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Aquaculture 243 (2005) 373–382

Aquaculture

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Stress responses in Atlantic salmon (*Salmo salar* L.) smolts during commercial well boat transports, and effects on survival after transfer to sea

Martin Iversen[a,*], Bengt Finstad[b], Robert S. McKinley[c], Robert A. Eliassen[a], Kristian Tuff Carlsen[d], Tore Evjen[d]

[a]*Nordland Research Institute, N-8049 Bodø, Norway*
[b]*Norwegian Institute for Nature Research, Tungasletta 2, N-7485 Trondheim, Norway*
[c]*University of British Columbia, West Vancouver, 4160 Marine Drive, Canada V7V 1N6*
[d]*Marine Harvest Norway AS, Pirterminalen, N-7488 Trondheim, Norway*

Received 25 April 2003; received in revised form 18 October 2004; accepted 22 October 2004

Abstract

Disease costs the Norwegian aquaculture industry approximately NOK 1 billion ($ 150 000 000) every year, and NOK 250 000 000 ($36 000 000) is spent on vaccines each year by the industry. Many of the disease outbreaks take place during the first months of transfer to the sea sites. Thus, the aim of this project was to monitor physiological stress parameters as plasma cortisol, glucose and lactate in salmon smolts before and after regular well boat transports. The loading process was observed to be a more severe stressor than the transport itself. Only minor plasma cortisol increases were observed during unloading. In four out of five transports, plasma cortisol had returned to resting level at arrival. However in one, which showed an unusually high mortality rate during the first month after transfer to the sea, the plasma cortisol level remained high during unloading. The study showed that well boat transports seemed to have an important recovery function. Without this ability to recover between the stressors, as on- and unloading, the ability for salmon smolts to handle multiple stressors was reduced. Further, the present study seems to strengthen the fact that some of the increased mortality experienced at sea sites in Norway may be explained by handling and transport prior to deliverance of smolts. Care should be taken during commercial boat transports. Planning of well boat routes, and avoidance of high seas could contribute to minimise mortality experienced after transports.

Keywords: Well boat transports; Salmon smolts; Stress; Plasma cortisol; Survival

* Corresponding author. Tel.: +47 75 51 74 98; fax: +47 75 51 74 10.
E-mail address: martin.iversen@nforsk.no (M. Iversen).

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doi:10.1016/j.aquaculture.2004.10.019

1. Introduction

Fish transportation is a common procedure in aquaculture and fisheries management. In 2001, approximately 135 000 000 Atlantic salmon (*Salmo salar* L.) smolts were transported to their respective sea sites on approx. 600 Well boats in Norway (Directorate of Fisheries, 2002). During transport, 13% of the total biomass (17 198 074 fish) died or were lost during the stay at the sea locations. Nearly 60% of the losses were due to disease, wounds or "lack" of smoltification (Directorate of Fisheries, 2002). The majority of these losses happened in the first months after transport.

Transportation consists of several traumatic events (stressors): capture, loading, transport, unloading and stocking. Handling and transport may initiate a severe stress response in anadromous salmonids (*Salmo* spp. and *Oncorhynchus* spp.) (Specker and Schreck, 1980; Nikinmaa et al., 1983; Robertson et al., 1987; Maule et al., 1988; Schreck et al., 1989; Barton and Iwama, 1991). Stress-related cortisol releases in fish can suppress immunological capacity (Ellis, 1981; Fries, 1986; Pickering, 1989; Maule et al., 1989; Schreck et al., 1993; Einarsdóttir et al., 2000), affect seawater tolerance (Redding and Schreck, 1983; Iversen et al., 1998), growth (Beitinger, 1990; Mommsen et al., 1999; Bernier and Peter, 2001) and survival (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Mommsen et al., 1999).

The objective of this study was to follow five different commercial well boat transports of smolts in Norway and Scotland, and to document and describe the effects of transport procedures on primary (plasma cortisol) and secondary (lactate and glucose) stress responses, and the potential effects on survival following transfer to sea.

2. Materials and methods

2.1. Experimental animals

The Atlantic salmon smolts (1+) used during the transport experiment were of the Aqua Gen strain (Sunndalsøra, in Norway) and Mowi Cross (in Scotland). The smolts were produced at five different commercial smolt plants (Marine Harvest and Bunes Fisk) in Norway and Scotland (Scottish Seafarms) following standard production procedures. The salmons were reared at natural water temperatures (2–20 °C), and were held at natural light conditions. Commercial dry feed, dispensed from automatic feeders was provided in excess. All smolts were vaccinated with commercial oil-adjuvanted vaccines as Lipogen Pentium vet, Pentium Forte vet or Norvax Compact 5 vet (Europharma, Norway).

Prior all transport, smolt status were confirmed by a 24–48-h seawater challenge test (Blackburn and Clarke, 1987), and the animals were fasted and without human contact or disturbance for 72 h before transport. The average size of the smolts ranged from 70 to 215 g (Table 1).

2.2. Transport and hauling

Fish transported in commercial well boats were monitored in Norway and Scotland between spring and summer 2002 (Tables 1 and 2). To ensure that the results were a "true" representation of stressors during the commercial transports, no instruction or advice was given to the handlers at the smolt plants, well boats or sea locations. Thus, the experiment contained all of the stressors present during a routine fish transport (Specker and

Table 1
Number of smolts, average weight, cargo space, stocking density, water temperature and oxygen levels during five different commercial smolt transports in Norway and Scotland

Transport	Number of smolts	Average weight (g±S.D.)	Cargo space[a] (m^3)	Stocking density (kg/m^3)	Water-temperature (°C±S.D.)	O_2 (mg/L±S.D.)
I	160 000	138 (±28)	540	41	8.0±0.9	9.0±1.2
II	60 000	215 (±110)	310	42	9.6±1.9	10.0±0.6
III	200 000	77 (±11)	600	26	9.5±1.4	10.6±1.9
IV	116 000	77 (±9)	310	29	8.5±1.0	9.0±0.8
V	160 000	70 (±10)	650	17	7.9±0.5	10.2±0.5

[a] Total cargo space (all well boats were divided into two compartments).

Table 2
Transport time and meteorological conditions during five different commercial smolt transports in Norway and Scotland

Transport	Time (date)	Transport time (h)	Average wind direction	Average wind speed (m/s)	Gust of wind (m/s)	Average wave height (m)[a]
I	03.06.00	6.5	NW	12	18	3.0–5.5
II	09.05.00	6.0	SW	6	9	1.0–2.0
III	04.06.02	40	SE to NW	5	7	0.4–1.0
IV	14.05.00	4.0	W	8	11	2.0
V	30.03.00	4.0	No wind	0	0	0

Source for weather observation for transports I, II and IV (DNMI, 2002), and transports III and V are based on ship logs (i.e. own observations).
[a] Average wave height is the probable wave height based on average wind speed and gust of wind. For closer description of wind force and its effects at the sea, see http://marine.cwb.gov.tw/CWBMMC/windwaveE.html or http://www.hotribs.com/04features/34-marinemetguide/01.htm.

Schreck, 1980; Weireich and Tomasso, 1991; Iversen et al., 1998).

Details describing the various parameters during the transport process are given in Tables 1 and 2. For transports I, II, and III, the onloading process contained reduction of the water level in the tanks (to 25% of normal level), crowding, vacuum-pumping, and gravity transport through a pipe line (700, 60, and 50 m, respectively) to the well boat. Transport IV experienced reduction of the water level in the tanks (to 50%), and gravity transport through a pipe gate (80 m) to the well boat. For transport V, the on loading process contained crowding, snailhouse-pumping to a transport bucket containing 5 ppt sodium chloride, 3 ppt calcium chloride and 150 ppm sodium bicarbonate, followed by 3-min-long helicopter transport, and mechanical on loading of the bucket to the well boat. The unloading process from the well boat to the sea pens contained several stressors as reduction in water level in the wells, crowding and vacuum-pumping.

2.3. Blood sampling procedures

In all five transports, blood samples were obtained from the fish before handling and transport (control), 1 h after start on loading (stage 1 loading), last group of fish being on loaded to the well boat (stage 2 loading), after transport (sea location arrival), and 1 h after start unloading (stage 1 unloading). In addition, in transports I, II and III, blood samples were collected from the last group of fish (<1000) unloaded from the well boat (stage 2 unloading). Fish were rapidly transferred to a bucket containing metomidate solution at 5 mg L^{-1}, Marinil™, Wildlife Labs., Fort Collins, CO, USA, a concentration was sufficient to induce rapid anaesthesia, and to prevent a blood plasma cortisol increase (Olsen et al., 1995; Iversen et al., 2003).

Blood samples from six to eight smolts at each sampling time were obtained from the caudal vein complex using size 0.50×16-mm heparinized syringes. Whole blood was used to measure glucose and lactate. The remaining blood was centrifuged at 5000 rpm for 5 min, plasma was removed and stored in cryo tubes at −20 °C until analyses were performed.

2.4. Analytical procedures

Plasma glucose and lactate were determined on whole blood immediately after sampling using a Precision Q.I.D™ sensor (Medisense™, Bedford, UK) and a Lactate Pro™ (Arkray, KDK, Japan). Radioimmunoassay (RIA) techniques were used to measure plasma cortisol concentrations as described by Iversen et al. (1998).

2.5. Registration of mortality

The mortality rates after transport were observed daily for 1 month at their respective sea locations. The mortality was expressed each day as % cumulative.

2.6. Statistical analysis

Statistical tests were done by use of a statistical program, SPSS 11.0 for Windows. All data sets were tested for normality using a Kolmogorov–Smirnov test, and for homogeneity of variance a Levene Test. A one-way ANOVA test was thereafter performed at each sampling time to test for differences among the groups. If the F-values were significant, a Bonferroni post hoc test was used to determine which group's differed (Sokal and Rohlf, 1987). A one-way ANOVA with Bonferroni post hoc within-group was also used to discover difference among all sampling times for each physiological parameter. A Kruskal–Wallis ANOVA (non-parametric) and a Mann–Whitney *U*-test with a Bonferroni adjusted significance level, were used when requirements for parametric statistics were not met. Significant differences were established at 0.05 levels. Results are given as means±standard deviation (S.D.).

3. Results

3.1. Plasma cortisol, lactate and glucose

The changes in plasma coritsol during onloading, transport and unloading of five commercial smolt transports are shown in Fig. 1. Plasma cortisol levels prior to handling and transport (control) ranged from 54 (±19) nM (transport IV) to 72 (±41) nM (transport V). After stages 1 and 2 of loading, all fish experienced a significant increase in the average plasma cortisol levels compared to their respective controls (from 327±59 to 482±178 nM). In transports II, III, IV and V, the average plasma cortisol levels returned to resting levels (control) upon arrival at the sea locations. During unloading, fish in transports IV and V showed a significant increase in plasma cortisol levels (Fig. 1). Fish in transport I had significantly elevated plasma cortisol levels after arrival at the sea location and during stages 1 and 2 of unloading. This was significantly higher than fish in transports II, IV and V.

Fig. 2 shows the average change in fish plasma lactate level during the transport. Plasma lactate levels prior to handling and transport (control) ranged from 1.2 (±0.8) mM (transport IV) to 5.1 (±1.2) mM (transport II). The average plasma lactate level only decreased significantly (compared to their respective controls) at stage 1 loading (transport I) and sea site arrival (transport V). Increased plasma lactate levels were detected after stage 2 loading in transport I, and at arrival at the sea location in fish in transport IV (Fig. 2).

Fig. 3 shows the average plasma glucose level during the five transports. Prior to handling and transport (control), average plasma glucose levels ranged from 3.8 (±0.4) mM (transport V) to 5.7 (±1.4) mM (transport IV). During transport III, the average plasma glucose levels showed a significant increase after stage 1 loading, stage 2 loading and stage 2 unloading, compared with the control. During transport IV, the average plasma glucose levels were significantly lower in comparison to the control, during all handling phases. Further, a significant increase in plasma glucose level was observed during stage 1 loading in transport V, compared to the control (Fig. 3).

3.2. Cumulative mortality

Daily cumulative mortality (%) for 30 days after transport, for the five commercial smolt transports, is shown in Fig. 4. The mortality observed transports II, III, IV and V ranged from 0.2% (transport V) to 1.6% (transport II). After transport I, a steady increase in cumulative mortality was observed, and had reached 12% 30 days after the transport (Fig. 4). The mortality for this group of salmon continued to increase, and 3 months after the transport it was 18%. After 4 months, the salmon fish were diagnosed with infectious pancreas necrosis (IPN) (T. Evjen, personal communication, Marine Harvest, Norway).

4. Discussion

Disease costs the Norwegian aquaculture industry approximately NOK 1 billion ($150 000 000) every year, and NOK 250 000 000 ($36 000 000) is spent on vaccines each year by the industry (Intrafish, 2002). Many of the disease outbreaks take place during the first months after transfer to the sea sites (Directorate of Fisheries, 2002). One possible explanation for this occurrence is poor handling procedures prior and after smolt transport to the sea sites.

In the present study, plasma cortisol levels were shown to return to initial levels in four out of five transports upon arrival at the sea locations, with only minor increases during unloading. The values for plasma cortisol after stage 2 loading in our study were similar in magnitude to those measured in juvenile walleyes (*Stizostedion vitreum*) 1 h after a 30-s handling stressor and post-loading (Barton and

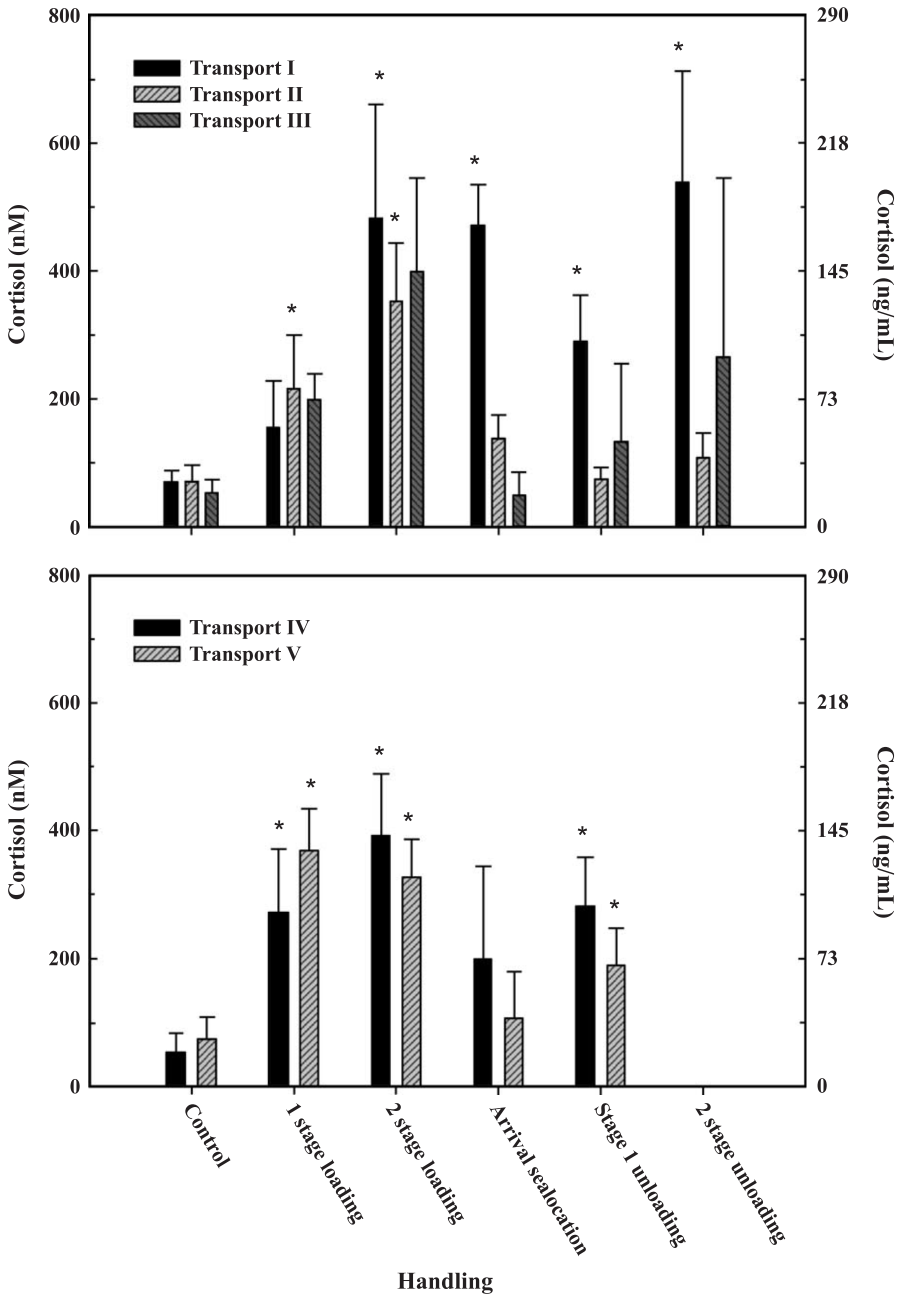

Fig. 1. Plasma cortisol levels (nM and ng/mL) in Atlantic salmon smolts before, during and after five commercial smolt transports in Norway and Scotland. Values are expressed in mean±S.D. (n=6–8). Significant changes (p<0.05) from control are indicated with asterisk (*). See further description in Materials and methods, and in Tables 1 and 2.

Zitzow, 1995; Barton et al., 2003), and in Atlantic salmon smolts after loading (Iversen et al., 1998; Sandodden et al., 2001), but were somewhat greater than those documented by Forsberg et al. (1999; 2001). The loading process elicited a greater stress response than the boat transport itself. Previous

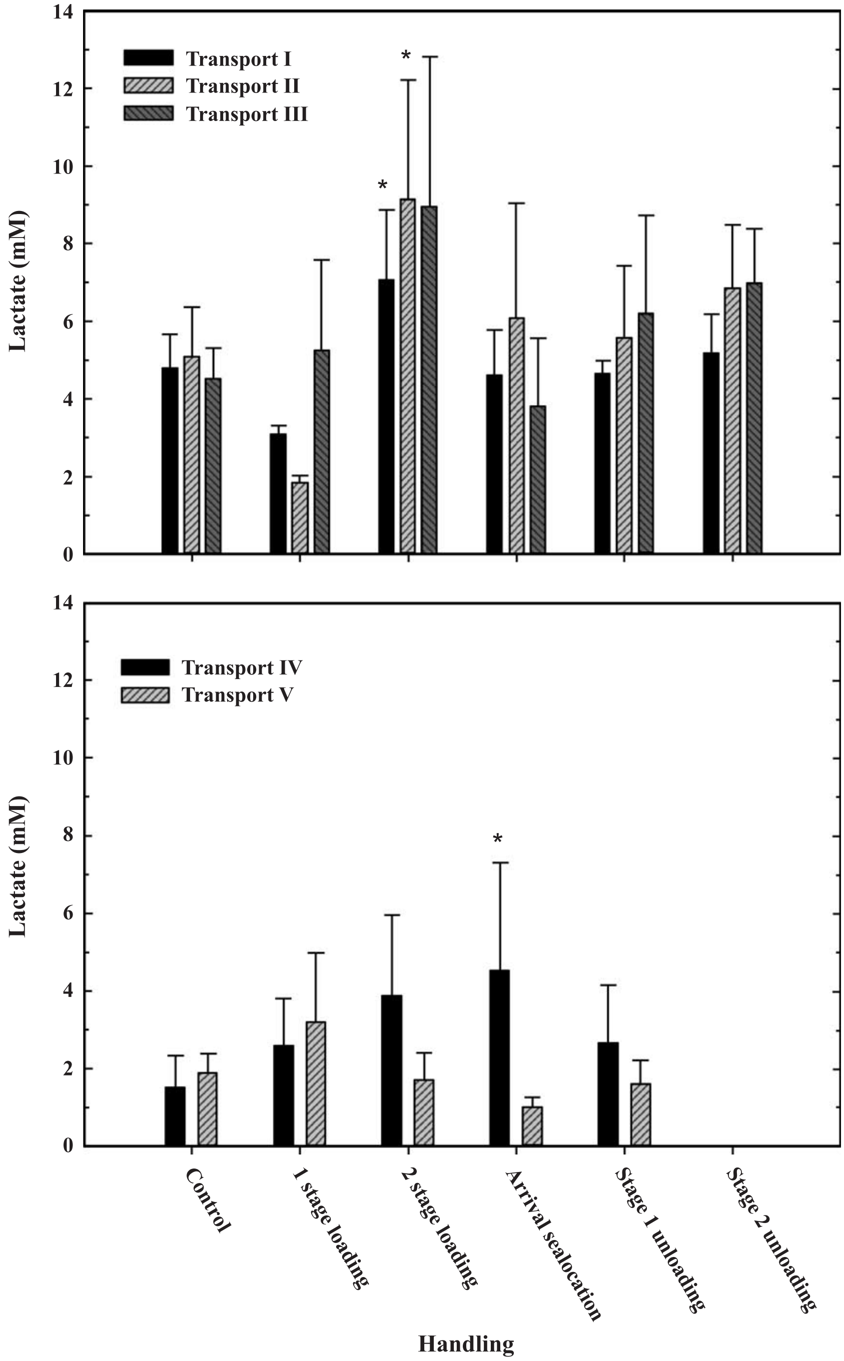

Fig. 2. Plasma lactate levels (mM) in Atlantic salmon smolts before, during and after five commercial smolt transports in Norway and Scotland. Values are expressed in mean±S.D. (n=6–8). Significant changes (p<0.05) from control are indicated with asterisk (*).

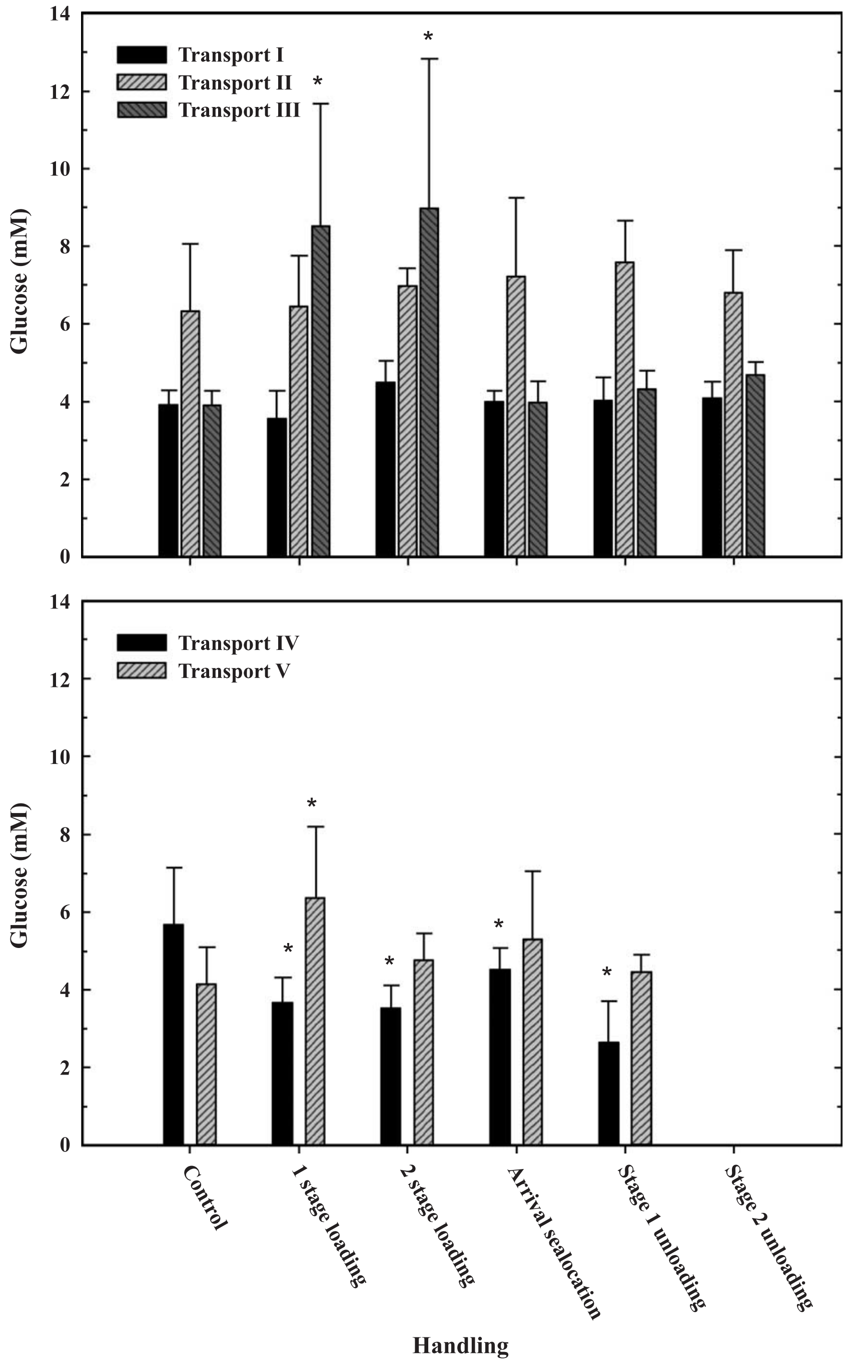

Fig. 3. Plasma glucose levels (mM) in Atlantic salmon smolts before, during and after five commercial smolt transports in Norway and Scotland. Values are expressed in mean±S.D. (n=6–8). Significant changes (p<0.05) from control are indicated with asterisk (*).

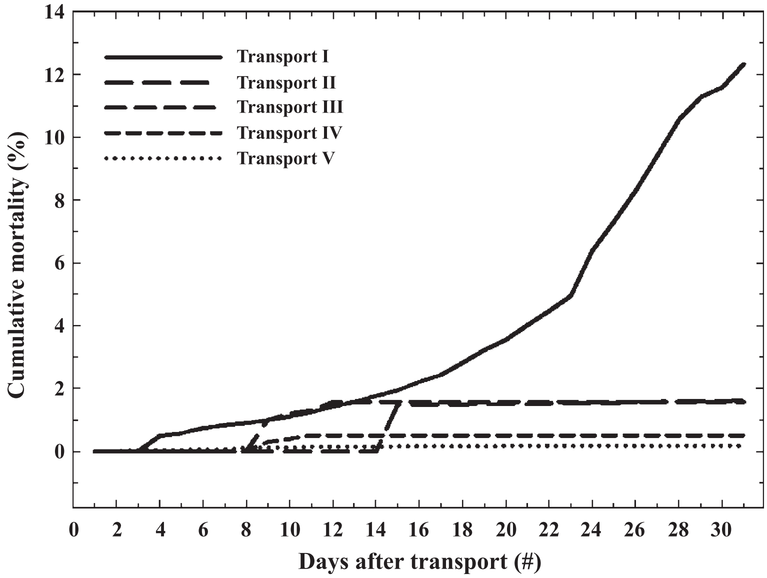

Fig. 4. Cumulative mortality (%) of Atlantic salmon smolts at the sea sites after five different commercial well boat transports in Norway and Scotland.

studies have shown that the initial moments of the capture-loading process are the major effector of the stress response (Barton et al., 1980; Specker and Schreck, 1980; Nikinmaa et al., 1983; Robertson et al., 1987; Maule et al., 1989; Schreck et al., 1989; Weireich and Tomasso, 1991; Iversen et al., 1998; Sandodden et al., 2001; Barton et al., 2003).

The duration of transport time did not affect the plasma cortisol levels. In fact, the longest transport time (Transport III; 40 h) showed the most complete recovery of plasma cortisol levels upon arrival at the sea site.

No consistent changes in secondary stress responses (plasma glucose and lactate levels) were observed during handling and transport. Significant hyperglycaemia can be evident within 16–32 min after stress (Laidley and Leatherland, 1988), but no such stress-related increase of plasma glucose was observed during these transports. The effects of cortisol on hepatic glycogen levels are inconsistent: increases as well as decreases have been described after cortisol administration (Wendelaar Bonga, 1997). Lactate increases in blood occur when insufficient oxygen is available for aerobic cell metabolism. This could be due to reduced ventilation, circulation or after heavy physical exercise (Houston et al., 1971; Iwama et al., 1989; Olsen et al., 1995). During handling and transport, the oxygen levels are monitored vigorously, and are kept at a relative high level. The inconsistency in the observed lactate levels may be an effect of this fluctuation in the oxygen level during transport. However, fasting of the fish (>3 days) prior to transport may have affected the "normal" plasma glucose and lactate levels in the fish.

During transport I, plasma cortisol levels did not return to resting levels, but remained high during unloading. The fish in this transport also suffered an unusual high mortality rate during the first month of transfer to sea. In addition, 4 months after transport, the fish were diagnosed with infectious pancreatic necrosis (IPN), although they had previously been vaccinated against the disease. The smolt plant that provided fish for transport I did not experience any disease outbreaks at other locations that they delivered to (fish from the same production batch). In addition, the sea location that experienced the IPN outbreak also had smolts delivered from other smolt plants, which displayed a normal mortality rate below 2% (T. Evjen, personal communication, Marine Harvest).

The main differences between transport I and the others in this study were the meteorological conditions. While the other transports were carried out during good weather conditions, transport I occurred during rough weather with strong breeze, with gale force winds and an average wave height of 3 to 5.5 m. The meteorological conditions may have been an additionally stressor for fish in transport I, resulting in cumulative or additive stress responses (Barton et al., 1980; Schreck, 1981; Barton et al., 1987; Pickering, 1992).

Transport in the well boat was observed to have an important recovery function; without this opportunity to recover between stressors (represented by loading and unloading) the ability of the salmon smolts to handle additional stressors was reduced. Multiple stressors are known to hinder basic performance in salmonids e.g. immunological capacity (Ellis, 1981; Fries, 1986; Pickering, 1989; Maule et al., 1989; Schreck et al., 1993; Einarsdóttir et al., 2000) seawater tolerance (Redding and Schreck, 1983; Iversen et al., 1998), growth (Beitinger, 1990; Mommsen et al., 1999; Bernier and Peter, 2001) and survival (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Mommsen et al., 1999).

The results of this study suggest that increased mortality experienced at sea sites in Norway may be explained as a result of handling and transport prior to deliverance of smolts at their sea locations. Further information on commercial transport of smolts and stress reduction methods are necessary, but avoidance of high seas could contribute to minimise mortality following commercial fish transports.

Acknowledgements

The authors wish to thank the staff of all contributing smolt production plants, well boats and salmon productions sites. Additional thanks are given to technician Roald Jakobsen at Nordland Research Institute for excellent support, and Dr. K. Fiona Cubitt for improving the manuscript by comments and criticism. Financial support was provided by the Norwegian Research Council (NRC) grants 133866/120 and 141666/120, Marine Harvest and the Norwegian Institute for Nature Research (NINA).

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