

# Swimming activity and energetic expenditure of captive rainbow trout *Oncorhynchus mykiss* (Walbaum) estimated by electromyogram telemetry

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## Abstract

Rainbow trout *Oncorhynchus mykiss* (Walbaum) are usually cultured at high densities to maximize production, but little is known about the physiological and behavioural consequences of high-density fish culture. The purpose of this study was to develop quantitative correlates of activity for fish held under conditions of increasing density. Fifteen hatchery-reared rainbow trout (mean fork length =  $432.3 \pm 9.2$  mm) were implanted with activity (electromyogram; EMGi) transmitters and randomly assigned to each of three replicate tanks. Original tank densities ( $15 \text{ kg m}^{-3}$ ) were then increased to 30 and finally to  $60 \text{ kg m}^{-3}$  at weekly intervals by adding additional fish. Remote telemetry signals indicated that activity increased with increasing stocking density. Fish were relatively inactive during the middle of the day, with diel activity patterns not differing among treatments. Fish were more active during periods of darkness, with activity increasing with increasing stocking density. Relationships between swimming speed, EMGi activity and oxygen consumption were developed using a respirometer and used to estimate oxygen consumption of the fish in the density treatments. Average oxygen consumption estimates increased with increasing density treatments as follows: low density =  $75.6 \text{ mg kg}^{-1} \text{ h}^{-1}$ ; medium density =  $90.0 \text{ mg kg}^{-1} \text{ h}^{-1}$ ; and high density =  $102.6 \text{ mg kg}^{-1} \text{ h}^{-1}$ . Telemetry permits quantifi-

cation of the effects of increasing density on fish activity. Physiological telemetry devices may provide a useful tool for remotely monitoring animal welfare correlates under controlled conditions for fish exposed to different husbandry conditions and may prove a valuable tool for the aquaculture industry.

## Introduction

Correct husbandry practices are important because they reduce physiological stress, which may exacerbate disease and repress growth (Pickering 1992). High stocking densities may have an adverse effect on growth of rainbow trout *Oncorhynchus mykiss* (Walbaum) (Refstie 1977; Trzebiatowski, Filipiak & Jakubowski 1981; Papoutsoglou, Papaparaskeva-Papoutsoglou & Alexis 1987; Baker & Ayles 1990; Holm, Refstie & Bo 1990; Alanärä 1996). However, Kebus, Collins, Brownfield, Amundson, Kayes & Malison (1992) reared rainbow trout at high density without significant adverse effects on growth, and they attributed the contradictory findings to water quality deterioration at high stocking densities in other studies.

The negative effects of crowding on salmonids have been attributed to increased 'stress' (Wedemeyer 1976; Vijayan & Leatherland 1988), but this has been difficult to quantify. Fish respond to stress with a series of defence mechanisms that

are generally energy demanding and, thus, costly in terms of metabolic resources (Barton & Iwama 1991). High stocking densities may have effects on energy partitioning, promoting gluconeogenesis and mobilization of triglycerides, but have little effect on protein metabolism (Vijayan, Ballantyne & Leatherland 1990).

Direct assessments of the activity levels of free-swimming fish are difficult to obtain (Beamish 1978; Scherer 1992; Tang & Boisclair 1993). However, the development of telemetric techniques has allowed activity of fish to be studied *in situ* (Lucas, Johnstone & Priede 1993). One of the best indicators of activity levels are integrated electromyograms (EMGi's) from axial musculature (Weatherley, Rogers, Pincock & Patch 1982; Weatherley & Gill 1987; Kaseloo, Weatherley, Lotimer & Farina 1992). EMGi activity is proportional to locomotory activity and can also be correlated to oxygen consumption in a respirometer. The relationships between swim speed and EMGi activity and oxygen consumption may be used to obtain estimates of the metabolic rates of free-swimming animals (Weatherley *et al.* 1982; McKinley & Power 1992; Briggs & Post 1997a,b). Despite the numerous possible applications of this technology to the aquaculture industry and related research, there are still limited examples in the scientific literature (see reviews by Holand 1987; Baras & Lagardère 1995).

The purpose of this study was to examine differences in locomotory muscle activity among hatchery-reared rainbow trout exposed to increasing tank biodensity, using axial muscle electromyogram signals. This novel use of EMGi telemetry may provide the basis for future studies of stress response and metabolic expenditures in fish culture facilities.

## Materials and methods

### Telemetry equipment

The transmitters (which are described in detail by Kaseloo *et al.* 1992 and Beddow & McKinley 1998) consisted of an epoxy-coated transmitter package with a pair of electrodes and a single antenna (EMGi, Lotek Engineering, Newmarket, Ontario, Canada). The electrodes (9 k gold,  $1 \times 7$  mm) detect electropotentials within the axial red muscle, and the pulse emitted from the transmitter provides information on integrated electrical activity (EMGi). The signal recorded by the receiver is an EMGi pulse

interval (ms), which is inversely related to muscular activity. Signals were detected and recorded automatically using an SRX\_400 radio receiver with W/20 software (Lotek Engineering). Data was downloaded to a computer as the storage banks approached capacity. The transmitters were 51 mm in length and 13 mm in diameter, and weighed 18.0 g in air. The transmitter weight was less than 2.2% of the weight of the fish in air.

### Surgical procedure

Rainbow trout were removed from a common stock tank, anaesthetized in an aerated solution of clove oil and ethanol ( $50 \text{ mg L}^{-1}$ ) (Anderson, McKinley & Colavecchia 1997; Keene, Noakes, Moccia & Soto 1998), weighed ( $\pm 1$  g), fork length recorded (mm) and a passive integrated transponder was inserted into the adipose tissue just posterior to the base of the dorsal fin. The fish were then placed ventral side up in a foam, V-shaped surgical table. During surgery, the gills were irrigated with temperature-regulated ( $8.5^\circ\text{C}$ ), oxygenated water containing clove oil and ethanol ( $30 \text{ mg L}^{-1}$ ).

A 3-cm incision was made in the ventral abdominal wall just posterior to the pelvic girdle, and the transmitter was inserted through the incision and pushed anteriorly into the body cavity. The antenna wire was wound in a small coil and also inserted into the coelom. The electrodes were inserted into the red axial musculature below the lateral line using 16.5-gauge rods (McKinley & Power 1992), electrode placement being standardized at the anterior portion of the dorsal fin (Beddow & McKinley 1999). Once in place, a plunger was used to secure the electrodes, and the rods were removed. The incision was closed using four braided silk sutures (2/0 Ethicon) and, before the last suture, tetracycline was injected intraperitoneally ( $1 \text{ mg antibiotic kg}^{-1}$  fish). The entire procedure took less than 5 min to complete, and fish recovered quickly when returned to fresh, oxygenated water.

### Increasing density experiment

Rainbow trout were held in three  $2 \times 2$  m fibreglass tanks at a density of  $7.5 \text{ kg m}^{-3}$ , and five fish from each tank were implanted with EMGi transmitters. Fish were allowed to recover for 7 days. Additional fish that had also been held at  $7.5 \text{ kg m}^{-3}$  were then introduced until densities of  $15 \text{ kg m}^{-3}$  had been

achieved. Simultaneously, other groups of fish were held in supply tanks at  $15 \text{ kg m}^{-3}$ . Seven days later, these fish were added to the experimental tanks to reach a density of  $30 \text{ kg m}^{-3}$ . Supply tanks were also stocked at  $30 \text{ kg m}^{-3}$ , and the final density ( $60 \text{ kg m}^{-3}$ ) was achieved by adding these fish to the experimental tanks 7 days later. During the experiments, fish were fed a maintenance ration of 6 Pt. commercial grower, floating trout food (Martin Feed Mills, Elmira, Ontario, Canada) from a belt feeder, which was distributed into tanks over 1 h.

Inflowing water had a temperature of  $8.5 \pm 0.1$  °C, dissolved oxygen of  $10.6 \text{ mg L}^{-1}$  and pH of  $7.6 \pm 0.1$ . The flows were set to  $50 \text{ L min}^{-1}$ , yielding an approximate tank turnover rate of three times per hour. Photoperiod was controlled and set to natural conditions for southern Ontario, Canada. Surgery was conducted on 20 November 1997, when sunrise was at 07:29 and sunset was 17:06 (11.5 h light:12.5 h dark). Lights were phased in and out over a 45-min period, beginning at sunrise and at sunset.

The recording of EMGi signals was started after the stocking density in the experimental tanks had reached  $15 \text{ kg m}^{-3}$ . Signals were then collected hourly throughout the 21-day study, except during times when the system was offline for downloading of data. Thirty sequential EMGi signals formed one 'record', of which there were 15 records collected in 1 h. After the receiver had completed scanning and recording data from the 15 transmitters, the receiver went offline and did not log signals until the next hour.

Experimental fish were held in a room with controlled access to minimize disturbance from human interaction. All entry and exits by personnel were recorded, but visits were limited to routine feeding activities and receiver downloadings. Water levels and flow rates were kept constant between tanks. At the end of the experiment, fish with transmitters were euthanized and post-mortem dissections were conducted to determine exact electrode placement.

#### **Relationships between muscle activity, swimming speed and oxygen consumption**

Calibrations were performed in a Blazka-type respirometer as described by Booth, McKinley, Økland & Sisak (1997) and pictured by Thorstad, Finstad, Økland, McKinley & Booth (1997). Fish were allowed to recover for at least 48 h after

surgery and were then loaded into the swim chamber and allowed to acclimate for 30 min at a swimming velocity of 1 body length (BL)  $\text{s}^{-1}$ . Fish were not fed for 48 h before surgery or between surgery and the respirometry trials. Fish swam in a post-absorptive state that did not incorporate the assimilative costs of food digestion. Water velocity was increased in a stepwise progression, with 15-min intervals and a  $0.2\text{-m s}^{-1}$  velocity increment. The swim chamber was operated on flowthrough for the first 3 min of each velocity increment. The water supply was then closed, and initial oxygen measurements were made (digital oxygen probe; Orion). After 10 min, final oxygen measurements were made, and the water flow resumed for the remainder of the 15-min time interval to allow oxygen to be replenished. Oxygen measurements were recorded at each successive velocity increment until fatigue was observed. EMGi signals were also recorded for each fish at each velocity. Fatigue was considered to have occurred when the fish could no longer maintain position in the chamber and was forced against the blocking screen twice.

#### **Statistical analysis**

EMGi signals were standardized for each fish in relation to the resting EMGi activity recorded while stationary. These recordings were obtained in the respirometer before exposing the fish to forced exercise. Activity was then assessed as the percentage increase from resting, and this measure of muscle activity is termed the 'activity index'.

The relationships between the muscle activity index and swimming speed, and between swimming speed and oxygen consumption, were generated using linear regression. These data were then used to develop an equation to describe the relationship between activity index and oxygen consumption.

Before pooling data, we tested for differences in fork length, weight and activity between tanks for each of the three treatments. Differences in activity between density treatments were tested on a weekly, daily and hourly basis using ANOVA (Systat Version 6) and, when differences were recorded, the Tukey–Kraemer HSD test was used to identify where specific differences occurred (Day & Quinn 1989). Sign tests were used to test for differences in activity during times of light (09:00–16:00) and darkness (18:00–07:00) for each of the densities. Activity recording during the periods when light was being phased in and out was omitted. The Kolmogorov–

Smirnov test was used to examine the weekly distribution of the activity index against the mean for each density treatment. All statistical tests were performed at an alpha of 0.05.

### Animal care

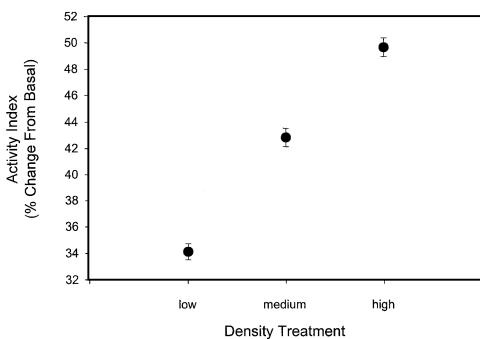
This study was conducted under the approval of the Animal Care Committee of the University of Guelph under the protocols of the Canadian Council on Animal Care 98R097.

### Results

Data from three of the 15 fish implanted with electromyogram transmitters were excluded because of technical problems and, for the remaining fish ( $n=12$ ), there were no differences in the fork length (mean fork length  $\pm$  SEM =  $432 \pm 9$  mm) or weight (mean weight  $\pm$  SEM =  $1204 \pm 87$  g) among tanks. All tagged fish survived the study. Post-mortem examination confirmed that electrodes were within 10 mm of each other in the axial red musculature, but were not touching. Recordings for the first day of each density treatment were eliminated because the receiver was offline at different times.

Activity increased as stocking density increased (Fig. 1), mean activity level being significantly different ( $P < 0.05$ ) between all three treatments. Within treatments, there were changes in mean levels of daily activity over time, but no clear trends were observed (Fig. 2).

Diel changes were also observed (Fig. 3), with hourly activity patterns over the study being



**Figure 1** Comparison of average activity indices recorded during each of the three density treatments: low ( $15 \text{ kg m}^{-3}$ ); medium ( $30 \text{ kg m}^{-3}$ ); and high ( $60 \text{ kg m}^{-3}$ ). Data are given as means ( $\pm$  SEM).

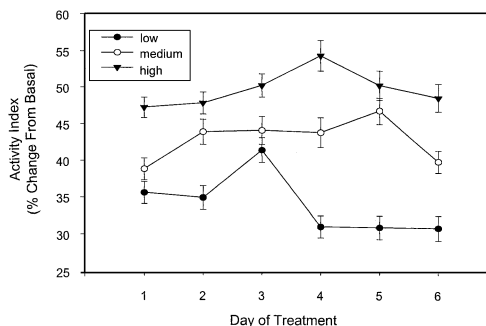
distinct and significantly different ( $P < 0.05$ ). Between 08:00 h and 17:00 h, EMGi activity was highly variable, with no significant differences observed between any of the three treatments. From 17:00 h until 07:00 h, there were differences in the activity levels among treatments. The fish were more active when lights were off than when lights were on ( $P < 0.05$ ), irrespective of treatment.

### Oxygen consumption

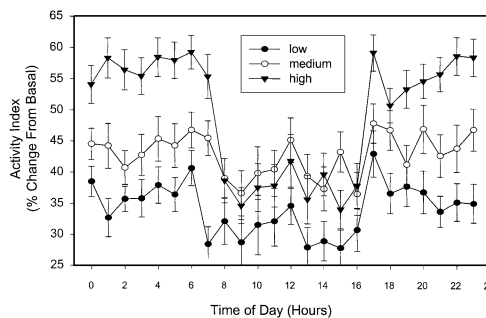
The muscle activity index was correlated with swimming speed, and the pooled data could be described by the following equation:

$$\text{Muscle activity index} = 90.7542 (\text{swimming speed}) + 17.345, r^2 = 0.978,$$

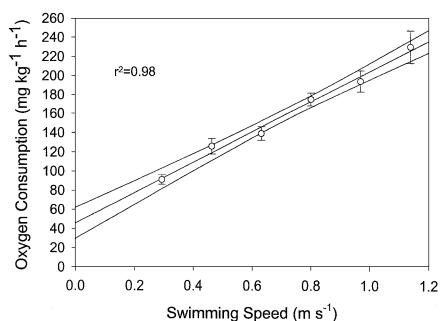
where muscle activity is the percentage increase from resting, and swimming speed is in  $\text{m s}^{-1}$ .



**Figure 2** Comparison of average percentage increase in activity from basal during each day of each of three consecutive density treatments: low ( $15 \text{ kg m}^{-3}$ ); medium ( $30 \text{ kg m}^{-3}$ ); and high ( $60 \text{ kg m}^{-3}$ ). Data are given as means ( $\pm$  SEM).



**Figure 3** Comparison of average percentage increase in activity from basal on an hourly basis for each weekly density treatment: low ( $15 \text{ kg m}^{-3}$ ); medium ( $30 \text{ kg m}^{-3}$ ); and high ( $60 \text{ kg m}^{-3}$ ). Data are given as means ( $\pm$  SEM).



**Figure 4** Oxygen consumption during forced swimming trials of rainbow trout ( $n = 12$  fish at each of six speeds). Confidence intervals are plotted at the 95% limits.

Oxygen consumption was also positively correlated with swimming speed (Fig.4) and was described by the equation:

$$\text{Oxygen consumption} = 158.445 (\text{swimming speed}) + 46.032, r^2 = 0.988,$$

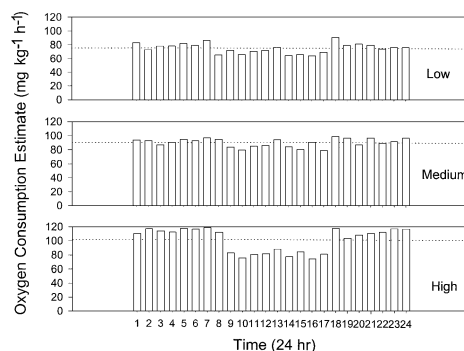
where oxygen consumption is in  $\text{mg O}_2 \text{kg}^{-1} \text{h}^{-1}$ , and swimming speed is in  $\text{m s}^{-1}$ .

The relationship between oxygen consumption and the muscle activity index could be described by the following equation:

$$\text{Oxygen consumption} = 1.738 (\text{muscle activity index}) + 16.115,$$

where oxygen consumption is in  $\text{mg O}_2 \text{kg}^{-1} \text{h}^{-1}$ , and muscle activity is the percentage increase from resting.

Thus, we were able to estimate the *in situ* oxygen consumption of the rainbow trout implanted with EMGi transmitters during routine activity while exposed to increasing density treatments. Oxygen consumption estimates based upon the average activity level at each density increased with increasing density. Our calculations yielded the following estimates of oxygen consumption: low density,  $75.6 \text{ mg kg}^{-1} \text{ h}^{-1}$ ; medium density,  $90.0 \text{ mg kg}^{-1} \text{ h}^{-1}$ ; high density,  $102.6 \text{ mg kg}^{-1} \text{ h}^{-1}$ . On a daily basis, the total oxygen consumption was estimated to be  $1814 \text{ mg kg}^{-1} \text{ h}^{-1}$  for the lowest density,  $2160 \text{ mg kg}^{-1} \text{ h}^{-1}$  for the medium density and  $2462 \text{ mg kg}^{-1} \text{ h}^{-1}$  for the highest density. Oxygen consumption estimates exhibited similar diel patterns to those observed for activity (Fig. 5).



**Figure 5** Comparison of daily patterns in oxygen consumption based upon estimates of EMGi activity. Mean hourly oxygen consumption for the 24-h period is represented by a horizontal dotted line.

### Discussion

Conditions reproduced in this study, whereby the density tolerance of rainbow trout were approached and exceeded, showed increased levels of locomotory activity and corresponding increases in oxygen consumption. These factors are important concerns in intensive aquaculture facilities (Wedemeyer 1997). Previously, Baker & Ayles (1990) and Miller, Wagner & Bosakowski (1995) also found that oxygen consumption was positively correlated with increasing density in rainbow trout. Low dissolved oxygen concentrations are known to reduce rainbow trout tolerance to ammonia (Thurston, Phillips, Russo & Hinkins 1981) and increase the probability of disease (Snieszko 1974). Generally, the increased activity that may occur at higher densities also results in higher oxygen consumption rates and an increased excretion of ammonia, which can be toxic (Linton, Reid & Wood 1997). In our study, however, the water flow and subsequent turnover rate was sufficient to preclude the effects of ammonia or low dissolved oxygen.

High densities, increased activity levels, lower dissolved oxygen and increased ammonia levels may contribute to an increase in the level of stress and to a reduction in feeding activity by rainbow trout (Smart 1981). Some studies, however, have suggested that the food intake and feeding behaviour of rainbow trout are not altered by density, indicating that reduced growth rate may not be solely attributable to a reduction in food intake (Wedemeyer 1976). Another reason for the reduced growth rate of fish under high-density conditions may be the increased energy expenditure associated

with elevated swimming activity (Niimi & Beamish 1974) and behavioural interactions (Fenderson & Carpenter 1971; Symons 1971; Li & Brocksen 1977; Holm *et al.* 1990; Alanära & Brannas 1996). This study observed considerable changes in locomotory activity occurring with increasing density, with fish held at high densities swimming on average  $\approx 60\%$  faster than those held at low densities.

Behavioural measures may be sensitive indicators of the complex biochemical and physiological changes that occur in response to stress (Schreck 1990; Scherer 1992; Schreck, Olla & Davis 1997). Changes in behaviour or activity can be adaptive, such as avoidance manoeuvres, which mitigate exposure to stressors. However, if such mitigation is not possible, induced changes in behaviour may reflect deleterious changes in how an animal senses, and responds to, its environment (Schreck *et al.* 1997). To date, these behavioural changes have been difficult to quantify (Scherer 1992). This study provides further quantitative evidence that increased stocking density and associated disturbances may influence swimming activity and, therefore, the metabolic expenditure of rainbow trout under hatchery conditions.

### Activity patterns

Activity was examined on a weekly basis (for each treatment) to see whether there was any acclimation effect, and also on a diel basis, to determine whether there were diurnal patterns of activity. No real trends in activity were observed over each 6-day treatment period, suggesting that fish either acclimated very quickly to the new treatment environment or that the time period was too short to assess the acclimatory phase. Longer term assessments might reveal different patterns of activity if fish are left to adjust for extended periods of time.

Activity patterns were also assessed on a diel basis: there was heightened nocturnal activity and reduced activity during the photophase coinciding with the 11.5:12.5 photoperiod conditions to which the fish were exposed. Circadian rhythms in locomotor activity have been observed for many species, e.g. white sucker *Catostomus commersoni* (Lacépède) (Kavaliers 1980), catfish *Silurus asotus* (L.) (Tabata, Minh-Nyo, Niwa & Oguri 1989) and goldfish *Carassius auratus* (L.) (Iigo & Tabata 1996), including rainbow trout (Mork & Gulbrandsen

1994; Iigo & Tabata 1997; Sanchez-Vazquez & Tabata 1998). Feeding of rainbow trout may be nocturnal when water temperatures are low and diurnal during warmer temperatures (Landless 1976). Water temperature in our study was  $8.5 \pm 0.1$  °C, and this may explain the nocturnal activity pattern. It is possible that the maintenance feeding regime and timing of feeding (daylight) may also have influenced the activity patterns that we observed.

Several obvious differences were observed when we compared the different density treatments with reference to diel activity patterns. Activity levels of fish exposed to increasing densities were significantly different during the dark phase, during which time fish in the highest density treatment were the most active, and fish in the lowest density treatment were the least active. This marked difference in nocturnal activity patterns between density groups probably occurred as a result of the fact that the fish are generally more active during the night and less active during the photophase, when activities of all density groups were consistently low.

During the photophase, all fish swam at speeds around  $20\text{--}25\text{ cm s}^{-1}$  or 0.5 body lengths. This swimming activity was generally a rheotactic response to the mild flows created from the inflowing water. These speeds are generally regarded as low and are categorized as sustained swimming activity that can be maintained indefinitely (Beamish 1978). During periods of heightened nocturnal activity, rainbow trout generally doubled their swimming speeds ( $\approx$  one body length  $\text{s}^{-1}$ ) compared with that observed during the day. Although this speed is still within the category of sustained swimming, this increase in activity also results in an additional energetic expenditure (Beamish 1978).

### Oxygen consumption and energy expenditure

It should be noted that the estimates of oxygen consumption obtained in this study represent minimum values. Spontaneous swimming activity (including turning and variable acceleration rates) is more energetically costly than unidirectional swimming in a respirometer (Krohn & Boisclair 1994; Tang & Boisclair 1995). Also, the fish exposed to forced swim trials in our study were in a post-absorptive state. This is the standard protocol for fish respirometry trials (Beamish 1978), but under normal hatchery conditions, fish would be fed

frequently to maximize production and would therefore rarely be in a post-absorptive state. Respiration rates of fish that are processing/digesting food would be higher than those of fish in the respirometer or those fed a maintenance ration (Beamish 1964; Alsop & Wood 1997), hence magnifying the underestimates of oxygen consumption determined by our study. A recent study (Alsop & Wood 1997) documented the effects of feeding on rainbow trout oxygen consumption during critical swimming trials and routine activity. Fish fed to satiation had elevated oxygen consumption during both measures over fasted fish, with those fed maintenance diets having intermediate oxygen consumption. Another potential source of error is that fish oxygen consumption can increase without any change in locomotory activity in response to alterations in cardiac activity induced by stress (Lucas 1994). Nonetheless, this approach is still robust enough to provide estimates of *in situ* activity in fish under different density conditions.

Our estimates of oxygen consumption show increases at higher densities, suggesting that energetic expenditures were lowest at the lowest density. Similar trends in oxygen consumption were found during crowding density experiments on Arctic charr maintained at 15 and 60 kg m<sup>-3</sup>; however, the higher oxygen consumption among high-density fish may be related to higher food intake and growth rates compared with the low-density fish (Jørgensen, Christiansen & Jobling 1993). Several other researchers have demonstrated that there is a metabolic cost and resultant increase in oxygen consumption for fish subjected to physical disturbances (e.g. Barton & Schreck 1987; Davis & Schreck 1997).

The effects of crowding and the associated energy expenditure can be viewed in the context of the organisms' aerobic 'scope for activity' (Fry 1947). When fish must devote a portion of their energy budget to coping with a 'stressor', such as crowding, presumably less energy will be available for other processes (Schreck 1982; Barton & Iwama 1991) and, in particular, growth (Pickering 1992).

Field studies using EMGi telemetry to monitor rainbow trout have reported regular diel patterns of activity that are both crepuscular and diurnal (Briggs & Post 1997a,b). Our activity patterns derived from EMGi telemetry are similar to both field studies using EMGi, and laboratory studies employing other techniques. Weatherley *et al.* (1982) were the first to estimate oxygen consump-

tion from electromyogram records. They reported good correlation coefficients between oxygen consumption and epaxial white musculature activity, although they did observe a difference between slopes of regression obtained from spontaneous activity and forced swimming.

In our study, electrodes were implanted in the red musculature, thereby primarily monitoring aerobic activity. Beddow & McKinley (1999) observed minimal activity in white fibres at low swimming speeds, whereas red musculature activity increased steadily with increasing swimming speeds until over 80% U-crit. For these reasons, and owing to the fact that our fish were always active, either swimming in place or moving about, we were able to use the relationship between oxygen consumption and EMGi activity derived in the respirometer to estimate the oxygen consumption of free-swimming fish. We did not have to adjust oxygen consumption estimates, as was suggested by Weatherley *et al.* (1982), when implanting electrodes in anaerobic white musculature.

Spontaneous activity associated with agonistic behaviour has been shown to cause elevated metabolic rates in juvenile salmonids (Brett 1964), which may also lead to decreased food utilization efficiency (Fagerlund, McBride & Stone 1981). Estimates of oxygen consumption obtained during this study can only provide minimum estimates of the energetic expenditures of the fish that we monitored. Christiansen, Jørgensen & Jobling (1991) report that Arctic charr under social stress had oxygen consumption rates similar to those forced to swim at moderate speeds.

Swimming levels in the density study were almost always at low speeds, including sustained and prolonged activity (as discussed above). Only in the respirometer as the fish approached exhaustion at high water velocities of  $\approx 1 \text{ m s}^{-1}$  was any consistent bursting activity observed. These activity levels were within the aerobic scope and are usually supported by red muscle. Burst swimming, which primarily uses anaerobic pathways in white musculature, was rarely observed in fish in our study (except during calibrations). Several small bursts were usually elicited during tank disturbances, which are revealed as spikes in EMGi activity. These activity spikes were rare in normal activity records. It is therefore unlikely that these fish ever incurred an oxygen debt, which is known to occur after strenuous activities (Gaesser & Brooks 1984; Scarabello, Heigenhauser & Wood 1991).

There are conflicting reports in the literature regarding the relationship between stocking density and oxygen consumption in aquaculture facilities determined using influent and effluent monitoring. In this study, over a 24-h period, fish at the highest density consumed an estimated 26% more oxygen than those at the lowest density, and 12% more than those at the medium density. Fish at the medium density consumed 17% more oxygen than those at the lowest density. Miller *et al.* (1995) and this study both report positive correlations between stocking density and oxygen consumption rates. Conversely, Kindschi, Smith & Koby (1991) report no density-dependent differences in oxygen consumption. Miller *et al.* (1995) reported that, in several sampling periods, dissolved oxygen consumption was comparable between low- and high-density treatments. Our results would support this conclusion, in that oxygen consumption is directly related to physical activity, and distinct patterns of such activity exist on a diel basis. During the day, oxygen consumption values are comparable, whereas diurnally, oxygen consumption increases as a result of heightened physical activity. Therefore, depending upon when oxygen consumption is measured, different results may be obtained. Kindschi *et al.* (1991) and Miller *et al.* (1995) obtained their oxygen readings during 15:00–16:00 h and 14:00–16:00 h, respectively, which would have underestimated overall oxygen consumption if the fish were exhibiting the same diel activity patterns as in this study. Also, considering that salmonids apparently alter diel activity based upon water temperature, this must also be considered as a cofactor when attempting to draw comparisons between studies. Similarly, the fish in the aforementioned studies were fed more than in our study, potentially magnifying such effects.

Our results also suggest that future studies of oxygen consumption should include numerous sampling intervals throughout the 24-h period in order better to reflect variability in physical activity. Given that oxygen consumption is strongly correlated with physical activity, such measures would more accurately represent the temporally variable energetic costs. This will be particularly useful if future EMGi studies attempt to integrate food processing/digestion costs and the energetics of non-linear swimming activity. As such, our results also provide insights and additional interpretations into previously published studies on oxygen con-

sumption (i.e. Kindschi *et al.* 1991; Miller *et al.* 1995).

## Conclusion

This study indicates that, overall, higher densities and the associated sequential disturbances yielded an increase in muscle activity levels, resulting in a concomitant increase in oxygen consumption. In addition, our study has provided some insight into the activity patterns of fish in captive facilities, which showed a period of heightened nocturnal activity, apparently mediated by temperature. The activity patterns observed in our study could also differ if fish were fed under conditions more similar to commercial aquacultural practice. Here, fish were held in a controlled access area, under a controlled photoperiod regime and were fed with belt feeders. The challenge for fish culturists is to identify and provide rearing conditions under which behaviours such as aggression or social dominance are no longer effective in acquiring space, favourable water quality, food and other resources (Wedemeyer 1997). This novel use of physiological telemetry provides one of the first quantitative measures of free-swimming fish activity in aquaculture facilities. As mentioned throughout the discussion, however, there are still many deficiencies with this methodology. Further studies are required that integrate several observation methods in an attempt to link behaviour and physiology. Future studies using this technology could provide additional information, which could help to minimize stress in aquaculture facilities, leading to increased production yields and improved animal welfare conditions if the deficiencies outlined above can be remedied. Some of the limitations in experimental design observed in this study will probably be ameliorated in the future as this type of technology becomes more widely available and, hence, more affordable.

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