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Richard J. Strange, Major Professor

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SLIGHT STRESS DOES NOT LOWER CRITICAL THERMAL MAXIMUMS IN HATCHERY-REARED RAINBOW TROUT

A Thesis

Presented for the

Master of Science

Degree

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Roger B. Petrie

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ABSTRACT

This study evaluated the physiological response of rainbow trout to various initial stressors, either confinement or electroshock, followed by a heat challenge in order to determine how the critical thermal maximum of the fish was affected by the intensity of the initial stressor. Plasma cortisol and glucose levels were determined following the initial stressor and the challenge stressor. The initial stressor induced a typical, but mild, response by increasing cortisol levels to 68 ng/mL for the electroshocked group and 112 ng/mL for the 60-minute confinement group. Although these increases are significantly different ($P < .05$) from the baseline group (9 ng/mL) they are lower than values observed in the literature for similar stressors. The challenge stressor did not induce any further cortisol increase in fish receiving an initial stress, and in fact decreased significantly ($P < .05$). The electroshock-challenged group had a significantly higher critical thermal maximum (30 C), but this was only 0.9 C higher than the lowest critical thermal maximum.

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CHAPTER I

INTRODUCTION

The widespread propagation of rainbow trout (Oncorhynchus mykiss) in fish hatcheries has led to extensive research concerning the effects of stress on this species. Routine management procedures involved in the culture and stocking of these fish have been shown to induce stress. Therefore, it is imperative that the physiological mechanisms and responses to these stressors be fully understood so that proper compensatory efforts can be taken to minimize the stress placed upon the fish.

The first comprehensive analysis of stress as it pertained to vertebrates was in the 1950's. Selye (1950) described stress, which he termed the General Adaptation Syndrome, as any one of a number of physiological changes in an organism which results from a noxious change in its physical environment. The General Adaptation Syndrome develops in three sequential stages. The first stage is the Alarm Reaction, which is marked in fish by the increase in levels of plasma cortisol, plasma glucose, and a decrease in plasma chloride, as well as changes in levels of other chemicals in the blood. The second stage is the Stage of Resistance, which is marked by a decrease in cortisol and glucose plasma levels and an increase in chloride plasma levels. During this stage

the organism is attempting to adapt to the new conditions. If the organism fails to adjust to these conditions then the third stage, which is the Stage of Exhaustion, follows. During this stage the organism exhausts itself trying to maintain a homeostatic state within noxious conditions. During this stage the cortisol and glucose plasma levels will increase again and the chloride plasma levels will decrease as the organism attempts to survive the stressor. In current terms this has become known as the stress response (Selye 1950).

Wedemeyer (1970) concluded that with stress comes a reduction in the overall fitness of a fish which in turn can make it vulnerable to epizootic infection and possible death. Due to the crowded conditions in hatcheries, these results indicated a need for further study into the causes and effects of stress in fish.

From the beginning, fry and fingerlings are subjected to stress in a hatchery. Handling and confinement have been shown to elicit a stress response in rainbow trout fingerlings (Barton et al. 1980) resulting in an increase in plasma cortisol levels. Other Salmonid species have been shown to exhibit a stress response when confined. Wedemeyer (1972, 1976) showed that plasma chloride levels were decreased and blood glucose levels were elevated when juvenile coho

salmon (O. kisutch) were confined. Confinement of brown trout (Salmo trutta) caused an elevation in plasma cortisol levels as well as plasma lactate levels followed by an increase in plasma glucose concentrations (Pickering et al. 1981). Similar treatment of juvenile chinook salmon (O. tshawytscha) caused elevation of plasma cortisol concentrations to over 200 ng/mL (Strange and Schreck 1978; Strange et al. 1978). Some warm water species have also been examined for responses to confinement. Channel catfish (Ictalurus punctatus) has exhibited increased levels of corticosteroids in response to net confinement (Tomasso et al. 1983). Carmichael et al. (1984a) elicited increases in corticosteroid and glucose levels and a decrease in chloride concentrations of largemouth bass (Micropterus salmoides) when they confined the fish for 60 minutes. Even marine fish have been shown to exhibit a stress response. Caldwell and Tomasso (1985) showed that red drum fingerlings (Sciaenops ocellatus) responded to confinement with elevated glucose and chloride levels. The chloride levels in marine fish respond in the opposite manner of those in freshwater fish due to the fact that in a marine situation the water has a higher osmolality than the fish, where as in freshwater the situation is the reverse.

Once the fish have been grown to the appropriate

size they are transported and stocked out in predetermined locations. Specker and Schreck (1980) showed that the effects of transportation on coho salmon smolts caused an elevation of both cortisol and glucose concentrations. Similar results were obtained when fingerling rainbow trout were subjected to transport and stocking stressors (Barton and Peter 1981). In this study plasma cortisol levels were measured and shown to be elevated after application of the stressor. Carmichael et al. (1984b) demonstrated that largemouth bass respond to transport stress with increased cortisol and glucose concentrations and decreased chloride concentrations. Red drum fingerlings were tested for a stress response to transport and showed elevated glucose and chloride levels (Caldwell and Tomasso 1985).

One stressor that affects both wild and hatchery fish is water quality. The presence of poor water quality has been shown to induce stress in fish. Barton and Peter (1981) found that the lower water quality associated with transportation of fish, notably the decrease in dissolved oxygen, caused increases of cortisol concentrations in fingerling rainbow trout. Decreased dissolved oxygen was also determined to elicit a stress reponse from largemouth bass by increasing the levels of cortisol and glucose and decreasing the levels of chloride (Carmichael et al. 1984a). A later study by

Matthews et al. (1986) determined that the increasing salinity of the water in the down stream migration of spring chinook salmon smolts was related to increases in their mortality during this time. Low concentrations of copper have been shown to elevate serum cortisol levels in juvenile coho salmon (Schreck and Lorz 1978). Hill and Fromm (1968) determined that exposure of rainbow trout to low concentrations of chromium caused increases in plasma cortisol levels. A sublethal dose of the insecticide endrin has been shown to increase plasma cortisol and plasma glucose in rainbow trout (Grant and Mehrle 1973). Yaron and Ilan (1974) showed that a sublethal dose of the insecticide o'p'-DDD caused and elevation of plasma cortisol in carp (Cyprinus carpio). Some naturally occurring events, such as spawning, have also been shown to increase cortisol concentrations in pink salmon (O. gorbuscha) as they migrate upstream. (McBride et al. 1986). Strange et al. (1977) demonstrated that rapid increases in temperature caused cortisol levels in juvenile cutthroat trout to become elevated.

Almost all sampling techniques induce some kind of stress. Seining subjects fish to handling and confinement stress which, as stated above, can cause a stress response. Other sampling techniques such as electroshocking can also cause cortisol concentrations to

become elevated in rainbow trout (Schreck et al. 1976; Woodward and Strange 1987). Wydoski et al. (1976) found that the hooking associated with angling stress caused increases in plasma glucose levels and a decrease in plasma chloride levels. This study has wide ranging implications due to the growing importance of "catch-and-release" fishing.

The effects of these stressors are just now beginning to be understood. Most research is presently being directed toward the measurement of hematological parameters to gauge stress. These measurements include glucose, chloride, and corticosteroid levels in the plasma.

Of the corticosteroids present, the level of cortisol in the blood is the most indicative of the stress response. Also, it is considered one of the most important steroids in fish (Seal and Doe 1965; Freeman and Idler 1966, 1971; Idler and Truscott 1972; Goodman and Butler 1976). This is due to the fact that cortisol levels in fingerling rainbow trout can be significantly elevated by as little as 90 seconds of handling and confinement (Barton et al. 1980). Since cortisol levels can remain elevated for as long as two days after the application of the stressor, it can be used to determine stress over a longer time period than some other methods, such as plasma glucose which usually returns to basal

levels more rapidly than plasma cortisol levels (Barton and Peter 1981).

Since cortisol is a glucocorticoid, it possesses the property of increasing energy metabolism in an individual (Leach and Taylor 1980). This increased energy metabolism is reflected by increased plasma glucose levels, which can be measured and used to determine stress in a similar manner to plasma cortisol levels (Chavin and Young 1970; Wedemeyer 1972, 1976; Wydoski et al. 1976; Woodward and Strange 1987).

A third blood parameter which can be used as an indicator of stress is plasma chloride level. Studies have shown that fish subjected to a stressor have lower plasma chloride levels than fish which have not been stressed (Mazeaud et al. 1977; Nikinmaa et al. 1983; Davis and Parker 1983; Redding and Schreck 1983). The chloride pump in the gills of fish are suspected to function at a lower efficiency than normal during stress. This would be compounded by the greater ventilation volume during stress which would increase the rate of water imbibition. This means that fish stressed in freshwater tend to gain water and lose electrolytes, which accounts for the lower levels of plasma chloride in stressed fish.

One area of stress physiology which has remained relatively unexplored is the cumulative effects of

stressors. This is the situation in which an initial stressor influences the performance of a fish subjected to a subsequent stressor. This is of practical importance due to the fact that in a natural environment a fish is likely to be affected by more than one stressor (Wedemeyer and McLeay 1981). The aim of this study is to determine whether sublethal stress, as measured by blood parameters, can be related to the ability to perform when a fish is subjected to a challenge. The following experiments will attempt to reveal a relationship between the initial stressor and the secondary, or challenge, stressor.

CHAPTER II

METHODS

Data Collection

The following experiments were based on a general design consisting of four groups. The baselines were fish that were unstressed. The group Treatment 1 were fish that received the initial stressor but not the challenge stressor. The group Treatment 2 did not receive the initial stressor but did receive the challenge stressor. The group Treatment 3 received both the initial stressor and the challenge stressor. In these experiments the initial stressor was either 30-minutes confinement, 60-minutes confinement, or electroshock, while the challenge stressor was a thermal challenge in all experiments (Table 1).

On 16 March 1989, 200 fingerling rainbow trout were obtained from the Tennessee Wildlife Resources Agency fish hatchery at Buffalo Springs, Tennessee. These fish were placed in a 850 liter circular fiberglass tank in the Fisheries Research Laboratory at the University of Tennessee, Knoxville. The water temperature in the tank was maintained at approximately 15 C throughout the 4 week acclimation period. Water flow through the tank was from two sources. One source was tap water filtered

Table 1. Experiment dates and treatment groups

Date	Treatment Group	Initial Stressor	Challenge Stressor
4/12/89	Baseline	None	None
	Treatment 1	30 minute confinement	None
	Treatment 2	None	Heat
	Treatment 3	30 minute confinement	Heat
4/13/89	Baseline	None	None
	Treatment 1	30 minute confinement	None
	Treatment 2	None	Heat
	Treatment 3	30 minute confinement	Heat
4/14/89	Baseline	None	None
	Treatment 1	Electroshock	None
	Treatment 2	None	Heat
	Treatment 3	Electroshock	Heat
4/18/89	Baseline	None	None
	Treatment 1	Electroshock	None
	Treatment 2	None	Heat
	Treatment 3	Electroshock	Heat
4/19/89	Control 1	None	None
	Control 2	30 minute confinement	None
	Control 3	90 minute confinement	None
11/8/89	Baseline	None	None
	Treatment 1	60 minute confinement	None
	Treatment 2	None	Heat
	Treatment 3	60 minute confinement	Heat
1/4/90	Baseline	None	None
	Treatment 1	60 minute confinement	None
	Treatment 2	None	Heat
	Treatment 3	60 minute confinement	Heat

through a charcoal filter to dechlorinate the water. The other source was from a biological filter which treated water drawn from the tank. Fish were fed a maintenance diet one half of that recommended for growth by Leitritz (1972). The feed was 40 mm pellets composed of 38% crude protein and 8% fat, and was obtained from Zeigler Brothers, Incorporated.

Blood samples were collected by caudal sectioning. Blood was drawn by placing a heparinized Caraway micro blood collecting tube up to the caudal artery and allowing blood to fill the Caraway tube. The Caraway tube was then drained into a Fisher 1.0 ml polystyrene centrifuge tube. Samples were centrifuged in a Fisher Model 59 centrifuge at 2500 g for 5 minutes. Plasma was drawn from the centrifuge tubes using diSPo Pasteur-type soda lime glass pipets and then placed into 1.2 ml Corning cryogenic vials. The samples were then frozen at -8 C.

On 12 April 1989, 15 fish were removed from the tank. Five of these fish were immediately placed in a 20 L cooler containing a solution of 400 ppm tricaine methanesulfonate (MS-222). These were then removed from the MS-222 and bled, weighed, and measured. The remaining 10 fish were placed in a confinement net. The net was adjusted so that the fish were in contact with each other and their dorsal fins were exposed just above

the water surface. The fish remained in the confinement net for 30 minutes. At the end of 30 minutes of confinement, 5 fish were placed in the MS-222 and then bled, weighed, and measured, as before, while the other 5 fish were placed in a 2.1 m long Living Stream tank (Frigid Units, Inc., Toledo, Ohio) containing 314 L of 15 C water which contained 6 Safe-Hete electric immersion-type water heaters (Electra, Inc., Chicago, Ill.). Five more fish from the acclimation tank were placed in the Living Stream, which had been divided so as to separate those fish which had been confined from those which had not been confined. Water in the Living Stream was circulated by means of a Coolarator unit (Frigid Units, Inc., Toledo, Ohio) placed in the Living Stream. Once the fish were in place in the Living Stream, the heaters were started; the original water temperature was 15 C. Water temperature was recorded every 5 minutes. As the temperature approached the critical thermal maximum, after approximately 50 minutes of heating, the fish began to lose equilibrium. As each fish exhibited a sustained loss of equilibrium, it was removed from the Living Stream and placed in the MS-222. The water temperature at which each fish lost equilibrium was also recorded. The fish were removed from the MS-222 and bled, weighed, and measured. After completion of this procedure the water in the Living Stream was replaced as was the MS-222

solution. A second replication was run on 12 April following the same protocol as in the first replication. On 13 April 1989, a third and fourth replication of the experiment were done using the same protocol as that of 12 April.

On 14 April 1989, 15 fish were removed from the acclimation tank. Five of these fish were placed into a 20 L cooler containing a solution of 400 ppm MS-222. These were bled, weighed, and measured. The remaining 10 fish were placed in the Living Stream, which contained 314 L of 15 C water, in two groups of 5 fish each. Both groups were electroshocked (220 V AC) followed 5 minutes later by another electroshock. The fish were allowed to recover for 25 minutes. After the recovery period, 5 fish were removed, placed in the MS-222, and then bled, weighed, and measured. The 5 remaining fish were left in the Living Stream. Five fish from the acclimation tank were placed in the Living Stream. The electroshocked fish and the fish from the acclimation tank were kept in separate compartments in the Living Stream. At this point, the heaters were turned on. Fish were removed from the Living Stream as each exhibited a sustained loss of equilibrium; the water temperature was recorded at that point. Once removed, each fish was placed in the MS-222 and bled, weighed, and measured. A second replication of this experiment was conducted using the

same protocol. Water in the Living Stream as well as the MS-222 solution was changed after each replication. On 18 April 1989, two more replications of the 14 April experiment were conducted following the same protocol as that of the 14 April experiment.

On 19 April 1989, an experiment was conducted to determine the effects of the Living Stream without heating. Five fish were removed from the acclimation tank and placed in a cooler containing a solution of 400 ppm of MS-222 then bled, weighed, and measured. Another 10 fish were removed from the acclimation tank and placed in the Living Stream. After 30 minutes, 5 fish were removed and placed in the MS-222 then bled, weighed, and measured. The remaining 5 fish were removed after 90 minutes and placed in the MS-222 then bled, weighed, and measured.

On 8 November 1989, 5 fish were removed from the raceways of the Buffalo Springs hatchery. They were placed in a 20 L cooler containing a solution of 400 ppm MS-222, then bled and measured. Another 10 fish were removed from the raceways and placed in a confinement net such that the fish were side by side and the dorsal fins were exposed just above the water surface. The fish were kept in the confinement net for 60 minutes, after which 5 of the fish were placed in the MS-222, then bled and measured. The remaining 5 fish were placed in the Living

Stream which contained 400 L of 15 C water. This extra volume of water caused the heating period to be extended and in turn decreased the rate of temperature increase. An additional 5 fish were removed from the raceways and placed in the Living Stream. Both groups of fish were kept in separate compartments of the Living Stream; at this point, the immersion heaters were turned on. After the fish exhibited a sustained loss of equilibrium, the water temperature was recorded and each fish placed in the MS-222, then bled and measured. Two additional replications of the experiment were done using the same protocol as the first replication. The MS-222 solution was changed after each replication as was the water in the Living Stream. Since bleeding took place prior to measuring, the lengths were rounded to the nearest 5 mm.

On 4 January 1990 another 3 replications of the 8 November experiment took place. The only difference was that the Living Stream contained 314 L of water instead of 400 L as in the previous replications.

Laboratory Analyses

Plasma samples were analyzed in the Animal Science Laboratory at the University of Tennessee, Knoxville. The plasma samples were analyzed to determine levels of cortisol, glucose, and chloride levels.

Plasma cortisol levels were determined using the

Diagnostic Products Corporation Coat-A-Count RIA kits (Diagnostic Products Corp., Cat. No. TKCO5). The assay was based on the competitive antibody binding between known quantities of radioactive-labeled cortisol and non-labeled indigenous cortisol in of the plasma samples. The assay used Iodine 125-labeled cortisol as the radioactive tracer to determine percent binding over a range of control standards of 10, 50, 100, 200, and 500 ng/mL. In addition to the provided standards, an internal standard consisting of pooled striped bass plasma (253 ng/mL $\pm$ 6.87, mean $\pm$ std. err.) was used to validate each assay. The mean intraassay coefficient of variation for the pooled sample was 4.5%. The interassay coefficient of variation was 9.4%. Radioactive levels and final cortisol concentrations were determined using a conventional gamma counter. Cross reactivity of the antibody with other steroids in fish, besides cortisol, was less than 1.6%, as determined by the manufacturer. The highest cross reactivity measured was with 11-deoxycorticiosterone, which was 1.5%. Corticosterone had a cross reactivity of 1.4%, cortisone was 0.6%, 11-deoxycortisol was 0.25%, and aldosterone was 0.01%. The lowest was with 17 -hydroxyprogesterone, which was nondetectable. Effects of anticoagulants, such as heparin and EDTA, were also shown by the manufacturer to have no significant interference with the assay. A

description of the laboratory procedure is included in Appendix 1.

Plasma glucose levels were determined using glucose kits (Sigma Diagnostics Corp., Cat. No. 510). This is a colormetric assay (460 nm) based on glucose oxidase/peroxidase reactions with o-dianisidine serving as chromogenic indicator. A description of the laboratory procedure is included in Appendix 2.

Plasma chloride levels were determined using chloride kits (Sigma Diagnostics Corp., Cat. No. 461). This is also a colormetric assay (460 nm) but is based on the reaction of chloride with mercuric thiocyanate. The chloride ions displace thiocyanate which then forms a red complex with ferric ions. A description of the laboratory procedure is included in Appendix 3.

Statistical Analyses

The statistical data analysis was performed on an IBM PS/2 using PC-SAS (SAS Institute Inc., Cary, NC). Each sample was assigned a day variable according to which day it was taken, a repitition variable depending on which repitition of that day it came from, and a treatment variable depending on what treatment it received. The data were processed using a General Linear Model on PC-SAS. The model used is as follows:

$$Y_{ijkl} = B_0 + B_1*DAY_i + B_2*REP_j + B_3*TRT_k + E_{ijkl}$$

where y is equal to temperature, cortisol concentration, glucose concentration, length, or weight. The variable DAY represents the day on which samples were taken. The variable REP signifies which repitition the sample was taken from, and the variable TRT represents which treatment the sample received. The effect of length was analyzed by substituting the variable LENGTH into the model in place of REP. To determine significant differences between treatment groups, orthogonal contrasts were run to compare groups of interest. The effect of weight data was not analyzed since values for this variable are incomplete.

CHAPTER III

RESULTS

Results were deemed to be significant at the 95% confidence level ($P < .05$). In some instances, where results were considered close to being significantly different, these were noted.

Analysis of the length data showed that there were significant differences between the fish sampled during April 1989 and those sampled on the two later dates. This can be attributed mainly to the fact that in April the fish were only about 6 months old while the fish on the later two dates were about 12 months old. No relationship was seen to exist between the length data and either the blood parameter data or the performance data. Analysis of the length data according to which day sampling took place showed that fish on 4 January 1990 were significantly longer than those on all other dates ($P < .05$) and that fish on 8 November 1989 were significantly longer than fish from the April 1989 sampling dates ($P < .05$). Fish sampled on 19 April were longer than those from 12-14 April. Fish from 12 and 18 April were significantly longer than those sampled on 14 April ($P < .05$). Raw data values for length are shown in Appendix 4.

Analysis of weight showed that no relationship

existed between treatments and mean weights. Comparisons of weights by day showed that fish from 19 April were heavier than those from 12-14 April and that the fish from 18 April were heavier than those from 13-14 April. Mean lengths and mean weights and the associated standard errors were calculated for each experiment date (Table 2). Raw data values for these are shown in Appendix 4.

Chloride levels for plasma samples were obtained and analyzed as described earlier. Raw data values for chloride concentrations are shown in Appendix 4. Values obtained were not consistent with known values in the literature. The concentrations measured were extremely high, and, as a result, there is little confidence in their validity. A possible explanation for the high values is sublimation. The plasma samples had been stored frozen for just over one year when the chloride assay was used. The small quantity present in the cryovials and repeated thawing and freezing over the course of the storage period would possibly account for this. Attempts were made to correct chloride concentrations on the basis of plasma glucose concentrations. These attempts proved to be inadequate since the correction factors neither brought the chloride concentrations down to normal levels nor did they have a noticeable pattern or trend that was consistent. In addition, any correction factor would introduce more

Table 2. Mean weights and mean lengths of sampled fish.

Date	N	Mean Weight[a]	Mean Length[b]
4/12/89	40	24.2 ± 1.58[c]	126 ± 2.9[c]
4/13/89	40	23.0± 1.58	125 ± 2.9
4/14/89	40	19.8± 1.58	117 ± 2.9
4/18/89	40	26.1± 1.58	127 ± 2.9
4/19/89	15	27.2± 2.60	130 ± 5.2
11/8/89	60	No Data[d]	165 ± 2.6
1/4/90	60	No Data[d]	181 ± 2.4

[a]Weight is measured in grams.

[b]Length is measured in millimeters.

[c]Standard error.

[d]Weights were not obtained for these fish.

variability into the analysis which already includes random sampling error, laboratory error and statistical error.

Chloride samples taken in November 1989 and January 1990 did not show any signs of sublimation or increased concentration. These samples had values which fell within norms established in the literature. Due to the fact that these comprised a small portion of the total number of samples, it was decided not to include analysis of any chloride data.

Plasma cortisol concentrations were analyzed using the GLM procedure. Samples were grouped according to the treatments they received; either baseline, 30-minute confinement-initial stress and no challenge, 60-minute confinement-initial stress and no challenge, electroshock-initial stress and no challenge, baseline and heat-challenged, 30-minute confinement-initial stress and heat-challenged, 60-minute confinement-initial stress and heat-challenged, or electroshock-initial stress and heat-challenged. Mean concentrations were determined for the four initial stress groups (Fig. 1) and also for the four challenged groups (Fig. 2) which included the groups from 8 November which were subjected to an extended heating period. Statistical analysis indicated that a significant difference existed between the baseline group (9 ng/mL $\pm$ 3.1) and all other treatment groups ($P < .05$)

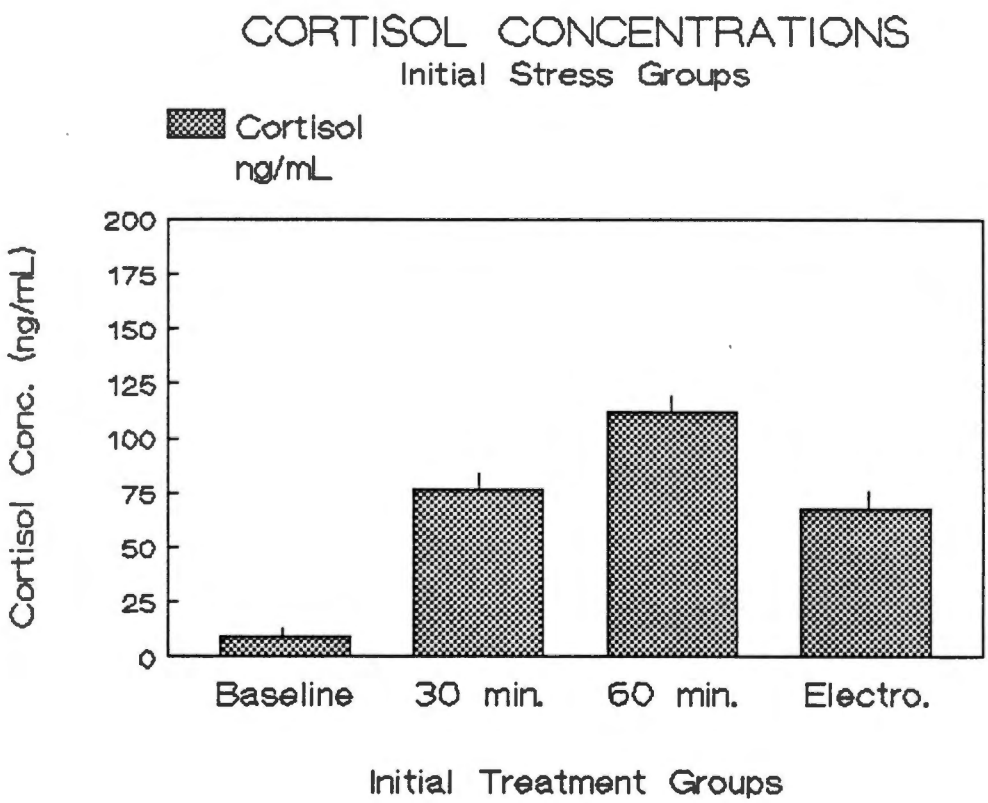

Figure 1. **Mean cortisol concentrations for unchallenged treatment groups after initial stressor. Baseline = no initial stressor, 30 min. = 30-minute confinement initial stressor, 60 min. = 60-minute confinement initial stressor, Electro. = electroshock initial stressor. (mean ± s.e.m.)**

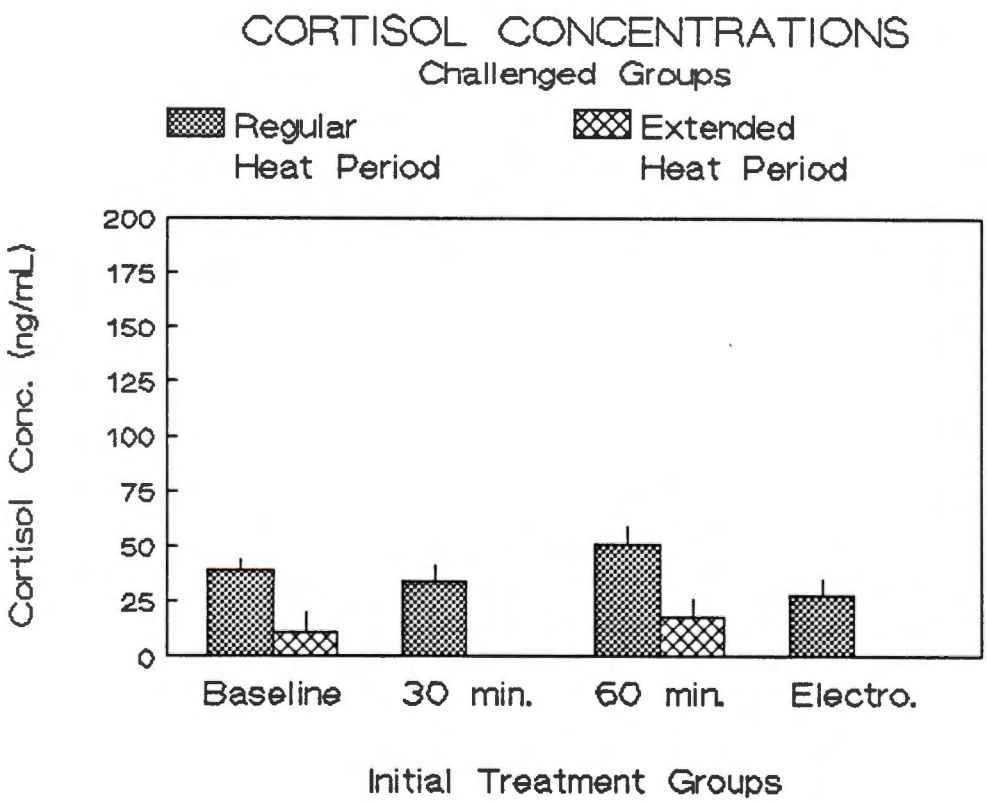

Figure 2. Mean cortisol concentrations for heat challenged treatment groups after critical thermal maximums were reached. Baseline = no initial stressor, 30 min. = 30-minute confinement initial stressor, 60 min. = 60-minute confinement initial stressor, Electro. = electroshock initial stressor. (mean ± s.e.m.)

except for the extended heat period group which received no initial stress (11 ng/mL ± 8.1). Analysis also indicated the cortisol concentration for the 30-minute confinement-initial stress group (76 ng/mL ± 6.5) was significantly lower than that for the 60-minute confinement-initial stress group (112 ng/mL ± 5.7). Furthermore, the cortisol concentration for the 30-minute confinement-initial stress group (76 ng/mL ± 6.5) was significantly higher ($P < .05$) than that for the 30-minute confinement-challenged group (34 ng/ml ± 6.5). The cortisol concentration for the 60-minute confinement-initial group (112 ng/mL ± 5.7) was significantly higher than that for the 60-minute confinement-challenged group (51 ng/mL ± 7.4) and the electroshock-initial group (68 ng/mL ± 6.6). The cortisol concentration for the 60-minute confinement-challenged group (51 ng/mL ± 7.4) was significantly higher ($P < .05$) than that for the same group that was exposed to the extended heat period (18 ng/mL ± 8.0). The cortisol concentration for the 60-minute confinement-challenged group (51 ng/mL ± 7.4) was also significantly higher ($P < .05$) than that for the electroshock-challenged group (27 ng/mL ± 6.7). Cortisol concentrations for the control groups were 104 ± 14.3 for the group confined in the Living Stream for 30 minutes and 84.5 ± 14.3 for group confined for 60 minutes. Both of these groups were significantly higher from all other

challenged groups. Raw data values for these are shown in Appendix 5.

Statistical analysis of glucose values was done following the same procedure as that described for the cortisol. Mean concentrations were determined for the initial stress treatment groups (Fig. 3) and for the four challenged groups (Fig. 4) which included the groups of 8 November which were subjected to the extended heat period. The baseline glucose levels (106 mg/dL $\pm$5.5) were found to be significantly lower ($P < .05$) than all other treatment groups. The 60-minute confinement-challenged group glucose level (234 mg/dL $\pm$ 13.1) was significantly higher than all other treatment groups except those that were subjected to the extended heat period (207 mg/dL $\pm$ 14.1). The glucose level for the group that received no initial stress but was challenged (161 mg/dL $\pm$ 6.9) was significantly lower than the group which received no initial stress but which was subjected to the extended heat period (207 mg/dL $\pm$ 14.1). Glucose concentrations for the group confined in the Living Stream for 30 minutes, Control 2, (122 mg/dL $\pm$ 25.2) was statistically different from all other groups except the 30-minute confinement-challenged group (145 mg/dL $\pm$ 11.5), the Control 3 group (119 mg/dL $\pm$ 25.3), and the baseline group (106 mg/dL $\pm$ 5.5). The glucose level for the group confined 60 minutes in the Living Stream,

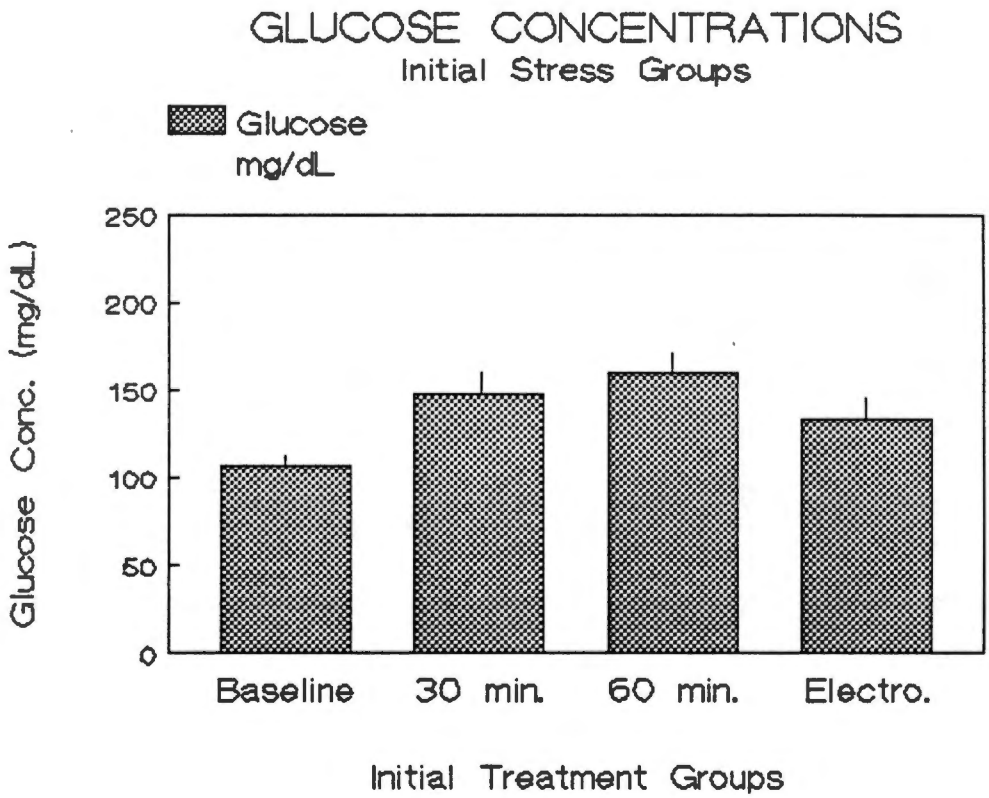

Figure 3. Mean glucose concentrations for unchallenged treatment groups after initial stressor. Baseline = no initial stressor, 30 min. = 30-minute confinement initial stressor, 60 min. = 60-minute intitial stressor, Electro. = electroshock initial stressor. (mean ± s.e.m.)

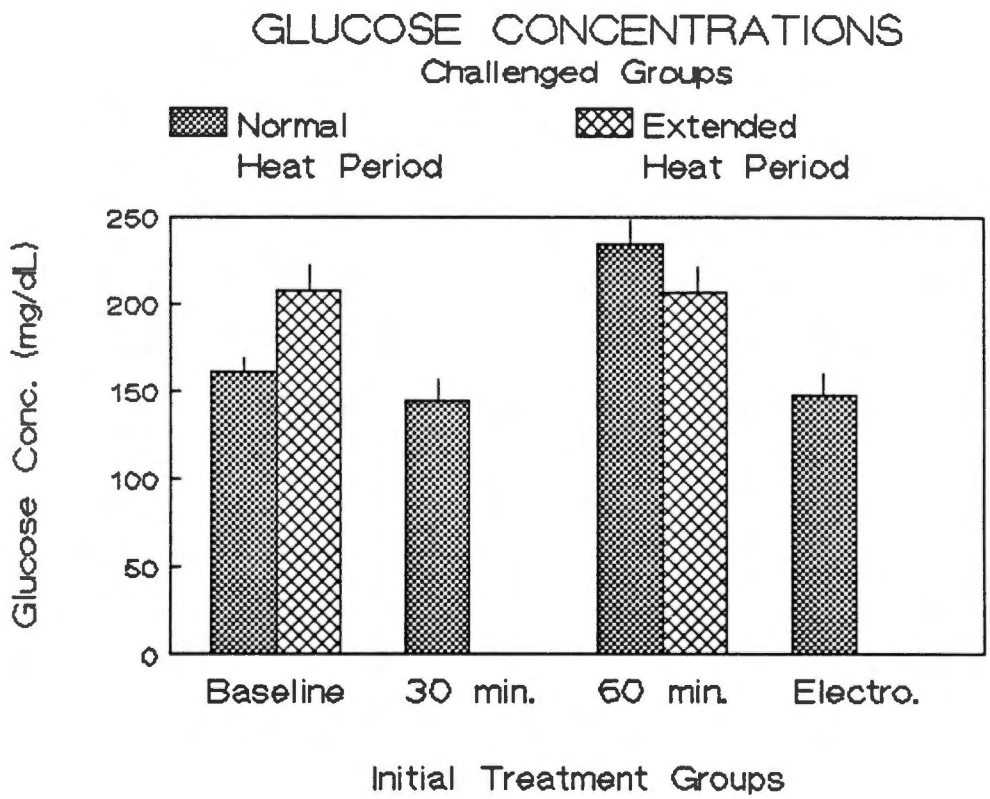

Figure 4. Mean glucose concentrations for heat challenged treatment groups after critical thermal maximums were reached. Baseline = no initial stressor, 30 min. = 30-minute initial stressor, 60 min. = 60-minute initial stressor, Electro. = electroshock initial stressor. (mean ± s.e.m.)

Control 3, (119 mg/dL ± 25.3) was significantly different from all other groups except the Control 2 group (122 mg/dL ± 25.3), and the Baseline group (106 mg/dL ± 5.5). Raw data values for these are shown in Appendix 5.

Temperature data were also analyzed to determine when the challenged fish totally lost equilibrium, the averages of which were considered to be critical thermal maximums (Fig. 5). This included temperature data for the groups exposed to the extended heat period. The critical thermal maximum for the electroshock-challenged group (30 C ± 0.13) was significantly higher ($P < .05$) than all other treatment groups; otherwise, there were no significant differences detected between critical thermal maximums for any of the treatment groups. Raw data values for these are shown in Appendix 5.

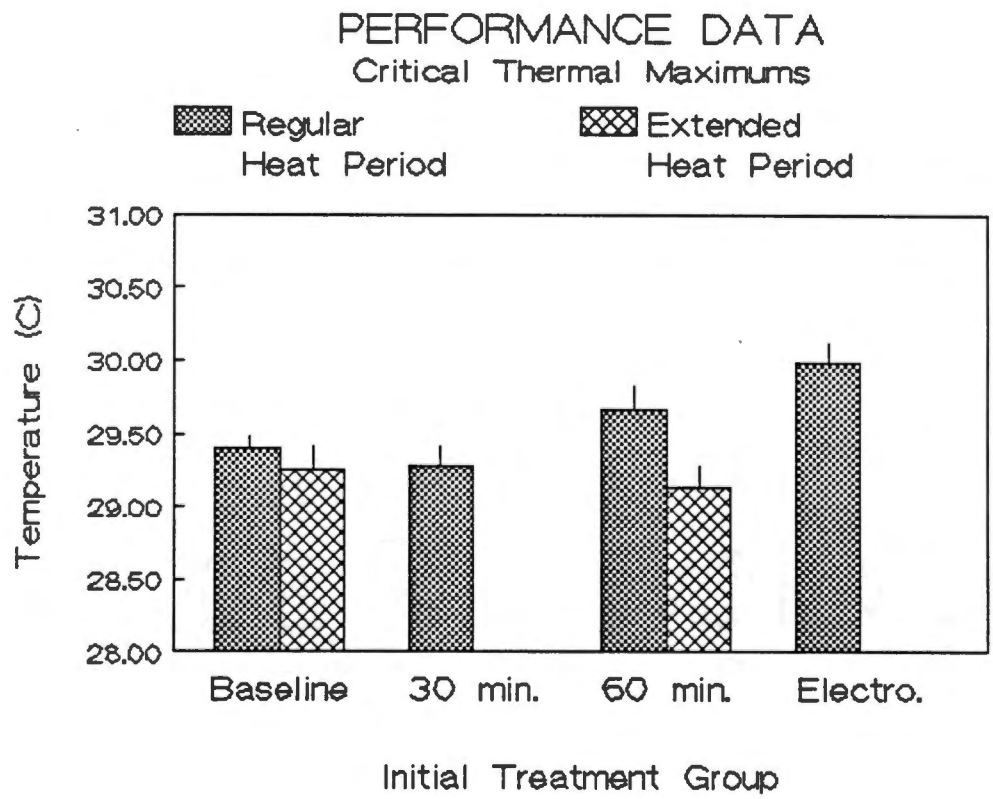

Figure 5. **Mean critical thermal maximums for heat challenged treatment groups. Baseline = no initial stressor, 30 min. = 30-minute confinement initial stressor, 60 min. = 60-minute confinement initial stressor, Electro. = electroshock initial stressor. (mean ± s.e.m.)**

CHAPTER IV

DISCUSSION

The baseline cortisol concentrations were below the 10 ng/ml level which is consistent with other studies (Mazeaud et al. 1977; Strange et al. 1978; Barton et al. 1979). The cortisol levels measured after the initial stressor of either 30-minutes confinement (76 ng/mL), or 60-minutes confinement (112 ng/mL) were comparable to earlier findings. Barton et al. (1980) found that 30-minutes of mild agitation caused cortisol levels to rise to approximately 35 ng/mL and that 60-minutes of mild agitation caused cortisol levels to rise to approximately 70 ng/mL in fingerling rainbow trout. Strange et al. (1978), utilizing confinement as a stressor, showed that under confinement conditions cortisol levels rise much more than this. Strange et al. (1978) found that 30-minutes of confinement caused an elevation in cortisol levels to over 200 ng/mL in juvenile chinook salmon and that 60-minutes of confinement caused an elevation in cortisol levels to approximately 300 ng/mL. These levels are much higher than those measured for the confined fish in this experiment. The large difference between these values is most likely due to the fact that fish in this study are fourteenth generation hatchery fish where as the fish used by Strange et al. (1978) retained many of

their wild traits. The increase in cortisol concentrations (68 ng/ml) due to electroshocking in this study was more in line with previous studies. Schreck et al. (1976) found that after one intitial electroshock that cortisol levels in rainbow trout were elevated to 38 ng/mL. Woodward and Strange (1987) found cortisol levels elevated to 70 ng/mL in hatchery-reared rainbow trout following an electroshock stress. Even though the procedure for this stressor was not the same, it incorporated two shocks instead of the one used in the previous studies, the value obtained (68 ng/ml) falls within a range of the other two.

Baseline glucose concentrations (106 mg/dL) were not consistent with those found in other studies. Normal baseline values for rainbow trout usually range anywhere from 25 mg/dL to 80 mg/dL (Schreck et al. 1976; Woodward and Strange 1987). The higher baseline value may be partly due to their pelleted diet which was relatively high in carbohydrates. The effects of various confinement times on blood glucoses were more toward the accepted norms. Wedemeyer (1972) found that after 30-minutes of confinement that glucose levels in juvenile steelhead trout were elevated to appproximately 100 mg/dl, compared to 148 mg/dL found here, and that after 60-minutes of confinement that the glucose levels were elevated to over 150 mg/dl, similar to that found in this

study (160 mg/dL). Although Wedemeyer's values are lower than those found in this study they are close enough to be comparable. The glucose levels of the electroshocked fish in this study (134 mg/dL) were appreciably higher than those found in other studies. Woodward and Strange (1987) found glucose levels to be less than 60 mg/dL 30-minutes after one initial electroshock, while Schreck et al. (1976) found glucose levels to be close to 70 mg/dL 1 hour after one initial shock. The difference between the previous studies and the current one is that this experiment used two electroshocks spaced 5 minutes apart then allowed the fish 25 minutes to rest before sampling took place. This time lag could have allowed the glucose levels to elevate more than in the previous studies in which blood samples were taken almost immediately following the electroshocking (Pickering et al. 1982).

The cortisol levels of the heat-challenged groups in this study do not show a consistent pattern. The use of heat as a stressor has been evaluated on juvenile cutthroat trout (Strange et al. 1977). The results of that study showed an increase in cortisol concentrations to about 70 ng/mL after 25 minutes of exposure to 26 C water, where in my study the baseline-challenged group had a cortisol level of only 39 ng/mL. The results of the current study show only a relatively slight elevation and in the case of the extended heat period (11 ng/mL)

the levels almost return to baseline values. One previous study has shown that it required at least 4 hours for cortisol concentrations to return to baseline levels after confinement (Pickering et al. 1982).

The cortisol values observed for the control groups, those that were confined in the Living Stream for either 30 minutes or 60 minutes without heating, were elevated to levels consistent with the levels seen in the groups that received the confinement as an initial stress but no heat challenge. At the same time these levels were significantly higher than any of the groups that were subjected to the heat challenge, including the group which received no initial stress but was subjected to the heat challenge. This may indicate that the higher temperatures could have an effect on lowering cortisol levels in stressed fish. The glucose levels of the control groups were significantly lower than the glucose concentration of the group which received no initial stress but was subjected to the heat challenge, suggesting that heat has an effect on the glucose concentration also.

There are several possible explanations for the decrease in cortisol levels during challenge stress following an increase during initial stress. Since the initial stressor elevated the cortisol concentrations only a moderate amount, compared to Strange et al.

(1978), less time would be required to recover, but this alone may not account for the drop observed in cortisol concentrations. The influence of the greater heat also has a possible effect. Strange (1980) noted in channel catfish that individuals at higher temperatures had increased rates of cortisol elevation. This higher rate of increase may also apply to the rate of recovery, the higher the temperature the faster the rate of recovery. Strange et al. (1977) observed after 70-minutes of confinement that juvenile cutthroat trout acclimated to 23 C had lower cortisol concentrations than those acclimated to 9 C. Cortisol concentrations returned to resting levels faster in juvenile chinook salmon acclimated to 21.0 C than those acclimated to 7.5 C (Barton and Schreck 1987). Another possible explanation is that since fish are poikilothermic, the cause of death by higher temperatures could be related to the the breakdown of enzymatic functions in the body. At higher temperatures this breakdown could affect any one of a number of the enzymatic systems involved in maintaining the fish in a homeostatic state. A combination of any of these effects could possibly lead to the decrease in cortisol levels that were observed.

Pickering et al. (1982) showed that glucose levels exhibit a time lag related to the cortisol concentrations. This lag would explain the high glucose

values of the challenged groups, that is the glucose levels have simply not had time to decrease. In the cases of the baseline-challenge group (161 mg/dL) and the 60-minute confinement-challenge group (234 mg/dL), the glucose levels are still increasing.

Analysis of the performance data showed that only the electroshocked group was significantly higher than any other challenged group, although this is only an actual difference of 0.9 C between the the highest and lowest critical thermal maximums. This significant differnce between the elcetroshock-challenged group and the other groups could be related to the fact that this group has one of the lowest values for all the other measured variables.

The rate of temperature increase may also have an effect on the cortisol levels. This is shown by the fact that the fish subjected to the extended heat period had lower critical thermal maximums than those which were put through the normal heating procedure.

Woodward and Strange (1987) showed that hatchery-reared trout exhibit more resistance to stress than do wild trout and that the hatchery fish recover faster than the wild fish. The stress hardiness of hatchery-reared fish has been related to genetic selection for low stress response as in other domesticated species of animals (Mazeaud et al. 1977). In this experiment, the lack of a

pronounced stress response to the initial stressor and the subsequent rapid recovery of the challenged groups can be mostly attributed to the use of hatchery-reared trout. The rapid recovery of the challenged groups, caused by the stress hardiness of the hatchery-reared trout coupled with the possible increased recovery rate caused by heat and the interference caused by heat in the enzymatic functions, could account for the lack of any apparent difference in the critical thermal maximums of the challenged groups.

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LITERATURE CITED

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APPENDIXES

APPENDIX 1

RADIOIMMUNOASSAY DETERMINATION OF CORTISOL IN FISH PLASMA

Cortisol Assay - Diagnostic Products Corp. Coat-A-Count

This assay is based on the competitive antibody binding between known quantities of Iodine 125-labeled cortisol, which acts as the radioactive tracer, and non-labeled indigenous cortisol in the plasma samples. Radioactivity is measured using a gamma counter from which percent binding is calculated.

1. Regular polypropylene tubes are labeled in duplicate: Total counts tube, Non-specific binding.
   Antibody coated tubes are labeled in duplicate: Standards 0 ng/mL, 10 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, and 500 ng/mL, Sample 1, 2, 3, 4, etc....

2. a) To Total counts tube add: 1 mL Iodine solution.
   b) To Non-specific binding tube add: 1 mL cortisol standard solution (0 ng/ml) + 1 mL Iodine solution.
   c) To standards add:
      i. 25 uL cortisol standard solution (0 ng/mL) + 1 mL Iodine solution.
      ii. 25 uL cortisol standard solution (10 ng/mL) + 1 ml Iodine solution.
      iii. 25 uL cortisol standard solution (50 ng/mL) + 1 mL Iodine solution.
      iv. 25 uL cortisol standard solution (100 ng/mL) + 1 mL Iodine solution.
      v. 25 uL cortisol standard solution (200 ng/mL) + 1 mL Iodine solution.
      vi. 25 uL cortisol standard solution (500 ng/mL) + 1 mL Iodine solution.
   d) To samples add: 25 uL plasma sample + 1 mL Iodine solution.

3. Incubate for 45 minutes at 37 C.

4. Decant thoroughly.

5. Count for 1 minute in a gamma counter.

6. Plot a standard curve and read sample concentrations from this.

APPENDIX 2

ENZYMATIC DETERMINATION OF GLUCOSE IN FISH PLASMA

Glucose Assay - Sigma Diagnostics Procedure No. 510

This procedure is an enzymatic test based on the oxidation of glucose in the blood by Glucose Oxidase, which results in Gluconic Acid and Peroxide. Peroxidase is added to catalyze the oxidation of Peroxide and the indicator o-dianisidine. This reaction produces a color change, which is relative to the concentration of glucose in the sample, that is measured with a spectrophotometer.

1. Tubes are labeled: Blank, Standard 40 mg/dL, 100 mg/dL, and 400 mg/dL, Sample 1, 2, 3 etc....

2. a) To Blank add 0.5 mL distilled water.
   b) To standards add:
      i. 10 uL of glucose standard (100 mg/dL) to the 40 mg/dL tube + 0.2 mL distilled water
      ii. 25 uL of glucose standard to the 100 mg/dL tube + 0.2 mL distilled water
      iii. 100 uL of glucose standard to the 400 mg/dL tube + 0.2 mL distilled water.
   c) To samples add: 10 uL plasma sample + 0.2 mL of distilled water.

3. To ALL tubes add 3.0 mL of the Combined Enzyme Color Reagent.

4. VORTEX

5. Place in 37 C water bath for 30 minutes.

6. After incubation read absorbances on a spectrophotometer adjusted to 460 nm.

7. Plot a standard curve and read sample concentrations from this.

APPENDIX 3

COLORMETRIC DETERMINATION OF CHLORIDE IN FISH PLASMA

Chloride Assay - Sigma Diagnostics Procedure No. 461

This procedure is based on the reaction of free chloride ions in plasma with mercuric thiocyanate. The chloride ions displace the thiocyanate which in turn forms a red complex with ferric ions producing a color change that is measured with a spectrophotometer.

1. Tubes are labeled: Blank, Standard 50 mEq/L, 100 mEq/L, and 300 mEq/L, Sample 1, 2, 3 etc....

2. a) To blank add 0.5 mL distilled water.
   b) To standards add:
      i. 5 uL chloride standard (100 mEq/L) to the 50 mEq/L tube + 1 mL chloride reagent.
      ii. 10 uL chloride standard to the 100 mEq/L tube + 1 mL chloride reagent.
      iii. 30 uL chloride standard to the 300 mEq/L tube + 1 mL chloride reagent.
   c) To samples add: 10 uL plasma sample + 1 mL chloride reagent.

3. VORTEX

4. Incubate tubes for 45 minutes at room temperature or at 37 C in a water bath for 30 minutes.

5. After incubation read absorbances on a spectrophotometer adjusted to 460 nm.

6. Plot a standard curve and read sample concentrations from this.

APPENDIX 4

RAW DATA VALUES FOR

LENGTH, WEIGHT, AND CHLORIDE CONCENTRATIONS

Table 3. Length, weight, and chloride values for individual fish.

FISH #	DAY	REP	TREATMENT GROUP	LENGTH (mm)	WEIGHT (grams)	CHLORIDE CONC. (mEq/L)
1	1	1	BASELINE[a]	121	20.7	193.10
2	1	1	BASELINE	137	31.2	282.93
3	1	1	BASELINE	147	34.8	238.47
4	1	1	BASELINE	130	27.7	303.66
5	1	1	BASELINE	142	32.7	298.85
6	1	1	CON_30[b]	133	24.0	209.92
7	1	1	CON_30	140	29.2	212.93
8	1	1	CON_30	126	23.6	247.78
9	1	1	CON_30	117	19.1	253.79
10	1	1	CON_30	129	22.7	136.92
11	1	1	HEAT[c]	120	20.0	206.01
12	1	1	HEAT	139	30.2	NO DATA
13	1	1	HEAT	118	17.5	103.27
14	1	1	HEAT	121	20.5	135.42
15	1	1	HEAT	148	21.1	210.53
16	1	1	C30_HEAT[d]	126	22.7	NO DATA
17	1	1	C30_HEAT	119	19.9	NO DATA
18	1	1	C30_HEAT	138	29.2	NO DATA
19	1	1	C30_HEAT	144	34.5	93.06
20	1	1	C30_HEAT	141	28.3	155.85
21	1	2	BASELINE	124	25.0	235.16
22	1	2	BASELINE	132	26.7	240.57
23	1	2	BASELINE	133	27.4	249.58
24	1	2	BASELINE	128	25.4	178.38
25	1	2	BASELINE	115	15.7	203.62
26	1	2	CON_30	125	23.8	257.69
27	1	2	CON_30	116	16.5	230.35
28	1	2	CON_30	122	20.6	240.87
29	1	2	CON_30	121	20.5	269.11
30	1	2	CON_30	109	13.8	249.28
31	1	2	HEAT	130	27.9	275.42
32	1	2	HEAT	110	15.0	251.98
33	1	2	HEAT	138	30.6	NO DATA
34	1	2	HEAT	122	19.8	228.55
35	1	2	HEAT	118	16.9	173.57
36	1	2	C30_HEAT	127	24.2	196.41
37	1	2	C30_HEAT	137	32.0	208.12
38	1	2	C30_HEAT	123	23.2	265.50
39	1	2	C30_HEAT	96	10.6	NO DATA
40	1	2	C30_HEAT	113	14.8	246.88
41	2	1	BASELINE	119	19.8	231.92

Table 3 (continued)

FISH #	DAY	REP	TREATMENT GROUP	LENGTH (mm)	WEIGHT (grams)	CHLORIDE CONC. (mEq/L)
42	2	1	BASELINE	134	29.0	129.74
43	2	1	BASELINE	126	22.3	256.46
44	2	1	BASELINE	113	15.4	192.53
45	2	1	BASELINE	110	16.4	192.53
46	2	1	CON_30	136	28.9	166.84
47	2	1	CON_30	135	29.9	181.68
48	2	1	CON_30	131	25.8	257.60
49	2	1	CON_30	109	12.5	NO DATA
50	2	1	CON_30	133	24.1	199.67
51	2	1	HEAT	125	21.7	226.49
52	2	1	HEAT	116	17.8	263.88
53	2	1	HEAT	132	27.0	223.35
54	2	1	HEAT	114	16.5	162.85
55	2	1	HEAT	133	22.8	204.23
56	2	1	C30_HEAT	142	34.4	152.00
57	2	1	C30_HEAT	130	22.9	265.02
58	2	1	C30_HEAT	117	16.0	168.56
59	2	1	C30_HEAT	122	19.0	230.49
60	2	1	C30_HEAT	118	16.2	148.58
61	2	2	BASELINE	118	16.4	155.14
62	2	2	BASELINE	145	36.2	187.96
63	2	2	BASELINE	128	22.2	180.54
64	2	2	BASELINE	127	23.4	228.78
65	2	2	BASELINE	119	17.8	231.06
66	2	2	CON_30	136	28.8	301.84
67	2	2	CON_30	127	20.0	259.31
68	2	2	CON_30	118	17.8	223.93
69	2	2	CON_30	115	16.6	212.51
70	2	2	CON_30	114	18.7	302.13
71	2	2	HEAT	126	24.7	292.99
72	2	2	HEAT	133	28.7	NO DATA
73	2	2	HEAT	114	16.9	179.97
74	2	2	HEAT	128	22.4	212.79
75	2	2	HEAT	134	26.4	233.91
76	2	2	C30_HEAT	137	29.0	242.48
77	2	2	C30_HEAT	142	30.6	211.37
78	2	2	C30_HEAT	121	17.7	246.76
79	2	2	C30_HEAT	123	18.4	244.19
80	2	2	C30_HEAT	124	20.0	229.35
81	3	1	BASELINE	150	NO DATA	155.38
82	3	1	BASELINE	150	NO DATA	154.15
83	3	1	BASELINE	175	NO DATA	127.86

Table 3 (continued)

FISH #	DAY	REP	TREATMENT GROUP	LENGTH (mm)	WEIGHT (grams)	CHLORIDE CONC. (mEq/L)
84	3	1	BASELINE	150	NO DATA	90.27
85	3	1	BASELINE	190	NO DATA	205.01
86	3	1	CON_60[e]	165	NO DATA	177.00
87	3	1	CON_60	165	NO DATA	183.88
88	3	1	CON_60	180	NO DATA	169.39
89	3	1	CON_60	160	NO DATA	125.40
90	3	1	CON_60	160	NO DATA	203.29
91	3	1	CON_SLOW[f]	170	NO DATA	88.79
92	3	1	CON_SLOW	160	NO DATA	163.98
93	3	1	CON_SLOW	150	NO DATA	87.81
94	3	1	CON_SLOW	170	NO DATA	105.26
95	3	1	SLOWHEAT[g]	165	NO DATA	83.58
96	3	1	SLOWHEAT	165	NO DATA	139.41
97	3	1	SLOWHEAT	170	NO DATA	96.16
98	3	1	SLOWHEAT	160	NO DATA	130.32
99	3	1	SLOWHEAT	160	NO DATA	144.57
100	3	1	CON_SLOW	185	NO DATA	116.31
101	3	2	BASELINE	165	NO DATA	183.64
102	3	2	BASELINE	150	NO DATA	127.62
103	3	2	BASELINE	160	NO DATA	190.52
104	3	2	BASELINE	170	NO DATA	131.06
105	3	2	BASELINE	195	NO DATA	253.67
106	3	2	CON_60	140	NO DATA	106.24
107	3	2	CON_60	145	NO DATA	95.92
108	3	2	CON_60	160	NO DATA	219.27
109	3	2	CON_60	170	NO DATA	155.63
110	3	2	CON_60	175	NO DATA	99.11
111	3	2	CON_SLOW	150	NO DATA	79.70
112	3	2	CON_SLOW	190	NO DATA	199.86
113	3	2	SLOWHEAT	170	NO DATA	100.34
114	3	2	CON_SLOW	165	NO DATA	92.23
115	3	2	SLOWHEAT	190	NO DATA	120.74
116	3	2	SLOWHEAT	145	NO DATA	90.76
117	3	2	CON_SLOW	155	NO DATA	101.32
118	3	2	SLOWHEAT	145	NO DATA	109.68
119	3	2	CON_SLOW	175	NO DATA	124.42
120	3	2	SLOWHEAT	150	NO DATA	109.43
121	3	3	BASELINE	155	NO DATA	176.76
122	3	3	BASELINE	165	NO DATA	122.70
123	3	3	BASELINE	150	NO DATA	212.63
124	3	3	BASELINE	175	NO DATA	143.83
125	3	3	BASELINE	150	NO DATA	148.01

Table 3 (continued)

FISH #	DAY	REP	TREATMENT GROUP	LENGTH (mm)	WEIGHT (grams)	CHLORIDE CONC. (mEq/L)
126	3	3	CON_60	145	NO DATA	129.34
127	3	3	CON_60	140	NO DATA	145.06
128	3	3	CON_60	165	NO DATA	206.49
129	3	3	CON_60	175	NO DATA	148.75
130	3	3	CON_60	160	NO DATA	125.40
131	3	3	CON_SLOW	165	NO DATA	87.81
132	3	3	SLOWHEAT	170	NO DATA	126.14
133	3	3	SLOWHEAT	160	NO DATA	121.96
134	3	3	SLOWHEAT	155	NO DATA	68.15
135	3	3	CON_SLOW	190	NO DATA	93.95
136	3	3	SLOWHEAT	160	NO DATA	144.32
137	3	3	SLOWHEAT	150	NO DATA	121.72
138	3	3	CON_SLOW	150	NO DATA	109.43
139	3	3	CON_SLOW	165	NO DATA	121.96
140	3	3	CON_SLOW	165	NO DATA	133.51
141	4	1	BASELINE	190	NO DATA	211.89
142	4	1	BASELINE	175	NO DATA	62.81
143	4	1	BASELINE	200	NO DATA	95.13
144	4	1	BASELINE	165	NO DATA	145.43
145	4	1	BASELINE	165	NO DATA	186.35
146	4	1	CON_60	170	NO DATA	93.56
147	4	1	CON_60	175	NO DATA	152.46
148	4	1	CON_60	175	NO DATA	105.29
149	4	1	CON_60	185	NO DATA	180.61
150	4	1	CON_60	145	NO DATA	144.12
151	4	1	HEAT	200	NO DATA	158.72
152	4	1	C60_HEAT[h]	200	NO DATA	137.87
153	4	1	C60_HEAT	180	NO DATA	164.45
154	4	1	HEAT	180	NO DATA	140.74
155	4	1	HEAT	185	NO DATA	114.14
156	4	1	HEAT	170	NO DATA	157.16
157	4	1	C60_HEAT	210	NO DATA	181.65
158	4	1	HEAT	180	NO DATA	181.91
159	4	1	C60_HEAT	165	NO DATA	188.95
160	4	1	C60_HEAT	185	NO DATA	151.68
161	4	2	BASELINE	185	NO DATA	192.08
162	4	2	BASELINE	185	NO DATA	200.42
163	4	2	BASELINE	190	NO DATA	163.93
164	4	2	BASELINE	160	NO DATA	79.49
165	4	2	BASELINE	170	NO DATA	160.80
166	4	2	CON_60	185	NO DATA	169.93
167	4	2	CON_60	175	NO DATA	170.45

Table 3 (continued)

FISH #	DAY	REP	TREATMENT GROUP	LENGTH (mm)	WEIGHT (grams)	CHLORIDE CONC. (mEq/L)
168	4	2	CON_60	180	NO DATA	164.45
169	4	2	CON_60	180	NO DATA	82.62
170	4	2	CON_60	155	NO DATA	94.87
171	4	2	C60_HEAT	175	NO DATA	145.43
172	4	2	C60_HEAT	165	NO DATA	136.57
173	4	2	C60_HEAT	165	NO DATA	166.54
174	4	2	C60_HEAT	190	NO DATA	175.92
175	4	2	HEAT	180	NO DATA	164.19
176	4	2	C60_HEAT	160	NO DATA	120.93
177	4	2	HEAT	185	NO DATA	135.26
178	4	2	HEAT	185	NO DATA	136.31
179	4	2	HEAT	200	NO DATA	121.19
180	4	2	HEAT	185	NO DATA	118.58
181	4	3	BASELINE	150	NO DATA	169.40
182	4	3	BASELINE	170	NO DATA	208.50
183	4	3	BASELINE	205	NO DATA	139.95
184	4	3	BASELINE	195	NO DATA	187.39
185	4	3	BASELINE	190	NO DATA	215.53
186	4	3	CON_60	200	NO DATA	205.63
187	4	3	CON_60	160	NO DATA	161.85
188	4	3	CON_60	180	NO DATA	127.18
189	4	3	CON_60	140	NO DATA	109.46
190	4	3	CON_60	200	NO DATA	147.77
191	4	3	C60_HEAT	175	NO DATA	122.75
192	4	3	HEAT	180	NO DATA	107.90
193	4	3	C60_HEAT	195	NO DATA	95.39
194	4	3	HEAT	175	NO DATA	92.78
195	4	3	HEAT	175	NO DATA	119.37
196	4	3	C60_HEAT	175	NO DATA	145.17
197	4	3	HEAT	195	NO DATA	94.87
198	4	3	C60_HEAT	175	NO DATA	52.65
199	4	3	HEAT	190	NO DATA	106.86
200	4	3	C60_HEAT	150	NO DATA	107.64
201	5	1	BASELINE	124	21.2	194.18
202	5	1	BASELINE	128	22.4	194.43
203	5	1	BASELINE	120	17.2	232.18
204	5	1	BASELINE	125	24.8	191.59
205	5	1	BASELINE	101	10.6	191.85
206	5	1	ELECTRO[1]	122	19.9	243.29
207	5	1	ELECTRO	108	13.6	240.19
208	5	1	ELECTRO	121	22.9	180.73
209	5	1	ELECTRO	115	17.2	205.03

Table 3 (continued)

FISH #	DAY	REP	TREATMENT GROUP	LENGTH (mm)	WEIGHT (grams)	CHLORIDE CONC. (mEq/L)
210	5	1	ELECTRO	143	35.1	207.36
211	5	1	HEAT	115	16.7	197.01
212	5	1	HEAT	127	26.3	192.63
213	5	1	HEAT	132	27.9	200.38
214	5	1	HEAT	130	32.0	161.86
215	5	1	HEAT	113	17.2	204.77
216	5	1	ELECHEAT[j]	110	15.5	231.14
217	5	1	ELECHEAT	121	16.4	228.30
218	5	1	ELECHEAT	131	23.6	174.53
219	5	1	ELECHEAT	104	9.4	216.92
220	5	1	ELECHEAT	120	18.1	237.86
221	5	2	BASELINE	104	11.9	230.88
222	5	2	BASELINE	111	15.6	192.88
223	5	2	BASELINE	131	23.2	221.06
224	5	2	BASELINE	106	11.7	NO DATA
225	5	2	BASELINE	105	13.4	91.55
226	5	2	ELECTRO	114	19.0	287.60
227	5	2	ELECTRO	126	24.8	233.99
228	5	2	ELECTRO	132	24.6	253.89
229	5	2	ELECTRO	122	22.8	153.59
230	5	2	ELECTRO	96	10.4	NO DATA
231	5	2	HEAT	127	22.2	167.81
232	5	2	HEAT	112	15.7	NO DATA
233	5	2	HEAT	114	17.1	196.76
234	5	2	HEAT	99	10.6	119.99
235	5	2	HEAT	134	26.4	204.00
236	5	2	ELECHEAT	135	27.9	214.34
237	5	2	ELECHEAT	137	25.2	153.59
238	5	2	ELECHEAT	156	41.8	198.05
239	5	2	ELECHEAT	127	20.0	238.90
240	5	2	ELECHEAT	96	8.1	NO DATA
241	6	1	BASELINE	149	44.0	266.72
242	6	1	BASELINE	131	27.5	303.92
243	6	1	BASELINE	127	24.8	337.49
244	6	1	BASELINE	106	14.3	218.42
245	6	1	BASELINE	119	19.0	234.18
246	6	1	ELECTRO	133	27.3	254.07
247	6	1	ELECTRO	135	34.0	160.05
248	6	1	ELECTRO	127	27.3	355.57
249	6	1	ELECTRO	128	26.0	350.15
250	6	1	ELECTRO	105	14.2	NO DATA
251	6	1	HEAT	128	25.3	209.64

Table 3 (continued)

FISH #	DAY	REP	TREATMENT GROUP	LENGTH (mm)	WEIGHT (grams)	CHLORIDE CONC. (mEq/L)
252	6	1	HEAT	135	30.0	196.21
253	6	1	HEAT	123	22.5	222.81
254	6	1	HEAT	148	38.3	215.84
255	6	1	HEAT	132	27.3	191.56
256	6	1	ELECHEAT	132	26.0	165.73
257	6	1	ELECHEAT	138	30.0	183.81
258	6	1	ELECHEAT	156	43.3	291.23
259	6	1	ELECHEAT	138	29.2	281.37
260	6	1	ELECHEAT	122	18.3	187.17
261	6	2	BASELINE	139	32.7	270.08
262	6	2	BASELINE	137	27.7	217.91
263	6	2	BASELINE	145	34.5	213.26
264	6	2	BASELINE	128	24.8	245.03
265	6	2	BASELINE	129	26.1	253.55
266	6	2	ELECTRO	142	32.4	243.22
267	6	2	ELECTRO	108	14.8	186.91
268	6	2	ELECTRO	122	25.9	160.31
269	6	2	ELECTRO	111	15.7	163.41
270	6	2	ELECTRO	145	38.7	196.47
271	6	2	HEAT	110	16.3	161.60
272	6	2	HEAT	108	13.7	NO DATA
273	6	2	HEAT	130	27.3	213.26
274	6	2	HEAT	125	22.8	176.06
275	6	2	HEAT	118	21.6	156.95
276	6	2	ELECHEAT	117	18.2	199.05
277	6	2	ELECHEAT	148	34.0	194.66
278	6	2	ELECHEAT	144	33.0	142.23
279	6	2	ELECHEAT	126	22.0	161.86
280	6	2	ELECHEAT	130	23.4	147.39
281	7	1	BASELINE	139	33.5	197.96
282	7	1	BASELINE	146	39.4	192.49
283	7	1	BASELINE	129	26.3	478.45
284	7	1	BASELINE	114	19.6	226.53
285	7	1	BASELINE	133	21.6	185.82
286	7	1	NOHEAT30[k]	145	32.5	201.77
287	7	1	NOHEAT30	115	16.3	197.25
288	7	1	NOHEAT30	162	51.6	209.62
289	7	1	NOHEAT30	152	40.9	213.68
290	7	1	NOHEAT30	149	37.7	178.68
291	7	1	NOHEAT60[l]	136	28.6	185.11
292	7	1	NOHEAT60	129	24.6	162.26
293	7	1	NOHEAT60	121	19.3	241.76

Table 3 (continued)

FISH #	DAY	REP	TREATMENT GROUP	LENGTH (mm)	WEIGHT (grams)	CHLORIDE CONC. (mEq/L)
294	7	1	NOHEAT60	119	18.0	167.50
295	7	1	NOHEAT60	114	15.6	163.93

[a]No initial stressor, no challenge.

[b]30-minute confinement, no challenge.

[c]No initial stressor, heat challenged.

[d]30-minute confinement, heat challenged.

[e]60-minute confinement, no challenge.

[f]60-minute confinement, extended heat challenge.

[g]No initial stressor, extended heat challenge.

[h]60-minute confinement, heat challenged.

[i]Electroshocked, no challenge.

[j]Electroshocked, heat challenged.

[k]30-minute confinement in Living Stream, no heat.

[l]60-minute confinement in Living Stream, no heat.

APPENDIX 5

RAW DATA VALUES FOR

CORTISOL CONCENTRATIONS, GLUCOSE CONCENTRATIONS,

AND CRITICAL THERMAL MAXIMUMS

Figure 4. Cortisol, glucose, and critical thermal maximum values for individual fish.

FISH #	DAY	REP	TREATMENT GROUP	CORTISOL CONC. (ng/mL)	GLUCOSE CONC. (mg/dL)	TEMP. (C)
1	1	1	BASELINE[a]	10.23	71.04	15.0
2	1	1	BASELINE	1.10	89.44	15.0
3	1	1	BASELINE	2.70	117.86	15.0
4	1	1	BASELINE	1.50	124.26	15.0
5	1	1	BASELINE	4.30	135.60	15.0
6	1	1	CON_30[b]	57.02	133.47	15.0
7	1	1	CON_30	37.40	100.12	15.0
8	1	1	CON_30	62.89	123.53	15.0
9	1	1	CON_30	58.44	94.45	15.0
10	1	1	CON_30	49.10	104.39	15.0
11	1	1	HEAT[c]	63.10	118.59	26.8
12	1	1	HEAT	2.50	77.37	27.6
13	1	1	HEAT	31.69	44.76	29.1
14	1	1	HEAT	52.38	101.52	29.5
15	1	1	HEAT	32.02	62.76	30.0
16	1	1	C30_HEAT[d]	41.78	147.01	27.7
17	1	1	C30_HEAT	4.10	75.97	27.9
18	1	1	C30_HEAT	42.00	93.71	28.3
19	1	1	C30_HEAT	24.29	195.23	28.8
20	1	1	C30_HEAT	56.33	80.97	28.9
21	1	2	BASELINE	7.40	155.48	15.0
22	1	2	BASELINE	21.69	153.34	15.0
23	1	2	BASELINE	8.30	123.53	15.0
24	1	2	BASELINE	2.20	101.52	15.0
25	1	2	BASELINE	7.40	66.77	15.0
26	1	2	CON_30	41.63	88.78	15.0
27	1	2	CON_30	63.71	104.59	15.0
28	1	2	CON_30	69.78	127.80	15.0
29	1	2	CON_30	80.61	143.41	15.0
30	1	2	CON_30	112.74	112.19	15.0
31	1	2	HEAT	119.88	188.16	27.9
32	1	2	HEAT	31.87	127.13	29.1
33	1	2	HEAT	1.80	NO DATA	29.4
34	1	2	HEAT	16.13	51.83	29.5
35	1	2	HEAT	24.70	190.30	30.0
36	1	2	C30_HEAT	18.59	88.04	29.6
37	1	2	C30_HEAT	17.20	152.68	29.8
38	1	2	C30_HEAT	33.40	98.72	30.3
39	1	2	C30_HEAT	12.23	75.24	30.4
40	1	2	C30_HEAT	83.68	157.61	30.6
41	2	1	BASELINE	7.90	141.29	15.0

Table 4 (continued)

FISH #	DAY	REP	TREATMENT GROUP	CORTISOL CONC. (ng/mL)	GLUCOSE CONC. (mg/dL)	TEMP. (C)
42	2	1	BASELINE	9.70	117.75	15.0
43	2	1	BASELINE	9.00	77.73	15.0
44	2	1	BASELINE	8.80	95.00	15.0
45	2	1	BASELINE	9.00	120.89	15.0
46	2	1	CON_30	111.99	165.61	15.0
47	2	1	CON_30	107.05	167.18	15.0
48	2	1	CON_30	38.73	136.58	15.0
49	2	1	CON_30	38.32	114.61	15.0
50	2	1	CON_30	85.93	192.29	15.0
51	2	1	HEAT	59.04	211.90	28.8
52	2	1	HEAT	35.41	134.23	29.3
53	2	1	HEAT	30.24	61.26	29.3
54	2	1	HEAT	28.98	135.01	30.3
55	2	1	HEAT	26.10	124.03	30.4
56	2	1	C30_HEAT	76.50	147.57	27.8
57	2	1	C30_HEAT	33.89	139.72	28.5
58	2	1	C30_HEAT	27.32	108.33	29.0
59	2	1	C30_HEAT	25.60	186.80	29.6
60	2	1	C30_HEAT	22.83	151.49	29.9
61	2	2	BASELINE	9.70	52.20	15.0
62	2	2	BASELINE	14.40	110.84	15.0
63	2	2	BASELINE	8.20	89.84	15.0
64	2	2	BASELINE	8.90	100.70	15.0
65	2	2	BASELINE	8.40	105.04	15.0
66	2	2	CON_30	136.68	151.37	15.0
67	2	2	CON_30	155.97	139.06	15.0
68	2	2	CON_30	116.69	147.03	15.0
69	2	2	CON_30	41.64	136.17	15.0
70	2	2	CON_30	22.60	162.95	15.0
71	2	2	HEAT	88.18	168.02	28.8
72	2	2	HEAT	8.30	124.59	29.7
73	2	2	HEAT	19.04	160.78	30.0
74	2	2	HEAT	23.80	168.02	30.0
75	2	2	HEAT	32.83	178.15	30.2
76	2	2	C30_HEAT	38.59	168.02	29.4
77	2	2	C30_HEAT	22.32	123.14	29.6
78	2	2	C30_HEAT	17.53	160.06	29.8
79	2	2	C30_HEAT	25.59	126.04	30.1
80	2	2	C30_HEAT	25.32	110.84	30.1
81	3	1	BASELINE	8.75	83.69	15.0
82	3	1	BASELINE	41.00	83.12	15.0
83	3	1	BASELINE	22.50	75.19	15.0

Table 4 (continued)

FISH #	DAY	REP	TREATMENT GROUP	CORTISOL CONC. (ng/mL)	GLUCOSE CONC. (mg/dL)	TEMP. (C)
84	3	1	BASELINE	22.50	91.05	15.0
85	3	1	BASELINE	21.25	99.55	15.0
86	3	1	CON_60[e]	127.50	109.18	15.0
87	3	1	CON_60	66.25	87.09	15.0
88	3	1	CON_60	115.00	176.60	15.0
89	3	1	CON_60	155.00	127.88	15.0
90	3	1	CON_60	102.50	134.11	15.0
91	3	1	CON_SLOW[f]	45.00	144.87	28.7
92	3	1	CON_SLOW	47.50	134.11	28.9
93	3	1	CON_SLOW	12.50	143.17	29.2
94	3	1	CON_SLOW	18.00	192.46	29.4
95	3	1	SLOWHEAT[g]	15.00	172.06	29.4
96	3	1	SLOWHEAT	12.50	185.66	29.5
97	3	1	SLOWHEAT	NO DATA	187.36	29.8
98	3	1	SLOWHEAT	6.25	231.99	29.8
99	3	1	SLOWHEAT	41.25	186.80	29.8
100	3	1	CON_SLOW	7.00	164.70	29.8
101	3	2	BASELINE	2.00	131.16	15.0
102	3	2	BASELINE	1.50	87.69	15.0
103	3	2	BASELINE	3.00	133.20	15.0
104	3	2	BASELINE	1.00	109.42	15.0
105	3	2	BASELINE	3.00	172.59	15.0
106	3	2	CON_60	177.50	111.46	15.0
107	3	2	CON_60	180.00	268.36	15.0
108	3	2	CON_60	102.50	277.87	15.0
109	3	2	CON_60	107.50	143.38	15.0
110	3	2	CON_60	70.00	165.12	15.0
111	3	2	CON_SLOW	5.00	224.21	29.0
112	3	2	CON_SLOW	42.50	355.52	29.0
113	3	2	SLOWHEAT	5.00	201.80	29.1
114	3	2	CON_SLOW	14.00	245.09	29.2
115	3	2	SLOWHEAT	13.00	356.21	29.3
116	3	2	SLOWHEAT	13.00	201.12	29.4
117	3	2	CON_SLOW	9.00	199.08	29.5
118	3	2	SLOWHEAT	12.50	256.13	29.6
119	3	2	CON_SLOW	7.00	205.19	29.7
120	3	2	SLOWHEAT	1.00	141.58	29.7
121	3	3	BASELINE	11.00	96.81	15.0
122	3	3	BASELINE	5.00	114.77	15.0
123	3	3	BASELINE	22.50	131.49	15.0
124	3	3	BASELINE	19.25	259.69	15.0
125	3	3	BASELINE	6.25	130.25	15.0

Table 4 (continued)

FISH #	DAY	REP	TREATMENT GROUP	CORTISOL CONC. (ng/mL)	GLUCOSE CONC. (mg/dL)	TEMP. (C)
126	3	3	CON_60	110.00	166.18	15.0
127	3	3	CON_60	80.00	220.68	15.0
128	3	3	CON_60	140.00	253.50	15.0
129	3	3	CON_60	90.00	112.91	15.0
130	3	3	CON_60	87.50	204.57	15.0
131	3	3	CON_SLOW	42.50	169.27	29.0
132	3	3	SLOWHEAT	26.25	160.60	29.2
133	3	3	SLOWHEAT	8.72	190.95	29.3
134	3	3	SLOWHEAT	17.75	205.19	29.4
135	3	3	CON_SLOW	17.75	198.38	29.5
136	3	3	SLOWHEAT	15.00	286.51	29.6
137	3	3	SLOWHEAT	10.00	323.77	29.6
138	3	3	CON_SLOW	7.50	322.39	29.8
139	3	3	CON_SLOW	11.25	260.93	29.8
140	3	3	CON_SLOW	29.00	314.80	30.0
141	4	1	BASELINE	0.00	65.28	14.0
142	4	1	BASELINE	0.00	109.40	14.0
143	4	1	BASELINE	6.80	51.58	14.0
144	4	1	BASELINE	7.30	53.10	14.0
145	4	1	BASELINE	2.70	71.36	14.0
146	4	1	CON_60	107.77	151.25	14.0
147	4	1	CON_60	128.40	101.03	14.0
148	4	1	CON_60	81.16	188.53	14.0
149	4	1	CON_60	161.58	149.97	14.0
150	4	1	CON_60	107.21	124.62	14.0
151	4	1	HEAT	67.31	136.79	28.3
152	4	1	C60_HEAT[h]	33.39	243.02	28.4
153	4	1	C60_HEAT	41.86	135.27	28.9
154	4	1	HEAT	92.79	169.51	29.0
155	4	1	HEAT	30.95	225.09	29.1
156	4	1	HEAT	62.33	120.82	29.2
157	4	1	C60_HEAT	36.25	265.80	29.3
158	4	1	HEAT	29.01	142.12	29.4
159	4	1	C60_HEAT	69.18	287.20	29.7
160	4	1	C60_HEAT	47.12	129.18	30.0
161	4	2	BASELINE	8.00	72.96	14.0
162	4	2	BASELINE	8.80	122.86	14.0
163	4	2	BASELINE	4.30	58.58	14.0
164	4	2	BASELINE	6.80	112.71	14.0
165	4	2	BASELINE	7.60	123.70	14.0
166	4	2	CON_60	162.99	169.37	14.0
167	4	2	CON_60	90.54	163.45	14.0

Table 4 (continued)

FISH #	DAY	REP	TREATMENT GROUP	CORTISOL CONC. (ng/mL)	GLUCOSE CONC. (mg/dL)	TEMP. (C)
168	4	2	CON_60	124.87	76.34	14.0
169	4	2	CON_60	143.36	122.01	14.0
170	4	2	CON_60	76.64	211.28	14.0
171	4	2	C60_HEAT	57.97	321.70	28.6
172	4	2	C60_HEAT	50.13	343.09	28.8
173	4	2	C60_HEAT	38.65	357.59	28.9
174	4	2	C60_HEAT	45.84	216.12	29.1
175	4	2	HEAT	44.32	225.78	29.2
176	4	2	C60_HEAT	33.80	182.92	29.3
177	4	2	HEAT	58.76	199.82	29.5
178	4	2	HEAT	28.18	171.07	29.5
179	4	2	HEAT	37.01	247.86	29.6
180	4	2	HEAT	35.75	185.44	29.6
181	4	3	BASELINE	19.00	66.67	14.0
182	4	3	BASELINE	13.87	52.39	14.0
183	4	3	BASELINE	10.66	92.84	14.0
184	4	3	BASELINE	5.20	116.63	14.0
185	4	3	BASELINE	6.80	133.88	14.0
186	4	3	CON_60	76.34	206.44	14.0
187	4	3	CON_60	190.36	225.47	14.0
188	4	3	CON_60	63.21	151.13	14.0
189	4	3	CON_60	106.47	227.26	14.0
190	4	3	CON_60	102.91	163.62	14.0
191	4	3	C60_HEAT	102.48	270.63	28.3
192	4	3	HEAT	50.68	133.28	28.4
193	4	3	C60_HEAT	86.74	183.25	28.5
194	4	3	HEAT	41.57	243.02	28.9
195	4	3	HEAT	35.78	167.78	28.9
196	4	3	C60_HEAT	35.74	143.40	29.1
197	4	3	HEAT	42.83	158.26	29.1
198	4	3	C60_HEAT	71.68	194.55	29.4
199	4	3	HEAT	19.82	160.64	29.6
200	4	3	C60_HEAT	46.52	246.29	29.9
201	5	1	BASELINE	10.29	126.77	15.0
202	5	1	BASELINE	0.00	112.97	15.0
203	5	1	BASELINE	0.00	86.18	15.0
204	5	1	BASELINE	27.89	65.88	15.0
205	5	1	BASELINE	7.40	88.61	15.0
206	5	1	ELECTRO[1]	111.20	129.21	15.0
207	5	1	ELECTRO	100.67	112.16	15.0
208	5	1	ELECTRO	55.62	112.16	15.0
209	5	1	ELECTRO	47.35	116.22	15.0

Table 4 (continued)

FISH #	DAY	REP	TREATMENT GROUP	CORTISOL CONC. (ng/mL)	GLUCOSE CONC. (mg/dL)	TEMP. (C)
210	5	1	ELECTRO	57.92	108.10	15.0
211	5	1	HEAT	45.66	104.04	29.0
212	5	1	HEAT	49.57	74.81	29.2
213	5	1	HEAT	27.62	157.63	29.7
214	5	1	HEAT	31.75	107.29	29.8
215	5	1	HEAT	39.45	161.69	29.9
216	5	1	ELECHEAT[j]	46.44	130.83	30.1
217	5	1	ELECHEAT	19.27	94.30	30.2
218	5	1	ELECHEAT	37.31	153.57	30.2
219	5	1	ELECHEAT	35.20	171.43	30.6
220	5	1	ELECHEAT	NO DATA	112.97	30.6
221	5	2	BASELINE	40.27	56.76	15.0
222	5	2	BASELINE	0.00	59.86	15.0
223	5	2	BASELINE	0.00	79.99	15.0
224	5	2	BASELINE	8.10	65.28	15.0
225	5	2	BASELINE	0.00	81.54	15.0
226	5	2	ELECTRO	102.44	95.47	15.0
227	5	2	ELECTRO	77.75	112.50	15.0
228	5	2	ELECTRO	87.87	97.79	15.0
229	5	2	ELECTRO	24.53	102.44	15.0
230	5	2	ELECTRO	NO DATA	121.02	15.0
231	5	2	HEAT	57.56	129.53	27.5
232	5	2	HEAT	20.03	122.57	29.5
233	5	2	HEAT	28.94	72.25	29.6
234	5	2	HEAT	26.40	134.18	30.0
235	5	2	HEAT	56.64	128.76	30.1
236	5	2	ELECHEAT	22.90	95.47	29.6
237	5	2	ELECHEAT	18.10	130.31	29.7
238	5	2	ELECHEAT	23.44	107.86	30.2
239	5	2	ELECHEAT	21.93	108.63	30.3
240	5	2	ELECHEAT	NO DATA	119.47	30.5
241	6	1	BASELINE	20.40	107.31	15.0
242	6	1	BASELINE	7.20	100.05	15.0
243	6	1	BASELINE	0.00	96.94	15.0
244	6	1	BASELINE	8.70	83.45	15.0
245	6	1	BASELINE	7.30	127.02	15.0
246	6	1	ELECTRO	87.43	163.34	15.0
247	6	1	ELECTRO	26.97	118.73	15.0
248	6	1	ELECTRO	66.49	148.81	15.0
249	6	1	ELECTRO	54.23	148.81	15.0
250	6	1	ELECTRO	57.78	100.05	15.0
251	6	1	HEAT	62.57	173.71	26.0

Table 4 (continued)

FISH #	DAY	REP	TREATMENT GROUP	CORTISOL CONC. (ng/mL)	GLUCOSE CONC. (mg/dL)	TEMP. (C)
252	6	1	HEAT	31.83	211.06	28.2
253	6	1	HEAT	37.35	190.31	28.9
254	6	1	HEAT	11.93	198.61	29.1
255	6	1	HEAT	33.04	175.78	29.3
256	6	1	ELECHEAT	37.88	139.47	28.7
257	6	1	ELECHEAT	24.74	155.04	28.8
258	6	1	ELECHEAT	16.01	156.07	29.0
259	6	1	ELECHEAT	27.18	149.85	29.1
260	6	1	ELECHEAT	18.62	161.26	29.3
261	6	2	BASELINE	24.87	90.88	15.0
262	6	2	BASELINE	13.65	98.72	15.0
263	6	2	BASELINE	10.87	117.01	15.0
264	6	2	BASELINE	0.00	76.06	15.0
265	6	2	BASELINE	7.90	117.88	15.0
266	6	2	ELECTRO	51.23	156.22	15.0
267	6	2	ELECTRO	75.07	131.82	15.0
268	6	2	ELECTRO	51.21	107.43	15.0
269	6	2	ELECTRO	56.33	111.79	15.0
270	6	2	ELECTRO	74.99	164.06	15.0
271	6	2	HEAT	42.64	236.37	29.8
272	6	2	HEAT	NO DATA	143.15	29.8
273	6	2	HEAT	35.69	166.67	30.0
274	6	2	HEAT	22.73	247.70	30.2
275	6	2	HEAT	29.41	137.05	30.5
276	6	2	ELECHEAT	24.19	100.46	30.5
277	6	2	ELECHEAT	15.00	159.70	30.5
278	6	2	ELECHEAT	27.29	171.03	30.6
279	6	2	ELECHEAT	26.95	153.60	30.7
280	6	2	ELECHEAT	24.95	174.51	31.0
281	7	1	BASELINE	14.64	108.83	15.0
282	7	1	BASELINE	11.39	112.90	15.0
283	7	1	BASELINE	10.92	96.62	15.0
284	7	1	BASELINE	12.28	130.81	15.0
285	7	1	BASELINE	12.01	87.66	15.0
286	7	1	NOHEAT30[k]	86.70	99.06	15.0
287	7	1	NOHEAT30	103.59	114.53	15.0
288	7	1	NOHEAT30	131.60	125.55	15.0
289	7	1	NOHEAT30	72.23	160.92	15.0
290	7	1	NOHEAT30	139.36	117.33	15.0
291	7	1	NOHEAT60[l]	235.85	125.11	15.0
292	7	1	NOHEAT60	13.74	110.46	15.0
293	7	1	NOHEAT60	73.49	143.02	15.0

Table 4 (continued)

FISH #	DAY	REP	TREATMENT GROUP	CORTISOL CONC. (ng/mL)	GLUCOSE CONC. (mg/dL)	TEMP. (C)
294	7	1	NOHEAT60	56.37	111.27	15.0
295	7	1	NOHEAT60	58.35	110.46	15.0

[a]No initial stressor, no challenge.

[b]30-confinement, no challenge.

[c]No initial stressor, heat challenged.

[d]30-minute confinement, heat challenged.

[e]60-minute confinement, no challenge.

[f]60-minute confinement, extended heat challenge.

[g]No initial stressor, extended heat challenge.

[h]60-minute confinement, heat challenged.

[i]Electroshocked, no challenge.

[j]Electroshocked, heat challenged.

[k]30-minute confinement in Living Stream, no heat.

[l]60-minute confinement in Living Stream, no heat.

VITA

Roger Petrie was born in Boston, Massachusetts on November 13, 1964. He attended primary schools in Oak Ridge, Tennessee and graduated from Oak Ridge High School in June 1983. The following September he entered Georgia Institute of Technology in Atlanta, Georgia. In September of 1984 he entered Roane State Community College in Harriman, Tennessee. In August of 1985 he entered the University of Tennessee, Knoxville. In August of 1988 he received a Bachelor of Science degree, cum laude, in Forestry, Wildlife, and Fisheries Science. In the fall of 1988 he began studies toward a Master's degree in Wildlife and Fisheries Science at the University of Tennessee, Knoxville. This degree was awarded in December 1990.

The author is a member of Gamma Sigma Delta, Xi Sigma Pi, Gamma Beta Phi, the Dean's list Roane State Community College and the Dean's List University of Tennessee, Who's Who Among Students in American Junior Colleges, Golden Key National Honor Society, and the National Dean's List.