Aquaculture Research, 2005, **36,** 1226–1238 doi: 10.1111/j.1365-2109.2005.01347.x

Use of electromyogram telemetry to assess the behavioural and energetic responses of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to transportation stress

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Abstract

Behavioural and energetic responses of domesticated rainbow trout *Oncorhynchus mykiss* (Walbaum) (mean fork length = 440 ± 45 mm) to a brief transportation episode were investigated. Fish implanted with radio transmitters measuring muscle activity (electromyogram; EMG*i*) were transported in a standard commercial shipping tank for 50 min by truck, and then allowed to recuperate for 48 h in stationary culture tanks. The EMG*i* telemetry data indicated that vigorous swimming activity occurred during transportation. Telemetry recordings also indicated that the fish's swimming activity returned to baseline levels within the 48 h period after transport. However, even beyond the 48 h resting period, the swimming performance (measured as critical speed and endurance) of transported fish was still impaired relative to non-transported controls ($P < 0.05$). Respirometry measurements of fish taken after transportation indicated that oxygen consumption (V_{O_2}) was significantly elevated. The rise in V_{O_2} of post-transport fish could be attributed to handling procedures, as well as the intense swimming behaviour observed during transportation. Therefore, the behavioural responses of fish during transportation produced physiological consequences that persisted long after the transportation event. This study demonstrates the potential for utilizing behavioural measures, in concert with biotelemetry technologies, as tools to assess the impacts of routine aquacultural procedures on the health and welfare of captive fish.

Keywords: telemetry, transportation, rainbow trout, respirometry, energetics, behaviour

Introduction

In aquaculture management, fish are regularly subjected to transportation, which is recognized as a potential physiological stressor (Specker & Schreck 1980; Schreck 1982; Carmichael 1984; Davis & Parker 1986; Maule *et al.* 1988; Robertson *et al.* 1988; Schreck *et al.* 1989; Barton 2000). Transportation protocols typically subject fish to initial crowding, followed by capture and loading, then a period of time spent in the transport tank, and finally, subsequent release to a new environment. A number of biological indicators of stress have been used to monitor the physiological sensitivities of fish at various points during the transportation procedure. The stress indicator most often used has been the concentration of circulating plasma cortisol. Cortisol is a hormone released into the bloodstream of fish following the perception of potentially harmful stimuli (Mazeaud *et al.* 1977). Using plasma cortisol, it has been determined that a stress response occurs in fish regardless of the transportation or handling method employed (Barton & Peter 1982), and that stress reduction may occur when fish are anaesthetized or sedated prior to, or during, the transportation events (Robertson *et al.* 1988; Sandodden *et al.* 2001).

As the stress responses of fish to transport cannot be completely avoided, researchers have proposed

pre- and post-transport protocols designed to increase the overall health and survival of transported fish. Strategies incorporated before transportation include either removing feed, feeding of immuno-stimulatory diets (Jeney *et al.* 1997), and pre-conditioning the fish to the stressful experiences associated with transport (Schreck *et al.* 1995). Post-transportation strategies include the incorporation of 'resting' periods immediately after transport (Carmichael 1984; Schreck *et al.* 1989; Johnson *et al.* 1990; Tipping 1998; Sandodden *et al.* 2001). Presumably, a post-transport resting period may permit fish to recover and therefore adapt better to subsequent environmental challenges. However, few studies have adequately identified the characteristics of a recovered state in fish after transportation, or determined the environmental factors that may facilitate this recovery process (Carmichael *et al.* 1984; Milligan *et al.* 2000). As plasma cortisol levels are typically elevated after transportation, it has been suggested that fish may be sufficiently recovered when cortisol values drop to pre-transport baseline levels (Specker & Schreck 1980; Schreck *et al.* 1989). However, a resting time recommendation based on cortisol values alone, or on any other single physiological or behavioural indicator, may not accurately reflect the overall recovery state of the transported fish (Adams 1990; Barton 2000). Some behaviours that presumably signal recovery in stressed fish can return to normal prior to cortisol returning to baseline levels (Olla *et al.* 1992), while other behaviours or performance measures remain adversely affected even after plasma cortisol concentrations have reached pre-transport levels (Maule *et al.* 1988). Hence, the return of one physiological stress variable to a baseline level may not be related to other, affected and unmeasured physiological pathways. In addition, different fish species, or domestication level, can influence the physiological responses or susceptibility to handling, transportation and the environments into which the fish may be released (Korovin *et al.* 1982; Davis & Parker 1986; McDonald *et al.* 1993; McDonald & Robinson 1993; Olla *et al.* 1995; Barton 2000; Congleton *et al.* 2000). Therefore, a wider variety of biological indicators, carefully selected according to species, life stage and future environment, should be used to monitor the recovery status of fish post transportation.

Many post-transport environments demand that fish have an unimpaired ability to swim and display appropriate behavioural activities. Therefore, the incorporation of biological indicators that reflect swimming performance or other behaviours relating to survival may improve the accuracy of indices that are used to determine the fish's recovery status (Adams 1990; Scherer 1992; Olla *et al.* 1995). Behavioural indicators may be able to show that fish are responding to a stressor even when clinical, physiological indicators such as plasma cortisol do not (Schreck 1990). The behavioural responses of fish may also reflect additional physiological and metabolic alterations within the animal (Mesa & Schreck 1989; Schreck 1990; Schreck *et al.* 1997). Altered swimming behaviour of fish in response to stressful husbandry stimuli has been observed when adding mild salt solutions to transportation containers (Barton & Peter 1982), or with the use of abrupt artificial lighting systems (Mork & Gulbrandsen 1994), in wavey conditions in fish cages (Srivastava *et al.* 1991), or with conspecific social (i.e. heirarchal) stress (Winberg & Nilsson 1993). Because of the practical difficulties inherent in field observations, swimming activity, or performance is often reported in a non-quantitative manner, which can preclude the use of these observations for behavioural tests. For example, Barton and Peter (1982) noticed hyperactivity and erratic swimming of rainbow trout in transport tanks at the time of stocking when the tank water contained 0.5% salt, although the behaviour was not quantified and no observations of this behaviour during transportation were reported. Garcia and colleagues (2000) reported that survival of milkfish *Chanos chanos* (Forsskal) was improved when struggling and locomotory responses of fish during handling and transportation were minimized. Again, these behaviours were not quantified. As the locomotory activity of free-swimming fish can now be objectively characterized and quantified using integrated electromyogram (EMG*i*) telemetry (Kaseloo *et al.* 1992; Briggs & Post 1997a, b; Cooke *et al.* 2000), the opportunity exists to gain a better understanding of the behavioural responses of fish to transportation.

The purpose of this study was to examine the swimming activity and behavioural responses of domesticated rainbow trout *Oncorhynchus mykiss* (Walbaum) during transportation using EMG*i* telemetry. In addition, a 48 h post-transport resting protocol was evaluated by comparing swimming activity, V_{O_2} and swimming performance of fish, with or without a resting period. This application of EMG*i* telemetry technology will provide insight into the behavioural and physiological impacts of transportation procedures on fish, and may clarify the value of post-transport resting periods.

Materials and methods

Experimental animals and animal care

A hatchery-reared, Ontario strain of domesticated rainbow trout, raised at the Alma Aquaculture Research Station (AARS) (University of Guelph, Alma, ON, Canada) were used in this study. Experimental fish ($n = 12$) were randomly chosen (mean weight = 1259 ± 370 g; mean fork length = 440 ± 45 mm) and surgically implanted with telemetry devices. All experimental and surgical protocols were conducted under the approval of the Animal Care Committee (98RO97) of the University of Guelph, under the guidance of the Canadian Council on Animal Care.

Telemetry equipment

The EMG*i* transmitters, telemetry radio receiver (SRX 400) and software (W/20) used in this study were manufactured by Lotek Engineering (Newmarket, ON, Canada). The EMG*i* transmitters used in this study were identical to those used, and previously described, by Cooke and colleagues (2000), and have also been described in detail by Kaseloo and colleagues (1992), Beddow and McKinley (1998) and Cooke and colleagues (2004).

Surgical procedure

The surgical techniques used to implant transmitters into fish were similar to those reported by Cooke and colleagues (2000), however, the anesthetic used in the current experiment was a solution of tricaine methanesulphonate (70 mg L^{-1}) as opposed to the clove oil and ethanol (50 mg L^{-1}) solution previously employed. The placement of electrodes was standardized in each fish by aligning the paired electrodes with the anterior portion of the dorsal fin (Beddow & McKinley 1999). Ten centimeter long, T-bar anchor tags were placed near the dorsal fin (Floy Tag & MFG, Floy Tag Inc., Seattle, WA, USA) to allow for easy identification of individual fish throughout the experiment. The surgical procedure took a maximum of 10 min to complete on each individual, after which the fish were placed into a 2 × 2 m semi-square fibreglass holding tank supplied with fresh, 85%+ oxygen-saturated water until they fully recovered.

Pre-transport period

Following surgery, the bio-density of fish in the holding tank containing the tagged individuals was adjusted to 30 kg m^{-3} (a target transport bio-density commonly used in the local industry) by the addition of non-tagged individuals. The fish were given a 48 h recovery period during which any disturbances because of lighting, human movement and feeding were minimized. At the same time, EMG*i* signals were monitored to ensure that all tags were functioning properly. These recovery period EMG*i* signals were not used for subsequent analysis. Over the next 4 days, EMG*i* signals were recorded from each tagged fish. The receiver was programmed to scan for each tag frequency and to record 30 sequential EMG*i* signals from each tag. This was done continuously over the 4-day period, except during times when the system was taken off-line to download data. The data collected during this period were used to establish the pre-transport levels of swimming activity. The fish were not fed at any time during the experiments. This was similar to the common industry practice of taking fish off feed approximately 48–72 hours before and during transport.

Transport by truck

Tagged fish were transported by truck from the AARS to the University of Waterloo's wet lab facilties (Waterloo, ON, Canada). Four EMG*i*-tagged fish were transported during each trip, and there were three separate trips made in total. At the AARS the fish were netted out of the holding tank and placed into a 20 L plastic container, and then transferred to the truck-mounted fibreglass transport tank. Additional fish were then added to the holding tank from which the tagged fish were taken, to maintain a constant bio-density in the pre-transport holding tank. The density of fish in the transport tank was approximately 5.9 kg m^{-3}; and aeration was not supplied. The transport tank (dimensions 1.47 × 0.97 × 0.60 m) was filled with fresh, 100% oxygen saturated water at the same temperature as the holding water, just prior to the fish being added. Fish were transported for ca. 50 min along a paved, secondary highway with light-to-moderate traffic density and stop-and-go conditions. The colour of the fibreglass transport tank was white, and it featured a locking cover that blocked out most of the daylight. During transport the SRX 400 radio receiver was mounted inside a weather resistant container, and was powered by a

DC adapter connected to the truck's cabin power source. The receiver was programmed to continuously record 15 sequential EMG*i* signals from each fish during the entire transportation event. Upon arrival at the University of Waterloo laboratory, the fish were again netted out and placed into 50 L plastic containers, and then transferred to a 2 × 2 m semi-square holding tank identical to those tanks used at the AARS.

Post-transport period

Within 1.5 h of arrival, two of the four EMG*i*-tagged fish (defined here as non-rested fish) were subjected to a tunnel respirometer protocol procedure, and the two remaining fish were allowed to rest for 48 h (defined here as rested fish). The SRX 400 receiver collected 30 sequential EMG*i* signals continuously over the 48 h period from each resting fish. The data collected during this period were used to determine post-transport levels of activity. The 'resting tank' contained other non-tagged rainbow trout, and was previously adjusted to maintain a bio-density of 30 kg m^{-3}. At the end of the 48 h rest period, the rested fish were also subjected to the tunnel respirometer protocol. The comparison of performance in the tunnel respirometer between the rested and unrested fish was used to assess the effect of the rest period after transport on activity level and swimming performance.

Tunnel respirometer protocol

At the appropriate time, each rested and non-rested fish was, in turn, forced to swim against water supplied at controlled velocities within a tunnel respirometer. During this procedure, EMG*i* signals were recorded, oxygen consumption (V_{O_2}) measurements were taken and swimming performance was assessed. All procedures were done in a 120 L Blazka-type, swim speed chamber/respirometer, as described in Thorstad and colleagues (1997). Fish were netted and placed into the chamber, and allowed to acclimate at a slow water velocity of 0.1 m s^{-1} for a 15 min period. The water velocity was then increased every 15 min by 0.17 m s^{-1} intervals, until the fish became fatigued. A fish was considered fatigued when it could no longer maintain position in the chamber and was impinged twice against the blocking screen. At this point, the water velocity was immediately turned down to close to zero. The time of fatigue was recorded and used to calculate the critical swimming speed (U_{crit}) of fish which is a measure of prolonged swimming ability (see Brett 1964). During each water velocity increment, EMG*i* signals were recorded when the fish held a constant position in the swimming tube and was actively swimming. These EMG*i* signals were later calibrated to the swimming speed as in Cooke and colleagues (2000). Oxygen readings were also taken at the beginning and end of each successive 15 min interval. Oxygen levels in the swim chamber were monitored by re-circulating a small fraction of the chamber water over a digital oxygen probe (Orion Inc., Fisher Scientific Limited, Nepean, ON, Canada), using a peristaltic pump fitted with gas-tight tubing. During the tests, the oxygen concentration of water in the swim chamber never fell below 80% saturation.

In a parallel study (Cooke *et al.* 2000) using fish from the same source, the fatigue times and V_{O_2} of 12, non-transported, EMG*i*-tagged rainbow trout were tested at the AARS. These fatigue times and V_{O_2} data obtained by Cooke and colleagues (2000) were used as benchmark controls in the present study, as the standard for such experiments was developed in this previous work. The EMG*i*-tagged fish (mean weight and fork length = 1204 ± 87 g and 432 ± 9 mm) tested in Cooke and colleagues (2000) were of the same source and genetic stock as used in the present study, and were reared at the AARS under identical environmental conditions. The initial behavioural and physiological state of fish tested in Cooke and colleagues (2000) and the present study, as determined by post-surgical recovery period EMG*i* signals, were indistinguishable. All equipment used in both experiments was identical. The testing protocols in the parallel experiment were slightly different in a number of respects. Fish tested in Cooke and colleagues (2000) were given a 30 min acclimation interval (the time a fish spends in the swim chamber before the stepwise increases in water velocity occur), instead of the 15 min acclimation interval used in the present study. A shorter acclimation interval was used in the present study in order to ensure that fish were truly in an immediate, post-transport and non-rested state at the time of testing. It has been previously shown that the duration of the acclimation interval does not affect U_{crit} in rainbow trout (Peake *et al.* 1997). Another difference in testing protocols was that the swim chamber used in Cooke and colleagues (2000) was flushed with freshwater periodically throughout the test procedure, while in the

present study the chamber was flushed with fresh, aerated water only during the 15 min acclimation period. As the oxygen concentration did not fall below 80% air saturation during any of the tests in either study, it was assumed that this difference in protocol was negligible.

Environmental conditions

Water used for all procedures at the AARS and during transportation had a temperature of 8.5 ± 0.1 °C, a dissolved oxygen concentration of 10.6 mg L^{-1}, and a pH of 7.6 ± 0.1. All transport containers were filled with fresh, aerated water immediately before use. The fresh, aerated well water used at the University of Waterloo was approximately 9 °C, and was similar in all other water quality characteristics. At both facilities, the tanks used to hold experimental fish were covered with a perforated plastic sheeting, and artificial lighting systems were used to provide a photoperiod that mimicked natural conditions for southern Ontario, Canada at that time (January–February 1998). Water was delivered to holding tanks at both facilities at a rate between 22 and 24 L min^{-1}, creating a circular flow within the tanks.

Statistical analysis

EMG*i* signals were standardized according to the method of Økland and colleagues (1997) for each fish in relation to its pre-transport EMG*i* activity levels. Fish activity during the transport and post-transport periods was therefore assessed as the percentage increase or decrease from these pre-transport activity levels. This measure of muscle activity is termed the 'activity index'.

All statistics were performed with Statistical Application Software (Version 6.1, SAS® Institute). Prior to combining data among any of the three replicates, tests for differences in weight, fork length and activity index were conducted. Differences in fish activity index during the pre-transportation and post-transport 48 h period (days 1 and 2) were tested using ANOVA. A *t*-test for unequal variances was used when testing pooled pre- and post-transport fish activity index with activity index during transport. The relationship between the activity index and swimming speed for both rested and non-rested fish during forced swim trials, was generated using linear regression. Differences in $\dot{V}_{O_2}$ at each swimming speed within and between rested and non-rested groups of fish were assessed using the SAS® Proc Mixed procedure. The critical swimming speeds for rested and non-rested fish were compared using a *t*-test. U_{crit}, weights and fork lengths among non-transported fish (Cooke *et al.* 2000) and transported fish (this study) were also compared with a *t*-test. All tests were performed at an α level of 0.05.

Results

All fish survived the surgery, transportation episodes and the tunnel respirometer protocols with the exception of two fish, which succumbed to a water failure condition present at the Waterloo laboratory after transportation during one replicate trial. This was an isolated incident that did not affect the remainder of the trials. During the third transportation event, EMG*i* transmitter output from one fish failed to be detected by the receiver, so EMG*i* data for that individual could not be obtained. There were no significant differences ($P > 0.05$) between the weights or fork lengths of fish among replicates in this study, or with the fish tested in Cooke and colleagues (2000).

Fish activity during transportation and resting events

The activity index of fish during transportation (Fig. 1) was significantly different from the activity index of fish recorded during their pre-transportation and 48 h post-transport resting periods ($P < 0.01$). During transportation, two general types of fish activity were distinguishable as assessed by EMG*i* records (Figs 1 and 2). Seven fish demonstrated an elevated activity index, while the remaining four showed a significant decrease in activity as compared with their pre-transport activity levels. The activity levels (activity index) of fish during the pre-transportation and 48 h post-transport resting periods were similar ($P > 0.05$). Within the first day of arrival, fish returned to pre-transport levels of activity and maintained those levels throughout the 48 h resting period. As EMG*i* signals were calibrated with swimming speed, the average swimming speed of fish during transport could be estimated. Regardless of activity type, all fish except one, demonstrated activity index levels indicative of fast swimming at some point ($\approx 70\%$ U_{crit}) during transportation (Fig. 2).

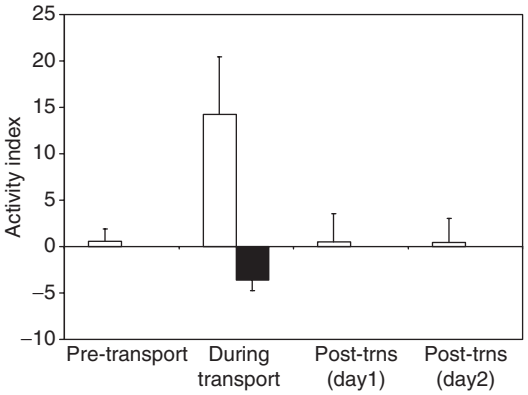

Figure 1 Rainbow trout activity as recorded by EMG*i* telemetry during pre-transport (i.e. baseline activity level; $n = 12$), transport ($n = 11$) and post-transport periods (post-trns days 1 and 2; $n = 6$). The activity of fish during transportation could be described as either elevated (white bar; $n = 7$) or lowered (black bar; $n = 4$) relative to baseline. Error bars are ± 1 SEM.

Respirometry estimates

Regression analyses indicated that the activity index was positively correlated to swimming speed (Fig. 3) for both non-rested fish ($r^2 = 0.95$, $P < 0.05$) and rested fish ($r^2 = 0.92$, $P < 0.05$). The regression-derived slope (mean slope) of the activity index by swimming speed for rested fish (29.86) was significantly elevated as compared with that of non-rested fish (16.76).

Swimming speed did not significantly affect the rate of V_{O_2} as measured during forced swim trials for either rested or non-rested, transported fish ($P = 0.53$; Fig. 4). This was clearly different from the positive correlation of those variables observed when testing non-transported fish (Cooke *et al.* 2000). The mean V_{O_2} for non-rested fish was higher than that of rested fish at each swimming speed tested, but these differences were not significantly different ($P = 0.62$). The average V_{O_2} during forced swim trials for all transported fish was 148 ± 4 mg kg^{-1} h^{-1} (mean ± SEM). At low-to-moderate swimming speeds (between 0.25 and 0.63 m s^{-1}), transported rainbow trout, on average, had a higher rate of V_{O_2} than non-transported fish (148 and 108 mg kg^{-1} h^{-1} respectively).

The critical swimming speeds of non-rested and rested trout were not significantly different ($P > 0.05$), and were therefore pooled to give a mean U_{crit} of 0.64 ± 0.04 m s^{-1} (± SEM). The mean U_{crit} of non-transported, rested, EMG*i*-tagged trout tested in Cooke and colleagues (2000) was 0.90 ± 0.05 m s^{-1} (± SEM). U_{crit} of transported and non-transported fish were significantly different ($P < 0.05$).

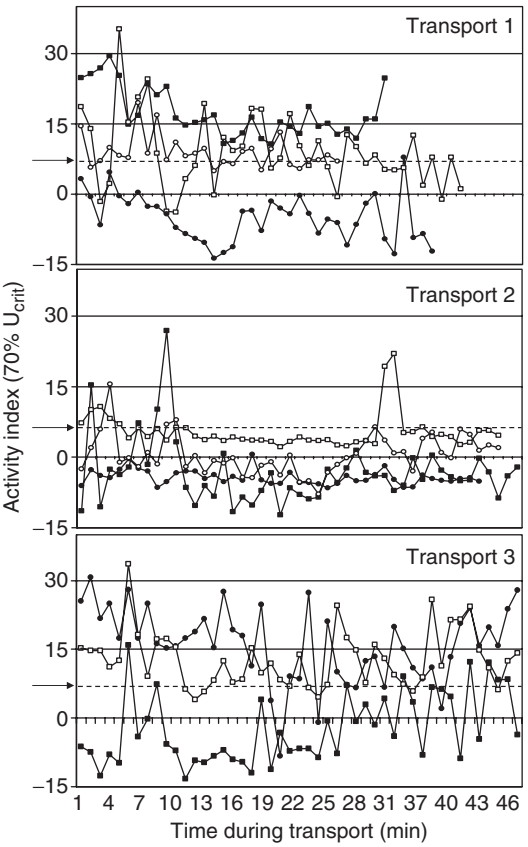

Figure 2 Rainbow trout activity, as recorded by EMG*i* telemetry, during a 50 min transportation episode by truck. Each lined data point represents the mean of 15–20 EMG*i* signals for a single fish; for clarity, error bars have been omitted. The dashed vertical lines represent the estimated activity index value for fish swimming at 70% U_{crit}. Calculations of 70% U_{crit} were as follows: the mean U_{crit} for all EMG*i*-tagged fish (Cooke *et al.* 2000, this study) subjected to swim trials was 0.765 ± 0.13 m s^{-1} (mean SEM). Seventy percent of this value yielded a swimming speed of 0.535 m s^{-1}. Using the regression formula developed for non-rested fish ($y = 24.85x - 6.54$), the activity index equivalent to a swimming speed of 0.535 m s^{-1} was calculated.

Discussion

Respirometry estimate variability

The V_{O_2} estimates determined in the present study as well as those reported in Cooke and colleagues (2000) were compared with tunnel respirometer-

derived estimates obtained from the literature (Dickson & Kramer 1971; Weatherley *et al.* 1982; Briggs & Post 1997a). Maximal V_{O_2} expression of the rainbow trout tested according to the current protocols was at the lower end of previously published values. There are two possible explanations for the lower V_{O_2} values reported in Cooke and colleagues (2000) and in this study. The lower V_{O_2} observed in the present study may be expected as the water temperature (8.5–9 °C) used during our determinations was lower in comparison with other studies. In addition, the V_{O_2} of a single fish, relative to a large volume respiration chamber, will result in a slower decline in oxygen concentration in the water (Steffensen 1989). Therefore, the 10–15 min sampling intervals used in the current and Cooke and colleagues (2000) study may have been insufficient to detect changes in V_{O_2}. Geist and colleagues (2000) also report variable V_{O_2} determinations of EMG*i*-tagged chinook salmon *O. tshawytscha* (Walbaum) due in part to the large-sized respirometer utilized in their study.

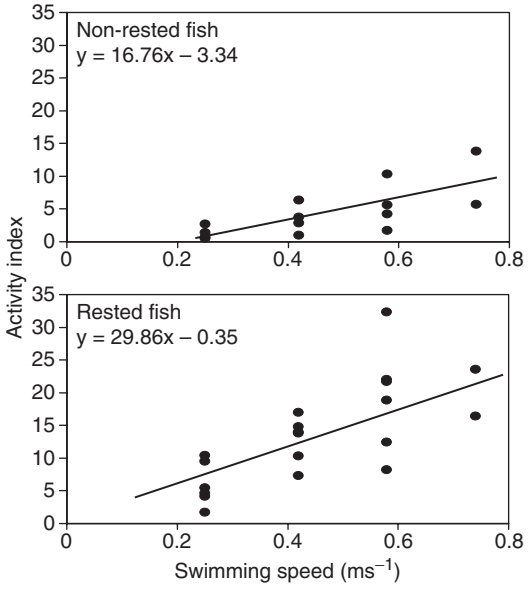

Figure 3 Activity index vs. forced swimming speed in non-rested ($n = 4$) and rested ($n = 6$) rainbow trout. Each data point is the mean of 15–40 EMG*i* signals recorded from an individual fish.

Fish activity and energy utilization during transport

Fish showed periodic bouts of elevated swimming activity during transportation. On average, these bouts of swimming could reach 70% of U_{crit} (Fig. 2). Swimming at this intensity requires the support of both red (aerobic), and white (anaerobic) musculature (Jayne & Lauder 1994; Burgetz *et al.* 1998). Swimming

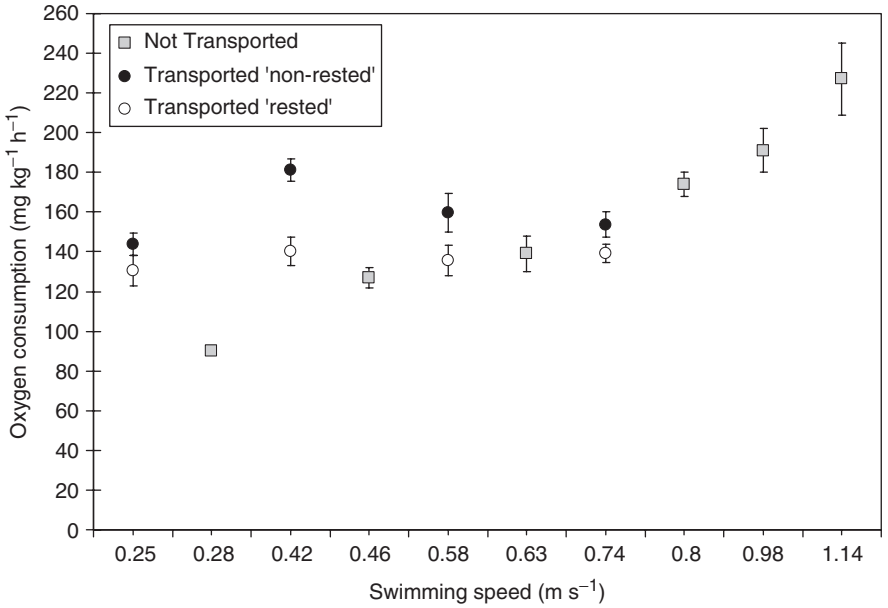

Figure 4 Comparison of the rates of oxygen consumption at successively increasing swimming speeds observed in non-rested ($n = 4$), rested ($n = 6$) and non-transported ($n = 12$; Cooke *et al.* 2000) fish. Data points plotted are the actual values measured in each study (mean ± SEM), so the *x*-axis is an overlay of both scales used in each study.

mechanics utilizing the white musculature in fish is typically associated with vigorous movements, such as burst-glide, or fast-start, swimming (Domenici & Blake 1997). The erratic pattern of EMG*i* signals recorded during transportation, in combination with high activity index values, suggests that the behaviour of fish during transportation encompassed 'burst-glide' swimming in combination with body-turns. These swimming movements may be simple random flight responses, or may have been performed in reaction to waves or other water movements generated within the moving transport tank. Four fish during transportation maintained activity below resting levels, with only occasional bursts of swimming speeds above 70% U_{crit}. Although lowered in magnitude, the erratic pattern of EMG*i* signals from those fish suggested that irregular, complex swimming was occurring during transport (i.e. spontaneous movements), perhaps to maintain a constant position within the carrier tank. Variation observed between individual fish within different parts of the transport tank was not unexpected, as complex swimming patterns have been described by Liao and colleagues (2003), showing that individual fish can exploit vortices in a microenvironment to conserve energy. Spontaneous swimming in fish is energetically expensive relative to a steady, undulatory swimming motion (Boisclair & Tang 1993). The current results support previous suggestions that reduced survival of fish released to the wild immediately after transportation is in part caused by reduced energy in the fish induced by the transportation (Schreck *et al.* 1989). The rate of energy utilization in fish is considerable when they swim at intensities approaching their U_{crit}, or when swimming involves numerous fast-starts and other erratic movements (Driedzic & Hochachka 1978; Dobson & Hochachka 1987). Furthermore, those swimming activities can promote the accumulation of the end-products of anaerobic metabolism, such as lactate, within various organs or tissues of fish (Burgetz *et al.* 1998). As lactate accumulation in fish has been measured immediately after transportation (Nikinmaa *et al.* 1983; Iversen *et al.* 1998), but also occurs as a result of routine handling stressors (Vijayan & Moon 1992; Davis & Schreck 1997), it would have been difficult in the past to attribute such findings to specific swimming behaviours during transport. However, the results of this study demonstrate how fish behaviour during the transport period could be responsible for a portion of the increased lactate measured in fish after transport. Similarly, other stress variables that are sensitive to moderate or vigorous swimming may also be affected.

It is not clear how the bio-density of fish or the water quality in the transport tank influenced fish behaviour during transportation. Given the low bio-density of fish in the transport carrier, and the fact that fish were not fed prior to transport, the build up of metabolic waste products over the 50 min transport period was considered negligible. As a build up of respiratory gasses, such as CO_2, and the use of water additives, such as salt, have sometimes been observed to cause erratic swimming behaviour in rainbow trout (Barton & Peter 1982), it is possible that water quality alterations common to transportation practices may cause additional behavioural reactions not observed during this study, particularly when fish are transported for extended time periods (Carmichael 1984). The high levels of fish activity observed during transportation might be specific to unidentified aspects of the current transport protocol.

Oxygen consumption of transported and non-transported fish

Respirometry measurements (V_{O_2}) of non-transported fish were obtained from the parallel study (Cooke *et al.* 2000) and were used for comparison. Fish used in the current and parallel study demonstrated similar baseline muscle activity and behaviour as determined by their post-surgical EMG*i* signals. In general, this comparison reveals that at low-to-moderate swimming speeds (i.e. between 0.25 and 0.63 m s^{-1}) the transported rainbow trout, on average, had a higher rate of V_{O_2} than non-transported fish (Fig. 4). Transported fish were subjected to a number of stressors that were likely to affect their metabolic activity. The fish were first briefly chased and netted (< 5 s), then subjected to a brief air exposure (< 10 s) during the transfer by dip-net, and then placed into the transport tank. After transportation, the fish were again netted, subjected to another brief air exposure, and then placed either in the resting tank or directly into the respirometer chamber. Physical disturbances, such as capture and handling can cause fish to struggle, and this has been shown to double the V_{O_2} of rainbow trout and coho salmon *O. kisutch* (Walbaum) (Barton & Schreck 1987; Davis & Schreck 1997). Large increases in V_{O_2} also occur during erratic, forced exhaustive exercise (Scarabello *et al.* 1991). Dickson and Kramer (1971) found that physical handling of rainbow trout followed by

forced swimming of those fish at high speeds was sufficient to illicit a full expression of V_{O_2} (i.e. the active metabolic rate). Another factor that may have influenced the rates of V_{O_2} of fish tested in this study was the brief instances of air exposure when transferring fish by dip net before and after transportation, which may exacerbate metabolic imbalances in fish that have been previously exercised. Ferguson and Tufts (1992) found that 30 s of air exposure after bouts of burst swimming in rainbow trout caused a greater degree of anaerobic end-product accumulation within the white musculature, as well as an increased mortality rate. Davis and Schreck (1997) observed that among a variety of experimental handling stressors, treatments that involved the exposure of *O. kisutch* (Walbaum) to air caused the greatest elevations in V_{O_2}. Therefore, it is expected that the several disturbances experienced by fish in this study may have caused an elevated level of V_{O_2}, relative to the non-transported fish.

As the fish in our study were also active at swimming speeds that have been correlated with the build up of anaerobic end-products (i.e. lactate) in rainbow trout (Wokoma & Johnston 1981; Jones 1982; Burgetz *et al.* 1998), we suggest that elevations of V_{O_2} post transport also involved the repayment of an oxygen debt acquired during transportation. The oxygen debt hypothesis states that after a period of intense exercise, there is an elevated, post exercise V_{O_2} (i.e. the oxygen debt) that is indicative of an adjustment and recovery from physiological alterations (i.e. metabolic recovery of lactate and other intracellular energy sources) because of strenuous white muscle activation (Scarabello *et al.* 1991; Schulte *et al.* 1992). Similarly, Barton and Schreck (1987) and Farrell and colleagues (1998) hypothesized that metabolic recovery could occur during low swimming speeds post exercise, and may be experimentally measured as an elevation of V_{O_2}.

The physiological responses to exercise in trout are often reported to include a positive correlation between V_{O_2} and increasing swimming speed (Brett 1964; Beamish 1979; Weatherley *et al.* 1982). In this study, forced swimming of trout, from very slow to higher, critical velocities, did not cause a step-wise increase in V_{O_2}. This was an unexpected, but reasonable result in light of the immediate histories of the fish prior to testing, and the characteristics of our respirometer. It is possible that the observed rates of V_{O_2} in transported fish did not increase with progressive swimming speeds because of the inability of the respirometer to resolve the relatively smaller change in V_{O_2} specific to swimming. Driedzic and Hochachka (1978) reported that the red musculature of fish (which powers swimming at low-to-moderate speeds) only accounts for approximately 50% of the total oxygen usage when the energy required for respiratory adjustments (Steffensen 1985) and cardiac metabolism (Jones & Randall 1978) are taken into account. Therefore, if the magnitude of the change in V_{O_2} specific to swimming at low-to-moderate speeds was relatively small as compared with the elevation in V_{O_2} caused by the transportation-invoked stressors and swimming activities, the tested respirometry protocol may not have been sensitive enough to detect these smaller changes.

Swimming performance of transported and non-transported fish

The swimming performance (indicated by U_{crit}) of transported fish was impaired when compared with the performance of non-transported fish in Cooke and colleagues (2000). Furthermore, this comparison shows that the maximum aerobic capacity of transported fish was not attained at U_{crit}. This is not a novel finding, as a number of studies have demonstrated that U_{crit} is not always coupled with maximum V_{O_2} in the Salmonidae (Alsop & Wood 1997; Farrell *et al.* 1998) and in other teleosts (Reidy *et al.* 1995). This finding is interesting, however, as it may be indicative of physiological alterations relating to aerobic activity that occur in fish when they are transported. The fishes' experience during transportation may be viewed as a bout of extended exercise and potentially highly stressful stimuli. Physical disturbances and extended exercise have significant physiological impacts affecting the swimming musculature and respiratory systems of trout (Jones & Randall 1978; Milligan & Wood 1982; Gonzalez & McDonald 1992; Butler & Day 1993). As U_{crit} and V_{O_2} are reflective of the biochemical and functional status of trout musculature and gill processes (Waiwood & Beamish 1978; Schreck 1990), alterations in those measures because of muscular or ionoregulatory disturbances should have been resolvable in the current respirometry tests. The reduced U_{crit} and elevated, but non-maximal V_{O_2} expression in transported fish may have been caused by an interaction of anaerobic, end-product accumulation within the fishes musculature, and disturbances in ionoregulation, i.e. the osmorespiratory compromise (Gonzalez & McDonald 1992). The relationships of prolonged swimming and

ionoregulatory disturbances to muscle fatigue and V_{O_2} have been examined in rainbow trout (Milligan & Wood 1982) and brown trout *Salmo trutta* (Linnaeus) (Butler & Namba 1992; Butler & Day 1993; Day & Butler 1996). Transportation-induced ionoregulatory disturbances have been observed in various *Salvelinus* species (McDonald *et al.* 1993), although the post-transport swimming ability of those fish was not tested.

Post-transport resting periods

As assessed by swimming activity, EMG*i* signals recorded post transport indicated that the fish were resting. Based on those results alone, however, it could not be concluded that the fish had achieved a metabolically 'recovered' state, as evidenced by the reduced swimming performance of those fish. Similarly, Maule and colleagues (1988) found that although plasma cortisol titers indicated that *O. tshawytscha* (Walbaum) were rested after transportation, their swimming performance was still impaired. Elevated plasma glucose levels in *S. salar* (L.) after a 48 h post-transport rest period led Sandodden and colleagues (2001) to suggest that longer than expected recovery times are needed for fish to return to a pre-stressed state. It is possible that the resting environment we provided could have induced stress in the fish, as we did not measure other stress indicators. In most studies, including the present one, in which the fish are rested after transportation, the resting container is designed to be relatively non-stressful (e.g. optimum water quality, darkened, covered tanks), but it is undoubtedly still an alien environment for the fish. McDonald and Robinson (1993) found that transported trout recovered much faster when returned to their own race-ways, rather than when introduced to a new environment that was designed to be non-stressful. Similarly, Tipping (1998) reported that allowing rainbow trout to rest for 24 h post transport in a new resting environment did not improve post-release survival of those fish.

Post-transport survival of fish released to natural environments is influenced by a number of factors, and is related to the physiological and behavioural factors involved in the acclimation processes (Munakata *et al.* 2000), genetic influences (McDonald & Robinson 1993), and the timing of release (Hansen & Jonsson 1989; Congleton *et al.* 2000). Therefore, post-transport resting periods may only be of value if they allow for an adequate time for fish to acclimate to novel circumstances in the release environment. Johnson and colleagues (1990) found that transported coho salmon, *O. kisutch* (Walbaum), that were acclimated to the release site for 6 weeks still showed higher rates of survival than fish immediately released. As acclimation to new, post-transport conditions can take many days or weeks (Korovin *et al.* 1982; Nikinmaa *et al.* 1983; Johnson *et al.* 1990) and involves a number of biological pathways, a multitude of biological indicators are necessary to successfully monitor the progression of acclimation. For example, swimming stamina tests and behavioural assays would be particularly useful to identify the later stages of acclimation, and indicators of the fish's response to acute stressors, such as cortisol and swimming activity measurements, would be more useful when testing in the early stages of acclimation. A finding which may be of interest for future research was the difference observed between the regression-derived slope of muscle activity vs. swimming speed among rested and non-rested fish (Fig. 3). The greater slope calculated for rested fish may be indicative of recovery and subsequent recruitment of red muscle during forced swimming.

Conclusions

The findings of this study demonstrate the merit of integrating behavioural measures with physiological estimates when assessing the impact of aquacultural practices on fish. Studies examining fish transportation protocols generally regard the capture and loading procedures as the major causes of stress, but disregard transportation *per se* as a stressor (Barton & Peter 1982; Maule *et al.* 1988; Robertson *et al.* 1988; Schreck *et al.* 1989; Iversen *et al.* 1998). Those conclusions are usually based on the fact that plasma cortisol levels in fish usually decrease during the transport period. However, the results of this study illustrate how the behavioural responses of fish during transportation may contribute to physiological changes observed in fish after transportation. Furthermore, the combinations of various biological indicators should be assessed when attempting to determine the recovery status of transported fish. Although resting intervals employed after transportation are certainly useful, they may only be effective in specific situations where the release environment allows for acclimation of fish to the novel aspects of their environment. Finally, the aerobic capacity which influences the swimming performance of fish,

may be impaired for longer periods after transportation than was previously expected.

Acknowledgments

We thank the staff of the Alma Aquaculture Research Station and the University of Waterloo Biotelemetry wet lab staff for their technical assistance provided throughout this study. Statistical advice was provided by I. McMillan and M. Quinton of the University of Guelph. Earlier versions of this manuscript benefited from reviews by I. J. H. Duncan, J. F. Leatherland and J. Hynes. Special thanks also to Mr John Brake for assistance during the final manuscript preparation. This manuscript constitutes a portion of the degree requirements for an MSc at the University of Guelph for K. P. C. Funding was provided by a grant from the Ontario Ministry of Agriculture and Food to R. D. M. Additional support was provided by an NSERC grant to R. S. M. Project support for S. J. C. was provided by a Julie Payette NSERC Fellowship.

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