Available online at www.sciencedirect.com
SCIENCE DIRECT®
Aquaculture
ELSEVIER
Aquaculture 239 (2004) 467–484
www.elsevier.com/locate/aqua-online

Can feeding status and stress level be assessed by analyzing patterns of muscle activity in free swimming rainbow trout (*Oncorhynchus mykiss* Walbaum)?

W.J. McFarlane[a], K.F. Cubitt[a,*], H. Williams[b], D. Rowsell[b], R. Moccia[c], R. Gosine[b], R.S. McKinley[a]

[a] *Centre for Aquaculture and the Environment, The University of British Columbia, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, British Columbia, Canada V7V 1N6*
[b] *C-CORE, St. John's, Newfoundland, Canada A1B 3X5*
[c] *Aquaculture Centre, The University of Guelph, Guelph, Ontario, Canada N1G 2W1*

Received 28 November 2003; received in revised form 21 May 2004; accepted 28 May 2004

Abstract

This study involved monitoring the activity levels of three groups of adult rainbow trout that were either fasted on a weekly cycle, fed to satiation, or fed to satiation and then acutely stressed by overcrowding twice weekly. A subsample of fish was implanted with electromyogram (EMG) radio-transmitters that broadcast a signal proportional to muscle activation levels, allowing for the continuous recording of swimming activity by a remote receiver. These EMG transmitters did not affect the swimming performance of fish, but did reveal variation in activity as a result of feeding status. The results of this study clearly illustrate differences in activity levels in fish of differing feeding and stress status in cultured fish. Ultimately, fish may be able to control feed availability using their behaviour patterns, with feed being presented upon demand. This type of technology may enhance the automation of intensive fish culture operations while simultaneously minimizing feed wastage as well as overall production costs.

Keywords: Aquaculture; Feeding; Swimming; Biotelemetry; Electromyogram

* Corresponding author. Tel.: +1-604-666-6065; fax: +1-604-666-3492.
E-mail address: kfcubitt@interchange.ubc.ca (K.F. Cubitt).

0044-8486/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2004.05.039

1. Introduction

The field of biotelemetry has made significant advances in the creation of devices that can remotely monitor physiological parameters in free swimming, salmonid fish. Measures such as heart rate (Lucas et al., 1991; Anderson et al., 1998), ventilatory rate (Rogers and Weatherley, 1983), tailbeat frequency (Ross et al., 1981) and locomotory muscle activation (Kaseloo et al., 1992; Thorstad et al., 2000) have been correlated to estimates of metabolic rate in order to predict energetic expenditures of various activities. Of these, perhaps the best estimator of whole body swimming effort is the electromyogram, or EMG (Weatherley et al., 1982), likely due to the large amount of discrete muscle tissue found in salmonids (>60% body mass; Johnston, 1981) and its involvement in a wide variety of physical activities. As muscles become activated, they generate electropotentials, detected as EMGs, which are proportional to the intensity and duration of muscle activity (Sullivan et al., 1963). EMGs are measured by small electrodes implanted within the muscle tissue. The coded EMG signal is sent to a wireless receiver, and these signals can then be correlated to oxygen consumption and swimming speed under controlled conditions (cf. McKinley and Power, 1992).

EMG transmitters have been used for a variety of field applications, including estimating routine activity (Demers et al., 1996), and evaluating swimming effort of fish during migration (Hinch et al., 1996), and spawning (Weatherley et al., 1996; Okland et al., 1997, 2000). Telemetric data has proven to be an accurate measure of swimming behaviour in fish, and has also previously been used to evaluate captive husbandry protocols such as lighting regimes and transportation stress (Chandroo et al., 2000) and rearing density (Cooke et al., 2000). However, there is a heightened need to evaluate the welfare status of fish under aquaculture conditions, particularly as pressure to increase rearing density becomes an issue. To date, relatively few telemetry studies have branched into this realm.

One research area where physiological telemetry has yet to be applied is in the evaluation of feeding requirements and the classification of hunger or feeding status and in-tank stress levels in captive fish. These situations are of obvious concern within the aquaculture industry, as they often relate to the need for decreasing mortality, increasing feed efficiency and minimizing food waste and the concomitant environmental pollution (Baras and Lagardere, 1995). By establishing the relationship between fish behaviour and feeding status, or stress levels, enhanced monitoring of fish stocks and prediction of their physiological needs may be possible. In order to assess levels of stress, it is necessary to identify a known stressor. Crowding is a common husbandry practice in aquaculture, as is reducing the water level. Therefore, many studies have investigated the effect of crowding on stress levels by reducing the space available to the individuals and measuring common stress indicators such as plasma cortisol, glucose and lactate (Barnet and Pankhurst, 1998; Frisch and Anderson, 2000; Ruane et al., 2002).

Our first hypothesis for this study was that hungry fish would exhibit different activity level patterns as compared to fish that are satiated, and that these patterns could be used to predict feeding status. For example, fish that are fasted for a number of days may become less active owing to an energy conservation strategy and lower metabolic rate associated with starvation. Alternatively, they may exhibit elevated activity levels, which could be indicative of foraging behaviour. A second hypothesis was that stressed fish would exhibit

characteristic types of swimming behaviours (e.g. erratic bursts of activity) in response to an acute stressor, and that the pattern of such behaviours could also be used to predict the onset of a stress response in fish. By collecting activity data of fish in controlled physiological states (e.g. fasted vs. fed vs. stressed), our overall objective was to see if inferences could be made about the effects of feeding status and stress level on some quantifiable estimate of swimming behaviour.

Ultimately, the hope is to integrate these analysis tools into the development of a reasoning system that can take behavioural data (e.g. EMG data) and make decisions regarding the physiological needs of cultured fish. These types of tools may be implemented to augment existing feeding and monitoring technologies in intensive aquaculture operations.

2. Materials and methods

2.1. Experimental animals

Adult, hatchery-reared rainbow trout ($N = 200$; 820.6 ± 8.7 g, 38.8 ± 0.1 cm) were obtained from the Alma Aquaculture Research Station (AARS; Elora, Ontario, Canada). Fish were held in four identical 2-m-diameter tanks continuously supplied with air-saturated freshwater at a flow rate of 41 l min^{-1} and volume set to 1.26 m^3. These tanks were located in a quarantine room, in order to control access, thereby ensuring that any observed changes in activity level were not the result of uncontrolled human interference. The tank biodensities were equalized as much as possible to achieve both similar fish densities and numbers; 50 fish making up densities of 41.8 ± 0.03 kg per tank, or an average density of 33 kg m^{-3}. Water flow and dissolved oxygen were measured once weekly, with flow adjusted as necessary. Temperature was monitored by a data logger (Optic StowAway Temp $-5+37$, Onset Computer, Pocasset, MA), with measurements logged every 5 min. Over the experimental period, mean dissolved oxygen was 8.95 ± 0.10 mg l^{-1} and temperature was 8.47 ± 0.07 °C. Photoperiod was maintained as a natural regime (between August and October), and was monitored using light intensity data loggers (StowAway LI, Onset Computer), that measured light levels every 2.5 min. Fish were maintained on commercial trout diet (9.0 mm sinking broodstock pellets; 45% protein, 12% fat, 15 MJ kg^{-1} digestible energy, 50 ppm Astaxanthin pigment; Martin Mills, Elmira, Ontario). Up until the start of the experiment, fish were fed a near-satiation ration, twice daily, using automated feeders (Sweeney AF7 vibrating feeders powered by a Sweeney AFT1-QA automatic controller). At the start of the experiment, fish were fed a satiation +20% ration via automated feeders, with feeding regularity on a week-to-week basis dependent on treatment group. The additional 20% increases the amount of food that is available to the fish, compensating for the uneaten food that falls into the drain. Feeders were set up to dispense feed over two 1-h periods, with the interval time 5 min and the duration of each feeding episode 1.1 s. Growth was monitored by measuring lengths and weights of all fish (both tagged and untagged) prior to and following the 10-week holding period to determine both the effects of treatment and tagging on growth. There were no initial differences in weight between tanks (Table 1). The above procedures were

conducted with the approval of the Animal Care Committees at both the University of Waterloo and the University of Guelph, under the protocols of the Canadian Council on Animal Care.

2.2. Classification of physiological state

In order to collect activity data from fish in known feeding states, fish in one tank were maintained on a feeding regime of 1 week with feed, 1 week without feed, for the duration of the 10-week monitoring period (“fasted”). The second group was fed a daily satiation ration (“fed”). The third group was fed in the same manner as the satiation fed group, but they were also exposed to an acute stressor twice weekly (“fed/stressed”). The stressor involved crowding fish through lowering the water level by half (in effect, producing a doubling of fish density), and allowing it to refill (a total duration of about 1 min). In each of these three groups, 10% of the population (i.e. 5 of 50 fish) were implanted with EMG transmitters in order to monitor routine activity levels. The last group of fish served as controls which were not implanted with transmitters, and were fed a satiation + 20% ration (“control”).

2.3. Telemetry equipment

Due to cost constraints associated with the telemetry equipment, it was not possible to replicate the experimental treatments. Therefore, five transmitters were used in each of the three treatments. The transmitters used were integrated electromyogram (EMG) transmitters ($N = 15$; model CEMG-R11-25, Lotek Wireless, Newmarket, Ontario) of dimensions 11×61 mm and weighing 12 g in air, approximately 1–2% of fish weight. The EMG tags consisted of an epoxy-coated transmitter package with a pair of Teflon-coated electrodes with 10 K gold muscle-anchoring tips (dimensions 1×7 mm) that are inserted into the red muscle band. The electrodes detect electropotentials within the axial red muscle tissue, with the amplitude and frequency of these pulses being directly correlated to the level of muscle activity. The CEMG model is equipped with a differential muscle probe, a signal conditioning circuit, a digitizer, a micro-controller and a radio transmitter. The voltage corresponding to muscle activity is rectified and sampled from the beginning to the end of each 3-s time interval. Individual samples are summed and temporarily stored. At the end of the time interval, the average value is calculated and assigned an activity level (EMG signal) ranging from 0 to 50. This calculated result is then transmitted to a receiver. The signal from each tag was detected and recorded by an SRX_400 radio receiver (Lotek Wireless), and data were downloaded to a computer every second day. Tags were programmed to transmit at one of five frequencies (i.e. three tags at each of five frequencies). Therefore, one transmitter from each of three tanks would transmit an activity sample to the receiver for each frequency. The receiver was set to scan all frequencies and record activity from fish of each treatment groups (fasted, fed and stressed). The gain was set to 15, and for half of the study (the first 5 weeks) the scan time was set to a 10-s interval (i.e. each frequency was scanned for 10 s). However, this configuration did not provide sufficient EMG data to be collected to allow for the mathematical pattern analysis. Therefore, we experimented with a shorter scan time of

3.5 s, a gain of 50 and a noise blank level of 50, which provided enhanced signal detection and more data for analysis. For this reason, we chose to focus our mathematical analysis on the EMG data collected in the latter half of the study.

2.4. Surgical procedure

Five fish from each of three treatment groups (fasted, fed and fed/stressed) of 50 fish were implanted with EMG transmitters, and an additional five fish were operated on, but had no transmitter inserted. These fish served as shams to evaluate the effects of the surgical procedures. The EMG surgical implantation was similar to that outlined in Beddow and McKinley (1999). In brief, fish were anaesthetized in 60 ppm clove oil until they reached stage 2 of anaesthesia (Iwama et al., 1989), after which they were placed on a wet piece of foam, and their gills were irrigated with a temperature regulated (8.5 °C), 30 ppm aerated clove oil:ethanol solution (1:9) to maintain sedation during the procedure. A 3-cm incision was made posterior to the pelvic girdle. The transmitter was inserted and pushed interiorly into the body cavity. A 16-gauge needle was used to make a small opening, posterior to the incision, for the antenna to exit and trail behind the fish. From within the body cavity, specially designed stainless steel rods containing the gold electrodes were inserted into the red muscle band, and a plunger within the rods helped to anchor the electrode tips into the muscle. The incision was closed with four separate surgical sutures. Once surgery was complete (approximately 10 min), fish were returned to tanks for a period of 48 h to recover from the procedure.

2.5. Calibration of EMG transmitter output and assessment of swimming performance

Calibrations of the EMG transmitters were made by performing critical velocity tests on a total of 15 fish at the end of the 10-week period. Blazka-type respirometers supplied continuously with aerated freshwater were used, as described by Booth et al. (1997). Ten fish (five tagged and five untagged) from each treatment group were fasted for at least 24 h prior to swimming performance assessment to ensure a post-absorptive state. The critical velocity test was similar to that outlined by Brett (1964). In brief, fish were placed, one at a time, into the respirometer and allowed to acclimate at a water velocity of 30 cm s^{-1} for a period of 30 min. Following this acclimation period, speed was incremented by 10 cm s^{-1} every 10 min until the fish fatigued. Fatigue was determined when fish could no longer maintain position in the chamber and became pinned against the back screen for longer than 10 s. U_{crit} was calculated according to Brett (1964). Even though fish are known to use glycolytically derived energy at speeds far lower than their critical swimming velocity, U_{crit} is the speed at which enough energy is produced via aerobic means to sustain the activity for long periods of time. We have called this a "threshold" as speeds higher than this would lead to rapid exhaustion; however, it is possible that there is glycolytic energy production at these speeds. For each treatment group, the relation between swimming speed and EMG signal output from tagged fish was plotted and used as a "standard curve" to estimate speeds in free swimming fish from collected EMG signals (see also McKinley and Power, 1992; Demers et al., 1996; Hinch and Rand, 1998). In addition to validating the EMG

transmitter output, the swim trials also served to estimate the maximum sustainable swimming speed (or U_{crit}) of fish from each of the treatment groups, to determine whether the transmitter implantation affected swimming abilities (e.g. see Robertson et al., 2003; Connors et al., 2002).

2.6. EMG signal collection

EMG activity levels were compared between groups to determine the effect of fasting on routine activity, the effect of food presence, and the effect of crowding stress on activity levels. These comparisons were made to determine whether activity levels resulting from each treatment could be visually distinguished, prior to mathematical analysis.

2.7. Statistical analysis

As EMG data were normally distributed during swim tests, values are reported as either means ± 1 S.E.M., or as individual points. EMG validation curves were fitted by non-linear regression and tested to identify differences between curves using Sigma Plot 2000 (SPSS). However, non-parametric statistics were used to compare differences in overall activity level between treatments as the data were not normally distributed. Kruskal–Wallace tests with Dunn's multiple comparison procedure were used to compare the EMG values of the five tagged fish in each treatment and a Mann–Whitney *U*-test was used to compare fed with fed/stressed fish. The experimental design of the described study was constrained by cost. This has resulted in a design that is open to error in terms of pseudoreplication, a result of replicating within treatments. In order to reduce this possibility, it was ensured that there were no differences between tanks and in all experimental procedures carried out during the experiment.

Table 1
Growth (initial and final mass) and swimming performance (U_{crit}) for tagged and sham-operated fish from each of the treatment groups over the experimental period

	Growth		Swimming performance	
	Initial mass (g)	Final mass (g)	U_{crit} (cm s^{-1})	U_{crit} (BL s^{-1})
Control	806.9 ± 17.0 (50)	1172.2 ± 28.3 (50)*	121.40 ± 9.90 (6)	2.92 ± 0.72
Fasted	827.8 ± 18.0 (50)	1020.0 ± 26.6 (50)*,†		
Sham	755.8 ± 68.7 (5)	881.7 ± 61.8 (5)	114.23 ± 7.69 (5)	2.77 ± 0.40
Tagged	880.0 ± 47.8 (5)	845.7 ± 71.4 (5)	115.72 ± 13.36 (4)	2.77 ± 0.53
Fed	826.2 ± 17.3 (50)	1153.3 ± 37.0 (50)*		
Sham	851.2 ± 55.3 (5)	1025.9 ± 68.5 (5)	113.92 ± 5.42 (5)	2.75 ± 0.30
Tagged	845.8 ± 40.8 (5)	943.6 ± 23.0 (5)	101.32 ± 5.34 (5)	2.52 ± 0.25
Fed/stressed	821.4 ± 17.4 (50)	1208.8 ± 37.5 (50)*		
Sham	816.8 ± 24.9 (5)	1259.9 ± 93.2 (5)‡	129.59 ± 7.21 (5)	2.95 ± 0.45
Tagged	813.3 ± 74.2 (5)	944.1 ± 86.6 (5)	104.17 ± 7.80 (5)	2.64 ± 0.48

* Significantly different from initial mass ($p < 0.05$).
† Significantly different from control, fed and fed/stressed ($p < 0.05$).
‡ Significantly different from tagged ($p < 0.05$).

3. Results

3.1. Growth performance

Fish from all treatment groups grew significantly over the experimental period, with approximately 30% increase in body mass in all groups that were fed to satiation daily (i.e. control, fed and fed/stressed; Table 1). However, the group that was intermittently fasted grew significantly less than any of the other treatments, exhibiting less than 20% increase in mass (Table 1). In the three treatment groups in which fish were tagged, a comparison of growth in sham vs. tagged fish illustrated no significant differences in initial mass between or within treatment groups (Table 1). At the end of the study, the only differences in growth between sham and tagged fish occurred within the fed/stressed group, in which sham fish grew significantly better than tagged fish (Table 1). However, it appears that in this group, the sham fish grew extraordinarily well, because the tagged fish grew equally well as tagged fish in the other treatment groups.

3.2. Swimming performance

The critical swimming speeds of 35 fish tested at the end of the 10-week experimental period ranged considerably, from 81 to 155 cm s^{-1}, and when expressed relative to body size, the range was 1.8–3.2 body lengths (BL) s^{-1}. One fish from the fasted group was not swim tested as it had sustained physical injury resulting from incomplete healing of the incision. Upon calculation of mean relative U_{crit} for each treatment group, there were no significant differences in swimming ability between the treatment groups (Table 1). Furthermore, the effect of the surgical procedure itself, and the presence of a transmitter did not impair swimming performance in any of the groups as compared to control fish (Table 1).

3.3. Calibration of the EMG signal

From EMG data collected during the U_{crit} tests, muscle activity levels were correlated with swimming speed over speeds ranging from 40 to 90 cm s^{-1} (Fig. 1). Although speeds greater than 90 cm s^{-1} were achieved, EMG data became less reliable at higher speeds due to swimming behaviours induced by the forced nature of the swim test. At high speeds, fish were observed to periodically rest their tails on the back grate (presumably as an energy saving strategy), periodically resuming high speed swimming. Up to and including 90 cm s^{-1} water velocities, all fish were swimming continuously. The slopes of the regression lines for the three treatments were not significantly different (Fig. 1). Absolute U_{crit} values were plotted along the regression lines for each group, in order to determine the EMG signal that corresponded to the maximal sustainable swimming speed (U_{crit}) for each group (open symbols and dotted lines, Fig. 1).

3.4. Monitored activity levels

EMG signals were continuously transmitted and recorded over 24-h periods; however, we focused on time periods throughout the day when there was no human access to the

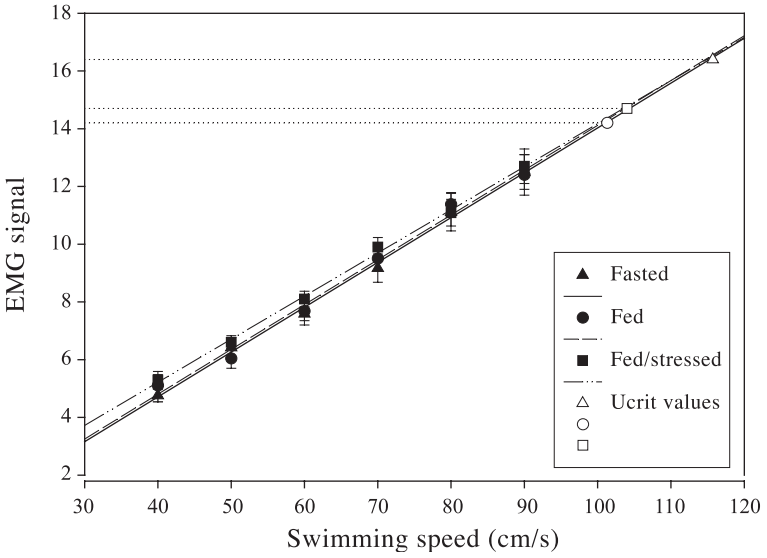

Fig. 1. Radio-transmitted electromyogram (EMG) signals in relation to swimming speed during a 10-min critical velocity protocol for fasted, fed and fed/stressed groups of fish. The testing temperature was 8.5 °C. Fish were swum individually, and the mean (± S.E.M.) reported for each group. Lines were fitted by linear regression: fasted fish: EMG = 0.16 (speed) − 1.5, $r^2 = 0.99$; fed fish: EMG = 0.16 (speed) − 1.4, $r^2 = 0.99$; fed/stressed fish: EMG = 0.15 (speed) − 0.76, $r^2 = 0.98$. Open symbols of the same shape represent the average critical velocity (in cm s^{-1}) for each group of fish.

holding room. During the remainder of the day, downloading of receiver data, water quality analysis, data logger retrieval, or filling of feeders could potentially disrupt normal fish activity. Therefore, representative data were chosen from time periods when the fish were not interrupted, with the exception of the crowding stress data, which necessitated human presence. However, when comparisons were made between treatment groups the same periods were used. The number of EMG data points collected for the five fish in each treatment group over each 1-h period examined ranged from 624 to 757. Differences in numbers between time periods were typically due to the number of error signals resulting from unavoidable transmitted signal collisions.

3.4.1. Routine activity

Routine activity levels were significantly different between fed, fasted and fed/stressed fish ($\chi^2 = 190.13$, $df = 2$, $p < 0.001$; medians and interquartile range: fasted = 4, 4; fed = 2, 3; fed/stressed = 3, 4) as illustrated by plots of the raw EMG data in Fig. 2a–c. Unlike the satiation fed groups, the fasted group exhibited periodic bursts of activity greater than their maximum aerobic swimming speed (i.e. U_{crit}), even doubling U_{crit} on some occasions (Fig. 2a). The high activity levels were not reported to come from any single fish, but were noted in all fish in this group. In addition, the overall level of activity in the fasted treatment was higher than that of the fed treatment. Furthermore, the fed/stressed group had routine activity levels between those of the fasted and fed group, and although fish

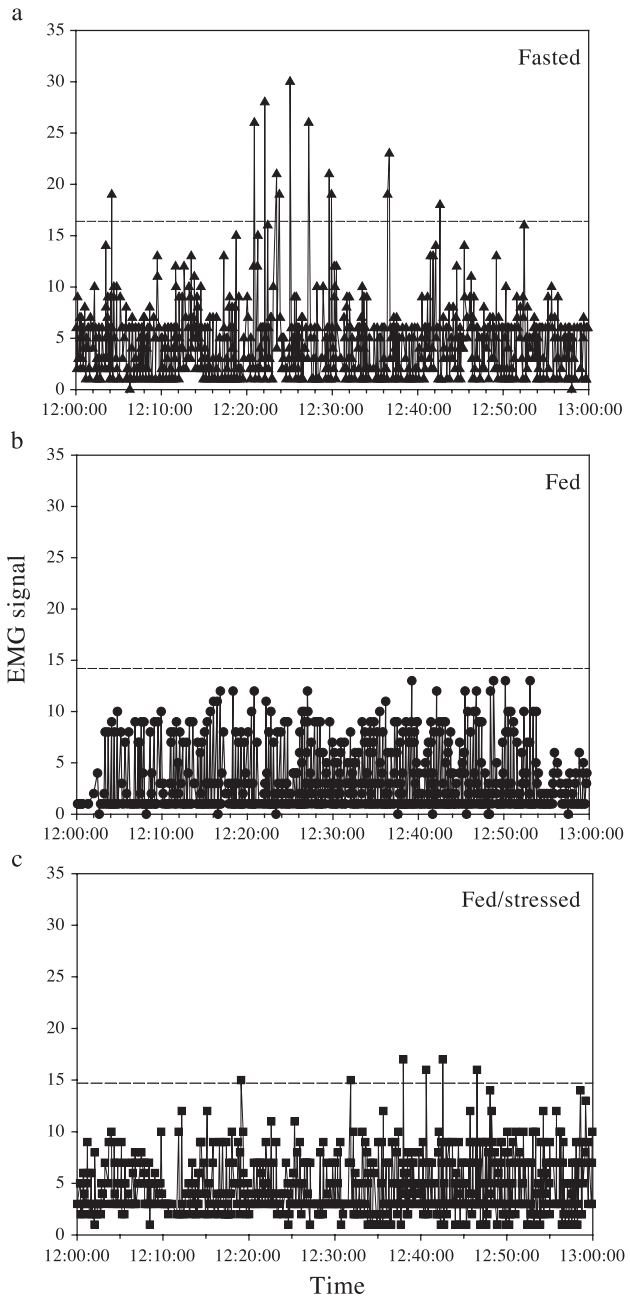

Fig. 2. Representative EMG profiles collected over a 1-h time interval from 12 nn to 1 pm, on day 6 of a fasting week. At this point, the fasted group had not been fed for 6 days, both of the fed groups had been fed at least 5 h earlier, and the fed/stressed group had undergone a crowding exposure 1 and 4 days prior to this point. The dashed lines represent the average critical velocity (or "U_{crit}") for each group.

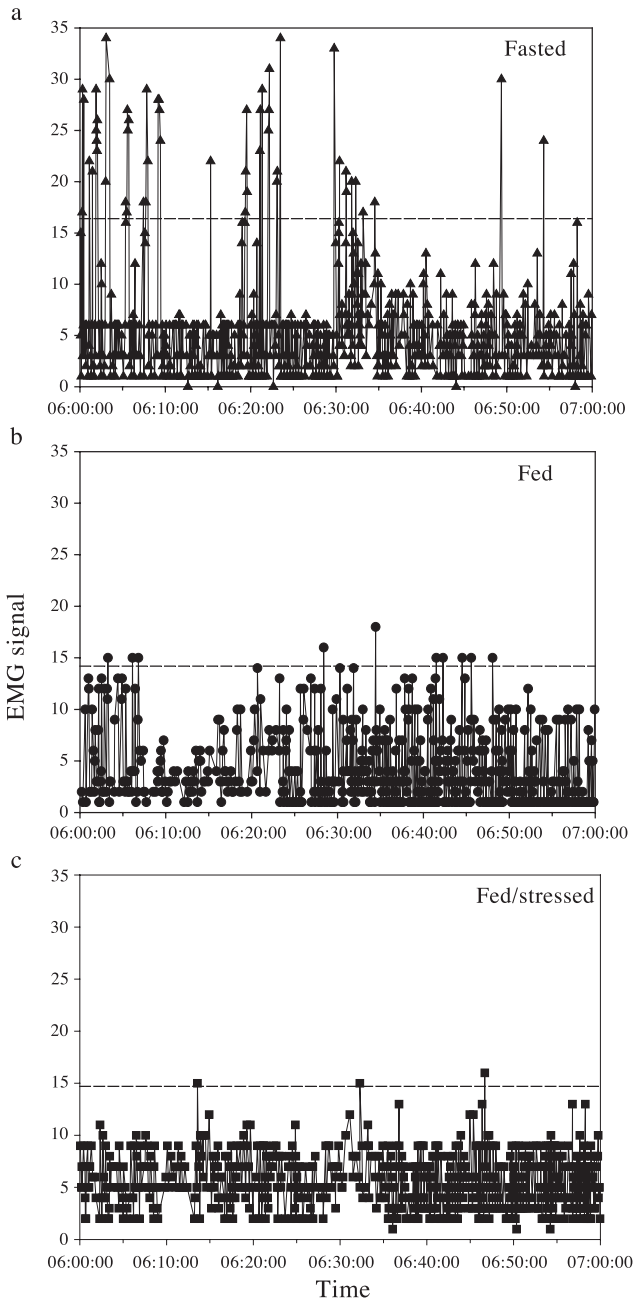

Fig. 3. Representative EMG profiles collected over a 1-h time interval from 6 to 7 am, on day 1 of a feeding week. At this point, the fasted group was being fed for the first time after a 7-day fasting period, while both of the fed groups had been 12 h earlier, and the fed/stressed group had undergone a crowding exposure 3 days prior to this point. The dashed lines represent the average critical velocity (or "U_{crit}") for each group.

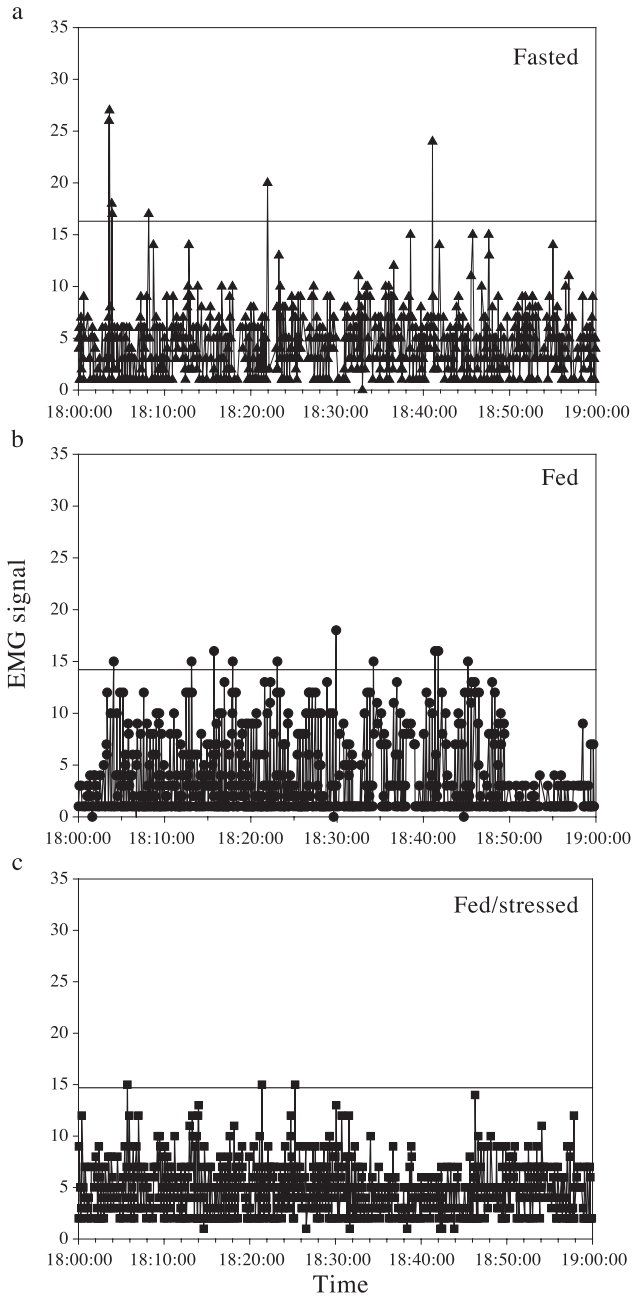

Fig. 4. Representative EMG profiles collected over a 1-h time interval from 6 to 7 pm, on day 4 of a feeding week. At this point, the fasted group had been fed to satiation for 4 days. The fed/stressed group had undergone a crowding exposure 3 days prior to this point. The dashed lines represent the average critical velocity (or "U_{crit}") for each group.

from the stressed group did show some activity greater than U_{crit}, results were less dramatic than in the fasted group (Fig. 2a–c).

3.4.2. During feeding

Similarly, during feeding there were significant differences between the three treatments ($\chi^2 = 140.28$, $df = 2$, $p < 0.001$; medians and interquartile range: fasted = 4, 4; fed = 3, 5; fed/stressed = 5, 4; Fig. 3a–c) Activity levels upon first re-feeding after a fasting period (i.e. day 1 of the feeding week) were intermittently very high and frequent in the fasted group, particularly at the beginning of the feeding period (Fig. 3a). Some of the highest swimming speeds of any fish were noted under these conditions, and were often far greater than the U_{crit}. However, the fish in the fed/stressed treatment exhibited significantly greater

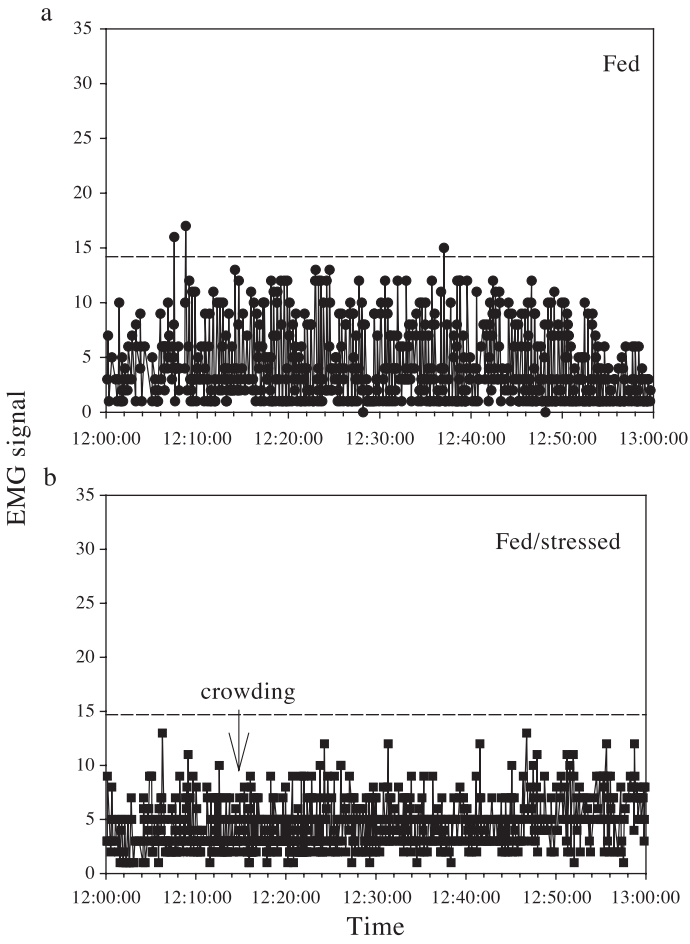

Fig. 5. Representative EMG profiles collected over a 1-h time interval from 12 nn to 1 pm, on a crowding day. The crowding period is indicated on the plot by an arrow, and the duration was approximately 1 min. The dashed lines represent the average critical velocity (or "U_{crit}") for each group.

activity levels overall. Interestingly, the activity level of the fasted fish did not change significantly between fasting and feeding (compare Figs. 2a and 3a), the median and interquartile range of EMG activity were both 4 after 6 days of fasting (Fig. 2a), on the first day of re-feeding (Fig. 3a) and following 4 days of re-feeding (Fig. 4a).

3.4.3. During crowding

The crowding procedure took approximately 1 min to complete, including the draining and refilling of the tank. During this period, fish in the crowded treatment showed a significantly higher EMG activity level than fish in the fed treatment ($U = 192{,}905.5$, $Z = -5.420$, $p < 0.001$; medians and interquartile range: fed = 3, 5; fed/stressed = 4, 3; Fig. 5a,b). In addition, the fish in the fed/stressed treatment exhibited the highest EMG activity levels at all but the first sampling points (see medians quoted above and Figs. 3c to 5c).

4. Discussion

The most significant and novel discovery of this study is that activity levels, as determined by EMG transmitters, can be used to predict feeding status in cultured fish. Significantly different activity levels were found in fasted, satiated and stressed fish, providing insight into relative energetic strategies, and their metabolic consequences. EMG transmitters were found to provide reproducible results, evident from the consistent calibration of signal output to swimming speed for 14 fish under controlled conditions. The transmitters also had no effect on mortality or swimming performance, when implanted for a relatively long experimental period of 10 weeks. This lack of performance-related effect has also been noted in previous telemetry studies (e.g. Kaseloo et al., 1992; Booth et al., 1997; Beddow and McKinley, 1998, 1999). Because the EMG transmitters provide valuable information regarding the condition of fish *in real time*, they become an attractive technology to incorporate into continuous daily monitoring systems for aquaculture operations.

Although EMG implantation itself did not affect growth in a consistent manner, intermittent fasting on a weekly cycle did influence growth over the 10-week period. Periods of starvation or food restriction are known to result in a deviation in the pattern of somatic growth. However, upon re-feeding, a response known as "compensatory growth" occurs, in which fish grow more rapidly than normal and can quickly re-establish a normal growth pattern (Jobling, 1994). This growth theory would suggest that all fish should have been nearly the same size at the end of the final feeding period, yet they were not. Given that the fasted group did exhibit periodic bursts of activity, particularly during fasting weeks, this suggests a higher energy expenditure by fish in the fasted group that was not adequately compensated for during the re-feeding periods.

Swimming performance was not negatively affected by the presence of electrodes within the red muscle band, or by the added weight of the transmitter carried within the body cavity. In this study, relative U_{crit} values were similar to those noted in previous studies on similar sized fish (e.g. 2.8–3.3 BL s^{-1}; Beddow and McKinley, 1998);

however, U_{crit} values were higher than in other studies using longer time intervals between velocity increments (e.g. 1.2–1.5 BL s^{-1}; Farrell et al., 1998). The elevated U_{crit} values in this study are likely an artefact of the 10-min time interval between velocity step, which has been reported to elevate U_{crit} estimates (Farlinger and Beamish, 1977). Regardless, a measure of relative U_{crit} performance between treatment groups provides a rough estimate of relative differences in aerobic capacity (Brauner et al., 1994), thought to reflect maximum O_2 consumption capability (Farrell and Steffensen, 1987). In this study, U_{crit} assessment allowed us to determine the approximate swimming threshold for each group of fish, and to determine that fasted fish appear to spend more time above this threshold than do satiated fish. If anything, the over-estimate of U_{crit} has led to an underestimate of time spent above this threshold by fasted fish.

The number of individuals utilized in this study was dictated by the cost of the equipment used. This resulted in an experimental design which could yield results that are influenced by pseudoreplication (replicating within treatments, but not at the treatment level). However, great care was taken to ensure that the experimental protocol was identical for individuals within all treatments throughout the study. Furthermore, it is highly unlikely that the results demonstrated in the study have arisen solely as a result of differences in tanks with no effect of treatment.

Periodic bursts of activity may be the main determinant of lower growth rate in fasted fish. Burst swimming, which relies on anaerobic pathways, is likely the type of activity noted during the spikes in EMG data. At this point, red muscle is maximally recruited (this occurs at approximately 80% of U_{crit}; Webb, 1971; Taylor et al., 1996), and white muscle must be recruited to some extent to provide the additional thrust. In a previous EMG study by Cooke et al. (2000), routine activity of fish was monitored; however, spikes in EMG data (i.e. burst activity) occurred only when fish were disturbed, and were rarely observed in normal activity records. The authors concluded that it was doubtful that fish incurred any oxygen (O_2) debt, which is known to occur as a result of strenuous activity (Gaesser and Brooks, 1984), under routine conditions. In contrast, in this study, some fish did exhibit regular bursts of activity, even when there was no human access to the quarantine room. Thus, fish are likely accruing periodic oxygen debt, which is metabolically expensive to repay (Scarabello et al., 1991), particularly when there is not a consistent food source. Exacerbating the potential effect of an oxygen debt, fish were held in large tanks with only enough current speed to allow for orientation (i.e. less than 1 BL s^{-1}). Therefore, fish were exposed to recovery conditions that may have actually impeded metabolic recovery, given the observation that recovering fish in current has been shown to enhance recovery rates following high intensity exercise (Milligan et al., 2000).

Two potential hypotheses that may explain why fasted fish are more active than fed fish are that they are actively hunting for food, or alternatively, that they are becoming more aggressive and territorial in the face of a restriction in ration (i.e. transition from a satiation ration to no ration). It is well known that fish become more aggressive on a restricted ration (Davis and Olla, 1987). Furthermore, spontaneous activity associated with agonistic behaviour has been linked to elevated metabolic rates in salmonids (Brett, 1964). Therefore, given that the differences in activity of the fasted fish were reflected by an increase in burst activity, it seems likely that fish were becoming aggressive in response to

reduced ration, since foraging behaviours are typically of a low, sustainable intensity (Beamish, 1978). In this study, fish were often accelerating from very low, maintenance speeds (e.g. an EMG signal of four translates to a swimming speed of about 1 BLs^{-1}) to high intensity bursts in a matter of seconds.

Higher overall metabolic rates may have led to decreased food utilization efficiency (Fagerlund et al., 1981), which would also help explain the lack of growth. For example, if we take an EMG signal of 30, and calculate the swimming speed based on the relation between EMG signal and speed (Fig. 1), this translates to almost 5 BL s^{-1} burst of activity, which is not unrealistic for chasing and eluding chases (Domenici and Blake, 1997). In fact, using respirometer-derived data to calculate the cost of this activity is likely providing an underestimate as it has been shown that the energetic cost of turning and varying acceleration is more costly than swimming uni-directionally within a respirometer (Krohn and Boisclair, 1994).

Fasting intermittently may itself be acting as a stressor due to its effects on interactions between fish. When fish are forced to devote energy to coping with stressors, there will obviously be less energy available for processes such as growth (Schreck, 1982; Barton and Iwama, 1991; Pickering, 1992). However, it is important to consider that a limitation to this type of data collection is that we are not able to record every *single* activity of every tagged fish. Furthermore, only a subsample of all fish are tagged. As the receiver is set to scan each frequency in sequence, while it is scanning one frequency, it is missing activity from the transmitters programmed from other frequencies. In addition, while signals are being transmitted, information cannot simultaneously be collected. Therefore, the energetic expenditure (i.e. number of high intensity activities) of free swimming fish may actually be higher than what has been documented.

Upon initial feeding following a fasting week, the elevation in swimming activity noted in fasted fish suggests voracious feeding, but may also represent enhanced territoriality. However, this behavioural response appears to dissipate somewhat (although not completely) with daily feeding. Together, these observations suggest that the activity levels seen in fasted fish were in fact due to a restriction in ration. With time, fasted fish begin to respond in a manner more similar to fish fed to satiation.

Our attempt to stress fish via crowding had no influence on growth, but did induce obvious changes in swimming behavior. This concurs with results noted by Cooke et al. (2000), who found that higher density did lead to higher routine activity levels. Indeed, individuals that were stressed had a higher activity level than those that were starved in most cases. This may be due to the fact that fish would encounter periods of starvation in the wild and so could have evolved mechanisms to deal with this. However, it is unlikely that wild fish would be exposed to such sudden decreases in water level as the experimental fish experienced in this study. A means by which we can quantify physiological stress level will also become important in the correlation of activity levels to stress levels in future studies.

Intelligent systems technology could eventually be used to enhance existing feeding operations. Currently, there are automated commercial feeding systems in use that incorporate sensors at the bottom of sea cages to detect falling pellets and transmit a signal to the feeder to stop dispensing feed. Smart tag technology would go one step

further, allowing the feeder to be turned off in response to patterns of activity that are indicative of satiated fish.

Conversely, when fish become hungry, the feeder will be turned on in response to "hungry" patterns of activity. In future studies, methods for distinguishing between stressed and non-stressed fish will be developed, thus creating the potential to incorporate the same type of technology into a monitoring system that would alert a producer of the presence of a stressor, such as degrading water quality or a predator. Another area of interest is determining whether other species of salmonids bred for high growth respond to these parameters in similar ways as rainbow trout, and on the development of technologies to assess welfare of smolts immediately following stocking into sea cages.

Acknowledgements

The authors thank Jennifer Wilson and Kris Hunter for their excellent technical assistance, and the staff of the Alma Aquaculture Research Station (especially Michael Burke) for their help in setting up and monitoring the study. This work was supported by NSERC, NCE in Aquaculture: Aquanet funding to RG and RSM and by the Ontario Ministry of Agriculture and Food to RDM. RSM is supported by the Canada Research Chair Program.

References

Anderson, W.G., Booth, R., Beddow, T.A., McKinley, R.S., Finstad, B., Okland, F., Scruton, D., 1998. Remote monitoring of heart rate as a measure of recovery in angled Atlantic salmon, *Salmo salar*. Hydrobiologia 371–372, 233–240.

Baras, E., Lagardere, J.-P., 1995. Fish telemetry in aquaculture: review and perspectives. Aquac. Int. 3, 77–102.

Barnet, C.W., Pankhurst, N.W., 1998. The effects of laboratory husbandry practices on the stress response of greenback flounder *Rhombosolea tapirina* (Günther, 1862). Aquaculture 162, 313–329.

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

Beamish, F.W.H., 1978. Swimming capacity. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. VII. Academic Press, New York, pp. 101–187.

Beddow, T.A., McKinley, R.S., 1998. Effects of thermal environment on electromyographical signals obtained from Atlantic salmon (*Salmo salar* L.) during forced swimming. Hydrobiologia 371/372, 225–232.

Beddow, T.A., McKinley, R.S., 1999. Importance of electrode positioning in biotelemetry studies estimating muscle activity in fish. J. Fish Biol. 54, 819–831.

Booth, R.K., McKinley, R.S., Okland, F., Sisak, M.M., 1997. In situ measurement of swimming performance of wild Atlantic salmon (*Salmo salar*) using radio transmitted electromyograms signals. Aquat. Living Resour. 10, 213–219.

Brauner, C.J., Iwama, G.K., Randall, D.J., 1994. The effect of short-duration seawater exposure on the swimming performance of wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. Can. J. Fish. Aquat. Sci. 51, 2188–2194.

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

Chandroo, K.P., Moccia, R.D., McKinley, R.S., 2000. Utilization of physiological telemetry to monitor behavioural responses of rainbow trout, *Oncorhynchus mykiss*, to captive culture conditions. Bull. - Aquac. Assoc. Can. 99-4, 34–35.

Connors, K.B., Scruton, D.A., Brown, J.A., McKinley, R.S., 2002. The effects of surgically-implanted dummy radio transmitters on the behaviour of wild Atlantic salmon smolts. Hydrobiologia 483, 231–237.

Cooke, S.J., Chandroo, K.P., Beddow, T.A., Moccia, R.D., McKinley, R.S., 2000. Swimming activity and energetic expenditure of captive rainbow trout *Oncorhynchus mykiss* (Walbaum) estimated by electromyogram telemetry. Aquac. Res. 31, 495–505.

Davis, M.W., Olla, B.L., 1987. Aggression and variation in growth of chum salmon (*Oncorhynchus keta*) juveniles in seawater: effects of limited rations. Can. J. Fish. Aquat. Sci. 44, 192–197.

Demers, E., McKinley, R.S., Weatherley, A.H., McQueen, D.J., 1996. Activity patterns of largemouth and smallmouth bass determined with electromyogram biotelemetry. Trans. Am. Fish. Soc. 125, 434–439.

Domenici, P., Blake, R.W., 1997. The kenimatics and performance of fish fast-start swimming. J. Exp. Biol. 200, 1165–1178.

Fagerlund, U.H.M., McBride, J.R., Stone, E.T., 1981. Stress-related effects of hatchery rearing density on coho salmon. Trans. Am. Fish. Soc. 110, 644–649.

Farlinger, S., Beamish, F.W.H., 1977. Effects of time and velocity increments on the critical swimming speed of largemouth bass. Trans. Am. Fish. Soc. 106, 436–439.

Farrell, A.P., Steffensen, J.F., 1987. An analysis of the energetic cost of the branchial and cardiac pumps during sustained swimming. Fish Physiol. Biochem. 4, 73–79.

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

Frisch, A.J., Anderson, T.A., 2000. The response of coral trout (*Plectropomus leopardus*) to capture, handling and transport and shallow water stress. Fish Physiol. Biochem. 23, 23–24.

Gaesser, G.A., Brooks, G.A., 1984. Metabolic bases of exercise post-exercise oxygen consumption: a review. Med. Sci. Sports Exerc. 16, 29–43.

Hinch, S.G., Rand, P.S., 1998. Swim speeds and energy use in upriver-migrating sockeye salmon (*Oncorhynchus nerka*): role of environment and fish characteristics. Can. J. Fish Aquat. Sci. 55, 1821–1831.

Hinch, S.G., Diewert, R.E., Lissimore, T.J., Prince, A.M.J., Healey, M.C., Henderson, M.A., 1996. Use of electromyogram telemetry to assess difficult passage areas for river-migrating adult sockeye salmon. Trans. Am. Fish. Soc. 125, 253–260.

Iwama, G.K., McGeer, J.C., Pawluk, M.P., 1989. The effects of five fish anaesthetics on acid–base balance, hematocrit, blood gases, cortisol and adrenaline in rainbow trout. Can. J. Zool. 67 (8), 2065–2073.

Jobling, M., 1994. Biotic factors and growth performance. Fish Bioenergetics. Chapman and Hall, New York, pp. 170–201.

Johnston, I.A., 1981. Structure and function of fish muscles. Symp. Zool. Soc. Lond. 48, 71–113.

Kaseloo, P.A., Weatherley, A.H., Lotimer, J., Farina, M.D., 1992. A biotelemetry system recording fish activity. J. Fish Biol. 40, 165–179.

Krohn, M., Boisclair, D., 1994. Use of a stereo-video system to estimate the energy expenditure of free-swimming fish. Can. J. Fish. Aquat. Sci. 51, 1119–1127.

Lucas, M.C., Priede, I.G., Armstrong, J.D., Gindy, A.N.Z., De Vera, L., 1991. Direct measurements of metabolism, activity and feeding behaviour of pike, *Esox lucius* L., in the wild, by the use of heart rate telemetry. J. Fish Biol. 39, 245–325.

McKinley, R.S., Power, G., 1992. Measurement of activity and oxygen consumption for adult lake sturgeon (*Acipenser fulvescens*) in the wild using radio-transmitted EMG signals. In: Priede, I.M., Swift, S.M. (Eds.), Wildlife Telemetry: Remote Monitoring and Tracking of Animals. Ellis Horwood, England, pp. 456–465.

Milligan, C.L., Hooke, G.B., Johnson, C., 2000. Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. J. Exp. Biol. 203, 921–926.

Okland, F., Finstad, B., McKinley, R.S., Thorstad, E.B., Booth, R.K., 1997. Radio-transmitted electromyogram signals as indicators of physical activity in Atlantic salmon. J. Fish Biol. 51, 476–488.

Okland, F., Fleming, I., Thorstad, E.B., Finstad, B., Einum, S., McKinley, R.S., 2002. EMG radio tags in recording the spawning behaviour of Atlantic salmon: effects, reliability and accuracy. In: Moore, I., Flemming, I. (Eds.), Proceedings for the Third Conference on Fish Telemetry in Europe, 20–25 June, Norwich, UK. Advances in Fish Telemetry, The Centre for Environment, Fisheries and Aquaculture Science, Suffolk, U.K., pp. 51–58.

Pickering, A.D., 1992. Rainbow trout husbandry: management of the stress response. Aquaculture 100, 125–139.

Robertson, M.J., Scruton, D.A., Brown, J.A., 2003. Effects of surgically implanted transmitters on swimming performance, food consumption and growth of wild Atlantic salmon parr. J. Fish Biol. 62, 673–678.

Rogers, S.C., Weatherley, A.H., 1983. The use of opercular muscle electromyography as an indicator of the metabolic cost of fish activity in rainbow trout, *Salmo gairdneri* Richardson, as determined by radiotelemetry. J. Fish Biol. 23, 535–547.

Ross, L.G., Watts, W., Young, A.H., 1981. An ultrasonic biotelemetry system for the continuous monitoring of tail-beat rate from free-swimming fish. J. Fish Biol. 18, 479–490.

Ruane, N.M., Carballo, E.C., Komen, J., 2002. Increased stocking density influences the acute physiological response of the common carp *Cyprinus carpio* (L.). Aquac. Res. 33, 777–784.

Scarabello, M., Heigenhauser, G.J.F., Wood, C.M., 1991. Glycogen depletion in juvenile rainbow trout as an experimental test of the oxygen debt hypothesis. Can. J. Zool. 69, 2562–2568.

Schreck, C.B., 1982. Stress and rearing of salmonids. Aquaculture 28, 241–249.

Sullivan, G.H., Hoefener, C., Bolie, V.W., 1963. Electronic systems for biological telemetry. In: Slater, L.E. (Ed.), Bio-Telemetry: The Use of Telemetry in Animal Behaviour and Physiology in Relation to Ecological Problems. Permagon Press, London, pp. 83–106.

Taylor, S.E., Egginton, S., Taylor, E.W., 1996. Seasonal temperature acclimatisation of rainbow trout: cardiovascular and morphometric influences on maximal sustainable exercise level. J. Exp. Biol. 199, 835–845.

Thorstad, E.B., Okland, F., Koed, A., McKinley, R.S., 2000. Radio-transmitted electromyogram signals as indicators of swimming speed in lake trout and brown trout. J. Fish Biol. 57, 547–561.

Weatherley, A.H., Rogers, S.C., Pincock, D.G., Patch, J.R., 1982. Oxygen consumption of active rainbow trout, *Salmo gairdneri* Richardson, derived from electromyograms obtained by radiotelemetry. J. Fish Biol. 20, 479–489.

Weatherley, A.H., Daseloo, P.A., Gare, M.D., Gunn, J.M., Lipicnik, B., 1996. Field activity of lake trout during the reproductive period monitored by electromyogram radiotelemetry. J. Fish Biol. 48, 675–685.

Webb, P.W., 1971. The swimming energetics of trout: I. Thrust and power output at cruising speeds. J. Exp. Biol. 55, 489–520.