

## **STRESS: PAIN AT A SAFE DISTANCE?**

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### **Problem**

Evolution has fixed the neuro-endocrine stress response in fishes; however, the adaptive nature of stress is puzzling, given its seemingly mal-adaptive effects in the near term--e.g. reduced disease resistance, reduced growth, and reduced survival.

We suggest that it may be useful to categorize environmental stressors into two general groups: (1) environmental factors that trigger the stress response directly, by physically changing an organism's internal environment (e.g. temperature, oxygen, salinity) and (2) those that initiate the stress response through the neurological senses (e.g. predators, competitors, absence of cover, the unknown). The adaptive nature in the latter case is poorly understood, especially considering that non-dangerous stimuli may elicit the stress response (e.g. handling, feeding).

### **The Model**

We have developed a conceptual framework for understanding the adaptive nature of stress in response to group-2 stressors. We expect that stress evolved in part as an adaptive neuro-endocrine response (to group 2 stimuli) that causes an organism to move away from the stressor. The advantage of such a system is

that the organism can avoid the risk of painful injury and mortality while learning not to waste energy in fleeing from non-dangerous stimuli.

Our model predicts that organisms generally behave to maximize available power (scope for activity, Figure 1a) and accordingly tend to select environments in proportion to available power (Kelsch, 1996). [Scope for activity is the difference between the maintenance power (standard metabolic rate) and maximum aerobic power that an organism can generate (active metabolic rate) under any set of environmental conditions (Fry, 1947).] We also predict that the stress response results in a temporary decrease in scope for activity in the presence of a stressor (Figure 1b), perhaps by shunting power to emergency (fight or flight) systems. This would result in a tendency for organisms to avoid stressors while maximizing scope for activity over the short-term (and power for functions such as locomotion, growth, reproduction, and survival).

Considering the first and second laws of thermodynamics, it makes sense that natural selection would favor phenotypes with greater available power. In sum, chemical and physical reactions naturally go from higher to lower energy (increasing entropy, second law) and the free energy (energy available for doing work) necessary for organisms to counter this effect is limited (first law). Phenotypes with greater available power would be better able to reverse their own entropy and would more likely be favored by natural selection.

Priede (1985) provided a rationale for natural selection favoring organisms with greater scope for activity. Such phenotypes would have surplus power that could be used for activities that enhance fitness such as survival and reproduction. Priede also predicted that organisms with greater scope would experience a reduced probability of mortality by being better able to withstand environmental challenges that result in loss of available power.

An important feature of our model is that the stress response may allow biotic factors such as predators and competitors, or vague abiotic factors such as lack of cover, to affect available power and accordingly behavior, performance, and fitness. In the case of a benign stressor (novel and non-dangerous) we predict that the level of stress would decrease with learning and that organisms would correspondingly change distribution through time (Figure 1c).

We test the model by comparing these qualitative predictions with observations from the literature.

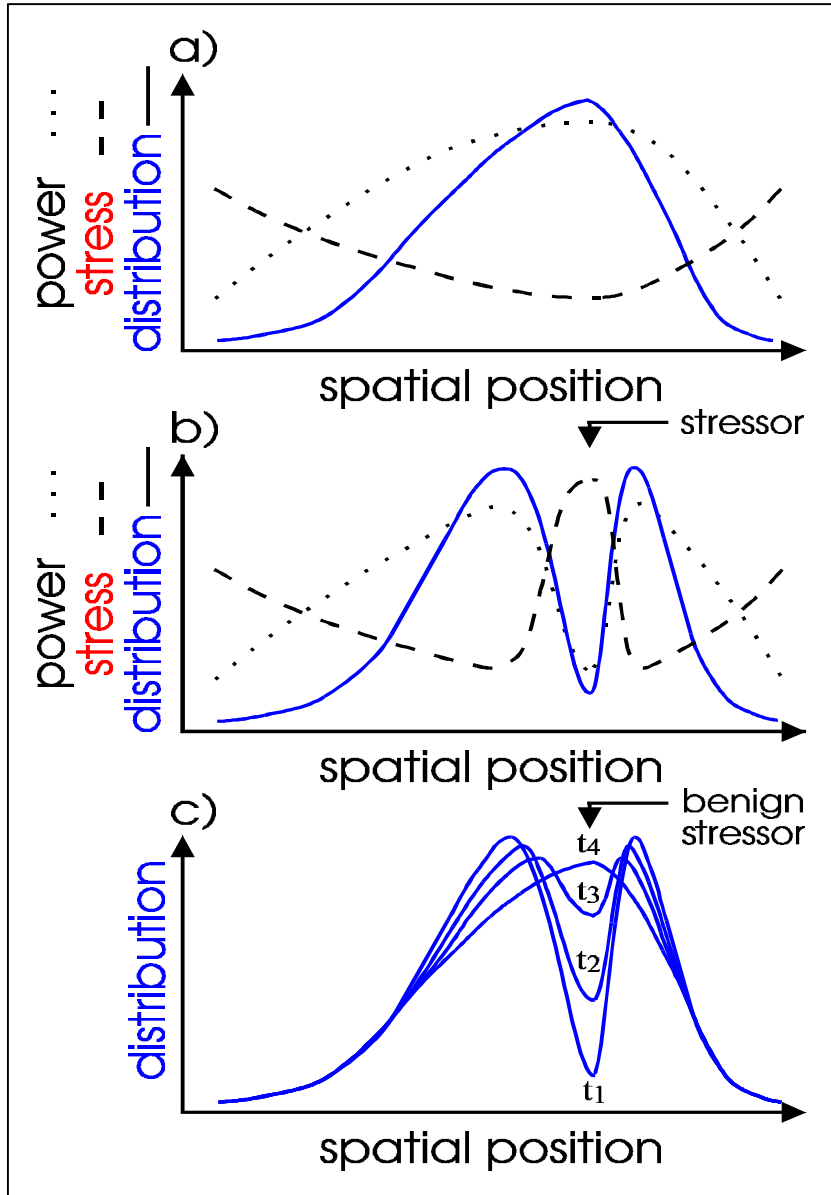


Figure 1. Hypothetical relationship (a) of available power and performance (powerin), tensity of stress response (stress), and frequency distribution and preference (distribution) in response to an abiotic environment with an optimum at the spatial position showing a mode of available power. Panel b shows the effect of a biotic stressor such as a predator or competitor at the environmental optimum and panel c shows how frequency distributions are predicted to change through time ( $t_1$  through  $t_4$ ) after exposure to a novel, benign stressor. The changes in distribution through time are in response to learning.

According to our model, stress is similar to pain--a better-understood neuro-endocrine response. Rather than having to wait for physical damage from a dangerous stimulus, pain results in a rapid retreat from the stimulus to avoid damage and future deleterious effects of that damage. The primary difference between pain and stress is that while pain is limited to stimuli that contact the body of the organism, stress can result in the avoidance of distant stimuli that are detected through the senses. Both allow individuals to avoid future risk of mortality by altering behavior in the present.

### *Conclusions*

We present and support a model that explains the adaptive nature of the neuro-endocrine stress response. We predict that fishes (and probably other organisms) behave to maximize available power—power necessary for activity, survival, growth and reproduction. We also predict that the stress response results in a decrease in available power in the vicinity of the stressor (perhaps by shunting power to emergency systems) and, therefore, avoidance of the stressor. This model makes sense of some of the seemingly mal-adaptive aspects of the stress response including reduced growth and survival.

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**HEAT SHOCK PROTEINS AND  
PHYSIOLOGICAL STRESS IN FISH**

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**Introduction**

Heat shock proteins (HSP) are expressed constitutively, and in response to a wide range of stressors in plant and animal cells. Some of their numerous intracellular functions include the facilitation of proper protein folding, repair of damaged protein, protein translocation, and modulating steroid-receptor interaction. Most of these functions have been elucidated in mammalian systems, and not much is known about the physiological significance of this cellular stress response in fishes.

This brief overview will summarize results from our lab on this topic, in the context of emerging data in the literature. HSP levels increase in concentration in response to a wide range of biotic (eg. bacterial kidney disease) and abiotic (eg. temperature, toxicants) stressors. Thus, HSPs have been called stress proteins in the literature. These responses have been shown in cell lines, primary cell cultures, and validated in whole animal studies. However this cellular stress response is not entirely a general response, but it is tissue- and HSP-specific. Therefore, given a stressor, certain HSP levels will be increased in select tissues. HSP70 levels increase in most tissues, in response to a wide range of stressors, and regulation is likely at the transcription level. The cellular stress response can be long-lasting. For example, after a 1h heat shock in coho salmon, HSP70 levels were elevated up to 3 weeks after that stress. It is likely that this cellular stress response is not closely linked to the familiar endocrine

(catecholamine & cortisol) stress response that has been well characterised for fishes. Handling stress does not cause HSP70 increase in liver tissue. Cortisol attenuates the increase in HSP70 in salmonid liver cells caused by toxicant exposure. Physiological and pharmacological levels of adrenaline cause increases in HSP70 in trout liver cells; this response is blocked by propranolol. This HSP response to stressors is the basis for the possibility of applying it as a biomarker for environmental quality in aquatic organisms. However, much more research is needed to better describe the relationship between this cellular stress response and physiological stress in the whole organism.



**THE HEAT-SHOCK PROTEIN RESPONSE  
IN ATLANTIC SALMON (*SALMO SALAR*)  
BRANCHIAL TISSUE**

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**Introduction**

Heat shock proteins (hsps) are a family of highly conserved intracellular proteins which, when induced by a stressor such as heat shock, act to confer protection against the cytotoxic consequences of protein denaturation (Lindquist and Craig 1988). The heat shock protein (hsp) response of juvenile Atlantic salmon was investigated in isolated branchial tissue exposed to heat shock or sodium arsenite (NaAsO<sub>2</sub>). The description of hsps in this report will serve as a basis for additional studies of the role of hsps in the adaptation of Atlantic salmon to the marine environment.

**Experimental Techniques**

Proteins were metabolically radiolabeled by incubating tissue in media containing <sup>35</sup>S-methionine, after which proteins were size-fractionated by 8% SDS-PAGE and transferred to a nylon membrane for western blotting or visualized by fluorography. Total RNA from heat shocked branchial tissue was size-fractionated on 1.25% MOPS-formaldehyde gels, transferred to nylon a membrane, and probed with <sup>32</sup>P-labeled cDNAs. Protein bands were quantified by scanning laser densitometry to yield an integrated optical density (IOD), and

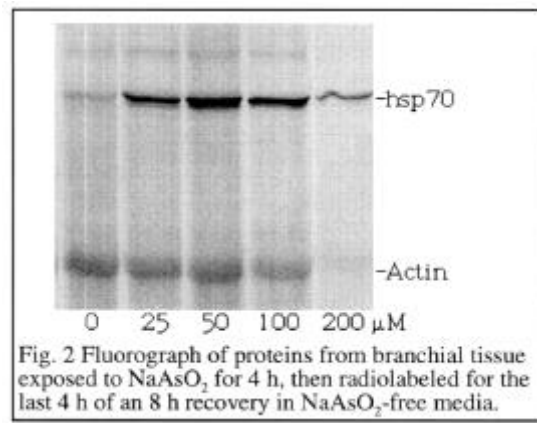
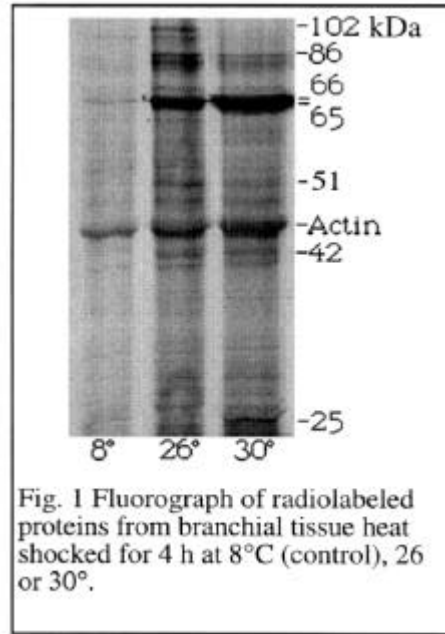
bands were normalized to the actin band in the same lane.

### **Heat shock**

Exposure to 26°C heat shock for 4 h with radiolabeling induced proteins from the major hsp families: hsps 100, 90, 70, 60 and small hsps (Fig. 1). This array of proteins is similar to those observed in other teleosts (Dyer et al. 1991, White et al. 1994, Currie and Tufts 1997). Western blotting with an hsc70/hsp70 antibody (StressGen Biotechnologies, #820) showed that two of the most prominent proteins induced by heat shock (MW<sub>65</sub> and 66 kDa) were antigenic homologues of mammalian hsp72/73. Further evidence that hsp70 was induced comes from northern blots probed with Atlantic salmon and human hsp70 cDNAs, which show a 2.6 kbp transcript upregulated by heat shock. To determine the kinetics of hsp70 induction, tissue samples were radiolabeled for successive 1 h intervals of a 4 h incubation at 26°. Hsp70 was induced in the first hour of heat shock, and synthesis was maximal by 3 h at about 3.5 times the control level. In contrast, branchial tissue of the eurythermal teleost *Fundulus heterclitus* exhibited a 15-fold increase in hsp70 after 1 h of heat shock (Dyer et al. 1991).

### **Sodium arsenite exposure**

Branchial tissue was also exposed to NaAsO<sub>2</sub> to provide a comparison with heat shock, and to see if stressor-specific proteins would be induced. Tissue was incubated in media containing NaAsO<sub>2</sub> for 4 h, and either metabolically radiolabeled during that incubation, or radiolabeled during a subsequent incubation in media without NaAsO<sub>2</sub>. In contrast to heat shock, only hsp70 was strongly induced by NaAsO<sub>2</sub>, and only during recovery from exposure (Fig. 4). This response is also contrary to that observed in fathead minnows (Dyer et al. 1993) and rainbow trout cells (Kothary et al. 1984), which synthesized an array of inducible proteins in response to NaAsO<sub>2</sub> exposure.



## Summary

This is the first description of the heat shock protein response in isolated salmonid tissues, and the first description of hsps in Atlantic salmon, a valuable aquaculture species worldwide. Atlantic salmon branchial tissue have a hsp response similar to other teleosts in the number and MW of proteins induced. However, two notable differences are the lower quantity of hsp70 synthesized by the stenothermal salmon as compared to the eurythermal *F. heteroclitus*, and the induction of only hsp70 by NaAsO<sub>2</sub> exposure. Additional studies are planned to determine if hsps are induced in response to osmotic shock.

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## RESPONSES OF PITUITARY HORMONES TO STRESS IN THE GILTHEAD SEA BREAM *SPARUS AURATA*

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### Introduction

The stress response in fish involves a number of neuro-endocrine activities, and specifically the activation of the hypothalamus-pituitary-interrenal (HPI) axis. This activation leads to secondary responses including alterations in osmoregulation, immunocompetence and energetics (Sunyer et al., 1995; Wendelaar-Bonga, 1997). Few reports on plasma pituitary hormones are available in fish due to the lack of specific and sensitive assays (Balm et al., 1994). This work shows for the first time the levels of four pituitary hormones ACTH, GH,  $\alpha$ -MSH and  $\beta$ -Endorphin in the gilthead sea bream after stress and their interaction with interrenal tissue activity. The sea bream *Sparus aurata* is mainly produced by countries around the Mediterranean sea and is the most important fish species in the marine aquaculture industry in south-Europe.

### Methods

Sexually immature sea bream of  $150.7 \pm 20.3$ g were obtained from a local fish farm and acclimated for 2 months (16°C) to laboratory conditions in recirculating seawater tanks. *Handling and confinement.* Groups of 8 fish were subjected to confinement stress by transfer to small net cages of 5L water volume. After 1h, 4h, 24h and 72h, a single net cage was removed and fish were anaesthetised and sampled. Two control fish groups (0h and 7 days) were used.

*Crowding.* After acclimation, crowding was reached by reducing water volume to 30-kg/m<sup>3</sup> density. Samples were taken after 1, 3, 7 and 23 days. Controls (10 kg/m<sup>3</sup>) were sampled simultaneously.

*Sampling and analytical.* After anaesthesia with phenoxyethanol, blood samples were obtained by caudal puncture and the head kidney and the pituitary gland were excised. After plasma separation, GH and pituitary POMC-derived peptides (ACTH,  $\alpha$ -MSH and  $\beta$ -Endorphin) and cortisol were analysed by specific Radioimmunoassays. *In vitro* release of cortisol was determined by superfusion of the excised head kidney with appropriated buffer and stimulation with ACTH.

## **Results**

### *Handling and confinement*

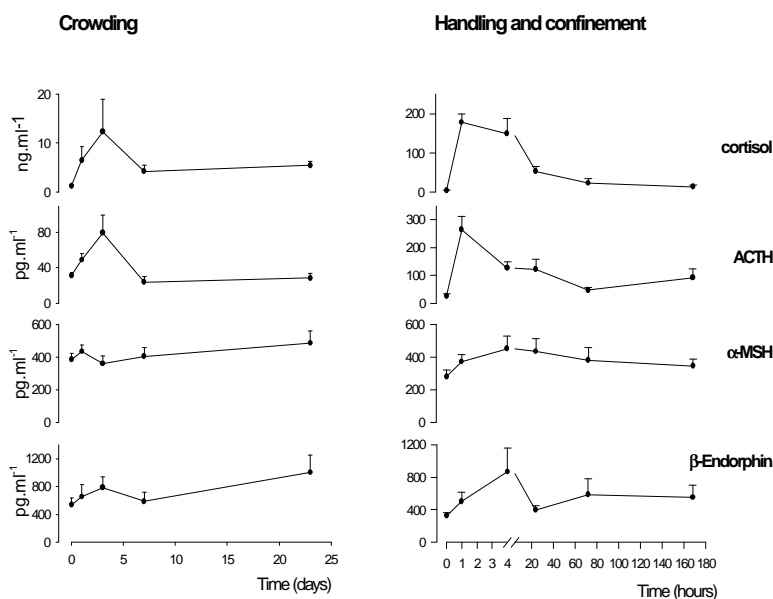
Plasma ACTH and cortisol rapidly increased after stress and a peak appeared after 1 hour. From that point, levels started to decrease and after 4 hours ACTH was reduced to half of the stressed levels whereas the reduction in cortisol was not significant. After 24h cortisol decreased significantly and after 72h plasma hormones approached to control levels. A significant increase was recorded in  $\alpha$ -MSH and  $\beta$ -Endorphin but the peak was observed at 4 hours in both peptides. GH showed a drastic reduction after 1 hours followed by a subsequent increase after 24 hours. Later samples showed recovery for  $\alpha$ -MSH along 7 days and quick recovery for  $\beta$ -Endorphin and GH after 24h. (See figure 1a). Regarding pituitary peptides in the gland, the results show important decrease after stress, but the recovery of the peptides in the gland is slow and irregular. ACTH recovery is observed after 7 days and levels of  $\alpha$ -MSH and  $\beta$ -Endorphin do not show complete recover by the end of the experiment.

### *Crowding.*

Plasma cortisol increased after stress and the levels were significantly higher after 1 and 3 days. Later samples (7, 23 days) showed recovery. Plasma ACTH showed coincident at the same time-points (Figure 1b). This pattern is verified through the hormone content by head kidney and pituitary glands. Regarding plasma  $\alpha$ -MSH and  $\beta$ -Endorphin, no significant changes were detected (Figure 1b), although a tendency to increased plasma levels and decreased gland levels is shown at 25 days. GH shows decreased levels compared to controls in most samples. The *in vitro* release of cortisol by ACTH administration in crowded fish showed a heavy decrease of ACTH stimulatory power.

## Discussion

The present studies demonstrate that in sea bream, stress induce the release of POMC-derived peptides in the pituitary and activation of HPI axis, as has been shown in trout and tilapia (Balm et al., 1995a,b). After acute stress, there is a release of hormones from pituitary and interrenal to the blood stream and a further recovery of plasma levels in 24 hours whereas gland levels are recovered after a longer period, thus indicating hormone clearance in plasma. This is coincident with a decrease of GH as described in other fish. Pituitary peptides show a similar trend but slower dynamics. After chronic crowding stress there is also an activation of the HPI axis. The recovery of plasma and gland hormone levels is taking place after several days. Crowded fish show an important decrease of the sensitivity of interrenal cells to ACTH but an increase of the basal release level, which may indicate a process of dissociation between the two hormones when the stressor is chronic. As the levels of  $\alpha$ -MSH and  $\beta$ -Endorphin and GH did not recover after 24 days they may be the source of further physiological imbalances.



### **Acknowledgements**

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**PHYSIOLOGICAL STRESS RESPONSES IN JUVENILE  
CHONDROSTEAN FISHES — DIFFERENCES FROM TELEOSTS**

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**EXTENDED ABSTRACT ONLY DO NOT CITE**

Physiological stress resulting from aquaculture-related practices is well documented in teleostean fishes but not in chondrosteans (Barton and Iwama, 1991; Iwama et al., 1997). Hatchery-reared juveniles of two chondrostean species, the paddlefish (*Polyodon spathula*) and hybrid pallid × shovelnose sturgeon (*Scaphirhynchus albus* × *S. platyrhynchus*) were subjected to acute physical disturbances associated with culture to evaluate their physiological responses relative to other fishes.

When paddlefish were given a 30-s aerial emersion handling stressor, plasma cortisol increased from  $2.2 \pm 0.6$  (mean  $\pm$  SE,  $n = 10-12$ ) to  $11 \pm 1.8$  ng/mL in 1 h but returned to the prestress level by 3 h. Addition of 0.5% NaCl to the recovery tanks had no effect on plasma cortisol changes. Plasma lactate rose from  $26 \pm 2.6$  to  $52 \pm 4.5$  mg/dL within 3 h. Plasma cortisol in hybrid sturgeon subjected to the same stressor rose from  $1.4 \pm 0.3$  to  $2.8 \pm 0.5$  ng/mL at 1 h but changes in plasma lactate concentrations from prestress values were negligible. Plasma cortisol increased to  $14 \pm 4.8$  ng/mL and plasma lactate rose to  $67 \pm 3.7$

mg/dL after paddlefish were continuously chased in their home tanks for 1 h; plasma lactate remained elevated for 5 h.

In paddlefish severely confined (all fish touching each other) for 6 h in small cages with intermittent handling, plasma cortisol rose from  $6.2 \pm 1.6$  ng/mL to its peak level of  $74 \pm 6.3$  ng/mL by 2 h but approached the prestress value after 24 h (18 h of recovery). Plasma lactate levels in confined paddlefish fish increased from  $27 \pm 2.7$  to  $73 \pm 6.9$  mg/dL by 3 h but returned to prestress levels by 24 h. Plasma cortisol reached a peak level of  $14 \pm 1.4$  from  $2.0 \pm 0.3$  ng/mL by 3 h in hybrid sturgeon when severely confined in the same manner. Plasma lactate levels in hybrid sturgeon rose from  $10 \pm 1.0$  to  $19 \pm 1.4$  mg/dL during the 6-h confinement period. By 24 h, these physiological features returned to prestress levels. In both species, plasma cortisol reached a plateau level during the 6-h confinement suggesting that these fish had reached their maximum interrenal secretory capacity. Changes in plasma glucose and chloride and hematocrit from all experimental treatments were negligible in both paddlefish and hybrid sturgeon.

The results suggest that these chondrosteian fishes exhibit physiological stress responses to physical disturbances much lower than those previously documented for many teleostean fishes, which can be up to one or two orders of magnitude higher (e.g., Figure 1). Moreover, the stress responses of hybrid sturgeon were appreciably lower than those of the paddlefish. Reasons for the differences in chondrosteians from teleosts are presently unknown but may relate to their evolutionary history.

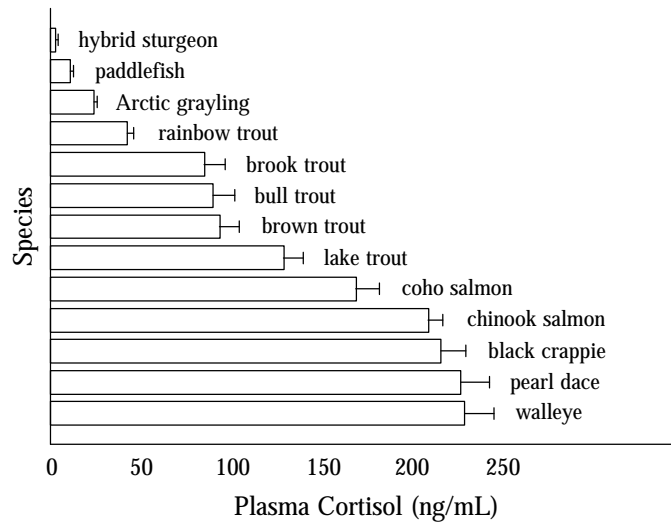


Figure 1. Examples of peak post-stress levels of plasma cortisol (mean + SE) in chondrosteian (paddlefish and hybrid pallid  $\times$  shovelnose sturgeon) and teleostean fishes measured at 1 h after being subjected to the same 30-s aerial emersion handling stressor in a dip-net (from unpublished and published data; contact author for references).

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**ACUTE STRESS RESPONSE IN  
TRIPLOID BROOK TROUT (*SALVELINUS FONTINALIS*)**

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**Introduction**

Triploid fish differ from diploids in three fundamental ways: they are more heterozygous, they have larger (but fewer) cells in most tissues and organs, and they are sterile (Benfey, 1998). Their sterility makes triploids of interest for aquaculture and fisheries management in instances where gametogenesis and/or spawning need to be prevented. Although it is widely accepted that triploids do not perform as well as conspecific diploids in a variety of natural and artificial conditions, little data are available to support this (Benfey, 1998). The purpose of the research summarized in this paper has been to examine the physiological responses of triploids to a variety of acute stressors, using a model salmonid species: the brook trout (*Salvelinus fontinalis*). In all cases, triploids were compared to diploids originating from the same egg lots (families) and reared under identical conditions. Triploids were produced by hydrostatic pressure treatment (5 minutes at 9500 psi) applied to eggs 200°C-minutes after fertilization, with triploidy confirmed by flow-cytometric measurement of erythrocytic DNA content.

**Acute Handling Stress (Biron and Benfey, 1994)**

Blood samples were collected prior to, during, and for up to 3 hours following acute handling stress, which consisted of capturing the fish, confining them in a

bucket, and returning them to their tank 5 minutes after first disturbance. Both diploids and triploids exhibited a rapid increase in plasma cortisol (within 5 minutes of first disturbance) followed by a sustained elevation (80 to 100 ng/ml) for 1 hour, and then a decline to pre-stress levels (10 to 20 ng/ml) after an additional 2 hours. Hematocrits were variable, but showed a rapid increase (to 40 to 43%) within 5 minutes of disturbance followed by a gradual decline to pre-stress levels (36 to 38%) by the end of 3 hours. Plasma glucose levels remained low (6.5 to 7.5 mmol/l) for the first hour after disturbance, and then gradually increased to 7.5 to 8.5 mmol/l by the end of the experiment. There were no statistically-significant differences between triploids and diploids in any of these responses.

#### **Acute Salinity Tolerance (MacLeod, 1998)**

Freshwater-adapted diploids and triploids were transferred directly to waters of various salinities (5, 10, 15, 20, 25, 30, and 35 ppt), where they were held for 24 hours prior to collection of blood samples for the measurement of plasma sodium and potassium levels. There was a trend of higher plasma ion levels at 25 to 35 ppt compared to freshwater controls, but this was only statistically-significant for sodium. There were again no statistically-significant differences between triploids and diploids.

#### **Critical Thermal Maxima (Benfey et al., 1997)**

Two age classes of diploids and triploids were exposed to two rates of temperature increase (2 or 15°C/hour) to determine the temperature at which they lost equilibrium (i.e., the critical thermal maximum), which is related to the thermal upper lethal limit. Younger fish and those fish exposed to the faster rate of temperature increase had higher critical thermal maxima than older fish and those fish exposed to the slower rate of temperature increase, with no statistically-significant differences between triploids and diploids.

#### **Critical Swimming Velocity (Stillwell and Benfey, 1997)**

Swimming tests with diploids and triploids were conducted using a Blazka respirometer. After a 1-hour habituation/training period with fish swimming at 0.5 body lengths (BL)/second, water speed was increased by 0.5 BL/second

every 30 minutes until the fish were fatigued. No statistically-significant differences were found between the critical swimming speeds of diploids ( $2.31 \pm 0.33$  and  $1.86 \pm 0.21$  BL/second for two strains of fish) and triploids ( $2.16 \pm 0.29$  and  $1.73 \pm 0.27$  BL/second for these same two strains).

### **Conclusions**

Results from the experiments summarized in this paper indicate that triploids are identical to diploids in their responses to, and tolerances of, a variety of acute stressors. Fundamental biological differences between triploids and diploids thus are not reflected in these sorts of tests. Further research should be directed towards examining the responses of triploids to chronic stressors, which may give a different perspective to understanding the tolerances of these fish to natural and artificial changes in their environment.

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**SEX STEROID REGULATION OF CORTISOL  
IN RAINBOW TROUT**

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**Introduction**

It is becoming apparent that the regulation of the teleost corticosteroid stress response is much more complex than formerly thought, and several non-classical factors are now suspected of playing key roles in determining the susceptibility of fish to the harmful effects of stress-induced hypercortisolism. Sex steroids, for example, can have a marked impact on interrenal activity in salmonid fishes (Pottinger et al. 1995, 1996; Young et al. 1996; Barry et al. 1997). Androgens reduce, and estrogens enhance, the corticosteroid stress response of immature rainbow trout (Pottinger et al. 1995, 1996; Young et al. 1996). Moreover, recent evidence from our laboratory suggests that specific cortisol target tissues in fish may be protected from hypercortisolism by their ability to enzymatically inactivate cortisol before it binds to cellular receptors.

**Methods**

The purpose of our investigation was to study the effects of sex steroids in modulating the corticosteroid stress responses of rainbow trout (*Oncorhynchus mykiss*). The specific objectives were to: (1) determine the effects of 17,20-P on cortisol production *in vitro* and *in vivo*, (2) document the tissue distribution of cortisol metabolizing activity, and evaluate the effects of sex steroids on cortisol

metabolism in specific tissues; and (3) characterize the effects of sex steroids on free (i.e., non-protein bound) cortisol levels in the serum of rainbow trout.

## Results

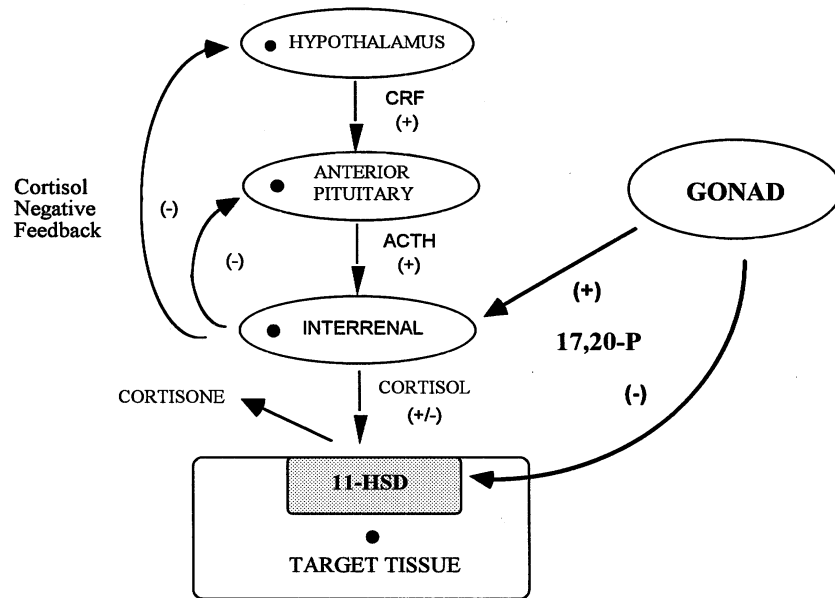
Physiological levels of  $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (17,20-P) stimulated time- and dose-dependent increases in cortisol production by rainbow trout interrenal tissue *in vitro* (Barry et al., 1997). Radiotracer studies indicated that 17,20-P is a substrate for cortisol biosynthesis (Barry et al., 1997). Rainbow trout administered 17,20-P *in vivo* had significantly higher cortisol levels than control fish.

The tissue distribution of cortisol metabolizing activity was determined in rainbow trout by measuring the disappearance of [ $^3$ H]cortisol from *in vitro* cultures. High cortisol metabolic activity was detected in the pyloric caeca, head kidney, skin, kidney, liver and brain. Liver had low activity. Sexually mature fish had lower overall tissue-specific cortisol metabolism than immature fish. The effects of sex steroids on cortisol metabolism was evaluated by incubating pyloric caeca in the presence of various levels of estradiol- $17\beta$  ( $E_2$ ) 11-ketotestosterone (11-KT), testosterone (T), and 17,20-P. Estradiol- $17\beta$ , 11-KT, and T had no effect on cortisol metabolism by the pyloric caeca of rainbow trout. Physiological concentrations of 17,20-P (30 and/or 300 ng/ml), however, significantly inhibited total cortisol metabolism.

Sexually immature rainbow trout implanted with T or 11-KT had reduced total cortisol stress responses compared to control fish. Free cortisol levels, however, were not different between control and androgen-implanted fish. Immature rainbow trout implanted with  $E_2$  had higher total cortisol stress responses than control fish. Free cortisol levels, however, were not different between control and estrogen-implanted fish.

## Conclusions

The results suggest the following: (1) the pre-spawning rise in 17,20-P may be at least partially responsible for the hypercortisolism associated with spawning



**Figure 1.** Proposed model of the regulation of the cortisol production and metabolism by  $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (17,20-P). Corticotropin-releasing factor (CRF) stimulates the secretion of adrenocorticotropin hormone (ACTH), which in turn stimulates the secretion of cortisol. Cortisol acts on numerous target tissues to induce an array of physiological responses. Our data suggest that 17,20-P may (1) stimulate corticosteroidogenesis (perhaps even when CRF and ACTH are inhibited by negative feedback), and (2) inhibit the activity of cortisol metabolizing enzymes such as  $11\beta$ -hydroxysteroid dehydrogenase (11-HSD) in specific target tissues. • cortisol receptors; (-) negative control/feedback; (+) stimulatory control.

in salmonid fishes; (2) specific tissues in rainbow trout may be protected from hypercortisolism by the activity of cortisol-metabolizing enzymes; (3) this protection may be lost at spawning when levels of 17,20-P are elevated; (4) sex

steroids can alter the levels of free cortisol in rainbow trout, probably by changing the concentrations of serum cortisol binding proteins.

Overall, similar results were obtained when parallel experiments were conducted with coho salmon (*Oncorhynchus kisutch*). Together the data are compatible with the hypothesis that the prespawning rise in 17,20-P could regulate the death of semelparous Pacific salmon. It is widely accepted that cortisol hypersecretion from the interrenal tissue mediates programmed death by causing tissue degeneration, suppressing the immune system, and disrupting homeostasis (Dickhoff 1989; Stein-Behrens and Sapolsky 1992). The mechanism causing this hypercortisolism, however, is not known. We propose the following model (Fig. 1). Under normal physiological conditions, cortisol feeds back at the brain and pituitary to inhibit CRF, ACTH, and further cortisol production. Normally, peripheral target sites are protected from hypercortisolism by cortisol metabolizing enzymes, even in the face of highly elevated stress-induced cortisol levels. During spawning, however, 17,20-P inhibits cortisol metabolizing enzymes in these tissues while simultaneously stimulating corticosteroidogenesis. Thus, cortisol receptors in specific target tissues are exposed to high concentrations of cortisol only when 17,20-P levels are elevated.

### **Acknowledgments**

We thank Lynne Procarione, Thomas Kuczynski, and James Held of the U.W. Aquaculture Program for their help and assistance. This research was supported in part by the University of Wisconsin-Madison College of Agricultural and Life Sciences and School of Natural Resources; the Wisconsin Department of Natural Resources; the University of Wisconsin Sea Grant College Program, National Oceanic and Atmospheric Administration, US Department of Commerce, and the State of Wisconsin (Project No. R/AQ-31). Eric Vandeloise was supported by the Belgian National Funds for Scientific Research (FNRS) and the Funds for Research in Industry and Agriculture (FRIA).

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**EFFECTS OF RAM-AIR VENTILATION  
DURING TRANSPORTATION  
ON WATER QUALITY AND PHYSIOLOGY  
OF WALLEYE FINGERLINGS**

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**Abstract**

The Iowa Department of Natural Resources (IDNR) maintains walleye (*Stizostedion vitreum*) populations in most Iowa lakes and reservoirs by stocking fry or hatchery-raised fingerlings. Some fingerlings must be transported 6-7 hours from hatchery to stocking sites. IDNR personnel estimate that post-stocking survival of fingerling walleye transported for 6-7 h is substantially lower than survival of walleye transported for 1 h or less. Our working hypothesis is that dissolved carbon dioxide (CO<sub>2</sub>) accumulates in tank water as a result of fish respiration and a lack of tank ventilation. After being transported 6 h, fish seemed dazed, perhaps sedated from CO<sub>2</sub>. An anesthetic effect may occur when CO<sub>2</sub> concentrations reach 100 mg/L (Wedemeyer 1996). Also, supersaturated oxygen (O<sub>2</sub>) concentrations were common, which can cause further increases in blood CO<sub>2</sub> levels by decreasing ventilation rate. If fingerlings are sedated upon stocking, they are at increased risk of predation.

We installed ram-air ventilators (RAV) in two hauling tanks and measured their effect on water quality during a 6-h haul. Two unvented tanks on the same truck

served as controls. Both tank types were equipped with mechanical agitators and supplied with pure O<sub>2</sub>. Water quality sensors were installed in each of the four tanks for continuous measurement of temperature, pH, and O<sub>2</sub>. Carbon dioxide concentrations were calculated from total alkalinity and pH (APHA 1995). During the haul, measurements were made automatically every 2-3 seconds and means were logged every 5 minutes. Mean O<sub>2</sub>, temperature, pH, and CO<sub>2</sub> for each treatment were calculated from the 5-minute mean values logged during the haul.

Mean CO<sub>2</sub> and O<sub>2</sub> concentrations in tanks equipped with RAV (35.25 ± 3.04 mg/L CO<sub>2</sub> and 8.42 ± 0.18 mg/L O<sub>2</sub>) were significantly lower than concentrations of CO<sub>2</sub> (104.33 ± 8.15 mg/L) and O<sub>2</sub> (10.31 ± 0.03 mg/L) in the control tanks (Figure 1). Mean pH of water in RAV-equipped tanks (6.82 ± 0.03) was significantly higher than pH in control tanks (6.47 ± 0.03) (Figure 1). Temperature increased about 1°C in both treatments during the haul, but mean water temperatures were similar in control (19.6°C) and RAV-equipped tanks (19.7°C). Ammonia and alkalinity were not significantly different between hauling-tank treatments; maximum un-ionized ammonia-nitrogen concentration was 0.0125 mg/L. Alkalinity was 100 mg/L CaCO<sub>3</sub> before and after the haul.

To determine if RAV reduced hauling stress experienced by walleye fingerlings, blood samples were taken for measurement of physiological stress and blood gases, and assessment of acid-base status. Blood samples were taken from walleye the evening before the haul (baseline), after loading onto the truck (postload), and upon arrival at Spirit Lake Hatchery (posthaul). Fish were also sampled during the 10 days following the haul to determine the length of time required for a return to baseline values. Individual fish were netted from raceways or tanks and immersed in buffered Finquel (250 mg/L). Blood was sampled from caudal vasculature with a heparinized syringe. A 150-μl portion of whole blood was immediately analyzed for pCO<sub>2</sub>, pO<sub>2</sub>, pH, sodium (Na<sup>+</sup>), and potassium (K<sup>+</sup>) using a portable blood-gas analyzer. The remaining blood was centrifuged to separate the plasma, which was frozen for later analysis of cortisol and chloride (Cl<sup>-</sup>).

At the end of the 6-h haul, blood pCO<sub>2</sub> had increased from 11.20 ± 0.35 (baseline) to 19.81 ± 0.75 mmHg and HCO<sub>3</sub><sup>-</sup> had increased from 10.17 ± 0.39 (baseline) to 21.48 ± 0.67 mmol/L (Figure 2). There were no significant differences among sample times (baseline, postload, or after the transportation event) for blood Na<sup>+</sup>, K<sup>+</sup>, pO<sub>2</sub>, pH and plasma Cl<sup>-</sup>. Although postload and posthaul cortisol levels of fish in ventilated tanks were lower than those from



fish in control tanks, differences were only significant at postloading (Figure 2). Postload and posthaul glucose concentrations in fish from RAV tanks were lower than those from fish in control tanks although the differences were not significant (Figure 2). With the exception of cortisol and glucose, which returned to baseline levels by day 4, all blood constituents had returned to baseline levels within 1 d following the haul.

In this study, tanks equipped with RAVs had superior water quality (lower CO<sub>2</sub>, higher pH, and normoxic O<sub>2</sub>) compared with control tanks without RAVs. Fish transported in RAV-equipped tanks had a significantly lower stress response (cortisol only) after loading than that of walleye in control tanks, but the differences were not significant after the haul. Measurement of blood pCO<sub>2</sub> and blood HCO<sub>3</sub><sup>-</sup> support the water quality findings that fish transported in the RAV tanks were exposed to less dissolved CO<sub>2</sub> than fish in the control tanks.

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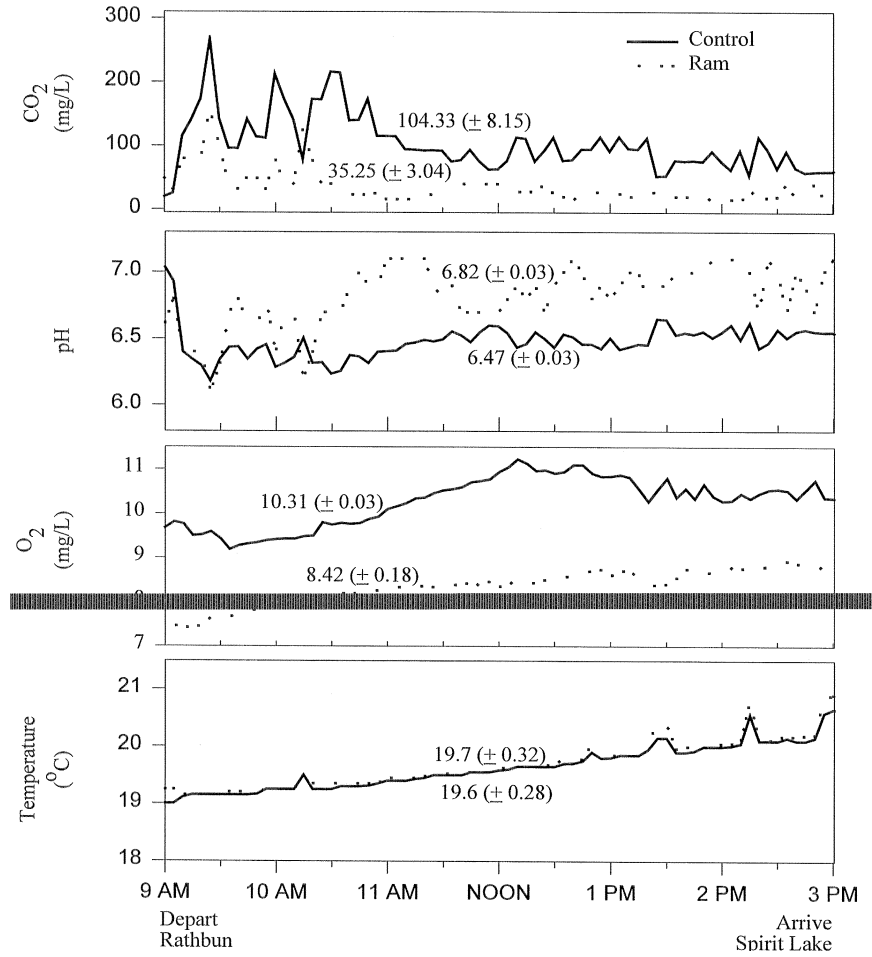


Figure 1. Water quality in hauling tanks during a 6-h transportation event. Measurements were taken every 2-3 seconds, and a mean value was logged every 5 minutes. Mean ( $\pm$  SE) values given on the graph are calculated from logged values.

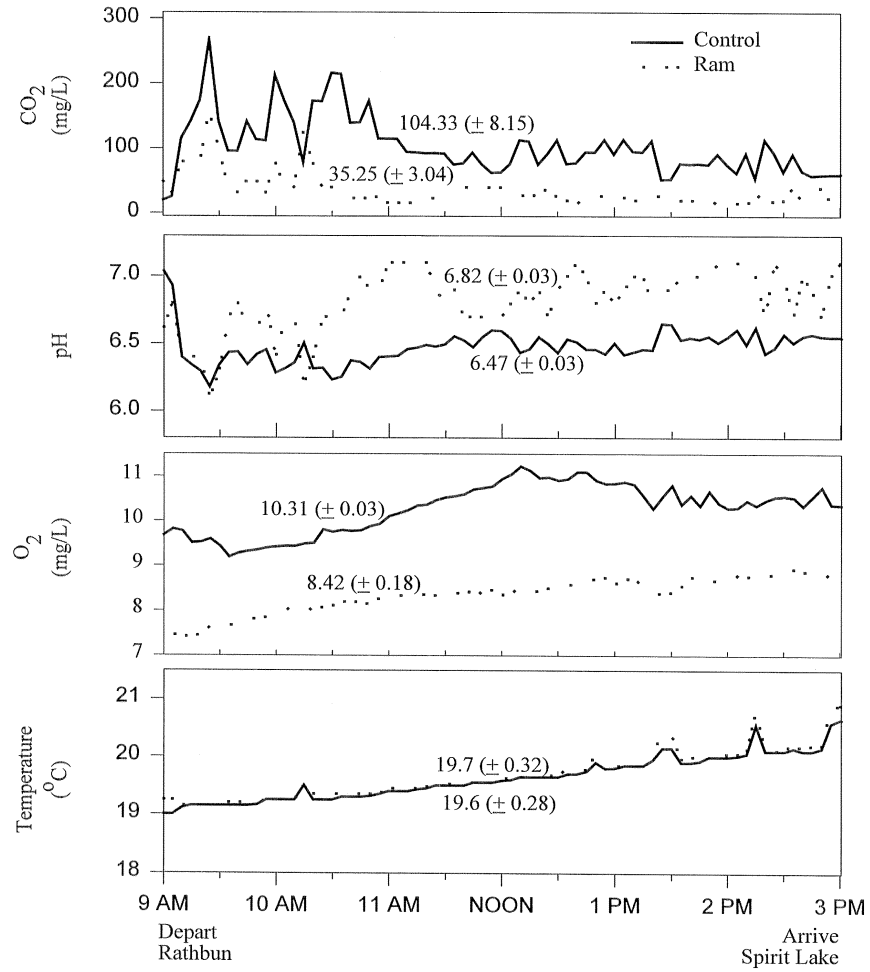


Figure 2. Blood chemistry values (Mean  $\pm$  SE) of walleye fingerlings before (baseline), after loading (postload), and after a 6-h transportation event (posthaul). Superscripted “a” denotes a significant difference from the baseline value. Superscripted “b” denotes a significant difference between ram-air ventilated tanks and control tanks.



**THE EFFECT OF PROLONGED SWIMMING,  
FASTING, AND PREDATOR PRESENCE  
ON ENERGY UTILIZATION AND CHRONIC STRESS  
IN JUVENILE WALLEYE (*STIZOSTEDION VITREUM*)**

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**Introduction**

Environmental and physical stresses can affect many physiological functions e.a. growth, reproduction, osmoregulation, homeostasis and mobilization of energy stores (Pickering, 1993; Wedemeyer et al., 1990). Walleye produced in intensive culture conditions and reared on formulated diets are stocked to many reservoirs and lakes in North America. Upon release YOY walleye need to adjust to altered conditions in order to survive. The quality of cultured walleye in terms of withstanding food deprivation, swimming capacity, and responses to physiological stress caused by natural predators have never been tested.

**Methods**

Juvenile walleye grown on two formulated diets were subjected successively to the same experimental conditions. The two diets fed to walleye prior to the experiments differed in protein source and added lipids (4 versus 10%). Groups of six fish were placed in triplicate rectangular wire cages and submerged randomly in three artificial stream compartments. Three treatments were designed: 1) water current plus predator, 2) water current and no predator, 3). no water current and no predator. Water velocity was maintained at 1.5 body lengths/s throughout the experiments. Six 1 year old muskellunge *Esox*

*masquinongy* were used as potential predators. They were allowed to swim freely outside walleye cages and were fed with YOY walleye. The water temperature was maintained at 14°C.

Fish were exposed to experimental condition for 6 weeks and were deprived of food to simulate poststocking period of fasting. Every two weeks, six fish per each treatment were removed, anaesthetized with MS-222 in less than one minute, blood sampled from the caudal vessel using heparinized syringe, weighed, measured, and frozen in liquid nitrogen for biochemical analysis. Blood plasma was separated by centrifugation at 1500 g for 10 min and stored at -20°C until assay. Total time to obtain all samples per treatment was less than 5 min to avoid stress associated with sampling procedure.

Following sampling, all remaining fish were weighed, measured and returned to the artificial stream. Plasma glucose were measured using commercial Glucose HK kit (Sigma Chemical Comp.). Other parameters such as plasma cortisol (determined by radioimmunoassay, validated for use with walleye), fatty acids in total body lipids (determined gas chromatography), and proximal analysis (proteins, lipids, and ash) of fish (performed following usual laboratory procedures) are in progress to evaluate the impact of swimming and/or presence of the predator on stress and nutrients expenditure in juvenile walleye.

Each parameter was first analyzed by one way ANOVA for changes that might have occurred during the experimental period. Then, each parameter was compared simultaneously among all treatments using two way ANOVA to test for treatment effects.

## **Results**

The weight of the fish grown on diet A (krill meal protein based, 10% lipids added) did not decrease significantly, regardless of the treatment (Fig. 1A). The weight of fish grown on diet B (fish meal based, 4% lipids added) decreased significantly ( $p < 0.05$ ) in all three treatments during the experiment (Fig. 1B). In both experiments, walleye forced to swim, coupled with the presence of potential predator, lost more weight than those subjected only to swimming or resting non-exercised controls. However, no significant differences were found among the three treatments.

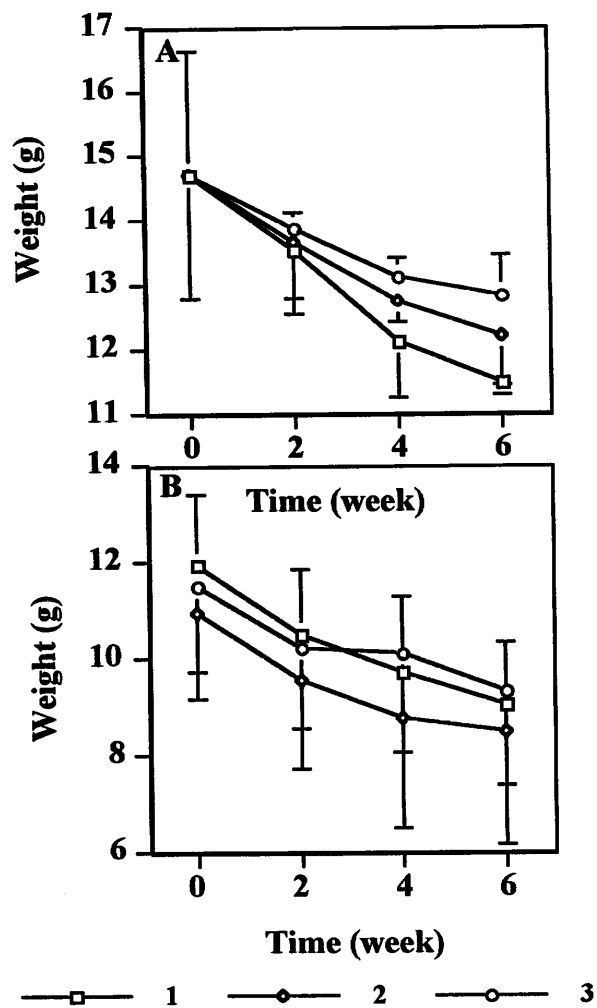


Fig. 1 : Changes in body weight of walleye grown on diets A (10% lipid) and B (4% lipid) during the experiment.

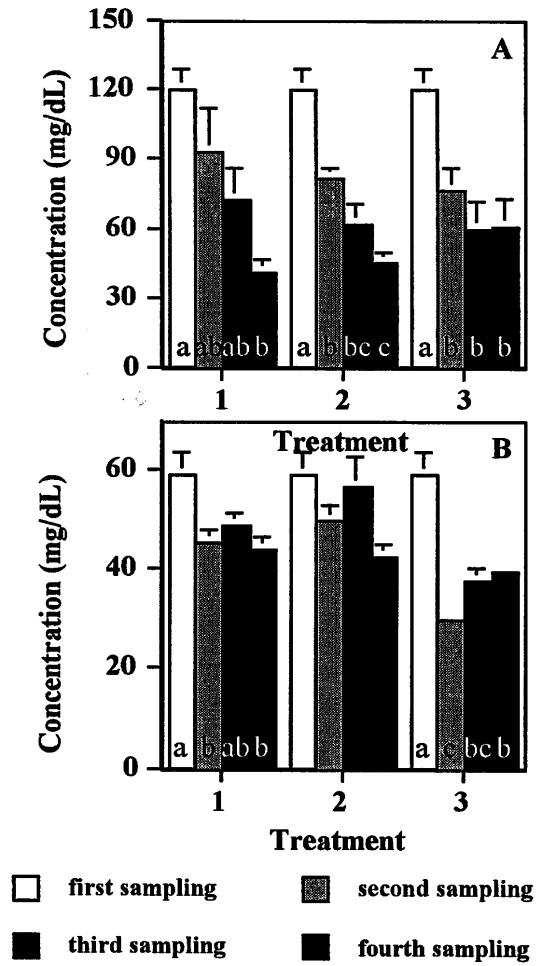


Fig. 2 : Changes in plasma glucose levels of the walleye grown on diets A and B. Means within the same treatment with the same letter are not significantly different from each other.



The plasma glucose levels in the fish grown on diet A decreased significantly ( $p < 0.05$ ) in each treatment during the experimental period (Fig. 2A). However, there were no significant differences between treatments. Alternative patterns of plasma glucose levels were observed in fish grown on diet B. After an initial drop (first 2 weeks), the glucose levels were maintained at similar levels in all three treatments (Fig. 2B). Noteworthy is the fact that the initial level of plasma glucose was twice as high in fish grown on diet A compared to fish grown on diet B.

### **Acknowledgements**

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**THE ROLE OF CORTISOL  
IN REGULATION OF GLYCOGEN METABOLISM  
IN WHITE MUSCLE OF RAINBOW TROUT**

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**Introduction**

In fish, high intensity exercise results in a near total depletion of white muscle glycogen stores. Glycogen replenishment and hence, restoration of sprint performance, is a slow process, requiring in excess of 6 hr. Exercise to exhaustion also results in an elevation of plasma cortisol, which can attain levels 5 times those at rest and remain elevated through 6 h after exercise. We have shown that preventing the rise in plasma cortisol, either by blocking its release or synthesis (Pagnotta *et al.*, 1994; Eros & Milligan, 1996) hastens the restoration of muscle glycogen stores.

**Methods**

The rate at which glycogen is synthesized is a function of the relative activities of glycogen synthase and phosphorylase. The activity of these enzymes is regulated by a complex phosphorylation/dephosphorylation cycle. Phosphorylation of glycogen phosphorylase leads to an increase in activity, whereas phosphorylation of glycogen synthase decreases its activity. Therefore, in order to gain insights into the role of cortisol in regulating muscle glycogen, we measured the changes in white muscle glycogen synthase and phosphorylase

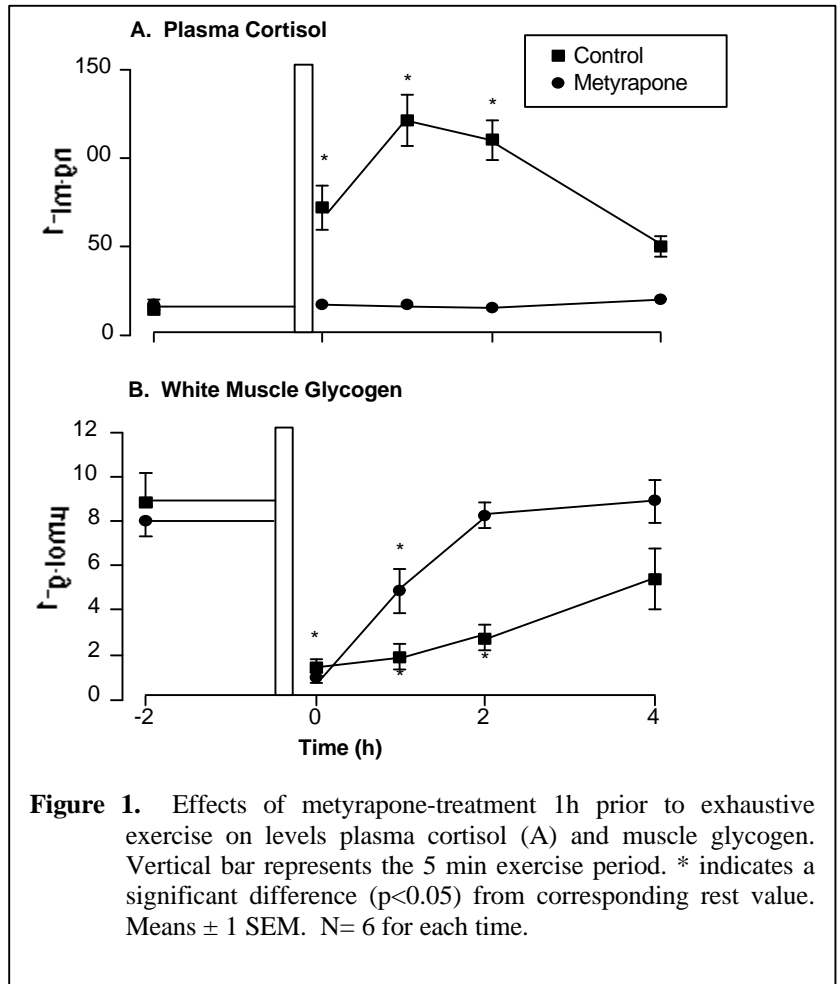
activities (according to the methods described by Schalin-Jäntti, et al., 1992 and Storey, 1991, respectively) in rainbow during recovery from exhaustive exercise. Fish were cannulated in the dorsal aorta, allowed to recover 48 hours and then injected with either saline (control) or metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone;  $3 \text{ mg } 100 \text{ g}^{-1}$ ) 1 hour prior to exercise. Fish were exercised to exhaustion by chasing them around a circular tank and blood and muscle samples were taken immediately after exercise (time 0), or 1, 2 or 4 h later. Resting fish were injected as described, but not exercised, and sampled 1 hour post-injection.

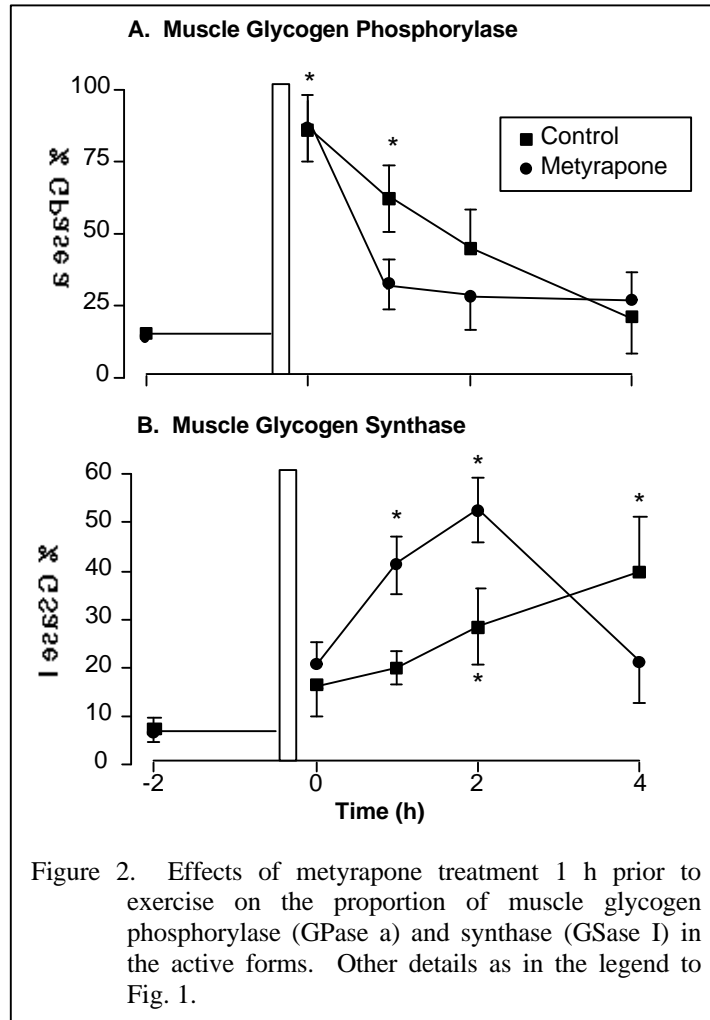
## Results and Discussion

Metyrapone treatment blocked the rise in plasma cortisol (Fig. 1A) associated with exhaustive exercise and hastened the recovery of muscle glycogen stores following exercise (Fig. 1B). These findings are consistent with previously reported effects of metyrapone treatment (Pagnotta et al., 1994; Eros & Milligan, 1996).

In resting fish, approximately 25% of muscle glycogen phosphorylase was in the active form (Fig. 2A) while only 10% of the glycogen synthase was active (Fig. 2B), in both the control and metyrapone-treated fish. Exercise resulted in a near total activation of muscle glycogen phosphorylase in both control and the metyrapone-treated fish (Fig. 2A), which coincided with the time of lowest glycogen levels (Fig. 1B). In control fish, the proportion of glycogen phosphorylase in the active form (GPase a) declined slowly, not returning to rest levels until 4 h post exercise; a time when plasma cortisol levels had returned to resting levels. In contrast, GPase a activity was turned off more rapidly in metyrapone-treated fish, with rest levels attained within 1 h after cessation of exercise.

In the control group, the proportion of active glycogen synthase (GSase I) slowly increased after exhaustive exercise, but not significantly until about 2-4 h post-exercise, again, following a similar time course to the decline in plasma cortisol levels. The increase in GSase I activity coincided with the onset of glycogen resynthesis. However in fish treated with metyrapone, muscle glycogen synthase activity is significantly elevated within 1 hour post-exercise, to levels higher than that seen in control fish. Again, this increase in GSase I activity paralleled the recovery of muscle glycogen (Fig. 1B). In neither group was there any significant changes in total synthase or phosphorylase activities.





The more rapid activation of glycogen synthase and inactivation of glycogen phosphorylase are consistent with the more rapid restoration of muscle glycogen seen in metyrapone-treated fish. These data strongly suggest that plasma cortisol is involved in the regulation of muscle glycogen synthesis, by regulating the activities of either glycogen synthase or phosphorylase or both. However, the nature of the regulatory role of cortisol is not at all clear.

### **Acknowledgments**

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**EFFECTS OF EXOGENOUS CORTISOL  
ON ION REGULATION  
IN DEVELOPING LARVAE  
OF TILAPIA (*OREOCHROMIS MOSSAMBICUS*)**

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**Introduction**

Embryos and larvae of several teleosts, whose gills or kidneys are poorly or under-developed, have been found to be able to maintain constancy in ion concentrations and osmolality of their body fluids (Alderdice, 1988). Recent studies have demonstrated the presence of cortisol in embryos and larvae whose interrenal glands are not developed yet (De Jesus et al., 1991; Hwang et al., 1992; Hwang and Wu, 1993). The object of the present paper was to study the role of cortisol in the mechanisms of ion regulation in the developing tilapia (*Oreochromis mossambicus*) larvae.

**Materials and Methods**

At 48 h post-fertilization, embryos or newly-hatched larvae of freshwater (FW) tilapia were immersed in 0 (control), 1, 5 or 10 mg l<sup>-1</sup> cortisol immersing solution for different periods (see below). Then, the animals were transferred to 20-30 g l<sup>-1</sup> seawater (SW), and changes in cumulative mortality, body Na content and yolk-sac epithelial ouabain binding of larvae after transfer to SW were examined.

## Results and Discussion

Immersion in 5 and 10 mg l<sup>-1</sup> cortisol for 3 days resulted in a significant increase, about 6.6 and 8.5 fold, respectively, in the tissue cortisol contents of tilapia larvae, indicating that exogenous cortisol does accumulate in the body of larvae. Immersion in 10 mg l<sup>-1</sup> cortisol for longer than 8 days was found to inhibit the growth of larvae based on the data of yolk-sac diameter. Therefore, shorter-term treatments without inhibition on larval growth were conducted in the following experiments:

48-h-post-fertilization embryos were treated with 1-10 mg l<sup>-1</sup> cortisol for 3 days, and after hatching, were transferred to 20-30 g l<sup>-1</sup> SW. Cortisol revealed dose-related effects to reduce the cumulative mortality of larvae in SW. Moreover, cortisol significantly diminished the degree of increase in body Na content of larvae after the transfer to SW. The significant effect of cortisol on body Na content of larvae occurred as early as 8 h after the SW transfer (Fig.1), while no significant difference was found in the ouabain binding of yolk-sac epithelia between control and cortisol-treated larvae even 12 h after the transfer (Fig.2).

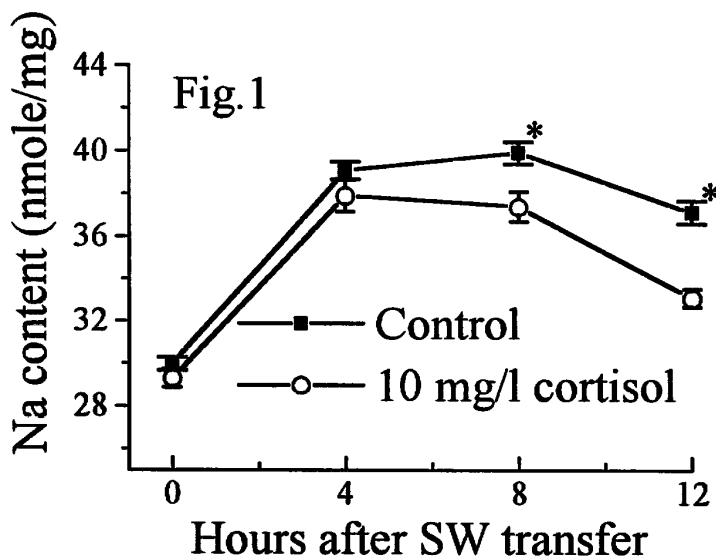


Fig.1. Changes in body Na content in cortisol-treated tilapia larvae after SW transfer. \* significantly different from the control group ( $p < 0.05$ , t-test).

Another SW experiment was conducted without any cortisol treatment on tilapia larvae in order to reconfirm the above results. The body Na content in larvae increased during the initial 8 h in SW (20 g l<sup>-1</sup>) and then declined to near the level of FW control. On the other hand, it was not until 24 h after the transfer that the yolk-sac epithelial ouabain binding in SW larvae was significantly higher than that in FW control. This indicated that the changes in body Na content did not coincide with those in yolk-sac epithelial ouabain binding upon SW transfer.

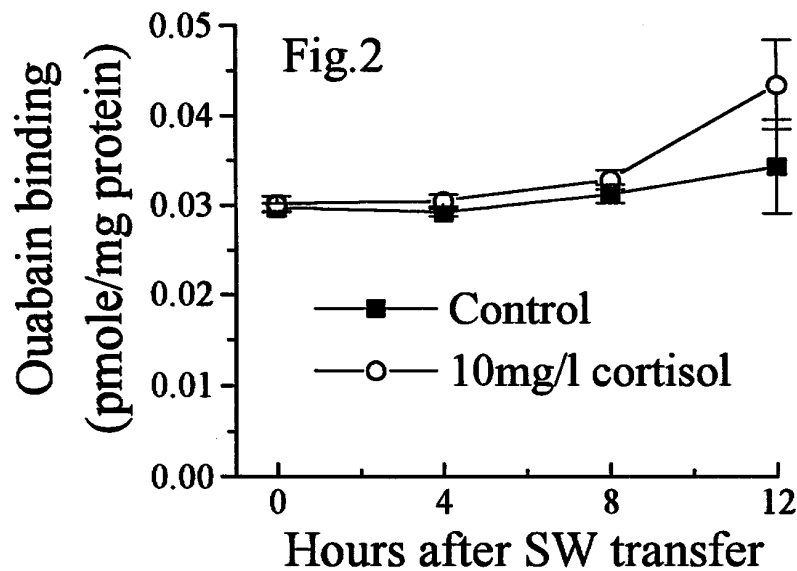


Fig.2. Changes in yolk-sac epithelial ouabain binding in cortisol-treated tilapia larvae after SW transfer. No significant difference between the two groups ( $p > 0.05$ , t-test).

It has been well documented that 1-2 weeks of cortisol treatment can enhance the salinity tolerance in several adult fish via stimulation of gill Na-K-ATPase and chloride cells (McCormick, 1995). On the contrary, Forrest et al., (1973) reported that American eels, which were treated with cortisol for 2 days and then transferred to SW, increased Na efflux prior to an increase in gill Na-K-ATPase activity.

A short-term (3 days) treatment of cortisol was also conducted in the present study, and a similar inconsistency was found in the changes of larval body Na content and yolk-sac epithelial ouabain binding (as described above). These results suggested that one of the early physiological effects of exogenous cortisol is to enhance the hypoosmoregulation ability in tilapia larvae, however this mechanism may be achieved via other pathways rather than by stimulating the Na-K-ATPase of yolk-sac epithelia.

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**EFFECTS OF CORTISOL  
ON WATER BALANCE  
IN DEVELOPING LARVAE  
OF TILAPIA (*OREOCHROMIS MOSSAMBICUS*)**

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**Introduction**

Cortisol is thought to be a seawater-adapting hormone because of its salt excretion in hypo-osmoregulating fish. However, little is known about its action on water balance in fish. Our previous study demonstrated that the presence of cortisol in embryos and developing larvae of tilapia, and suggested that exogenous cortisol can promote the maintenance of tissue osmolality during seawater adaption (Hwang and Wu, 1993). The purpose of the present report was to study the role of cortisol in water balance of developing tilapia larvae.

**Materials and Methods**

*Experiment I*

4-day-old larvae of freshwater (FW) of seawater (SW) tilapia were immersed in 0 (control), 10 mg l<sup>-1</sup> cortisol solution for different periods, thereafter drinking rate, water content, and body size of the larvae were measured.

### Experiment II

4-day-old FW larvae were immersed in 0 (control), 10 mg l<sup>-1</sup> cortisol for 8 h and then transferred to 20 g l<sup>-1</sup> SW. Changes in the drinking rate and water content were measured after the transfer.

### Results and Discussion

After 24 h cortisol immersion, the wet weight, water content and drinking rate of SW larvae were about 10%, 15% and 33%, respectively, lower than those of control larvae (Table 1.). The decreased scale of wet weight was equal to that of water content, indicating that the decreased proportion of wet weight is due to the water loss. On the other hand, cortisol did not affect the other parameters including dry weight, total length and yolk-sac diameter of larvae, indicating that no inhibition on larval development occurred. In the case of FW larvae, the drinking rate of cortisol-treated group was also lower (about 50%) than that of control after 8 h of cortisol immersion, however no difference was found in wet weight and water content between control and cortisol group.

Table 1. Changes in the body size, water content and drinking rate of 4-day-old SW larvae immersed in 10 mg l<sup>-1</sup> cortisol for 24 h.

	Wet weight (mg)	Water content (mg)	Dry weight (mg)	Total length (mm)	Yolk-sac diameter (mm)	Drinking rate (nl h <sup>-1</sup> larva <sup>-1</sup> )
Control	6.97 ± .45	5.17 ± .31	1.80 ± .22	8.42 ± .31	1.51 ± .11	240 ± 20
Cortisol	6.24 ± .29*	4.43 ± .15*	1.81 ± .81	8.37 ± .22	1.53 ± .18	160 ± 30*

All the data were obtained from one brood of tilapia larvae. Values shown are mean ± SD (n=10). Student's *t*-test analysis was made between control and treated group in each parameter. \* significant difference, *P* < 0.05.

In experiment II, FW larvae were immersed in cortisol solution for 8 h and then were transferred to SW. The drinking rates of both cortisol-treated and sham group showed dramatically increase after transfer and reached to the value of

SW control (larvae were adapted well to SW) at 8 h after transfer to SW (Figure 1.). It was noted that the drinking rate of cortisol-treated group increase faster than sham group within 4 h after transfer to SW.

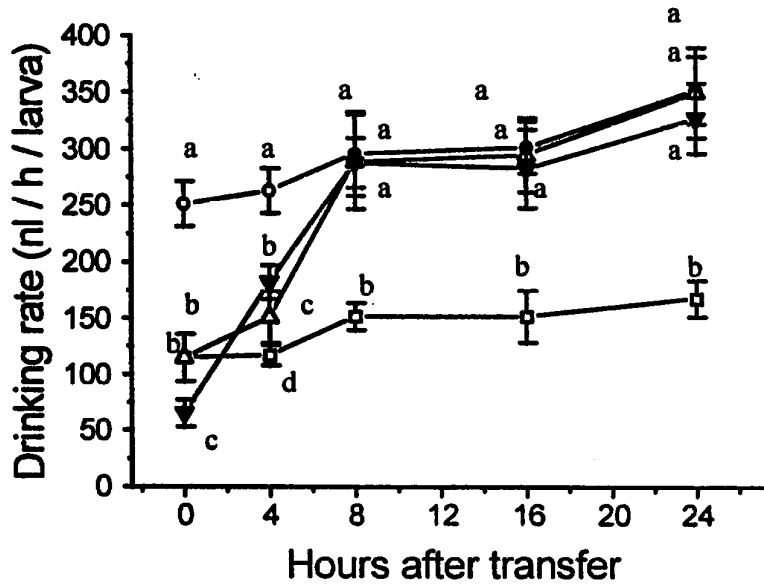


Figure 1. Changes in the drinking rates of cortisol-treated larvae (solid triangle) and sham larvae (open triangle) in FW and after transfer to SW. The drinking rates of SW control (circle) and FW control (square) are also shown. One way ANOVA (Tukey's pairwise comparisons) analysis was made among different groups at the same time. Different letters indicates significant difference.

It has been well documented that exogenous cortisol can enhance the salinity tolerance in several adult fish via stimulation of gill Na-K-ATPase and chloride cells (McCormick, 1995). And recent reports extended its function to stimulating intestinal water absorption in salmon smolts (Veillette et al. 1995). The present finding showed that cortisol may be also involved in the control of water drinking in tilapia larvae. As Fuentes et al. (1996) suggested, cortisol may promote drinking during SW challenge in salmon presmolts and juvenile

rainbow trout. On the other hand, the inhibition on drinking by cortisol in both FW and SW larvae suggested that additional exogenous cortisol would interfere the normal regulation of drinking activity in the larvae which were adapted well to SW or FW.

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**CHANGES IN TAURINE PLASMA  
AND TISSUE CONCENTRATIONS  
DURING PERIODS OF OSMOTIC STRESS  
IN THE EURYHALINE TILAPIA,  
*OREOCHROMIS MOSSAMBICUS***

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**Abstract**

These studies were undertaken to examine the effects of changes in environmental salinity on blood and tissue taurine levels in the euryhaline tilapia, *Oreochromis mossambicus*. The amino acid taurine is an important intracellular osmolyte which has been shown to move across cell membranes in response to changes in extracellular osmolality. Tilapia adapted to seawater (SW) were transferred to fresh water (FW) and FW-adapted tilapia were transferred to SW. At intervals, blood, pituitary and hypothalamus samples were collected. Blood plasma osmolality and taurine levels were measured. Pituitary and hypothalamic tissues were sonicated and taurine levels in the supernatant were measured. Compared with control levels at time 0, plasma

taurine levels in SW-adapted tilapia transferred to FW increased significantly ( $p < 0.05$ ) over the first 6 hrs and remained elevated for the duration of the study (24hrs). By contrast, pituitary taurine levels in these animals decreased significantly ( $p < 0.05$ ) within one hour after transfer and remained lowered for the duration of the study. In general, the opposite was seen in these parameters when FW-adapted tilapia were transferred to SW. Plasma taurine levels in these fish at 1 and 3 hr after transfer were significantly lower ( $p < 0.05$ ) than those of animals at time 0. After 3hr, however, there was an upward trend. Taurine levels in the pituitary of FW fish transferred to SW increased significantly ( $p < 0.05$ ) by 1 hr and remained elevated at 3 and 6 hrs. Beyond 6 hrs all animals that were transferred from FW to SW succumbed to osmoregulatory failure. Hypothalamic taurine levels in FW-adapted fish transferred to SW did not vary at any time from that of animals at time 0. Hypothalamic taurine levels in SW-adapted animals were significantly lower ( $p < 0.05$ ) 1 hr after transfer to FW than those of animals at time 0, but these levels rebounded by 3 hrs and did not vary significantly thereafter. The results of our study are consistent with a possible role for taurine as a signal to fine-tune the release of prolactin, the FW osmoregulatory hormone in the euryhaline fishes.

## **Introduction**

Taurine is a  $\beta$ -sulphonated analogue of  $\beta$ -alanine. Generally classed as a free amino acid, it is not incorporated into proteins, but may be found in some simple peptides (McMahon *et al.*, 1996). It differs from classical amino acids by the location of the amino group on the  $\beta$  carbon and the substitution of a sulphate for the  $\alpha$  carboxyl group. The sulfonic group is substantially more negative than the carboxylic acid group. This makes taurine highly reactive with cations, readily forming salt bridges and it significantly decreases the pK of taurine's amino group, allowing 2.8% of taurine to remain in its deprotonated state at physiological pH (Schaffer *et al.*, 1995). It is present in high concentrations in many tissues and organs, especially the brain of both invertebrates and vertebrates, where it is located in both glial and neuronal fractions (Huang *et al.*, 1996, Stummer *et al.*, 1995). Taurine is implicated in many functions, including olfactory transduction (Sung *et al.*, 1996), neuromodulation (Oja and Saransaari, 1996), insulin secretion (Cherif *et al.*, 1996), and antioxidation (Trachtman and Sturman, 1996) and is a recognized major intracellular osmoregulatory compound (Oja and Saransaari, 1996).

Different groups of organic osmolytes are used by different groups and species of fish during osmoregulatory adaptation (Van der Boon *et al.*, 1991, Goldstein

and Davis, 1994, Assem and Hanke, 1983, Sakaguchi *et al.*, 1988). Only three classes of organic compounds play major roles in cellular osmoregulation: free amino acids, polyols, and the methylamines (Goldstein and Davis, 1994). Among the amino acids, the most common appear to be taurine, glycine, and both  $\alpha$ - and  $\beta$ alanine (Assem and Hanke, 1983). The role of taurine in osmoregulation in the euryhaline tilapia, *Oreochromis mossambicus*, remains unclear. Van der Boon *et al.* (1991) reported that taurine is the most common amino acid in carp and goldfish blood and that no taurine could be detected in tilapia blood. They also noted that glycine (with threonine) occurs at very high levels in tilapia whole blood, accounting for nearly 75% of the total free amino acids, with 96% or 14 mmol/l of the glycine contained inside the red blood cells. By contrast, Assem and Hanke (1983) report that taurine is the most abundant amino acid in tilapia liver and muscle cells. In fish transferred directly from fresh water (FW) to 27 ppt seawater (SW) there is a gradual increase in the intracellular content of taurine that is mirrored by a decrease in blood taurine concentration. After 9 hours of acclimation, cell size begins to increase back to normal volumes as taurine moves back into the blood. During the 9 hours of acclimation, blood serum taurine levels drop by half from 0.90 mmol/l in FW to 0.46 mmol/l in 27 ppt SW. Evidence from the literature also suggests that taurine is biologically active as a chemical messenger (Sung *et al.*, 1996, Oja and Saransaari, 1996, Scheibel *et al.*, 1980, Scheibel *et al.*, 1984, Makinen *et al.*, 1993). Its dual roles in intracellular osmoregulation and chemical signaling suggest that it might play a role, through either short or long cycle feedback, in whole body osmoregulation.

A highly selective and sensitive HPLC procedure, developed by McMahon *et al.* (1996), for measuring taurine in human blood plasma was used to measure taurine levels in tilapia blood plasma and the supernatant from hypothalamic and pituitary homogenates. Plasma taurine levels were found to vary inversely and tissue supernatant taurine levels to vary directly when compared with changes in the osmolality of the environment to which the tilapia were exposed.

## **Materials and Methods**

### *Animals.*

Tilapia, *O. mossambicus*, used in this study were collected from brackish water streams and ponds on the north shore of the island of Oahu, Hawaii and maintained under natural photoperiod conditions in flowing water at 23-25°C in

5,000 l (3 m diameter, 1 m depth) fiberglass tanks at the Hawaii Institute of Marine Biology. Fish had been held in either FW or SW for about two years and were fed Purina trout chow twice daily to satiety.

#### *Experimental Protocol.*

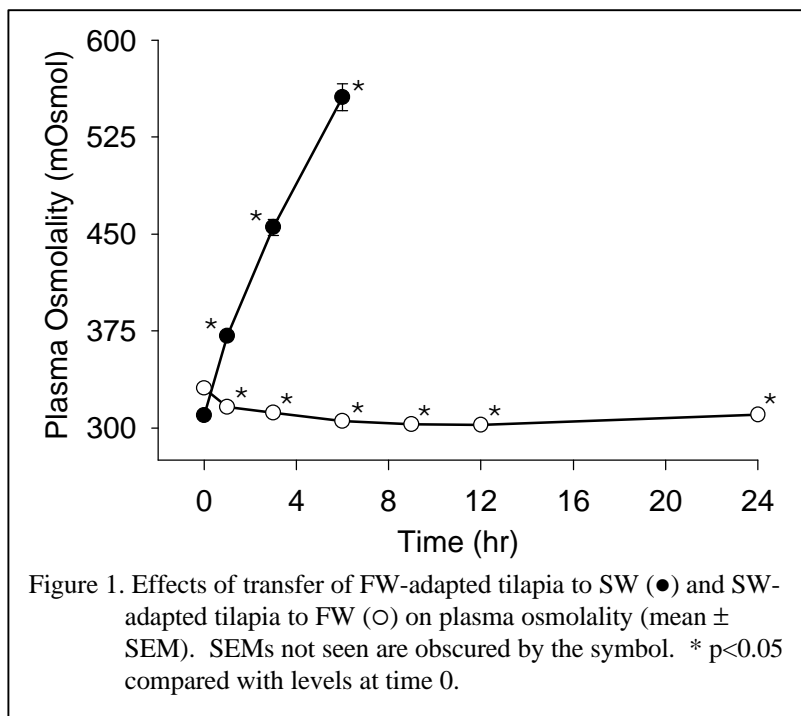
A total of 108 (75 male, 33 female) fish, weighing between 137g-410g (males  $260\pm 7$ g, females  $177\pm 6$ g) were used in this study. There were 18 FW controls and 36 SW controls. Experimentals consisted of groups of 6 each that were transferred either from SW to FW for 1, 3, 6, 9, 12, or 24 hours or from FW to SW for 1, 3, or 6 hours. The remaining FW-adapted fish transferred to SW succumbed to osmoregulatory failure by 7 hours. Pituitary, hypothalamus and blood samples were collected from 6 fish at each time point. Blood was collected in heparinized syringes from the caudal vein and placed immediately on ice prior to centrifugation and freezing of the plasma at  $-80^{\circ}\text{C}$ . Pituitaries and hypothalami were immediately sonicated in 100  $\mu\text{l}$  of HPLC water, vortex-mixed with 150  $\mu\text{l}$  of acetonitrile, centrifuged at 5800g for 10 min, and the supernatant frozen at  $-80^{\circ}\text{C}$ .

Blood plasma samples were tested for osmolality and taurine concentration. Osmolalities were measured with a Wescor 5100c vapor pressure osmometer. The procedure used to measure taurine concentration was developed by McMahon, *et al.*, (1996) to provide a simple, efficient, and rapid method for the determination of taurine in human blood plasma. Samples (100  $\mu\text{l}$ ) were mixed with 150  $\mu\text{l}$  of acetonitrile and centrifuged at 5800g for 10 min prior to the addition of 50  $\mu\text{l}$  of 10mM borate buffer (pH 9.2) to the supernatant. Fifty  $\mu\text{l}$  of 5 mM fluorescamine were added to the supernatant and the mixture immediately vortex mixed. A 20  $\mu\text{l}$  sample was then injected into a reversed-phase HPLC system using a 300 X 3.9 mm analytical column and carried by a tetrahydrofuran-acetonitrile-phosphate buffer (15 mM, pH 3.5)(4:24:72, v/v/v) mobile phase. UV absorbance at 385 nm was used to measure the taurine derivative. Frozen supernatant samples from pituitaries and hypothalami were treated as above beginning with the addition of 50  $\mu\text{l}$  of borate buffer.

## Results

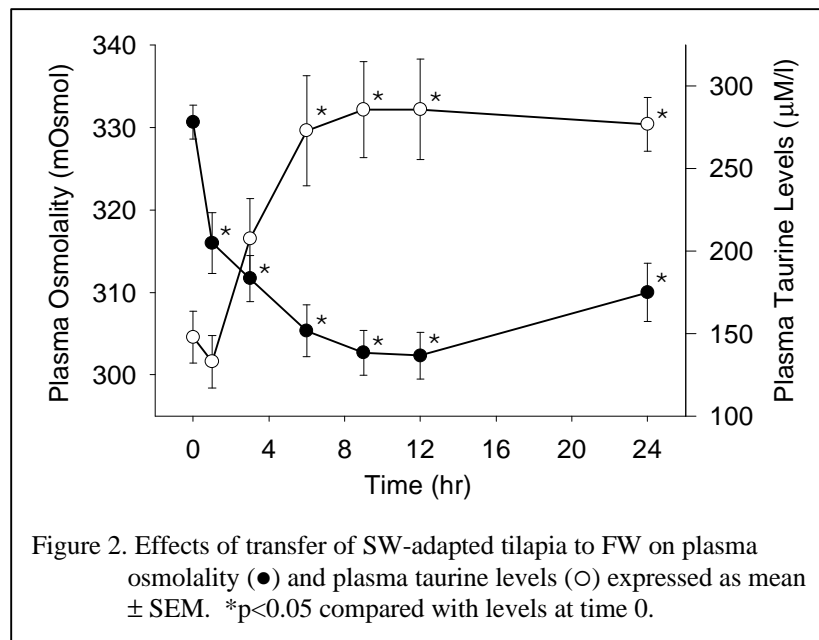
### *Blood Osmolality Changes in Tilapia Moved to Anisotonic Environments.*

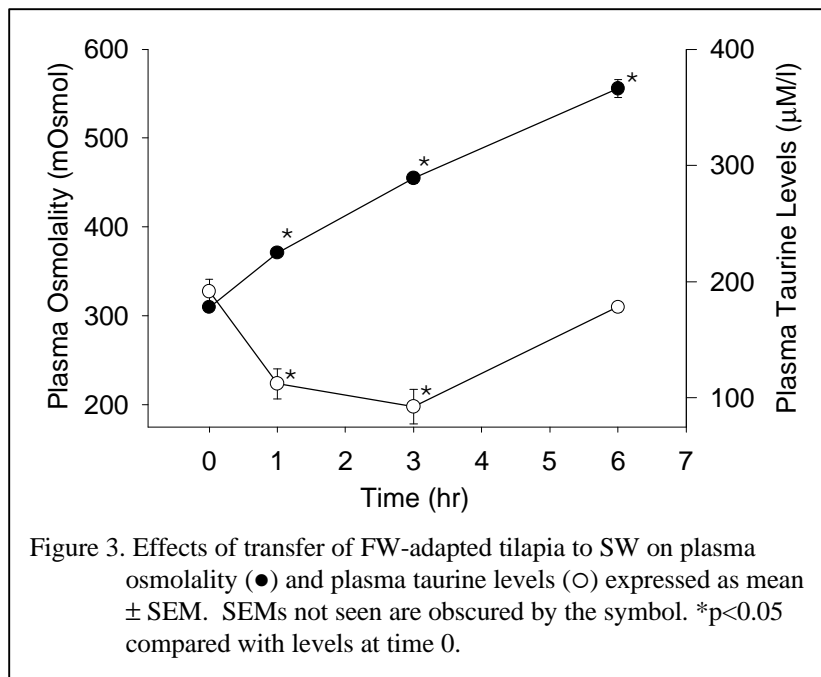
The effects of anisotonic environments on tilapia blood osmolalities are shown in Figure 1. Tilapia that were moved directly from SW to FW successfully adjusted to their new environment. Initially, the blood osmolalities of these fish dropped rapidly from about 330 mOsmols, reaching a low of 302 mOsmols between 9 and 12h hours of exposure to FW, before rising to 310 mOsmols, a level normally found in tilapia acclimated to FW. Without preadaptation to an intermediate concentration, tilapia moved directly from FW to full-strength SW did not adapt. In this study, the tilapia were overwhelmed by osmotic stress and died within 7 hours of exposure to full-strength SW. Blood plasma osmolality rose rapidly until it reached an osmolality in excess of 500 mOsmols after only 6 hours of exposure to SW.



*Taurine Levels (µM/l) in the Blood Plasma of Osmotically Stressed Tilapia.*

Coincident with changes in blood plasma osmolality were changes in plasma taurine levels. In SW-adapted tilapia transferred to FW, taurine levels increased significantly ( $p < 0.05$ ) from about 150 µM/l at time 0 to nearly 290 µM/l during the first 9 hours of FW adaptation and remained at this level to the end of the experiment. As can be seen in Figure 2, these changes closely mirrored the significant drop ( $p < 0.05$ ) in plasma osmolality compared with that of fish at time 0. An inverse relationship between blood plasma osmolality and taurine concentration was seen initially in FW-adapted tilapia transferred to SW, but the pattern changed after 3 hours (Figure 3). Blood osmolality rose significantly ( $p < 0.05$ ) from the level at time 0, about 310 mOsmols, to 555 mOsmols within 6 hours of transfer to SW. During the first 3 hours in SW, taurine levels dropped significantly ( $p < 0.05$ ) from around 190 µM/l at time 0 to nearly 90 µM/l and then returned to around 190 µM/l over the next 3 hours. Fish began to die shortly after 6 hours in SW with none surviving beyond 7 hours of exposure.





#### *Hypothalamic and Pituitary Taurine Levels in Osmotically Stressed Tilapia.*

Taurine levels in tissue extracts from both the hypothalamus and pituitary of SW-adapted tilapia averaged about 240  $\mu\text{M/l}$ . Both tissue extracts from SW-adapted fish showed a significant drop ( $p < 0.05$ ) in taurine levels after one hour of exposure to FW compared with that of animals at time 0. Pituitary taurine concentrations remained low throughout the remainder of the 24 hour experiment. This was in contrast to the taurine levels in the hypothalamic tissue, which returned by 3hr to levels that were not different from the control value at time 0 (Figure 4). Changes in taurine levels in the pituitary extracts from SW-adapted tilapia transferred to FW showed an inverse distribution compared with that of the blood plasma (compare Figures 2 & 4). Hypothalamic and pituitary supernatants from FW-adapted tilapia contained taurine at about 360 and 295  $\mu\text{M/l}$ . During the first hour of exposure to SW pituitary taurine concentration significantly rose ( $p < 0.05$ ), above levels at time 0, to 459  $\mu\text{M/l}$ .

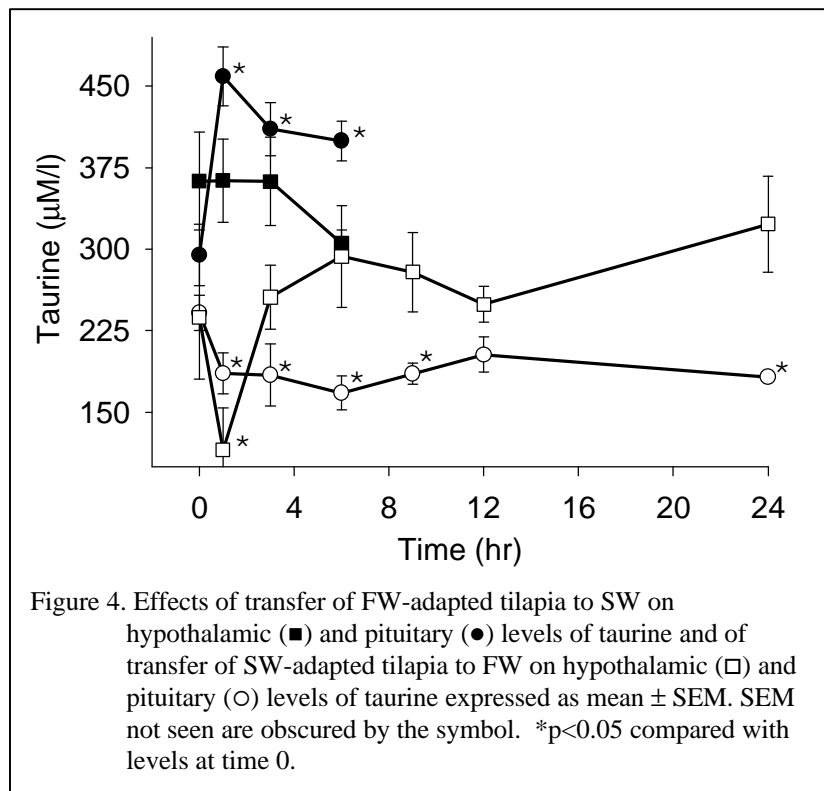


Figure 4. Effects of transfer of FW-adapted tilapia to SW on hypothalamic (■) and pituitary (●) levels of taurine and of transfer of SW-adapted tilapia to FW on hypothalamic (□) and pituitary (○) levels of taurine expressed as mean ± SEM. SEM not seen are obscured by the symbol. \*p<0.05 compared with levels at time 0.

Hypothalamic taurine levels remained constant for 3 hours and then showed a decreasing trend at 6 hours of exposure to SW (Figure 4).

### Discussion

Data presented in this report and by Assem and Hanke, (1983) demonstrate that taurine is present in the blood and tissues of the euryhaline tilapia, *O. mossambicus* and that both intra- and extra-cellular taurine concentrations fluctuate during adaptation to FW and SW environments. The results of this investigation indicate that extracellular (blood and tissue fluid) taurine



concentrations vary inversely with the osmolality of the environment to which the tilapia is exposed. As fish move into hypoosmotic environments, water moves osmotically into the fish, diluting the blood and causing cells to swell due to the osmotic uptake of water. Cells respond with a regulatory volume decrease initiated by releasing both electrolytes and organic osmolytes (Hall *et al.*, 1996, Ballatori and Boyer, 1996, Goldstein *et al.*, 1995, Strange and Jackson, 1995). It is under these conditions that we found a significant increase in plasma levels and a significant decrease in pituitary levels of taurine in tilapia. Upon exposure to a hypertonic environment, fish osmotically lose water and their cells shrink. Shrunken cells regain volume through a regulatory volume increase that includes the selective uptake of organic osmolytes (Assem and Hanke, 1983, Tamai *et al.*, 1995, Beetsch and Olson, 1996). We observed, following exposure of FW tilapia to a hypertonic environment, a significant decrease in plasma levels and a significant increase in pituitary levels of taurine at 1 and 3 hr. Although these fish failed to survive beyond 7 hr, plasma osmolality at 3 hr had not exceeded 450 mOsmol, a level from which tilapia may recover under certain condition (Yada *et al.*, 1994). Beyond 3 hrs, plasma taurine levels increased coincident with elevated plasma osmolality.

It is generally accepted that cells respond to hypoosmotic conditions by releasing electrolytes and organic osmolytes. This is the response we have observed in pituitaries. The hypothalamus, after an initial first hour drop in taurine, retained high levels of taurine, especially at 6 and 24 hours after tilapia were moved to FW. Since levels in whole tissue extracts were measured, it is unclear whether the increased taurine levels were from:

- the cellular uptake of taurine or
- from increased taurine levels in hypothalamic blood and tissue fluids or
- from increased taurine levels in the third ventricle of the brain or
- some combination of these possibilities.

Previous investigations provide evidence that elevated levels of taurine in the hypothalamus stimulate the release of prolactin (PRL) from the pituitary (Scheibel *et al.*, 1980; 1984). They demonstrated that the intraventricular infusion of taurine elicited a significant increase in PRL secretion in male rats. Since taurine was ineffective in stimulating PRL secretion in an *in vitro* pituitary preparation, the authors postulated that the taurine effect appears to be mediated centrally, presumably at the hypothalamus. Their argument is strengthened with a follow-up investigation in which it was shown that taurine stimulated PRL secretion only when microinfused into the arcuate nucleus of the rat hypothalamus (Scheibel *et al.*, 1984). In a similar study, Makinen *et al.* (1993)

were able to show that taurine, as well as hypotaurine and gamma amino butric acid, stimulates the release of PRL after intracerebroventricular administration and that there are gender-related differences in serum PRL levels induced by these three compounds in rats. This correlates with the observation in the present investigation (data not shown) that plasma and hypothalamic levels of taurine are higher in female than in male tilapia. Pituitary levels of taurine in male and female tilapia, however, do not differ (data not shown).

Evidence from the present investigation and an earlier report from our laboratory suggest that there may be a relationship between plasma taurine levels and serum PRL levels in tilapia. The results of the previous investigation show that plasma levels of both tilapia PRLs (PRL<sub>177</sub> and PRL<sub>188</sub>) decline in fish moved from FW to SW and that they both rise in fish moved from SW to FW (Yada *et al.*, 1994). The direction of change in the circulating levels of PRL<sub>177</sub> and PRL<sub>188</sub> with changes in environmental salinity are similar in direction to those of plasma taurine levels observed in the this study. This similarity is consistent with the notion that plasma taurine levels may contribute to the control of the release of tilapia PRLs from the pituitary. Investigations are underway to determine if taurine has a direct effect on pituitary PRL-releasing cells and/or if there is taurine-stimulated hypothalamic intervention in prolactin release.

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**BLOOD CHEMISTRY AND PERFORMANCE INDICES  
FOR JUVENILE CHINOOK SALMON AND STEELHEAD  
DESCALED EXPERIMENTALLY AND DURING PASSAGE  
THROUGH FISH BYPASSES AT DAMS  
ON THE SNAKE RIVER, WASHINGTON**

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**Introduction**

Fish culturists and fishery biologists must often sample, move, or otherwise handle fish. These procedures can result in the loss of scales and damage to the skin. Because the skin, scales, and associated mucus layer impede ion and water exchange with the external environment and prevent the entry of potential pathogens, scale loss is believed to be deleterious, and qualitative or semi-

quantitative estimates of descaling are frequently used to evaluate the damage done by handling procedures. Despite the wide acceptance and use of descaling as an index of physical damage, few studies have examined the physiological responses of salmonid fish to scale loss or the effects of descaling on subsequent performance and survival. The study reported here examined blood chemistry changes in migrating chinook salmon and steelhead smolts descaled to varying degrees during passage through juvenile fish diversion and bypass systems at hydroelectric dams on the Snake River, Washington. The survival of descaled fish relative to undamaged control fish was determined by holding fish in tanks of running river water at the dams for several weeks. In addition, laboratory trials were performed to determine the effects of scale removal on physiological responses and disease resistance under controlled conditions.

Enzymes used as markers of tissue damage (alanine aminotransferase, ALT; aspartate aminotransferase, AST; creatine kinase, CK) occurred in higher concentrations in the plasma of more highly descaled chinook salmon sampled at Snake River dams (Figure 1), and in addition, the incidence of internal bruising and other injuries was higher in more highly descaled fish. These results indicate that, on a population basis, descaling is useful as an index of total tissue damage.



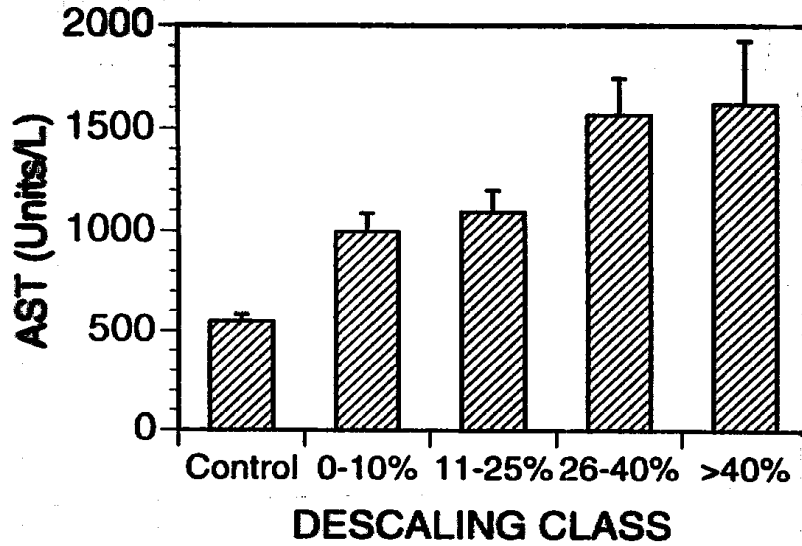


Figure 1. Plasma AST levels in steelhead descaled to varying degrees during passage through bypass systems at Snake river dams.

Mortality rates for descaled chinook salmon and steelhead held in tanks at the Lower Granite fish facility for 2 to 4 weeks ranged from 0 to 44% and did not differ significantly from mortality rates for undamaged control fish. Of fish that died (both descaled and control fish), 75% of chinook salmon and 44% of steelhead developed fungal infections of the skin prior to death. Fungal infections occurred with equal frequency in descaled and control groups (Figure 2.), and usually appeared on the fins rather than on descaled areas or elsewhere on the body. In these trials, descaling did not predispose fish to fungal infections.

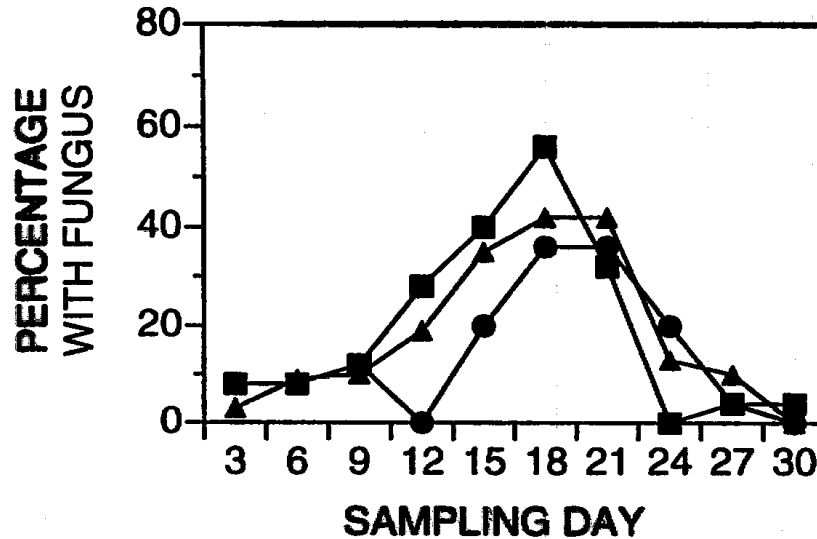


Figure 2. Percentages of control (circles; minimal scale loss), descaled (squares; scales missing over >10% body surface) and experimentally descaled (triangles; scales removed over 10% body surface) steelhead developing fungus infections. The fish were examined at 3-d intervals during a 30-d holding period at the Lower Granite Dam fish collection facility.

In the laboratory, descaling did not result in increased susceptibility of juvenile chinook salmon to the bacterial pathogen *Vibrio anguillarum*. Similarly, descaling of chinook salmon previously infected with *Renibacterium salmoninarum* did not increase the prevalence or severity of infection or the mortality rate relative to control fish. Exposure to a handling stressor alone did, however, increase susceptibility to a *V. anguillarum* challenge if the challenge immediately followed the stressor. Moderate descaling did not affect the growth rate of juvenile chinook salmon in seawater.

In laboratory tests with juvenile steelhead, tissue damage indices (ALT, AST, CK) were elevated to a greater extent if descaling injuries affected the deeper layers of the skin, but were unaffected by the site of the injury (dorsal or ventral surface). Both descaling (20% of total body area on dorsal surface) and exposure to a stressor (water level reduced to 3 cm for 15 min) elicited increases in

plasma cortisol, ALT, AST, CK, and lactate dehydrogenase. Cortisol and enzyme responses to a stressor were not accentuated in descaled fish, but were similar in descaled and control fish following exposure to a low-water stressor at intervals of 12, 24, 48, or 96 h after descaling. Cortisol levels returned to resting levels within 24 hours in both descaled and control groups, but ALT, AST, and CK levels were still elevated in descaled fish at the end of the experiment (96 h). Plasma  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Ca}^{++}$  concentrations declined slightly after handling, but were similar in descaled and control groups.

In summary, the stress responses (plasma cortisol and electrolytes) of juvenile chinook salmon and steelhead to moderate descaling ( $\leq 20\%$  of body surface) in fresh water were transitory and similar to the responses to an acute handling stressor. Descaling fish held for up to 30 d in river water did not develop fungal skin infections at a higher rate than undamaged control fish. Descaling injuries more severe than those investigated in this study may result in immediate, life-threatening osmoregulatory disturbances, but no evidence was found for longer-term adverse effects of descaling on fish viability.



**FOREST HARVESTING INCREASES STREAM TEMPERATURE:  
POTENTIAL IMPACTS ON JUVENILE CHINOOK?**

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**Introduction**

Juvenile chinook salmon from the upper Fraser River in British Columbia reside in or near their natal streams for a year after emerging from the streambed (Healey 1991). An understanding of the freshwater life history of *stream type* chinook from this region of BC is lacking, however, making it difficult to predict the impacts of habitat change. Habitat changes associated with timber harvesting adjacent to streams have been shown to occur. Riparian vegetation adjacent to streams serves to supply large woody debris and increase habitat complexity, contribute allochthonous food and organic nutrients, moderate the hydrological regime and moderate stream temperature (Barton et al. 1985). Stream temperature plays a central role in the aquatic ecosystem because it influences all biological processes including metabolic rates, growth, behavior, and survival of fish populations. Clear cutting is a common logging practice in British Columbia. Recognition of the importance of the riparian zone to the aquatic environment has resulted in the development of the Forest Practices Code (FPC) by the BC government that details prescriptions for harvest methods adjacent to streams. Riparian Reserve Zones (RRZ) must be left adjacent to streams, but the dimensions are determined by stream size, gradient and presence of fish

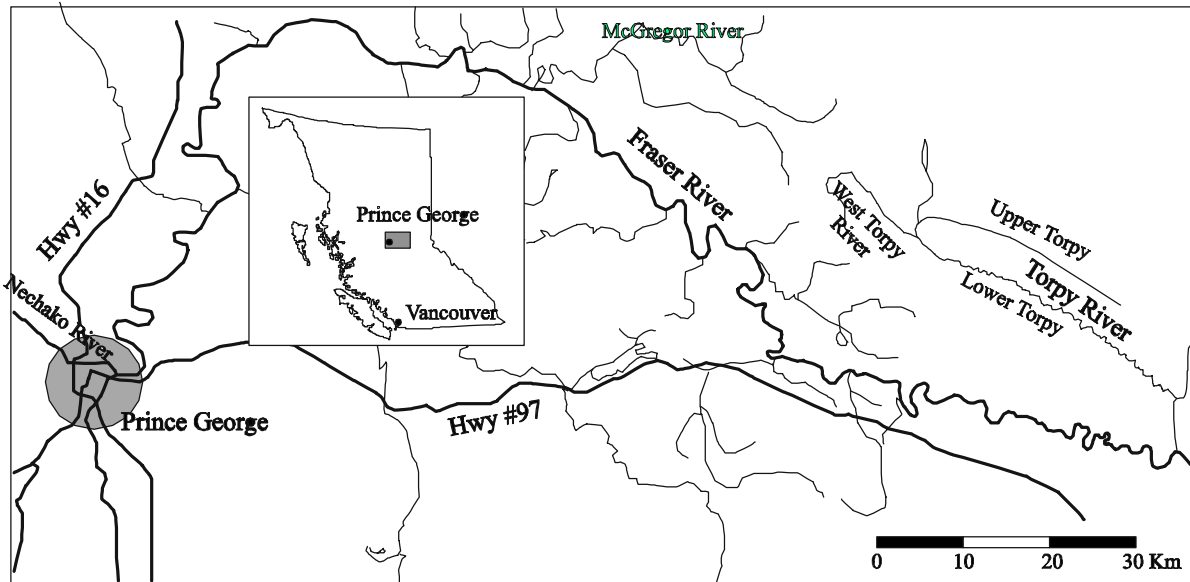


Figure 1 Location of the Torpy River in central British Columbia. Sites of timber harvesting and fish sampling is in tributaries running into the Lower Torpy River.

## **Study Plan**

The first objective has been to examine the seasonal temperature changes for a river and tributaries in central BC. The Torpy River and tributaries is the site of our study and is located in the central interior of BC. The river is approximately 120 km long and flows into the Fraser River 140 km east of Prince George (Figure 1). The Torpy River has a population of chinook salmon and forest harvesting is presently being conducted in the river drainage. Our second objective has been to determine the relative distribution of fish within this river system. The third objective of our studies is to evaluate whether riparian leave strips as specified in the BC Forest Practices Code (FPC) are effective in providing protection to adjacent aquatic environments and their constituent biota. Our final goal is to assess the effect on growth potential and stress physiology of fish residing in streams impacted by harvesting.

## **Analysis**

The Torpy River is much warmer and exhibits a greater daily variation in temperature during the summer months than many of its tributaries. Maximum daily temperatures during August can exceed 18 °C. Over the winter, the water temperature difference is reversed and the mainstem is colder (slightly above freezing) than the tributaries (ranging from 1 to 2.5 °C). Not only do the tributaries show less seasonal variation in temperature, but also they exhibit less daily variation in temperature (Figure 2).

No fish were caught by trapping or electrofishing during the height of runoff in the mainstem or tributaries. During the latter part of June, we caught increasing numbers of fish in the tributaries. We assume that this temporal pattern reflects the movement of juveniles into small streams after emergence from the gravel of the mainstem. Juvenile chinook less than one year old were the dominant species and age group caught. Numbers of chinook are significantly greater in the tributaries suggesting these non-natal streams are important juvenile rearing habitat. Fish older than one year have also been caught in the tributaries. These fish must have spent the entire previous summer in the river or its tributaries, and overwintered successfully. We continued to capture chinook in some of the tributaries until mid December of 1997.

We have also documented that changes in stream temperature occur due to forest harvest practices, and that the presence of riparian vegetation moderates

these changes. The experimental removal of riparian zone was performed to determine the impact on stream temperature and to compare with the FPC mandated riparian reserve zones widths. Water that flows through the creeks where the RRZ was removed heats more than the water in the creeks with a RRZ. As a consequence the diurnal change in temperature is greater and daily maximum temperatures are higher in the unbuffered creeks.

Within the tributaries that flow through harvested and non-harvested areas size of juvenile chinook caught during the late spring was not significantly different, but was larger than the fish captured in the mainstem or the side channels. Bradford and Taylor (1997) documented that two days after capture of newly emerged fry, larger fry distributed themselves downstream, while smaller fry held position. Movement of fry from natal rivers into smaller non-natal tributaries has been observed during the spring (Scrivener et al. 1994). The size difference between fish in the tributaries and mainstem may, therefore, be a result of movement by the larger fry into the tributaries. Over the summer months, the fish in the non-harvested stream reaches grew significantly larger than the fish inhabiting stream reaches within cutblocks, the mainstem or the side channels of the mainstem. The temperature of streams flowing through the cutblocks were warmer than streams flowing through non-harvested regions, but cooler than the Torpy River (Figure 2). Mean temperature cannot account for the differences observed in growth. Thomas et al. (1986) presented evidence that fluctuations in daily temperature were stressful and impacted on the energy reserves of coho salmon. Whether the daily temperature variation or other factors associated with timber harvest can account for the differences, remains to be determined.



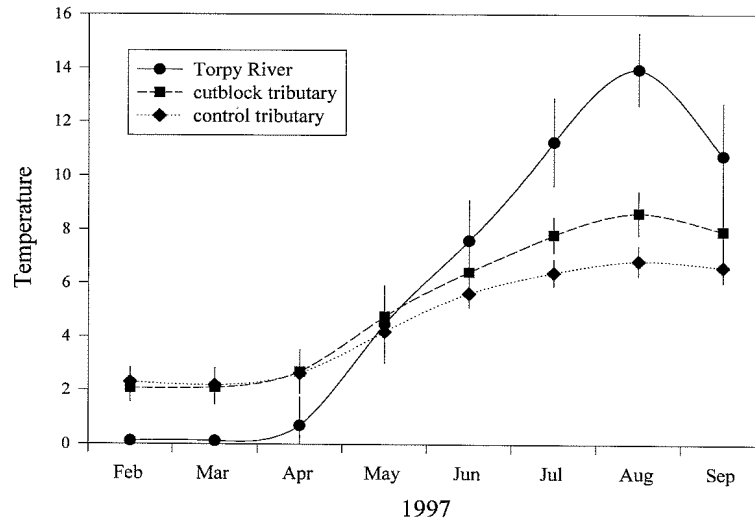


Figure 2. Water temperature of the Torpy River and two tributaries from February to September of 1997. Timber was harvested from the cutblock tributary in February 1997. The control tributary flows through an unharvested stand that is approximately 800 m upriver of the harvested block. Data presented represents monthly mean temperatures  $\pm$  1 standard deviation.

### **Acknowledgements**

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**PHYSIOLOGICAL INDICATORS OF STRESS  
AMONG FISHES EXPOSED TO  
CONTAMINATED SEDIMENTS  
FROM LAKE CHAMPLAIN**

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**Introduction**

From 1992 to 1998, we conducted a series of investigations to determine whether fishes exposed to contaminated sediments in Lake Champlain showed physiological evidence of stress. We focused on macrophage aggregates in the spleen because several studies have shown that fishes from contaminated sites exhibited greater macrophage aggregate area than fish from less contaminated reference sites (see, for example, Wolke *et al.* 1985, Macchi *et al.* 1992, Couillard and Hodson 1996).

We compared the percent area displaced by splenic macrophage aggregates of yellow perch (*Perca flavescens*), brown bullhead (*Ameiurus nebulosus*), and rock bass (*Ambloplites rupestris*) from known contaminated sites to reference

sites with lower contaminant levels. Previous studies by researchers from the University of Vermont showed that inner Cumberland Bay, inner Burlington Harbor, and the area south of the mouth of the LaChute River showed elevated levels of PCBs, PAHs, and some heavy metals. We, therefore, selected these areas for study, as well as reference sites with known or suspected lower levels of contaminants.

## **Methods**

Fishes were captured either by electrofishing or angling. We measured length and weight, and removed scales or spines to determine fish age. Spleens were removed and fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. We compared mean macrophage aggregate area between fish of similar age to control for any change in macrophage aggregate area with age (see Blazer *et al.* 1987). We also removed and weighed livers to determine the hepatosomatic index, or HSI (liver size expressed as a percent of overall body weight), which may be higher in fish exposed to toxic environments (Goede and Barton 1990). Statistical comparisons were made using a t-test.

## **Results**

Data from yellow perch and brown bullhead have been published elsewhere (see Blazer *et al.* 1994). Yellow perch had no significant differences in macrophage aggregate parameters among sites, probably because they are highly mobile fish and are not likely to spend much time in any single location. Brown bullhead from inner Cumberland Bay did have significantly higher macrophage aggregate area and significantly larger HSI than similarly aged fish from a reference site. Brown bullhead from the contaminated site also had high rates of external lesions and barbel deformities not observed at the reference site.

Three-year-old rock bass from Burlington Harbor in 1992 had a significantly larger area of spleen occupied by macrophage aggregates than did fish from one reference site (Table 1). HSI also was significantly larger than in fish from the less contaminated reference site. Four-year-old rock bass from Burlington Harbor in 1992 also had a significantly greater area of splenic macrophage aggregates than did rock bass from two reference sites (Table 1). There also

was a significant difference in the HSI, with fish from the cleanest site (Sunset Lake) having the smallest mean HSI.

Both three- and four-year-old rock bass collected in 1997 from Burlington Harbor had significantly less splenic area occupied by macrophage aggregates and lower HSI values than had been found in 1992 (Table 1). There was no significant difference in macrophage aggregate area between the 1997 Burlington Harbor fish and similar aged fish captured from any of the reference areas, although the very small sample of four-year-olds from the Inland Sea site makes this comparison difficult. In 1997, three-year-olds from the Inland Sea site did, however, have significantly larger mean HSI than fish from Burlington Harbor.

The decline in splenic macrophage aggregate area and HSI seen among Burlington Harbor fish from 1992 to 1997 suggests that the environmental quality of Burlington Harbor improved during the five years between samples. During this time the city of Burlington completed a substantial upgrade of its main sewage treatment facility, including a significant improvement in the degree of treatment and an extension of the outlet pipe so that the treated effluent no longer is released in the inner harbor. Although our results are based on rather small samples, they suggest that this treatment upgrade is paying off with improved water quality and less physiological stress to rock bass in the inner harbor.

## **Conclusion**

Our results suggest that:

1. macrophage aggregates are good biomarkers of fish exposure to contaminants;
2. fishes from areas of Lake Champlain known to have contaminated sediments did show increased area of macrophage aggregates in their spleens; and

3. efforts to improve water quality at one contaminated site seem to have resulted in a decrease in splenic macrophage aggregate area in fish from that site.

### **Acknowledgments**

Special thanks to Madeleine Lyttle and the U. S. Fish and Wildlife Service (Essex Junction, Vermont) for the use of their electrofishing boat and to Kristen Bartlett, Lori Pyzocha, and Darlene Bowling for many hours of excellent laboratory work. Funding was provided by the U. S. Geological Survey, Vermont EPSCoR, and St. Michael's College.

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Wolke, R. E., R. A. Murchelano, C. D. Dickstein, and C. J. George. 1985. Preliminary evaluation of the use of macrophage aggregates (MA) as fish health monitors. *Bulletin of Environmental Contamination and Toxicology* 35: 222-227.

Table 1. Mean ( $\pm$  one standard deviation) percent area of spleen tissue displaced by macrophage aggregates and mean HSI ( $\pm$  one standard deviation) in 3 and 4 year old rock bass from Burlington Harbor and three reference sites. For t-test comparisons within age groups: \* indicates values significantly lower than those from Burlington Harbor 1992 ( $P < 0.05$ ); \*\* indicates values very significantly lower than those from Burlington Harbor 1992 ( $P < 0.01$ ); and # indicates that mean HSI for Inland Sea 1997 fish was significantly higher than mean HSI from Burlington Harbor 1997 ( $P < 0.05$ ).

	3 year olds			4 year olds		
	% area of splenic MA s	Hepatosomatic Index	n =	% area of splenic MA s	Hepatosomatic Index	n =
Burlington Harbor 1992	3.77 ( $\pm$ 2.49)	1.66 ( $\pm$ 0.33)	6	11.44 ( $\pm$ 9.87)	1.37 ( $\pm$ 0.27)	8
Malletts Bay 1992	1.36 ( $\pm$ 1.01) *	1.06 ( $\pm$ 0.19) **	8	2.98 ( $\pm$ 2.44) *	1.09 ( $\pm$ 0.19) *	11
Sunset Lake 1994	n/a	n/a	0	2.02 ( $\pm$ 1.82) **	0.80 ( $\pm$ 0.19) **	10
Burlington Harbor 1997	0.39 ( $\pm$ 0.53) **	1.08 ( $\pm$ 0.36) *	6	1.72 ( $\pm$ 0.89) *	1.12 ( $\pm$ 0.13) *	7
Inland Sea 1997	1.09 ( $\pm$ 0.36)	1.81 ( $\pm$ 0.48) #	6	2.13 ( $\pm$ 0.65)	1.18 ( $\pm$ 0.06)	2



## INTERSPECIFIC HYBRIDIZATION AND RECOVERY FROM ECOLOGICAL STRESS

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### Introduction

The June Sucker (*Chasmistes liorus* Jordan) is a federally listed endangered species endemic to Utah Lake, Utah. Utah Lake has experienced repeated historical drought conditions, most recently in the mid-1930's. Overlap in spawning timing and location due to decreased habitat may result in hybridization.

### Methods

Nuclear and mitochondrial DNA from contemporary and museum preserved (late 1800's) June suckers and the sympatric Utah Sucker (*Catostomus ardens*) was analyzed for the purpose of identifying the genetic integrity of contemporary June suckers. Random amplified polymorphic DNA (RAPD) analysis was used to assess nuclear DNA sequences. Restriction fragment length polymorphism (RFLP) analysis of two mitochondrial DNA regions and

DNA sequencing of three mitochondrial DNA regions was used to assess mitochondrial DNA sequences.

## **Results**

No genetic differences between contemporary or museum preserved June suckers and the sympatric Utah sucker (*Catostomus ardens*) found in Utah Lake were detected. Although the genetic characters that distinguish a June sucker are enigmatic, the distinct morphological and behavioral characteristics of June sucker persist, albeit in a decreasing number of adult individuals.

This interspecific hybridization may allow survival of genetic capacity during periods of ecological stress. In contrast, a parallel analysis of the sympatric Cui-ui (*Chasmistes cujus*) and Tahoe sucker (*Catostomus tahoensis*) of Pyramid Lake, Nevada, yielded explicit and multiple differences in the nuclear and mitochondrial DNA of each species.

Together, these data suggest a unique genetic and natural history of Utah Lake fishes, particularly June sucker, as compared to the Bonneville and surrounding remnant pluvial lake basins.