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Carl B. Schreck

Various aspects of the energetic response to handling stress in juvenile coho salmon (*Oncorhynchus kisutch*) were examined. Fish were subjected to four different handling stressors in a Blazka-style respirometer. Stressed fish had rates of oxygen consumption that were higher than controls. The magnitude of the increase ranged from 139 to 198% of the control value, and appeared roughly related to the severity of the stressor. The post-stress increase in oxygen consumption also appeared to vary seasonally, with less of a stress effect on respiration observed in the spring as compared to the fall. Elevation in oxygen consumption following stress was largely eliminated within 1 h post-stress, but metabolic rate may have remained slightly elevated for an additional 2 h.

Plasma cortisol and lactate titers also increased significantly following handling stressors. Oxygen consumption was positively correlated with both plasma cortisol and lactate after a moderate stressor, but no correlation was found after more severe stressors. Whole body lactate concentration was significantly elevated following stress, reaching levels almost 500% higher than controls. By 5 h post-stress whole body lactate had returned to control levels.

The mechanism of excess post-stress oxygen consumption remains unclear. Fish given exogenous cortisol did not experience an increase in oxygen consumption, so it is unlikely that cortisol alone has a major effect on metabolic rate. Similarities between the energetic responses to both stress and exercise suggest that the results of exercise physiology may provide a basis for understanding the energetic response to stress.

THE ENERGETIC RESPONSE TO HANDLING STRESS IN JUVENILE COHO SALMON (*Oncorhynchus kisutch*)

by

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THE ENERGETIC RESPONSE TO HANDLING STRESS IN JUVENILE COHO SALMON (*Oncorhynchus kisutch*)

INTRODUCTION

It has been proposed that stress in fish results in an increase in energy consumption, and that this extra energy demand could have a detrimental effect on subsequent performance (Schreck, 1981; 1982; 1991). A stress-related energy drain could have important adaptive consequences in certain situations, such as when hatchery fish are released into the natural environment following the stress of transportation. A primary goal of this study was to estimate the potential for negative energetic effects of stress by measuring the magnitude and duration of elevations in metabolic rate following different types of stressors.

Although information is scarce, limited evidence indicates the existence of an energetic cost of stress. Oxygen consumption was found to increase in fish that had been handled and were in the process of acclimating to a respirometer (Saunders, 1963; Holeton, 1974). Brett (1964) observed that restlessness or excitement in sockeye salmon (*Oncorhynchus nerka*) could increase oxygen consumption rates by a factor of 2 to 2.5 times the rate in calm fish.

Barton and Schreck (1987) found that steelhead trout (*Oncorhynchus mykiss*) given a handling stressor experienced a 220% increase in oxygen

consumption, which corresponded to approximately one fourth of the scope for activity. Similarly, Mohamed (1982) found that freshwater mullet (*Rhinomugil corsula*) subjected to a handling in a dipnet experienced a 260-290% increase in oxygen consumption compared to controls. A 200-270% increase in oxygen consumption was seen in juvenile rainbow trout (*Oncorhynchus mykiss*) after they had been chased with a dip net for 6 min (Goolish, 1989).

These studies indicate that a significant amount of energy may be used in the immediate post-stress response but say nothing about the duration of this energetic cost. A goal of this study is to provide an estimate of the time required for metabolic recovery from an acute stressor. Some measure of recovery period is necessary to understand the true adaptive consequences of stress, since the overall energetic cost is a function of both the magnitude and duration of the elevation in metabolic rate.

Recovery period is usually defined as the time from onset of stress until the response of the variable of interest has returned to normal or control levels. For plasma variables such as cortisol, glucose, and lactate, changes resulting from stress can be eliminated in 24 h or less (Donaldson, 1981; Hille, 1981). Complete recovery from an acute stress (i.e. a return of all parameters affected by the stress response to control or basal levels) may take much longer, and Pickering et al. (1982) suggests a period of at least 2 weeks.

Reports of recovery from post-handling elevations in metabolic rate

are largely anecdotal and vary widely. Holeton (1974) found it took about 48 h for oxygen consumption to stabilize in arctic fishes (*Gymnelis viridis, Boreogadus saida*) after capture and handling. An even longer period of 3-4 days was needed for oxygen consumption to return to normal after handling in young carp (*Cyprinus carpio*; Korovin et. al., 1982). In contrast, a recovery period of only 3-5 h was reported for fasted cod (*Gadus morhua*; Saunders, 1963).

A possible source of this variation in the metabolic response to stress is the quality of the stressor. Leach and Taylor (1980) found that different types of stressors elicited different corticosteroid stress responses in *Fundulus heteroclitus,* which consisted of a variation in the length of plasma cortisol elevation. In rainbow trout, Barton et al. (1980) noted that the severity of the stressor affected the magnitude of the plasma cortisol elevation. A cumulative corticosteroid and hyperglycemic effect in response to multiple stresses has also been observed in juvenile chinook salmon (*O. tshawytscha*; Barton et al., 1986). By measuring oxygen consumption following different types of stressors, I hope to determine if metabolic rate following stress is influenced by the quality of the stressor. If the quality of the stressor influences the metabolic response, then changes in handling procedures could result in a smaller energy drain on the stressed individual, minimizing any negative adaptive effects.

In addition to evaluating the magnitude and duration of the energetic

response to stress, a second goal of this work is to elucidate the mechanism by which oxygen consumption is increased following stress. Such an investigation provides an opportunity to more clearly establish the links between the primary (neuroendocrine) and secondary (metabolic) responses to stress.

How oxygen consumption increases during the stress response is unknown. It is possible that the glucocorticoid hormone cortisol may play some role in this phenomenon. Elevated plasma cortisol is a widely recognized indicator of stress in fish (Donaldson, 1981; Schreck, 1981; Wedemeyer & McLeay, 1981), and cortisol may influence metabolic rate in fish. Chan and Woo (1978) observed that treatment of hypophysectomized eels (*Anguilla japonica*) with cortisol increased oxygen consumption by 79% over controls, and suggested a possible functional relationship between cortisol and metabolic rate. In Barton and Schreck's (1987) study, a positive correlation was found between plasma cortisol titer and oxygen consumption in stressed fish, but not controls. The influence of cortisol on metabolic rate will be determined by measuring the effect of exogenous cortisol on oxygen consumption.

A second hypothesis to be tested is that the increase in oxygen consumption during stress represents the repayment of an oxygen debt, similar to the situation following exercise. The classical oxygen debt hypothesis states that most of the increase in oxygen consumption seen

following exercise is used to remove lactate, primarily via oxidation with some conversion to glycogen (Gaesser & Brooks, 1984). A rise in plasma lactate is a typical response to physical disturbances in many fish (Pickering et al.; 1982, Fraser and Beamish; 1969, Sovio and Oikari, 1976; Schwalme and MacKay, 1985). The twofold increase in plasma lactate of stressed fish seen by Barton and Schreck (1987) was attributed to an anaerobic response to the handling stress. They theorized that part of the increase in oxygen consumption seen during stress was the result of a repayment of the oxygen debt incurred during anaerobic metabolism.

If the metabolic responses to both stress and exercise are similar, then similar amounts of lactate should be observed following stress as is seen after exercise. A measurement of whole-body lactate concentration before and after stress will be compared to published post-exercise lactate values to test this hypothesis. Whole-body lactate concentration is used because it is a more accurate measure of total lactate production than is plasma lactate.

METHODS AND RESULTS

General Methods

Fish acquisition and rearing

Coho salmon (*O. kisutch*) were obtained as parr from the U.S. Fish and Wildlife Service's Eagle Creek National Fish Hatchery and held at Oregon State University's Fish Genetics and Performance Laboratory, Smith Farm. Two groups of fish were used: one hatched in the winter of 1988, and the other hatched in the winter of 1990. All fish were post-smolts at the time of their use in experiments. Size of the animals varied from experiment to experiment, therefore this data is given separately. The animals were held in aerated well water ranging in temperature from 12.5-14.0 ^{0}C and exposed to the natural photoperiod. A semi-dry pelleted feed (Bio-Diet) was fed once daily.

Fish to be used in experiments were removed from the natural photoperiod 1-2 months before use and placed under an artificial photoperiod of 12 h light:12 h dark. These animals were fed to satiation once every other day between the hours of 1500-1800. This standardization of feeding time and photoperiod was intended to limit diurnal variation in activity and metabolic rate. Variations in photoperiod and entrainment to a feeding schedule are known to affect activity and metabolic rate in fish (Brett and Groves, 1979; Spieler and Noske, 1984).

Measurement of metabolic rate

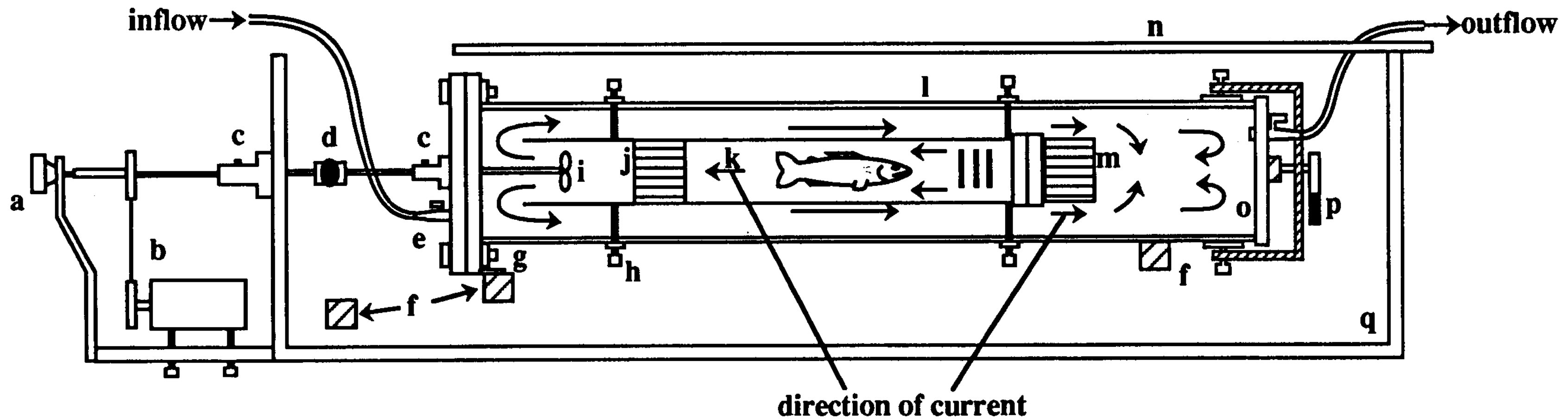
Throughout the study metabolic rate was estimated by measuring oxygen consumption. The equipment and procedures used were based on those described in Barton and Schreck (1987). Two Blazka-type respirometers (Blazka et al., 1960) with the modifications described in Barton and Schreck (1987) were used to determine aerobic metabolic rate (Figure 1). These devices were further modified to include a tachometer attached to the driveshaft (for fine control of water velocity within the respirometer). Approximately 3/4 of the box containing the swimming tube was covered to provide partial darkness.

Fish were fasted for 48 h prior to being placed into the respirometers to ensure a post-absorptive state (Davis, et al., 1963) since specific dynamic action costs can account for as much as 50% of total inactive metabolism in fish (Beamish, 1964). Single fish were removed from the stock tank and anesthetized in 50 mg/l MS222 buffered with 100 mg/l $NaHCO_3$, then loaded into each of the two respirometers between the hours of 1700-1800. The fish were then acclimated within the respirometers overnight. The minimum acclimation period was determined via a pilot experiment where I found no observable difference in oxygen consumption between fish acclimated within the respirometers either 24 h or three days.

At 0730 the next morning an 8 h swim adjustment [forced swimming at a rate of 0.2-0.3 BL/S (body lengths/second)] was initiated to accustom the

Figure 1: Diagram of the Blazka-type respirometer used to measure oxygen consumption in juvenile coho salmon. This figure is adapted and redrawn from Barton (1986).

Closed System Blazka-type Fish Respirometer



a. tachometer

b. motor and pulley

c. sealed bearings

d. driveshaft with detachable coupling

e. stopcock

f. wooden supports

g. hinge

h. set screw with rubber seal

i. 3-bladed propellor

j. baffle

k. inner swimming tube

l. outer swimming tube

m. removable baffle

n. wooden lid

o. outer swim tube lid (removable)

p. tube lid hold-down screw

q. plywood box

fish to swimming within the respirometer. After the swim adjustment (1530 hours) the animals were exposed to the appropriate treatment with the respirometry trial immediately following. The respirometry trial consisted of forced swimming at a rate of 0.5 BL/S for 1 h. The time of day for swimming adjustments and respirometry trials was kept as constant as possible to minimize diurnal variation in metabolic rate.

During the acclimation and swim adjustment water was allowed to pass once through the respirometers. The inflow was shut off during respirometry trials, when the tubes were operated as a closed system. Water samples were collected immediately before and after each trial. In the early part of the study dissolved oxygen was measured via the azide modification of the Winkler method (APHA et. al., 1980). Later, an air-calibrated YSI model 54A oxygen meter was used. Oxygen consumption measurements were divided by the weight of each fish to standardize for the effect of size on oxygen consumption.

After the respirometry trial fish were quickly removed from the swim tubes and killed with a lethal dose of anesthetic (200 mg/l MS222 buffered with 500 mg/l $NaHCO_3$). The fish were then weighed and bled by caudal severance. Plasma was separated from whole blood by centrifugation and stored at -80^{0}C for later analysis. Plasma cortisol concentration was determined via radioimmunoassay (Foster and Dunn, 1974) as modified for use with salmonid plasma (Redding et. al., 1984). Plasma glucose was

determined by the spectrophotometric method described in Wedemeyer and Yasutake (1989) and plasma lactate by the method of Passonneau (1974).

Statistical analysis was done using the STATGRAPHICS (Statistical Graphics Corporation) statistical software package. Specific analyses are described in the section for each experiment.

Metabolic Effect of Various Stressors

Methods

The first experiment consisted of subjecting fish to different handling stressors and then measuring the resulting effect on oxygen consumption. All animals were acclimated to the respirometers and given swim adjustments as described earlier. Groups of 10 individual fish were then subjected to one of the following treatments:

1) Forcing the fish to struggle by lifting the swim tube into a vertical position and holding it there for 2 min.

2) Forcing the fish to struggle as described above, but repeating this procedure three times; each stressor separated by a 30 min period of swimming at 0.2-0.3 BL/S. This is the same stressor Barton and Schreck (1987) used in their study.

3) Removal of the fish from the respirometer, exposing it to the air for 30 s, and then returning it to the swim tube. This was accomplished

by removing the inner swim tube of the respirometer (with the fish inside), holding it just out of the water, and then replacing it.

4) Removal of the fish from the respirometer and placing it in a live well for a period of 30 min, then returning it to the respirometer. Water depth in the live well was maintained at a level just covering the back of the fish.

5) A control group was exposed to the full respirometry protocol, but not subjected to any additional stressor.

These stressors were designed to approximate some of the different types and intensities of experiences (mild handling, intense handling, confinement) that might be encountered in a culture situation.

The individuals used in this experiment were 1988 brood 2+ year fish with weights ranging from 66.7-154.9 g ($\bar{X}$=97.0). Water temperature ranged from 13.0-13.2 ^{0}C during the study period of 16 October to 26 November 1990.

Differences between groups in oxygen consumption and plasma variables were analyzed with a one-way analysis of variance (ANOVA). If significant differences in mean values were indicated by the ANOVA then the means were compared using a Tukey multiple comparisons procedure ($p \leq 0.05$). Correlation between variables was investigated using a simple regression analysis, with each comparison made separately.

Results

Coho salmon exposed to various handling stressors all had significantly higher rates of oxygen consumption than controls (ANOVA $p<0.0001$). Controls had a mean oxygen consumption rate of 140 $\pm$ 4.7 mg $O_2kg^{-1}hr^{-1}$ (SE) while stressed groups had means ranging from 195 $\pm$ 16.0 to 277 $\pm$ 8.0 mg O_2 $kg^{-1}hr^{-1}$ (Figure 2), an increase over controls of 139-198%. Statistically, the results could be divided into three different groups (in order of increasing magnitude): controls, those subjected to one or three upright positionings of the swim tube, and those removed from the respirometer for 30 s or confined (Fig. 2).

Mean plasma cortisol titer in the removal and confinement groups was significantly higher than in controls (ANOVA $p=0.03$) but cortisol was not significantly elevated in the other two groups with less severe stress (Table 1). There was no correlation between plasma cortisol level and oxygen consumption for the control, removal, and confinement groups, but a significant correlation was observed for the one upright ($p=0.01$, $r=0.76$) and three upright groups ($p=0.002$, $r=0.85$).

Plasma lactate level was also significantly higher in removal and confinement groups compared to controls (ANOVA $p=0.0005$, Table 1). Lactate concentration was significantly correlated to oxygen consumption for the one upright ($p=0.02$, $r=0.73$) and three upright treatments ($p<0.01$, $r=0.81$). There was no difference in mean plasma glucose concentration of the fish

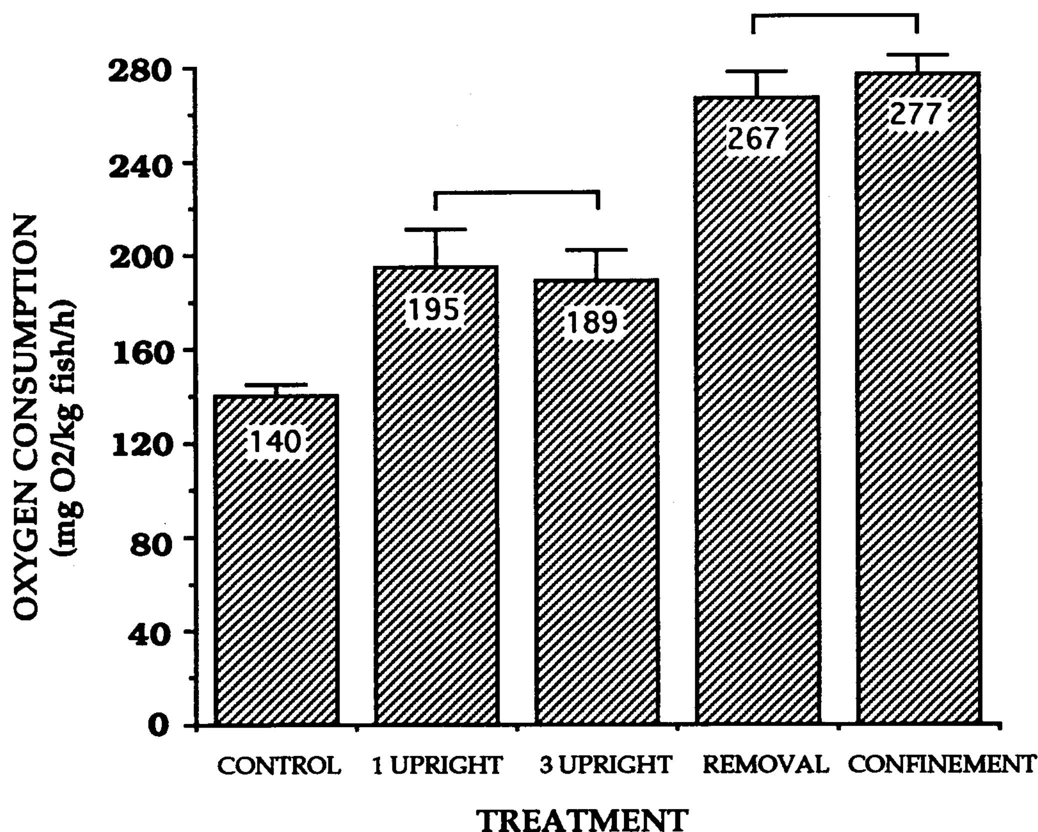


Figure 2: Mean (and SE) rates of oxygen consumption of juvenile coho salmon that were exposed to one of the following treatments:

Control: no handling stressor given

1 Upright: swim tube lifted into an upright position for 2 min

3 Upright: three episodes of lifting the swim tube upright for 2 min, separated by 30 min intervals

Removal: holding the fish out of the water for 30 s

Confinement: removal from the respirometer and confinement within a live well for 30 min

Mean values (n=10) are shown within each column. Treatments that are not statistically different ($p>0.05$) are connected by brackets.

Table 1: Mean (and SE) plasma cortisol, glucose, and lactate of juvenile coho salmon that were exposed to one of the following treatments:

Control: no handling stressor given

1 Upright: swim tube lifled into an upright position for 2 min

3 Upright: three episodes of lifting the swim tube upright for 2 min, separated by 30 min intervals

Removal: holding the fish out of the water for 30 s

Confinement: removal from the respirometer and confinement within a live well for 30 min

Mean values (n=10) are shown within each column. Treatments that are not statistically different ($p>0.05$) are connected by brackets.

Treatment	Plasma Cortisol (ng/ml)	Plasma Glucose (mg/dL)	Plasma Lactate (mg/dL)
Control	**78 ±14**	**177 ±21**	**28.2 ±3.6**
1 Upright	**86 ±17**	(n=9) **196 ±27**	**41.9 ±4.8**
3 Upright	**99 ±20**	**212 ±24**	**36.2 ±5.5**
Removal	**132 ±15**	**212 ±22**	**56.1 ±6.3**
Confinement	**140 ±12**	**233 ±21**	**60.6 ±6.5**

subjected to the different treatments (Table 1); there was also no correlation between oxygen consumption and plasma glucose level.

Aerobic Recovery From a Stressor

Methods

A second experiment was conducted to determine the time required for the increased oxygen consumption resulting from a stress response to disappear. One group of 10 fish was used to establish a control measurement of metabolic rate. These fish were individually exposed to the usual respirometry protocol, but not exposed to any additional handling stressor. Four other groups of 10 fish each were also allowed to acclimate as usual, but in contrast were given a removal stressor (treatment #3 as described in the previous experiment). This occurred immediately prior to the time oxygen consumption would normally be measured. The difference between these four groups was the amount of time allowed to elapse between the completion of the stressor and the measurement of oxygen consumption. Respirometry trials were conducted on individual fish at times 0 h (immediately following), 4 h, 24 h and 48 h post-stress. Plasma cortisol, glucose, and lactate were determined for all 10 individuals in each group, except those at 24 h where 9 fish were sampled.

Trials were conducted from 1 April 1991 to 15 July 1991 using 1988

brood 3+ year individuals. Fish weights ranged from 69.1-205.5 g ($\bar{X}$=139.2), with most individuals average in weight. Water temperature varied from 12.4-13.1^{0}C; there was no correlation between temperature and oxygen consumption over this range. Statistical analysis was conducted in the same manner as the previous experiment.

There was some concern at the outset of the experiment that confinement within the respirometer for long periods could in itself result in significant stress, confounding any attempt to measure recovery. This theory was tested when the acclimation period was being determined; no difference was seen in routine (no extra handling stress) oxygen consumption between fish held in the respirometer for either one or three days. To further investigate the effect of confinement an experiment was conducted in which fish were held in the respirometers for 3 days, then given a handling stress and oxygen consumption measured. There was no difference in oxygen consumption between these fish and another group held in the respirometers the usual amount of time (24 h) and then stressed. Therefore, confinement within the respirometer for up to three days did not effect the respiratory response to stress.

Results

Elevation in metabolic rate resulting from the removal stressor was eliminated by 4 h post-stress (Figure 3). Mean oxygen consumption was

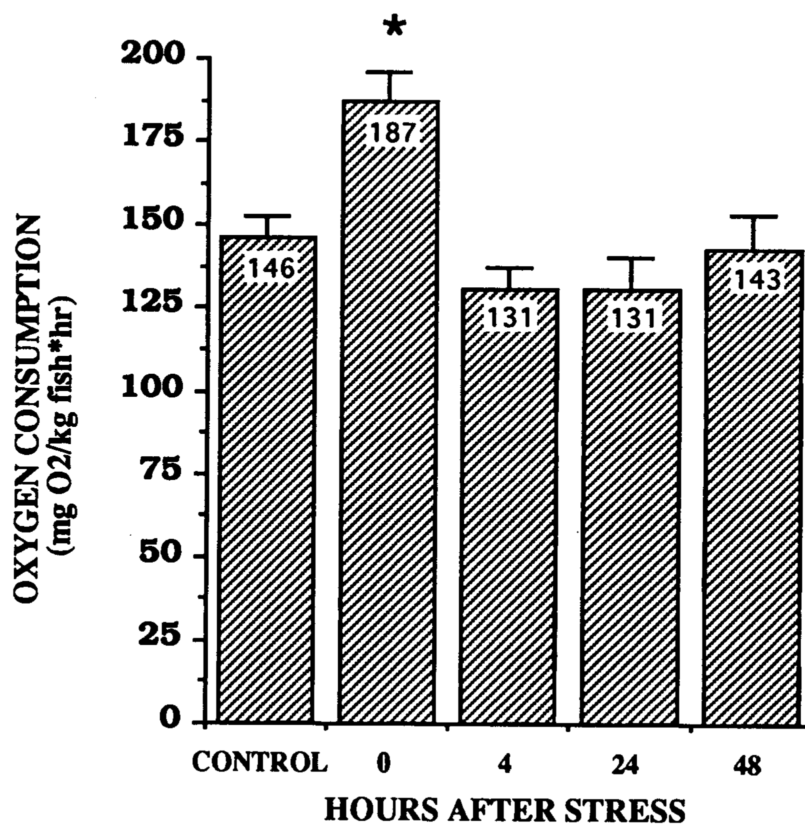


Figure 3: Mean (and SE) rates of oxygen consumption of juvenile coho salmon 0, 4, 24, and 48 h following exposure to a handling stressor. Controls were not given the handling stressor. Mean values (n=10) are shown in each column. The presence of an asterisk indicates that the mean is significantly different ($p \leq 0.05$) from the control mean.

significantly elevated immediately following the stressor to 187 $\pm$ 9.4 $mgO_2kg^{-1}hr^{-1}$, compared to 146 $\pm$ 7.0 $mgO_2kg^{-1}hr^{-1}$ for unstressed fish (ANOVA p=0.0002, Fig. 3). Oxygen consumption returned to control levels by 4 h post-stress and remained at this level until the final sampling at 48 h. The stress group sampled at time zero was given the same treatment as the removal stress group in the previous experiment, but mean oxygen consumption of the stressed fish in this experiment (conducted during spring) was much less than in the previous (fall) experiment (187 vs 267 $mgO_2kg^{-1}hr^{-1}$).

There was no significant difference in plasma cortisol concentration at any sampling time although values ranged from 154 $\pm$ 8.6 ng/ml to 190 $\pm$ 25.1 ng/ml (Table 2). I cannot account for the extremely high cortisol titers observed in the control fish. Immediately following stress there was a significant correlation between plasma cortisol and metabolic rate (p=0.02, r=0.70) but there was no correlation at any other time.

Plasma glucose varied significantly between groups (ANOVA p=0.02). Mean plasma glucose was significantly higher at 4 h post-stress than at 48 h (218 vs 132 mg/dl), but no other pairwise comparisons were significant (Table 2). There was no correlation between glucose and oxygen consumption at any time.

No difference in plasma lactate was observed between groups (Table 2).

Table 2: Mean (and SE) values for plasma cortisol, glucose, and lactate of juvenile coho salmon sampled at 0, 4, 24, and 48 h following exposure to a handling stressor. For cortisol and lactate there are no significant differences among means. For glucose, asterisks indicate the only two means that are significantly different from each other. Sample size for each cell is ten, except for the 24 h sample in which n=9.

Sampling Time (hrs post-stress)	Plasma Cortisol (ng/ml)	Plasma Glucose (mg/dL)	Plasma Lactate (mg/dL)
Control	190 ±25	180 ±24	27.1 ±3.3
0 h	180 ±15	173 ±13	38.2 ±5.4
4 h	171 ±23	218 ±19 *	33.8 ±4.1
(n=9) 24 h	154 ±8.6	179 ±14	36.2 ±2.0
48 h	160 ±24	132 ±10 *	30.1 ±3.3

Lactate Production During Stress

Methods

Measurements of whole body lactate content following a handling stressor were conducted to assess total anaerobic metabolism. Lactate was sampled immediately following and 5 h after the imposition of a removal stressor in two different groups of 8 fish each. A third group of 8 control fish did not receive any additional handling, but were held for 5 h before sampling. Whole body lactate could not be sampled in one control fish, but oxygen consumption data was collected for this individual.

A method was developed to measure whole body lactate, based on the techniques of Bennet and Licht (1972) and Lackner et al. (1988). Fish were killed by injecting concentrated solutions of MS222 and $NaHCO_3$ directly into the swim tube. Delivery volume and concentration of these solutions were regulated to achieve final concentrations of 200 mg/l MS222 and 500 mg/l $NaHCO_3$ within the respirometer. Anesthetizing the animals in this manner helped to limit lactate production resulting from struggling during removal from the swim tube. (A pilot study indicated that fish anesthetized within the respirometer had lower plasma lactate levels then fish removed and then anesthetized. There was no difference in plasma cortisol or glucose between the groups.)

Two minutes after injecting the anesthetic each fish was quickly removed from the respirometer and weighed. Immediately after weighing,

the carcass was homogenized in 500 ml of ice-cold 0.6N perchloric acid using a laboratory blender. It was assumed that the density of the carcass was 1 g/ml, and fish weight (g) was added to the acid volume (ml) to calculate the total homogenate volume. Triplicate samples of homogenate were placed in 1.5ml microfuge tubes and frozen in liquid nitrogen. The samples were stored at -80 ^{0}C until they could be processed further.

Determination of lactate concentration in the homogenates was obtained by thawing the samples and then centrifuging them at 12,800 g for 10 min at a temperature of approximately 5 ^{0}C. Lactate concentration in the supernatant was then measured via the spectrophotometric procedure of Passonneau (1974). Once the lactate concentration of the homogenate had been established, whole body lactate concentration was calculated by the formula:

$$\frac{\text{homogenate lactate concentration X total homogenate volume}}{\text{fish weight}}$$

Oxygen consumption was measured before stress and at 1 h intervals during recovery in order to more precisely measure the period of aerobic recovery from stress and to estimate the excess oxygen consumed following a stressor. After acclimation and swim adjustment the inflow was shut off and water velocity was increased to 0.5 BL/S. Oxygen consumption was measured over the next 30 min, followed by a 30 min period during which

water was flushed through the swim tube. At this point, two treatment groups were given a removal stressor, with controls left undisturbed. Fish in the first stress group were immediately removed from the respirometer and homogenized. Fish in the other stress group were replaced into the respirometer and their oxygen consumption measured over the next 30 min, followed by a 30 min period of flushing. This pattern of 30 min of measuring oxygen consumption followed by 30 min of flushing was repeated until 5 h post-stress. Oxygen consumption in controls was measured at hourly intervals over a similar 5 h period. During this 5 h period water velocity within the respirometer was maintained at 0.5 BL/S. Immediately following the last dissolved oxygen measurement fish were processed for the lactate determination. Two fish were run each day, both from the same treatment group.

Differences in whole body lactate concentration were analyzed with a one-way ANOVA, followed by a Tukey multiple comparison procedure ($p \leq 0.05$). The oxygen consumption data were analyzed using a STATGRAPHICS multiple ANOVA procedure modified for use with a repeated measures design. Following this procedure, mean oxygen consumption of stress and control groups were compared at each sampling time, for a total of seven pairwise comparisons. These comparisons were made manually by using the mean square error term from the multiple ANOVA in a Bonferroni multiple comparison procedure modified for use

with multiple ANOVA (Neter et al., 1985).

Trials were conducted from 24 August 1991 to 4 October 1991 at a temperature of 14 ^{0}C. All fish used in this and the following experiment were 1+ individuals from the 1990 brood. Fish weights ranged from 81.9-156.8 g ($\bar{X}$=118.0).

Results

Fish sampled immediately following a handling stressor had a mean whole body lactate concentration of 6.25 $\pm$ 0.84 μmol lactate/g fish, a concentration nearly five times the control level of 1.27 $\pm$ 0.17 μmol lactate/g fish (Figure 4). This was a highly significant difference (ANOVA $p<0.0001$, data log transformed due to unequal variances). Whole body lactate in individuals stressed but allowed to recover for 5 h was not different from the control level (Fig. 4).

After exposure to the handling stressor the fish experienced rates of oxygen consumption that were typically about 250% higher than before the stress and those observed in the control fish (multifactor ANOVA overall $p<0.0001$, treatment main effect $p<0.0001$; Figure 5). There was no difference in oxygen consumption between control fish and treatment fish measured just prior to the onset of stress. At 1 h post-stress, mean oxygen consumption was no longer significantly different from controls (Fig. 5).

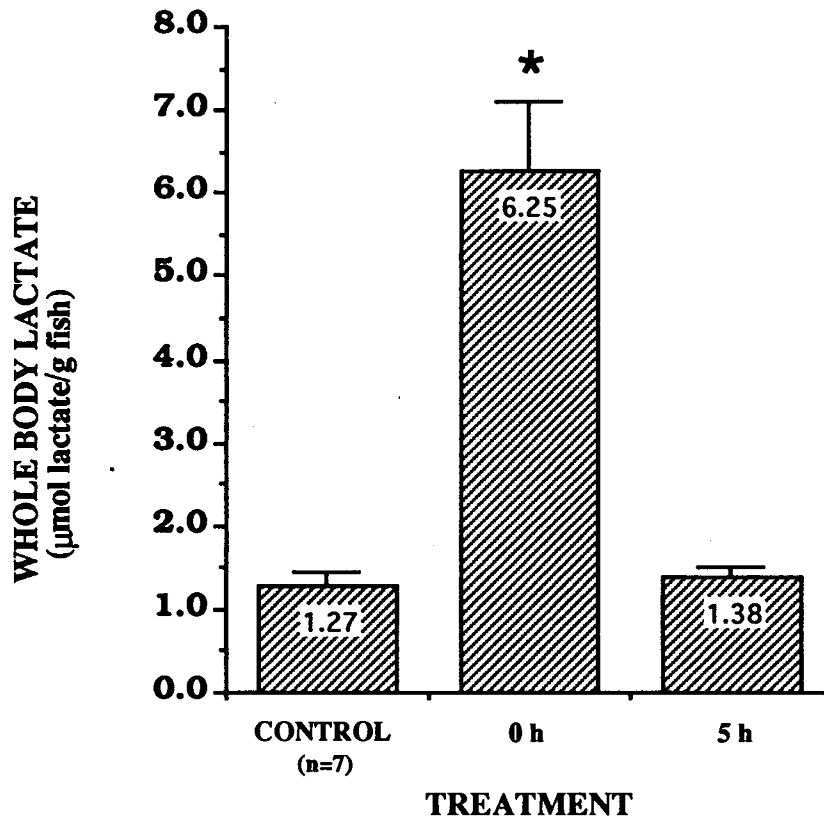


Figure 4: Mean (and SE) whole body lactate concentration in juvenile coho salmon subjected to one of three protocols:

Control: no handling stressor given

0 h: given a handling stressor and sampled immediately

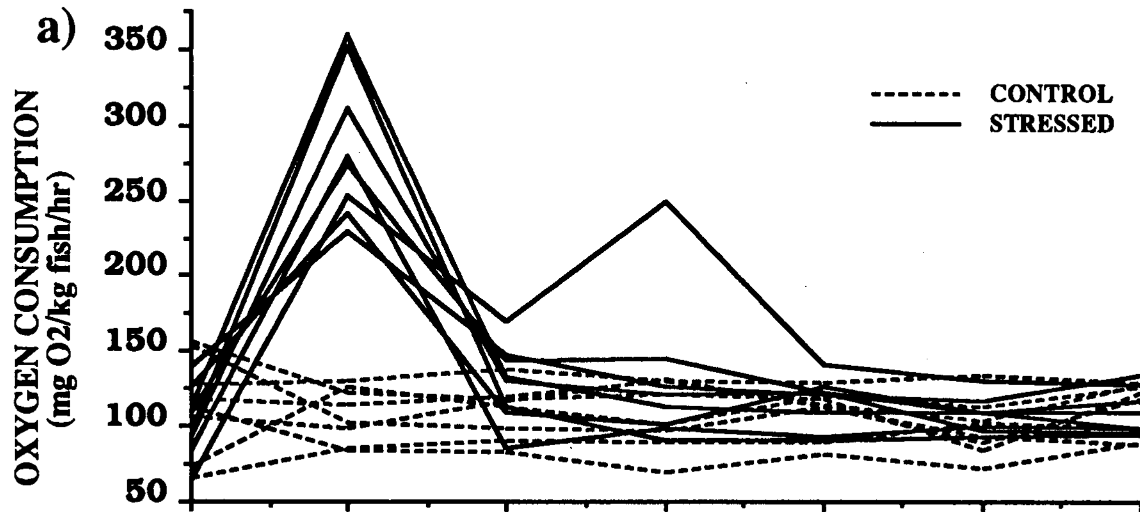
5 h: given a handling stressor and sampled 5 h later

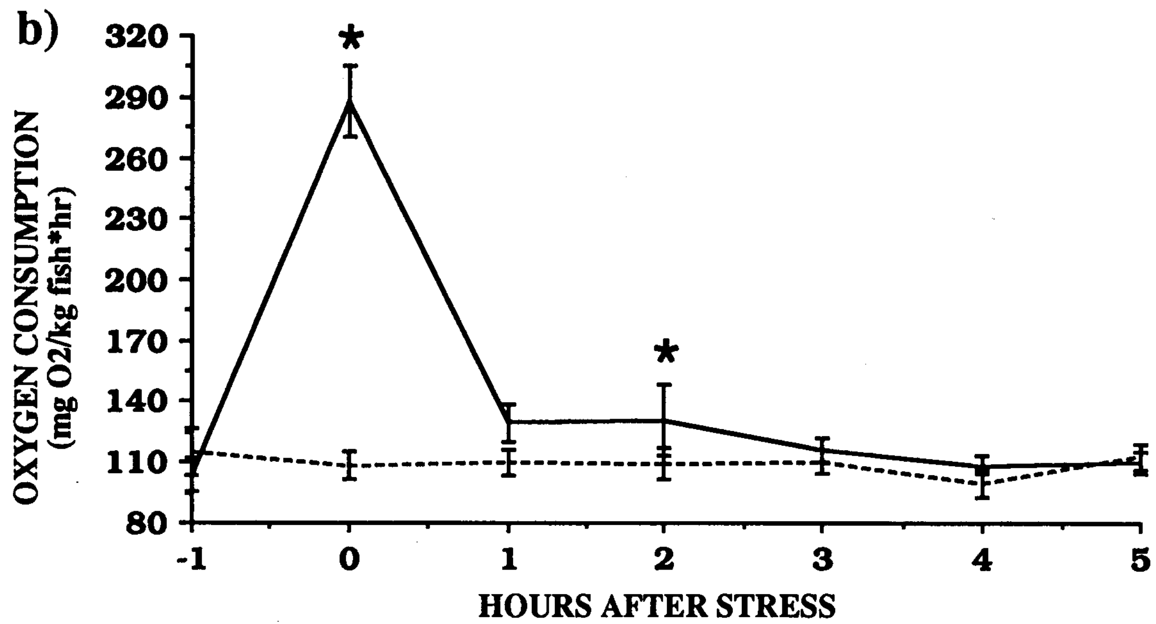
Mean values are shown in each column; the presence of an asterisk indicates that the mean is significantly different ($p \leq 0.05$) from the control value. Sample size is eight unless marked otherwise.

Figure 5:

a) Rates of oxygen consumption of individual juvenile coho salmon sampled at hourly intervals beginning 1 h before and ending 5 h after the imposition of a brief handling stressor. Controls did not recieve a handling stress.

b) Mean (and SE) rates of oxygen consumption of the juvenile coho salmon shown in part (a). Means (n=8) marked with an asterisk are significantly higher ($p \leq 0.05$) than the corresponding control mean.


a)
OXYGEN CONSUMPTION
(mg O2/kg fish/hr)
350
300
250
200
150
100
50
CONTROL
STRESSED


b)
OXYGEN CONSUMPTION
(mg O2/kg fish*hr)
320
290
260
230
200
170
140
110
80
*
*
-1
0
1
2
3
4
5
HOURS AFTER STRESS

Conversely, at the next sampling time mean oxygen consumption of stressed fish was significantly higher than controls (2 h post-stress, Fig. 5). This observation may have been the result of a high value seen in a single individual at this sampling time (Fig. 5a). After 3 h post-stress there were no significant differences in oxygen consumption between stressed and control groups.

Influence of Cortisol on Oxygen Consumption

Methods

Exogenous cortisol was administered in an attempt to quantify any direct effect of cortisol on oxygen consumption. One group of 11 fish received an implant of 30 mg cortisol/kg fish while a second group of eleven received a sham implant. A cortisol dosage of 30 mg/kg was chosen following a dose response experiment and was designed to elevate plasma cortisol to levels in the range of 200-300 ng/ml.

Cortisol was administered via a coconut oil implant injected into the body cavity. Crystalline cortisol was combined with liquid coconut oil to form a solution/suspension. Controls received a sham implant of pure coconut oil. Each fish received 0.1 ml of liquid (temperature 35-40^{0}C) implant media/kg body weight, which solidified within seconds after injection.

Implantation occurred at the time when they were loaded into the respirometers, and the fish were acclimated and given a swim adjustment as usual. No additional handling stressor was given to the fish, and oxygen consumption was measured in a 1 h respirometry trial. After the trial the fish were killed and bled.

Previous experiments had suggested that the variation in oxygen consumption was smaller between fish run on the same day compared to fish run on different days, so one treated and one control fish were run each day. This way, if the data were found to be paired according to the date of trial, a pairwise statistical analysis could be used.

A pairwise t-test was used for analysis of the oxygen consumption data, since a scatter plot analysis indicated it was appropriate. An unpaired t-test gave the same results, however. Plasma cortisol, glucose, and lactate were all compared using a simple t-test, as the data did not appear to be paired. Correlations between variables were determined using a simple regression analysis.

Trials were conducted from 20 November 1991 to 7 December 1991, with temperature varying from 13.0-13.2 ^{0}C. Fish weights ranged from 78.2-196.2 g ($\bar{X}$=130.5 g).

Results

There was no difference in mean oxygen consumption between fish implanted with cortisol and those given a sham implant (Table 3). No correlation between plasma cortisol titer and metabolic rate was seen in either group. Plasma cortisol was significantly elevated in implanted fish, with levels ranging from 153-547 ng/ml ($\bar{X}$=299), as compared to an average level of 71.2 ng/ml in the control group (Table 3).

Plasma glucose and lactate appeared unaffected by the cortisol treatment (Table 3), and neither variable was correlated to oxygen consumption.

Table 3: Mean (and SE) values for oxygen consumption, and plasma cortisol, glucose, and lactate for juvenile coho salmon implanted with 30 mg/kg cortisol, or given a sham implant conataining no cortisol. Means ($n=11$) marked with an asterisk are siginificantly different than the corresponding sham implant value ($p<0.05$).

Treatment	Oxygen Consumption (mgO2/kg*hr)	Plasma Cortisol (ng/ml)	Plasma Glucose (mg/dL)	Plasma Lactate (mg/dL)
Sham Implant	**110 ±2.3**	**71 ±16**	**144 ±15**	**24 ±1.8**
Cortisol Implant	**130 +11.1**	**299 ±36 ***	**139 ±22**	**21 ±1.7**

DISCUSSION

Exposure to acute handling stressors increased oxygen consumption in coho salmon. The magnitude of this response was dependent on the nature of the stressor, but all of the stressors significantly elevated metabolic rate. The average increase in oxygen consumption following various stressors ranged from 55 to 137 $mgO_2kg^{-1}hr^{-1}$, which represented 12% to 30% of the scope for activity as estimated for other juvenile salmonids of similar size and acclimation temperature (Dickson & Kramer, 1971; Dwyer and Kramer, 1975). It appears that immediately following a stressor the cost of elaborating a stress response accounts for a significant fraction of the total energy available for non-basal function.

It is unknown what qualities of a stressor are most important in influencing the increase in oxygen consumption, but removal from the water may be a factor. All four of the stressors used in the "various stressors experiment" differed in both length and intensity of struggling induced in the fish, but statistically only two groups were recognized: the treatments in which the swim tube was lifted into an upright position (one or three times), and the treatments in which the fish were removed from the water (for 30s or placed in a live well). One important difference between these two groups was that the second involved removing the fish from the water and the first did not. The intensity of struggling during the stressor may also be a factor since individuals removed from the water typically struggled much harder

than those remaining submerged.

Oxygen consumption did not differ in fish subjected to either one or three upright positionings of the swim tube, indicating repeated stressors had no cumulative effect on metabolic rate. This finding may be the result of the experimental procedure, however. A thirty minute period separated each of the three repeated stressors, and this may have been enough time to allow recovery from one stressor to the next. If the time between stressors had been shorter, it is possible that a cumulative effect on oxygen consumption might have been observed.

The magnitude of elevation in oxygen consumption following stress appears to vary seasonally. The first two experiments were separated by a period of several months; the first was from mid-October to late November, and the second from the beginning of April to mid-July. Although mean oxygen consumption of control groups was similar in both the fall and spring (140 vs 146 $mgO_2kg^{-1}hr^{-1}$), stress-induced elevation in oxygen consumption was lower in the springtime experiment (267 vs 187 $mgO_2kg^{-1}hr^{-1}$). The increase in oxygen consumption due to the removal stressor was 127 $mgO_2kg^{-1}hr^{-1}$ in the fall, but 41 $mgO_2kg^{-1}hr^{-1}$ in the spring.

This effect may be associated with smoltification. The fish used had smolted the year before, but may have gone through a second smoltification process during the course of the spring experiment. Mean concentration of plasma cortisol of controls in the springtime was much higher than that in

the fall (190 vs 78 ng/ml). Increase in cortisol titer is typical of the smoltification process (Specker & Schreck, 1982; Barton et al., 1985). The increase in standard metabolic rate during smoltification observed in Atlantic salmon (*Salmo salar*) by Maxime et al. (1989) and Higgins (1985), and the decrease in swimming stamina seen by Flagg et al. (1983) further suggests the possibility of a change in the energetic response to stress during smoltification. It is possible that the energy used for the parr-smolt transformation is not available for use in elaborating an energetic response to stress (Schreck, 1982), or that the metabolism of smolting fish is altered in some way that limits the change in metabolic rate.

Although oxygen consumption increased dramatically after an acute stressor, the effect was short lived. The increase in oxygen consumption was largely eliminated during the first hour post-stress, with oxygen consumption back to control levels within 3 h post-stress. This recovery period is brief compared to plasma variables, which may remain elevated 24 h or more following an acute stressor (Hille, 1982).

It is possible that the increased oxygen consumption resulting from stress is eliminated in two phases, with oxygen consumption rapidly decreasing during the first hour post-stress, and more slowly decreasing over the next two or more hours. Although the data are equivocal as to whether oxygen consumption actually remains elevated beyond the first hour post-stress, biphasic recovery curves have been seen in other studies. Wieser

et al. (1989) reported a two-stage aerobic recovery in rainbow trout exposed to a 60 s electroshock. In addition, a biphasic reduction in oxygen consumption is characteristic of recovery from strenuous exercise in mammals and reptiles (Gaesser and Brooks, 1984).

Lactate production following exposure to a stressor is similar to that seen after exercise. We observed that immediately following an acute handling stressor, whole body lactate concentration increased to nearly five times the value seen in controls. Whole body lactate also increases dramatically following exercise. After exhaustive exercise, whole body lactate in untrained 1-2 g chub (*Leuciscus cephalus*) increased 16 times beyond control levels (Lackner et al., 1988). The mean post-exhaustion lactate level seen in Lackner et al.'s study was nearly identical to the post-stress mean seen by me (6.28 vs 6.25 μmol/g). Another study found that 10 minutes after 60 s of mild electroshock (accompanied by intense exercise) whole body lactate increased by 262% in 80-1000 mg rainbow trout (Wieser et al., 1985). Similar increases in lactate have been seen in a number of reptile and amphibian species following exercise (Bennett, 1978).

When the amount of lactate produced as a result of stress is compared to the excess oxygen consumed, a situation similar to exercise is again seen. The classical studies of post-exercise oxygen consumption observed that the excess oxygen consumed following exercise could account for the oxidation of 1/6 to 1/3 of the lactate produced (reviewed in Gaesser and Brooks, 1984). I

estimate that the increase in oxygen consumption following stress could oxidize 18%, or somewhat more than 1/6 of the lactate produced. This estimate was derived by first calculating the average amount of oxygen consumed as a result of stress (2.677 μmol O_2/g). This value was multiplied by a conversion factor of 1μmol lactate oxidized/3μmol O_2 consumed to get 0.892 μmol lactate/g that could potentially be oxidized. Finally, the total amount of lactate that could possibly be oxidized was divided by the amount of lactate actually produced following a stressor (4.98 μmol lactate/g - the difference between mean control and stressed whole body lactate concentrations). The result is the fraction of lactate produced that could be potentially oxidized (0.18, or 18%).

The mechanism of the stress-induced increase in oxygen consumption remains unclear. From my results, it seems unlikely that cortisol in itself plays a major role in this phenomenon. Although plasma cortisol was significantly correlated to oxygen consumption following the relatively mild stressors, there was no correlation for the more severe stressors. Elevating plasma cortisol through the use of implants did not cause significant changes in oxygen consumption. This lack of significance may be due in part to an insufficient sample size, or to the stress associated with confinement within the respirometer. Plasma cortisol levels in the control groups were typical of that seen in response to a moderate stressor. It is possible that a maximal

respiratory response to cortisol was already present in both control and implant groups; further increases in plasma cortisol would then have no effect. At this time, however, it still seems unlikely that cortisol alone has a major effect on oxygen consumption.

Although only a small amount of data concerning the energetics of stress is available, it seems possible that stress and exercise induce generally similar energetic responses, and could therefore have analogous mechanisms. Unfortunately, the metabolic basis of excess post-exercise oxygen consumption (EPOC) is not well understood. It has long been believed that the excess oxygen was used to remove lactate generated during exercise, but mounting evidence indicates that the EPOC is not directly linked to lactate (Gaesser & Brooks, 1984).

Gaesser and Brooks (1984) argue that the cause of the EPOC must be found among those factors that influence mitochondrial respiration following exercise. They suggest that direct control of mitochondrial respiration is via concentrations of organic phosphates, particularly adenosine triphosphate (ATP), adenosine diphosphate (ADP), inorganic phosphate (Pi), and creatine phosphate. Indirect control of respiration may be mediated by many factors including catecholamines, thyroxine, glucocorticoids, fatty acids, calcium ions, and temperature. An analysis of mitochondrial energetics under the conditions of stress might expand our understanding of the metabolic basis of increased oxygen consumption

following handling.

In conclusion, I found that short-term acute stressors can result in a doubling in oxygen consumption, with the magnitude of the increase related to the severity of the stressor. The metabolic response to stress appeared to vary seasonally, with less of an elevation in metabolic rate as seen in the spring as compared to the fall. Increases in metabolic rate following an acute stressor were of short duration, being largely eliminated within an hour after the initiation of stress. I estimate that the increase in oxygen consumption following stress may account for as much as one third the scope for activity, indicating stressed fish could have reduced energy available for other activities like finding food or avoiding predators. In light of this possibility I suggest that following stress a recovery period of no less than 1 h be allowed before placing fish in a hostile environment. This practice could be especially helpful in increasing the survival of hatchery fish released into the natural environment.

The mechanism of excess post-stress oxygen consumption requires more study. Similarities between the energetic responses to both stress and exercise suggest that the results of exercise physiology may provide a basis for understanding the energetic response to stress.

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