir &amp; Nilssen 1996</td>
</tr>
<tr>
<td>110</td>
<td>203.2</td>
<td>12 h</td>
<td>48 h (150.8)</td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Handeland et al. 1996</td>
</tr>
<tr>
<td>115 ~ 120</td>
<td>145</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Brauner et al. 2000</td>
</tr>
<tr>
<td>122.1 ~ 126.1</td>
<td></td>
<td></td>
<td></td>
<td>Natural smoltification</td>
<td>Atlantic smolts</td>
<td>Poole et al. 2003</td>
</tr>
<tr>
<td>127</td>
<td>148</td>
<td>24 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Smith et al. 1991</td>
</tr>
<tr>
<td>129 ~ 132</td>
<td></td>
<td></td>
<td></td>
<td>Natural smoltification</td>
<td>Atlantic smolts (wild)</td>
<td>Poole et al. 2003</td>
</tr>
<tr>
<td>129 ~ 138</td>
<td>110 ~ 120</td>
<td>&gt; 24 h</td>
<td>&gt; 48 h</td>
<td>Loading &amp; truck transport (0.5 ~ 4.5 h)</td>
<td>Atlantic smolts</td>
<td>Iversen et al. 1998</td>
</tr>
<tr>
<td>~ 130</td>
<td>140 / 120</td>
<td>30 min / 4 h</td>
<td>&gt; 12 h</td>
<td>Swimming to exhaustion (6 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>Wood et al., 1983</td>
</tr>
<tr>
<td>130</td>
<td>165</td>
<td>48 h</td>
<td>96 h (160)</td>
<td>SW Challenge (4.6°C)</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Handeland et al. 2000</td>
</tr>
<tr>
<td>130</td>
<td>175</td>
<td>12 h</td>
<td>96 h (145)</td>
<td>SW Challenge (9.1°C)</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Handeland et al. 2000</td>
</tr>
<tr>
<td>130 ~ 132</td>
<td>155 ~ 158</td>
<td>48 h</td>
<td></td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Maxime 2002</td>
</tr>
<tr>
<td>130.5 ~ 131.8</td>
<td>140 ~ 142</td>
<td>8 h</td>
<td>14 d</td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Singer et al. 2002</td>
</tr>
<tr>
<td>133 ~ 138</td>
<td>151 ~ 181</td>
<td>24 h</td>
<td></td>
<td>SW challenge following truck transport</td>
<td>Atlantic smolts</td>
<td>Iversen et al. 1998</td>
</tr>
<tr>
<td>133</td>
<td>NS</td>
<td></td>
<td></td>
<td>Hypoxia (90 min)</td>
<td>Rainbow trout (cannulated)</td>
<td>van Raaij et al., 1996</td>
</tr>
<tr>
<td>138</td>
<td></td>
<td></td>
<td></td>
<td>Nutrition study</td>
<td>Atlantic adults</td>
<td>Waagbe et al. 1994</td>
</tr>
<tr>
<td>139</td>
<td>124</td>
<td>3 h</td>
<td>24 h</td>
<td>Acute handling + 3 h confinement</td>
<td>Atlantic smolts</td>
<td>Carey &amp; McCormick 1998</td>
</tr>
<tr>
<td>140.5</td>
<td>155.0</td>
<td>48 h</td>
<td>14 d</td>
<td>SW Challenge</td>
<td>Atlantic smolts (fw-sw)</td>
<td>Nonnotte &amp; B0euf 1995</td>
</tr>
<tr>
<td>145</td>
<td>128</td>
<td>4 h</td>
<td>&gt; 48 h</td>
<td>Loading &amp; transport (2 h, fw)</td>
<td>Atlantic parr</td>
<td>Sandodden et al. 2001</td>
</tr>
<tr>
<td>146</td>
<td>122</td>
<td>8 h</td>
<td></td>
<td>Continuous swimming (8 h)</td>
<td>Atlantic smolts</td>
<td>Virtanen &amp; Forsman, 1987</td>
</tr>
<tr>
<td>165 ~ 170</td>
<td>190</td>
<td>1 h</td>
<td>48 h</td>
<td>Netting confinement (9 min)</td>
<td>Atlantic adults (cannulated)</td>
<td>Waring et al. 1992</td>
</tr>
</tbody>
</table>
References


BCMAL (British Columbia Ministry of Agriculture and Lands), 2007b. Province issues decisions on three aquaculture site licenses. media release 2007AL0044-001032, Parliament Buildings, Victoria, BC, Canada 29 August.


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CHAPTER 2: PHYSIOLOGICAL RESPONSES OF ATLANTIC SALMON (Salmo salar) SMOLTS DURING COMMERCIAL TRANSPORT ON THE WEST COAST OF CANADA

2.1 Introduction

Salmon aquaculture in British Columbia (BC) is economically important for many coastal communities, and is the largest reported agricultural export product for the province (BCMAL 2006). Farmed salmon from BC have been reported to contain low levels of contaminants and provide a highly nutritious food for humans (Ikonomou et al., 2007), and so as the wild salmon fishery continues to decline, farmed salmon will continue to play an important role in the world seafood market (Tacon et al., 2006).

In BC, most salmon aquaculture operations occur around the northern half of Vancouver Island and along the Central Coast. Juvenile salmon are produced and raised at inland freshwater hatcheries or farms, which in many cases are located far from the saltwater grow-out sites. Therefore, each year, approximately 15 million commercially raised smolts (as reported by Farrell 2006) are transported to sea farms en masse, using either trailer trucks over land or live-haul vessels at sea. Good fish welfare during transport is essential as it ensures that mortality is minimal and growth quickly resumes in the sea cages. In addition, good fish welfare provides for greater public confidence in farmed salmon (Ellis et al., 2002; Ashley, 2007).

Fish welfare is commonly assessed in terms of stress physiology (Ellis et al., 2002; Braithwaite and Huntingford, 2004; Huntingford et al., 2006; Portz et al., 2006; Ashley, 2007). The majority of work in this area has focused on the three levels of the generalized stress response in animals, which apply to fish. Elevated plasma catecholamines and cortisol, which are released in direct response to a stressor, represent the primary stress response (Mazeaud et al., 1977; Barton

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and Iwama, 1991; Barton, 2002). Secondary stress responses, triggered by the aforementioned primary hormones, include changes in metabolic activity, plasma glucose levels, osmotic and ionic balance, immune function and heat shock proteins (Wedemeyer, 1996; Wendelaar Bonga, 1997; Iwama et al., 2004). Tertiary stress responses at the whole-animal level include changes in growth rate, disease susceptibility and behaviour (Hemre and Krogdahl, 1996; Duncan et al., 1998; McCormick et al., 1998; Gregory and Wood, 1999; Arnesen et al., 2003; Handeland et al., 2003).

A considerable amount of work has focused on transport stress in fish (e.g. Cooke, 2004; Chandroo et al., 2005; Iversen et al., 2005). The majority of these studies has focused on changes in plasma cortisol, glucose and ions as indicators of primary and secondary stress in fish (Barton and Iwama, 1991; Wedemeyer, 1996; Wendelaar Bonga, 1997). However, studies on salmonid smolts are confounded by plasma cortisol being naturally elevated during the fish’s preparation for seaward migration (e.g. Shrimpton et al., 2000; Quigley et al., 2006). Cortisol mobilizes liver glycogen stores (thereby increasing plasma glucose) and is associated with increased chloride cell numbers and gill Na\(^+\), K\(^+\)-ATPase activity (Langhorne and Simpson, 1986; Mommsen et al., 1999; Singer et al., 2003). Although abrupt transfer of smolts from freshwater to seawater is standard management practice in commercial operations, little is known about the physiological changes that take place during such large-scale operations.

To my knowledge there has only been one study that has examined the effects of transport on the physiological response of commercial smolts using live-haul vessels (also called well boats). Iversen et al. (2005) measured plasma cortisol, glucose and lactate levels in Norwegian Atlantic salmon (*Salmo salar*) smolts. Although each of the five transports that were followed in that study varied in terms of vessel size, duration of haul and number of fish transported, the authors concluded that the greatest stressor was the process of loading the fish into the live-haul vessels. For the most part, transport aboard the vessels appeared to calm the smolts such that by the time they arrived at the sea farms 4 to 40 h later, their mean plasma cortisol had returned to
pre-transport levels. Plasma glucose and lactate, the secondary stress responses, were found to be extremely variable across transports. Interestingly, plasma cortisol levels of smolts hauled in one vessel became elevated at the end of the trip and this physiological response was followed by a 12% cumulative mortality at the sea farm after 30 days, unlike the more normal cumulative mortalities of 0.2 - 1.6% for the other vessels during the 30 days post-transport. An increased background plasma cortisol level due to smoltification is reported to intensify a fish’s response to acute stressors such as handling, crowding or changes in water quality (Woodward and Strange, 1987; Hunn and Greer, 1991; Shrimpton and Randall, 1992; Carey and McCormick, 1998; Congleton et al., 2000). It is, therefore, not surprising that transport-related stress was suggested as a possible factor for high post-delivery mortality rates (Iverson et al., 2005).

Since BC aquaculture producers are continuously looking for ways to improve fish survival as well as the final product quality, I assessed changes in water quality and fish stress physiology during routine live-haul smolt transport conditions used by one of BC’s largest salmon producing companies to identify any points of concern. In addition to plasma cortisol, glucose and lactate, I measured plasma ions (sodium, chloride and potassium levels), and obtained post-transport mortality and growth records. Based on the work of Iversen et al. (2005) and other studies on smolt handling and transport (Specker and Schreck, 1980; Carey and McCormick, 1998; Iversen et al., 1998), I predicted that the initial loading process and transport by truck would elicit physiological stress responses, and that recovery would follow on board the live-haul vessel.

### 2.2 Materials & Methods

#### 2.2.1 Experimental animals

Atlantic salmon (Salmo salar) smolts were raised commercially by Marine Harvest Canada, formerly Stolt Sea Farms, BC, Canada. I examined smolts from two of their freshwater rearing sites on northern Vancouver Island (Figure 2.1). One site was a land-based facility (Dalrymple)
that raised fish in circular, 9 - 10 m fibre-glass tanks with re-circulated groundwater, and required a 30 min truck journey to reach the dock where the smolts were transferred to the sea going vessel. The second site (Georgie Lake) had square net pens measuring 50' x 50', similar in design to those used at saltwater grow-out farms, and required a 90 min truck journey to the saltwater dock. A brief description of the fresh water facilities is given in Table 2.1. The smolts were either Mowi or McConnell x Mowi (“McMowi”) strains, mixed together into a general population, and were aged between 10 - 12 months post hatch at the time of this study. Shipments occurred when mean gill Na\(^+\), K\(^+\)-ATPase rose above 10 \(\mu\)mol ADP h\(^{-1}\) mg protein\(^{-1}\) (as determined by Marine Harvest Canada operations).

2.2.2. Transport process

Smolt transports followed Marine Harvest Canada standard operating protocols, and always began at 07:00. Smolts moved early in the season were all from Dalrymple, where 24-hour light was used—thus all transports began in light. At Dalrymple, fish were vacuum-pumped through a drain at the bottom of the fibreglass tanks to an elevated platform and on to a computerized smolt counter. At Georgie Lake, fish were crowded with a seine net before being vacuum-pumped in the same manner. After counting, fish were allowed to drain by gravity into transport tanks aboard truck trailers to a density of 60 - 80 kg m\(^{-3}\). I chose to sample from a tanker-type trailer, which had two compartments: 12 m\(^3\) in the front, and either 18 m\(^3\) or 16 m\(^3\) in the back. Both groups were transported in their respective source waters. Air and pure O\(_2\) gas were supplied, but no chemical additives were used. O\(_2\) saturation of the water was checked at the beginning, midway, and at the end of each trip and adjusted if necessary, to maintain 110 - 120% saturation (\(~13 - 15\) mg O\(_2\) l\(^{-1}\) ). Upon arrival at the dock, fish were immediately drained by gravity through a 6”-diameter hose into one of the two cargo holds of the live-haul vessel, which were initially half-filled with seawater. Smolts were added to the cargo holds along with their transport
water, so that water in the cargo hold tended to be brackish, particularly near the surface where a brackish water layer remained until loading was completed for that hold.

The *Sterling Carrier* is a 40 m long, state-of-the-art live-haul vessel with two identical cargo holds, each with a water volume of 325 m$^3$. As illustrated in Figure 2.2, each of these holds has front and rear valves below sea level, which can be opened to allow ambient seawater to flow through, as well as cargo pumps to circulate the water inside (see Farrell, 2006). While waiting for the smolt trucks to arrive, the valves were kept open at 50% and the pumps operated at about 60%, resulting in a complete turnover of seawater approximately every hour. Pure O$_2$ gas was added, and dissolved O$_2$ was continuously monitored and adjusted to maintain ~8 mg O$_2$ l$^{-1}$.

Four specially designed fish transport trucks, each capable of carrying 20 - 30 m$^3$ of water, shuttled back and forth between the freshwater farms and the dock where the *Sterling Carrier* was moored. Typically, it took 4 - 6 truckloads of smolts to fill each cargo hold, with 30 - 60 min intervals between loads. One hold was filled at a time, each taking 2.5 - 4 h to fill. For this study the total vessel loading time ranged between 5.5 - 7.5 h. The *Sterling Carrier* left the dock as soon as the last truck finished unloading. Consequently, at departure, each hold contained fish that had arrived at different times, and those in the first hold to be filled had been onboard (on average) twice as long as the fish in the second hold. If, as I assumed, each new truckload of fish disturbed those that were already in the hold, proper recovery of the fish in the second hold could not begin until loading was complete and the vessel got underway. Similarly, I also assumed that the fish contained in the first hold had had a 3 - 4 h recovery period as the *Sterling Carrier* remained at dockside during loading of the second hold (before the vessel got underway). Figure 2.3 summarizes a typical smolt loading procedure with 8 truckloads of smolts.

During sea transport, the valves of the *Sterling Carrier* remained 50% open, so that at the cruising speed of 9 knots, seawater was replenished in each hold at a rate of ~25 m$^3$ min$^{-1}$. The hatch covers of the holds on the deck of the boat were left open unless rough water was expected.
Each voyage was approximately 2.5 h long, with the exception of one 7.5-h trip. Table 2.2 summarizes the conditions for each of the transport shipments that were followed in this study.

2.2.3 Sampling procedures

Fish and water samples were taken a) at the freshwater facility one day before transport, b) from the trucks at the end of truck transport, and c) from the holds of the *Sterling Carrier* at completion of loading, and every 2 h thereafter, as described below.

2.2.3.1 Water sampling

Water was collected directly from the tanks and pens at the freshwater facilities, from the rear compartment of the tanker trucks, and the *Sterling Carrier*’s holds. Temperature, pH, salinity and dissolved oxygen were measured on site using handheld meters (Symphony SP301, VWR, Buffalo Grove IL; YSI Model 30, YSI Incorporated, Yellow Springs OH; OxyGuard Handy, Point Four Systems, Richmond BC). Samples for dissolved CO$_2$ were transferred to 250 ml ground glass stoppered BOD bottles, spiked with 2.5 ml of saturated aqueous HgCl$_2$, and then sealed with vacuum grease and Parafilm$^\text{TM}$ for later analysis. Samples for total ammonia (NH$_3$-N) were placed in 15 ml plastic tubes with screw tops, acidified with 2 - 3 drops of 6 M aqueous HCl and frozen at -20°C for later analysis.

2.2.3.2 Fish sampling

To obtain control values for the plasma variables, fish were sampled at the freshwater rearing sites on the day preceding shipment in order to allow the remaining fish time to recover from any disturbances and to avoid disturbances from the general activity associated with fish transfer. Sampling was always done as quickly and quietly as possible. At Dalrymple 3 - 5 fish were caught directly by dipnet, usually with a single scoop from each tank identified for transport the
following day. Fish were collectively placed into the anaesthetic bucket, typically within a few seconds (always < 1 min) from initial disturbance of the tank. At Georgie Lake the fish were first gently crowded by a seine net because of the lower rearing density (see Table 2.1) before dipnetting as above, and this procedure added a full minute before fish were placed in the anaesthetic bucket. Fish were sampled on 3 separate days at Dalrymple (10 tanks in total) and 2 days at Georgie Lake (10 pens in total). I assumed that the measurements taken at the freshwater facilities were the best possible representation of the routine state for these smolts, acknowledging the possibility for sampling stress.

Fish were uniformly sampled at the end of truck transport by removing them as they were discharged from the rear compartment of the 1st, 5th and sometimes 9th truckloads arriving at the dock. Half way through discharge of the rear compartment (after ~ 8 min), a dipnet was held under the hose to remove 5 fish. By sampling the last compartment on the truck at the end of truck transport, I assumed that all fish were as stressed as much as they would be by truck transport, and that the method of sampling fish minimised any additive sampling effect. A total of 7 trucks transporting smolts from Dalrymple were sampled on 3 different sampling dates, for a total of 35 fish, and 6 trucks transporting smolts from Georgie Lake were also sampled on 3 different sampling dates for a total of 30 fish. All trucks could not be sampled as they were arriving as quickly as 30 min apart, and sampled fish needed to be measured and processed for plasma, gill and brain samples.

The identical holds on board the *Sterling Carrier* provided two potentially different recovery regimes. For the first hold that was filled, the fish had several hours to recover while the *Sterling Carrier* remained dockside (Figure 2.3); whereas, fish recovery in the second hold could begin only after the *Sterling Carrier* got underway. Fish (n = 10) were sampled once the loading of each hold was completed, and this was repeated every 2 h thereafter for each hold. Therefore, as Figure 2.3 indicates, the time 0 samples for the first hold always occurred while the *Sterling Carrier* was docked,
and time 0 samples for the second hold took place just as the vessel got underway. Thus, by comparing the two holds, it was possible to determine the degree of fish recovery at dockside. Although there was seawater exchange while the vessel was stationary due to waves and the vessel's pumps, the water current through the holds at dockside was much less than when the vessel was underway. Using either a 3-m long dipnet or a brailer, I captured 3 or 4 fish at a time from the hold as quickly as possible to minimize handling effects. The large size of the holds and the motion of the vessel at sea resulted in capture times varying from 30 s to 5 min, and only fish in the top 2 m of water could be effectively sampled. Because the fish in the holds were likely from different truckloads and thus potentially variable in their response, the sample size was increased to 10 fish at a time.

2.2.3.3 Fish tissue handling

Fish were euthanized in water containing 400 mg l\(^{-1}\) of clove oil, prepared as described by Holloway et al. (2004). Clove oil is effective in suppressing post-capture blood cortisol release (Cho and Heath, 2000; Iversen et al., 2003; Cooke et al., 2004). Blood was removed by caudal puncture using syringes with 23-gauge needles pre-coated with lithium heparin (Sigma-Aldrich, St. Louis MO), and kept on ice for a maximum of 15 min before centrifugation for 10 min at 1,163 x g (BD-Adams™ Compact II Centrifuge, Franklin Lakes, NJ). Plasma was removed and frozen immediately in liquid nitrogen for transport, and stored at -80° C until analysis. The fish sampling procedures were approved by the University of British Columbia Animal Care Committee.

2.2.4 Analytical procedures

2.2.4.1 Water quality

The total CO\(_2\) in sample waters was measured in triplicate. 2 ml aliquots of sample water were combined with 4 ml of 0.1 M HCl saturated with N\(_2\) gas and 4 ml of N\(_2\) gas, and allowed to
mix for 4 min. The resulting gas phase passed through a dehydrating filter and into a Carle Model III gas chromatograph connected to a chart recorder, as described by Tang et al. (in prep.). Total CO$_2$ was calculated from the height of the peaks using bicarbonate (Sigma-Aldrich, St. Louis MO) standards that were prepared daily. The total CO$_2$ values were then converted to partial pressure of CO$_2$ using the Henderson-Hasselbach equation (eq. 5.8, Cameron, 1986). Total ammonia (NH$_3$-N) in water was determined with the method described by Verdouw et al. (1977), using sodium nitroprusside instead of potassium ferricyanide. Processed samples were transferred in duplicate to a 96-well plate and read using a microplate spectrophotometer (SpectraMax 340PC, Molecular Devices Corporation, Sunnyvale CA). Assays were repeated for duplicates whose coefficient of variance exceeded 10%.

### 2.2.4.2 Plasma chemistry

Plasma glucose and lactate were measured using an immobilized enzyme biosensor analyzer, chloride was measured using silver chloride titration, and cortisol concentrations were measured using commercial ELISA kits (Neogen Corp., Lexington, KY). All methods are described in detail in Farrell et al. (2001). Plasma sodium and potassium concentrations were measured with a single channel digital flame photometer (Model EW-02655-00, Cole-Parmer, Vernon Hills IL) calibrated with a four-point standard curve. A 5-µl aliquot of plasma was diluted 1:1000 for sodium and 1:200 for potassium with deionized water for analysis. Assays were repeated if duplicate samples were more than 6 mM apart for sodium, and 0.6 mM apart for potassium.

### 2.2.5 Growth and mortality

Marine Harvest Canada provided records on mortality and growth rates of fish for up to 30
days following each smolt shipment. Specific growth rate was calculated as described by Rowe and Thorpe (1990):

\[
SGR = 100 \times (\log M_n – \log M_1)/(D_n – D_1)
\]

where SGR is specific growth rate, M is fish mass at day 1 and day \( n \), and \( D_n – D_1 \) is number of days between days 1 and \( n \).

2.2.6 Statistical analysis

Statistical analyses were carried out using SigmaStat 3.0.1a for Windows (Systat Software, Inc., San Jose CA). The Kolmogorv-Smirnov test was used to test for normality, and a one-way ANOVA was performed among sample points with \( \alpha = 0.05 \). When warranted, the Holm-Sidak procedure was used for post hoc multiple comparisons. If data sets were not normally distributed, a Kruskal-Wallis One Way Analysis of Variance on Ranks Test and Dunn's Test were used to determine significance. Unless otherwise noted, results are presented as sample mean ± standard deviation.

2.3 Results

2.3.1 Water quality

Water temperature at the two freshwater facilities was similar (8.5 ± 0.3 °C for Dalrymple and 4.6 ± 1.4 °C for Georgie Lake) during the 3-month period when smolts were transported for this study, and salinity was at the limit of detection (0.1 – 0.2 ppt). As shown in Figure 2.4, rearing water pH at Dalrymple was 6.96 ± 0.01, while the lake water at Georgie Lake was 6.12 ± 0.08.

Water pH, \( CO_2 \) and \( NH_3-N \) did not change during closed truck transport for Dalrymple fish, but for Georgie Lake fish, which were subjected to a longer drive, there were significant increases in \( CO_2 \) and \( NH_3-N \), but water pH was unchanged.

Aboard the Sterling Carrier, water pH increased while \( CO_2 \) and \( NH_3-N \) decreased for both
groups. As smolts were added to the cargo holds along with their transport (fresh) water, salinity varied during loading between ~20 ppt just below the surface to full strength (~30 ppt) at 3 m below the surface. All water variables converged to ambient seawater values by 2 h after loading was completed.

2.3.2 Plasma chemistry

Figures 2.5 and 2.6 summarize the changes in plasma chemistry before and during the course of transport, the details of which are described below.

2.3.2.1 Routine status prior to transport

Fish at Dalrymple hatchery were at sufficiently high densities (35.9 ± 4.5 kg m⁻³) to allow for quick capture with minimal disturbance. As shown in Figures 2.5 and 2.6, for fish taken from the Dalrymple tanks, plasma cortisol was 29.3 ± 26.4 ng ml⁻¹, glucose was 4.4 ± 1.1 mM, lactate was 3.9 ± 0.9 mM, chloride was 127.2 ± 3.4 mM, sodium was 145.1 ± 9.2 mM and potassium was 4.1 ± 0.9 mM. In contrast, fish at Georgie Lake were stocked at much lower densities (5.8 ± 2.0 kg m⁻³) and had to be crowded with a seine net before capture. Despite the potential for greater capture stress, plasma cortisol, sodium and potassium (18.6 ± 9.2 ng ml⁻¹, 148.2 ± 5.2 mM and 3.9 ± 0.5 mM, respectively) concentrations were similar (P > 0.243) to those obtained for Dalrymple fish. Plasma glucose (6.2 ± 1.2 mM), lactate (5.5 ± 1.6 mM) and chloride (136.2 ± 6.0 mM) concentrations, however, were significantly higher (P ≤ 0.016) for Georgie Lake versus Dalrymple fish.

2.3.2.2 Plasma chemistry after truck transport

Plasma cortisol (Figure 2.5a) increased four- to six-fold at the end of truck transport compared with the respective routine (pre-transport) plasma cortisol values obtained at the rearing
farms. There was no difference (P = 0.656) between the Dalrymple group (132.5 ± 45.0 ng ml⁻¹) and the Georgie Lake group (122.6 ± 29.3 ng ml⁻¹) despite the longer truck transport for the latter. In contrast, plasma glucose, lactate, chloride, sodium and potassium concentrations did not change significantly (P > 0.05) as a result of truck transport. Again, there were no significant differences between Dalrymple and Georgie Lake groups for plasma lactate, sodium and potassium concentrations despite different driving times and the corresponding changes in water quality. Only glucose and chloride were different (P ≤ 0.004) between groups, but both parameters were already higher for Georgie Lake fish before transport.

2.3.2.3 Plasma chemistry aboard the Sterling Carrier

There were no significant differences (P > 0.05) in the plasma chemistry values between the two holds (Table 2.3), even though sampling began (t= 0) while the Sterling Carrier was still dockside for the first hold. This implies that the physiological recovery described below occurred similarly for fish whether the vessel was stationary at dockside, or underway. Therefore, the data for the two holds were pooled (Figures 2.5 and 2.6) in order to increase statistical power for the analysis of post-truck transport recovery.

For Georgie Lake fish, plasma cortisol concentrations remained elevated at load completion (165.8 ± 7.9 ng ml⁻¹; P < 0.001) compared to routine values, but no higher than the post-trucking values. In contrast, plasma cortisol of Dalrymple fish at t = 0 h was not significantly different (P > 0.05) from either the routine value or the post-truck value, suggesting an earlier onset of recovery for these fish. For both fish groups, plasma cortisol at t = 2 h was similar (P > 0.05) to routine values, suggesting that there were no additional primary stressors to the smolts onboard the Sterling Carrier. Plasma glucose reached a peak value at t = 0 h for both groups, with both groups increasing by 1 - 2 mM (to 5.1 ± 0.8 mM for Dalrymple fish and to 8.6 ± 1.4 mM for Georgie Lake fish). Plasma glucose returned to routine levels at t = 2 h, again suggesting that there were no
additional stressors to the smolts onboard the *Sterling Carrier*. Plasma chloride rose significantly from post-truck values for Dalrymple fish at $t = 0$ h ($P = 0.018$), and for Georgie Lake fish at $t = 2$ h ($P = 0.002$), changes that possibly reflected the abrupt transfer of smolts into seawater. Nevertheless, sodium and potassium values were unchanged ($P > 0.128$) from routine values during transport.

### 2.3.3 Growth and survival data from farms

Figure 2.7 summarizes mortality and growth data from the saltwater farms. Mortality data were converted to percent mortality per pen whenever a single pen contained fish from one hold of the *Sterling Carrier* (pens that received fish from multiple freshwater sites were omitted). Percent mortality ranged between 0.74 and 1.19\% over the 30 days, except in one pen containing Dalrymple fish where a disease outbreak caused mortality to increase to 1.93\%. Growth per pen was similarly estimated based on the amount of feed consumed, and ranged between 0.8 - 1.5 g fish$^{-1}$ day$^{-1}$, which equates to 0.9 - 1.4 \% mass day$^{-1}$ or a specific growth rate of between 0.8 - 1.2.

### 2.4 Discussion

Handling, chasing or confinement can trigger a stress response in fish (Wedemeyer, 1976; Strange et al., 1977; Laidley and Leatherland, 1988; Waring et al., 1992; Erikson et al., 1997; Sharpe et al., 1998; Skjervold et al., 2001; Barton et al., 2005). Studies that consider catecholamine or cortisol responses to stressors are often confounded by the effect of sampling, which can cause a physiological response in the study animal, particularly when attempting to obtain control or pre-treatment values (Barton and Peter, 1982; Hunn and Greer, 1991; Poole et al., 2003; Turnbull et al., 2005). In this study, small fish were sampled from large tanks, net pens and and live-holds. My challenge was to minimize the sampling effect when capturing fish (i.e., preventing an overestimate of the level of stress), as well as minimizing the stress to the fish that remained (i.e.,
preventing additional stress for fish that could be subsequently sampled), while providing a representative fish sample from a large and varied population. Secondly, fish had to be processed quickly after capture and this necessarily limited the number of fish samples that could be processed during a time-sensitive commercial operation. Thirdly, the effects of smoltification and abrupt seawater transfer on plasma cortisol and ions (Folmar and Dickhoff, 1980; Specker and Schreck, 1982; Carey and McCormick, 1998, Brauner et al., 2000; Shrimpton et al., 2000; Poole et al., 2003) were confounding effects. Fewer replicates onboard the Sterling Carrier was also a concern in terms of statistical power; however, tests in which $1 - \beta < 0.8$ had $P > 0.13$ (where $\alpha = 0.05$), thus statistical significance was not an issue.

2.4.1 Water quality

Both CO$_2$ and NH$_3$-N are metabolic waste products, which can accumulate to high concentrations in intensive culture systems, and particularly during live-haul transport in a closed container of water. Dissolved CO$_2$ can dissociate and react with water to form bicarbonate, carbonate and hydrogen ions, thereby lowering pH (Sanni and Forsberg, 1995). Ammonia is produced as a toxic by-product of protein catabolism and normally diffuses into the ambient water through the gills, but high environmental ammonia concentrations can interfere with this excretion—and ammonia accumulation in the organism can affect the central nervous system (Brockway, 1950; Meade, 1985; Randall and Tsui, 2002). Commercial freshwater aquaculture operations rely on the industry threshold standards of 10 mg l$^{-1}$ CO$_2$ (total carbonate) and 0.02 mg l$^{-1}$ ammonia for water quality (Wedemeyer, 1996), which convert roughly to about 5 mm Hg dissolved CO$_2$ (Fivelstad et al., 1998; Fivelstad et al., 1999; Fivelstad et al., 2003) and 225 µmol l$^{-1}$ NH$_3$-N. In this study, dissolved CO$_2$ rose to near the threshold levels for the Georgie Lake smolts during transport by truck, but remained similar to routine values during the rest of the transport aboard the Sterling Carrier. Throughout the study, NH$_3$-N remained below 50 µmol l$^{-1}$; therefore
this was not considered a stress factor.

2.4.2 Routine status at the freshwater farms

Routine plasma chemistry of smolts was established before transport and, despite the potential difficulties of sampling smolts quickly and without major disturbances, plasma variables were largely within the expected routine values for salmon smolts. In particular, the mean values for the primary stress hormone cortisol for the two groups were well within the range of resting plasma cortisol concentrations reported by other studies on salmonid smolts (Specker and Schreck, 1980; Virtanen and Forsman, 1987; Iversen et al., 1998; Shrimpton et al., 2000; Iversen et al., 2003; Poole et al., 2003; Iversen et al., 2005). The slightly longer sampling procedure used for the Georgie Lake fish did not result in elevated plasma cortisol levels. Moreover, 24% of the individual cortisol values for Dalrymple fish and 38% for Georgie Lake fish were under 10 ng ml\(^{-1}\), a level considered normal for non-smolting, non-spawning salmonids (Pickering and Pottinger, 1989). Sampling order of smolts did not appear to affect cortisol values; it was likely not a factor as fish were caught together in one dip per tank or pen and immediately anaesthetized.

To my knowledge this study is the first to compare plasma cortisol levels for smolts raised in lake-pens versus land-based tanks. Considering that wild fish are reported to have a greater response to handling stress than hatchery fish (Woodward and Strange, 1987; Shrimpton and Randall, 1992; Congleton et al., 2000), and adult Atlantic salmon in sea cages have been reported to have higher plasma cortisol concentrations (Hemre and Krogdahl, 1996) than those in tanks (Waring et al., 1992; Einarsdóttir and Nilssen, 1996; Veiseth et al., 2006), I cannot discount the possibility that the Georgie Lake fish had even lower basal cortisol before seining. Furthermore, routine plasma glucose, lactate and chloride concentrations were significantly higher for the Georgie Lake fish compared to Dalrymple fish.

Plasma lactate, considered a secondary stress response, was higher than resting levels
reported for salmonids (Pickering et al., 1982; Milligan, 1996; Carey and McCormick, 1998) for both groups. Plasma lactate is produced by anaerobic metabolism in the white muscles and released into the blood almost immediately following vigorous exercise and typically continues to rise for about 2 h after burst activity (Pickering et al., 1982; Waring et al., 1992; Milligan 1996, McDonald and Milligan, 1997). Thus, the modest difference in plasma lactate between groups is likely to be the result of the slightly longer sampling time for lake fish, allowing lactate release from muscle to blood to have progressed somewhat more.

A similar explanation for the elevated plasma glucose in Georgie Lake fish seems unlikely. Glucose is a secondary stress response that rises more slowly and does not peak for several hours post-stress, and takes longer to recover (Pickering et al., 1982; Barton and Iwama, 1991; Waring et al., 1992; Hemre and Krogdahl, 1996; Sandodden et al., 2001). Plasma glucose for the Georgie Lake group was at the upper limit of the normal range reported for Atlantic salmon smolts (Carey and McCormick, 1998; Iversen et al., 1998; Iversen et al., 2005), and it remained significantly higher than the Dalrymple group at all stages of this transport study despite the clear recovery of other plasma variables. Thus the difference in plasma glucose concentrations between the two smolt-rearing sites may be attributed to differences in nutrition, health status or temperature, which are known to affect plasma glucose levels (Pickering et al., 1982; Galloway and Kieffer, 2003; Suski et al., 2006); however, these were not characterized for the present study.

Plasma chloride concentrations were significantly higher for Georgie Lake fish than for the Dalrymple fish. Chloride is often used as an indicator of stress as it is relatively easy to measure, and it typically decreases after a stress event in freshwater fish as osmoregulation is compromised (McDonald and Milligan, 1997; Carey and McCormick, 1998). The range of routine plasma chloride values reported for Atlantic salmon smolts in freshwater is very broad: 110 mM (Handeland et al., 1996) to 138 mM (Carey and McCormick 1998, Iversen et al. 1998); both fish groups fall within this range.
Both plasma sodium and potassium concentrations were comparable to freshwater control values for Atlantic salmon smolts as reported by Brauner et al. (2000) and Singer et al. (2002). However, other studies have reported much higher sodium concentrations in the range of 155 - 165 mM (Smith et al., 1991; Carey and McCormick, 1998; Iversen et al., 1998; Quigley et al., 2006) and potassium in the 2.0 - 3.5 mM range (Carey and McCormick, 1998; Quigley et al., 2006; Fridell et al., 2007). Minor hemolysis that can occur during caudal puncture and blood collection can result in higher plasma potassium and lower plasma sodium concentrations (Blackburn and Clarke, 1987; Yucel and Dalva, 1992). Low plasma sodium levels could also reflect regional differences in water chemistry (Blackburn and Clarke, 1987). However, since plasma sodium and potassium values did not vary either between groups or during transport, they are not discussed further.

2.4.3 Status after truck transport

Given the expectation that the loading of smolts into closed containment and transportation for 30 - 90 min along gravel roads and/or highways to the dock would result in severe stress, I was surprised to find that changes in plasma chemistry were limited to cortisol. Plasma cortisol typically rises immediately after a stress event, peaking in 15 - 60 min, depending upon the magnitude and duration of the stressor (Barton and Iwama, 1991; Einarsdóttir and Nilssen, 1996; Wedemeyer, 1996; Wendelaar Bonga, 1997). The transport protocol used in this study allowed sufficient time for this peak response to manifest during truck transport. Each tank or pen at the freshwater facilities was drained or seined over a period of 15 - 30 min, and then it took ~30 min to count and load each truck. The main difference between the two groups was driving time (30 min vs. 90 min), which also resulted in a greater accumulation of dissolved CO₂ for the Georgie Lake fish. Plasma cortisol may have peaked earlier on in the transport journey and begun declining towards the end of trucking, but this explanation assumes that crowding, confinement and elevated CO₂ during trucking did not impact cortisol levels. Plasma cortisol
values of over 750 ng ml\(^{-1}\) have been reported for smolts subjected to acute stress (Virtanen and Forsman, 1987), and this value is almost 6-times higher than the mean plasma cortisol values observed at the end of trucking and more than twice the highest individual value of 334 ng ml\(^{-1}\) measured in the present study. In view of this result, I conclude that while loading and truck transportation elicited a primary stress response, the magnitude of this response was well below the maximum primary stress response reported in the literature for smolts.

The delay in the secondary stress response of elevated plasma glucose, usually 1 - 2 h, is well documented (Wedemeyer, 1990; Barton and Iwama, 1991). In the present study, plasma glucose had not increased significantly at the end of truck transport, suggesting that the modest primary stress response had not elicited a secondary stress response at this time. Plasma glucose increased later for both groups aboard the Sterling Carrier.

2.4.4 Status aboard the Sterling Carrier

Transfer to the live-haul vessel requires additional handling as well as an abrupt introduction to seawater. Interpretation of the data is confounded by each hold containing fish that had arrived on 3 - 4 different occasions, which likely increased data variability if, as anticipated from Iverson et al. (2005), the live-holds on the Sterling Carrier promote recovery. As predicted, both groups had lower mean plasma cortisol concentrations at the completion of loading, approximately 4 h after the first fish were introduced into the holds. Given the normal trajectory of plasma cortisol following acute stress, this result suggests that primary stresses subsequent to loading onto the Sterling Carrier were in fact minor. Furthermore, plasma cortisol levels remained at this lowered level during sea transport.

While the results suggest that fish did not fully recover to routine or pre-transport levels of plasma cortisol onboard, it is important to note that, of all the fish sampling procedures used in this study, sampling fish rapidly from the hold of a sea-going vessel was the most challenging.
Therefore, I cannot exclude the probability that fish were variably stressed during capture on board the *Sterling Carrier*. It is also possible that as transportation progressed and the majority of fish recovered, weaker fish near the surface of the live-hold were more likely to be sampled. This potential sampling bias would result in an overestimation of the recovery time experienced by the majority of the load. However, for the present study, 7 - 9% of individual fish at 4 h post-load completion had plasma cortisol levels under 10 ng ml\(^{-1}\), indicating that sampling and transport were no longer major stressors for those fish. Some studies have suggested that cortisol decreases when properly smolted fish are put in seawater (Langhorn & Simpson 1986, Brauner et al. 2000); in any case, my interpretation that recovery continued through 2 and 4 h is supported by plasma glucose, a secondary stress response, which peaked later than cortisol at load completion in the holds and then decreased to routine levels during the 4 h of sea transport. For Atlantic salmon, it has been previously reported that plasma glucose does not change much in response to capture because it responds more slowly than plasma cortisol or lactate (Hunn and Greer, 1991). The maximum value reported for Atlantic salmon smolts exceeds 12 mM; as the mean values in the present study never exceeded 8 mM and individual values never exceeded 10 mM, I concluded that the secondary stress response was modest throughout transport.

Mean plasma lactate concentration was not significantly different from routine values for the Georgie Lake group. However, for the Dalrymple group, the lactate concentration declined 2 h after load completion aboard the live-haul vessel compared to post-truck, 0 h and 4 h after load completion. This decrease, and the subsequent rise in lactate concentration, can be explained using the same reasoning described above for plasma cortisol, i.e., that there was a sampling effect while aboard the *Sterling Carrier*. Ferguson et al. (2007) recently reported that juvenile chinook salmon passing through a hydroelectric dam bypass system had elevated plasma lactate concentrations following passage through the system compared to fish that were released downstream of the system, and that lactate for both groups had decreased to similar levels by 4 h. The fish in this
study were held in tanks after initial capture; thus it may be that subsequent sampling was relatively quick and a plasma lactate response had not time to manifest itself.

Plasma chloride concentrations were measured in part to assess osmoregulatory stress. Mean chloride was unchanged during truck transport, and increased significantly by 2 h post-load for both groups. In a 24-h seawater challenge, plasma chloride in smolted Atlantic salmon measure on average 170 mM (Handeland et al., 1996; Shrimpton et al., 2000), but other studies report values of only 140 - 150 mM after 6 to 12 h in seawater (Handeland et al., 1996; Handeland et al., 2000; Singer et al., 2002). My chloride values at 4 h post-load (136.7 ± 3.8 mM for Dalrymple and 142.4 ± 3.1 mM for Georgie Lake fish) are consistent with the latter. Marine Harvest Canada conducts gill Na\(^+\), K\(^+\) ATPase testing for smolts prior to shipping, and since the majority of the smolts appeared to ionoregulate adequately, I conclude that the ATPase test was an effective tool in confirming smolt status.

2.4.5 Post-transport growth and survival

Although there were differences in the original freshwater rearing conditions, water quality changes during truck transport and, most notably, higher plasma glucose in the Georgie Lake group throughout the transport process, there were no differences in either mortality or average growth rate (with the exception of one sea pen, containing Dalrymple fish, that had a disease outbreak). Iversen et al. (2005) reported that at Marine Harvest Norway, a mortality rate of less than 2% in the 30 days following transport is normal and so our mortality rate of 0.74 - 1.93% during the first 30 days post-transport can be considered excellent. Growth rates for Atlantic salmon under 150 g in sea cages have been estimated to be about 1.3 % mass day\(^{-1}\) at a temperature of 8 °C (Austreng et al. 1987), but more recent studies using specific growth rate report control values for smolts following transfer to seawater in the range of 0.9 - 1.1 % day\(^{-1}\) (Fivelstad et al., 1998; 1999; 2003), so our smolts, at 0.8 - 1.2 % day\(^{-1}\), were growing well during the
first 30 days post-transfer to the seapens.

### 2.5 Conclusions

As predicted, primary stress (as assessed by plasma cortisol values) was greatest immediately following truck transport. However, primary and secondary stresses were only moderate when compared with other work cited on fish and stress physiology. Once on board the live-haul vessel, all of the physiological parameters indicated that the smolts were recovering, demonstrating the benefits of a live-haul vessel with flow-through holds. As I found no differences between 30-min and 90-min transports, the most stressful step in the truck transport procedure may have been the initial crowding, counting and loading process. With the exception of plasma glucose levels in the Georgie Lake group, maximum measurements of all parameters were well within industry-accepted limits. Further field investigations are encouraged to confirm and quantify the condition of fish immediately after loading onto the trucks, followed by a comparison of different methods or speeds in loading.
Table 2.1  A comparison of the two freshwater facilities.

<table>
<thead>
<tr>
<th></th>
<th>Land-based (Dalrymple)</th>
<th>Lake (Georgie Lake)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water source</strong></td>
<td>well (pH 6.96 ± 0.01)</td>
<td>lake (pH 6.12 ± 0.08)</td>
</tr>
<tr>
<td><strong>Water temperature</strong></td>
<td>8.5 ± 0.3 °C (in tanks)</td>
<td>4.6 ± 1.4 °C</td>
</tr>
<tr>
<td><strong>Rearing container</strong></td>
<td>9 m or 10 m diam. circular fibreglass tanks</td>
<td>50’ x 50’ square pens with 1 cm gauge netting, suspended in lake</td>
</tr>
<tr>
<td><strong>Vaccinations</strong></td>
<td>Forte</td>
<td>Aqua Health</td>
</tr>
<tr>
<td><strong>Rearing density at shipment</strong></td>
<td>35.9 ± 4.5 kg m⁻³</td>
<td>5.8 ± 2.0 kg m⁻³</td>
</tr>
<tr>
<td><strong>Average fish mass at shipment</strong></td>
<td>99.5 ± 29.7 g</td>
<td>73.6 ± 25.4 g</td>
</tr>
<tr>
<td><strong>Average fork length at shipment</strong></td>
<td>20.6 ± 2.0 cm</td>
<td>18.7 ± 1.9 cm</td>
</tr>
<tr>
<td><strong>Condition factor, K at shipment</strong></td>
<td>1.11 ± 0.09 g cm⁻³</td>
<td>1.07 ± 0.22 g cm⁻³</td>
</tr>
<tr>
<td><strong>Pre-transport treatment</strong></td>
<td>Starved 48 h, 24 h lighting for 30 days prior to shipment</td>
<td>Starved 36 h, natural light</td>
</tr>
<tr>
<td><strong>Typical loading density for transport</strong></td>
<td>61.4 ± 19.4 kg m⁻³</td>
<td>75.0 ± 7.1 kg m⁻³</td>
</tr>
<tr>
<td><strong>Drive time to dock</strong></td>
<td>30 min</td>
<td>90 min</td>
</tr>
</tbody>
</table>
Table 2.2  Smolt transports using the *Sterling Carrier*, spring 2006.

<table>
<thead>
<tr>
<th>Date</th>
<th>Source</th>
<th>Number of smolts moved</th>
<th>Time spent loading (h)</th>
<th>Length of voyage (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 9, 2006</td>
<td>Dalrymple</td>
<td>158,382</td>
<td>5.5</td>
<td>2.5</td>
</tr>
<tr>
<td>March 16</td>
<td>Dalrymple</td>
<td>182,031</td>
<td>7.5</td>
<td>2.5</td>
</tr>
<tr>
<td>March 22</td>
<td>Georgie Lake</td>
<td>203,040</td>
<td>7.0</td>
<td>2.5</td>
</tr>
<tr>
<td>March 27</td>
<td>Georgie Lake</td>
<td>195,581</td>
<td>7.0</td>
<td>2.5</td>
</tr>
<tr>
<td>April 20</td>
<td>Georgie Lake</td>
<td>209,269</td>
<td>6.5</td>
<td>7.5</td>
</tr>
<tr>
<td>April 27</td>
<td>Dalrymple</td>
<td>137,705</td>
<td>6.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 2.3  Summary of plasma measurements taken aboard the *Sterling Carrier*, by hold.

Sample means ± S.D. are shown (Samples with N=1 have no SD).

<table>
<thead>
<tr>
<th></th>
<th>Dalrymple fish</th>
<th>Georgie Lake fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First hold</td>
<td>Second hold</td>
</tr>
<tr>
<td><strong>Cortisol</strong> (ng ml(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 0</td>
<td>91.2 ± 6.8</td>
<td>110.7</td>
</tr>
<tr>
<td>t = 2</td>
<td>40.6 ± 0.5</td>
<td>72.1 ± 27.6</td>
</tr>
<tr>
<td>t = 4</td>
<td>86.4 ± 34.9</td>
<td>64.1</td>
</tr>
<tr>
<td><strong>Glucose</strong> (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 0</td>
<td>6.1 ± 8.9</td>
<td>5.2</td>
</tr>
<tr>
<td>t = 2</td>
<td>4.8 ± 0.4</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>t = 4</td>
<td>3.7 ± 1.7</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>Lactate</strong> (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 0</td>
<td>4.2 ± 1.5</td>
<td>3.9</td>
</tr>
<tr>
<td>t = 2</td>
<td>3.1 ± 0.6</td>
<td>3.7 ± 1.6</td>
</tr>
<tr>
<td>t = 4</td>
<td>4.7 ± 0.3</td>
<td>4.7</td>
</tr>
<tr>
<td><strong>Chloride ion</strong> (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 0</td>
<td>133.0 ± 2.8</td>
<td>130.0</td>
</tr>
<tr>
<td>t = 2</td>
<td>135.5 ± 5.1</td>
<td>134.1 ± 4.6</td>
</tr>
<tr>
<td>t = 4</td>
<td>137.7 ± 4.7</td>
<td>134.6</td>
</tr>
<tr>
<td><strong>Sodium ion</strong> (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 0</td>
<td>155.8 ± 3.3</td>
<td>158.9</td>
</tr>
<tr>
<td>t = 2</td>
<td>160.1 ± 5.2</td>
<td>158.2 ± 7.4</td>
</tr>
<tr>
<td>t = 4</td>
<td>160.2 ± 9.6</td>
<td>145.5</td>
</tr>
<tr>
<td><strong>Potassium ion</strong> (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t = 0</td>
<td>3.7 ± 0.4</td>
<td>4.6</td>
</tr>
<tr>
<td>t = 2</td>
<td>4.9 ± 2.9</td>
<td>4.6 ± 1.4</td>
</tr>
<tr>
<td>t = 4</td>
<td>4.2 ± 1.6</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Figure 2.1 Map of study area showing locations of freshwater (FW) and saltwater (SW) facilities as well as transport route by land (dotted lines).
**Figure 2.2** Diagram illustrating the shape and dimensions of the *Sterling Carrier*’s holds. Each cargo hold is approximately 22 m long x 3 m wide x 5 m deep. The forward and aft valves can be adjusted to control the flow of ambient seawater through the holds. Smolts are drained in through the open hatch during loading, and unloaded via the rear valve using a specially designed pump.
Figure 2.3  A schematic of a typical smolt transport aboard the *Sterling Carrier*, with eight truckloads of smolts coming from the freshwater facility. Sample points are indicated with a dipnet icon.
Figure 2.4  Mean ± S.D. values of pH (a), dissolved CO$_2$ (b) and NH$_3$-N (c) in rearing or transport water at the freshwater facility one day before transport, immediately after truck transport, and at t hours after load completion aboard the Sterling Carrier. Dissimilar letters indicate statistically significant ($\alpha = 0.05$) differences within the same group (from the same freshwater facility); asterisks indicate significant differences between groups at a given sample point.
Figure 2.5  Mean ± S.D. values of plasma cortisol (a), glucose (b) and lactate (c) concentrations in smolts at the freshwater facility one day before transport, immediately after truck transport, and at t hours after load completion aboard the Sterling Carrier. Dissimilar letters indicate statistically significant ($\alpha = 0.05$) differences within the same group (from the same freshwater facility); asterisks indicate significant differences between groups at a given sample point.
**Figure 2.6**  Mean ± S.D. values of plasma chloride (a), sodium (b) and potassium (c) concentrations in smolts at the freshwater facility one day before transport, immediately after truck transport, and at t hours after load completion aboard the *Sterling Carrier*. Dissimilar letters indicate statistically significant ($\alpha = 0.05$) differences within the same group (from the same freshwater facility); asterisks indicate significant differences between groups at a given sample point.
Figure 2.7  Mortality (a) and growth (b) rates of smolts, per pen, for 30 days post-transport. Each line represents one pen at one of three saltwater farms. Each pen usually contains all of the fish from one hold of the Sterling Carrier; pens which contained fish from multiple freshwater sites have been omitted.
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CHAPTER 3: QUANTIFYING BEHAVIOURS OF ATLANTIC SALMON (Salmo salar) SMOLTS DURING COMMERCIAL LIVE-HAUL TRANSPORT, AND CORRELATIONS WITH PHYSIOLOGICAL MEASURES

3.1 Introduction

Aquaculture in Canada is a growing industry that brought in CAD$968.7 million in 2006, up almost 25% from the previous year (Statistics Canada, 2007). BC-farmed salmon made up almost half of this income, with a farm gate sales of CAD$407.4 million (BCMoE, 2007). This rapid growth has raised concerns amongst environmental and consumer groups in terms of environmental impact and food safety, but unlike in Europe, there has been little attention or research on the welfare of the farmed fish in Canada. Good animal welfare generally goes hand-in-hand with good productivity, so I embarked on a two-part study to investigate indices of welfare for commercially raised Atlantic salmon (Salmo salar) smolts as they are transported from freshwater farms to the sea cages via truck and live-haul vessel. Chapter 2 of my thesis investigated the physiology, and this chapter investigates the behaviour of smolts during live-haul.

Marine Harvest Canada moves approximately 200,000 smolts on a single commercial live-haul trip. It is logistically impossible to mark or separate individual fish within such large numbers for observation without affecting their behaviour, so any behavioural study on a commercial smolt transport must be based on group behaviours. Atlantic salmon parr are territorial, but with smoltification they take up more of the water column and begin to school with conspecifics (Bakshtanskiy et al., 1988). Schooling behaviour of smolts therefore provides an opportunity to assess fish welfare.

Early studies defined schools as aggregates of fish that move together (Breder and Halpern 1946), and much work has described the relationships between fish size and shape,
packing density or nearest neighbour distance (NND), and school size and shape for commercially important species such as anchovy, mackerel and herring (Breder 1954; Serebrov, 1966; Pitcher and Partridge 1979). More recently, there are emerging studies on the dynamics of schooling behaviour, utilizing computer simulation models (Vabø and Nøttestad, 1997; Hoare and Krause, 2003; Viscido et al., 2005) or capturing and analyzing the interactions between schools of pelagic fish and large predators or vessels (Gerlotto and Fréon, 1992; Nøttestad et al., 2002).

When confronted by a stressor, the typical behavioural response by prey species is avoidance or escape. When escape is not possible, for example if fish are either exposed to a toxic chemical or subjected to handling stress by humans, deviations in behaviour may occur that may in turn affect the response to subsequent or additional stressors (Ryer and Olla, 1996; Schreck et al., 1997). Thus, it follows that when a school is threatened, it can undergo avoidance behaviour by either scattering briefly or becoming more compact (Keenleyside 1955; Partridge 1981; Misund 1993). Where the stressor is unavoidable, school structure may be affected such that fish are unable to maintain order (Birtwell and Kruzynski, 1989; Ryer and Olla, 1996).

As salmonids are important in fisheries, aquaculture and research, a wealth of behavioural studies exist, particularly in ecology (McCormick et al., 1998; Bremset, 2000; Olsen et al., 2004), exercise physiology and locomotion (Brett, 1964; Hinch and Rand, 2000; Enders et al., 2004), and social behaviour (Gibson 1978; Ryer and Olla, 1991a; Sloman et al., 2001). Very few, however, have studied salmonid schooling behaviour (Ryer and Olla 1991b; Ryer and Olla 1996), and only a handful have studied their behaviour during transport. Iguchi et al. (2002) found that forcing ayu (Plecoglossus altivelis) to school using an artificial current during truck transport decreased transport stress, and Chandroo et al. (2005) used electromyogram telemetry to record rainbow trout (Oncorhynchus mykiss) behaviour during truck transport and discovered that fish swam vigorously during the transports.

Chapter 2 dealt with the physiological measures of welfare for Atlantic salmon smolts
during commercial transport. I discovered that the smolts were most stressed immediately following truck transport, but that time spent on the live-haul vessel allowed recovery, as judged by changes in plasma cortisol and glucose concentrations. This chapter deals with the use of non-invasive, behavioural criteria that are easily observed using existing equipment, to assess stress levels during transport.

3.2 Materials & Methods

3.2.1 Experimental animals and transport procedures

The details of the fish and transport process are presented in sections 2.1 and 2.2 of Chapter 2. To summarize, commercially produced Atlantic salmon smolts were transported by truck from two different freshwater farms, Dalrymple and Georgie Lake, to a saltwater dock, and then by live-haul vessel to various saltwater farms. The two locations resulted in two truck transport times and two different loading times on the vessel. A summary of the characteristics of each farm is presented in Table 2.1 of Chapter 2.

3.2.2 Video recording and analysis

Video recording of fish behaviours was limited to control observations made at the freshwater farms the day before transport and on the vessel during loading and transport. At the freshwater farms, three submersible black and white CCD cameras (Lorex Pro Model CVC6990, Strategic Vista Corp., Markham ON) were each fixed to a 1 m long metal pole with clamps and lowered simultaneously and directly into either the tanks or the net pens. The cameras were connected to a Swann digital video recorder (DVR4-Net, Richmond, Victoria, Australia) and left to record for 1 - 2 h both before and during water and fish sampling, as described in Chapter 2. Recordings were later scanned continuously, and sections of footage containing fish capture by dipnet for physiological sampling were excluded from control behaviours. At the land-based
Dalrymple hatchery, I recorded from three tanks simultaneously on each of 2 trips. At the Georgie Lake net pen facility, I recorded in one pen during each of 2 trips. The physical setup of the pens and availability of power limited further measurements.

Onboard the Sterling Carrier, a generic VHS video cassette recorder (VCR) was connected to an existing black and white CCD camera system, and was set to record continuously during the loading and sea-going transport process. As illustrated in Figure 3.1, the existing system had two cameras in each of the two cargo holds, affixed at the rear (aft) and slightly forward of the middle (forward). The four camera outputs were fed into a splitter and monitor on the bridge of the Sterling Carrier, enabling the captain and crew to observe fish at all times. Separate and continuous recordings were made from both holds for 2 trips out of Dalrymple and 3 trips out of Georgie Lake, for a total of 4 replicates for Dalrymple and 6 replicates for Georgie Lake.

The Sterling Carrier’s cameras captured footages in PAL format because the system was installed in Norway. North American VCRs, on the other hand, record in NTSC format. As a result, viewable footage for analysis was only from the aft (rear) cameras. The VCR recordings were digitized into MPEG-4 format using a DVD video recorder (Panasonic, DMR-E100H, Osaka, Japan), and all footage was viewed, edited and converted into AVI format using Microsoft® Windows® Movie Maker Version 5.1 (Microsoft Corporation, Redmond, WA). AVI footage was split into a series of still frames and analysed for observed density using the image processing program ImageJ (National Institutes of Health, Bethesda, MD).

3.2.3 Behavioural analyses

Behavioural observations were made on 10 - 15 min samples of footage preceding and including the physiological sampling points described in Chapter 2: at the freshwater farms, and aboard the Sterling Carrier at load completion, and every 2 h thereafter. These samples were scanned in their entirety, and smaller segments (20 - 30 s long) containing appropriate footage (i.e.,
unobstructed view, single fish visible onscreen for at least 5 s to allow tailbeat counts) were taken and used as units of analysis. Each sample (N) yielded 5 - 12 such segments (n); these segments were then pooled for each sample before statistical analysis.

3.2.3.1 Fish orientation

I used the criteria used by Koike and Tsukamoto (1994) in their study of juvenile masu salmon: “aggregation” to describe a gathering of fish that is not aligned, and “school” as a group of fish that are aligned in the same direction. I described orientation in terms of the proportion of fish in view that were facing the same direction (alignment, or schooling) and their approximate distance to camera (vertical distribution), estimated proportionally against the largest fish visible on a stopped frame.

3.2.3.2 Observed fish density

As an estimate of schooling behaviour, NND was used as a measure of fish density that was comparable across all samples, and which could account for a single camera observing fish at various angles and depths/distances. To do this, the number of visible fish of a certain size (between 1 and 0.5 body lengths of the largest smolt visible) in a randomly chosen frame was counted. This was then divided by the area that those fish occupied, in square body lengths. An example of this procedure is shown in Figure 3.2. This method tends to underestimate fish density, as water quality, lighting or crowding of fish near the camera often limited the depth (distance) from the camera to which I could confidently differentiate details. The purpose, however, was for a robust measure for comparison across different types of footage.

3.2.3.3 Swimming effort

Swimming effort was measured using tailbeat frequency (tb s⁻¹). One tailbeat consisted of a
full left-and-right movement of the tail, and tailbeats were counted for at least 5 s for any smolts whose tail movements were visible on the 20-s or 30-s segments. This method may have underestimated the average tailbeat frequency for the population, as I was unable to sample fish that were moving quickly across the screen (but see below for darting and flashing behaviours). Swim speed was not estimated, due to fish being too close to the camera or swimming at an angle relative to the camera.

3.2.3.4 Darting and flashing behaviours

The number of fish darting across the screen or turning suddenly (flashing) was counted in each of the 20-s or 30-s video segments, and the numbers were expressed as events min\textsuperscript{-1}. If one erratic fish collided with another, causing additional fish to dart or flash as well, each fish was counted as a separate darting event.

3.2.4 Statistical analysis

Statistical analyses were carried out using SigmaStat 3.0.1a for Windows (Systat Software, Inc., San Jose CA). The Kolmogorov-Smirnov test was used to test for normality, and a one-way ANOVA was performed among sample points with $\alpha = 0.05$. When warranted, the Tukey test was used for post-hoc multiple comparisons. If data sets were not normally distributed, a Kruskal-Wallis One-Way ANOVA on Ranks Test and Dunn’s Test were used to determine significance. Unless otherwise noted, results are presented as sample mean ± standard deviation.

3.3 Results

3.3.1 Behaviour at the freshwater farms

At Dalrymple, the rearing density was 35.9 ± 4.5 kg m\textsuperscript{-3} and mean fish mass was 99.5 ±
29.7 g, such that each cubic meter would contain approximately 360 fish if they were evenly distributed. Video recordings confirmed that fish generally occupied the entire water column, were evenly distributed and swam in circles, with 73 ± 22% swimming counter-clockwise. Swimming effort was 1.2 ± 0.4 tb s⁻¹.

At Georgie Lake, the rearing density (5.8 ± 2.0 kg m⁻³) was 6-fold lower than Dalrymple. Mean fish mass was 73.6 ± 25.4 g resulting in approximately 80 fish m⁻³. The lake water was humic and brown, limiting visibility from above to almost nil. Underwater footage nevertheless revealed that fish were swimming at a depth of about 1.5 m or lower; most (85 ± 14%) swam in a counter-clockwise direction and the rest swam clockwise. Swimming effort (1.1 ± 0.1 tb s⁻¹) was not significantly different when compared with smolts in the tanks at Dalrymple (P = 0.562).

The observed fish density is plotted against rearing density in Figure 3.3. There were no significant differences in observed density between Dalrymple (5.3 ± 1.4 fish bl⁻²) and Georgie Lake (5.0 ± 0.1 fish bl⁻², P = 0.748), although rearing density differed quite considerably (P < 0.001).

Darting and flashing were minimal at either facility. The Dalrymple fish moved erratically at a rate of 1.3 ± 1.7 min⁻¹, and Georgie Lake fish at 0.9 ± 1.8 min⁻¹. These values were not significantly different (P = 0.606).

### 3.3.2 Behaviour on board the Sterling Carrier

Smolts from Dalrymple were trucked at a density of 61.4 ± 19.4 kg m⁻³ for 30 min before unloading into the Sterling Carrier's holds to a density of 25.4 ± 5.9 kg m⁻³. Smolts from Georgie Lake were trucked at a density of 75 ± 7.1 kg m⁻³ for 90 min and density was 23.3 ± 1.8 kg m⁻³ onboard the Sterling Carrier. Onboard densities were not statistically different (P = 0.433).

When smolts were first drained into the cargo hold, both groups tended to avoid surface water and aggregated near the bottom of the hold. At the end of loading for a cargo hold (about 4
Dalrymple fish continued to aggregate near the bottom and away from the camera, and only 5 ± 5% of fish were aligned. In contrast, Georgie Lake fish at completion of loading were 80 ± 16% aligned in the same direction and occupied more of the water column (closer to the camera). As shown in Figure 3.6, swimming effort was not significantly different between the two groups either at completion of loading, or when compared with the pre-transport data for the freshwater farm (P > 0.05). However, erratic behaviour was nearly 4-fold higher (P < 0.001) for Dalrymple fish at 4.2 ± 1.8 min⁻¹ compared to 1.1 ± 1.1 min⁻¹ for Georgie Lake fish (Figure 3.5). Observed fish density did not change (P > 0.05) while onboard the Sterling Carrier, although for Dalrymple fish, observed fish density did decrease significantly below the freshwater farm value at 2 h after load completion (Figure 3.3; P = 0.008).

During the voyage, there were no observed changes in any of the measured behavioural parameters for either group of smolts compared with their behaviours at the completion of loading. The significant differences in alignment and erratic behaviour between Dalrymple and Georgie Lake fish remained throughout the voyage (Figures 3.3, 3.5, 3.6). There also appeared to be a difference in swimming activity for Georgie Lake fish when the Sterling Carrier was docked compared to when it was underway, however this was simply due to the lack or presence of an appreciable current in the water. As no significant differences were found for any of the behavioural data at completion and during the voyage, these were re-grouped as “onboard” data, as shown in Figure 3.4.

Behaviour changed when smolts were sampled with a dipnet or brailer (see chapter 2 for description of sampling procedures). Dalrymple fish continued to aggregate near the bottom but erratic behaviour increased significantly (P < 0.001) and almost 10-fold to 37.5 ± 36.6 min⁻¹. Georgie Lake fish also moved towards the bottom of the hold and schooling decreased from 80 ± 16% to 40 ± 26% (P = 0.029) while erratic behaviour increased 8-fold to 9.4 ± 18.7 min⁻¹ (P < 0.001).
3.4 Discussion

3.4.1 Social density

At Georgie Lake, rearing density in the lake-pens was 4-fold lower than in the circular tanks at Dalrymple. Despite this the Georgie Lake smolts did not disperse themselves evenly throughout the available space in the lake-pens, and this is in contrast to the smolts reared in tanks. Thus, the “social” density chosen by the Georgie Lake fish was considerably higher than their rearing density. Due to the simplicity of the experimental setup, it was not possible to accurately measure NND on the video footages; however, the relative observed fish density (number of fish per square body length) on-screen did not differ significantly in the tanks and in the lake-pens. This supports my assumption that lake fish choose to maintain a social density that is higher than the overall rearing density. On the Sterling Carrier, loading density was similar for the two groups, so that compared with respective freshwater rearing densities, density onboard remained unchanged for Dalrymple smolts but increased for Georgie Lake. Still, there were no significant differences in observed density between groups at any point during the transport, suggesting that there is a homeostasis of sorts for social density.

It is also possible that, because Georgie Lake smolts were raised in humic lake water where visibility was low, they deliberately assumed a higher social density in order to maintain visual contact. Studies have shown that Atlantic salmon in sea cages swim as a school during the day but tend to aggregate after dark, when they can no longer see each other (reviewed by Juell, 1995).

3.4.2 Schooling behaviour

Some differences in activity between groups became apparent when the fish were aboard the Sterling Carrier. The majority of Dalrymple-reared fish, reared at a higher density than Georgie Lake fish, stayed near the bottom of the vessel holds and aggregated. Georgie Lake fish, however, were more aligned and utilized more of the water column, except when disturbed.
Smoltification in salmonids is associated with a change in behaviour, which includes rising up from the substrate and schooling (Bakshtanskiy et al., 1988; Koike and Tsukamoto, 1994). Schooling has been defined as “an aggregation of fish which face the same direction and move together” (Breder and Halpern, 1946; Camazine et al., 2001, Parrish and Viscido, 2005). This definition certainly applied to the behaviour of these smolts, and particularly those from Georgie Lake. The difference between Dalrymple and Georgie Lake fish could come about because Dalrymple fish were either not properly smolted, or were more stressed. It is worth noting that both groups behaved similarly when stressed during fish sampling procedures: they aggregated near the bottom of the live-hold. In view of this, I suggest that because the Dalrymple fish were accustomed to a higher rearing density, they may have attempted to maintain a similar social density aboard the Sterling Carrier. The same line of reasoning can be used for Georgie Lake fish; the improved visibility in the light-coloured holds compared to the dark lake-pens may have caused them to spread out a little and to utilize more of the vertical space.

Studies have shown that swimming helps to reduce stress in fish (Milligan et al., 2000; Iguchi et al., 2002; Veiseth et al., 2006), however there were no differences in plasma cortisol concentration between the two groups at all sample points. In fact, there were no significant differences in swimming effort for the two groups at any point, even though schooling in Georgie Lake fish gave the impression that they were swimming faster than Dalrymple fish. Georgie Lake fish appeared to swim faster while the Sterling Carrier was docked; however, this was due to a lack of appreciable current through the cargo holds. Once the vessel left the dock, Georgie Lake fish faced the current, which was estimated as 8 cm s^{-1}, and swam to maintain position so that individual fish tended to stay on-screen for longer periods of time.

It seems unlikely that a difference in activity level can help to explain the higher plasma glucose concentrations in Georgie Lake fish. While an advantage of schooling is energy savings (Herskin and Steffensen, 1998; Svendsen et al., 2003), differences in swimming effort in terms of
tailbeat frequency were not observed between the two groups at any time during transport.

Tailbeat frequency can be converted into swim speed and thence to metabolic rate (Maxime et al., 1989; Brett, 1964; Steinhausen et al. 2005), and this may have been useful had I found any differences.

Lastly, the direction of schooling at both freshwater farms was counter-clockwise. Similar observations were made in a study on the schooling behaviour of juvenile masu salmon (Oncorhynchus masou), and the authors speculated that this had to do with the direction of migration once the fish reached the sea (Koike and Tsukamoto, 1994).

Because there were no differences in plasma cortisol concentration or post-transport growth and mortality rates for the two groups of smolts, I conclude that the differences in their schooling behaviour are not due to transportation, handling or osmotic stress, but likely caused by differences in freshwater rearing conditions or in the genetic composition at the two sites that were not measured.

3.4.3 Erratic behaviour

As long as fish were undisturbed in the Sterling Carrier's holds, there was no appreciable change in either group's behaviour during the 2 - 4 h sea voyage. Darting and flashing were minimal. During sampling, however, fish responded by increasing erratic behaviours. Georgie Lake fish in particular moved towards the bottom of the hold, and their percentage alignment decreased. Once sampling finished, fish returned to their previous states within minutes. These observations point to a minimal expression of avoidance behaviours, caused by sampling stress during live-haul transport of smolts on board the Sterling Carrier. These results are consistent with the physiological data that I collected concurrently, which were reported in Chapter 2.

Physiological measurements point to initial loading and transport by closed containment aboard the trucks as the most stressful portion of the commercial transport process for the smolts,
as indicated by significant increases in the primary and secondary stress indicators (plasma cortisol and glucose concentrations) (Iverson et al. 2005; Chapter 2). Physiological recovery occurred onboard the Sterling Carrier, both at the dock and underway, and recovery of both primary and secondary stress responses were completed after about 6 h (4 h loading plus 2 h after load completion). Behavioural response to stress, though considered a tertiary response, can be immediate and transitory; as such it can easily go undetected. Thus, any behavioural stress response that the smolts may exhibit during loading at the freshwater farm, transport aboard the trucks and upon entry into to saltwater aboard the Sterling Carrier, would only be observed if the fish continued to display the behaviour for an extended period of time. As smolts arrive in several truckloads per cargo hold, I assumed that each time a load is added, the smolts that were already in the hold were disturbed. This assumption was supported by the observation that Georgie Lake smolts display “disturbed” behaviour during loading but resume schooling by the time loading is completed from each truck.

The physical activity of darting and flashing, which are burst activities, could be associated with elevated plasma lactate and potassium concentrations (Milligan and McDonald, 1988; McDonald and Milligan, 1997). Both parameters were assayed in the physiology study, however they did not change significantly over the entire transport process. Darting fish are also difficult to catch with a large dipnet, so they were likely under-represented in the samples. Consequently, the conclusion that the majority of fish were not observed to be darting is supported by the physiological data.

3.5 Conclusions

Video monitoring systems are becoming standard equipment in live-haul vessels in the United Kingdom and in Norway, and are a requirement for Freedom Food certification (RSPCA 2007). It is a convenient and non-invasive tool for assessing the behaviour of transported fish;
therefore I attempted to come up with easily measured criteria that were comparable to physiological measurements. The criteria that were most promising, and these are already in use anecdotally by on-site staff, were rates of schooling (% alignment) and darting/flashing. It is important to determine, however, what the behaviour is for a given group of smolts as there can be differences in behaviour depending upon strain and rearing conditions.
Figure 3.1  Diagram of the *Sterling Carrier*’s cargo holds, indicating the flow of water and position of the cameras.
Figure 3.2 A screen capture of ImageJ, showing an example of an observed fish density measurement. Fish 1 is the largest whole fish visible in this particular frame, and it is used as the standard, bodylength (bl) = 1. 14 whole fish, between 0.5 and 1 bl, are discernible in this frame. The yellow square measures the area that contains the 14 fish, 1.998 bl × 1.882 bl. The observed density in this frame is 14 fish per (1.998 bl × 1.882 bl), or 3.71 fish bl\(^{-2}\).
Figure 3.3  Box plot of observed density and a line plot of the mean ± S. D. of actual rearing/loading density for (a) Dalrymple smolts and (b) Georgie Lake raised smolts. Dissimilar small case letters indicate significant differences ($\alpha = 0.05$) in observed densities within the same group (from the same freshwater farm) and between groups, while dissimilar upper case letters indicate significant differences in actual densities within and between groups.
Figure 3.4  Box plot of observed density and a line plot of the mean ± S. D. of actual rearing/loading density for (a) Dalrymple-raised smolts and (b) Georgie Lake raised smolts. Dissimilar small case letters indicate significant differences ($\alpha = 0.05$) in observed densities within the same group (from the same freshwater farm) and between groups, while dissimilar upper case letters indicate significant differences in actual densities within and between groups.
Figure 3.5 Mean ± S. D. values for fish alignment, or schooling (a), and erratic behaviour (b) at the freshwater farm and aboard the *Sterling Carrier*. Dissimilar superscript letters indicate statistically significant ($\alpha = 0.05$) differences within the same group (from the same freshwater farm), and asterisks indicate significant differences ($p < 0.05$) between groups at a given sample point.
Figure 3.6  Mean ± S.D. values of tailbeat frequency at the freshwater farm and aboard the *Sterling Carrier*. No statistical differences were found between or among sampling points.
References


CHAPTER 4: SUMMARY AND CONCLUSIONS

In order to assess the welfare of Atlantic salmon smolts during commercial transfer from fresh to seawater, I set myself three objectives: (1) quantify plasma cortisol, glucose, lactate, potassium, sodium and chloride concentrations during the transport procedure; (2) compare these results with literature values in order to assess welfare in relation to physiological stress as an objective measure; and (3) define and test behavioural criteria that can be used to visually assess welfare during live-haul transport of smolts. These objectives allowed me to address the smolts’ freedom from discomfort, pain and distress, and freedom to express normal behaviour as outlined by FAWC (1979).

Objective 1

The total number of trips examined was limited as commercial smolt shipments were few to begin with, and the schedule was continually modified due to logistics associated with the availability of the Sterling Carrier, the weather conditions and gill ATPase test results. These factors resulted in my being able to follow just seven shipments of smolts from two different freshwater farms, the land-based Dalrymple farm and the lake-pen facilities at Georgie Lake. The smolts were transported first by truck and then by Sterling Carrier to the saltwater cages. I found that prior to transport the two groups differed only in their plasma glucose, lactate and chloride concentrations, but were statistically similar in their plasma cortisol, potassium and sodium concentrations. Loading procedure and drive time to the dock differed between the two farms, likely explaining the greater accumulation of metabolic waste in the transport water (decrease in water pH, increases in partial pressure of CO₂ and concentration of NH₃-N) following truck transport for the Georgie Lake smolts. This was expected because this part of the transport was by closed containment, and the loading density of fish was similar for the two farms. Even so, the
physiological measures were not significantly different between the two groups immediately
following truck transport, with the exception of plasma glucose and chloride, which were already
different before transport. Plasma cortisol concentration increased significantly for both groups
after truck transport compared with pre-transport values, but recovered to pre-transport values by
2 h after load completion aboard the Sterling Carrier. Plasma glucose reached a peak at load
completion for both groups, a result that is consistent with it being a secondary stress response,
and recovered fully by 2 h post-load completion. Plasma glucose remained significantly higher in
Georgie Lake fish compared to Dalrymple fish (by ~2 mM) throughout the transport procedure.
Lactate, potassium, and sodium concentrations did not change significantly compared with
pre-transport values during the course of transport for either group. Plasma chloride increased
significantly from freshwater farm values for both groups at 2 h after load completion, a response
consistent with the abrupt introduction from freshwater to seawater. Smolts from both groups had
similar mortality rates (<2.0%) and growth rates (0.8 - 1.2 % mass day\(^{-1}\)) over the 30 days
post-delivery.

Objective 2

Despite the challenges of sampling smolts quickly from large, commercial-scale tanks and
Georgie Lakes, the pre-transport values for cortisol, glucose, lactate, potassium, sodium and
chloride concentrations were all within the range of routine values reported in literature. Plasma
glucose for Georgie Lake smolts, and plasma lactate and potassium for both groups were in the
high end of the ranges; while plasma chloride for Dalrymple fish was in the lower range. Notably,
plasma cortisol concentration, a primary stress hormone, was 29.3 ± 26.5 ng ml\(^{-1}\) for Dalrymple
fish and 18.7 ± 9.2 ng ml\(^{-1}\) for Georgie Lake fish. While these values are high for a resting,
non-smolting fish sampled via an indwelling arterial cannula, they are well within the range for
smolting fish and certainly provide evidence that a “non-stressed” physiology existed before
trucking from which to make comparisons concerning the physiological stress of live-haul.

Pre-transport plasma lactate was $3.9 \pm 0.9$ mM for Dalrymple fish and $5.47 \pm 1.6$ mM for Georgie Lake fish, and these did not change significantly for either group throughout transport. This is likely reflective of the capture and handling involved in obtaining the blood sample; for instance, at the freshwater farm, the Dalrymple fish have significantly lower lactate levels compared to Georgie Lake fish, and this was presumably because Georgie Lake fish were seined before sampling, adding about 1 min to the capture process. That lactate did not change significantly during the course of transport suggests that capture stress for the fish was consistent at all sampling points. In order to minimize capture stress and to obtain true “resting” plasma lactate values, fish should be cannulated and well rested (Houston, 1990), but this was not possible for the present study.

Immediately following truck transport, cortisol peaked at $132.5 \pm 45.0$ ng ml$^{-1}$ for Dalrymple fish and $122.6 \pm 29.3$ ng ml$^{-1}$ for Georgie Lake fish. Handling stresses reported in the literature are usually over 150 ng ml$^{-1}$ (see Chapter 1) suggesting that most fish during this study were only moderately stressed by the crowding, loading onto trucks and transport for 30 - 90 min in water that had declining quality. Fish are transported according to strict company guidelines, and their protocol seems to be effective in limiting the primary and secondary stress responses of smolts to a moderate level. This contention was further supported by the rapid recovery of plasma cortisol once the fish were transferred to the live-haul vessel. As a secondary stress response, plasma glucose peaked somewhat later (at load completion), but even these peak values ($4.4 \pm 1.1$ mM for Dalrymple and $6.2 \pm 1.2$ mM for Georgie Lake fish) were well below peak plasma glucose levels associated with severe stress, which can exceed 10 mM (Hemre and Krogdahl, 1996). The elevated glucose for Georgie Lake fish is likely due to differences in rearing conditions such as temperature or nutrition, and is probably unrelated to transport stress. Plasma sodium and potassium values did not change significantly during transport, again indicating that stress was not
severe. Interestingly, plasma chloride concentrations for Dalrymple fish were at the lower end of the reported range, but these values converged with those of the Georgie Lake fish following transfer to seawater onboard the *Sterling Carrier* where plasma chloride rose gradually, as would be expected in a successful saltwater challenge test. Mortality rate for 30 days post-transport was comparable to numbers reported by Iversen et al. (2005), and growth for the same period was comparable to those reported by Fivelstad et al. (1999; 2003). Overall, the transfer and truck transport of smolts produced moderate physiological stress, and recovery began aboard the *Sterling Carrier* and was completed 2 h after loading.

**Objective 3**

To the best of my knowledge, there has been no prior study of collective smolt behaviour during a large-scale transport. Use of the existing video surveillance system aboard the *Sterling Carrier* required the definition of some simple and direct behavioural criteria that could be measured on low-resolution, black and white footage. Picture quality limited the details to which data could be collected (e.g., ventilation rate could not be observed for Dalrymple fish while aboard the *Sterling Carrier* because they tended to aggregate too far from the camera), thus I decided to observe schooling (alignment and social density), swimming effort (tailbeat frequency) and erratic behaviour (flashing and darting). As with the physiology study, data obtained at the freshwater farm were assumed to be unstressed, pre-transport values against which data obtained during transport aboard the *Sterling Carrier* were compared. Unfortunately, I was logistically unable to make recordings of fish during truck transport.

Dalrymple and Georgie Lake fish were reared at substantially different densities, but were loaded onto the trucks (61.4 - 75.9 kg m⁻³) and onto the *Sterling Carrier* (23.3 - 25.4 kg m⁻³) at similar densities, so that Dalrymple fish faced an acute decrease in loading density (from 35.9 ± 4.5 to 25.4 ± 5.9 kg m⁻³) and Georgie Lake fish were subjected to an acute increase (from 5.83 ± 2.0...
to $23.3 \pm 1.8 \, \text{kg m}^{-3}$). Despite this, observed density and swimming effort remained similar between and among groups throughout the transport process, suggesting that smolts chose to be closer to each other even when space was available.

There were characteristic differences in behaviour between the two groups of smolts, perhaps due to the different rearing densities at the freshwater farms. Dalrymple fish tended to aggregate (rather than being aligned in the same direction) near the bottom of the cargo holds while aboard the live-haul vessel, and darting/flashing behaviour was more prevalent ($4.8 \pm 1.9 \, \text{min}^{-1}$) than in Georgie Lake fish ($1.1 \pm 1.3 \, \text{min}^{-1}$). Georgie Lake fish, on the other hand, tended to swim together quite readily using more of the water column; if there was no appreciable current then they would swim around the cargo hold, or if the vessel was moving, they would face the current and maintain position. Interestingly, when fish were disturbed (i.e., sampling by dipnet), they behaved alike regardless of site of origin. Characteristically, they aggregated near the bottom, and darting and flashing increased significantly. Dalrymple fish behaved as if they were more stressed than Georgie Lake fish aboard the *Sterling Carrier*, although I found that plasma cortisol concentrations were not significantly different for the two groups, and plasma glucose, which was significantly and consistently higher for Georgie Lake fish, suggested the exact opposite. Because subsequent growth and mortality rates at the saltwater farm were similar between groups, I concluded that the difference in behaviour was due in large part to freshwater rearing conditions, not transport stress.

**Conclusions and industry recommendations**

Transport conditions for Atlantic salmon smolts in British Columbia by Marine Harvest Canada follow strict corporate protocols. The protocols used by Marine Harvest during the present study to transfer smolts to seacages resulted in only moderate physiological stress during loading and closed-containment trucking. Recovery aboard the live-haul vessel was verified by
physiological and behavioural observations, indicating that the current methods used appear to be best practices. Changes in fish behaviour, particularly an increase in darting and flashing, could be routinely used by operators to monitor acute disturbances, such as changes in water quality, disturbances (predators, people, equipment); however it is important to understand what normal behaviour is for a particular group of smolts before making any assumptions.

Future studies

My personal wish was for this study to lead to behavioural criteria as alternatives for invasive and terminal sampling for stress effects. Darting and flashing appear to be the best options. I was unable to find correlations between physiology and behaviour, perhaps because of low stress levels and because I was able to observe and measure behaviour during the recovery phase only. Chandroo et al.’s (2005) telemetry study reported that trout swam vigorously during closed truck transport, thus future research should attempt to visually monitor fish behaviour during truck transport.

Improved video quality may have allowed for the quantification of tailbeat or ventilation frequency, which in turn could be used to ascertain \( MO_2 \) (Herskin and Steffensen, 1998; Steinhausen et al. 2005). This measure could provide valuable information on the physiological status of the smolts (Maxime et al., 1989). A more accurate method of measuring social density, perhaps by angling the cameras differently (Laurel et al., 2005; Dunbrack, 2006) or developing appropriate software for image tracking and analysis (Tillett et al., 2000; Kane et al., 2004), may also have allowed me to discern differences where my current methods could not.
References


