AN ABSTRACT OF THE THESIS OF

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Title: Changes in the Swimming Performance, Behavior, and Physiology of Juvenile Chinook Salmon (*Oncorhynchus tshawytcha*) after Exposure to One, Two or Three Acute Handling Stresses

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The performance of an organism or organismic subsystem is the result of the interaction between the performance capacity of the system and its environment. Environmental conditions can stress an organism and thus affect it's performance. In this study, three whole organism performances were examined: critical swimming speed, fatigue time and response time to a sudden bright light. In addition, subsystem performances were examined by measuring changes in hematocrit and plasma levels of cortisol, glucose, lactic acid, osmolarity, sodium and potassium. Performance tests were made on juvenile chinook salmon stressed 0, 1, 2 or 3 times, with 1 or 3 h between stresses, and on fish allowed to recover 1, 3, 6, 12 and 24 h after each level of stress. A stress consisted of holding the fish in a dip net in the air for 30 sec. The physiological responses and the swimming tests were conducted on salt water adapted fish while the behavioral response was measured with fish in fresh water.

Plasma levels of cortisol, lactic acid, osmolarity and sodium increased cumulatively following several acute handling stresses spaced 1 h apart, though each parameter returned to control levels in 6-12 h. Plasma glucose rose significantly by 1 h after the first

stress and remained higher than control levels at all levels of stress and through 24 h after stress. Plasma potassium increased initially following one and two stresses, dropped below control levels within 1-6 h after the last stress, and then increased above control levels for the remainder of the 24 h. Following three stresses potassium was lower than controls initially and then was similar to the levels for one and two stresses throughout the rest of the 24 h recovery period. There was a decrease in hematocrit 3-6 h after each level of stress followed by a return to control levels within 12 h of the last stress.

Critical swimming speed was measured by increasing the water velocity in a flow-through swim tube and noting the velocity at which each fish stopped swimming. Critical swimming speeds after handling were highly variable and no differences were found between stressed fish and unstressed fish at any level of stress or any recovery time.

Fatigue time was measured as the time a fish can maintain position in a swim tube at a given constant water velocity (60 cm/sec). Following each fatigue test, fish were killed and blood samples were obtained. Unlike unstressed fish, which all fatigued within13 min, the times to fatigue of stressed fish varied with some fish fatiguing within a few minutes and some fish swimming the 60 min period. There was a depression in fatigue times immediately following one and three handling stresses spaced 1 h apart. Immediately after two stresses and with all groups given time to recover from stress, fatigue times were similar to or higher than for unstressed fish.

Plasma levels of cortisol, glucose, osmolarity and sodium were higher in swimming fish than in non-swimming controls. Plasma concentrations of cortisol, glucose and lactic acid were all highly variable in fish following fatigue and no differences were found betweeen fish handled in a dip net and unhandled fish at any level of stress or any time after stress. Plasma osmolarity and sodium levels in fatigued fish immediately after one stress were higher than levels in unstressed fatigued fish. Plasma potassium

was higher in fatigued fish than in unstressed fatigued controls at several time periods after one and three stresses.

The behavior test consisted of exposing groups of salmon in fresh water to a sudden bright light and measuring the time it took each fish to reach cover. Unstressed fish reached cover within 15 sec. Stressed fish took longer to reach cover, with the greatest delay immediately after stress and a gradual decrease in response time with recovery from stress. Exposure to two and three consecutive stress with 3 h between stresses increased the response times and the recovery times indicating that the effects of stress were cumulative.

Changes in the Swimming Performance, Behavior and Physiology of Juvenile Chinook Salmon (*Oncorhynchus tshawytcha*) after Exposure to One, Two or Three Acute Handling Stresses

by

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INTRODUCTION

Definition and History of Stress

The concept of stress was first used by Selye (1973, 1936) to describe the nonspecific response of an organism to any demand placed upon it. He exposed rats to many types of nocuous agents including physical injury, heat, cold, and injections of diverse substances such as epinephrine or formaldehyde. In all cases, he observed a generalized response which included 1) an enlargement of the adrenal cortex, 2) atrophy of the thymus, spleen, lymph nodes and other lymphatic structures and 3) the formation of ulcers in the stomach and upper gut. Further experimentation, led him to propose the general adaptation syndrome (GAS) (Selye 1973, 1956). In rats exposed to continuous stress, there is first the alarm reaction in which the adrenals release a large amount of hormone and become depleted. There is also hemoconcentration and general tissue catabolism. At this stage, resistance is low. For example, rats exposed to cold and then to an even colder temperature are more sensitive than control rats placed directly in the colder temperature. If the animal survives the alarm reaction and stress is continued to be applied, the second stage, called the stage of resistance, is reached in which the adrenals have a large supply of secretory granules, the blood returns to normal concentration and there is a trend toward normal body weight. At this stage, rats can survive in a colder temperature than control rats. Eventually, with continuous exposure, the stage of exhaustion is reached with symptoms similar to the alarm reaction. At this point, rats could not even survive at the cold temperature they had been in for several months.

Factors such as age, sex, diet, physical condition and nature of the nocuous agent can modify the stress response which led some investigators to question Selye's definition of stress as a non-specific response. In the current literature, the term stress is sometimes used to describe the response and sometimes to describe the causative agent. Both definitions have merit since both a stimulus and subsequent reaction are involved and their relative merit is arguable (Pickering 1981). However, since the stimulus definition does not have the problem of the specific actions of the stimulus superimposing on the non-specific response, it is preferred by many authors. Thus, for the purposes of this study, stress will be considered the causative agent.

Current theories on stress in fish divide the response to stress into three levels (Mazeaud and Mazeaud 1981, Wedemeyer and McLeay 1981). The primary response is the release of catecholamines and cortisol into the blood stream. These elicit several secondary responses. Energy and oxygen are made available to the fish by increased heart rate and gill blood flow, a breakdown of liver glycogen and increased blood glucose. Gill permeability, drinking rates and excretion rates are altered leading to changes in plasma electrolyte levels. In addition, the immune system is depressed. Tertiary effects of stress include changes in growth, disease resistance, smoltification, spawning and migratory behavior.

Stress and Performance

Warren *et al.* (1979) helped to conceptualize the relationship between an organism's performance and its environment. Each organism has a potential capacity or inherent ability to do certain performances that is determined by its genetic constitution. For example, a newly hatched salmon parr has the potential capacity to develop, grow, reproduce, osmoregulate in fresh and salt water, and to migrate between the ocean and fresh water. At any particular time, it can not do all of these things. For example, the newly

hatched parr cannot reproduce, but if it survives and the environment is suitable, it will develope this capacity (Warren and Liss 1980). An organism's capacity at any given time is termed the realized capacity. It is determined by the environment, the potential capacity and past realized capacities. The realized capacity determines the range of performances possible for the organism while the environment will determine the actual performance. For example, a salmon smolt can osmoregulate over a range of salinities. It's actual osmoregulation at any particular time will depend on the salinity of the environment.

Stress can be considered as part of an organism's environment and thus has the ability to affect performance (Fig. 1). An organism in a relatively non-stressful environment may have a hypothetical performance (1), while the same organism in a relatively stressful environment may have performance (2). As the environment changes over time, the performance may also change. An organism stressed once and then placed in a less stressful environment may have yet another performance (3a) than an organism exposed to a second stressful environment (3b).

Swimming is one type of performance that is of interest to fisheries biologists because it is a necessary activity for survival. It can be measured in the laboratory by several methods. One common test is step-acceleration in which fish are exposed to increasing increments of water velocity, each for a constant time interval. Experiments by different investigators vary widely as to type of apparatus used (recirculating tunnel, flow-through tunnel, rotating circular troughs, oval trough with paddlewheel, tube within a tube driven by a propellor), size of velocity increment (2-24 cm/sec), lenth of time interval (1-75 min), length of time that fish are allowed to adjust to the apparatus before the test (0-24 h), whether or not a swimming acclimation period (0-1 h) is used at the minimum velocity at which fish must swim to maintain position, and the number of fish per trial (1-100). The most common end-point for this type of test is the critical swimming speed, defined by Brett (1964) as the final velocity at which the fish maintained for the entire time

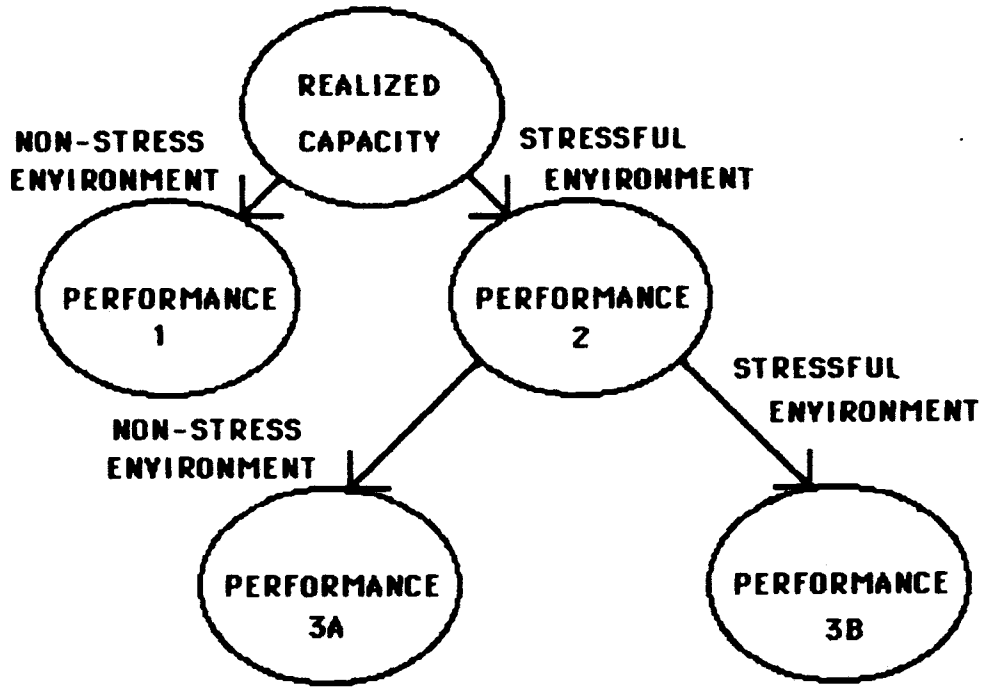


Figure 1: The effects of environment on organism performance.
(Adapted from Warren and Liss 1980)

interval plus the fraction of time at the quitting velocity multiplied by the size of the velocity increment. Other endpoints include the final velocity endured for the entire time period (Fry and Cox 1970), the velocity at which each fish quit swimming (Butler and Milleman 1971, Brett 1965), the velocity of first or second failure (Davis *et al.* 1963), total length of time swimming (Lagasse *et al.* 1980) or a performance index consisting of the sum of the times at which 25% and 75% of the fish quit swimming (Thomas *et al.* 1964).

Results of step-acceleration tests vary with the experimental set-up (Hartwell and Otto 1978, Farlinger and Beamish 1977), species (Beamish 1984,1981, Freadman 1979, Jones *et al.* 1974) and experimental variables considered. Changes in environmental conditions such as temperature, oxygen concentration, carbon dioxide concentration, salinity and pH all influence critical swimming speed (Turnpenney 1983, Schneider and Connors 1982, Grahm and Wood, 1981, Glova and McInerny 1977, Jones 1971, Dahlberg *et al.* 1968, Brett 1967, Brett 1964, Davis *et al.* 1963). Variations in fish condition including fish size, feeding levels, parasite infestations, rearing conditions and training also affect swimming speed (Besner and Smith 1983, Russell 1980, Boyce 1979, Farlinger and Beamish 1978, McNeish and Hatch 1978, Laurence 1972, Butler and Millemann 1971, Fry and Cox 1970, Brett 1965). Environmental pollutants and physical damage tend to adversely affect critical swimming speed (Cripe *et al.* 1984, Kumaguru and Beamish 1983, Duthie and Hughes 1982, Waiwood and Beamish 1978, Howard 1975).

Some investigators have used variations of the step-acceleration method. Kutty and Sukumarau (1975), Schiewe (1974), Kutty and Saunders (1973) and Kutty (1968) tried raising or lowering the water temperature or oxygen concentration in steps to the point where fish stop swimming. Rimmer *et al.* (1985) measured the critical holding velocity which is where a stationary fish can no longer hold position without swimming.

Another common test of swimming performance test is

fatigue time in which fish are exposed to a given constant water velocity and the time at which each fish stops swimming is noted. This test uses the same type of apparatus as the critical swimming speed tests. Again, experiments among different investigators vary widely as to apparatus used, velocity for measurement (2-5 body lengths/sec), number of fish per trial (1-10), amount of time fish are allowed to adjust to the apparatus (0-24 h), and the presence or absence of a swimming acclimation period (0-1 h). Results of fatigue tests are usually reported as the actual minutes the fish swam. Often the data is plotted on a graph of cumulative percent fatigued on a probit scale versus fatigue time on a log scale, and the time to fifty percent fatigue is determined from the regression line. Another method of reporting the data is to report the number or percent of fish fatigued after a given time at a given velocity (Brett 1967).

Fatigue times of fish vary from seconds to hours depending the experimental set-up (McCleave 1980, Hartwell and Otto 1978, Rulifson 1977), species (Brett 1982, Dorn *et al.* 1979) and treatment factors considered. Environmental factors such as temperature and oxygen concentration affect fatigue time (Rulifson 1977, Schiewe 1974, Brett 1967, Brett 1964, Katz *et al.* 1959). Variations in fish including phenotype (Klar *et al.* 1979), strains (Tsuyuki and Williscroft 1977), fish size (Houde 1969, Vincent 1959), fish conditioning (Hammond and Hickman 1966) and parasite infestations (Russell 1980, Butler and Millemann 1971, Klein *et al.* 1969) also affect time to fatigue. Toxicants and physical damage or handling of fish tend to reduce fatigue times (Adams 1975, Horak and Klein 1967, Clancy 1963).

Behavior is anotyher type of performance of interest to fisheries biologists. Changes in behavior are often the most sensitive indicator that something has affected an organism (Sprague 1971). Behavior tests such as predator-prey interactions, social interacitons and avoidance responses are being used frequently in toxicity studies on fish (Hedke and Norris 1980, Hatfield and Anderson 1972, Woltering *et al.* 1978).

The simplest type of behavior test is a stimulus-response reaction. Webb (1975) has developed an elaborate system for studying stimulus-response in which he subjects fish to a brief electrical shock and then determines the response time and acceleration of the fish using high-speed cinematography. He has found variations with species (Webb 1983, 1975), temperature (Webb 1978), schooling (Webb 1980), fin amputation (Webb 1977), fish size (Webb 1976) and developmental stage (Webb and Corolla 1981). He discusses how both reponse time and acceleration are important in determining the outcome of a predator-prey interaction (Webb1976).

A simpler type of stimulus-response experiment with an inexpensive apparatus was developed for this thesis. It looks at the ability of fish to seek cover after exposure to a sudden bright light. This type of test would have application to fish survival in the wild since seeking cover would enable fish to avoid predation and other dangers.

In addition to looking at the response of the whole fish, performances of subsytems can also be measured. There are many investigations in the literature that show the effects of stress on the endocrine system (Barton and Peter 1982, Barton *et al.* 1980, Tomasso *et al.* 1981, Strange and Schreck 1978, Strange *et al.* 1978, 1977), circulatory system (Fletcher 1984, Fletcher 1975, Wells *et al.* 1984, Wardle 1972), osmoregulatory system (Redding and Schreck 1983), respiratory system (Korovin *et al.* 1982) and various combinations of the above (Brown *et al.* 1984, Giles *et al.* 1984, Carmichael *et al.* 1983, Nikinmaa *et al.* 1983, Sovio and Nikinmaa 1981, Specker and Schreck 1980, Mazeuad *et al.* 1977, Umminger and Gist 1973, Wedemeyer 1972).

Performance as the Interactive Performance of Subsystems

The performance of an organism can be considered the result of the interactive performances of the organisms subsystems (Warren *et al.* 1979) (Fig. 2). For example, in order for a fish to

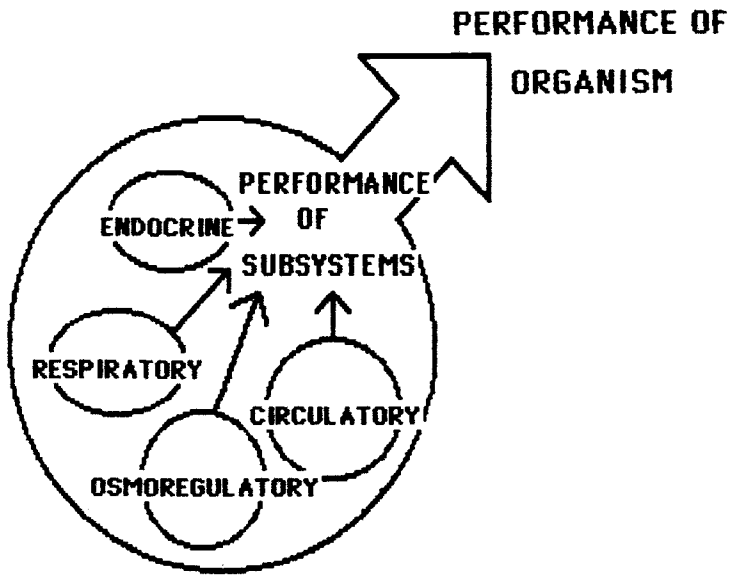


Figure 2: The performance of the whole organism is a result of the interacting performances of the organismic subsystems (Adapted from Warren *et al.* 1979).

swim, the nervous sytem must stimulate the muscles to contract rythmically, the circulatory system must deliver oxygen to the muscles and carry away wastes, the respiratory system must obtain oxygen from the environment and get rid of carbon dioxide and other wastes, and the nervous and endocrine systems must coordinate these activities. The performance of the subsystem also depends on its environment. For example, the environment of the heart is not the same as the environment of the fish. The heart is enclosed in the pericardial sac in osmotically controlled fluid and receives electical stimuli from nerves and hormones in the blood all of which are part of its environment and will determine its performance (Warren and Liss 1980). It is all of the organ systems working together and affecting each other's environments that leads to the overall performance of the organism.

Several studies note some of the changes in organ systems during swimming. These changes include an increase in heart activity (Hanyu *et al.* 1979, Priede 1974), changes in pH (Black *et al.* 1959), accumulations of lactic acid (Driedzic and Kiceniuk 1976, Black *et al.* 1962, 1960, 1959, 1957a,b,c,1955), changes in the endocrine system (Higgs and Eales 1971), changes in the muscles (Tsukamoto 1981, Johnston and Goldspink 1973a,b, Pritchard *et al.* 1971), changes in respiration (Bushnell *et al.* 1984, Kiceniuk and Jones 1977) and changes in blood chemistry/osmoregulation (Giles 1984, Wood *et al.* 1983, Turner *et al.* 1983a,b, Farlinger and Beamish 1978, Wood *et al.* 1977, Wood and Randall 1973a,b,c, Black *et al.* 1959).

If stress changes some of the performances of the organism subsystems, then the result of these changes will be manifested in the performance of the organism. Thus, since stress affects many of the systems used in swimming, it may also affect the swimming performance.

Definition of Problem and Objectives

Fish are exposed to stress from many natural and man-made

sources. Natural stresses include disease, predation and competition among individuals. Man-made stresses include fishing pressure, water pollution, hydroelectric dams, and industrial water intakes. Man also imposes stress by handling fish involved in hatchery or fish farming operations. Since stress is a part of a fish's life history, it is of interest to know how stress affects fish at both the whole organism and physiological levels. Furthermore, since exposure to two or more successive stresses is likely, information is needed as to the effects of repeated stress on the ability of fish to respond and survive.

The goal of this research is to determine if acute stress reduces the performance capacity of juvenile chinook salmon from immediately after stress to up to 24 h following stress. If so, do additional stresses, such as two or three consecutive stresses, further reduce performance capacity. Specifically, the questions that will be investigated are:

1. How is the critical swimming speed of salt water adapted juvenile chinook salmon affected during the 24 h period following an acute stress? Do two or three acute handling stresses further affect critical swimming speed during the 24 h recovery period?

2. How is the fatigue time of salt water adapted juvenile chinook salmon affected during the 24 h period following an acute stress? Do two or three acute handling stresses further affect fatigue time during the 24 h recovery period?

3. How is the cover-seeking response of salt water adapted juvenile chinook salmon affected during the 24 h period following an acute stress? Do two or three acute handling stresses further affect the response during the 24 h recovery period?

4. How are hematocrit and plasma concentrations of cortisol, glucose, lactic acid, osmolaritiy, sodium and potassium in salt water adapted juvenile fall chinook salmon affected during the 24 h period after an acute stress? Do two or three handling stresses further affect these concentrations over the 24 h recovery period?

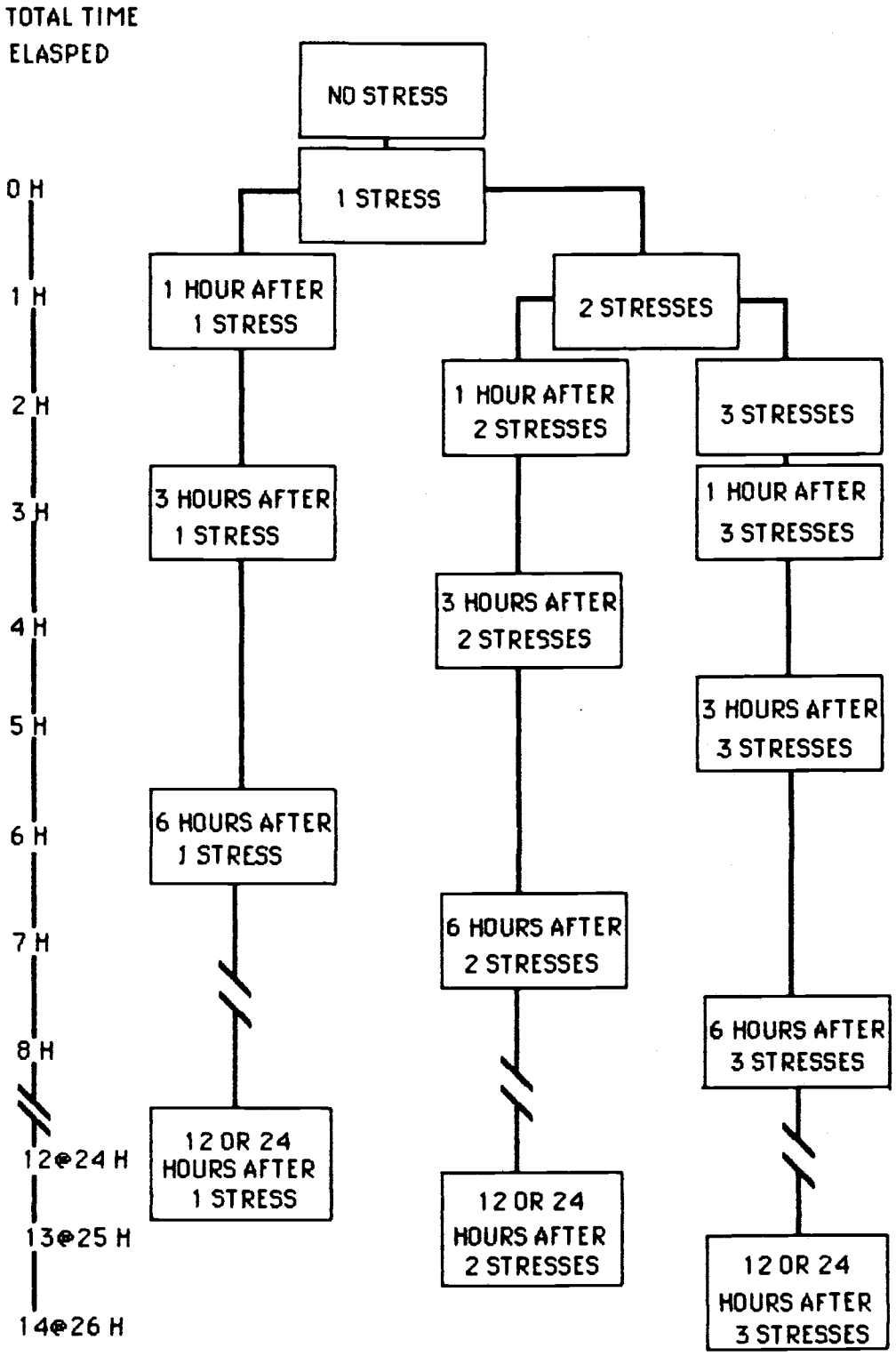
5. How does acute handling stess in conjunction with

swimming fatigue affect the hematocrit and plasma concentrations of cortisol, glucose, lactic acid, osmolaritiy, sodium and potassium of salt water adapted juvenile fall chinook salmon?

Experimental Design

All of the experiments in this study will follow the same basic design outlined in Fig. 3. Experiments will vary only in the type of performance test conducted. Fish will be stressed 1, 2, or 3 times and performances will be looked at immediately after stress and at 1, 3, 6, 12 and 24 h after the final stress. Performance tests will include critical swimming speed, fatigue time, response to light, and physiological measurements.

Figure 3: Flow diagram of groups of fish used in performance tests. Each block represents a different naive group of fish used in the test.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1 STRESS
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5 H
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6 HOURS AFTER 2 STRESSES
12 OR 24 HOURS AFTER 2 STRESSES
3 STRESSES
1 HOUR AFTER 3 STRESSES
3 HOURS AFTER 3 STRESSES
6 HOURS AFTER 3 STRESSES
12 OR 24 HOURS AFTER 3 STRESSES

Figure 3

HEMATOCRIT, PLASMA OSMOLARITY AND PLASMA CONCETRATIONS OF CORTISOL, GLUCOSE, LACTIC ACID, SODIUM AND POTASSIUM IN SALT WATER ADAPTED JUVENILE FALL CHINOOK SALMON (*ONCORYHCHUS TSHAWYTSCHA*) SUBJECTED TO ONE, TWO OR THREE ACUTE HANDLING STRESSES

ABSTRACT

Hematocrit, plasma osmolarity and plasma concentrations of cortisol, glucose, lactic acid, sodium and potassium were measured in salt water adapted juvenile fall chinook salmon immediately following 1, 2, or 3 handling stresses, with 1 h between stresses, and at 1, 3, 6, 12 or 24 h after each level of stress. Plasma osmolarity and plasma concentrations of cortisol, lactic acid and sodium increased cumulatively with repeated stress though each parameter returned to control levels in 6-12 h. Plama glucose rose significantly by 1 h after the first stress and remained higher than in controls at all levels of stress and through 24 h after stress. Plasma potassium increased initially following one and two stresses, dropped below control levels at 1-6 h post-stress and then increased above control levels for the remainder of the 24 h. Following three stresses, plasma potassium concentration was lower than in controls immediately after stress and then was similar to the levels for one and two stresses throughout the rest of the 24 h recovery period. There was a decrease in hematocrit 3-6 h after each level of stress followed by a return to control levels within 12 h of the final stress.

INTRODUCTION

Much work has been done on the physiological responses of salmonids in fresh water to single acute stresses (Pickering *et al.* 1982, Barton *et al.* 1980, Strange and Schreck 1978, Wydoski *et al.* 1976, Wedemeyer 1972). However, information on the stress response of salmonids or other species in salt water is scarce. In addition, until recent work by Barton *et al.* (1985) on fresh water adapted fall chinook, little was known about the effect of several acute stresses applied in succession though the simultaneous exposure to two different stresses, for example, close confinement and heavy metal exposure, had been shown to affect the stress response (Schreck and Lorz 1978). In this study, the responses of salt water adapted fall chinook smolts to 1, 2 or 3 handling stresses were measured immediately after stress and for a 24 h recovery period. Parameters measured were hematocrit, plasma osmolarity and plasma concentrations of cortisol, glucose, lactic acid, sodium and potassium.

MATERIALS AND METHODS

Fish

Juvenile fall chinook (15.9 cm mean fork length, 45.7 g mean wet weight) were obtained from Oregon Department of Fisheries and Wildlife Fall Creek Hatchery and transported to the Oregon State Universiy Marine Science Center at Newport, Oregon in October of 1984. Fish were held in a 1.8 m circular flow through fresh water tank for several weeks and vaccinated against *Vibrio*. Fish were then gradually acclimated to 24-28 ppt sea water over a two day period and held for several weeks at 12 C before the start of the experiment. The fish were obtained a few days before the hatchery release date and were considered to be smolts. The plasma sodium concentrations obtained on the fish were in the normal range for a sea water fish, verifying that the fish were able to handle the salt water.

Stress Test

Juvenile fall chinook were stressed 1, 2, or 3 times with 1 h between stresses by dip-netting the fish from the tank; suspending them in the air for 30 sec, and then placing them in another tank. Blood samples were collected from fish immediately after stress and from fish allowed to recover 1, 3, 6, 12 and 24 h following the last stress.

Blood sampling consisted of transferring the fish immediately upon removal from the tank into a bucket containing 250 mg/l tricaine methanesulfonate (MS-222). As soon as the fish were immobilized, the caudal peducle was severed and blood was collected in heparinized 0.25 ml capillary tubes. Blood samples were centrifuged and the plasma was removed and stored at -20 C. Additional blood was taken in heparinized 1.1 mm capillary tubes for hematocrit determination. These samples were centrifuged immediately in an hematocrit centrifuge and hematocrit was read

directly from a card reader.

Thawed plasma samples were analyzed for cortisol, glucose, lactic acid, osmolarity, sodium and potassium. Cortisol was measured using a radioimmunoassay (Redding *et al.*, 1984), glucose using the O-toluidine method (Hyvarinen and Nikkila 1962 as cited by Wedemeyer and Yasutake 1977) and lactate using a fluorimetric, enzyme reaction (Passonneau 1974). Osmolarity was determined using a vapor pressure osmometer (Westcor). Sodium and potassium were determined using a microelectrode sodium-potassium analyzer (Nova 1). For the sodium and potassium determination, plasma samples were diluted with a known standard and actual concentration was determined by the standard addition method (Skoog and West 1976).

Ten fish were sampled for each experimental group. Mean plasma concentrations were calculated for each parameter for each experimental group. Bartlett's test (Sokal and Rohlf 1969) indicated that variances were heterogeneous in many of the groups which violated one of the assumptions for analysis of variance. Log transformations were used on the glucose, lactic acid, and hematocrit data to reduce the heterogeneity so the assumptions of analysis of variance could be met. One way analyzes of variance were then performed on the transformed data to determine the effect of stress over time and to determine whether repeated stresses had a cumulative effect. Since a log transformation did not reduce the heterogeneity of variances for the cortisol, osmolarity, sodium or potassium data, the nonparametric Kruskal Wallis test was used. A non-parametric multiple comparison test suggested in Daniel (1978) and the STP multiple comparison test for equal sample sizes (Sokal and Rohlf 1969) were used to compare means when levels were significantly different ($P<.05$). For the few comparisons where Bartlett's test was not significant, a one way analysis of variance was used to verify the non-parametric results.

RESULTS

Mean circulating levels of cortisol, glucose, lactic acid, osmolarity, hematocrit, sodium and potassium for each group of fish are presented in Table 1. (Levels for individual fish are listed in appendix 1).

Plasma cortisol in juvenile chinook significantly increased within 1 h after one stress, remained high at 3 h after one stress and returned to control levels by 6 h post-stress (Fig. 4). After two and three stresses, peak cortisol levels occurred immediately (t=0 h) after the last stress, remained near peak levels at 1 h after the final stress and then declined to control levels by 6 h. The cortisol levels immediately following two and three stresses were significantly higher than after a single stress though similar to each other. At 1,12 and 24 h after the final stress, cortisol levels were not significantly different among the 1, 2 and 3 stress groups. At 3 and 6 h after the final stress, cortisol was significantly higher in the three stress group than in the one stress group.

Plasma glucose levels increased significantly within 1 h after the first stress, peaked at 3 h and remained significantly higher than control levels through 24 h after one stress (Fig. 5). Following two and three stresses, glucose levels were higher than control levels immediately (t=0 h) after the last stress and peak levels occurred 1-6 h after the final stress. Again, glucose levels did not return to control levels by 24 h after stress. Immediately after the final stress, fish stressed three times had a higher plasma glucose level than fish stressed twice which in turn had greater levels than fish stressed once. At 1 h after the final stress, glucose in the groups with two and three stresses was significantly higher than in the group with one stress. At 3,12 and 24 h after the final stress, there were no significant differences between the groups with 1, 2 or 3 stresses. At 6 h after the final stress, the fish stressed three times had significantly greater glucose levels than fish stressed one or two times.

Table 1: Length, weight, LeCren's condition factor, hematocrit, plasma osmolarity and plasma concentrations of cortisol, glucose, lactic acid, sodium and potassium (mean +/- standard error for n=10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. The groups are listed as x+y where x indicates the number of stresses and y indicates the hours after stress (e.g. 1+0 indicates 1 stress with 0 h recovery time).

Table 1

GROUP (X+Y)	LENGTH (CM)	WEIGHT (G)	CONDITION FACTOR	HEMAT-OCRIT	CORTISOL (NG/100ML)
CONTROL	15.6+/-0.5	43.4+/-3.7	0.98	48+/-2	68+/-20
1+0	16.1+/-0.2	49.9+/-2.1	1.08	46+/-1	86+/-9
1+1	15.8+/-0.2	43.4+/-3.2	0.96	46+/-2	222+/-21
1+3	15.9+/-0.3	47.0+/-2.6	1.03	49+/-2	136+/-5
1+6	15.2+/-0.3	40.9+/-2.5	1.00	38+/-1	58+/-9
1+12	16.4+/-0.2	49.7+/-2.0	1.04	47+/-1	50+/-6
1+24	15.6+/-0.3	43.0+/-3.0	0.97	45+/-1	62+/-7
2+0	16.0+/-0.2	46.7+/-1.6	1.02	45+/-1	288+/-26
2+1	15.7+/-0.4	44.9+/-3.6	1.00	44+/-1	279+/-26
2+3	15.6+/-0.5	44.0+/-4.2	0.99	40+/-1	179+/-20
2+6	15.8+/-0.3	44.1+/-2.8	0.98	43+/-1	77+/-8
2+12	16.4+/-0.2	49.6+/-2.0	1.04	48+/-1	74+/-11
2+24	15.8+/-0.2	45.5+/-1.4	1.00	45+/-1	70+/-9
3+0	16.5+/-0.2	52.9+/-2.0	1.10	46+/-2	329+/-29
3+1	15.6+/-0.2	43.6+/-2.1	0.98	42+/-1	304+/-30
3+3	15.8+/-0.3	45.3+/-2.7	1.00	42+/-1	223+/-21
3+6	15.6+/-0.3	41.9+/-2.7	0.94	41+/-1	128+/-21
3+12	16.1+/-0.2	47.3+/-2.0	1.02	45+/-1	58+/-4
3+24	15.8+/-0.3	44.8+/-2.7	0.99	47+/-1	52+/-8

GROUP (X+Y)	GLUCOSE (MG/100ML)	LACTIC ACID (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
CONTROL	73+/-3	32.2+/-3.4	301+/-5	157.6+/-1.2	1.99+/-0.15
1+0	70+/-3	34.9+/-2.0	307+/-4	156.0+/-2.1	2.93+/-0.20
1+1	94+/-4	65.2+/-5.2	318+/-6	166.6+/-4.1	2.67+/-0.17
1+3	139+/-6	64.7+/-6.9	324+/-3	163.4+/-3.9	0.82+/-0.12
1+6	120+/-4	43.0+/-3.1	313+/-3	157.8+/-4.3	1.15+/-0.15
1+12	122+/-10	39.4+/-4.4	309+/-7	162.4+/-5.8	3.52+/-0.41
1+24	114+/-10	31.2+/-3.6	310+/-5	155.3+/-2.1	3.53+/-0.21
2+0	108+/-4	81.9+/-5.6	340+/-3	168.9+/-1.8	4.39+/-0.12
2+1	133+/-7	104.3+/-11.2	356+/-6	178.2+/-1.3	0.49+/-0.07
2+3	132+/-4	87.6+/-12.4	337+/-5	171.8+/-2.2	1.96+/-0.25
2+6	128+/-6	42.4+/-6.1	322+/-2	160.9+/-1.4	3.98+/-0.22
2+12	136+/-10	43.9+/-4.0	317+/-2	155.0+/-8.7	3.33+/-0.30
2+24	134+/-9	35.6+/-4.2	310+/-3	159.2+/-2.8	4.03+/-0.20
3+0	142+/-8	114.3+/-15.5	351+/-5	177.8+/-1.4	0.52+/-0.08
3+1	140+/-7	98.5+/-5.7	365+/-5	178.3+/-2.2	0.62+/-0.06
3+3	139+/-6	60.4+/-5.5	348+/-5	168.1+/-5.7	0.89+/-0.09
3+6	146+/-7	38.4+/-4.0	342+/-4	163.1+/-4.1	4.10+/-0.14
3+12	132+/-7	38.1+/-3.8	316+/-2	159.2+/-2.3	3.89+/-0.30
3+24	119+/-7	43.2+/-3.3	313+/-2	155.7+/-1.7	3.97+/-0.33

Figure 4: Plasma cortisol levels (mean +/- SE for n=10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. Stars indicate the samples taken immediately (t=0 h) after stress and adjacent roman numerals indicate the number of stresses. Open circle and C indicate unstressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of the multiple comparison tests ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h:	1+0 < 2+0 3+0	6 h:	1+6 2+6 3+6
1 h:	1+1 2+1 3+1	12 h:	1+12 2+12 3+12
3 h:	1+3 2+3 3+3	24 h:	1+24 2+24 3+24

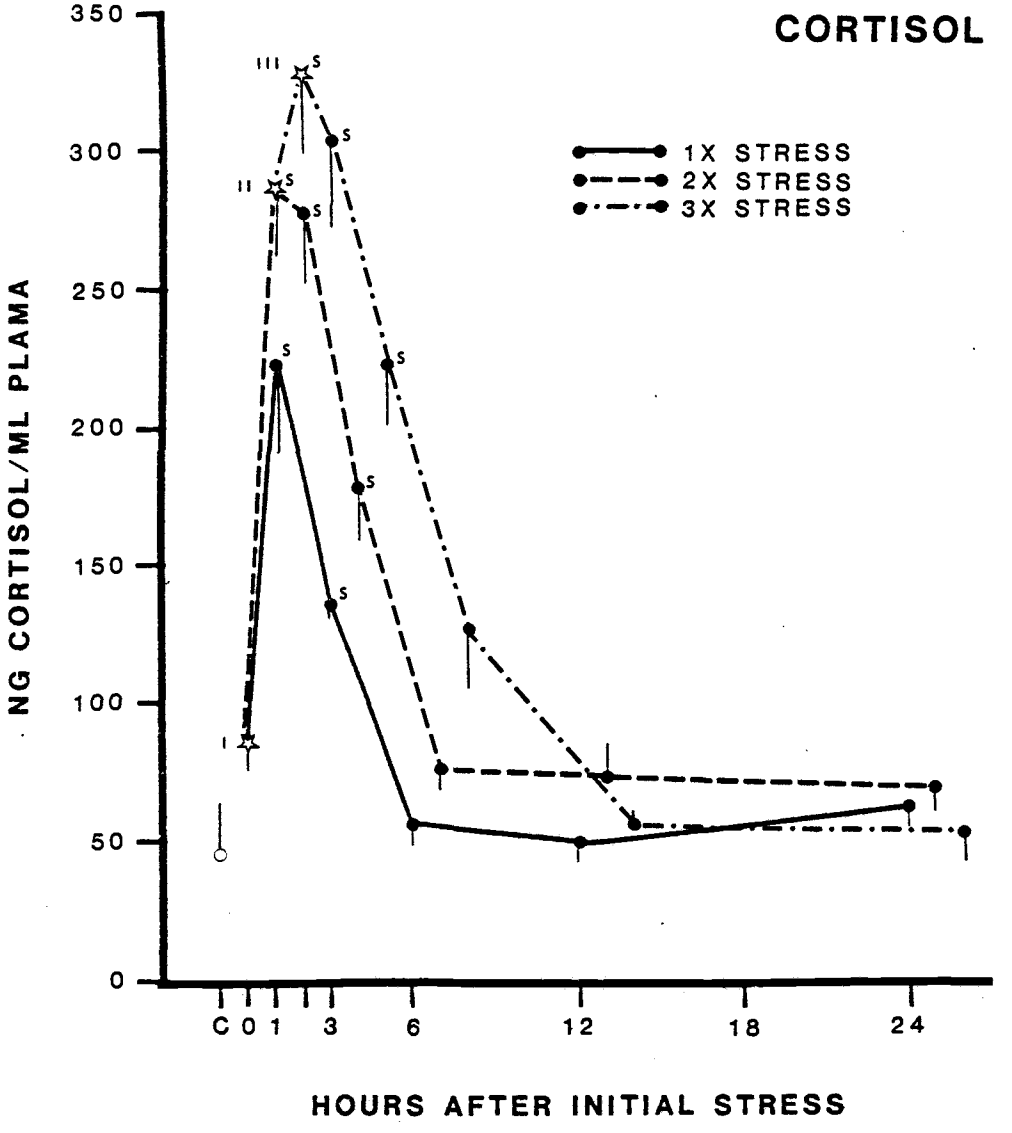
CORTISOL
1X STRESS
2X STRESS
3X STRESS
NG CORTISOL/ML PLAMA
350
300
250
200
150
100
50
0
C 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 4

Figure 5: Plasma glucose levels (mean +/- SE for n=10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. Stars indicate the samples taken immediately (t=0 h) after stress and adjacent roman numerals indicate the number of stresses. Open circle and C indicate unstressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of the multiple comparison tests ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h:	1+0 < 2+0 < 3+0	6 h:	1+6 2+6 < 3+6
1 h:	1+1 < 2+1 3+1	12 h:	1+12 2+12 3+12
3 h:	1+3 2+3 3+3	24 h:	1+24 2+24 3+24

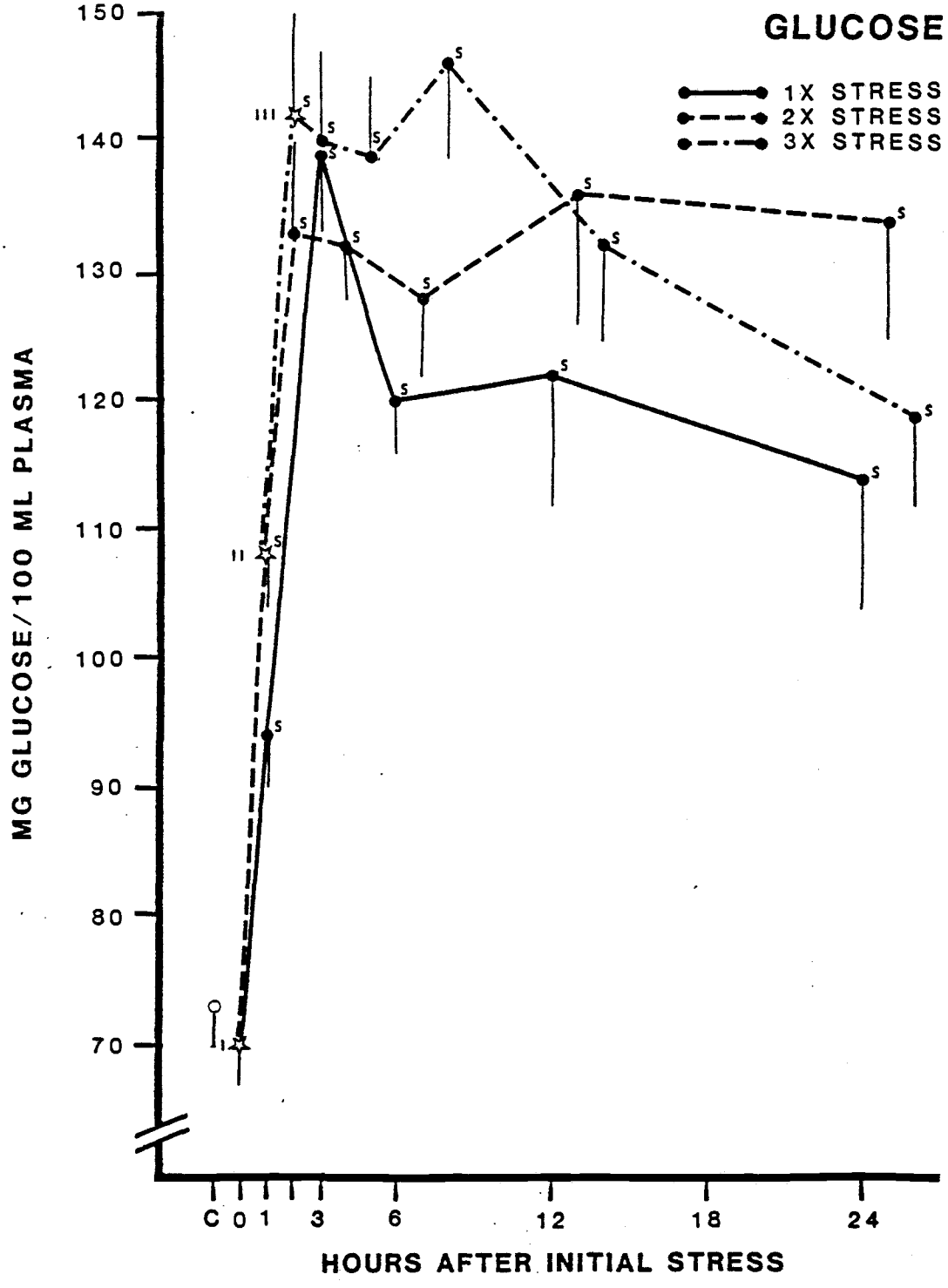
GLUCOSE
1X STRESS
2X STRESS
3X STRESS
MG GLUCOSE/100 ML PLASMA
150
140
130
120
110
100
90
80
70
C 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 5

Plasma lactic acid increased within 1 h after one stress, remained high at 3 h and returned to control levels by 6 h after one stress (Fig. 6). After two and three stresses, lactic acid levels were significantly higher than control levels at 0 h following the last stress but still returned to control levels by 6 h. Peak lactic acid levels occured at 1 and 0 h after the final stress for two and three stresses respectively. Lactic acid levels were higher in fish stressed two and three times than in fish stressed once at 0 and 1 h after the final stress. At 3, 6, 12, and 24 h after the last stress, lactic acid was not significantly different between the three groups.

Hematocrits were significantly lower than control levels in fish 6 h after a single handling stress (Fig. 7). Following two stresses, hematocrits were significantly lower than controls at 3 h after the final stress. After the third stress, hematocrits were lower than controls from 1 to 6 h. There were no significant differences between hematocrits of fish stressed 1, 2 or 3 times at 0, 1,12 and 24 h after the final stress. At 3 h after the last stress, the two and three stress groups had significantly lower hematocrits than the group stressed once, while at 6 h, the two and three stress groups had higher hematocrits than the one stress group.

Total plasma osmolarity increased significantly within 1 h after a single stress, peaked at 3 h and returned to control levels by 6 h (Fig. 8). In the fish stressed two times, osmolarity was greater than in control fish at 0 h after the second stress, peaked at 1 h and returned to the level of control fish at 6 h. Fish stressed three times had their peak osmolarity at 1 h after the third stress and continued to have significantly higher plasma osmolarities than controls for 6 h after stress while returning to control levels by 12 h. In comparing fish stressed 1, 2 and 3 times, osmolarities after two and three stresses were greater than after one stress at 0 and 1 h after the final stress. At 3 h after the final stress, osmolarity in fish with one stress was significantly greater in fish given three stresses than in fish

Figure 6: Plasma lactic acid levels (mean +/- SE for n=10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. Stars indicate the samples taken immediately (t=0 h) after stress and adjacent roman numerals indicate the number of stresses. Open circle and C indicate unstressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of the multiple comparison tests ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h:	1+0 < 2+0 3+6	6 h:	1+6 2+6 3+6
1 h:	1+1 < 2+1 3+1	12 h:	1+12 2+12 3+12
3 h:	1+3 2+3 3+3	24 h:	1+24 2+24 3+24

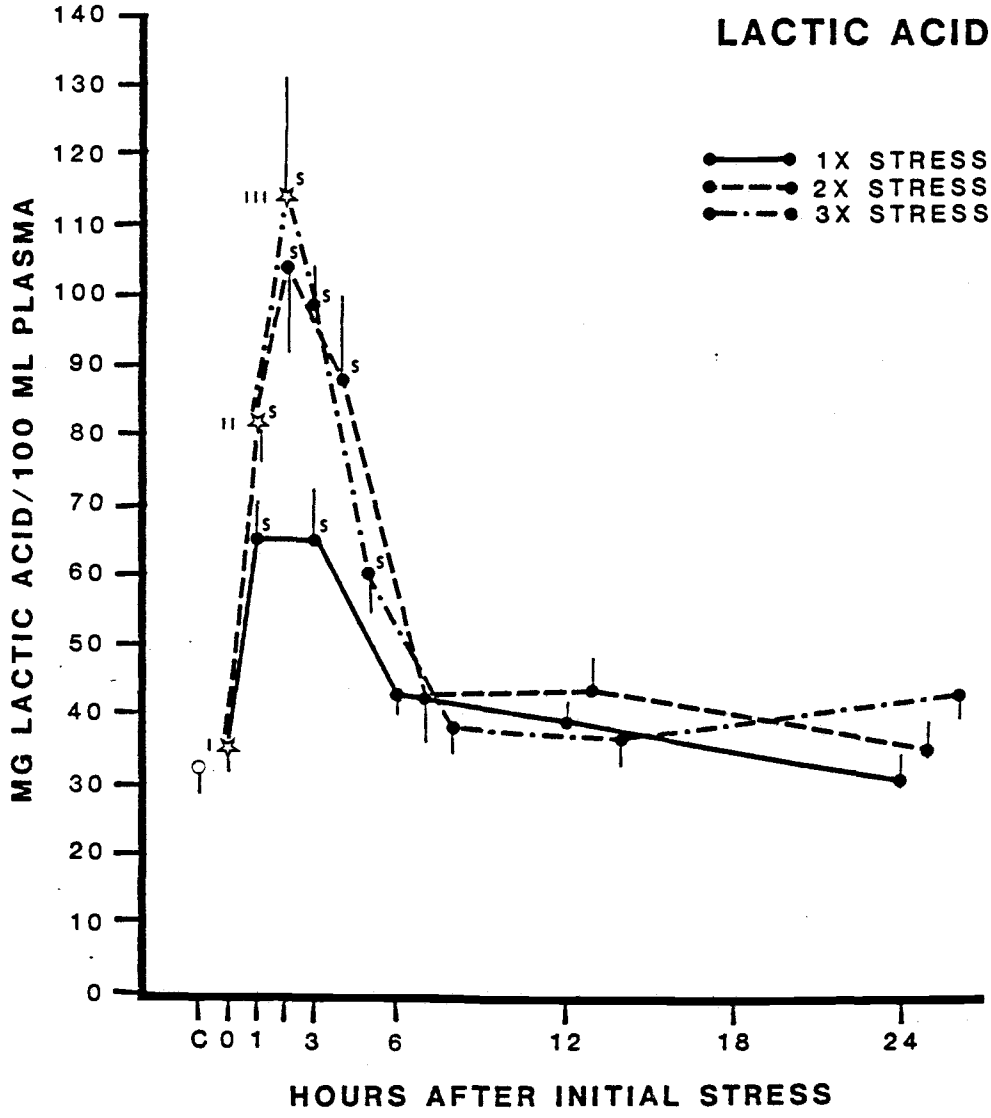
LACTIC ACID
1X STRESS
2X STRESS
3X STRESS
MG LACTIC ACID/100 ML PLASMA
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
C 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS
I
II
III
S

Figure 6

Figure 7: Hematocrit (mean +/- SE for n=10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. Stars indicate the samples taken immediately (t=0 h)after stress and adjacent roman numerals indicate the number of stresses. Open circle and C indicate unstressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of the multiple comparison tests ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h:	1+0 2+0 3+0	6 h:	1+6 < 2+6 3+6
1 h:	1+1 2+1 3+1	12 h:	1+12 2+12 3+12
3 h:	1+3 < 2+3 3+3	24 h:	1+24 2+24 3+24

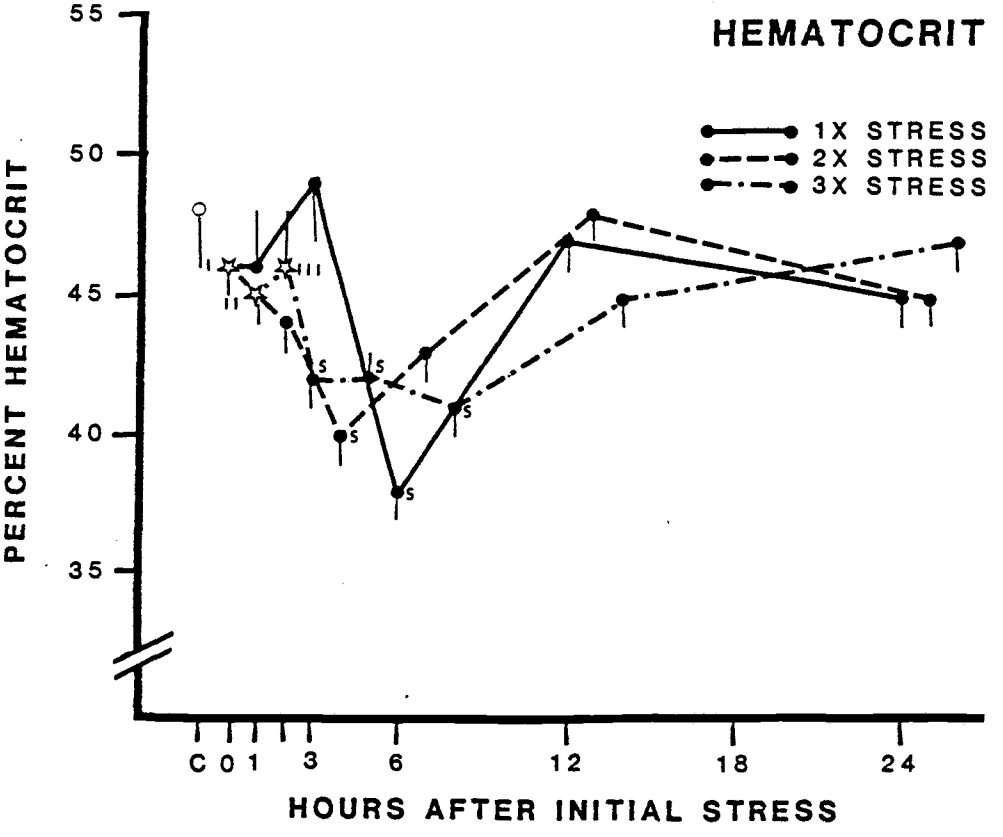
HEMATOCRIT
1X STRESS
2X STRESS
3X STRESS
PERCENT HEMATOCRIT
55
50
45
40
35
C 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 7

Figure 8: Plasma osmolarity (mean +/- SE for n=10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. Stars indicate the samples taken immediately (t=0 h) after stress and adjacent roman numerals indicate the number of stresses. Open circle and C indicate unstressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of the multiple comparison tests ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h:	1+0 < 2+0 3+0	6 h:	1+6 2+6 < 3+6
1 h:	1+1 < 2+1 3+1	12 h:	1+12 2+12 3+12
3 h:	1+3 2+3 3+3	24 h:	1+24 2+24 3+24

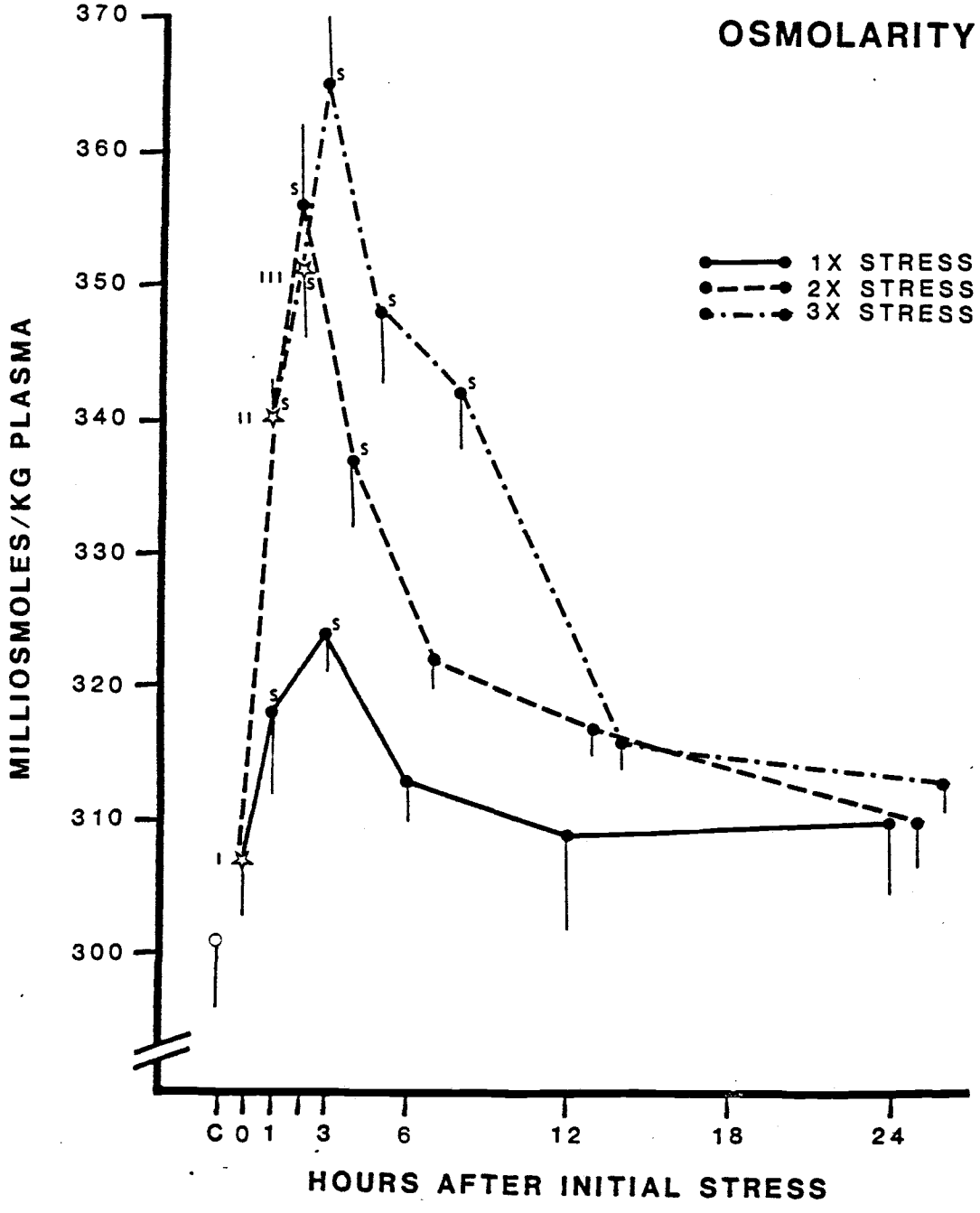
OSMOLARITY
1X STRESS
2X STRESS
3X STRESS
MILLIOSMOLES/KG PLASMA
370
360
350
340
330
320
310
300
I
II
III
S
C 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 8

given three stresses. At 6 h after the final stress, osmolarity was significantly greater in fish given three stresses than in fish given one or two stresses. There were no significant differences among groups at 12 and 24 h after the last stress.

Plasma sodium levels did not change significantly from control levels following a single stress (Fig. 9). Following a second stress, plasma sodium was higher than in controls at 0 h, peaked at 1 h and returned to control levels by 6 h. Fish stressed three times had significantly higher plasma sodium levels than controls only at 0 and 1 h after the final stress. At 0 h after the final stress, plasma sodium was significantly greater in fish stressed three times than in fish stressed twice which in turn was greater than in fish stressed once. At 1 h after the final stress, fish stressed two and three times had higher plasma sodiums than fish stressed once. At 3, 6, 12 and 24 h after the final stress, there were no significant differences between the groups with different numbers of stresses.

Plasma potassium in fish stressed once rose during the first hour, dropped to levels significantly lower than controls at 3 and 6 h post-stress and then rose to levels significantly higher than controls at 12 and 24 h post-stress (Fig. 9). After a second stress, potassium levels rose initially, dropped to levels significantly lower than controls at 1 h, rose to levels significantly higher than controls by 6 h and remained there throughout 24 h after stress. After three stresses, potassium was significantly lower than in controls for 3 h and was higher than in controls from 6-24 h after the last stress. At 0 h after the final stress, all groups are significantly different from each other. At 1 h after the final stress, fish stressed two and three times had lower potassium levels than the fish stressed once. At 3 h after the final stress, the one and three stress groups had lower potassium levels than the two stress group. At 6 h after the final stress, potassium was higher in the two and three stress group than in the one stress group. There were no significant differences among groups at 12 and 24 h.

Figure 9: Plasma sodium and potassium levels (mean +/- SE for n=10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. Stars indicate the samples taken immediately (t=0 h) after stress and adjacent roman numerals indicate the number of stresses. Open circles and C indicate unstressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of the multiple comparison tests ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

Na:	0 h:	1+0 <	2+0 <	3+0	K: 3+0 < 1+0 < 2+0
	1 h:	1+1 <	2+1	3+1	2+1 3+1 < 1+1
	3 h:	1+3	2+3	3+3	1+3 3+3 < 2+3
	6 h:	1+6	2+6	3+6	1+6 < 2+6 3+6
	12 h:	1+12	2+12	3+12	1+12 2+12 3+12
	24 h:	1+24	2+24	3+24	1+24 2+24 3+24

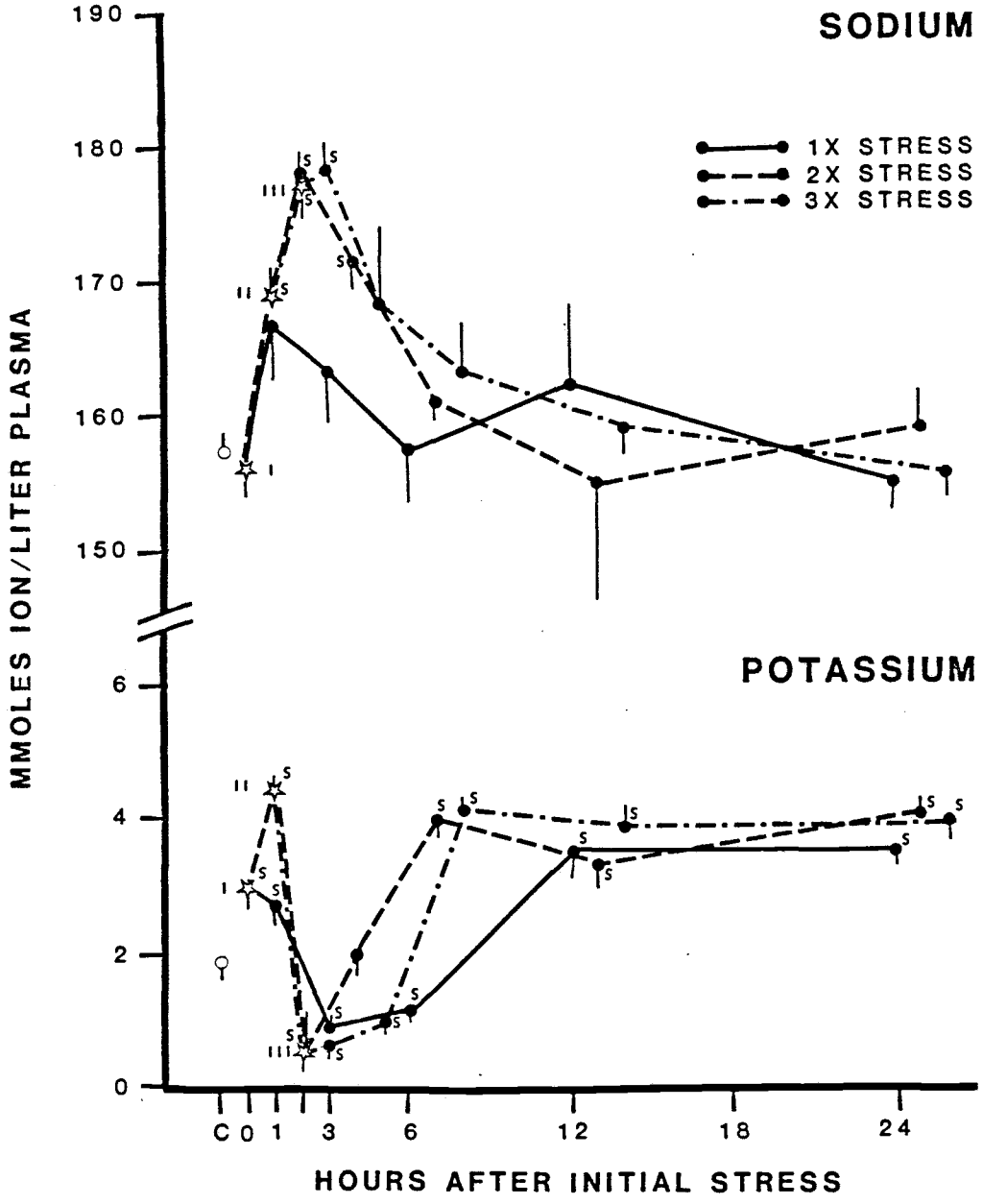
SODIUM
1X STRESS
2X STRESS
3X STRESS
190
180
170
160
150
POTASSIUM
6
4
2
0
MMOLES ION/LITER PLASMA
C 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 9

DISCUSSION

Plasma cortisol concentrations in salt water adapted fall chinook after a single stress followed a pattern similar to that found for salmonids in fresh water (Barton and Peter 1982, Pickering *et al.* 1982, Barton *et al.* 1980, Strange *et al.* 1978, Strange and Schreck 1978) which show peak cortisol levels of 45-200 ng/ml at 0.25-3 h after a single stress followed by a return to control levels in 2-12.5 hours depending on the severity of the stress. The plasma cortisol levels for the salt water adapted chinook after a single stress in this study closely paralleled the values obtained by Barton *et al.* (1985) for fresh water adapted fall chinook subjected to a similar handling stress of the same duration. Following two and three stresses, salt water adapted fall chinook showed a tendency for a general increase in the cortisol response with a corresponding increase in the time to recover, indicating that multiple stresses were cumulative. Barton *et al.* (1985) found a similar cumulative response with freshwater adapted fall chinook stressed three hours apart. They obtained peak cortisol levels of 190, 300 and 480 ng/100 ml for 1, 2, and 3 stresses respectively as compared to 215, 290, and 330 ng/100 ml in this study. The greater separation between the two and three stress peaks in the fresh water study may be due to variations in cortisol secretion and/or clearance (Strange *et al.* 1977) in fresh water versus salt water adapted chinook. Redding *et al.* (1984) found that salt water acclimated coho cleared cortisol 30% faster than those acclimated to fresh water indicating that cortisol may not accumulate as much in salt water. The greater separation may also be attributed to the different time delays used between stresses. However, in another experiment, Barton and Schreck (personal communication, Oregon State University, Corvallis, OR 97331) found that peak plasma cortisol levels in fall chinook following two stresses were the same regardless of whether 1, 3 or 12 h were used between stresses. However, the time delay between stresses may not

cause a greater separation between peaks until after three stresses. Other factors that may have contributed to the difference between fresh and salt water adapted chinook are variations in the age or in the stocks of the fish. Barton (personal communication, Oregon State University, Corvallis, OR 97331) has found differences in magnitude of the stress response with different stocks of fish.

Plasma glucose in salt water adapted chinook peaked at 3 h after a single stress and then began to decrease, though it did not return to control levels in 24 h. However, in fresh water adapted chinook (Barton *et al.* 1985), as well as in other fresh water salmonids (Pickering *et al.* 1982, Soivio and Nikinmaa 1981, Wedemeyer 1972, Nakano and Tomlinson 1967), plasma glucose generally returns to control levels within 24 h. An exception to this is a study by Wydoski *et al.* (1976), who, using a five minute hooking stress on fresh water adapted rainbow trout, obtained results nearly identical to the salt water adapted chinook in this study. Salt water species such as plaice, winter flounder, sand dabs and sculpins also show variations in the duration of the hyperglycemia (Fletcher 1984, Fletcher 1975, Wardle 1972, McCormick and MacLeod 1925), though it is generally longer than in fresh water species. The differences between studies may be due to variations in species, strains, rearing conditions, diet, or type of stress applied. Barton (personal communication, Oregon State University, Corvallis, OR 97331) has seen variability in the resting levels and magnitude of the glucose response in salmonids with stocks, species, diet and temperature.

Following two and three stresses, glucose in salt water adapted chinook did not rise higher than with one stress though glucose after two and three stresses did tend to decrease at a slower rate. Fresh water adapted chinook tested by Barton *et al.* (1985) did get larger glucose peaks with increasing number of stresses with peaks at 80, 130, and 210 mg/100 ml for 1, 2, and 3 stresses, respectively. Barton *et al.* (1985) found that glucose returned to control levesl in 24 h after one and two stresses and

was decreasing at 24 h after three stresses. In an additional study, Barton and Schreck (personal communication, Oregon State Universiy, Corvallis, OR 97331) found no differences in the glucose response of fish stressed twice with 1, 3, or 12 h between stresses. Thus, it appears the discrepancy between fresh and salt water adapted chinook was not due to the different amounts of time between stresses. It may be due to stock differences or to differences in glucose mobilization or clearance rates between fresh and salt water adapted fish.

One hypothesis to explain the more prolonged increase in glucose after stress in salt water than after stress in fresh water is the higher metabolic cost of osmoregulation in salt water. Even though the duration of the handling stresss was similar for fresh water and salt water adapted chinook, the larger osmotic difference between fish blood and sea water than between fish blood and fresh water may cause a greater osmotic stresss and thus prolonged glucose elevations to cope with it. Redding and Schreck (1983) showed that coho chronically confined in salt water had greater plasma osmolarities than a similar group of coho chronically confined in fresh water. Also, there was a greater increase in sodium in salt water adapted chinook in this study than the decrease in sodium found in stressed fresh water adapted chinook by Barton *et al.* (1985). Rao (1969) found that rainbow trout exercised in salt water had a greater increase in plasma osmolarity than those exercised in freshwater. Farmer and Beamish (1969) found similar results for rainbow trout. Nordlie and Leffler (1975), Rao (1968) and Farmer and Beamish (1969) found that oxygen consumption was greater at 30 ppt salt water than in fresh water. By subtracting the standard metabolic rate from the active metabolic rate, they found that the difference, which is the cost of swimming, was the same at all salinities. Thus, the differences in metabolic rate were attributed to a higher cost of osmoregulation. Bergstom (1971) found there was a correlation between the increase in plasma glucose and the decrease in plasma sodium when salmon were placed in deionized

water. In November, when the glycogen reserves were highest, the highest glucose levels were found and more fish survived the deionized water.

Plasma lactic acid in salt water adapted chinook following one stress peaked by 1 h at a level of 60 mg/100 ml and returned to control levels by 6 h. This pattern is the same as that found with fresh water adapted chinook (Barton *et al.* 1985) and other fish (Nikinmaa *et al.* 1983, Grahm *et al.* 1982, Pickering *et al.* 1982, Sovio and Nikinmaa 1981, Turner *et al.* 1983a,b, Sovio and Oikari 1976, Kirk 1974). The build-up in lactic acid is due to the increased anaerobic metabolism and the oxygen debt caused by the handling stress (Turner *et al.* 1983a). After two and three handling stresses, salt water adapted chinook had a cumulative plasma lactic acid build-up with peaks of 102 and 112 mg/100 ml respectively. In freshwater adapted chinook, Barton *et al.* (1985) obtained peak lactic acid concentrations of 50, 60 and 80 mg/100 ml plasma. The greater overall increase in lactic acid concentration in salt water versus fresh water may be due to the differences in delay time between stresses. In another experiment, chinook stressed twice with 1 h in between stresses had a higher peak lactate (110 mg/100 ml) than chinook stressed twice with 3 h in between stresses (Barton and Shreck, personal communication, Oregon State University, Corvallis, OR 97331).

Plasma osmolarity, electrolyte levels and hematocrit are all affected by stress. The catecholamines released after stress increase the permeability of the gills causing a net osmotic gain in fish in salt water and a net osmotic loss in fish in fresh water (Mazeaud *et al.* 1977). Catecholamines also inhibit drinking in salt water fish presumably because of gastric muscular contraction which further reduces the fishes water uptake and contributes to the net osmotic gain (Mazeaud *et al.* 1977, Pic *et al.* 1975). Concurrent increases in plasma glucose, lactic acid and cortisol following stress would also contribute to an osmotic gain in salt water.

As expected, plasma osmolarity in salt water adapted chinook showed an increase from 300 to 323 mosmoles/kg at 3 h after a single stress followed by a decrease by 12 h post-stress to control levels. Increases in osmotic concentration have been documented with stress in salt water. Farmer and Beamish (1969) observed an increase in osmotic pressure in *Tilapia nolitica* exercised in salt water. Turner *et al.* (1983b) observed an increase in osmolarity in sole, *Hippoglossoides elassodon*, after a 10 min chasing stress in which the increased osmolarity persisted for at least 6 h. Stevens (1972) oberseved a decrease in body weight, indicating water loss, in salt water acclimated *Tilapia mossambica* after handling or exercise. Redding and Schreck (1983) saw an increase in plasma osmolarity in coho chronically confined in salt water. Following two and three stresses, the osmotic concentrations of salt water adapted chinook increased cumulatively after repeated stress from 300 to 357 and 366 mosmoles/kg respectively.

Plama sodium followed a similar pattern to changes in osmolarity. After a single stress, there were no significant differences from control levels though there was a slight increase within an hour after stress. However, following two and three stresses, there was a significant increase above control levels. An increase in plasma sodium is expected in view of the gill permeability changes after stress; sodium entering from the sea water and water diffusion outward would tend to increase sodium in the plasma. Repeated stress seems to accentuate the effect. Work by Barton *et al.* (1985) showed the opposite effect in fresh water with a slight decrease in sodium following one stress and greater decreases following two and three stresses. This is expected due to the differences in the diffusion gradients between the fish and the external environment in salt water versus fresh water adapted fish.

Other investigators have found changes in sodium levels with stress. Redding and Schreck (1983) found that chronically confined coho salmon had an increased plasma sodium level in salt

water and a decreased plasma sodium concentration in fresh water. The same pattern occurred with alewifes, *Alosa pseudoharengus* , exposed to cold shock (Stanley and Colby 1971). In fresh water, goldfish (Umminger and Gist 1973), rainbow trout (Soivio and Nikinmaa 1981), brown trout (Nikinmaa *et al.* 1983) and smallmouth bass (Carmicael *et al.* 1983) experienced a decrease in plasma sodium after handling. In salt water, Fletcher (1975) observed an increase in plasma sodium of the winter flounder following handling.

The plasma potassium of stressed salt water adapted chinook followed an unexpected pattern with an initial increase in potassium, followed by a drop below control levels and then an increase above control levels. Following a single stress, the initial increase in potassium may have been due to the metabolic acidosis from lactic acid build-up shortly after stress and from respiratory acidosis brought on by handling (Turner *et al.* 1983a,b). Acidosis causes a shift in potassium from the intracellular to the extracellular fluids in order to maintain cellular electroneutrality. Turner *et al.* (1983a,b) obtained initial potassium rises in trout stressed in fresh water and flathead sole stressed in salt water.

The decrease in potassium in stressed salt water adapted chinook generally corresponded with the increase in sodium. Perhaps as sodium increases in the plasma and diffuses into cells, the activity of the cell's sodium-potassium pumps increase to maintain intracellular sodium levels at a normal level. As a consequence of the cell pumping sodium out, the plasma potassium is pumped into the cells which would cause a decrease in plasma potassium. Two studies support this hypothesis. Fletcher (1975) reported increases in intracellular sodium in salt water adapted flounder following stress. Houston *et al.* (1971) reported an increase in tissue potassium in brook trout following handling and a corresponding decrease in plasma potassium. There may be changes in excretion rates of potassium at the kidney as well. Sculpins, which, like other fish in salt water, produce little urine

when unstressed, increase urine production after handling (Eddy 1981). Thus, potassium may be lost in the urine causing the drop in plasma potassium concentration. Kobayashi and Wood (1980) have noted increased renal excretion of sodium and potassium following hypoxia.

The drop in plasma potassium is followed by an increase in potassium above control levels at a time when osmolartiy, sodium, cortisol and lactic acid are returning to control levels. This may be related to the changes in hematocrit. At the points where potassium begins to increase, hematocrit is decreased. Perhaps the fish are drinking sea water to compensate for earlier losses of water, and potassium is coming from the environment. If this is true, sodium regulation mechanisms must be working efficiently to prevent a concurrent increase in sodium which is not seen. Also, if the fish is drinking and sodium is entering the bloodstream, the pumping out of sodium at the gills by the sodium-potassium pumps may lead to an increase in potassium due to the sodium-potassium exchange. Maetz (1974) reported that the excretion of sodium in eels, *Anguilla anguilla*, is linked to the amount of potassium in the external environment to the extent that in potassium-free sea water, sodium excretion stops. This supports the idea that sodium regulation may lead to potassium build-ups. Another possible explanation for the hematocrit decrease and potassium increase would be lysis of red blood cells which would release cell contents into the plasma. Hattingh and Van Pletzen (1974) noted increased erythrocyte fragility after capture. However, Turner *et al.* (1983a) did not observe any cell lysis in fresh water adapted rainbow trout though they did observe increases in potassium. Also, Pickering *et al.* (1982) and Pickford *et al.* (1971) did not find changes in erythrocyte levels with stress. Further experimentation measuring red blood cell counts concurrently with hematocrit and water content in salt water adapted fish following stress would be needed to examine this hypothesis.

Changes in levels of potassium following stress have been

reported by several investigators. Turner *et al.* (1983a), Wood *et al.* 1983, Grahm *et al.* (1982) and Sovio and Nikinmaa (1981) observed a significant elevation in plasma potassium in fresh water adapted rainbow for 3-8 h following stress. Carmichael *et al.* (1983) found a similar response with smallmouth bass. Barton *et al.* (1985) also found elevations in potassium in fresh water adapted fall chinook after stress with a prolonged increase when subjected to several stresses. This increase in potassium in fresh water is believed to be due to intracellular acidosis (Turner *et al.* 1983a,b). In salt water, Turner *et al.* (1983b) observed an increase in potassium in sole, following exhaustive exercise, then a return to control levels in 8 h. Again this is explained as being due to the metabolic acidosis. The difference between Turner's observation and the changes in salmon observed in this study may be due to species and/or life style differences since flatfish are a relatively sedentary species compared with salmon. Redding and Schreck (1983) also report an increase in potassium in coho salmon chronically confined in salt water. The difference in this case may be due to the duration of the stress or, since the potassium readings were taken at 1 and 7 h in the chronically confined coho, the dip in potassium may have been missed.

Changes in hematocrit have also been noted after stress. Generally, it seems that there is an initial hemoconcentration and increase in hematocrit after stress due to a shift in water to the intracellular compartment to compensate for build-up of lactic acid (Turner et al. 1983a, 1983b). In fresh water this is often followed by a decrease in hematocrit probably due to the hemodilution resulting from the osmotic imbalance (Soivio and Oikari 1976, Sovio and Nikinmaa 1981). In salt water a hemoconcentration would be expected due to the water loss. Fletcher (1975) found an initial increase in hematocrit in flounder followed by a return to normal hematocrit within 24 h. No decreases were noted. However, Fletcher did not sample between 4 and 20 h after stress which was where the dip in hematocrit occured for chinook. Redding and Schreck (1983) did obtain a

slight drop in hematocrit after 7 h of confinement in salt water adapted coho which is similar in timing to that of chinook subjected to an acute stress. Turner *et al.* (1983b) also observed a decrease in hematocrit in sole from 1-12 h after stress.

Much more experimentation involving frequent sampling will be necessary to explain the changes in potassium and hematocrit that occur after stress. Factors such as 1) intracellular versus extracellular electrolyte and water levels, 2) electrolyte and water effluxes and influxes at the gills, gut and kidneys and 3) red blood cell counts, sizes and distribution will need to be examined.

The cumulative nature of the physiological response to stress, as evidenced in the cortisol, lactic acid, osmolarity and sodium data, has important implications in the study of fish biology as well as in fisheries management. The cumulative increases in cortisol indicate that the fish is capable of an additional corticosteroid response after additional stress in spite of the existing levels of cortisol in the blood. Generally, cortisol in the bloodstream would cause a negative feedback to the hypothalmus that would suppress further secretion of adrenocorticotropic hormone (ACTH) by the pituitary which in turn would prevent cortisol from continuously increasing in circulation (Fryer and Peter 1977). A second acute stress, especially after a short recovery time, may override this negative feedback loop and cause further secretions of ACTH and thus further elevations of plasma cortisol. The cumulative increases in lactic acid may be related to the slow clearance of lactic acid from the blood of fish. Following the build-up of an oxygen debt in the tissues with stress, lactic acid slowly diffuses into the blood stream, peaks several hours after stress, then returns to normal in 12-24 h (Jones and Randall 1978). Additional stress and subsequent build-up of lactic acid in the tissues before the plasma lactic acid levels from the previous stress have been cleared would cause cumulative increases in plasma lactic acid. This view is supported by the cumulative stress data in Barton *et al.* (1985) in which a 3 h delay between stresses caused smaller cumulative

increases in lactic acid than the 1 h delay used in this study. The cumulative increases in osmolarity and sodium are probably a consequence of the cumulative increases in cortisol since cortisol affects osmoregulation in fish (Eddy 1981).

The fact that the stress response is cumulative is of consequence to the fish. A stress, which alone may not be very harmful to the fish, may become a problem when it occurs after another stress. Also, since the performance of an organism depends on the combined performances of the organismic subsystems (Warren et al. 1979), the cumulative physiological changes associated with stress may lead to cumulative changes in performances such as swimming that can affect the organism's chances for survival. Thus, in management situations, fish should be given time to recover from stress before being subjected to additional disturbances.

CONCLUSIONS

Repeated stress in salt water adapted chinook caused cumulative changes in the plasma osmolarity and concentrations of cortisol, lactic acid and sodium indicating that the effects of stress are cumulative. Repeated stress also caused significant, though not cumulative, changes in hematocrit, plasma glucose and plasma potassium. The physiological reponses to stress in salt water adapted chinook were not quite the same as those seen in similarly stressed fresh water adapted fish in regards to duration, direction and/or maginitude of the changes. The differences in direction of the changes for osmolarity and sodium were likely due to differences in the diffusion gradients between the two media. Differences in the other physiological parameters may be due to variability between stocks or the potential higher cost of osmoregulation in salt water.

CHANGES IN THE SWIMMING PERFORMANCE OF SALT WATER ADAPTED JUVENILE FALL CHINOOK SALMON (*Oncorhynchus tshawytscha*) AFTER EXPOSURE TO ONE, TWO OR THREE ACUTE HANDLING STRESSES

ABSTRACT

Two types of swimming tests, critical swimming speed and fatigue time, were used to determine the effects of handling stress on the swimming performance of chinook salmon (*Oncorhynchus tshawytscha*) smolts. Tests were done on fish stressed 0, 1, 2 or 3 times with one hour between stresses and on fish 1, 3, 6, 12 and 24 h after stress.

Critical swimming speed was measured by increasing the water velocity in a flow-through swim tube and noting the velocity at which each fish stopped swimming. Critical swimming speeds after handling were highly variable and no differences were found between stressed fish and non-stressed fish at any level of stress or recovery time.

Fatigue time was measured as the time a fish could maintain position in a swim tube at a given constant water velocity (60 cm/sec). Following each fatigue test, fish were killed and blood samples were obtained. Unlike non-stressed fish which all fatigued within 13 min, the time to fatigue of stressed fish varied with some fish fatiguing within a few minutes and some fish swimming the 60 min period. There was a slight depression in fatigue times immediately following one and three stresses. Immediately after two stresses and with all groups given time to recover after stress, fatigue times were similar to or higher than for non-stressed fish.

Plasma osmolarity and concentrations of cortisol, glucose, and sodium were higher in swimming fish than in non-swimming controls. Plasma cortisol, glucose and lactic acid were all highly variable in fish after fatigue and no differences were found between stressed and unstressed fish at any level of stress or any time after stress. Plasma osmolarity and sodium levels were higher than controls in fatigued fish immediately after one stress. Plasma potassium was higher in fatigued fish than in fatigued controls at several time periods after one and three stresses but not at any of the time periods after two stresses.

INTRODUCTION

Fish are exposed to stress from many natural and man-made sources. Natural stresses include disease, predation and competition among individuals. Man-made stresses include fishing pressure, water pollution, hydroelectric dams, and industrial water intakes. Man also imposes stress by handling fish involved in hatchery or fish farming operations. Since stresss is a part of a fish's life history, it is of interest to know how stress affects fish at both the whole organism and physiological levels. Furthermore, since exposure to two or more successive stresses is likely, information is needed as to the effects of repeated stresses on the ability of fish to respond and survive.

Various physiological changes have been measured in fish after exposure to stress. These include changes in plasma hormone levels, elevation of plasma glucose and lactic acid, changes in plasma pH and electrolyte levels, histological changes, depletion of liver glycogen, and depression of the immune system (Mazeaud and Mazeaud 1981). Barton *et al.* (1985) has examined some the above responses after multiple stresses and found a cumulative affect.

Knowing that stress affects a fish physiologically, the next step is to determine how stress affects the performance of the organism. Swimming is one type of performance of interest to fisheries biologists because it is involved in many of the daily activities of fish including territory defense, staying or getting to a favorable environment, obtaining food and avoiding predation. In this study, two swimming tests, critical swimming speed and fatigue time, were used to evaluate the performance of fish following 1, 2, or 3 stresses. Physiological changes in the fish after stress and swimming were also examined.

MATERIALS AND METHODS

Fish

Juvenile fall chinook (20.9 cm mean fork length, 105.1 g mean wet weight) were obtained from Oregon Department of Fisheries and Wildlife Trask River Hatchery and transported to the Oregon State University Marine Science Center in Newport, Oregon in October of 1983. Fish were held in a flow-through 1.8 m circular tank in fresh water for several weeks and vaccinated against *Vibrio*. Fish were then gradually acclimated to 22-27 ppt sea water over a two day period and held at 10-14 C for several weeks before the start of the experiment. The fish were obtained shortly before the normal hatchery release date and were considered to be smolts. The plasma sodium concentrations, obtained on the fish after transfer to salt water,were in the normal range for a sea water fish, verifying that the fish were able to handle the salt water. The fish also fed and grew well in the salt water environment further indicating that they were smolts.

Apparatus

Swimming performance was measured in three flow-through swim tubes (Fig. 10). The test portion of the tubes in which fish swam was 7.6 cm in diameter and 1.5 m in length with screening at both ends to contain the fish. The tubes were equipped with a capped T for placing fish in the apparatus and a rotating end screen for removing impinged fish. The test portion of the swim tubes was enclosed in black plastic to reduce the effects of movement by the investigator on fish performance. The enclosed portion had a fluorescent light so fish were swimming in the light. The inflow end of the tubes had a baffling screen to eliminate turbulence. A flow meter was positioned on the inflow end of the

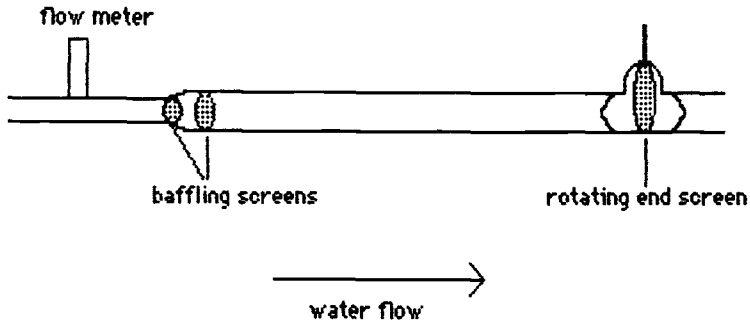

Figure 10: Diagram of swim tube used in critical swimming speed and fatigue time tests. The test section is 7.6 cm in diameter and 1.5 meters in length.

tubes, behind the baffling screen, so as not to interfere with the fish in the tubes. Water was supplied through the Marine Science Center sea water line and velocities in excess of 105 cm/sec could be achieved in the test section of the swim tubes.

Critical Swimming Speed

Critical swimming speed was measured by subjecting fish to stepwise increases in water velocity and noting the water velocity and time that each fish stops swimming. Preliminary experiments were done to determine the optimum conditions for the following parameters: length of time required to acclimate fish to the tube, number of fish to be used per trial, magnitude of the velocity increments, length of time at each velocity increment and the minimum water velocity at which fish must actively swim.

Three fall chinook smolts were introduced to the swimming tube and allowed to adjust for 18-24 h at a water velocity of 5 cm/sec. Fish were then either tested for swimming speed or stressed by draining the water from the tube for 30 sec. Fish were stressed 1, 2, or 3 times with a 1 h delay between stresses. After each stress, the water velocity was maintained at 5 cm/sec until the next stress or until the swimming trial was begun at 0, 1, 3, 6, 12, or 24 h after the last stress. A swimming trial consisted of increasing the water velocity to 25 cm/sec for 30 min then increasing the velocity by 10 cm/sec every 5 min. The time and water velocity at which each fish stopped swimming was noted. Critical swimming speed (CSS) as defined by Brett (1964) was then calculated as:

$$CSS = V_{max-1} + (I*t/T)$$

where:

T = length of time at each velocity, here equal to five minutes

V_{max-l} = maximum water velocity achieved and maintained for T

t = the length of time the fish swam at the maximum water velocity achieved (V_{max})

l = increment at which water velocity was increased, here equal to 10 cm/sec.

Four replicates of three fish were made for each experimental group and results were pooled. Mean critical swimming speed was calculated for each group. One-way analyzes of variance were done to examine the effects of stress over time and to examine whether repeated stress had cumulative effects.

Fatigue Time

Fatigue time was measured by subjecting fish to a constant water velocity and noting the time that the fish stopped swimming. Preliminary experiments were done to determine the water velocity to be used for measuring fatigue time.

Experiments to determine fatigue time were conducted in much the same way as the critical swimming speed experiments. Three juvenile fall chinook were acclimated overnight and stressed either 0, 1, 2, or 3 times as in the critical swimming speed tests. Fatigue trials were run at 0, 1, 3, 6, and 24 h after the last stress. A swimming trial consisted of immediately increasing the water velocity to 25 cm/sec for 30 min then increasing the velocity to 60 cm/sec. The time at which each fish stopped swimming was noted during a total test time of 60 min. As each fish became impinged on the end screen, or at 60 min, the fish was immediately removed from the tube, anesthetized, measured and a blood sample was taken. Blood samples were also obtained from unstressed fish not exposed to the swim tubes. Three replicates of three fish were tested for each experimental group and the data was pooled. The time to 50% fatigue was calculated for each group by plotting cumulative percent fatigued

on a probit scale versus time to fatigue on a logarithmic scale and extrapolating the 50% fatigue time from the regression line.

The data from the fatigue time test indicated two groups of fish, one group that fatigued within 60 min and the other group that did not. In order to determine if the prescence of fish that swim 60 min in a treatment group was due to the stress or due to random chance, chi square tests were performed on the data.

Blood sampling consisted of transferring the fish immediately upon removal from the swim tube into a bucket containing 250 mg/l tricaine methanesulfonate (MS-222). As soon as the fish were immobilized, the caudal peduncle was severed and blood was collected in heparinized 0.25 ml capillary tubes. Blood samples were centrifuged and the plasma was removed and stored at -20 C. Additional blood was taken in heperinized 1.1 mm capillary tubes for hematocrit determination. These samples were centrifuged immediately in an hematocrit centrifuge and hematocrit was read directly from a card reader.

Plasma samples were analyzed for cortisol, glucose, lactic acid, osmolarity, sodium and potassium. Cortisol was measured using a radioimmunoassay (Redding *et al.*, 1984), glucose using the O-toluidine method (Hyvarinen and Nikkila 1962 as cited by Wedemeyer and Yasutake 1977) and lactate using a fluorimetric, enzyme reaction (Passonneau 1974). Osmolarity was determined using a vapor pressure osmometer (Westcor). Sodium and potassium were determined using a microelectrode sodium-potassium analyzer (Nova 1). For the sodium and potassium determination, plasma samples were diluted with a known standard and actual concentration was determined by the standard addition method (Skoog and West 1976).

Mean plasma concentrations were calculated for each parameter measured for each experimental group. One way analyzes of variance were examined to determine the effect of stress over time and to determine whether repeated stresses had a cumulative effect. Analysis of variance was followed by the sum of squares simultaneous test procedure (Sokal and Rohlf 1969) for

comparison of means when appropriate. The choosen level of significance was $P<0.05$. In the case of sodium and some of the potassium comparisons, Bartlett's test (Sokal and Rohlf 1969) indicated that the variances were heterogeneous which violates one of the assumptions of the analysis of variance. Since a log transformation did not remedy this situation, the nonparametric Kruskal-Wallis test was used in place of the analysis of variance and a non-parametric multiple comparison test suggested in Daniel (1978) was used to compare means when levels were significantly different.

A t-test or an approximate t-test for samples whose variances are assumed to be unequal (Sokal and Rohlf 1969) was used to compare plasma concentrations of each of the parameters between swimming and non-swimming controls.

Mean plasma concentrations for each parameter were calculated for all fish that fatigued early (<20 min) and for all fish that swam almost or the complete time period (>40 min). A t-test was used to determine if the means were significantly different between the two groups for any of the blood parameters measured.

RESULTS

Critical Swimming Speed

Critical swimming speeds of fish stressed 1, 2 and 3 times and of fish tested 1, 3, 6, 12 and 24 h after stress are listed in Table 2 and represented graphically in Figure 11. (Critical swimming speeds of individual fish are listed in appendix 2.)

The critical swimming speeds of stressed fish tended to be slightly below that of non-stressed fish. However, these differences were not significant. Analyzes of variance showed no significant differences between fish stressed 1, 2, or 3 times with 1 h between stresses or between fish given 0, 1, 3, 6, 12 or 24 h to recover.

Fatigue Time

Fatigue times of stressed fish were highly variable (Fig. 12, Table 3). All unstressed fish quit swimming within 13 min. However, in each of the stressed groups, there were fish that quit swimming early (21 min or less) as well as fish that swam the 60 min period. The time to 50% fatigue was depressed immediately following the first stress. With one or more hours of recovery after one stress, the time to 50% fatigue was similar to or greater than in unstressed fish. Immediately following two stresses, the time to 50% fatigue increased from 3.8 to 6.4 min. With 1 through 24 h recovery after two stresses, the fatigue times generally remained near to or greater than in the non-stressed fish. The exception to this was the two stress plus six hours recovery group which had a time to 50% fatigue of 2.4 min. However, in this group, 33% of the fish swam longer than 60 min. Following three stresses, there was another drop in time to 50% fatigue to 1.1 min. With one through 24 h of recovery after three stresses, the times to 50% fatigue were similar to or greater than in unstressed fish.

Table 2: Critical swimming speeds and fork lengths (mean +/- standard error for n=12) of fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. Critical swimming speeds are listed in actual velocity (cm/sec) and velocity in terms of body lengths/sec (cm/sec/fork length). The groups are listed as x+y where x indicates the number of stresses and y indicates the hours after stress.

GROUP (X+Y)	LENGTH (CM)	CRITICAL SWIMMING SPEED (CM/SEC)	CRITICLE SWIMMING SPEED (BODY LENGTHS/SEC)
NO STRESS	19.6+/-0.3	62.9+/-6.5	3.2+/-0.4
NO STRESS+24H	19.6+/-0.2	60.4+/-6.6	3.1+/-0.3
1 STRESS	18.7+/-0.6	51.7+/-9.3	2.8+/-0.5
1 STRESS+1H	19.9+/-0.5	47.7+/-6.3	2.4+/-0.3
1 STRESS+3H	18.1+/-0.3	49.5+/-6.3	2.7+/-0.3
1 STRESS+6H	19.2+/-0.4	48.3+/-7.9	2.5+/-0.4
1 STRESS+12H	19.3+/-0.4	55.7+/-6.6	2.9+/-0.3
1 STRESS+24H	18.0+/-0.3	47.9+/-4.7	2.7+/-0.3
2 STRESSES	17.9+/-0.3	41.9+/-8.8	2.3+/-0.5
2 STRESS+1H	19.2+/-0.4	65.2+/-6.7	3.4+/-0.3
2 STRESS+3H	18.9+/-0.3	54.3+/-9.6	2.9+/-0.5
2 STRESS+6H	18.5+/-0.4	45.8+/-5.6	2.5+/-0.3
2 STRESS+12H	19.8+/-0.3	54.0+/-5.7	2.8+/-0.3
2 STRESS+24H	19.2+/-0.4	56.9+/-6.1	3.0+/-0.3
3 STRESSES	18.9+/-0.4	49.4+/-7.6	2.6+/-0.4
3 STRESS+1H	18.7+/-0.3	44.8+/-8.0	2.4+/-0.4
3 STRESS+3H	19.7+/-0.4	66.9+/-6.5	3.4+/-0.3
3 STRESS+6H	17.8+/-0.5	57.2+/-6.3	3.2+/-0.3
3 STRESS+12H	19.1+/-0.3	43.0+/-8.1	2.3+/-0.4
3 STRESS+24H	19.0+/-0.3	56.7+/-6.4	3.0+/-0.4

Figure 11: Critical swimming speeds (mean +/- SE for n=12) of fish exposed to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12 and 24 h after the final stress. Stars indicate the samples taken immediately after stress and adjacent roman numerals indicate the number of stresses. Open circles indicate unstressed fish.

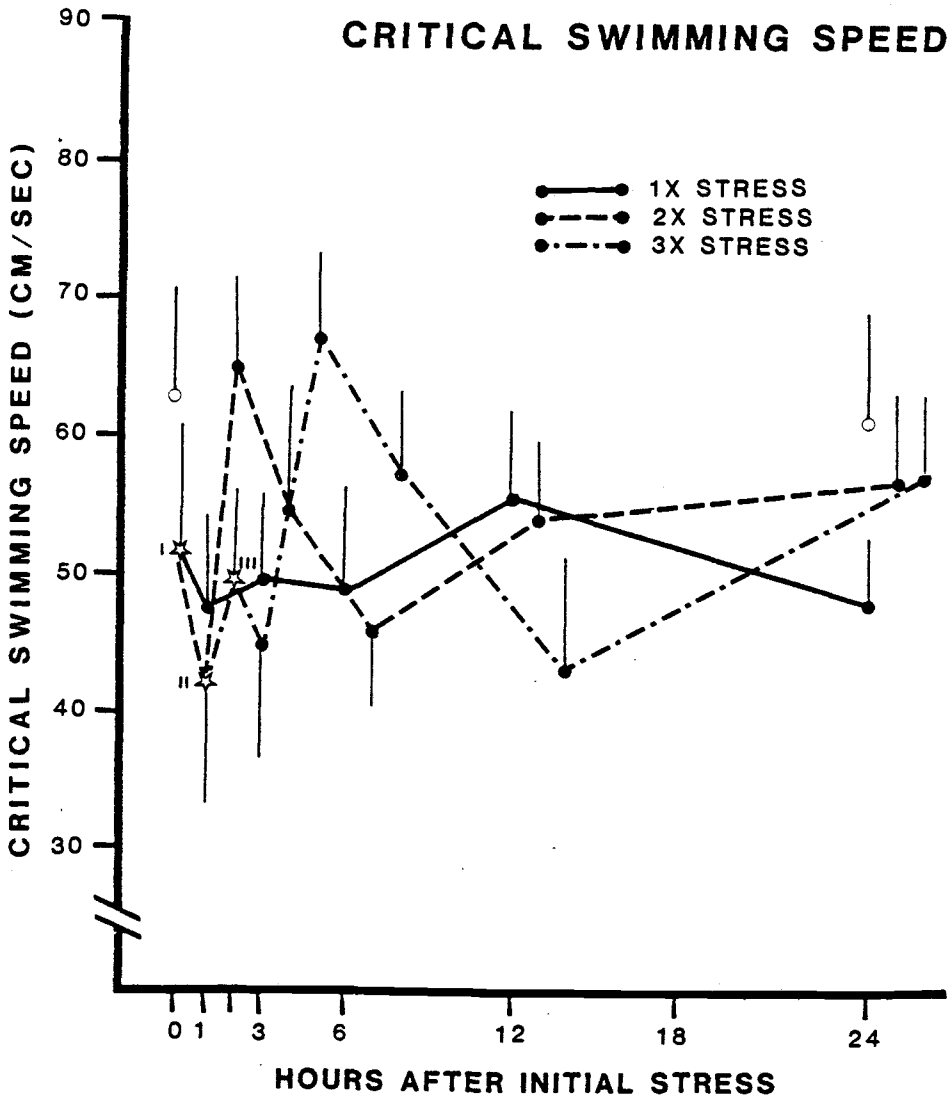
CRITICAL SWIMMING SPEED
1X STRESS
2X STRESS
3X STRESS
CRITICAL SWIMMING SPEED (CM/SEC)
90
80
70
60
50
40
30
I
II
III
0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 11

Figure 12: Fatigue times (min) of individual fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, and 24 h after the final stress. Stars indicate the time to 50% fatigue for each group.

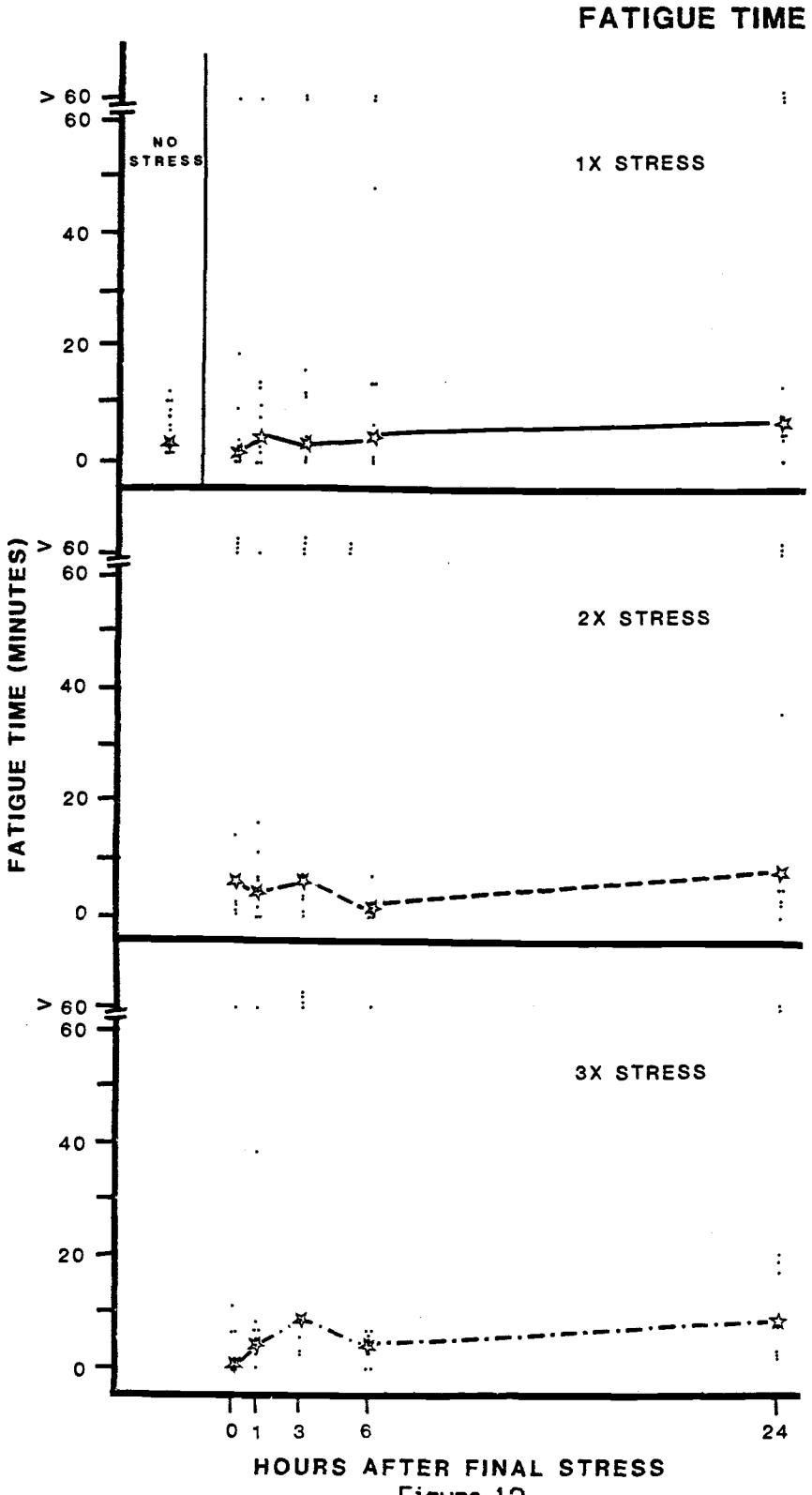
FATIGUE TIME
NO STRESS
1X STRESS
2X STRESS
3X STRESS
FATIGUE TIME (MINUTES)
> 60
60
40
20
0
0 1 3 6 24
HOURS AFTER FINAL STRESS

Figure 12

Table 3: Fatigue time (time to 50% fatigue for n=9) and mean (+/- SE for n=6-9) fork lenght, weight, LeCren's condition factor, hematocrit, plasma osmolarity and plasma concentraions of cortisol, glucose, lactic acid, sodium and potassium of fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6 and 24 h after the final stress. The groups are listed as x+y where x indicates the number of stresses and y indicates the hours after stress (e.g. 1+0 indicates one stress with 0 h recovery time).

Table 3

GROUP (X+Y)	FT_{50}	LT(CM)	WT(G)	HEMAT-OCRIT	CONDITION FACTOR	CORTISOL (NG/ML)
CONTROL		20.6+/-0.4	97.9+/-7.6	41+/-1	0.99	30+/-7
NO STRESS	3.8	20.9+/-0.3	109.3+/-6.2	40+/-2	1.06	139+/-29
1+0	2.0	20.2+/-0.6	92.3+/-9.3	42+/-4	1.00	159+/-23
1+1	4.8	20.5+/-0.3	96.9+/-7.2	37+/-3	1.00	112+/-22
1+3	3.5	21.8+/-0.5	122.6+/-8.0	45+/-2	1.02	161+/-23
1+6	5.1	21.0+/-0.4	108.6+/-8.0	47+/-2	1.03	149+/-30
1+24	7.9	21.4+/-0.6	113.0+/-10.4	42+/-3	1.01	114+/-18
2+0	6.4	21.1+/-0.5	111.7+/-9.7	42+/-3	1.04	148+/-26
2+1	4.2	20.7+/-1.6	103.2+/-8.5	46+/-2	1.03	223+/-30
2+3	5.8	21.5+/-0.3	116.2+/-6.2	37+/-3	1.02	134+/-40
2+6	2.4	20.2+/-0.5	92.4+/-7.3	39+/-2	1.00	168+/-47
2+24	8.3	21.2+/-0.4	107.8+/-6.9	48+/-2	1.00	156+/-23
3+0	1.1	19.9+/-0.5	86.7+/-8.8	34+/-4	0.99	160+/-22
3+1	4.5	20.5+/-0.3	98.2+/-5.4	40+/-2	1.01	130+/-27
3+3	9.3	20.8+/-0.5	105.8+/-8.5	44+/-2	1.04	91+/-14
3+6	4.6	21.3+/-0.2	116.7+/-4.0	34+/-3	1.06	154+/-38
3+24	9.1	21.2+/-0.6	107.3+/-8.2	41+/-2	0.99	116+/-27

GROUP	GLUCOSE (MG/100ML)	LACTIC ACID (MG/100 ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
CONTROL	79+/-3	39.9+/-3.0	315+/-4	154.2+/-1.5	1.93+/-0.34
NO STRESS	100+/-8	60.1+/-9.2	335+/-8	167.7+/-2.7	1.14+/-0.41
1+0	109+/-6	64.8+/-6.8	369+/-13	192.9+/-7.7	3.38+/-0.58
1+1	109+/-6	60.2+/-8.1	340+/-5	168.8+/-1.5	3.31+/-0.64
1+3	107+/-10	69.1+/-9.0	334+/-9	165.8+/-3.4	2.75+/-0.38
1+6	94+/-7	70.2+/-9.0	329+/-7	160.7+/-1.9	3.34+/-1.02
1+24	92+/-5	62.2+/-5.1	322+/-5	152.9+/-7.8	1.54+/-0.35
2+0	107+/-8	68.7+/-7.1	347+/-9	171.4+/-5.2	2.86+/-0.53
2+1	116+/-6	67.5+/-10.4	349+/-11	175.0+/-5.5	2.58+/-1.20
2+3	107+/-9	60.5+/-5.4	336+/-8	162.9+/-2.9	3.12+/-0.30
2+6	90+/-7	54.2+/-6.1	323+/-8	158.1+/-2.7	3.16+/-0.69
2+24	90+/-7	61.7+/-9.1	328+/-4	160.2+/-1.7	2.52+/-0.82
3+0	119+/-10	68.5+/-11.2	372+/-14	178.2+/-9.1	2.16+/-0.50
3+1	115+/-5	68.0+/-7.5	341+/-6	179.2+/-6.6	5.67+/-1.40
3+3	134+/-15	65.0+/-7.0	349+/-8	164.1+/-5.0	2.54+/-0.39
3+6	104+/-12	68.0+/-3.3	332+/-7	163.8+/-3.1	3.91+/-0.62
3+24	107+/-10	63.7+/-8.5	327+/-8	165.1+/-4.7	3.07+/-0.40

The results of the chi-square test for the fatigue time data are presented in Table 4. In comparing groups with 0, 1, 2 and 3 stresses, the chi-square value was significant indicating that stress does increase the number of fish swimming greater than 60 min. A significant chi-square was also found when comparing the three stress group over recovery times. The rest of the chi-square values were not significant.

The fatigue times and corresponding physiological levels of hematocrit, plasma osmolarity and concentrations of cortisol, glucose, lactic acid, sodium and potassium for each group of fish are presented in Table 3. (Fatigue times and blood parameters for individual fish are listed in appendix 3.)

Plasma cortisol was significantly higher in unstressed swimming fish than in unstressed, non-swimming controls (Fig. 13). The analysis of variance comparing unstressed fish, fish stressed one time, and fish allowed 1, 3, 6 or 24 h to recover after the single stress showed no significant differences. The same was true for two and three stresses. Analyzes of variance comparing 1, 2, and 3 stresses at each of the time periods after stress also showed no significant differences except at 1 h after the final stress when the fish with two stresses had significantly higher cortisol levels than the fish with one stress.

Glucose was significantly greater in swimmimg fish than in unstressed, non-swimming controls (Fig. 14). Analyzes of variance showed no significant differences between unstressed swimming fish and fish stressed 1, 2, or 3 times at any time after stress. However, fish stressed three times tended to have slightly higher glucose levels. There were also no significant differences between fish stressed 1, 2 or 3 times at any of the time periods after stress.

Lactic acid was variable in all groups of fish (Fig. 15) and no significant differences were found between groups of swimming fish at any level of stress or any recovery time. There was also no significant difference between swimming and non- swimming fish though swimming fish tended to have higher lactic acid levels.

Table 4: Results of Chi-Square test on fatigue time data. Groups are listed as x+y where x represents the number of stresses and y represents the hours after stress.

GROUPS BEING COMPARED	CHI-SQUARED	PROBABILITY
0+0, 1+0, 1+1, 1+3, 1+6, 1+24	5.19	0.1<p<0.5
0+0, 2+0, 2+1, 2+3, 2+6, 2+24	8.87	0.1<p<0.5
0+0, 3+0, 3+1, 3+3, 3+6, 3+24	12.45	0.025<p<0.05
0+0, 1+0, 2+0, 3+0	8.27	0.025<p<0.05
1+1, 2+1, 3+1	0.00	0.90<p<.98
1+3, 2+3, 3+3	2.15	0.1<p<0.5
1+6, 2+6, 3+6	1.29	0.5<p<0.9
1+24, 2+24, 3+24	0.36	0.5<p<0.9

Figure 13: Plasma cortisol levels (mean +/- SE for n=8-9) after fatigue of fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6 and 24 hours after the final stress. Stars indicate the sampless taken immediately after stress and adjacent roman numerals indicate the number of stresses. Open circles indicate unstressed fish. C indicates unstressed, non-swimming control fish. S indicates unstressed swimming control fish. Results of the sum of squares simultaneous test procedure ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h: 1+0 2+0 3+0

1 h: 1+1 3+1 < 2+1

3 h: 1+3 2+3 3+3

6 h: 1+6 2+6 3+6

24h: 1+24 2+24 3+24

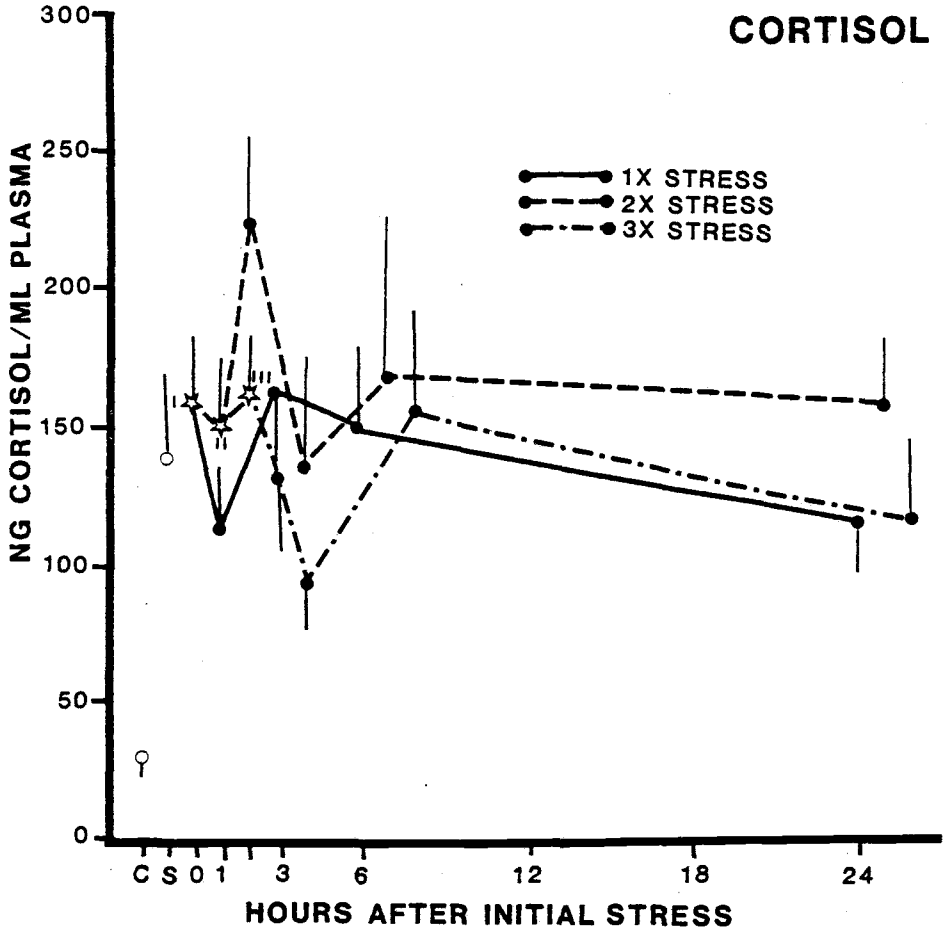
CORTISOL
1X STRESS
2X STRESS
3X STRESS
NG CORTISOL/ML PLASMA
300
250
200
150
100
50
0
C S 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 13

Figure 14: Plasma glucose levels (mean +/- SE for n=6-9) after fatigue of fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6 or 24 h after the final stress. Stars indicate the samples taken immediately after stress and adjacent roman numerals indicate the number of stresses. Open circles indicate unstressed fish. C indicates unstressed, non-swimming control fish. S indicates unstressed swimming control fish. Results of the sum of squares simultaneous test procedure ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h: 1+0 2+0 3+0

1 h: 1+1 2+1 3+1

3 h: 1+3 2+3 3+3

6 h: 1+6 2+6 3+6

24 h: 1+24 2+24 3+24

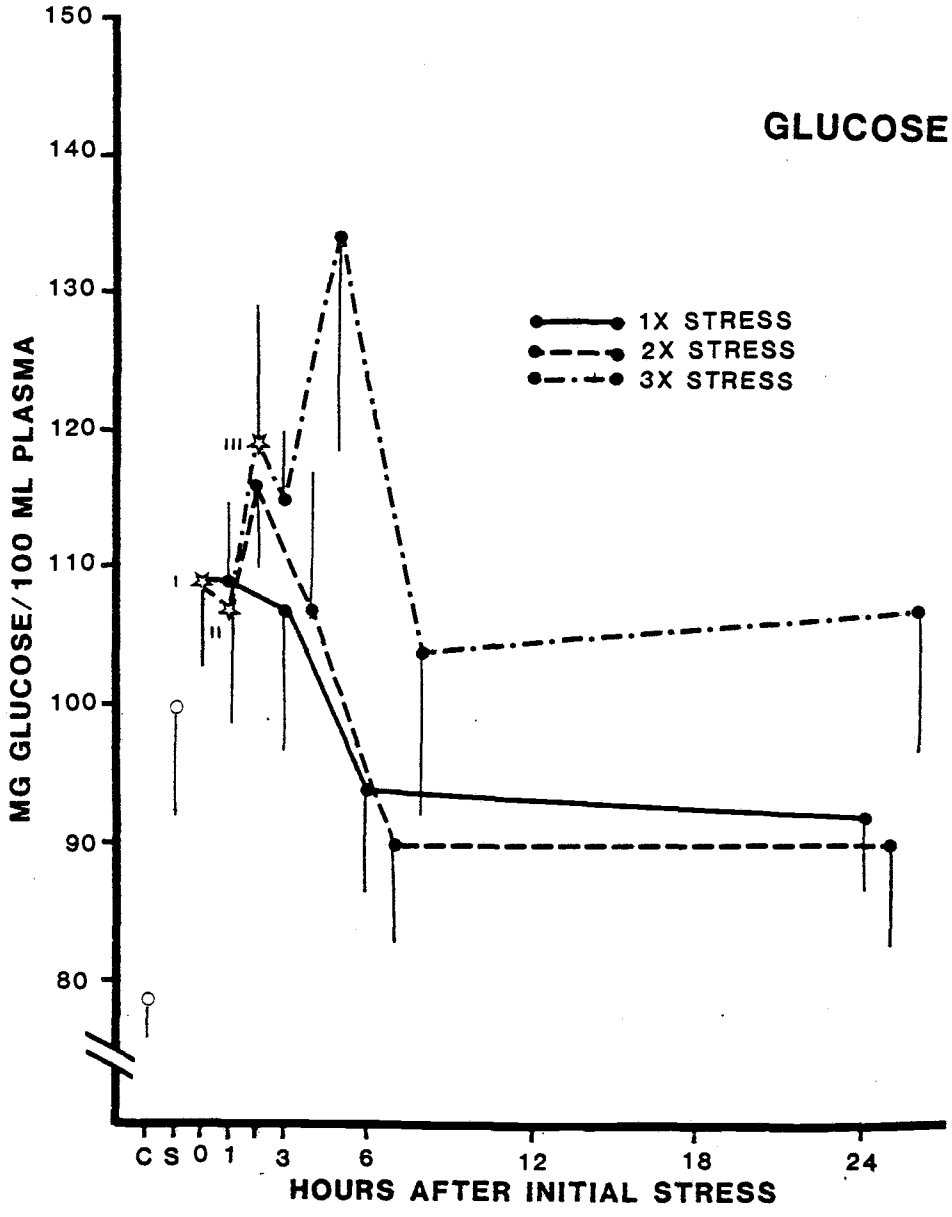
GLUCOSE
1X STRESS
2X STRESS
3X STRESS
MG GLUCOSE/100 ML PLASMA
150
140
130
120
110
100
90
80
I
II
III
C S 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 14

Figure 15: Plasma lactic acid levels (mean +/- SE for n=6-9) after fatigue of fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6 and 24 h after the final stress. Stars indicate the samples taken immediately after stress and adjacent roman numerals indicate the number of stresses. Open circles indicate unstressed fish. C indicates unstressed, non-swimming control fish. S indicates unstressed swimming control fish. Results of the sum of squares simultaneous test procedure ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h: 1+0 2+0 3+0

1 h: 1+1 2+1 3+1

3 h: 1+3 2+3 3+3

6 h: 1+6 2+6 3+6

24 h: 1+24 2+24 3+24

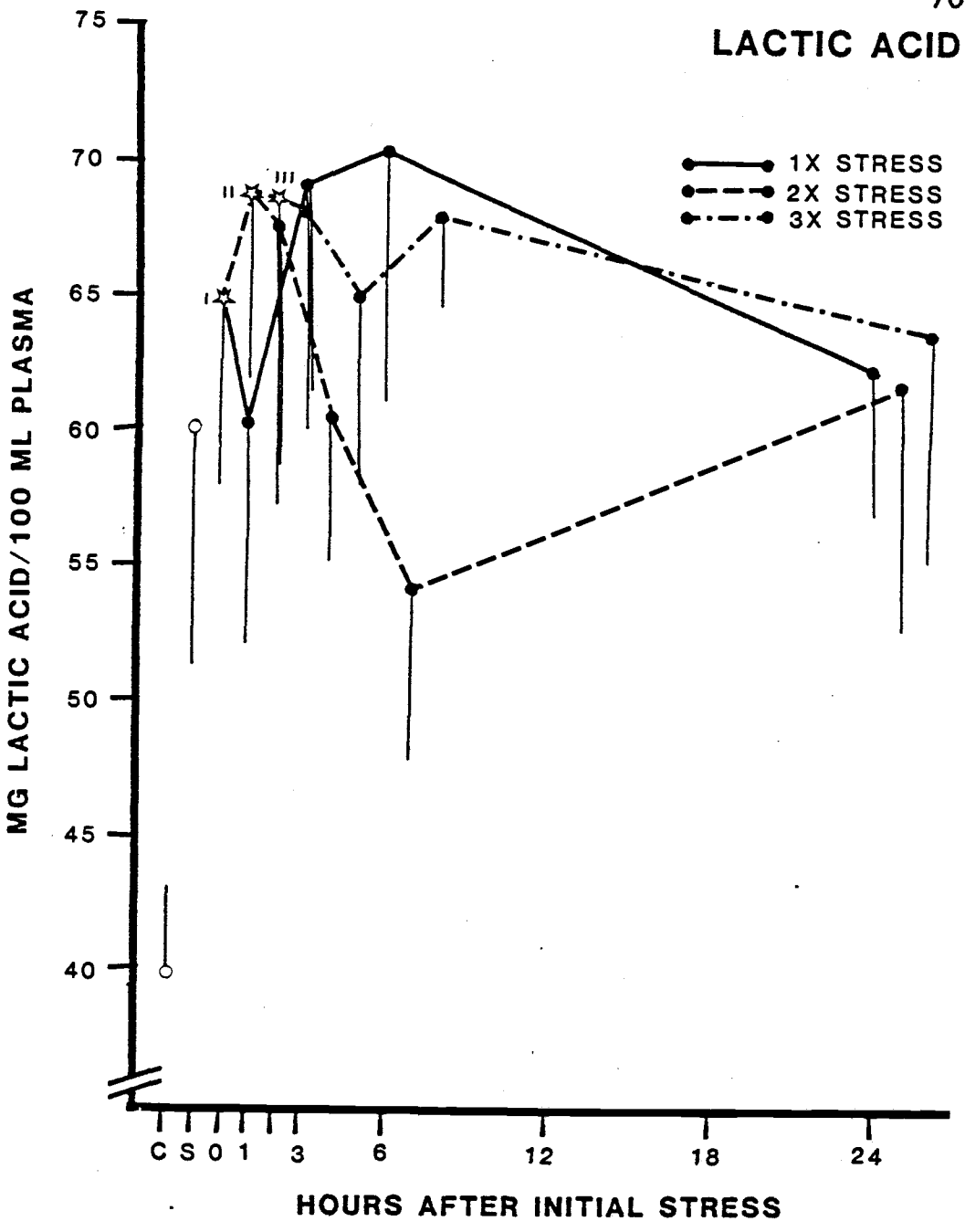
LACTIC ACID
1X STRESS
2X STRESS
3X STRESS
MG LACTIC ACID/100 ML PLASMA
75
70
65
60
55
50
45
40
I
II
III
C S 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 15

Plasma osmolarity was greater in swimmimg, unstressed fish than in non-swimming controls (Fig. 16). Osmolarity was also significantly greater in fish immediately after one stress and three stresses than in the unstressed swimming controls. There was no significant increase after two stresses or at any of the recovery times after stress. There were no significant differences in osmolarity between fish stressed 1, 2 or 3 times at any of the time periods after stress.

Swimming fish had a higher plasma sodium than non-swimming fish though potassium levels were similar in the two groups (Fig. 17). Only the fish sampled immediately after the first stress had a significantly higher plasma sodium than swimming controls. However, potassium levels were significantly higher than controls in fish from the following groups: one stress, one stress plus 1 h, two stresses, three stresses plus 1 h and three stresses plus 6 h. There were no significant differences in plasma sodium or potassium between 1, 2 or 3 stresses at any of the recovery times.

Hematocrits were similar in swimmimg and non-swimming fish (Fig. 18). Only the two stress plus 24 h group had a significantly higher hematocrit than controls. Analyzes of variance indicated a significant different between 1, 2 and 3 stresses at 1, 3 and 6 h after stress, but a posteriori comparisons yelded a significant difference only between the one stress plus 3 h and the three stresses plus 3 h groups.

Plasma samples from fish that fatigued early (<21 min) were compared to plasma samples from fish that swam greater than 35 min (Table 5). No significant differences were found between the means of any of the blood parameters tested though lactic acid and potassium tended to be higher in the group that swam longer.

Figure 16: Plasma osmolarity (mean +/- SE for n=6-9) after fatigue of fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6 and 24 h after the final stress. Stars indicate the samples taken immediately after stress and adjacent roman numerals indicate the number of stresses. Open circles indicate unstressed fish. C indicates unstressed, non-swimming control fish. S indicates unstressed swimming control fish. Points with an adjacent (s) indicate that they are significantly different from swimming controls. Results of the sum of squares simultaneous test procedure ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h: 1+0 2+0 3+0

1 h: 1+1 2+1 3+1

3 h: 1+3 2+3 3+3

6 h: 1+6 2+6 3+6

24h: 1+24 2+24 3+24

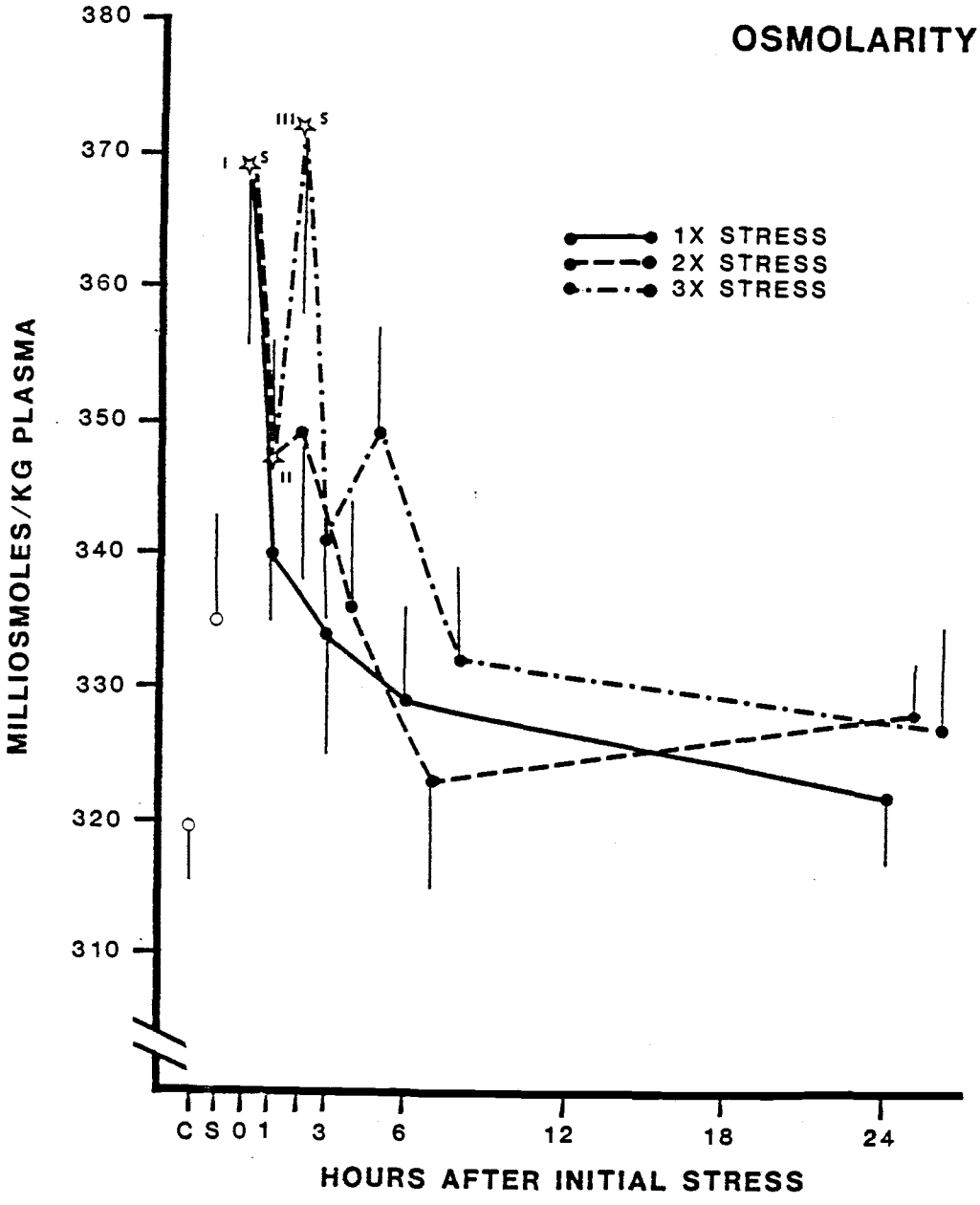
OSMOLARITY
380
370
360
350
340
330
320
310
MILLIOSMOLES/KG PLASMA
1X STRESS
2X STRESS
3X STRESS
I S
II
III S
C S 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 16

Figure 17: Plasma sodium and potassium levels (mean +/-SE for n=6-9) after fatigue of fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6 or 24 h after the final stress. Stars indicate the samples taken immediately after stress and adjacent roman numerals indicate the number of stresses. Open circles indicate unstressed fish. C indicates unstressed, non-swimming control fish. S indicates unstressed swimming control fish. Points with an adjacent (s) indicate that they are significantly different from swimming controls. Results of the sum of squares simultaneous test procedure ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

Na:		K:	
0 h:	1+0 2+0 3+0	0 h:	1+0 2+0 3+0
1 h:	1+1 2+1 3+1	1 h:	1+1 2+1 3+1
3 h:	1+3 2+3 3+3	3 h:	1+3 2+3 3+3
6 h:	1+6 2+6 3+6	6 h:	1+6 2+6 3+6
24 h:	1+24 2+24 3+24	24h:	1+24 2+24 3+24

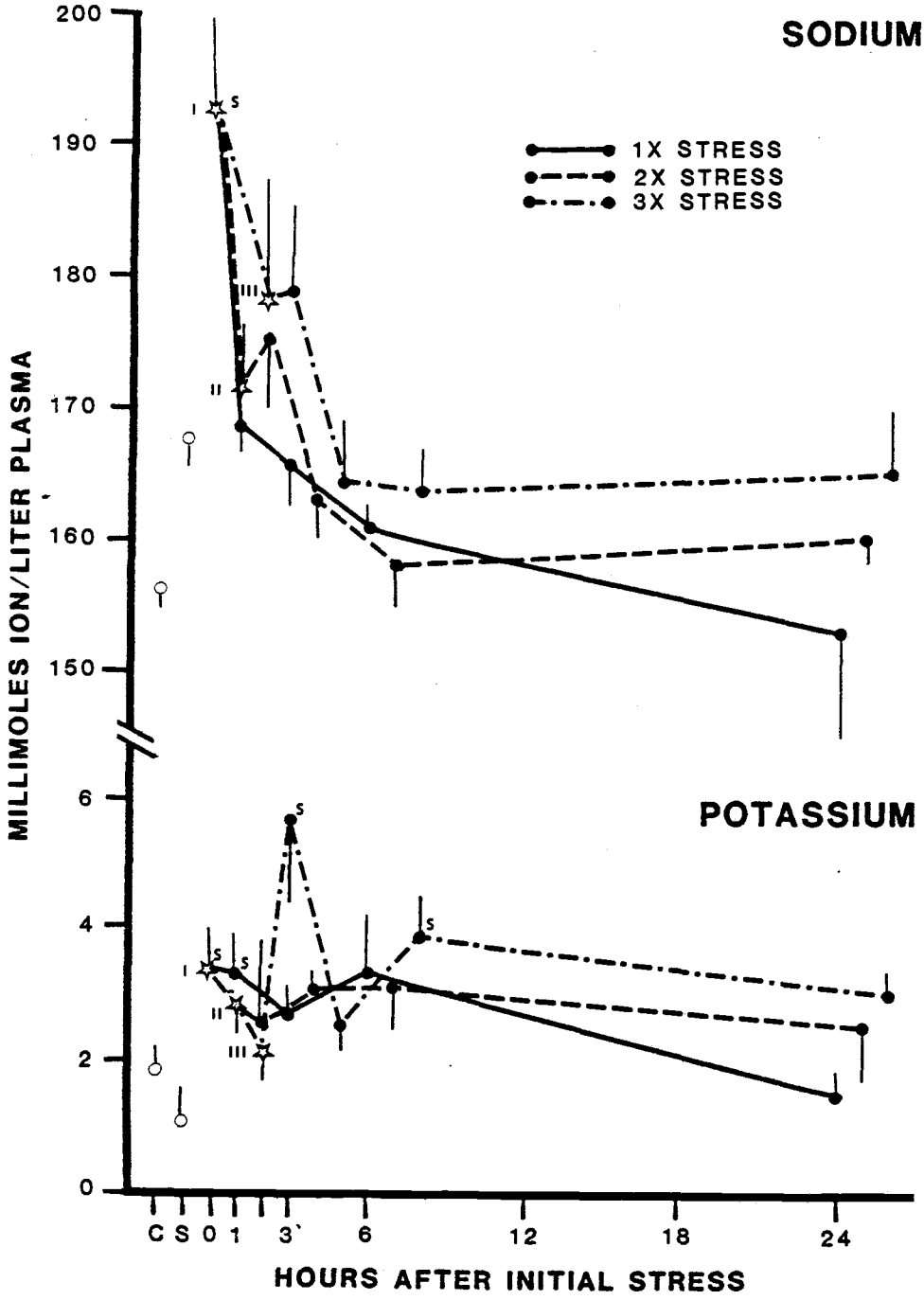
SODIUM
1X STRESS
2X STRESS
3X STRESS
POTASSIUM
MILLIMOLES ION/LITER PLASMA
200
190
180
170
160
150
6
4
2
0
C S 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 17

Figure 18: Hematocrits (mean +/- SE for n=6-9) after fatigue of fish subjected to 1, 2, or 3 handling stresses (30 sec in the air in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6 or 24 h after the final stress. Stars indicate the samples taken immediately after stress and adjacent roman numerals indicate the number of stresses. Open circles indicate unstressed fish. C indicates unstressed, non-swimming control fish. S indicates unstressed swimming control fish. Results of the sum of squares simultaneous test procedure ($p<.05$) are summarized below in the form of x+y where x=the number of stresses and y=the hours post-stress (underlined values are not significantly different):

0 h: 1+0 2+0 3+0

1 h: 1+1 2+1 3+1

3 h: 1+3 2+3 3+3

6 h: 1+6 > 2+6 3+6

24h: 1+24 2+24 3+24

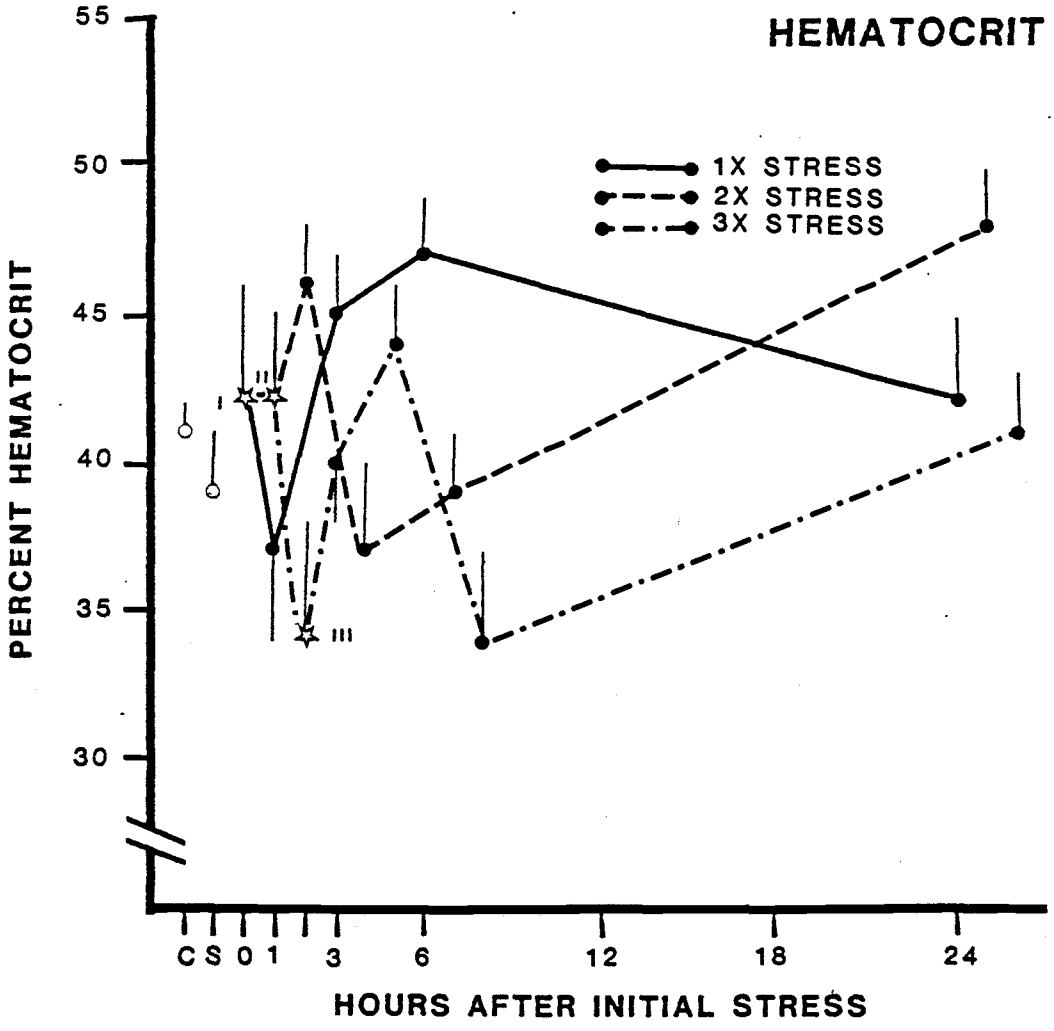
HEMATOCRIT
1X STRESS
2X STRESS
3X STRESS
PERCENT HEMATOCRIT
55
50
45
40
35
30
I
II
III
C S 0 1 3 6 12 18 24
HOURS AFTER INITIAL STRESS

Figure 18

Table 5: Hematocrit, plasma osmolarity and plasma concentrations of glucose, cortisol, lactic acid, sodium and potassium (mean +/- SE (sample size)) of fish that fatigued early (<=21 min) and of fish that swam at or near to the 60 min time period (>35 min).

	EARLY FATIGUE	LATE OR NO FATIGUE
Fatigue time	5.3+/-0.5 (110)	>60 (37)
Cortisol	148+/-9 (101)	135+/-11 (37)
Glucose	107+/-2 (96)	104+/-6 (37)
Lactic Acid	62.4+/-2.1 (95)	69.3+/-4.0 (37)
Hematocrit	41+/-1 (94)	42+/-1 (36)
Osmolarity	341+/-3 (94)	335+/-4 (37)
Sodium	169.1+/-1.6 (92)	164.4+/-3.1 (37)
Potassium	2.74+/-0.23 (92)	3.42+/-0.36 (37)

DISCUSSION

Critical Swimming Speed

Acute handling stresses did not affect the critical swimming speed of juvenile chinook salmon. However, reductions in critical swimming speed of salmonids after exposure to an environmental stressor have been reported by several investigators. Pollutants such as Kraft pulpmill effluent (Howard 1975), copper (Waiwood and Beamish 1978) and insecticides (Kumaraguru and Beamish 1983, Peterson 1974) significantly decrease critical swimming speed. Salmonids infested with certain parasites have lower critical swimming speeds than non-infested fish (Boyce 1979, Butler and Millemann 1971, Smith and Margolis 1970). Fish subjected to adverse conditions of temperature, oxygen, carbon dioxide and pH also have a reduction in critical swimming speed (Bushnell *et al.* 1984, Schneider and Connors 1982, Grahm and Wood 1981, Jones 1971, Dahlberg *et al.* 1968, Brett 1967, Davis *et al.* 1963). In addition, poor physical condition of the fish such as gill damage (Duthie and Hughs 1982) or anemia (Jones 1971) reduces swimming speed.

The mean critical swimming speed for unstressed fish in this study (3.2 body lengths/sec) was similar to the mean speeds of salmon of similar size at the same temperature (Schneider and Connors 1982, Brett and Glass 1973). The absence of a reduction in critical swimming speed with handling stess, despite the fact that other environmental stresses cause a reduction, may be due to the specific nature of the other environmental stresses. With many of the non-handling stresses, there is a reduction in the amount of oxygen available to the swimming muscles. For example, Kraft pulpmill effluent, copper, and physical damage to the gills decrease oxygen exchange at the gills (Howard 1975, Waiwood and Beamish

1978, Duthie and Hughs 1982) and thus the amount of oxygen the fish can consume. A low environmental oxygen would also limit the amount of oxygen available to the fish. High carbon dioxide concentrations, low pH, and pulpmill effluent reduce the blood's affinity for oxygen (Grahm and Wood 1981, Howard 1975, Dahlberg *et al.*1968) while anemia would limit the oxygen carrying capacity of the blood (Jones 1971) thus reducing the amount of oxygen that gets from the blood to the muscles. High environmental temperatures would decrease the oxygen dissolved in the water and increase the metabolic rate of the fish so it would require more oxygen. The insecticide permethrin has an energy cost to detoxify and eliminate (Kumaraguru and Beamish 1983) thus leaving less oxygen and energy available for swimming. Permethrin also is highly toxic to fish and would have some direct effects that could affect swimming. Some of the other environmental stresses decrease swimming performance by damaging the tissues. The salmon poisoning trematode (Butler and Millemann 1971) and the cestode *Eubothrium salvelini* (Boyce 1979, Smith and Margolis 1970) encyst in the muscles causing damage which would hinder tissue oxygen exchange and swimming ability. Fenitrothion is believed to act on the muscles or the nervous system controlling the muscles and thus reduces swimming ability (Peterson 1974).

With handling stress, on the other hand, there is no actual damage to the respiratory system or muscles and no change in availability of environmental oxygen. An acute stress actually increases heart rate and gill blood flow and elevates blood sugar thus making energy and oxygen available to the muscle for swimming. Thus, a reduction in swimming performance may not occur.

Sprague (1971) states that a reduction in swimming speed is often a symptom of an interference with gas exchange either at the gills or the muscles. This is consistent with the above results. In

studies where there were no direct interference with gas exchange, no reductions were reported. Russell (1980) found no change in critical swimming speed in rainbow trout exposed to an intestinal parasite. Webb and Brett (1973) reported no effect of sodium pentachlorophenate, a general metabolic poison, on the swimming performance of sockeye salmon.

Fatigue Time

Handling stress caused a marked reduction in fatigue time. Immediately after a single stress, fatigue time was reduced from 3.8 min for unstressed fish to 2.0 min for stressed fish. At the start of the one stress with no recovery fatigue time test, the fish were experienceing an oxygen debt from being out of water and the physiological stress response was just beginning to occur. Many fish may not have been able to recover quickly from this oxygen debt and a reduction in fatigue time occurred. By 1 h after the first stress, epinephrine and cortisol were released, glucose was elevated and the fish have had some time to recover from the oxygen debt. A swimming fatigue test at this time yeilded a time to 50% fatigue of 4.8 min which was slightly higher than that of the unstressed fish. The 50% fatigue time continued to be similar to or higher than controls throughout the recovery time of the fish stressed once. With the fish tested for fatigue immediately after two stresses, a time to 50% fatigue of 6.4 min, which was greater than that of the controls, occurred with 44% of the fish swimming greater than 60 min. Though these fish would also have had an oxygen debt from being in the air, it occurred at a time when cortisol and glucose and thus available energy were high and many of the fish were able to swim long periods of time. Throughout the recovery time of the two stress group there was a high proportion of fish swimming longer than 60 min, and generally high times to

50% fatigue. The data from the handling stress study in the previous chapter indicate that glucose remains elevated throughout the 24 h recovery period after stress and this continued elevation of glucose may have contributed to the elevation in time to 50% fatigue. Following three stresses, though cortisol and glucose were still high, a reduction in time to 50% fatigue from 3.8 to 1.1 min occurred immediately after stress. Perhaps with three stresses within 2 h, many fish were unable to cope with the oxygen debt despite the increase in energy available. The time to 50% fatigue was back near control level by 1 h after stress and remained at or above the control level for the duration of the 24 h recovery period. Again, this was probably due to the continued elevation of blood glucose throughout the recovery period from acute handling stresses (see chapter on physiological response to a handling stress).

In all groups of stressed fish, there were individuals that quit swimming early and ones that swam for 60 min, while in the no stress group no fish swam for 60 min. The chi square test between fish stressed 0, 1, 2 or 3 times and tested immediately after stress indicated that stress increased the number of fish swimming greater than 60 min and that the distribution of good swimmers was not due to random chance placing good swimmers in the stress groups. Thus it seems that some individuals were better "adapted" to cope with stress. However, when cortisol, glucose, hematocrit, osmolarity, lactic acid, sodium and potassium were compared between the fish that fatigued early and the fish that kept swimming, no significant differences were found. It is likely that there is some other parameter not measured, perhaps hemoglobin concentration, epinephrine, muscle glycogen, muscle lactic acid or some factor affecting the supply of oxygen to or the removal of wastes from the gills or muscles (Jones 1971), that caused the differences in the two groups of individuals. White

muscle glycogen depletion, which was associated with fatigue in jack mackeral (Pritchard *et al.* 1971), is a likely candidate.

It is difficult to compare fatigue times used in this study to absolute values of fatigue times in the literature due to differences in water velocity used, fish size, species and temperature. Since velocity is the most significant factor influencing fatigue time, only studies using a velocity similar to this handling stress experiment will be compared. The time to 50% fatigue of 3.8 lengths/sec obtained on control fish in this study was similar to that obtained by Russell (1980) and Clancy (1963) for rainbow trout at a similar temperature though with smaller fish. The fatigue times in this study tended to be shorter than those obtained by Brett (1964) for smaller sockeye at a higher temperature but longer than those obtained by Adams (1975) for smaller brook trout at a lower temperature. Though the absolute values may be different, the general spread of data was similar to Brett's (1967, 1964). Brett (1967) comments that variability among fish is not uncommon in time mortality type curves due to differences in limiting factors such as capacity to deliver oxygen to and remove wastes from tissues, ability to provide substrate, and ability to activate cellular enzymes.

Several studies have shown reductions in fatigue time of salmonids after environmental stresses such as pollutants, gas supersaturation and muscle parasites (Kovacs and Leduc 1982, Adams 1975 Schiewe 1974, Butler and Millmann 1971). As with critical swimming speed, factors that affect gas exchange and waste removal at the gills and tissues generally cause a reduction in fatigue time (Sprague 1971, Brett 1967). Other factors such as intestinal parasites (Klein et al. 1969, Russell 1980), which do not affect gas exchange, do not cause a reduction in swimming time. Handling causes an oxygen debt in the tissues which may affect oxygen exchange there and lead to a reduction in fatigue time. The

fact that fatigue time is affected by handling stress in this study and in studies by Horak and Klein (1967) and Clancy (1963), while critical swimming speed is not reduced by handling stress, may be due to the different nature of the tests. In critical swimming speed tests, the velocity is increased gradually giving the fish some time to recover from the oxygen debt brought on by the handling before maximum velocities are reached. Driedzic and Kiceniuk (1976) noted that there is no increase in blood lactate until 93% of the critical velocity. In the fatigue time tests, fish are exposed to a sudden, near critical velocity and must cope with it at a time when they are still recovering from the oxygen debt that would increase the acidity of the blood and lower oxygen carrying capacity. Thus it is logical that fatigue time is reduced immediately after handling. The stress response of increased heart rate, gill blood flow and elevated blood glucose within an hour after handling (Mazaeud and Mazaeud 1981) might allow for a quick recovery of fatigue time as was seen in this study.

Physiological Changes

Cortisol was higher in fatigued fish than in non-fatigued controls indicating that swimming to fatigue is stressful. However, among the stressed fish, results were variable and no differences were seen with time after stress or number of stresses. Also, peak cortisol levels in fish handled and then exposed to swimming were considerably less than those of fish exposed to handling stress alone in the previous experiment. The stress of swimming may have masked any effect of the handling stress on cortisol levels. However, the previous experiment showed that two or more stresses caused a cumulative increase in cortisol. A more likely explanation is that swimming fish have a higher metabolic rate (Brett 1964) and may be clearing cortisol

faster than fish not exposed to a swimming test.

Glucose was also higher in fatigued fish than in non-swimming controls. In other investigations where fish have been subjected to 10-15 min of exhausting exercise (Wendt and Saunders 1973, Nakano and Tomlinson 1967, Burrows 1969, Black *et al.* 1960, Black 1957a,b), an elevation in blood glucose was not seen until 1 to 2 h after swimming which is similar to the delay after a handling stress. However, in the fatigue time test of unstressed fish, there was a 30 min swimming acclimation and then up to 12 min at the final swimming velocity before the fish fatigued and blood samples were taken. This may have been enough time for a glucose elevation. The mean glucose value of 100 mg/100 ml for swimming fish was similar to the mean value of 95 mg/100 ml obtained on handled, non-swimming fish in the previous study at one hour after stress. The levels of glucose after handling stress plus swimming were generally less than those obtained after handling stress alone in the previous study. It is likely that the glucose is being used for energy by the swimming muscles resulting in lower plasma concentrations.

Lactic acid was higher in fatigued fish than in controls though it was not significant due to a high variability. Plasma lactic acid increases of similar magnitude immediately following 6-15 min of exhausiting exercise have been reported for various species by many investigators (Turner *et al.* 1983a, Wood *et al.* 1983, 1977, Burrows 1969, Black *et al.* 1966, 1962, 1960, 1959, Black1957a,b,c, 1955). The lactic acid is a result of the anaerobic metabolism of glycogen in the white muscles as evidenced by a decrease in muscle glycogen and an increase in muscle lactic acid following exhausting exercise (Turner *et al.* 1983a, Tsukamoto 1981, Johnston and Goldspink 1973a,b, Black *et al.* 1962). Generally, the blood lactic acid does not peak until approximately 2 h after activity due to its slow diffusion from muscles (Turner

et al. 1983a, Wood *et al.* 1977, Black *et al.* 1966, 1962, 1960, Black 1959, Black1957a,b,c, 1955). It then appears to be used for metabolism instead of synthesis into glycogen as in mammals (Black *et al.* 1966, 1962). The levels of lactic acid obtained after handling stress plus swimming are generally lower than the peak levels obtained after handling stress alone in the previous study. This may be due to the oxidation of lactic acid for energy requirements of swimming since lactic acid excretion is generally negligible (Kobayashi and Wood 1980).

The plasma osmolarity of swimming fish was greater than that of non-swimming controls. Byrne *et al.* (1972) also found an increase in osmolality in plasma of Atlantic salmon after 2 h of exercise at 3-4 body lengths/sec in sea water. The increase in osmolarity with swimming is greater than that from a single handling stress in the previous study. It may be that the continual high heart rates and gill blood flows during swimming, coupled with the increase in gill permeability due to catecholamines released during swimming, allow greater diffusion of water in or salts out than in the case of stress alone (Wood and Randall 1973a). Immediately after one and three handling stresses, the plasma osmolarities at fatigue were significantly higher than in swimming controls as well as higher than the fish exposed to handling stress alone in the previous experiment. These two times correspond with the two lowest time to 50% fatigue values indicating that a low swimming performance may be correlated with osmotic stress. Flagg *et al.* (1983) suggested that ionic imbalance reduces swimming performance by inhibiting the neuromuscular system. The fish tested immediately after two stresses do not have as high an osmolarity at fatigue and have a relatively long time to 50% fatigue. Thus, as was discussed with the fatigue time data, the stress response at 1 h after the first stress, associated with increased energy availblity and an

increase in cortisol which decreases gill permeability, may protect fish from high osmotic gains during swimming after two stresses. However, with three stresses this capacity is overloaded and osmotic problems are again seen. With the remainder of the groups of fish that had time to recover from stress before swimming, the plasma osmolarity at fatigue was not significantly different than swimming controls.

Sodium was also higher in swimming controls than non-swimming controls and followed a pattern similar to osmolarity except that it lacked the peak at three stresses with no recovery. Thus, something in addition to sodium was affecting osmolarity at the three stress point. Byrne *et al.* (1972) also reported an increase in plasma sodium in atlantic salmon exercised in salt water. Turner *et al.* (1983b) obtained similar results for fatigued flatfish. Plasma sodium was higher after swimming than after handling stress alone only in the samples taken immediately after the first stress. Afterwards, the curves were nearly identical. It appears that swimming plus stress causes a large initial increase in sodium, but that the changes that occur after stress, such as the increases in cortisol which decreases gill permeability, prohibit further effects of swimming on sodium concentration. Wood and Randall (1973a) found that exercise for 1 h increased sodium efflux rate in fresh water adapted rainbow trout while sodium influx rate remained unchanged. When the trout were forced to swim for up to 8 h, Wood and Randall (1973b) observed that sodium efflux rate declined during the second hour of swimming and was lower than influx rates at 3-8 h of swimming. They felt that these changes were due to a series of hormone actions. It is reasonable that a similar response occurs in salt water but in the reverse direction which is consistent with the results of the current experiment.

Potassium was not higher in swimming fish than in controls

which is consistent with Byrne *et al.*'s (1972) results for atlantic salmon. There were a few groups of stressed fish in which potassium levels were significantly greater than in the swimming controls. These included the one stress with zero recovery, the one stress with 1 h recovery, the three stresses with 1 h recovery and the three stresses with 6 h recovery. Potassium levels in the other groups of stressed fish were higher, though not significantly so, than the swimming controls. The increase in potassium immediately after one stress agrees with the increased ion influx seen with the sodium data. The other increases may be a result of changes in intracellular and extracellular acidity that affect potassium fluxes between the cells and the plasma. Lactic acid production by the swimming muscles causes an intracellular acidosis which causes an increase in plasma potassium (Turner *et al.* 1983a). Turner *et al.* (1983a,b) and Wood *et al.* (1983) have reported increases potassium in fish subjected to severe exercise.

Hematocrits were very variable in swimming fish with none being significant from the control. Other investigators have observed increases in hematocrit immediately after swimming which they attribute to osmotic shifts of water to the cells to compensate for the build-up of lactic acid in the muscles or to diuresis (Turner *et al.* 1983a,b, Farlinger and Beamish 1978).

Biological Significance

Since swimming is a necessary survival skill for fish in terms of obtaining food and avoiding environmental dangers, any reductions in swimming ability can adversly affect the fish. This study indicated that fish have a reduced swimming ability in terms of fatigue time immediately after an acute handling stress and that fatigue time will recover with one or more hours of recovery. Furthermore, this study indicated that fish subjected to

several acute disturbances within a short time span have different fatigue times than fish subjected to a single stress. In the case of three acute stresses, the reduction in fatigue time was even more severe than following a single stress. Thus, fisheries managers should allow fish that have been handled some time to recover before exposing them to other disturbances or challenges.

CONCLUSIONS

The following conclusions are supported by the results of this study:

1. Critical swimming speed is not affected by handling.

2. Fatigue time is affected by handling. Immediately after one and three stresses, fatigue time is depressed presumably due to the changes associated with the oxygen debt experienced by the fish. Immediately following two stresses, fatigue time is increased. When recovery time is allowed between the stress and the swimming test, fatigue time is at or above unstressed values. The long fatigue times after two stresses and after recovery from stress may be due to the high levels of glucose available when the fish begin swimming as noted in the previous experiment on handling stress without swimming.

3. The physiological factors that separate fish that fatigue early and those that do not fatigue were not identified in this study though osmoregulatory problems may have contributed to fatigue in those that were adversly affected.

4. Hematocrit and plama cortisol, glucose and lactic acid levels were generally lower in fish subjected to swimming fatigue and handling than in fish subjected only to handling. Plama osmolarity and sodium levels after stress plus swimming were similar to those after handling alone except immediately after the first and third stress. Plasma potassium concentrations were higher during the first 6 h of recovery in fish subjected to handling plus swimming than fish given only the handling stress. The physiological changes resulting from swimming seem to interact with the changes resulting from handling stress to cause the discrepancies.

CHANGES IN THE RESPONSE OF FRESH WATER ADAPTED JUVENILE SPRING CHINOOK SALMON (*Oncorhyncus tshawytscha*) TO A SUDDEN BRIGHT LIGHT AFTER EXPOSURE TO ONE, TWO OR THREE ACUTE HANDLING STRESSES

ABSTRACT

A simple behavioral test was devised to determine the effects of stress on the response of the whole organism. Groups of juvenile chinook salmon were exposed to a sudden bright light and the amount of time it took each of them to reach cover was noted. Tests were done on fish stressed 0, 1, 2 or 3 times with 3 h between stresses and on fish 1, 3, 6 or 24 h after each level of stress.

Unstressed fish reached cover within 15 sec. Stressed fish took longer to reach cover, with the greatest delay immediately after stress and a gradual decrease in response time with recovery from stress. Exposure to two or three consecutive stresses increased the response time and the recovery time indicating that the effects of stress were cumulative.

INTRODUCTION

Juvenile salmon are subjected to handling stresses while being raised in the hatchery, during transportation to release sites, and in certain by-pass operations for getting salmon around hydroelectric dams. Physiological studies have shown that exposure to a single handling stress causes changes in plasma hormone levels, elevations of plasma glucose and lactic acid, changes in pH and electrolyte levels, histological changes, depletion of liver glycogen and suppression of the immune system (Mazeaud and Mazeaud 1981). Barton *et al.* (1985) examined some of the above responses in fresh water adapted chinook after two or three consecutive stresses and found that stress has a cumulative physiological effect. A similar cumulative response in salt water adapted chinook was described in an earlier chapter.

Knowing that stress affects a fish physiologically, it is important to determine how stress affects the performance of the fish as a whole. The effects of stress on swimming performance were described in the previous chapter. Behavioral tests are another way of determining sublethal effects at the whole organism level and are being used frequently in toxicity testing (Sprague 1971). A response to a stimulus is a simple type of behavior to monitor. A response that may be of interest to fisheries biologists is the ability of fish to go to cover after exposure to bright light such as might be experienced by fish as they are released from a transport truck or pass over a spillway. A quick response would enable fish to avoid predation by aerial or water predators and would thus increase their chance of survival.

The objective of this study is to determine if stress reduces the ability of juvenile chinook salmon to respond to a stimulus such as a sudden bright light. A second objective is to determine if two or more acute stresses in succession cause cumulative changes in response.

MATERIALS AND METHODS

Fish

Juvenile spring chinook (15.0 cm mean fork length, 41.4 g mean wet weight) were obtained from Eagle Creek National Fish Hatchery and transported to the Oregon State University Smith Farm facility in Corvallis in December of 1985. The fish were held in a fresh water flow-through 1.8 m circular tank at 11-12 C for 2 months prior to the start of the experiment.

Apparatus

Behavior experiments were carried out in three Y-troughs measuring 0.7 meters in width and 2.5 meters in length (Fig. 19). The troughs were equipped with several gates to compartmentalize the fish. A permanent 44 cm long black plastic cover was placed on the center portion of each trough. Two 20 watt fluorescent lights were positioned 38 cm above the Y-troughs: one over the arm area and the other over the leg area. The arm areas of each trough were fitted with removable black plastic sheets that completely covered the arms.

Response Time

Response time was measured by exposing fish to a sudden bright light and measuring the amount of time it took for each fish to swim under cover. Preliminary experiments were done to determine the number of fish to use per trial, the size and distance to cover,the type of cover, and the position of the light source.

Six juvenile spring chinook were introduced to each arm of the Y-troughs, covered, and allowed to adjust overnight. The fluorescent light at the leg of the Y was kept on during daylight hours. The next morning, fish were either tested for response

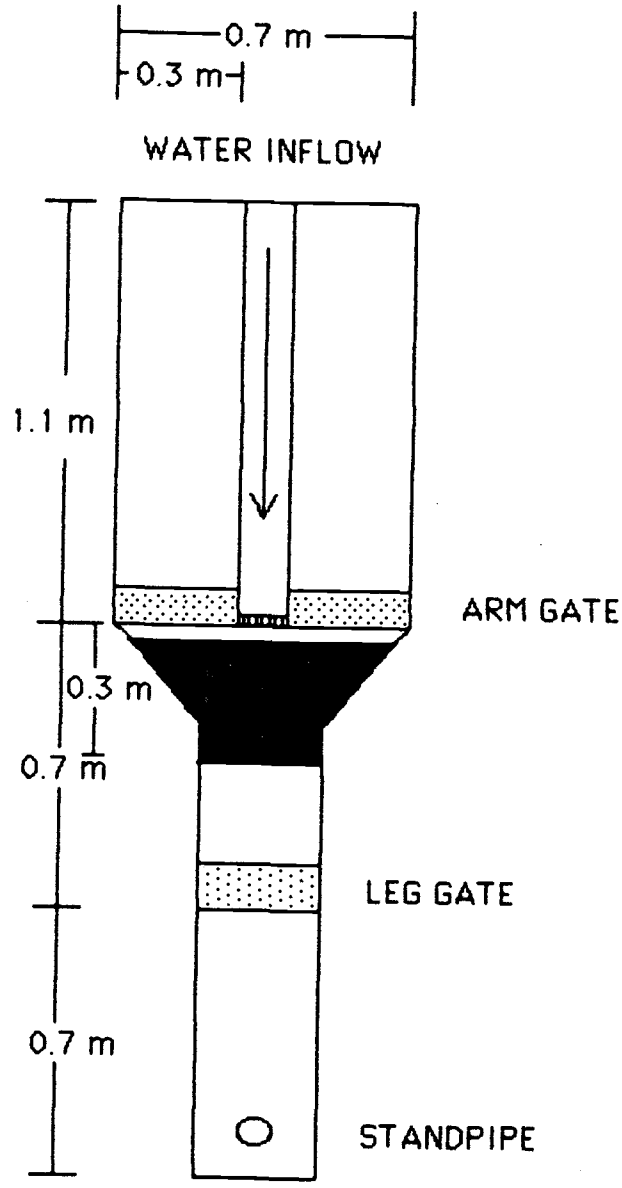

Figure 19: Diagram of apparatus used in response time experiment.

time or stressed by holding them in a dip net for 30 sec. Fish were stressed 1, 2, or 3 times with a 3 h delay between stresses. After each stress, the fish were returned to the apparatus and covered until the next stress or until the behavior trial was begun at 0, 1, 3, 6, or 24 h after the last stress. A trial consisted of turning on the bright fluorescent light above the arms of the Y, simultaneously removing the cover and opening the gate and noting the time it took for each fish to swim under the permanent cover at the center of the trough. A trial was terminated when the last fish reached cover or at 30 min, whichever came first. Some aspects of the behavior of the fish during the trials were also noted such as whether the fish came out of cover again, whether the fish would explore the tank or just sit still, and the position of the fish at the top or bottom of the water column.

Two or three replicates were tested for each experimental group and the data was pooled. Since occasionally fish escaped from the apparatus and since three replicates were done on some groups when two replicates were slightly different, sample sizes varied from 10-17 fish. The median response time was calculated for each group of fish. Median response time was used since data was skewed to the right and since some fish did not respond in the time period so calculation of mean could not be exact (Sokal and Rohlf 1969). The nonparametric Kruskal-Wallis test was used to examine the effect of stress over time and to determine whether repeated stresses had a cumulative effect. A non-parametric multiple comparison test suggested in Daniel (1978) was used to compare medians when responses were significantly different.

Fish not exposed to a handling stress were also tested individually in the apparatus. Their reponse times were compared to the reponse times of grouped fish using the non-parametric Mann-Whitney U-test.

RESULTS

The response times of stressed fish were greater than the response times of unstressed fish (Table 6, Fig. 20). (Response times of individual fish are listed in appendix 4.) All points except one stress plus 24 h and two stresses plus 6 h are significantly greater than the controls. No significant differences were found between 1, 2 and 3 stresses at 0, 1, 3, and 6 h after stress. However, at 24 h after stress, fish stressed two or three times had significantly higher response times than fish stressed once. Though the differences in response time between 1, 2 and 3 stresses were not significant, there was a tendency for an increase in response time with increasing numbers of stress.

The fish stressed only once tended to stay under cover for the duration of the run. Occasionally, a fish would make a quick dart from cover and go right back. Following two and three stresses, more fish tended to leave cover and remain out for longer periods of time. This was particularly pronounced at two stresses plus 3 h, three stresses plus 1 h and three stresses plus 6 h recovery. Since experimental trials were ended when the last fish entered cover and since unstressed fish and fish stressed once all went to cover sooner than the fish stressed two or three times, a total time out of cover quantitative comparison could not be made.

Prior to entering cover, stressed fish tended to remain motionless either on the bottom or top of the water column, and usually against a side. In a few cases, fish remained belly up for several minutes after stress. Stressed fish tended to move around slowly if at all. Some of the fish appeared oblivious to movement of other fish around them even if bumped by the swimming fish. Some of the stressed fish swam at the surface with their snout out of water. Preliminary experiments also showed that stressed fish were less likely to respond to a second stimulus such as a shadow passing over the water or a hand splashing the water.

There was some delayed mortality in the experimental trials and in the post-experimental recovery tanks (Table 7). The highest

Table 6: Median response times (i.e. time to swim to cover after exposure to a sudden bright light) of fish stressed 1, 2 or 3 times with 3 h between stresses and of fish allowed to recover 1, 3, 6, or 24 h after stress.

HOURS AFTER FINAL STRESS	NO STRESS	1X STRESS	2X STRESS	3X STRESS
0	0.05	1.57	5.90	15.97
1		0.72	1.98	3.45
3		0.35	3.10	2.80
6		0.43	0.25	0.35
24		0.07	1.12	1.00

Figure 20: Median response times (i.e. time to swim to cover after exposure to a sudden bright light) of fish stressed 1, 2 or 3 times with 3 h between stresses and of fish allowed to recover 1, 3, 6, or 24 h after stress. Stars indicate the samples in which fish were tested immediately after stresss and adjacent roman numerals indicate the number of stresses. C and open circles indicate control fish. Points with an (s) indicate that they are significantly different from swimming controls. Results of the multiple comparison tests are summarized below in the form of x+y where x=the number of stresses and y=the number of hours after stress (underlined values are not significantly different):

0 h: 1+0 2+0 3+0
1 h: 1+1 2+1 3+1
6 h: 1+6 2+6 3+6

6 h: 1+6 2+6 3+6
24 h: 1+24 < 2+24 3+24

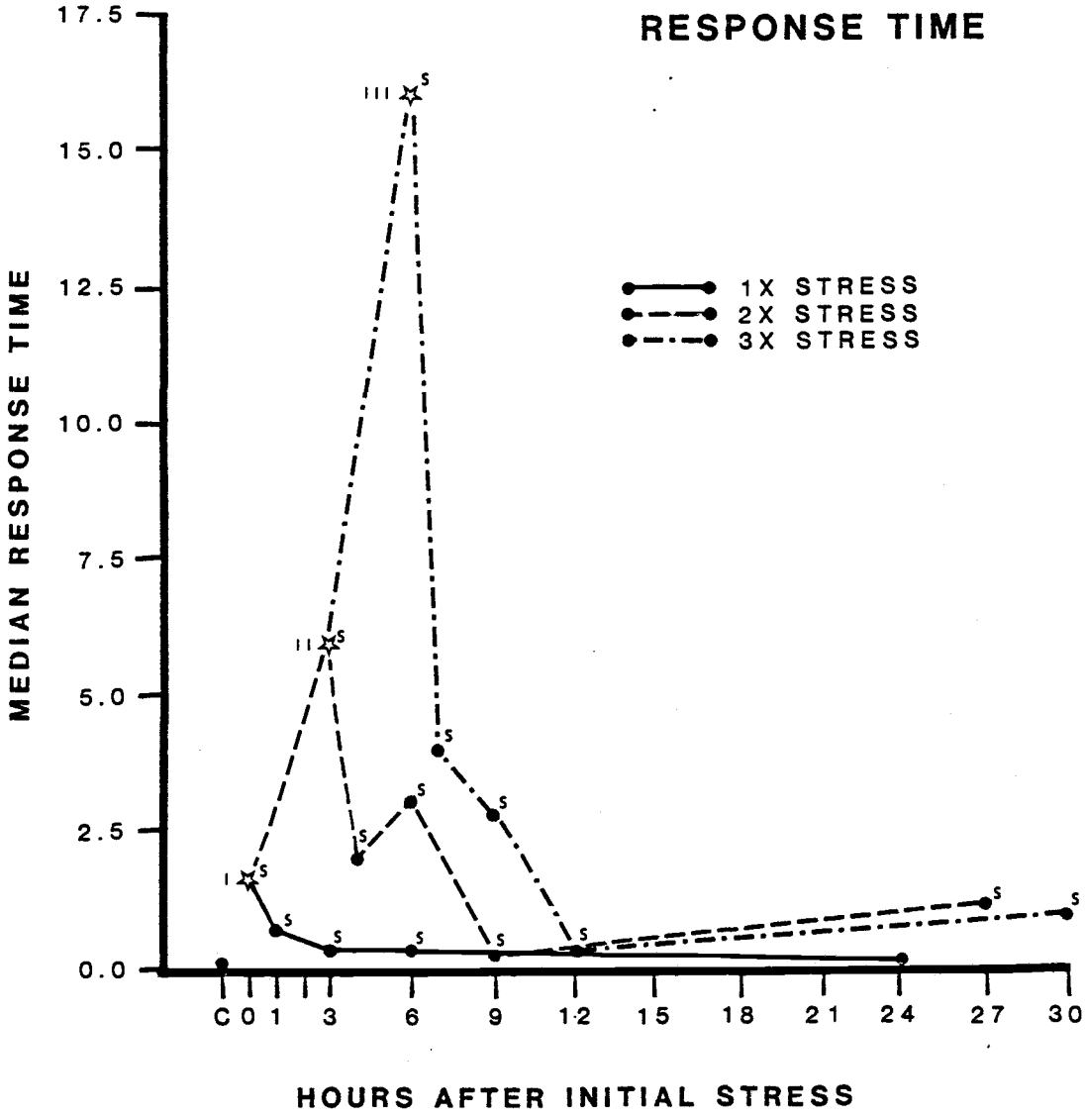
RESPONSE TIME
MEDIAN RESPONSE TIME
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
1X STRESS
2X STRESS
3X STRESS
C 0 1 3 6 9 12 15 18 21 24 27 30
HOURS AFTER INITIAL STRESS

Figure 20

Table 7: Percent mortality in response time experimental trials.

HOURS AFTER FINAL STRESS	NO STRESS	1X STRESS	2X STRESS	3X STRESS
0	0	0	0	0
1		9	0	0
3		0	0	17
6		7	8	22
24		5	9	26

delayed mortalities occurred in the groups of fish given three stresses in which 3-6 fish were dead before the starts of the 3, 6, and 24 h trials. In the groups of fish given one or two stresses, there were a few cases in which one fish died. The above mortalities plus the mortalities in the post-experimental tanks during the first 48 h accounted for a 14% total delayed mortality.

The fish that were tested individually had a median response time of 1.99 min with a range from .04-6.22 min (n=10). (Individual values can be found in appendix 4.) This was significantly different from unstressed fish that were tested in groups, but is not significantly different from fish with one stress tested in groups.

DISCUSSION

The results of this study indicated that acute handling stresses increase the time that it takes fish to respond to a stimulus. Acute handling stresses also elicited general lethargic behavior. Other investigators have noted similar changes in the behavior of fish after a handling stress. Bouck and Ball (1966) observed that rainbow trout stressed by hooking were lethargic and stopped feeding. Herting and Witt (1967) found that seined bluegill behaved sluggishly and did not exhibit darting avoidance reactions when confronted with a predator causing increased vulnerability to predation. Other stresses including thermal stress (Coutant 1973, Sylvester 1972) and various pollutants (Hedke and Norris 1980, Henry and Atchison 1979, Woltering *et al.* 1978, Kleerekoper 1976, Bull and McInerney 1974, Hatfield and Anderson 1972) also cause decreased activity, stuporous behavior and increased susceptibility to predation.

The results of this study demonstrated that the reduction in the ability of fish to respond to a stimulus was cumulative and that fish subjected to two or more stresses required longer recovery times. This indicates that the severity of a given stress increases when it occurs shortly after a previous stress. Thus, in management situations, fish should be given time to recover from stress before being subjected to additional disturbances.

Changes in behavior of an organism are often correlated with physiological changes. For comparative purposes, the timing of stresses and recovery times used in this response time study were chosen to coincide with the timing of physiological measurements made by Barton *et al.* (1985). The longest response times to a sudden bright light occurred immediately after stress followed by a decline in response time with increasing recovery time. However, physiologically, the peak responses occurred, depending on the number of stresses, at 0.5-1 h after stress for cortisol and lactic acid, at 3-6 h after stress for glucose, at 1-12 h after stress for sodium and at 0.5-3 h after stress for potassium. Thus,

the longest response times are occurring before the peak physiological stress responses. The high response times immediately after stress could be a result of the oxygen debt experienced by the fish. Perhaps the lack of oxygen to the brain is causing a stunning and disorientation similar to that documented for mammals (Ganong 1983, Selkurt 1982). As the physiological stress response peaks and the fish recover from the oxygen debt, their response time to light declines. This is consistent with the view by Redding and Schreck (1983) that the increase in cortisol and the secondary responses it causes are adaptive responses to compensate for the energetic costs of stress. However, the response times do not completely return to pre-stress levels, though the physiological levels recover in 6-12 h. This indicates that stressed fish have an increased sensitivity to environmental factors even though many of the physiological factors have recovered. Other studies indicate increased sensitivity to a second stress. Specker and Schreck (1980) observed that transportration reduced the ability of coho to withstand a second stress of crowding confinement as indicated by increased mortality and higher cortisol levels. Patino (personal communication, Oregon State University, Corvallis, OR 97331) found that the interrenal tissue of salmon has a greater sensitivity than normal 24 h after the first stress indicating a greater ability to respond to a second stress.

The fish in this study exhibited some delayed mortality in the experimental trials, particularly in the three stress group, and in the post-experimental recovery tanks. Though high delayed mortalities during the first 24 h after handling have been associated with hooking and seining (Herting and Witt 1967, Bouck and Ball 1966), no mortalities were seen in the experimental trials in the handling stress and swimming performance experiments reported in the previous chapters. Likewise, Barton *et al.* (1985) did not obtain significant mortality in their multiple stress experiment except when dealing with diseased fish. Perhaps the fish used in the response time study had some latent

diseasese that was not detected during holding but that did increase the susceptibility of the fish to the handling stress.

CONCLUSIONS

The increase in response time of stressed fish to a stimulus and the general changes in the behavior of the fish indicate that stress does affect the performance of the whole organism. The further increase in response time with two or three consecutive stresses indicates that the effect of stress on performance is cumulative. The most likely consequences of a delay in response time and general lethargic behavior after stress are increased exposure and vulnerability to predation or other environmental hazards. Since these changes in behavior can adversly affect the survival of the fish, these behavior changes should be considered in conjunction with physiological changes when determining the degree of stress to an organism and in setting management policies.

DISCUSSION

The performance of organismic systems was considered in the introduction to be the result of the environment acting on the inherent capacity of the organismic system. Any change in the environment could thus change the performance of the organism. Stress was considered to be an environmental factor that might change performance.

In the three chapters presented here, three whole organism responses-critical swimming speed, fatigue time and response time to a sudden bright light-were considered. The performances of various organismal subsystems including the endocrine, circulatory, neuromuscular and respiratory systems were also examined after stress and after stress plus swimming by measuring certain of their products in the blood stream including hematocrit and plasma osmolarity and plasma concentrations of cortisol, glucose, lactic acid, sodium and potassium.

Of the whole organism performances measured, fatigue time and response time were affected by stress. The response time to a simulus was increased which is likely to reduce the ability of the fish to avoid environmental dangers. The lethargic behavior after stress might also interfere with the fish's ability to obtain food or undergo migratory activities until they recover from the stress. The changes in fatigue time were more difficult to interpret. Stress seemed to enhance the swimming ability of some fish while reducing the performance of others. In addition, the fatigue times of the group of fish as a whole tended to be reduced after one and three stresses but enhanced after two stresses. The differences between individuals can be explained by differences in the realized capacities of each individual. Each individual has a genetic ability to carry out the necessary processes for swimming. For example, individuals have genes for producing lactate dehydrogenase which breaks down the lactic acid produced by anaerobic metabolism. Certain phenotypes of LDH are more efficient and fish with that phenotype have been shown to have higher swimming performances

(Klar 1979). The performance of the individual is further affected by its past history or environment (Schreck 1981). For example, a fish that has been eating well would have higher stores of glycogen and thus more energy available for swimming. A fish that has been starving would not have the energy for swimming even though it may have a high genetic capacity to swim. Also, some fish may have a greater muscle tone than others due to prior conditioning and may swim better. Thus, even though the fish in each group used in this study were stressed in the same manner, the individual performances were different. The actual physiological factor that reduced performance in some fish in this study was not identified. There is some indication, namely that the groups of fish with the lowest fatigue times also have the highest mean increases in osmolarity, that osmotic stress may contribute to the decrease in swimming performance.

I feel that the reduction in time to 50% fatigue in the group of fish tested immediately following one stress was likely the result of the oxygen debt and the physiological problems associated with it. The physiological stress response, that occurs after handling, probably reduced some of the problems, such as osmotic imbalance and energy availability, and thus allowed the swimming performance to be near or above control levels by 1 h after stress. Similarly, these changes, associated with the stress response, may have improved the swimming performance in the groups tested after two stresses. In the group of fish tested immediately after three stresses, the swimming performance was again reduced, presumably due to an overload on the system. With at least 1 h recovery after handling, the changes associated with the stress response again allow swimming performance to return to control or higher levels in the three stress group. The changes in fatigue time with increasing numbers of stress follow a pattern similar to Selye's General Adaptation Syndrome though the changes are shorter in time span. The initial stress would cause an alarm reaction which is the stage in which the organism is more susceptible to a second stress. Thus a reduction in swimming

when exposed to a stress such as high water velocity is logical. By two stresses, the organism may have reached the stage of resistance in which there is increased ability to withstand a stress. Finally, with three stresses, the organism reaches the stage of exhaustion and swimming ability is again reduced.

Critical swimming speed performance was not reduced by stress. Even though the fish in the critical swimming speed tests after stress would be experiencing similar problems to those in the fatigue time tests, they were subjected to a more gradual increase in water velocity. At low water velocities, fish primarily use their aerobic red muscles for swimming (Hudson 1973, Johnston and Goldspink 1973a,b). At higher water velocities near to the critical swimming speed, the anaerobic white muscles are primarily used (Hudson 1973, Johnston and Goldspink 1973a, Pritchard *et al.* 1971). Driedzic and Kiceniuk (1976) reported that there is no release of lactic acid from the white muscle until 93% of the critical velocity is reached. Pritchard *et al.* (1971) observed that fatigue was associated with an almost complete depletion of glycogen from the white muscle only. Thus, when critical swimming speed was measured immediately after stress, there was time, almost an hour by the method used in this study, for the white muscle to at least partially recover from the effects of the stress before these fibers were needed for swimming and a reduction in critical swimming speed was not seen. As with the fish in the fatigue time tests, there was a great deal of individual variation in critical swimming speeds in each group due to variation in the realized capacities of the individuals.

In the introduction, the performance of an organism was also considered to be the outcome of the interactive performances of the organism's subsystems. Changes in the performance of organism subsystems were noted after stress. Plasma cortisol, lactic acid, osmolarity and sodium had cumulative increases with increasing number of stresses. Glucose, hematocrit and potassium had significant, though not cumulative, changes. These performances were examined again after stress and swimming and

further changes were noted, again illustrating that any change in the environment can change the performance of a system. When these parameters were compared betweeen fish that fatigued in 60 min and those that did not, no significant differences were observed. However, these changes in performance after stress may have changed the environment of another organismic subsystem thus affecting it's performance and the performance of the whole organism. It is also possible that the tests used in this experiment were not sensitive enough to detect the differences between fish that fatigued in 60 min and those that did not.

Some of the consequences of changes in whole organism performance are obvious. The individuals that have enhanced swimming ability or quick response times are more likely to escape or cope with an environmental challenge. Those that have reduced performances are less likely to survive and reproduce. The consequences to the population are somewhat less obvious. Natural selection will weed out those individuals with poorer performances. But natural selection does not operate on seperate traits, but on the entire genotype (Dobzhansky 1956). Thus when we impose man-made stresses on organisms and select for or against a certain trait, we may be selecting for or against other traits that may be desirable. For example, the gill net fishery on the Columbia River can select against larger fish. An intensive fishery at a certain time can also select against early or late timing of salmon runs. However, large size or a particular timing may have been an adaptation for long migrations. Thus, even though smaller fish or fish that have their runs at a different time will survive the fishing pressure, they may be less fit as a spawning population (Warren and Liss 1980). Similarly when we select for fish that have better performances after stress by our hatchery operations or management schemes, we may or may not be selecting for the most fit populations as a whole. The consequences of this may not be seen until the environmental conditions become exceptionally severe (Warren and Liss 1980).

In summation, stress does affect certain performances of

organisms and organismic subsystems. The lethal and sublethal effects of stress on the organism is the outcome of the lethal and sublethal effects on its subsystems and in turn will determine the lethal and sublethal effects on the population. The potential and realized capacity of the individual will determine the actual performance change experienced by the individual for a given amount of stress.

CONCLUSIONS

The following conclusions are supported by the results of this study:

1. Exposure to repeated handling stresses caused cumulative increases in the plasma osmolarity and plasma concentrations of cortisol, lactic acid, and sodium indicating that the effects of stress are cumulative.

2. Exposure to repeated handling stresses caused significant, though not cumulative, changes in hematocrit, plasma glucose and plasma potassium.

3. Critical swimming speed was not affected by handling stress.

4. Fatigue time was affected by handling. Immediately after one and three stresses, fatigue time was depressed presumably due to the changes associated with the oxygen debt experienced by the fish. Following two stresses, fatigue time was increased. When recovery time was allowed between the stress and the swimming test, fatigue time was generally at or above unstressed values. The longer fatigue times after two stresses and following recovery from stress may be due to the high levels of available gluscose at the start of the swimming run as evidenced by the data on handling stress alone.

5. The physiological factors that separate fish that fatigue early and those that do not fatigue in 60 min were not identified in this study though osmoregulatory problems may have contributed to fatigue in those that were adversly affected.

6. Hematocrit and plasma cortisol, glucose and lactic acid levels were generally lower in fish subjected to swimming fatigue and handling than in fish subjected only to handling. Plasma osmolartiy and sodium levels after stress plus swimming were similar to those after handling alone except immediately after the first and third stress. Plasma potassium concentrations were higher during the first 6 h of recovery in fish subjected to handling plus swimming than in fish given only the handling stress.

The physiological changes resulting from swimming seem to interact with the changes resulting from handling stress to cause the discrepancies.

7. The increase in response time of stressed fish to a stimulus such as light and the general changes in the behavior of the fish indicate that stress did adversly affect the performance of the fish. The further increases in response time with two or three consecutive stresses indicated that the effect of stress on performance was cumulative.

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APPENDICES

APPENDIX 1

Length, weight, hematocrit, plasma osmolarity and plasma concentrations of cortisol, glucose, lactic acid, sodium and potassium from individual fish in the salt water multiple stress experiment for each level of stress and for each time period after stress.

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
CONTROL	12.7	22.8	41	0	16.1	52	288	152.2	2.12
	14.9	33.3	54	14	32.0	71	298	157.7	2.12
	14.7	33.4	43	165	37.5	71	309	155.3	2.95
	15.7	44.0	47	33	29.6	71	317	162.1	2.45
	15.7	47.0	54	110	35.2	76	303	155.5	1.35
	17.6	59.2	53	141	49.5	82	334	164.3	1.67
	17.5	52.8	52	11	31.8	93	298	161.0	1.46
	15.6	40.9	43	105	16.5	68	277	156.6	1.79
	15.3	41.9	48	5	28.0	70	301	157.7	2.23
	16.8	59.0	48	101	45.6	75	288	153.1	1.85
X	15.6	43.4	48	68	32.2	73	301	157.6	1.99
SE	0.5	3.7	2	20	3.4	3	5	1.2	0.15
1 STRESS	16.1	50.4	41	94	31.0	65	298	146.5	1.52
	15.5	43.5	45	34	25.4	62	293	155.5	2.67
	15.7	47.6	43	67	35.1	68	321	157.5	3.61
	16.3	51.0	48	112	46.6	80	311	160.8	2.51
	15.5	44.9	42	144	28.0	67	304	159.7	3.50
	16.8	56.8	43	83	30.2	69	283	142.3	3.33
	15.7	44.9	50	69	37.0	68	315	161.0	2.67
	17.3	65.6	49	93	41.8	64	316	163.2	2.89
	16.2	49.3	47	75	38.9	70	315	156.6	3.00
	15.6	45.3	50	86	35.4	91	316	156.4	3.61
X	16.1	49.9	46	86	34.9	70	307	156.0	2.93
SE	0.2	2.1	1	9	2.0	3	4	2.1	0.20

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
1 STRESS	15.4	42.2	43	286	80.1	77	296	158.6	2.40
+ 1 HOUR	15.4	37.7	44	250	76.0	84	322	158.8	1.90
	15.0	32.8	47	325	43.8	90	316	161.0	2.89
	15.9	47.2	48	171	81.4	98	338	168.7	2.56
	15.8	40.7	39	257	72.8	94	330	169.6	3.28
	15.5	40.3	50	215	86.1	116	353	175.1	2.73
	15.4	34.0	38	180	54.5	106	314	166.3	2.29
	15.3	36.6	44	111	50.0	91	297	195.1	3.22
	17.0	59.5	48	155	43.1	86	302	167.6	2.01
	17.0	63.0	54	265	64.1	93	317	145.4	3.39
X	15.8	43.4	46	222	65.2	94	318	166.6	2.67
SE	0.2	3.2	2	21	5.2	4	6	4.1	0.17
1 STRESS	16.0	42.6	44	121	32.0	127	308	158.6	0.42
+3 HOURS	17.0	59.7	45	119	43.5	109	323	137.7	1.08
	15.0	38.5	45	157	52.0	146	327	171.8	1.30
	14.7	33.6	46	157	112.4	162	336	169.8	0.80
	15.8	49.0	47	146	68.8	119	325	172.9	0.64
	17.2	57.3	51	144	57.0	142	310	157.7	0.36
	15.2	41.4	59	139	74.5	175	332	157.7	1.35
	16.0	48.5	--	131	73.7	144	326	184.1	0.80
	15.8	48.1	53	129	59.7	138	326	163.0	1.08
	16.2	51.1	53	117	73.2	130	325	161.0	0.35
X	15.9	47.0	49	136	64.7	139	324	163.4	0.82
SE	0.3	2.6	2	5	6.9	6	3	3.9	0.12

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
1 STRESS	14.3	31.8	34	61	29.4	123	303	164.3	0.58
+ 6 HOURS	13.4	25.5	36	136	43.5	134	307	163.2	0.69
	15.2	39.8	38	52	39.9	120	325	158.6	0.64
	14.7	35.8	37	68	32.0	124	314	166.5	1.02
	15.6	43.7	40	42	44.8	113	301	159.9	0.91
	15.9	43.2	39	37	32.3	97	309	121.4	1.02
	15.8	45.3	37	42	51.1	121	311	171.8	1.74
	15.6	48.9	37	40	56.6	118	315	159.9	1.90
	16.0	49.9	--	44	56.8	114	320	161.9	1.30
	15.6	45.5	40	53	43.5	138	323	159.9	1.68
X	15.2	40.9	38	58	43.0	120	313	158.7	1.15
SE	0.3	2.5	1	9	3.1	4	3	4.3	0.15
1 STRESS	17.4	58.7	42	43	21.1	100	258	126.9	1.46
+ 12 HOURS	15.4	43.9	45	23	33.3	94	306	156.6	2.34
	16.7	54.7	47	53	24.0	77	298	191.8	3.22
	15.6	44.0	47	53	30.4	131	303	152.2	3.00
	16.2	46.9	47	51	44.2	93	325	159.9	3.11
	16.7	50.7	48	67	33.3	121	314	155.5	3.33
	15.4	40.1	47	63	39.8	156	313	159.9	3.44
	16.7	54.8	49	63	52.2	119	328	187.4	4.32
	16.3	47.1	52	10	49.7	165	330	166.5	4.76
	17.1	56.4	46	69	66.4	165	311	167.5	6.17
X	16.4	49.7	47	50	39.4	122	309	162.4	3.52
SE	0.2	2.0	1	6	4.4	10	7	5.8	0.41

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
1 STRESS	14.7	32.3	43	58	17.5	103	288	148.9	2.67
+ 24 HOURS	14.3	32.5	44	49	25.9	104	310	157.7	3.11
	14.3	31.6	43	20	20.7	74	311	156.8	3.55
	16.2	48.7	49	78	22.1	88	283	140.1	2.45
	14.7	33.8	43	75	25.0	72	311	163.2	3.55
	15.9	45.8	43	42	40.8	138	324	153.3	3.55
	16.3	48.0	45	53	25.9	122	311	157.7	4.10
	16.8	53.2	46	85	44.0	121	306	161.0	3.88
	16.2	49.8	45	68	39.1	158	323	155.5	4.43
	16.6	53.9	48	97	51.1	162	328	158.8	4.21
X	15.6	43.0	45	62	31.2	114	310	155.3	3.55
SE	0.3	3.0	1	7	3.6	10	5	2.1	0.21
2 STRESSES	16.0	47.9	40	236	86.0	132	346	161.3	4.77
	15.3	39.4	48	413	112.4	98	336	172.9	4.71
	15.7	44.7	44	235	64.9	120	327	166.3	4.71
	16.5	50.4	48	387	74.4	104	336	177.5	4.10
	15.5	39.5	46	253	71.3	96	337	165.4	4.21
	16.4	50.5	46	247	87.4	125	342	165.2	4.16
	16.5	51.1	43	302	108.4	103	333	163.0	4.16
	15.2	44.1	47	389	83.7	110	347	175.1	4.82
	16.8	55.1	47	253	72.4	102	362	167.5	3.65
	15.6	44.1	44	163	58.4	91	335	175.2	4.64
X	16.0	46.7	45	288	81.9	108	340	168.9	4.39
SE	0.2	1.6	1	26	5.6	4	3	1.8	0.12

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
2 STRESSES	14.7	39.5	42	420	155.5	138	349	173.1	0.24
+ 1 HOUR	13.7	25.8	37	312	65.1	122	385	178.5	0.46
	17.8	65.5	49	157	93.8	126	333	177.5	0.47
	15.5	40.6	41	277	83.4	99	352	174.0	0.64
	15.4	42.6	42	232	106.3	112	327	171.8	0.42
	15.6	42.7	49	158	50.4	119	360	180.7	0.41
	16.1	45.2	44	345	116.4	149	358	179.5	0.42
	14.8	38.0	42	265	131.2	129	376	183.3	0.37
	15.7	47.0	48	279	86.9	157	358	179.5	1.08
	17.5	61.9	50	345	153.9	176	363	184.1	0.36
X	15.7	44.9	44	279	104.3	133	356	178.2	0.49
SE	0.4	3.6	1	26	11.2	7	6	1.3	0.07
2 STRESSES	15.2	36.4	36	238	81.7	122	330	164.1	1.63
+ 3 HOURS	13.5	27.2	39	285	59.3	111	342	173.1	1.35
	14.8	35.9	41	142	69.0	129	317	177.3	1.74
	14.7	36.6	43	108	56.6	133	328	158.8	1.02
	14.5	34.2	42	131	74.4	147	350	182.8	2.18
	18.8	73.0	38	149	39.1	121	320	167.5	1.89
	15.7	42.1	39	175	157.5	140	331	170.9	1.79
	16.5	53.3	41	128	103.0	149	360	171.8	1.63
	16.0	46.8	40	272	84.3	121	363	174.1	2.44
	16.7	54.8	41	163	150.8	146	333	177.5	3.88
X	15.6	44.0	40	179	87.6	132	337	171.8	1.96
SE	0.5	4.2	1	20	12.4	4	5	2.2	0.25

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
2 STRESSES	15.8	36.1	39	100	41.8	96	319	158.8	2.78
+6 HOURS	15.0	36.1	42	121	31.3	120	322	166.5	3.22
	15.0	36.1	42	70	31.1	109	314	151.1	3.55
	16.2	48.5	41	62	24.0	133	325	161.0	4.42
	16.4	50.2	45	76	30.2	124	321	159.9	3.66
	17.3	58.9	44	66	41.3	139	337	164.3	4.32
	14.8	38.6	43	40	33.7	119	319	161.0	3.88
	16.8	55.7	44	56	38.2	124	312	159.9	4.65
	15.7	46.0	44	73	86.5	155	323	161.0	4.32
	14.9	34.6	--	102	66.4	159	327	165.4	4.98
X	15.8	44.1	43	77	42.4	128	322	160.9	3.98
SE	0.3	2.8	1	8	6.1	6	2	1.4	0.22
2 STRESSES	15.3	41.1	49	149	26.5	111	315	191.8	3.55
+ 12 HOURS	17.0	56.9	46	91	29.1	104	315	155.5	2.45
	15.6	43.1	48	27	41.6	108	312	82.9	1.35
	15.9	44.0	44	70	40.1	124	310	161.0	3.22
	16.4	46.2	45	73	51.6	134	324	153.3	3.22
	16.7	51.5	48	85	32.1	108	310	159.9	3.00
	16.7	50.9	48	66	50.6	135	328	164.3	3.99
	16.3	50.6	49	63	57.3	202	312	157.7	3.77
	16.3	49.4	56	29	44.0	161	319	161.0	3.99
	17.7	62.0	47	87	66.0	172	327	162.1	4.76
X	16.4	49.6	48	74	43.9	136	317	155.0	3.33
SE	0.2	2.0	1	11	4.0	10	2	8.7	0.30

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
2 STRESSES	15.0	38.4	47	52	19.3	126	309	157.7	3.55
+ 24 HOURS	15.4	44.3	43	95	27.4	145	299	155.5	3.55
	15.3	42.5	46	39	35.4	105	309	162.1	3.44
	16.3	46.1	44	76	36.3	145	305	147.8	3.99
	15.7	43.8	42	79	42.6	126	318	168.7	4.10
	16.2	49.8	46	42	27.2	97	302	161.0	4.21
	16.2	49.4	--	111	37.9	167	319	169.8	4.21
	16.1	49.6	47	67	58.3	163	318	151.1	5.20
X	15.8	45.5	45	70	35.6	134	310	159.2	4.03
SE	0.2	1.4	1	9	4.2	9	3	2.8	0.20
3 STRESSES	15.3	42.7	42	350	84.0	116	326	168.5	0.53
	15.8	51.0	53	333	182.5	159	346	178.6	0.91
	16.7	55.9	47	239	77.8	109	333	178.6	0.36
	16.6	54.3	48	428	213.0	195	380	181.9	0.60
	16.3	52.4	42	450	75.6	133	365	184.0	0.13
	17.6	59.7	44	432	125.6	127	349	174.2	0.47
	15.7	42.4	--	231	124.6	142	348	175.2	0.57
	16.4	53.5	--	371	107.5	157	373	181.9	0.90
	17.4	62.8	--	237	62.6	142	352	179.6	0.13
	16.9	54.6	46	219	89.7	145	339	175.3	0.58
X	16.5	52.9	46	329	114.3	142	351	177.8	0.52
SE	0.2	2.0	2	29	15.5	8	5	1.4	0.08

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
3 STRESSES	15.0	35.4	44	448	102.9	116	342	172.9	0.75
+ 1 HOUR	15.8	47.6	44	440	119.0	118	337	166.5	0.58
	15.6	38.8	40	224	106.7	117	374	184.1	0.36
	15.4	39.7	42	304	84.4	111	370	176.2	0.42
	14.9	38.5	47	270	81.0	154	380	177.5	0.58
	16.0	43.5	40	273	82.8	151	390	192.9	0.47
	15.2	40.4	42	390	91.0	138	367	179.7	0.69
	15.8	45.3	43	297	119.6	148	361	178.4	0.64
	16.1	48.4	43	246	122.8	177	356	176.4	0.69
	16.7	58.3	38	150	74.5	167	369	178.4	1.04
X	15.6	43.6	42	304	98.5	140	365	178.3	0.62
SE	0.2	2.1	1	30	5.7	7	5	2.2	0.06
3 STRESSES	15.0	37.2	37	231	65.1	134	352	133.4	0.79
+ 3 HOURS	16.1	53.5	39	299	60.8	105	323	169.6	0.86
	15.0	36.8	40	346	44.0	151	355	182.8	0.71
	14.8	37.0	47	266	67.6	158	348	177.3	0.75
	14.9	40.8	40	179	45.6	117	343	171.8	0.86
	15.2	40.8	44	189	44.5	145	335	135.6	0.68
	15.7	41.4	42	243	52.7	142	351	176.2	0.97
	17.0	54.5	46	139	45.9	140	342	174.1	0.57
	17.1	58.2	42	153	87.3	170	378	181.8	1.23
	16.8	52.9	38	186	90.6	128	352	178.4	1.52
X	15.8	45.3	42	223	60.4	139	348	168.1	0.89
SE	0.3	2.7	1	21	5.5	6	5	5.7	0.09

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
3 STRESSES	14.9	33.3	37	237	30.0	155	349	172.0	3.77
+ 6 HOURS	15.0	34.3	39	131	29.1	130	352	174.2	4.43
	14.6	37.4	38	250	46.5	117	336	167.6	4.54
	14.6	31.3	41	115	41.1	159	346	166.5	3.99
	15.6	41.8	44	76	31.5	167	341	142.3	3.22
	15.8	44.3	43	105	25.7	143	326	161.0	3.88
	15.5	39.8	41	134	43.4	185	354	174.2	4.43
	16.2	46.9	43	69	30.2	115	334	142.3	3.66
	16.8	53.5	44	67	37.0	136	322	154.4	4.43
	17.3	56.0	42	93	69.0	151	357	176.4	4.54
X	15.6	41.9	41	128	38.4	146	342	163.1	4.10
SE	0.3	2.7	1	21	4.0	7	4	4.1	0.14
3 STRESSES	14.7	35.3	43	70	18.8	109	309	150.0	2.67
+ 12 HOURS	16.2	49.3	43	45	28.9	121	308	159.9	2.78
	15.8	42.9	45	74	40.3	117	311	155.5	3.00
	16.4	49.0	44	77	28.9	129	316	157.5	3.55
	16.5	50.7	52	42	41.3	123	307	159.9	3.99
	17.3	59.0	44	51	28.4	116	324	159.9	3.99
	15.7	42.6	--	48	39.4	128	321	156.6	4.65
	16.1	45.2	47	52	43.7	176	330	178.6	5.75
	16.2	51.2	46	68	54.9	163	317	157.7	4.21
	16.4	47.4	45	54	56.5	134	318	156.6	4.32
X	16.1	47.3	45	58	38.1	132	316	159.2	3.89
SE	0.2	2.0	1	4	3.8	7	2	2.3	0.30

SAMPLE	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MMOLE/L)	POTASSIUM (MMOLE/L)
3 STRESSES	15.3	37.5	46	48	40.8	76	302	153.3	2.78
+ 24 HOURS	15.3	39.2	--	21	43.7	110	315	156.6	3.77
	15.2	38.1	49	70	31.0	134	313	147.8	3.00
	15.4	43.6	45	77	25.1	104	307	163.2	3.44
	15.5	41.5	46	85	59.1	148	315	146.7	3.22
	16.5	46.7	49	27	38.2	94	325	159.9	3.33
	18.0	65.9	51	37	52.6	134	301	157.7	4.21
	15.8	47.8	47	82	54.9	144	323	159.9	4.54
	15.1	37.9	46	47	43.7	111	314	157.7	5.75
	16.4	50.1	44	27	42.1	135	315	154.4	5.64
X	15.8	44.8	47	52	43.2	119	313	155.7	3.97
SE	0.3	2.7	1	8	3.3	7	2	1.7	0.33

APPENDIX 2

Lengths and critical swimming speeds of individual fish for each level of stress and each time period after stress.

GROUP		LENGTH(CM)	CRITICLE SWIM SPEED (CM/SEC)	CRITICLE SWIM SPEED (BODY LENGTHS/SEC)
NO STRESS		19.0	36.0	1.9
		19.0	49.5	2.6
		21.0	79.0	3.8
		19.0	57.0	3.0
		20.5	57.0	2.8
		19.5	105.0	5.4
		17.0	57.0	3.4
		19.5	62.5	3.2
		21.0	98.0	4.7
		19.3	0.0	0.0
		20.0	55.0	2.8
		20.8	99.2	4.8
	X	19.6	62.9	3.2
	SE	0.3	8.5	0.4
NO STRESS		20.0	49.0	2.4
+ 24 HOURS		20.5	54.0	2.6
		19.5	66.7	3.4
		19.0	38.0	2.0
		20.0	56.7	2.8
		19.0	95.8	5.0
		18.5	36.0	1.9
		19.3	83.2	4.3
		20.0	104.5	5.2
		20.5	35.0	1.7
		21.0	50.5	2.4
		18.7	56.0	3.0
	X	19.6	60.4	3.1
	SE	0.2	6.6	0.3
1 STRESS		16.5	0.0	0.0
		16.3	35.0	2.1
		16.4	85.0	5.1
		16.5	37.0	2.2
		20.5	46.5	2.4
		17.5	77.5	4.4
		19.5	0.0	0.0
		20.5	50.0	2.4
		18.5	105.0	5.7
		21.0	46.0	2.2
		20.0	56.2	2.8
		21.0	81.0	3.9
	X	18.7	51.7	2.8
	SE	0.6	9.3	0.5

GROUP		LENGTH(CM)	CRITICLE SWIM SPEED (CM/SEC)	CRITICLE SWIM SPEED (BODY LENGTHS/SEC)
1 STRESS		18.5	57.5	3.1
+ 1 HOUR		18.5	56.0	3.0
		17.5	78.0	4.5
		19.0	0.0	0.0
		20.5	48.7	2.4
		20.5	57.3	2.8
		23.0	27.3	1.2
		20.5	45.0	2.2
		20.5	56.2	2.7
		19.5	25.0	1.3
		19.0	46.7	2.5
		22.0	75.0	3.4
	X	19.9	47.7	2.4
	SE	0.5	6.3	0.3
1 STRESS		18.0	38.5	2.1
+ 3 HOURS		18.5	46.0	2.5
		19.0	60.0	3.2
		15.5	0.0	0.0
		18.5	47.5	2.6
		19.5	76.0	3.9
		18.0	36.0	2.0
		17.5	37.0	2.1
		20.0	86.0	4.3
		18.0	50.0	2.8
		17.5	57.6	3.3
		17.5	59.0	3.4
	X	18.1	49.5	2.7
	SE	0.3	6.3	0.3
1 STRESS		18.5	15.0	0.8
+ 6 HOURS		17.5	33.0	1.9
		17.0	68.0	4.0
		17.5	33.0	1.9
		20.5	44.0	2.2
		19.0	105.0	5.5
		19.5	36.0	1.9
		19.5	38.5	2.0
		20.5	88.5	4.3
		19.5	15.9	0.8
		20.5	46.7	2.3
		20.5	56.3	2.7
	X	19.2	48.3	2.5
	SE	0.4	7.9	0.4

GROUP		LENGTH(CM)	CRITICLE SWIM SPEED (CM/SEC)	CRITICLE SWIM SPEED (BODY LENGTHS/SEC)
1 STRESS		17.5	25.0	1.4
+ 12 HOURS		18.0	47.0	2.6
		17.5	62.3	3.6
		19.5	37.0	1.9
		20.5	55.0	2.7
		19.0	87.5	4.6
		19.0	35.0	1.8
		19.0	50.5	2.6
		19.0	105.0	5.5
		22.0	41.3	1.9
		19.0	55.0	2.9
		22.0	68.0	3.1
	X	19.3	55.7	2.9
	SE	0.4	6.6	0.3
1 STRESS		15.5	27.0	1.7
+ 24 HOURS		17.7	52.0	2.9
		17.2	72.3	4.2
		18.3	39.0	2.1
		17.4	43.0	2.5
		18.5	53.0	2.9
		20.0	15.0	0.8
		19.0	59.0	3.1
		17.5	71.0	4.1
		18.5	43.0	2.3
		19.0	48.0	2.5
		17.5	53.0	3.0
	X	18.0	47.9	2.7
	SE	0.3	4.7	0.3
2 STRESSES		18.0	0.0	0.0
		17.0	8.3	0.5
		17.5	45.0	2.6
		18.5	17.3	0.9
		20.5	61.7	3.0
		17.0	77.3	4.5
		17.0	30.5	1.8
		17.5	49.5	2.8
		18.0	57.0	3.2
		17.0	0.0	0.0
		18.0	66.5	3.7
		19.0	90.3	4.8
	X	17.9	41.9	2.3
	SE	0.3	8.8	0.5

GROUP		LENGTH(CM)	CRITICLE SWIM SPEED (CM/SEC)	CRITICLE SWIM SPEED (BODY LENGTHS/SEC)
2 STRESSES		17.0	45.0	2.6
+ 1 HOUR		17.5	51.0	2.9
		19.7	89.0	4.5
		18.0	57.5	3.2
		21.0	70.0	3.3
		20.0	88.0	4.4
		19.5	45.0	2.3
		19.0	95.0	5.0
		20.5	105.0	5.1
		18.0	41.0	2.3
		20.0	43.0	2.2
		20.3	52.7	2.6
	X	19.2	65.2	3.4
	SE	0.4	6.7	0.3
2 STRESSES		18.0	36.0	2.0
+ 3 HOURS		17.5	93.0	5.3
		19.5	105.0	5.4
		18.0	47.5	2.6
		21.0	75.0	3.6
		19.0	105.0	5.5
		18.5	0.0	0.0
		19.5	37.5	1.9
		19.0	50.0	2.6
		18.5	26.0	1.4
		17.5	29.7	1.7
		21.0	47.3	2.2
	X	18.0	54.3	2.9
	SE	0.3	9.6	0.5
2 STRESSES		16.2	0.0	0.0
+ 6 HOURS		17.0	45.0	2.6
		18.0	77.0	4.3
		16.8	25.0	1.5
		18.5	44.5	2.4
		18.5	56.0	3.0
		20.0	35.0	1.8
		19.5	55.0	2.8
		19.5	61.0	3.1
		19.5	46.0	2.4
		20.5	47.0	2.3
		18.0	58.0	3.2
	X	18.5	45.8	2.5
	SE	0.4	5.6	0.3

GROUP		LENGTH(CM)	CRITICLE SWIM SPEED (CM/SEC)	CRITICLE SWIM SPEED (BODY LENGTHS/SEC)
2 STRESSES		19.0	60.0	3.2
+ 12 HOURS		19.0	71.5	3.8
		19.0	77.0	4.0
		19.5	36.0	1.8
		19.5	47.7	2.4
		21.0	79.5	3.8
		19.5	33.0	1.7
		20.0	43.0	2.2
		20.5	63.0	3.1
		22.5	25.0	1.1
		18.5	35.0	1.9
		19.0	77.0	4.1
	X	19.8	54.0	2.8
	SE	0.3	5.7	0.3
2 SRESSES		15.0	36.0	2.4
+ 24 HOURS		19.0	50.7	2.7
		19.0	70.0	3.7
		19.0	30.0	1.6
		19.5	44.7	2.3
		18.0	105.0	5.8
		20.0	43.3	2.2
		20.0	53.5	2.7
		20.0	77.0	3.8
		19.5	46.0	2.4
		21.0	51.8	2.5
		20.4	75.0	3.7
	X	19.2	56.9	3.0
	SE	0.4	6.1	0.3
3 STRESSES		17.0	55.0	3.2
		20.5	64.0	3.1
		19.0	83.5	4.4
		17.5	57.0	3.2
		16.5	58.0	3.5
		18.0	66.3	3.7
		20.0	25.0	1.2
		20.5	58.5	2.8
		20.5	63.5	3.1
		19.2	0.0	0.0
		19.0	0.0	0.0
		19.0	61.5	3.2
	X	18.9	49.4	2.6
	SE	0.4	7.6	0.4

GROUP		LENGTH(CM)	CRITICLE SWIM SPEED (CM/SEC)	CRITICLE SWIM SPEED (BODY LENGTHS/SEC)
3 STRESSES		19.0	40.0	2.1
+ 1 HOUR		16.6	49.5	3.0
		17.9	75.5	4.2
		18.0	0.0	0.0
		18.5	30.5	1.6
		19.0	46.0	2.4
		17.5	0.0	0.0
		19.5	47.0	2.4
		20.0	77.0	3.8
		18.5	25.0	1.4
		19.5	64.0	3.3
		20.5	83.3	4.1
	X	18.7	44.8	2.4
	SE	0.3	8.0	0.4
3 STRESSES		19.0	47.0	2.5
+ 3 HOURS		20.5	62.0	3.0
		19.5	76.5	3.9
		16.5	42.0	2.5
		20.0	48.0	2.4
		19.5	88.0	4.5
		20.0	47.7	2.4
		20.0	49.7	2.5
		19.0	67.0	3.5
		21.0	64.5	3.1
		20.5	105.0	5.1
		21.0	105.0	5.0
	X	19.7	66.9	3.4
	SE	0.4	6.5	0.3
3 STRESSES		17.7	25.0	1.5
+ 6 HOURS		16.8	45.0	2.7
		13.0	58.0	4.5
		18.0	37.0	2.1
		17.0	47.3	2.8
		18.0	52.0	2.9
		17.5	45.0	2.6
		17.5	64.5	3.7
		19.0	68.2	3.6
		18.5	53.7	2.9
		19.5	86.3	4.4
		20.5	105.0	5.1
	X	17.8	57.2	3.2
	SE	0.5	6.3	0.3

GROUP		LENGTH(CM)	CRITICLE SWIM SPEED (CM/SEC)	CRITICLE SWIM SPEED (BODY LENGTHS/SEC)
3 STRESSES		18.0	0.0	0.0
+ 12 HOURS		17.0	37.5	2.2
		19.0	58.0	3.1
		19.0	30.0	1.6
		20.5	35.0	1.7
		18.0	46.0	2.6
		20.0	0.0	0.0
		20.0	37.7	1.9
		20.5	56.0	2.7
		19.0	49.0	2.6
		19.0	62.0	3.3
		19.0	105.0	5.5
	X	19.1	43.0	2.3
	SE	0.3	8.1	0.4
3 STRESSES		16.7	15.0	0.9
+ 24 HOURS		18.5	58.0	3.1
		18.5	60.3	3.3
		20.0	49.5	2.5
		20.0	71.2	3.6
		17.5	105.0	6.6
		19.0	45.0	2.4
		19.0	55.0	2.9
		19.5	55.0	2.8
		18.0	44.3	2.5
		20.4	57.2	2.8
		20.5	75.0	3.7
	X	19.0	56.7	3.0
	SE	0.3	6.4	0.4

APPENDIX 3

Fatigue time, length, weight, hematocrit, plasma osmolarity and plama concentrations of cortisol, lactic acid, glucose, sodium and potassium of individual fish from fatigue time experiment at each level of stress and each time period after stress.

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
CONTROL	--	19.6	82.6	44	12	35.4	81	305	148.5	1.38
NO SWIM	--	20.2	84.5	46	47	--	93	328	156.2	1.78
	--	19.9	84.8	41	61	41.4	92	319	150.7	2.55
	--	21.4	112.1	40	11	30.9	75	308	152.9	3.43
	--	19.2	72.1	39	43	29.8	74	301	154.5	0.48
	--	20.5	88.8	39	11	40.1	74	324	151.5	1.24
	--	21.6	122.5	39	5	38.4	71	331	155.1	1.89
	--	22.7	142.3	45	50	45.2	79	314	154.8	1.24
	--	20.0	91.3	36	28	58.0	74	306	163.9	3.40
X	--	20.6	97.9	41	30	39.9	79	315	154.2	1.93
SE		0.4	7.6	1	7	3.0	3	4	1.5	0.34
NO STRESS	0.8	21.5	120.8	--	--	--	--	--	--	--
	0.8	21.3	99.8	35	144	21.0	72	302	155.1	0.68
	0.8	18.8	74.9	42	221	91.0	103	369	181.2	3.44
	3.0	20.6	93.1	38	41	34.6	96	350	171.6	0.13
	3.5	22.2	139.1	38	155	65.2	136	363	170.5	0.13
	5.5	19.0	80.5	34	33	64.3	99	325	164.7	1.57
	6.5	20.5	101.7	38	261	42.5	129	--	--	--
	8.0	21.5	106.6	44	135	83.4	75	314	163.6	2.01
	9.0	22.9	141.2	40	39	99.9	114	328	171.3	0.14
	10.5	20.6	105.7	51	221	38.9	79	327	163.3	1.02
	10.5	20.7	117.7	--	--	--	--	--	--	--
	12.8	21.4	130.7	--	--	--	--	--	--	--
X	4.1	20.9	109.3	40	139	60.1	100	335	167.7	1.14
SE		0.3	6.2	2	29	9.2	8	8	2.7	0.41

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
1 STRESS	0.0	21.3	99.8	38	142	94.5	116	372	182.3	2.01
	0.0	18.6	60.5	36	302	32.7	93	448	232.9	3.22
	1.0	19.3	87.1	61	188	74.3	125	365	198.8	2.56
	1.5	23.5	152.2	--	--	--	--	--	--	--
	2.0	20.0	90.2	44	166	65.5	99	345	191.4	6.07
	4.0	18.6	69.3	44	137	56.6	122	391	186.7	1.68
	9.5	19.4	84.6	58	131	82.1	134	329	--	--
	19.2	19.0	71.8	26	112	60.6	100	369	192.5	4.75
	>60.00	22.0	115.1	33	94	51.9	82	335	166.9	2.89
X	1.3	20.2	92.3	42	159	64.8	109	369	192.9	3.38
SE		0.6	9.3	4	23	6.8	6	13	7.7	0.58
1 STRESS	0.0	19.7	71.1	46	162	38.7	142	352	166.1	1.67
+ 1 HOUR	0.0	20.0	84.8	41	106	25.2	100	350	167.2	1.12
	2.5	21.0	113.1	39	192	--	97	--	--	--
	3.0	21.7	129.2	--	--	--	--	--	--	--
	8.0	20.4	85.3	31	98	82.5	96	342	163.2	3.22
	10.0	21.2	109.6	45	26	79.1	106	340	170.2	2.56
	13.5	20.5	90.2	31	32	74.1	115	342	168.0	4.43
	14.3	18.5	69.4	22	103	55.8	98	336	175.7	4.21
	>60.0	21.1	119.4	39	175	66.1	120	315	170.5	5.96
X	2.1	20.5	96.9	37	112	60.2	109	340	168.8	3.30
SE		0.3	7.2	3	22	8.1	6	5	1.5	0.64

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
1 STRESS	0.0	21.1	105.9	56	185	59.6	111	328	153.7	1.57
+ 3 HOURS	0.3	21.5	114.9	44	138	88.2	92	321	162.5	2.55
	0.5	19.3	85.5	--	99	37.8	98	317	160.4	1.89
	4.5	20.5	97.0	48	213	64.7	90	320	162.1	2.66
	11.5	21.8	120.3	--	--	--	--	--	--	--
	12.2	22.9	146.1	45	193	61.1	102	339	167.2	2.66
	16.5	23.4	155.1	45	228	90.6	157	360	176.8	2.45
	>60.0	23.3	145.7	38	39	40.4	72	302	159.2	3.44
	>60.0	22.3	132.8	42	194	110.2	134	383	183.4	4.98
X	3.4	21.8	122.6	45	161	69.1	107	334	165.8	2.75
SE		0.5	8.0	2	23	9.0	10	9	3.4	0.38
1 STRESS	0.0	19.1	78.6	39	187	67.2	84	340	165.0	3.21
+ 6 HOURS	0.3	20.4	89.3	50	62	39.9	79	306	153.7	2.56
	0.7	21.9	127.9	59	213	42.3	79	314	159.2	1.03
	7.2	23.0	149.2	53	20	90.0	90	323	159.2	1.79
	14.2	20.6	100.1	47	194	73.7	140	355	165.8	4.10
	14.2	21.8	119.9	46	118	--	96	--	--	--
	49.3	21.0	102.6	48	265	89.9	102	356	163.7	9.99
	>60.0	21.9	127.8	37	235	110.4	98	333	166.1	2.88
	>60.0	19.5	81.9	43	43	48.6	76	306	152.6	1.13
X	4.3	21.0	108.6	47	149	70.2	94	329	160.7	3.34
SE		0.4	8.0	2	30	9.0	7	7	1.9	1.02

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
1 STRESS	0.5	18.9	76.7	36	80	56.4	79	301	149.3	1.46
+ 24 HOURS	4.5	23.4	139.5	38	49	54.1	74	323	152.9	2.55
	5.2	23.6	155.8	48	130	76.1	98	347	174.6	2.23
	5.2	21.1	98.7	34	38	54.7	79	304	157.8	1.01
	8.2	21.2	107.5	48	134	59.2	79	318	154.0	0.46
	14.0	23.2	138.2	39	97	83.7	99	322	169.4	2.77
	>60.0	19.0	78.1	50	213	55.1	108	333	164.9	0.37
	>60.0	22.5	141.8	57	138	82.9	120	335	165.0	0.26
	>60.0	19.5	80.9	30	145	38.0	92	312	95.0	2.88
X	10.5	21.4	113.0	42	114	62.2	92	322	152.9	1.54
SE		0.6	10.4	3	18	5.1	5	5	7.8	0.35
2 STRESSES	0.3	19.4	85.3	37	192	60.1	120	344	172.7	2.66
	1.5	23.2	154.8	56	247	69.0	117	340	172.4	0.03
	2.0	21.5	124.8	--	--	--	--	--	--	--
	2.2	20.6	91.1	29	202	76.8	113	373	170.5	3.10
	14.5	18.4	67.8	34	64	47.1	120	333	161.7	4.31
	>60.0	21.8	117.9	46	36	36.5	78	324	152.9	1.89
	>60.0	23.0	151.5	51	179	82.0	74	334	188.1	3.65
	>60.0	21.4	108.6	45	160	80.1	93	327	157.0	2.45
	>60.0	21.0	103.7	38	106	98.0	142	400	195.8	4.75
X	9.0	21.1	111.7	42	148	68.7	107	347	171.4	2.86
SE		0.5	9.7	3	26	7.1	8	9	5.2	0.53

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
2 STRESSES	0.0	19.0	76.9	47	382	98.4	131	375	196.6	5.09
+ 1 HOUR	0.0	20.6	101.0	49	271	37.6	96	325	161.7	0.46
	1.8	19.0	81.4	54	301	71.1	134	314	163.6	0.00
	4.2	19.7	83.5	39	229	38.4	103	358	184.8	0.68
	6.2	19.5	90.6	38	245	61.4	137	411	194.4	9.38
	6.5	20.9	102.8	49	121	58.2	106	342	177.1	0.57
	11.5	20.7	103.0	--	210	--	--	--	--	--
	16.2	24.7	151.3	53	148	52.1	116	329	162.8	0.35
	>60.0	22.6	138.0	38	96	122.6	101	336	159.2	4.10
X	1.9	20.7	103.2	46	223	67.5	116	349	175.0	2.58
SE		0.6	6.5	2	30	10.4	6	11	5.4	1.20
2 STRESSES	0.0	20.8	110.1	43	165	76.9	156	352	179.0	1.13
+ 3 HOURS	0.8	20.4	87.0	27	228	46.8	138	366	173.5	3.22
	3.3	22.0	131.7	29	22	69.6	77	302	158.4	3.43
	3.7	21.3	98.6	47	388	43.8	84	370	160.3	2.45
	7.2	21.1	119.7	32	13	67.2	96	334	162.5	3.55
	>60.0	21.1	107.1	42	117	49.6	105	349	166.1	2.77
	>60.0	21.3	113.9	40	153	65.7	93	321	154.8	3.44
	>60.0	22.2	128.8	--	55	85.2	122	323	159.2	3.99
	>60.0	23.4	148.5	34	69	37.4	90	308	152.6	4.10
X	5.9	21.5	116.2	37	134	60.5	107	336	162.9	3.12
SE		0.3	6.2	3	40	5.4	9	8	2.9	0.30

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
2 STRESSES	0.0	18.3	69.0	53	425	73.7	122	322	172.4	0.00
+ 6 HOURS	0.0	19.9	81.0	34	315	77.8	106	379	147.1	3.99
	1.2	20.0	85.0	40	14	33.3	73	323	159.5	4.31
	1.5	19.2	80.3	38	15	48.2	67	312	157.0	3.88
	2.5	19.3	79.8	39	162	57.6	109	327	163.6	2.45
	7.0	20.2	88.3	34	140	43.4	102	318	165.8	3.22
	>60.0	21.9	115.4	45	260	39.1	80	307	152.6	0.14
	>60.0	22.8	140.0	33	45	35.6	80	297	155.1	6.62
	>60.0	20.4	92.4	36	134	78.7	75	319	149.6	3.87
X	2.1	20.2	92.4	39	168	54.2	90	323	158.1	3.16
SE		0.5	7.3	2	47	6.1	7	8	2.7	0.69
2 STRESSES	0.0	19.0	76.9	53	130	29.1	130	327	160.6	2.99
+ 24 HOURS	2.7	20.3	94.5	58	224	58.2	75	318	157.3	4.20
	3.1	23.2	147.7	47	180	61.9	113	322	159.2	0.25
	5.2	21.0	116.7	--	212	--	--	330	--	--
	5.2	20.4	90.7	38	108	88.3	85	327	151.8	1.56
	36.5	21.3	101.7	49	239	63.0	86	348	162.5	7.29
	>60.0	22.0	115.4	49	178	21.8	73	307	157.8	0.37
	>60.0	21.2	105.1	48	106	77.8	75	343	165.0	1.78
	>60.0	22.5	121.5	44	30	93.3	82	330	190.3	1.45
X	6.4	21.2	107.8	48	156	61.7	90	328	160.2	2.52
SE		0.4	6.9	2	23	9.1	7	4	1.7	0.82

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
3 STRESSES	0.0	19.0	70.4	45	132	56.0	125	328	167.2	0.48
	0.0	18.8	70.2	32	151	37.4	86	357	144.1	2.33
	0.2	21.3	113.7	--	167	--	--	411	--	--
	0.7	18.0	60.1	29	79	120.7	151	445	210.9	1.79
	1.8	19.6	72.9	25	149	61.1	121	330	161.4	2.89
	6.8	22.3	136.4	--	--	--	--	--	--	--
	6.8	18.9	72.6	--	300	97.7	138	359	187.8	0.58
	11.3	21.8	110.7	27	164	46.3	135	362	171.6	4.09
	>60.0	19.1	72.9	44	134	60.5	79	384	204.6	2.99
X	1.0	19.9	86.7	34	160	68.5	119	372	178.2	2.16
SE		0.5	8.8	4	22	11.2	10	14	9.1	0.50
3 STRESSES	0.2	19.4	79.1	37	121	63.1	100	356	174.6	2.89
+ 1 HOUR	3.0	20.1	88.4	35	31	36.8	110	320	155.1	0.68
	4.5	21.6	115.0	40	106	76.8	132	324	152.6	3.11
	5.5	20.8	105.7	49	240	52.3	137	--	198.8	13.34
	7.0	20.8	96.4	--	283	115.3	104	330	193.6	5.41
	7.0	19.4	74.0	44	75	58.4	117	328	158.4	1.12
	8.5	19.3	91.7	29	62	54.0	99	350	192.2	11.14
	38.8	21.6	117.8	41	136	76.7	108	358	201.0	6.30
	>60.0	21.7	116.0	42	114	78.7	126	359	186.7	7.17
X	6.2	20.5	98.2	40	130	68.0	115	341	179.2	5.67
SE		0.3	5.4	2	27	7.5	5	6	6.5	1.40

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
3 STRESSES	2.8	21.3	105.5	42	56	35.4	88	324	160.3	2.12
+ 3 HOURS	3.0	17.8	50.2	--	83	46.6	110	349	174.9	4.31
	5.5	20.3	116.6	38	75	60.4	124	325	141.9	3.21
	9.0	19.8	90.9	47	80	69.8	144	392	185.9	1.89
	>60.0	22.1	127.8	48	184	88.0	149	351	144.6	2.68
	>60.0	22.3	136.4	54	124	62.4	127	363	173.8	0.68
	>60.0	21.0	97.3	39	88	61.1	100	368	177.1	2.77
	>60.0	20.5	106.3	50	89	105.9	240	348	159.5	1.34
	>60.0	22.1	121.2	34	39	55.8	124	318	159.2	3.88
X	19.0	20.8	105.8	44	91	65.0	134	349	164.1	2.54
SE		0.5	8.5	2	14	7.0	15	8	5.0	0.39
3 STRESSES	0.0	20.9	110.3	48	273	51.9	92	332	168.0	2.56
+ 6 HOURS	0.0	20.8	104.6	39	42	72.3	74	335	169.1	5.75
	3.2	21.0	110.0	18	--	--	--	--	--	--
	4.2	20.7	106.0	34	136	69.6	112	309	149.6	4.20
	5.5	21.9	117.3	32	70	--	--	--	--	--
	6.5	21.3	106.9	23	304	--	--	--	--	--
	7.0	20.9	130.8	37	19	72.6	136	318	162.5	3.99
	7.0	22.1	130.8	35	166	71.4	135	361	169.4	1.78
	>60.0	22.1	134.0	39	220	70.2	74	334	163.9	5.19
X	1.7	21.3	116.7	34	154	68.0	104	332	163.8	3.91
SE		0.2	4.0	3	38	3.3	12	7	3.1	0.62

SAMPLE	FATIGUE TIME(MIN)	LENGTH (CM)	WEIGTH (G)	% HEMAT-OCRIT	CORTOSOL (NG/ML)	LACTIC ACID (MG/100ML)	GLUCOSE (MG/100ML)	OSMOLARITY (MMOLE/KG)	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)
3 STRESSES	2.0	21.6	123.9	--	1	31.1	66	296	143.8	2.12
+ 24 HOURS	2.5	19.7	89.2	36	51	56.0	74	296	155.1	3.21
	3.0	22.1	116.3	48	126	39.7	102	328	183.7	1.34
	8.0	22.3	118.7	47	137	38.4	105	356	177.1	1.89
	17.4	18.0	59.0	36	87	98.8	124	339	170.5	3.10
	19.5	23.2	139.1	38	48	67.9	98	340	173.5	4.87
	21.0	22.5	125.6	43	252	97.3	115	351	176.8	4.76
	>60.0	20.0	87.2	33	225	60.1	117	296	148.2	3.00
	>60.0	21.4	106.7	46	121	84.4	165	342	159.2	3.33
	X 11.3	21.2	107.3	41	116	63.7	107	327	165.3	3.07
	SE	0.6	8.2	2	27	8.5	10	8	4.7	0.40

APPENDIX 4

Response times for individual fish in the behavior experiment.

HOURS AFTER FINAL STRESS	RESPONSE TIME TO SUDDEN LIGHT (MINUTES) NO STRESS	1X STRESS	2X STRESS	3X STRESS
0	0.02	0.13	0.02	2.75
	0.02	0.20	0.37	3.13
	0.02	0.23	0.70	4.27
	0.03	0.45	1.22	4.37
	0.03	1.47	1.68	4.77
	0.05	1.57	3.88	15.97
	0.05	4.30	4.67	16.07
	0.07	18.88	5.90	18.33
	0.08	18.95	14.38	18.85
	0.10	20.68	14.95	>30.00
	0.12	30.20	19.08	>30.00
	0.12		20.62	
	0.18		23.05	
	0.23		>30.00	
			>30.00	
1		0.12	0.15	0.07
		0.45	0.30	0.12
		0.55	0.32	0.35
		0.67	0.38	0.40
		0.70	0.50	0.70
		0.75	0.57	0.70
		0.80	3.40	0.88
		0.85	5.77	1.68
		0.95	8.03	3.45
		5.85	8.27	4.02
			23.22	4.28
			>30.00	4.92
				6.97
				8.92
				9.07
				24.97
				>30.00
3		0.02	0.03	0.02
		0.05	0.03	0.03
		0.05	0.10	0.03
		0.08	0.12	0.07
		0.10	1.70	0.07
		0.10	2.13	0.28
		0.23	4.08	0.72
		0.27	6.78	2.80
		0.35	13.23	3.12
		0.80	19.98	3.17
		0.87	>30.00	3.77
		1.50	>30.00	4.03
		3.05		5.72
		3.32		7.73
		5.90		23.08
		6.55		
		12.13		

RESPONSE TIME TO SUDDEN LIGHT (MINUTES)

HOURS AFTER FINAL STRESS	NO STRESS	1X STRESS	2X STRESS	3X STRESS
6		0.05	0.03	0.02
		0.05	0.03	0.07
		0.08	0.05	0.12
		0.12	0.07	0.18
		0.13	0.23	0.27
		0.25	0.25	0.28
		0.25	0.38	0.32
		0.62	0.38	0.38
		0.68	0.60	0.38
		1.17	0.80	0.95
		1.40	23.77	1.73
		1.45		3.28
		1.68		8.47
		2.18		>30.00
24		0.02	0.63	0.02
		0.03	0.65	0.02
		0.03	0.75	0.12
		0.05	0.82	0.17
		0.05	1.05	0.45
		0.05	1.20	0.82
		0.05	1.28	0.90
		0.07	1.42	1.00
		0.07	3.08	1.00
		0.07	7.33	1.90
		0.52		2.17
		0.57		3.77
		0.58		4.30
		0.60		7.82
		0.63		12.48
		1.00		>30.00
		1.03		
		2.27		

RESPONSE TIMES OF FISH TESTED INDIVIDUALLY-NO STRESS

0.03
0.08
0.27
0.64
0.86
3.13
3.40
5.52
6.11
6.23