Temperature Effects on Recovery of Pacific Cod Following Exhaustive Exercise

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Abstract

We examined post-exhaustive exercise recovery in Pacific cod acclimated to either 2°C or 7°C. We collected blood samples prior to an exhaustive swimming protocol, immediately after and up to four hours of recovery. Plasma concentrations of cortisol, glucose, lactate, Na⁺, and Cl⁻ were determined. Concentrations of cortisol, glucose, and lactate significantly increased post-swim as compared to samples collected pre-swim irrespective of temperature treatment. For either temperature treatment, sample concentrations of cortisol, glucose, and lactate of cod remained elevated and did not return to pre-swim levels after four hours of recovery.

Introduction

Swimming performance is a critical aspect of the natural history of fish because it relates to migration, predator avoidance, and prey capture

(Beamish 1978) and many studies have sought to examine swimming performance in fishes, such as coho (*Oncorhynchus kisutch*, Davis et al. 1963, Lee et al. 2003), chinook (*Oncorhynchus tshawytscha*, Davis et al. 1963, Lee et al. 2003), and sockeye salmon (*Oncorhynchus nerka*, Brett 1964, Farrell et al. 1998), Atlantic cod (*Gadus morhua*, Nelson et al. 1996, Martinez et al. 2004, Herbert and Steffensen 2005), and cut-throat trout (*Oncorhynchus clarki clarki*, MacNutt et al. 2004). A number of metrics of performance can be assessed using swim trials including sprint speed, rate of acceleration, and endurance swimming (see review by Wood 1991). Critical swimming speed (U_{crit}) is an accepted evaluation of prolonged swimming performance (Beamish 1978) and has been used widely to determine the effects of environmental change on the physiological performance of fish (Farrell et al. 1998, Lee et al. 2003, MacNutt et al. 2004). In general, swim trials used to assess U_{crit} force a fish to swim in a flume against an increasing current for pre-set durations until it can no longer maintain its position in the flume. Additionally, repeated determination of U_{crit} has been used to assess the general health or condition of a fish (Jain et al. 1998, Farrell et al. 1998). In repeat swim trials, fish are forced to swim to exhaustion (U_{crit1}), allowed to rest for a specified period of time, and then forced to swim to exhaustion again (U_{crit2}). A reduced U_{crit2} suggests that the fish did not have sufficient time to recover from the initial exercise. The ability to recover from exhaustion can have profound impacts on fish in the wild and may limit subsequent swimming performance (Milligan 1996), impact survival, and ultimately affect fitness.

Fish exposed to exhaustive exercise in the laboratory exhibit increased concentrations of lactate in plasma and white muscle, decreased glycogen reserves in white muscle and a disturbance of osmotic balance (Wood 1991). Lactate from the tissue moves into the blood, although peak levels in the blood may not be reached until 2 hours after exhaustion (Milligan 1996). Recovery from exhaustion involves the replenishment of tissue glycogen reserves, decrease of lactate concentrations, and the normalization of tissue pH to pre-exercise values (Milligan and Wood 1986, Wang et al. 1994). Although recovery times differ among species and studies, an exhausted fish will generally clear lactate to pre-swim levels within 12 hours (Kieffer 2000) and will recover glycogen reserves within 24 hours (Milligan and Wood 1986). However, there is increasing evidence that glycogen replenishment and lactate removal post-exercise are inhibited by increased cortisol concentrations in the blood due to the exercise trial (see review by Milligan 1996).

Metabolic recovery rate is influenced by temperature (Galloway and Kieffer 2003), but the specific relationship is not always clear. In Atlantic salmon (*Salmo salar*), rates of glycogen synthesis, lactate catabolism, and restoration of pH balance following exhaustive exercise were higher

for fish acclimated at warm (18-23°C) compared to cool temperature (12°C) (Wilkie et al. 1997). However, Kieffer et al. (1994) found that rainbow trout (*Oncorhynchus mykiss*) acclimated to a higher temperature (18°C) had similar glycogen and lactate recovery times compared to fish acclimated to a lower temperature (5°C). These contrasting findings illustrate the need for further investigation into the effects of acclimation temperature on metabolic recovery post-exercise.

In a previous experiment we found that Pacific cod (*Gadus macrocephalus*), acclimated to two different temperatures (4°C and 11°C), showed no significant difference between U_{crit1} and U_{crit2} when swum to exhaustion twice with a one hour rest period in between swims (Hanna 2006) suggesting that one hour was an ample period for fish to recover between swim trials. Whereas analyses of survey catch data indicate that Pacific cod preferentially inhabit waters slightly above 7°C (Perry et al. 1994), Paul et al. (1988) examined oxygen consumption rates of Pacific cod held at different temperatures and found differences in fish held between 3.5°C and 7°C but no differences in fish held between 7°C and 12°C. This suggests that these fish can acclimate to maintain metabolic rate between 7°C and 12°C but adjust their metabolic rate at temperatures between 3.5°C and 7°C. We set out to determine temperature effects on rates of metabolic recovery of wild-caught Pacific cod held at 2°C and 7°C. To assess metabolic recovery we collected blood samples both before and after swim trials and after two and four hours of recovery. Samples were analyzed for hematocrit and plasma concentrations of cortisol, lactate, glucose, total protein, Na^+ , and Cl^- . Because Pacific cod swum at different temperatures did not differ in their U_{crit1} and U_{crit2} , we hypothesized that they would not differ in their rates of recovery.

Materials and methods

In June 2005, we captured adult Pacific cod (weight, 0.9-3.6 kg; total length, 44-68 cm) near Kodiak Island with pots (145 × 130 × 70 cm) at a depth of approximately 20 m and transported them back to the Kodiak Fisheries Research Center wet lab facilities. We measured each fish for total length (± 1.0 cm), weight (± 0.01 kg), and width and height (just posterior to the pectoral fins; ± 0.1 mm). Each fish was marked using a unique number coded tag (T-bar tag FD-68B, Floy Tag®, Seattle, WA) prior to being placed into one of two 6 m³ tanks supplied with sand filtered seawater drawn from Trident Basin at a depth of 25 m.

In November 2005, two weeks before the initiation of swim trials, six male and eight female fish were placed into a single tank maintained at 2°C and five males and three females were placed into another tank maintained at 7°C. All fish were fed Pacific herring (*Clupea pallasii*) to satiation every three days.

After the two week acclimation period, we captured each fish from the tanks using a low abrasion dipnet and immersed them in a 30 L solution of anesthetic (tricaine methansulfonate; 100 ppm) for up to two minutes. Once each fish was anesthetized, we placed it on a V-board and collected 0.5 ml of blood from the caudal vasculature using a 21-gauge needle and 3 ml syringe. We again measured fish for total length, standard length, width, height, and weight as described above. We then placed the fish back into their respective tanks for an average of 27 days prior to swim trials.

We placed individual blood samples into labeled vials coated with an anticoagulant, ammonium heparin, for the preparation of plasma samples. We determined hematocrit of each sample in duplicate by drawing blood into micro-hematocrit capillary tubes (Fisherbrand®, Pittsburgh, PA) followed by centrifugation in a micro-hematocrit centrifuge (Unico®, Dayton, NJ) at 12,000 RPM for 10 minutes. The remainder of the blood sample was separated via centrifugation (2,500 rpm for 15 minutes, Fisherbrand® accuSpin™ Micro R) ≤ 30 min after collection. We drew off plasma using a Pasteur pipette and placed it into a labeled vial, which was stored at -50°C for later analysis.

Immediately preceding each swim trial, fish were fasted for either four days (high temperature group) or five days (low temperature group) to minimize the effect of digestion and assimilation on metabolism (Paul et al. 1988). We captured fish from tanks using a low abrasion dipnet and placed them into a 370 L Brett-type swim flume maintained at the acclimation temperature of the fish. The swimming section of the flume was constructed of Lexan® and measured 1.87 m long with a diameter of 20 cm. Measures of total length, width, and height were used to calculate solid blocking effect of individual fish in the swim flume (Bell and Terhune 1970). Before the actual swim trial, we trained fish through exposure to stepwise increases in water velocity until the fish could no longer swim away from the back of the flume. We then allowed the fish to acclimate to 0.5 BL s^{-1} for 36 hr and then performed two U_{crit} trials with a 1 hr rest period in between trials. During each U_{crit} trial we increased water velocity 0.1 BL s^{-1} every 30 min until the fish failed to swim away from the back of the flume. Immediately after determination of the second U_{crit} , we removed the fish from the swim flume and collected a blood sample (as above) and placed the fish back into its respective tank but separated from the other fish via netting. We allowed the fish to recover for either two or four hours. Half of the fish in each temperature group (seven low temperature and four high temperature fish) were allowed a two hour recovery and the other half were allowed a four hour recovery. A slow, circular current ($<0.5 \text{ BL s}^{-1}$) was maintained in the tanks allowing the fish to swim during the recovery period. After the recovery period, we removed the fish and killed it with a sharp blow to the head. We obtained a 1 ml blood sample and weighed and measured the fish for standard morphometrics (as above).

We assayed plasma for concentrations of glucose, lactate, and protein using enzymatic assay kits (Glucose: Wako Pure Chemical Industries, Richmond, VA.; Lactate: Trinity Biotech, St. Louis, MO.; Protein: Pierce, Rockford, IL) according to manufacturers protocols adapted for use in 96-well microplates. A SPECTRAmax® microplate spectrophotometer and SOFTmax® PRO software (Molecular Devices, Sunnyvale, CA) were used. We assayed samples in triplicate to determine a mean optical density. A standard curve of optical density to concentration of each constituent was created.

We determined plasma Na^+ and Cl^- concentrations using a Medica EasyElectrolytes ion meter (Medica Corporation, Bedford, MA). We diluted samples with deionized water in the ratio 11:5 to fit plasma concentrations into the working range of the instrument. We assayed all samples in duplicate. The mean intra-assay variation was 0.2% determined by running a control every 15 samples.

Concentrations of plasma cortisol were determined using radioimmunoassay techniques originally described by Foster and Dunn (1974) and further modified by Redding et al. (1984) and verified for Pacific cod (courtesy of G. Feist, Oregon State University). Samples were assayed in duplicate.

We determined differences in blood parameters over time (pre-swim to recovery) and between temperatures using two-way repeated measures analysis of variance. Separate tests were performed for the two hour recovery group and the four hour recovery group. Multiple comparisons were made using the Holm-Sidak method. A t -test was used to test for differences in U_{crit1} between temperature groups and paired t -tests were used to determine differences between U_{crit1} and U_{crit2} . All tests were performed at $\alpha = 0.05$. Data are presented as mean $\pm$ standard error of measure. SigmaStat 3.0 statistical software (Systat Software, Inc., Richmond, CA) was used for all statistical analyses.

Results

The mean U_{crit1} for low ($0.97 \pm 0.03 \text{ BL s}^{-1}$) and high ($0.99 \pm 0.04 \text{ BL s}^{-1}$) temperature acclimated Pacific cod was not significantly different between groups ($P = 0.772$). U_{crit2} was significantly lower than U_{crit1} in the low temperature group ($0.94 \pm 0.03 \text{ BL s}^{-1}$, $P = 0.022$) but not in the high temperature group ($0.96 \pm 0.05 \text{ BL s}^{-1}$, $P = 0.093$).

Plasma cortisol concentrations increased significantly from pre- to post-swim in fish held at both temperature treatments at both sampling schedules (2 hr: $P < 0.001$; 4 hr: $P < 0.001$; Fig. 1). Concentrations of cortisol did not significantly differ between temperature treatments in either the two hour ($P = 0.907$) or four hour ($P = 0.925$) sampling intervals nor did they differ between fish sampled immediately post-swim and either samples collected two hours (low temperature: $P = 0.813$,

Figure 1. Interrelationships among swim state, acclimation, temperature, and circulating levels of blood constituents $\pm$ SE for Pacific cod acclimated to 2°C (14 fish) and 7°C (8 fish). Blood samples were taken pre-swim, immediately post-swim, and at 2 and 4 hr of recovery post-exhaustion.

high temperature: $P = 0.260$) or four hours (low temperature: $P = 0.496$, high temperature: $P = 0.460$) after swimming in either temperature treatment. The intra-assay variation was $<5\%$ and the inter-assay variation was $<10\%$.

Likewise, concentrations of plasma lactate increased significantly from pre- to post-swim in both the high and low temperature acclimated fish (2 hr: $P < 0.001$; 4 hr: $P < 0.001$) but did not differ between temperature treatments at two hours ($P = 0.249$) or four hours ($P = 0.233$) after the swim test (Fig. 1). Plasma lactate concentrations remained elevated in both temperature groups during recovery and were not significantly different from samples collected immediately post-swim after two hours (low temperature: $P = 0.629$, high temperature: $P = 0.330$) or four hours (low temperature: $P = 0.660$, high temperature: $P = 0.907$). Plasma protein concentrations did not significantly differ between temperature groups for fish sampled at two hours ($P = 0.517$) and four hours ($P = 0.426$) after the swim test, nor was there any difference in plasma protein concentrations over time (2 hr: $P = 0.108$; 4 hr: $P = 0.939$; Fig. 1). Plasma glucose concentrations increased significantly from pre- to post-swim and further increased during recovery in both the two hour ($P = 0.006$) and four hour ($P < 0.001$) recovery groups (Fig. 1). Concentrations of plasma glucose were consistently higher in the low temperature group

Figure 2. Interrelationships among swim state, acclimation temperature, and circulating levels of blood constituents $\pm$ SE for Pacific cod acclimated to 2°C (14 fish) and 7°C (8 fish). Blood samples were taken pre-swim, immediately post-swim, and at 2 and 4 hr of recovery post-exhaustion. Significant interactions existed between temperature and time for plasma Na⁺ concentrations and hematocrits in the 2 hr recovery group and for plasma Cl⁻ concentrations in the 4 hr recovery group.

than the high temperature group at both two and four hours post-swim. Glucose concentrations were significantly different between temperature groups in the two hour recovery group ($P = 0.008$) but not in the four hour ($P = 0.107$) recovery group. The mean intra-assay variation for the glucose, lactate, and protein assays were <4% and the mean inter-assay variations were <10%.

A statistically significant interaction existed between temperature and time for plasma Na⁺ concentrations in the two hour recovery group ($P = 0.023$). Plasma Na⁺ concentrations increased in both temperature groups in the four hour recovery group from pre- to post-swim and during recovery ($P < 0.001$, Fig. 2). Plasma Cl⁻ concentrations were consistently higher in the low temperature group than the high temperature group but only significantly higher in the two hour recovery group ($P < 0.001$). A statistically significant interaction existed between temperature and time for plasma Cl⁻ concentrations in the four hour recovery group ($P = 0.011$). Cl⁻ concentrations significantly increased from pre- to post-swim and during recovery in the two hour recovery group ($P = 0.023$).

A statistically significant interaction existed between temperature and time for hematocrit in the two hour recovery group ($P = 0.022$) and did not significantly differ between temperature groups in the four hour recovery group ($P = 0.836$). Additionally, hematocrit values significantly decreased from pre-swim to recovery in the four hour recovery group ($P = 0.020$).

Discussion

Pacific cod acclimated to 2°C and 7°C and exercised to exhaustion exhibited marked physiological disturbances as evidenced by plasma cortisol, glucose, lactate, and ions. These constituents remained elevated up to four hours post exhaustion and showed no signs of returning to pre-swim values in either temperature group. Based on these data we concluded that the temperatures utilized in this experiment have no significant effect on short term recovery following exhaustive exercise in Pacific cod and are consistent with previous studies we have conducted that indicate no significant difference in U_{crit1} and U_{crit2} between temperature treatments (Hanna 2006).

Although physiological recovery was incomplete four hours after exhaustion, it is interesting to note that swimming performance decreased only slightly during the second swim. With only a one hour rest period between swim trials, fish were able to perform almost as well as during the first swim with only a slight decrease in the low temperature group. Similar results were found in juvenile rainbow trout in which fish maintained swimming performance after only a 45 minute recovery period (MacFarlane and McDonald 2002). Therefore, it seems that complete physiological recovery is not necessary to maintain swimming performance in this species based on this particular test of performance.

Also consistent with our results, rainbow trout exhibit increased concentrations of plasma glucose post-exercise and during recovery and remained elevated four hours after exhaustion (Milligan and Wood 1986, Wood et al. 1990). Additionally, glucose concentrations in this study are similar to those for Atlantic cod in which pre-swim plasma glucose was between 4 and 7 mmol L⁻¹ and after four hours of recovery was between 8 and 10 mmol L⁻¹ (Nelson et al. 1996). Glucose concentrations increase in stressed fish due to combined effects of glycogenolysis and gluconeogenesis (Wendelaar Bonga 1997) and may be mediated by increases in plasma cortisol. Cortisol aids in the mobilization of energy stores and stimulates release and synthesis of glucose (see review by Mommsen et al. 1999) and can result in increased concentrations of plasma glucose. However, the differences in glucose concentrations between temperature groups found in this study are not consistent with a previous study on Pacific cod (Hanna 2006) and are only significant within the two hour

recovery group and not the four hour recovery group. This may be indicative of higher rates of glycogenolysis/gluconeogenesis in the low temperature acclimated fish or a reduced usage of glucose in these fish compared to high temperature acclimated fish. Similar differences in plasma Cl^- concentrations between temperature groups combined with differences in plasma glucose concentrations suggest low temperature acclimated fish may be more stressed than the higher temperature acclimated fish.

High concentrations of plasma cortisol pre-swim suggest that both temperature groups were stressed prior to the swim trials. The stress may have been due to the acclimation temperatures or simply captivity. In a previous experiment we found that Pacific cod acclimated to 4°C had higher plasma cortisol concentrations than those acclimated to 11°C (Hanna 2006). However, Pacific cod are known to inhabit waters slightly above 7°C (Perry et al. 1994). Therefore, we might expect the 2°C fish to be stressed from the low temperatures but not the 7°C fish. Although these increased levels of cortisol may not have affected swimming performance (Gregory and Wood 1999), the role of cortisol in slowing the recovery process is well known (Milligan 1996) and therefore may have inhibited recovery in these fish.

Cortisol, lactate, glucose, and ion concentrations did not return to resting levels after four hours of recovery in this study. Cortisol plays a key role by slowing the recovery process through influences on lactate metabolism and glycogen resynthesis (see review by Milligan 1996). When cortisol synthesis is blocked, rates of lactate clearance and glycogen resynthesis are increased (Pagnotta et al. 1994). Cortisol levels may remain elevated for several hours (see review by Milligan 1996) or days to weeks (Haukenes and Buck 2006) following exposure to a stressor. Cortisol does not increase in rainbow trout that are allowed to swim at low velocity after exhaustive exercise and thus lactate is cleared within two hours of recovery (Milligan et al. 2000). This suggests that either the fish in this study may not have been swimming fast enough to limit cortisol accumulation and thus metabolic recovery may have been delayed or that increased rates of clearance or inhibition of synthesis of cortisol due to exercise during the recovery period is not manifested in Pacific cod as in rainbow trout. It would also be interesting to examine the effects of high concentrations of cortisol on tissue glycogen and lactate recovery in Pacific cod. However, due to our location on Kodiak Island, we did not have access to liquid nitrogen for freeze clamping and so were unable to properly collect muscle samples from fish.

Plasma Cl^- and Na^+ concentrations were consistently higher in the low temperature fish than in high temperature fish throughout the experiment indicating greater osmoregulatory disturbance in the low temperature fish (Redding and Schreck 1983). Fish in the low temperature group were not able to ionoregulate as well as their high tempera-

ture counterparts possibly due to the stress associated with the lower temperatures. Redding and Schreck (1983) also indicate decreases in hematocrit associated with stress; yet, this effect was not observed in our study. The increases in plasma lactate and glucose in association with exercise has been observed in rainbow trout (Milligan and Wood 1986, Wood et al. 1990). These data, combined with the lack of change in plasma total protein concentrations, suggest that there was little net movement of water between the extracellular fluid and muscle of the fish (Wang et al. 1994).

Because neither temperature group experienced any measurable amount of metabolic recovery in any variable we assessed, we cannot say that temperature does not affect rates of recovery. However, the temperatures chosen do not seem to affect short term recovery after exhaustion in Pacific cod. Differences in recovery rate seen in other studies occurred over wider temperature ranges than those examined in this experiment and included three temperature groups. Wilkie et al. (1997) acclimated Atlantic salmon to 12, 18, and 23°C and Galloway and Kieffer (2003) examined Atlantic salmon at 6, 12, and 18°C. To effectively demonstrate a temperature effect on recovery, one would need to greatly increase the observation time post-exhaustion. Thus, adding more and broader temperature treatments might help to elucidate a relationship between recovery rate and ambient temperature in Pacific cod.

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