Environmental Stress and Health in Fish

SYMPOSIUM PROCEEDINGS

S. Marshall Adams

Bruce Barton

Don MacKinlay

International Congress on the Biology of Fish
University of British Columbia, Vancouver, CANADA
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PREFACE

This symposium summarizes our current understanding and use of biological indicators of stress and general health in fish. Invited paper topics cover physiological responses, immunosuppression, and condition-based indices, and how these indicators can be integrated to evaluate the health and well-being of fish populations. These synthesis papers describe the responses of fish to environmental stressors using various stress indicators and the advantages and limitations of their application. Contributed paper and poster presentations focus on the effects of stress on different levels of biological organization, ranging from molecular and cellular responses to those at the whole-organism level.

Symposium Organizers:

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Don MacKinlay, Fisheries & Oceans Canada
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I would like to extend a sincere ‘thank you’ to the many organizers and contributors who took the time to prepare a written submission for these proceedings. Your efforts are very much appreciated.

Don MacKinlay
Congress Chair
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Many physiological changes that occur in response to environmental disturbances are now used routinely for assessing stressed states in fish. Stress responses are mediated through neuronal and endocrine pathways, known as the primary response, following initial perception of the stressor. These, in turn, can influence secondary physiological features and tertiary or whole-animal performance characteristics in the fish, which could result in stress-induced alterations in fish populations. The initial stress response is considered adaptive, one designed to help the fish overcome the disturbance and regain its normal or homeostatic state. If the stressor is severe or long-lasting, however, the fish may no longer be able to cope with it and, as a result, enters a maladaptive or distressed state leading to decreased performance, a pathological condition or possibly death.

While many of the physiological changes documented in fish during stress still remain within the realm of experimental research and need further study, some have proven useful for quantifying stress in fish resulting from human activities, such as water pollution and habitat alteration, and in aquaculture. Typical primary responses used for evaluating stress in fish include determining circulating levels of cortisol and, to a lesser extent, catecholamines. Secondary responses include measurable changes in blood glucose, lactate, major ions (e.g., chloride, sodium) and osmolality, tissue levels of glycogen and lactate and, at the cellular level, heat-shock proteins. However, many other apparent non-stress factors influence characteristic physiological stress responses in fish that biologists need to be aware of in order to properly interpret data; these include genetic (e.g., species, strain, stock), developmental (e.g., life history stage) and
environmental (e.g., temperature, nutrition, water quality) factors, as well as the fish’s prior experience to stressors.

A number of secondary physiological indicators can be measured relatively easily with portable meters (e.g., glucose, hemoglobin) or easy-to-use assay kits (e.g., lactate). Some of these devices are suitable for field use but should be calibrated with established lab methods to validate accuracy and repeatability. Other indicators, such as specific ions and, especially, circulating hormones, require more sophisticated protocols and laboratory equipment, and many need special handling in the field to maintain the integrity of the sample prior to analysis.

Physiological measurements provide a useful approach to evaluate responses of fish to acute stressors but may not necessarily be so for monitoring fish experiencing sublethal chronic stress. Unless the stressors are severe enough to challenge the fish’s homeostatic mechanisms beyond their capacity to adjust, physiological mechanisms will generally adapt to compensate for the stress. In these cases, blood chemistry features, such as plasma cortisol, may appear normal and other approaches, such as evaluating the fish’s response to an additional acute stressor, may be needed to determine its physiological status.

Further information may be found in the following paper from which this abstract was extracted.

FISH HEALTH CHALLENGE AFTER STRESS. INDICATORS OF IMMUNOSUPPRESSIVE STATUS

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EXTENDED ABSTRACT ONLY - DO NOT CITE

The immune system of fishes is characterised by a number of features related to the evolutive status of fish and the water environment they inhabit. Thus, the occurrence of microorganisms and the nature of an aqueous habitat results in a high level of contact with foreign molecules. As a result, a significant feature of the piscine immune response is the range of non-specific immune response, its wide repertoire and also the notably high immune activity found on external surfaces, especially in freshwater fish. The potency of the non-specific responses in fish is opposite from that found in higher vertebrates, wherein memory and specific immunoglobulin responses are more effective.

Stressors activate the alarm responses in all physiological compartments and neuro-endocrine mediators are mobilised as a first response. These molecules may be used as markers for immune activation. The stressed status and the occurrence of hormones generate physiological and metabolic changes and when the effects become chronic one of the consequences of maladaptation is the depression or suppression of immune mechanisms due to the concentration of resources to overcome energetic needs. Therefore, two types of indicators may be considered regarding the time course of immune responses, early-immediate or short-term markers, that will indicate the initiation of immune mechanisms which may be adaptative, and the long term markers that will often indicate a diseased status and/or a drop in immunocompetence.

Amongst the first grouping, the cytokines are key players. Thus detection of pleiotropic interleukins such as TNFα or interleukin-1β allows for detection of primary immune be applied including those based upon specific cellular responses such as marker gene expression, cell proliferation/apoptosis, cell functions (phagocytosis) and cell migration/localisation. In addition, lysozyme
activity, complement activity, lectins and immunoglobulin production may be also be utilized.

In terms of applied work only a select number of indicators can be transferred and used to test immunocompetence/immunosuppression on site where the scientific/analytic equipment is often scarce. Of clear importance for quality analysis is sampling technique, especially in blood and tissue sampling, and subsequent sampling thereof. In the near future the development of simple kit-based formats for key markers such as cytokines for the assessment of immunological status will be highly desirable.
OVERVIEW OF CONDITION-BASED INDICATORS
OF ENVIRONMENTAL STRESS IN FISH

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Physiological responses to stress in fish can be assessed by laboratory-based techniques, however many of these require sophisticated instrumentation and are not practical for most field situations. Although sample test kits are available for some secondary stress indicators (see Barton, this session), there are also a number of condition-based indices that can be used to assess tertiary or whole-animal responses to stress. These condition-based indicators are relatively easy to measure, require less training to perform, and are usually less expensive than primary and secondary stress indicators. This makes them attractive to monitoring agencies and they have been widely used in fisheries management. They tend to be relatively insensitive to short-term (i.e., acute) changes in environmental conditions, however they can contribute appreciably toward understanding long-term trends in fish populations exposed to chronic environmental stressors.

Condition factor (K) and other length-weight relationships are commonly used as indicators of robustness as length and weight data are easy to collect without invasive procedures. Declines in condition factor can indicate changes in energy storage, metabolism and feeding activity due to environmental stressors. However these changes may also occur for reasons other than stress (e.g., seasonal and developmental changes) and therefore the use of condition factors in these cases should be interpreted with caution.

Ratios of the mass of particular organs or tissues relative to total body mass can also be used as indices of change in nutritional and energy status. Commonly used organosomatic indices include: hepatosomatic (HSI) or liver-somatic (LSI) index, gonadosomatic index (GSI), viscerosomatic index (VSI), and
splenosomatic index (SSI). The assumption that is generally made with these indices is that lower than normal values indicate a diversion of energy away from organ or tissue growth in order to combat a stressor. Similar to condition factors, the various organosomatic indices may vary naturally with food availability, state of sexual maturation and life history stage, often in concert with season. These factors should therefore be taken into account when using organosomatic indices in stress assessment studies.

The necropsy-based condition assessment, developed by Goede and Barton (1990) and further quantified by Adams et al. (1993), has been used to detect changes in the health and condition of fish populations. The necropsy method is based on a systematic examination of the appearance and condition of external and internal tissues and organs. A departure in the appearance of an organ or tissue system from the normal condition is assumed to reflect a response to a chronic stressor. The necropsy method is not meant to be a diagnostic tool, but it has been shown to be useful in detecting health problems in fish populations exposed to environmental contaminants, particularly in bottom-feeding species.

Further information may be found in Barton et al. (2002), from which this abstract was extracted.

References


Environmental stressors such as contaminants can cause a variety of biological responses in fish ranging from the biomolecular and biochemical to population and community-level effects. To assess fish health, the bioindicators technique utilizes a suite of biological responses both as integrators of stress effects and as sensitive response (early-warning) indicators of existing and past environmental conditions (Adams 1990). Short-term indicators, such as biomolecular and biochemical responses, and longer-term ecologically relevant indicators, such as population and community responses, are included in this approach to provide measurement endpoints that can be used in environmental management or in the regulatory decision and ecological risk assessment (ERA) process.

Biomarkers of environmental stress at lower levels of biological organization such as the mixed function oxidase (MFO) enzymes and DNA integrity provide direct evidence of exposure to stressors while intermediate-level responses such as histopathological, bioenergetic, immunological, and reproductive changes can help predict stress effects at the individual, population, and community levels. Responses at the lower levels of biological organization have the primary advantage of being relatively sensitive (short-term response) to stressors thus serving as early warning indicators of impaired fish health. Conversely, responses at higher levels of organization (populations, communities) are relatively insensitive (long-term response) to stressors but have higher ecological relevance and are therefore more directly applicable to the ERA process and for addressing environmental management and regulatory issues (Fig. 1). Biomarkers, however, cannot be considered useful bioindicators of fish health unless they are causally linked to
ecologically relevant responses such as population or community-level endpoints (McCarty and Munkittrick 1996).

Figure 1. Hierarchical responses of organisms to environmental stressors illustrating the sensitive early-warning responses at the lower levels of biological organization.
To determine possible causal relationships between lower (i.e. biomarkers) and higher level effects, responses in fish populations at several levels of biological organization were measured along a downstream gradient in a contaminated stream. The downstream response patterns for several biomarkers along this contaminant gradient were similar to higher-level response patterns indicating that these biomarkers could be potentially useful in the ERA process because of their sensitivity and relationships to ecologically relevant endpoints.

A suite of biomarkers and bioindicators were also used to demonstrate biological recovery over several years in a contaminated stream following environmental cleanup activities. Short-term response indicators such as the MFO enzymes and physiological parameters provided early indications of recovery, while the longer-term and slower-responding indicators (i.e., population, community indices) demonstrated delayed improvements in health. Using all the individual biomarker and bioindicator responses together in a multivariate discriminant analysis procedure provided an integrated assessment of fish health and identified a reduced set of measurement endpoints most responsible for distinguishing between stressed and healthy fish populations. Depending on the particular system and the types of stressors involved, only 4-7 response variables were needed to assess recovery and discriminate between healthy and unhealthy systems. These distinguishing variables generally represent different levels of biological organization and functional dynamics in organisms including MFO enzymes, organ dysfunction, bioenergetic, and reproductive competence. These results illustrate the importance of utilizing multiple-response endpoints at different levels of biological organization when assessing the health and recovery of organisms exposed to multiple environmental stressors.

Proper experimental design of bioindicator-based field studies involves measurement of responses which (a) range from the biomolecular/biochemical level (e.g., biomarkers of exposure) to the population and community levels (bioindicators of effects), (b) represent responses along gradients of both ecological relevance and response time (reflecting degrees of sensitivity) (Fig. 1), and © include measurements of both specific and nonspecific responses. Biomarkers, however, cannot be considered bioindicators of effects unless they are causally linked or related to ecologically relevant responses such as reproductive, population, or community level endpoints. Understanding mechanisms of stress response by establishing relationships between exposure biomarkers and
Bioindicators of effects should provide for more informed environmental regulatory decisions regarding ecosystem integrity and the effectiveness of cleanup activities.

Application of biomarkers and bioindicators in environmental stress studies involves measuring a suite of selected stress responses at each of several levels of biological organization in order to (1) assess the effects of environmental stressors on organisms, (2) predict future trends (early warning indicators of change), (3) obtain insights into causal relationships (mechanisms) between stress and effects at higher levels of biological organization, and (4) evaluate the effectiveness of remedial (cleanup) actions. Application of biomarkers and bioindicators in field studies are not without their limitations (Table 1), even though they may be most effectively utilized within an integrative framework to assess the health of fish populations.

Table 1. Major features of biomarkers and bioindicators relative to their advantages and limitations for use in field bioassessment studies.

<table>
<thead>
<tr>
<th>Major Features</th>
<th>Biomarkers</th>
<th>Bioindicators</th>
</tr>
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<tbody>
<tr>
<td>Types of response</td>
<td>Subcellular, Cellular</td>
<td>Individual Through Community</td>
</tr>
<tr>
<td>Indicators of exposure</td>
<td>Exposure</td>
<td>Effects</td>
</tr>
<tr>
<td>Sensitivity to stressors</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Relationship to cause</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Response variability</td>
<td>High</td>
<td>Low-Moderate</td>
</tr>
<tr>
<td>Specificity to stressors</td>
<td>Moderate-High</td>
<td>Low</td>
</tr>
<tr>
<td>Time scale of response</td>
<td>Short</td>
<td>Long</td>
</tr>
<tr>
<td>Ecological relevance</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

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STRESS-INDUCED DIFFERENTIAL GENE EXPRESSION
IN THE BRAINS OF JUVENILE STEELHEAD TROUT

(ONCORHYNCHUS MYKISS)

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EXTENDED ABSTRACT ONLY- DO NOT CITE

In response to stimuli, vertebrate brains display amazingly complex inter/intracellular interactions between neuroactive substances resulting in gene expression. Stressors, factors causing perceived fear or harm, are one group of stimuli that affect gene expression; however, information on brain-level genetic mechanisms of stress remains limited. Even less research describes stressor-induced gene expression in the fish brain. Previous research focused on genes known to be associated with stress. However, recently developed molecular tools allow the simultaneous capture of numerous genes unique to a particular treatment, providing a more complete description of the brain during stress. We utilized suppression subtractive hybridization to identify genes in the brains of
juvenile steelhead trout (*Oncorhynchus mykiss*) exposed to confinement and handling stressors.

We reared winter steelhead (*Oncorhynchus mykiss*) parr at Oregon State University's Fish Performance and Genetics Laboratory, Corvallis, OR, prior to experimentation. After completion of triplicate 0-hour (control N=10), 3-hour (N=9 or 10), or 48-hour (N=8 or 9) handling and continuous confinement treatments, we lethally anesthetized the fish and collected blood from the dorsal artery. Brain removals occurred within minutes of bleeding, and were stored in RNase free cryo-vials and frozen on liquid N. We immediately extracted brain total RNA (stored at -80ºC) which was pooled upon demonstration that plasma stress indicators (cortisol and glucose) across each triplicate were not different. Immediately after polyA⁺-selection, double stranded cDNAs were reverse transcribed using the CLONTECH PCR-select™ cDNA Subtraction Kit according to the manufacturer's protocol. Then, utilizing the above subtraction kit, we identified forward and reverse subtracted cDNAs representing up and down-regulated genes, respectively. We cloned genes using the TOPO TA Cloning® kit available from Invitrogen, PCR amplified positive-insert colonies, sequenced the genes, and grew-up and stored colonies (-80ºC) in liquid media. Sequenced genes were queried using the web-based BLASTN and BLASTX 2.2.1 algorithms. Genes resulting in significant identities were confirmed differentially expressed by Northern hybridization using the North2South® Direct HRP Labelling and Detection Kit available from Pierce Endogen according to the manufacturer's specifications.

Plasma indicators of stress demonstrated that the fish were undergoing a stress response at 3 hours. Forward and reverse subtractions from the 0 and 3-hour groups resulted in 58 genes which were sequenced. Of the 58, 11 were selected for further analysis, and 4 were confirmed differentially expressed. The sequenced genes fell into the following categories: those associated with metabolic pathways/oxidative stress, neuro-protection, apoptosis regulation, osmoregulation, and second messenger systems. Details of gene expression will be discussed. Funding was provided by the US Army Corps of Engineers. Special thanks to Wilfrido Contreras-Sánchez, Beth Siddens, Rob Chitwood, Janine La Paz, Amarisa Marie, Shaun Clements, Ruth Milston, Molly Webb, Grant Feist, Sam Bradford, Tammy Black, Chris Whipps, Marta Alonso, Estela Thoman, and Davis Sequencing, Inc.
The Atlantic salmon (*Salmo salar*) are one of Canada’s most important recreational fish, and are now classified as an endangered species in parts of Canada and the United States. Reasons for the observed population declines are believed to be many, and include variables such as overfishing, pollution, habitat degradation and temperature stress. Water temperature is known to be one of the most important environmental variables affecting fish. High stream temperatures between 23°C and 25°C have been observed to cause mortality in trout populations (Bjornn and Reiser 1991). Although Atlantic salmon can tolerate slightly higher temperatures, 27-28°C, depending on their life stage (Garside 1973), the sub-lethal impacts of temperature on molecular and physiological processes within the various life history stages of Atlantic salmon are less understood. Previous studies have shown that behavioural changes in Atlantic salmon start to occur at temperatures well below their lethal maximum, in the range of 22-24°C, when they start searching for refuge (Cunjak et al. 1993). While these behavioural changes are helpful in determining stressful temperatures for fish, changes at the molecular level may be the first “early warning signals” of sub-lethal heat stress that will later be manifested at an organismal or population level.
One of the most common molecular indicators, or biomarkers, of temperature stress in all organisms is the heat shock response. This response is characterised by a dramatic change in the pattern of gene expression resulting in a rapid induction of heat shock mRNA and protein translation, and simultaneous repression of synthesis of other cellular proteins (Lindquist 1986). Despite the fact that many North American fish populations may currently be affected by warm temperatures in their aquatic ecosystems, few studies have examined both the thermal behaviour of these systems and the biological impacts of warm temperatures on resident species. The goal of the present study was therefore to further examine this issue on one of Canada’s most important Atlantic salmon river systems, the Miramichi. This study combines laboratory experiments with temperature monitoring and fish sampling in the wild to determine whether Atlantic salmon parr from the Miramichi River in New Brunswick are currently experiencing significant sub-lethal heat stress during the warm summer months.

Experiments done on wild Atlantic salmon parr under controlled laboratory conditions indicated that Hsp 70 mRNA and protein and Hsp 30 mRNA were all significantly induced between 22 and 25°C. Field sampling was done within two different Miramichi rivers, Catamaran Brook and the Little Southwest Miramichi River, chosen to reflect the range of thermal characteristics observed within the Miramichi River basin. Salmon parr were sampled from both of these rivers during moderate spring temperatures and a high temperature event in summer. This field data further indicated that the threshold for mRNA induction of both Hsp 70 and 30 is around 23°C, but Hsp 70 protein levels were only significantly elevated in the field at 27°C, a temperature that was observed in the more wide and shallow Little Southwest. Hsc 70 mRNA and protein levels were not significantly increased during heat stress under laboratory conditions. In the field, however, Hsc 70 mRNA was significantly increased at 23°C and both Hsc 70 mRNA and protein levels were elevated at 27°C.

Analysis of temperature data collected in the present study indicates that Atlantic salmon in the Miramichi River system may have to cope with water temperatures that frequently exceed 23°C. Records showed that water temperatures in the Little Southwest exceeded 23°C for an average of 29 days each summer over the past ten years. Since denaturation and aggregation of proteins is believed to be the primary signal for hsp mRNA induction, our combined results can be viewed as strong evidence that Miramichi River Atlantic salmon parr are probably experiencing significant protein damage in the wild for a significant portion of the summer. This issue clearly warrants further investigation since induction of a heat shock response has been shown to suppress the synthesis of other proteins (Parsell and Lindquist 1993).
In summary, the combination of heat shock mRNA and protein induction profiles obtained from both laboratory experiments and field sampling, as well as river temperature records, in the present study clearly show that Miramichi Atlantic salmon parr are commonly exposed to significant heat stress during the summer months. These results provide further evidence that the hsp response does commonly occur in some wild populations. Although this hsp response is an important adaptation contributing to the survival of organisms under extreme environmental conditions, it can also be viewed as an “early warning” that a given population may be experiencing significant sub-lethal thermal stress. In this regard, our findings also suggest that any further increases in water temperatures as a result of climate change could have profound consequences for one of Canada’s most productive Atlantic salmon rivers.

References


LIPOXYGENASE AS AN INDICATOR OF FISH STRESS
FROM THE AQUATIC ENVIRONMENT

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Three types of lipoxygenases (LOX) were identified in the gill of grey mullet (Mugil cephalus). The bacteria-infected mullet sampled from the culturing ponds that showed exophthalmia, hemorrhaged eyes, and pink-discolored gills have LOX activity 5 fold of that in the normal fish. Activity of 5-LOX was higher in the infected fish than in the normal ones. However, in both cases, 5-hydroxyeicosatetraenoic acid (5-HETE), the product of 5-LOX was not found in gill tissue.

Content of 12-HETE was lower in the infected fish, while 15-HETE was found not being affected by the infection. In addition, our in vitro studies showed that three inhibitors of 5-LOX, diphenyl sulfide, nordihydroguaiaretic acid (NDGA), and green tea extract inhibited leukotriene A₄ synthase at the same time, indicating that both enzyme activities are from a single protein. Therefore, 5-LOX is expected to function in fish gill in a two-step process similar to that found in the leukotriene biosynthetic pathway in mammals. It first catalyzes the dioxygenation of arachidonic acid to hydroperoxyeicosatetraenoic acid (5-HpETE), then catalyzes the dehydration of 5-HpETE to form leukotriene A₄. In mammals, this unstable intermediate is further converted to leukotriene B₄ which has potent pro-inflammatory property.

Physiological responses of fish to environmental stress from heavy metal were
also studied. When tilapia and grey mullet were exposed to acute toxicity concentration of cadmium (Cd), accumulation of Cd increased continuously in liver with duration of exposure, while Cd in gill also increased initially then reached a plateau. No accumulation was found in the muscle or blood. Discoloration of liver, saprolegniosis, fin rot and lesions of scales appeared in these Cd-intoxicated fish. Hematological indices including leukocytes, erythrocytes, hematocrit, and hemoglobin decreased with increased Cd concentration or prolonged exposure. Mean corpuscular hemoglobin concentration (MCHC) decreased upon exposure to Cd.

In tilapia, after 19 h of exposure, activity of 5-LOX in gill increased to 23 times and 12-LOX increased to 17 times. Both of them decreased afterwards. 15-LOX decreased in activity with exposure time to 25% of the initial activity after 49 h. Similar trend of changes in LOX activities was found in grey mullet gill. Both tilapia and grey mullet gill showed that activities of 5-LOX and leukotriene A₄ synthase changed in a similar pattern with duration of exposure to Cd. Leukotriene metabolites were identified in gill tissues of both fishes. This evidence again indicated that 5-LOX was induced by the stress from the heavy metal pollution and produced 5 HpETE which was immediately transformed to leukotriene A₄. Other metabolites were formed possibly via the same mechanism as found in human and rats.

In medicinal studies, evidence has shown that leukotrienes play a putative role in human diseases while leukotriene antagonists and lipoxygenase inhibitors especially that against 5-LOX have therapeutic potentials. Our observations showed that fish bear similar lipoxygenase and leukotriene mechanisms to those in humans. Natural antioxidants, e.g., flavonoids, inhibited lipoxygenase and improved significantly the blood fluidity and low temperature adaptation for grey mullet to maintain growth through winters.

For fishes, the environmental stress factors may come from chemical or microbial pollutants, each of which may fall under action levels. But abnormality or mortality may come as a result of the collective effects. Therefore, we propose that the overall stress developed in the fish habitat may trigger the defense mechanisms reflected by the synthesis of LOX and the accumulation of the 5-LOX catalyzed leukotriene and the further metabolized products. Our objective is to find a feasible stress indicator to serve as an “environmental watch” before outbreak of mass mortality, and human health hazard by consumption of the marine organisms under stress.
THE CRH RESPONSE TO STRESS AND TO BACTERIAL LIPOPOLYSACCHARIDES IN TILAPIA

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Corticotropin-releasing hormone (CRH) is a key hypothalamic factor controlling the activity of the hypothalamo-pituitary-interrenal (HPI) axis. Under stressful conditions CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary, which in turn stimulates the release of cortisol from the interrenals. At least in mammals CRH also plays an important role in the regulation of feeding behaviour and in immune-endocrine interactions.

Our previous results revealed that the tilapia CRH peptide shares a low homology with mammalian CRH, but at least in vitro tilapia CRH stimulated α-MSH and ACTH release from the tilapia pituitary (Van Enckevort et al., 2000). Recently, combined immunohistochemical and RIA results demonstrated that CRH occurs widespread throughout the brain (including the forebrain) of tilapia, which suggests that CRH is involved in many non-pituitary related functions (Pepels et al., 2002).

To investigate whether CRH is also involved in immune-endocrine interactions in fish, tilapia were first treated in vivo with lipopolysaccharides (LPS; endotoxins) from either Escheria coli or Vibrio cholerae. For teleosts it has been demonstrated that LPS treatment in vivo activates the PI-axis (Wedemeyer 1969, White & Fletcher 1985), but no investigations have been done concerning the role of CRH in these regulations. Previous in vitro studies in tilapia (Balm et al., 1994) showed that LPS treatment affects the cortisol release from the headkidneys and the α-MSH / ACTH release from the pituitary.

As quantitative CRH changes in the present study were induced by the in vivo LPS treatments, both under basal conditions as in response to confinement, brain...
tissues were subsequently incubated in vitro with LPS to study the mechanisms of action involved.

Overall, the treatments altered CRH levels in brain regions which do not directly regulate the pituitary, which is consistent with the notion that also in fish CRH exerts pituitary independent functions following activation of the immune system and during stress.

References


LIFE AND DEATH IN A TOILET BOWL: THE EFFECTS OF
CHRONIC SUB-LETHAL AMMONIA EXPOSURE
ON IMMUNOPHYSIOLOGY OF RECENTLY SMOLTED
CHINOOK SALMON

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Ammonia is an unusual toxicant in that it is produced naturally by animals. It is released into the environment through production of industrial fertilizers and biological wastes. It is a compound that is toxic to animals if accumulated in the
body tissues and fish rely mainly on diffusion down the concentration gradient between body and water to eliminate ammonia waste. The current North American saltwater standards are based on a limited marine database and the toxicity tests follow standard guidelines using static water conditions, unfed, unstressed, resting animals. It is under these unrealistic conditions that internal ammonia production is minimal. In addition, the available literature generally expresses concern primarily with growth, survival, and reproduction in fish. We were interested in the applicability of these standards to more realistic conditions (fed fish in culture situations and in nature) and asked the question… “….is the health status of these animals impacted?” The data we accumulated points to significant effects on health parameters and a reduction in disease resistance in fish exposed to acceptable environmental ammonia levels.

This study was designed to consider the effects of a sub-lethal chronic ammonia exposure on physiological and cellular stress responses of recently smolted chinook salmon and to determine if such a low-grade ammonia exposure affected disease susceptibility. Unvaccinated chinook salmon juveniles weighing approximately 20g obtained from Big Tree Creek Hatchery were acclimated and smolted at the Bamfield Marine Station in outdoor tanks containing seawater at 11.1°C, pH 7.8, salinity 31 ppt and fed a daily ration of approximately 2% body weight per day. Ammonia (as NH₄Cl) was introduced into the tanks at two concentrations, 2.5 and 10 mg/L (2.5 ± 1.1 and 12.1 ± 1.9 mg/L N actual dose as determined from water samples (indophenol blue)). Both test levels are below the acute standard (2.5 mg/L is also below the chronic standard) and resulted in increased internal levels of ammonia in the fish. Neither treatment level affected feeding rates and, during the course of the exposure, there was no mortality in any of the tanks. At four time periods (6 hrs, 48 hrs, 96 hrs, and 244 hrs) fish were terminally sampled from each of five tanks (two tanks per treatment, one tank controls).

Lysozyme is a bacteriolytic enzyme produced by phagocytic cells that is involved in the destruction of invading pathogens and has been shown to increase in the plasma following an acute stress (Demers and Bayne, 1997). Lysozyme activity in plasma was determined by a modification of Litwack (1955) according to (Maule et al. 1996). Plasma lysozyme activity increased significantly (p<0.05, n=12-16) in both treatment groups compared to control fish. At 96 h post exposure both treatment groups had significantly higher levels than controls (mean value 2.33 ± 0.42 µg HEWL Eq) and fish exposed to 10mg N/L (mean value 4.05 ± 0.29 µg HEWL Eq) had significantly higher activity than did fish exposed to 2.5 mg N/L (mean value 3.18 ± 0.20 µg HEWL Eq). By
244 h, both treatment groups had lower lysozyme activity levels than controls with fish exposed to 2.5 mgN/L (1.99 ± 0.14 µg HEWL Eq) having significantly lower activity levels than both control fish (2.74 ± 0.31 µg HEWL Eq) or fish exposed to 10 mg N/L (2.54 ± 0.18 µg HEWL Eq).

Preparation of tissues, and dilution and determination of hsp70 was carried out by ELISA according to Ackerman and Iwama (2001) as a modification of Forsyth et al (1997). There were significant differences in liver hsp70 levels between the treatment groups at 48 h (p<0.05, n=6) (Figure 1). Fish exposed to 2.5 mg N/L had levels that were significantly higher than control fish or fish exposed to 10 mg N/L. Hsp70 levels in the head kidney tissue showed no significant differences over time or between treatments at p<0.05.

Following treatments, all fish were moved to clean water and challenged by i.p injection with the pathogen *Vibrio anguillarum* (1.7x10⁶ cfu/mL). The challenge method and *V. anguillarum* isolate are outlined in Ackerman and Iwama (2001). Control fish had the lowest mortality at 87%. Fish exposed to 2.5 mg N/L had a total cumulative mortality of 92% which was

![Figure 1. Liver and head kidney tissue levels of hsp70 in recently smolted chinook salmon exposed to ammonia (0, 2.0, and 10.0 mg N/L at pH 7.8 for 6, 48, 96, and 244 hours. Significant differences (p<0.05, n=6) between treatments within a sampling period are indicated by an asterisk. Letters indicate differences between time periods within treatment.](image)
not statistically different from control fish. Exposure to 10 mg N/L for 244 hours resulted in 100% cumulative mortality when pathogenically challenged. This was a statistically significant difference from control fish (Figure 2.)

In addition to the above data, we also saw significant effects on respiratory burst activity of glass adherent white blood cells, plasma cortisol and glucose concentrations, and differential blood cell numbers. The data we accumulated points to significant effects on health parameters and a reduction in disease resistance in fish exposed to currently acceptable environmental ammonia levels. We believe that we have indicated the need for the re-evaluation of the existing ammonia standards for the marine environment.

References


Acknowledgments

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PHYSIOLOGICAL EFFECTS OF LIVE-RELEASE ANGLING TOURNAMENTS

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EXTENDED ABSTRACT ONLY – DO NOT CITE

In recent years, the practice of “catch and release” angling has been an important tool for the management and conservation of recreational fish species. One area where “catch and release” has played an extremely important role is in competitive fishing. In recent years, most high profile competitive fishing events (ie tournaments) in North America have adopted a “live-release” format. In response to the growing popularity of live release tournaments for largemouth bass, numerous studies have attempted to monitor survival following these events. To date, however, most research concerning survival at tournaments has focussed on the correlation of environmental variables with mortality. These studies have produced some valuable information, but many important issues in this area have yet to be resolved because of the limitations of the approaches that are traditionally used in this area (ie main focus on monitoring immediate and/or delayed mortality).

We are now involved in a large-scale project to examine the physiological impacts of live-release tournaments on three important recreational species: largemouth bass, smallmouth bass and walleye. This project has several objectives. First, we are determining the physiological status of these species following a number of tournaments in order to gain insight into the nature of
their physiological disturbance following these events. In addition, we are conducting experimental tournament simulations with these species in which we sample fish after the different stages of a tournament (e.g., angling, livewell confinement, weigh-in). These tournament simulation experiments will provide important information about the relative contribution of different tournament practices towards the overall physiological disturbance. Finally, we are also conducting many experiments that examine other significant tournament issues and explore ways to reduce the physiological disturbance in fish during live-release tournaments. Taken together, the results of these studies will be used to minimize the biological impact of live-release tournaments on these important recreational species.

This presentation will mainly focus on the physiological changes that occur in largemouth bass during live release angling tournaments. During the summer of 2000, several live release bass tournaments in Ontario were visited to obtain tissue and blood samples from fish following weigh-in procedures, and a number of physiological parameters were compared in tournament-caught bass and resting control bass. Analyses showed that the plasma cortisol concentrations of tournament-caught fish were much greater than control individuals. Interestingly, however, the plasma cortisol concentrations in a group of control bass sampled after being held for several days in our aquatic facility were considerably higher than those in bass sampled immediately after angling in the wild. These results indicate that plasma cortisol levels in largemouth bass are very responsive to any type of disturbance. Plasma osmolarity was elevated in bass following tournaments and there was no evidence of significant ion losses that are known to occur following severe chronic stresses. The mRNA levels for an inducible heat shock protein (hsp 70) were not changed in tournament fish and differences in creatine phosphokinase levels between tournament-caught bass and control bass were not significantly different. These results seem to indicate that tournament fish do not normally experience significant cell or tissue damage.

Muscle variables were also very different between tournament and control bass. The muscle energy reserves, phosphocreatine, ATP and glycogen in tournament bass were reduced by 92%, 60% and 75%, respectively, relative to those in control bass. Lactate concentrations in the muscle and blood of tournament bass were also significantly elevated. The results for muscle energy reserves and lactate are very similar to those following exhaustive exercise and explain why largemouth bass are often very lethargic following live-release tournaments.
Taken together, these results suggest that, following a live-release angling tournament, largemouth bass have experienced a large decline in muscle energy reserves, an accumulation of lactate, and changes in osmotic balance, but have probably not experienced significant cell or tissue damage. These events also cause an increase in traditional indicators of stress such as plasma cortisol and glucose levels. Although there is a relatively large physiological disturbance in largemouth bass after tournaments, these physiological changes are normally reversible and we observed very little mortality in largemouth bass after tournaments. Indeed, largemouth bass that were transported to the laboratory after tournaments almost always survived.
EFFECTS OF CONFINEMENT STRESS AND CORTISOL ON THE 
SUSCEPTIBILITY OF CHANNEL CATFISH TO INFECTION BY 
ICHTHYOPTHERIUS MULTIFILIIS AND CHANNEL CATFISH VIRUS

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Many diseases of fish are referred to as opportunistic to indicate that the pathology caused by the infectious agent usually occurs after the protective mechanisms of the fish have been suppressed. Immunosuppression and disease outbreaks often follow an episode of stress, which can be induced by a variety of conditions frequently encountered in aquaculture. One of the primary stress responses is the secretion of cortisol. Cortisol has many biological activities including the induction of gluconeogenesis and the suppression of the inflammatory response. The role of cortisol in increasing disease susceptibility is difficult to assess when fish are stressed by physical means (such as handling) due to the other physiological responses which also occur. The objective of the present studies was to determine if confinement stress and the chronic administration of cortisol resulted in similar changes in susceptibility to the
protozoan parasite *Ichthyophtherius multifiliis* (ich) and to channel catfish virus (CCV).

**Handling Stress**- Yearling channel catfish were exposed for 15 minutes to 2,500 theronts/l of *I. multifiliis* after a low-water confinement stress of 0, 2 or 6 hours. Six days after exposure to ich, the trophont density on the skin was determined and reported as trophonts per cm². A second group of fish was used to determine the physiological stress response of fish exposed to the low-water confinement stress. Blood samples were taken before, 2 and 6 hours after confinement. Plasma cortisol, glucose, and chloride concentrations were measured. Channel catfish fry about 4 weeks old were challenged with 0, 3.3X10⁴, 3.3X10⁵ or 3.3X10⁶ plaque forming units of CCV in 400 ml water after 0, 2 or 6 hours of confinement stress. Mortality was determined during the 14 days after exposure.

Plasma cortisol and glucose, but not chloride, were significantly higher after 2 and 6 hours of confinement. This is a typical response, which has been documented several times previously. Fish exposed to 6 hours of stress prior to ich exposure had a significantly higher infection rate than control fish. Fish stressed for 2 hours had an intermediate infection rate not significantly different from the non-stressed fish or those stressed for 6 hours. Mortality after CCV exposure was related to the dose of the virus but was not affected by confinement stress.

**Dietary Cortisol**- Yearling channel catfish were fed for 9 days at 1% of the body weight per day with feed containing 200 mg cortisol per kg of feed. Plasma samples were taken immediately before the last feeding and at 2, 4, 6 and 12 hours after the last feeding and analyzed for cortisol.

Yearling channel catfish were offered feed containing 0, 100 or 200 mg cortisol per kg feed for 13 days. An initial blood sample was taken 24 hours after the last feeding. Fish were then exposed to a low-water confinement as above for 0, 2 or 6 hours and blood samples were taken and analyzed as above. A second group of fish, fed the same diets was exposed to ich theronts and the infection determined as above. Channel catfish fry offered cortisol-containing feed were exposed to CCV and mortality evaluated as above.

Plasma cortisol concentrations of cortisol-fed unstressed fish were significantly higher than controls 2, 4, and 6 hours after feeding. The highest concentration occurred six hours after feeding. When fish were fed cortisol and exposed to a
confinement stress 24 hours after the last feeding, fish fed the control diet displayed a typical cortisol stress response. Stressed fish had cortisol concentrations about four times that of unstressed fish. However, fish fed 100 or 200 mg cortisol per kg feed displayed no increase due to confinement. The hepatosomatic indices of fish fed cortisol were significantly smaller than controls under all experimental conditions.

**Conclusion**

These data suggest that confinement-induced stress and orally administered cortisol increase the susceptibility of channel catfish to infection by ich, but not to mortality due to CCV. The defense mechanisms which protect fish from parasite infections may differ from defense mechanisms against virus infection and cortisol may differentially affect these defense mechanisms. The fish used in these studies had no previous history of exposure to ich or CCV, therefore, likely both innate and acquired immunity systems were involved. Several papers have shown both handling stress and exogenous cortisol increase the sensitivity of fish to bacterial infections. Plasma cortisol concentrations from cortisol-fed fish were highly variable and likely reflect different feeding success among the fish. Cortisol fed once daily likely results in a large single spike in the blood each day which clears the blood by the next feeding. Cortisol-fed fish were not able to mount a confinement-stress-induced increase in cortisol likely due to a negative-feedback-induced suppression of ACTH or CRF.
THE EFFECT OF STRENUOUS EXERCISE AND β-ADRENERGIC BLOCKADE ON THE VISUAL PERFORMANCE OF JUVENILE RAINBOW TROUT, ONCORHYNCHUS MYKISS

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EXTENDED ABSTRACT ONLY- DO NOT CITE

A wide variety of stressors are known to increase the susceptibility of fish to predation. The underlying causal mechanisms remain poorly understood but a stress-related disruption to the visual sense has been hypothesised (Mesa et al., 1994).

Rainbow trout, in common with many other teleosts, possess Root effect haemoglobins that have reduced oxygen-carrying capacities under conditions of low pH. The physiological significance of this effect is strongly linked with the choroid rete mirabile that generates an extremely high partial pressure of oxygen within the ocular fluids (PO$_2$ = 445 mmHg)(Pelster and Randall, 1998).

An extreme bout of strenuous exercise can significantly affect the blood-oxygen transport of trout because the partial pressure of oxygen and pH of arterial blood is reduced (Milligan and Wood, 1987). A significant drop in arterial Hb-O$_2$ binding (27 - 32 %) is expected in the absence of β-adrenergic responses. It is hypothesised that these post-exercise responses will be a liability to high ocular PO$_2$ due to the restricted binding of oxygen at the gills, premature Hb-O$_2$ dissociation within the arterial blood supply of the choroid rete, and the diffusive shunt of unbound oxygen to the venous capillaries involved in counter-current exchange (Pelster and Randall, 1998).
Given that high levels of ocular PO$_2$ are necessary for trout retinal functions (Fonner et al., 1973), the present study has examined the effect of strenuous exercise on the visual performance of the rainbow trout. β-adrenergic blockade was also used as a tool to assess the potential role of red cell volume regulation in maintaining Hb-O$_2$ affinity and hence the visual performance of fish post-exercise.

The ability to resolve moving objects, as an ecologically relevant form of visual performance, was determined behaviourally using the optomotor response. Fish that are able to resolve high contrast objects on a moving background exhibit an optomotor response by behaviourally orientating with the background in an attempt to stabilise an image on the retina (Douglas and Hawryshyn, 1990). Fish were contained within a clear, circular holding tank and were exposed separately to 5 visual backgrounds that encircled the tank at 4 revs min$^{-1}$. The high contrast backgrounds consisted of alternating black and white bars that subtended different visual angles (i.e. 60, 120, 180, 240 and 300 min of arc) on the fishes’ eye. Fish were also exposed to a control white background (i.e. 0 min of arc). The behavioural response of fish to the moving backgrounds was monitored over a 3 min period and the magnitude of the optomotor response was quantified according to angular swimming velocity (net revolutions min$^{-1}$). The optomotor response threshold was defined as the minimum visual angle required to induce a significant optomotor response (with respect to control (i.e. 0 min of arc) reactions).

Two experiments were conducted to examine the effect of strenuous exercise (Experiment 1) as well as β-adrenergic blockade (Experiment 2) on the optomotor response threshold of juvenile trout (Mean wt ± S.D., 14.0 ± 1.8 g). The behavioural optomotor response of individual fish was therefore screened in a relative “resting”, “post-exercise” and “post-exercise/propranolol” state. These states were monitored physiologically by collecting blood (via caudal venepuncture) and measuring haematocrit (Hct), haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCHC as an indicator of red cell swelling) and blood lactate.

Strenuous exercise induced a metabolic acidosis (8.0 mmol l$^{-1}$ blood lactate) and a significant red cell swelling response but no change in the optomotor response threshold (120 min of arc) was observed between pre- and post-exercise fish (Figure 1).
Figure 1. The effect of subtended angles on the optomotor response of trout both pre-exercise (open symbols) and post-exercise (closed symbols). The dashed line indicates the angular velocity of the moving background in a positive (clockwise) direction. Error bars represent 95% confidence intervals. $T_1$ and $T_2$ represent the optomotor response thresholds for the pre-exercise and post-exercise fish respectively.

Beta-adrenergic blockade (propranolol) abolished post-exercise red cell swelling but optomotor response thresholds were still maintained at 120 min of arc despite a significant blood lactate load (7.8 mmol l$^{-1}$)(Figure 2). Surprisingly, exercise-propranolol fish exhibited an enhanced optomotor response at 240 - 300 min of arc.
Figure 2. The effect of subtended visual angles on the optomotor response of resting (open symbols) and exercise-propranolol (closed symbols) trout. The dashed line indicates the angular velocity of the moving background in a positive (clockwise) direction. Error bars represent 95% confidence intervals. $T_1$ and $T_2$ represent the optomotor response thresholds for the pre-exercise and exercise-propranolol fish respectively. † represents a significant difference between resting and exercise-propranolol reactions at the respective angle.

We suggest that the treatment of post-exercise fish with propranolol may have induced “tunnel vision” and strengthened optomotor reactions as a result of a restricted field of view being predominantly exposed to moving visual stimuli. It is possible that adrenergic regulation of Hb-O$_2$ affinity may possibly maintain high gradients of ocular PO$_2$, which satisfies the metabolic demand of peripheral retinal cells, after strenuous exercise. We conclude that strenuous exercise does not affect the ability of trout to resolve high contrast moving objects but future work should examine the possibility that stressed trout have impaired peripheral vision.
References


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THE EFFECTS OF CHRONIC STRESS ON SWIMMING PERFORMANCE, STANDARD METABOLIC RATE AND METABOLIC SCOPE FOR ACTIVITY IN GREEN STURGEON, ACIPENSER MEDIROSTRIS.

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Introduction

Identifying accurate and inclusive indices of fish stress and health that are reliably and practically measured has interested fish biologists for decades. Investigators have examined alterations in multiple physiological parameters from the molecular to the organismal levels. There are several physiological parameters that can serve as indicators of stress or poor health in fish including hematocrit, and circulating lymphocyte, hemoglobin, and red blood cell concentrations (Schreck, 1996). Attenuation of the reproductive system during times of stress or declining health has also been reported (Haddy and Pankhurst, 1999). In addition, acid-base and osmoregulatory perturbations are very common measures of fish health and stress. Although these physiologically...
based measures inform the investigator that specific systems are impacted during times of poor health, most of these reveal little information about the energetic status or potential long-term survivability of fishes, when considered alone.

Investigations into organismal indices of fish health and stress circumvent the specific nature of physiological or molecular measures, due to their inherent level of integration. Examples include growth rate and body composition measures, which provide estimates of how an organism is utilizing available resources. More short-term measures of an animal’s energetic expenditures and available metabolic resources would be the standard metabolic rate (SMR) and metabolic scope for activity (MSA), respectively. Swimming performance (e.g., as critical swimming velocity, $U_{crit}$) could also be utilized to estimate aerobic capacity and general health in fish. However, significant variation among individual fish regarding acclimation to experimental devices and willingness to swim in laboratory settings can present drawbacks to using organismal measures of fish health and stress.

**Approach and Methods**

We investigated the impacts of chronic stress on the SMR, MSA, and $U_{crit}$ in green sturgeon, *Acipenser medirostris*. The research model theorized that repeated acute stressors sum to result in “chronic stress.” Our approach was based upon a bioenergetic relationship, which hypothesizes that chronic stress will increase maintenance requirements for the fish to maintain homeostasis. The increased energy requirements would result in a significantly increased SMR, decreased MSA, and decreased energy resources for the immune system, reproductive system, growth and general health. If the stressors are severe enough or prolonged, the animal is thought to enter a pre-pathological state, leaving the animal more susceptible to pathogens (Moberg, 1985; Fig. 1).

To simulate chronic stress, groups of three young-of-the-year green sturgeon were placed into one of five identical, flow-through, in-door tanks and maintained on a natural photoperiod. Fish were stressed twice a day (1000 and 1600 h) for 28 consecutive days by exposure to two of three randomized stressors: a 5-min confinement stressor, a 5-min chasing stressor, or a 10-min water depth reduction stressor (to the dorsal surface of the fish), prior to metabolic and swimming experiments. Control animals were undisturbed prior to their experiments.
Measurements of SMR, MSA, and $U_{\text{crit}}$ were conducted using a closed, variable speed, Brett-type respirometer (Brett, 1964). The SMR was measured at approximately 0800-1000 h with the velocity in the respirometer at 10 cm s$^{-1}$. This velocity adequately circulated the respirometer water without inducing the fish to swim. The velocity was then increased to 35 cm s$^{-1}$ for 45 min, the respirometer water PO$_2$ was measured at the beginning and end of the 45-min period, and the O$_2$ consumption rate was calculated following Cech (1990).
respirometer water velocity was then increased 5 cm s\(^{-1}\) in a step-wise manner at 1-h intervals, with corresponding \(\text{O}_2\) consumption rates measured, until the fish fatigued. The MSA was calculated by subtracting the SMR from the highest (“active”) metabolic rate measured, and the \(U_{\text{crit}}\) was calculated following Brett (1964).

**Results and Conclusions**

Exposure to the chronic stress regime resulted in an increased SMR and a trend suggesting a reduced MSA, consistent with the hypotheses that acute stressors sum to simulate chronic stress and that chronic stress affects the SMR and MSA. Interestingly, there was no difference in \(U_{\text{crit}}\) between the stressed and control fish, and the stressed fish displayed positive growth throughout the 28-day regime. We conclude that our chronic stress regime resulted in a significant metabolic cost to green sturgeon, as indicated by the SMR and MSA measurements, but may not have jeopardized the overall health of these fish, as indicated from the \(U_{\text{crit}}\) and growth measurements.

**References**


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THERMAL TOLERANCE, SWIMMING PERFORMANCE, AND METABOLIC PHYSIOLOGY OF WILD SALMONIDS – LESSONS FROM REDBAND TROUT IN SOUTHEASTERN OREGON

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Introduction

Redband trout (Oncorhynchus mykiss ssp.) inhabit high elevation streams in southeastern Oregon, with extreme variability in seasonal flow and periods of high stream temperatures during midsummer. Given the strong influence and potential limitations exerted by temperature on fish metabolic demands and management interests in this subspecies, the objective of this study was to determine how acute temperature change, thermal history, and geographic separation affect the physiological capabilities and biochemical characteristics of these trout.

Study Sites, Experimental Animals, and Methods

During July and August of 2000, we conducted streamside measurements of critical swimming speed ($U_{crit}$), critical thermal maximum (CTM), and oxygen consumption at environmentally relevant temperatures for wild redband trout in Bridge, Rock, and 12-mile Creeks. These 3 streams contain genetically distinct populations (Gamperl et al. submitted), and both Rock and 12-mile Creeks are much warmer in the summer months than Bridge Creek. We also collected
samples for subsequent measurements of morphometrics, genetic variability, and biochemical indices of energy metabolism in the heart (ventricle), axial white skeletal muscle, and blood. To minimize capture stress and injury, fish were collected by anglers using dry flies and barbless hooks. All fish were held for 2-5 days prior to metabolic studies or sampling of tissue.

\( U_{\text{crit}} \) and metabolic capacity was determined by swimming fish in Blazka swim tunnel respirometers at increasing water velocities until they reached exhaustion. For CTM, water temperature in respirometers was initially set to 14°C and increased by 2°C h\(^{-1}\) until the fish lost equilibrium and was unable to swim. This rate of temperature increase approximated the maximum rate of heating that redband trout experience during a diurnal temperature cycle. Water velocity was maintained at approx. 0.5 bl sec\(^{-1}\). We also measured swimming performance of fish at 24°C. Metabolic Power was calculated as maximum \( \text{MO}_2 \) \( (\text{MO}_2\text{max}) \) measured at maximum swimming speed) minus routine \( \text{MO}_2 \) \( (\text{RMO}_2 \text{ at } 0.5 \text{ bl sec}^{-1}) \).

Additional trout were anesthetized and blood samples were drawn for measurement of plasma osmolality, electrolytes, hemoglobin and circulating lipid substrates. We weighed the cardiac ventricle and excised epaxial white muscle for measurement of maximal activities of citrate synthase (aerobic capacity) and lactate dehydrogenase (anaerobic capacity). ANOVAs (one-way and repeated measures) plus ANCOVAs were used to examine whether variables differed between trout within and between streams. Differences were considered significant when \( P<0.05 \).

**Results and Discussion**

*Adult versus Juvenile: Bridge Creek*

\( \text{RMO}_2 \), \( \text{MO}_2\text{max} \), and metabolic power all scaled allometrically with body mass at 24°C and there was no significant difference in relative \( U_{\text{crit}} \) values between adult (400-1400 g, 3.2 bl s\(^{-1}\)) and juvenile (40-100 g, 3.9 bl s\(^{-1}\)) redband trout. However, adult trout swam more efficiently than juveniles. \( \text{RMO}_2 \) in adult and juvenile redband trout increased at similar rates as water temperature was raised from 14° to 22°C, however, the metabolic response of these two groups between 22° and 26°C suggests that only adults have a high thermal sensitivity and may not withstand environmental challenges at temperatures above 24°C (Fig. 1). Surprisingly, the CTM of both size classes at Bridge Creek, and the other populations of redband trout was approximately 29°C.
Fig. 1. Routine metabolic rate (MO$_2$) vs. water temperature for large (400-1400 g; n = 5) and small (40-140 g; n = 7) redband trout as measured during critical thermal maximum (CTM) tests at Bridge Creek. Vertical bars show ± 1 SE Numbers next to the symbols indicate reduced sample size due to variability in the CTM or exclusion of trout exhibiting high activity levels.

Comparisons Between Populations: Juveniles
RMO$_2$, MO$_2$ max., metabolic power, and $U_{crit}$ at 24°C were not significantly different between juvenile trout in Bridge, Rock, and 12-mile Creeks. However, analysis of the relationship between COT and swimming speed revealed that Bridge Creek trout swam more efficiently than those from 12-mile or Rock Creeks (Fig. 1). The minimum COT of Bridge Creek trout was 30% lower than for trout from the other two locations, and the COT of these fish was markedly lower at swimming speeds in excess of 40 cm s$^{-1}$. 
Bridge Creek redband trout had 20% smaller ventricles than the other populations. Animals from 12-mile Creek, the warmest stream, were hyperkalemic and had significantly lower levels of plasma triglyceride and free fatty acids compared to Bridge Creek trout. These data provide evidence that the health and nutritional status of redband trout from 12-mile Creek were compromised. Other hematological variables were identical between populations. Lactate dehydrogenase activity in white skeletal muscle scaled with animal size and we did not see interpopulation differences in muscle enzyme activities. In summary, redband trout in southeastern Oregon display differences in thermal sensitivity and swimming efficiency according to body size and stream population, respectively. It is noteworthy that redband trout were capable swimmers at even 24°C, although their CTM values were similar to those in the literature. The take-home message is that redband trout in southeastern Oregon are not uniquely thermally-tolerant compared with other salmonids, but they may display phenotypic variability that promotes performance at warmer temperatures.
Figure 2. (A) Absolute critical swimming velocity vs. metabolic power for redband trout as measured at 24°C. The regression lines and the 95% confidence intervals for the relationships are shown. (B) Total cost of transport vs. swimming velocity for small redband trout from Bridge, Rock, and Twelve-mile Creeks at 24°C. Lines show fitted least-squares regression curves with 95% confidence intervals.

Acknowledgements

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FISH-PERFORMANCE ECOASSAY OF URBANIZING STREAMS IN

THE SAN ANTONIO RIVER BASIN, TEXAS

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EXTENDED ABSTRACT ONLY- DO NOT CITE

The San Antonio River Basin of Texas is characteristic of watersheds that are heavily impacted by urban, industrial, and agricultural development. Bluegill (Lepomis macrochirus) were used to perform an ecological assay of stream health in urbanizing watersheds of the upper San Antonio River Basin, during summer 1999 and 2000, and during winter 2000-2001. In each of the three ecoassays, caged bluegill were exposed continuously for 10 – 22 days, to in-stream conditions at mid- and lower-watershed sites both on Leon Creek and Salado Creek. While in the cages, fish were fed freshly chopped earthworms or whole, live mealworms. In-stream temperature, dissolved-oxygen concentration (DO), pH, and conductivity were measured near each set of cages and recorded every 15 min. Observed weight-change rate (treatment medians, −0.56 to 3.01 %·day⁻¹) and average daily mortality rate (0 to 2.96 %·day⁻¹) were used as in-stream measures of fish performance. Complementing these performance measures were laboratory estimates of metabolic capacity obtained via automated routine respirometry (Springer and Neill 1988), using some surviving
fish from each stream/site/year/season treatment. Respirometry was performed with fish immediately after they had completed the in-stream phase of the ecoassay, and in water collected from their stream site and at the same time.

Although fish performance varied from place to place and from time to time, those differences were not clearly associated with “stream.” Bluegill had significantly greater rates of weight-change during summer and during the year 2000, as described by the following regression model ($R^2 = 0.44; P < 0.0001$):

$$\text{Weight-change rate (\%·day}^{-1}) = 0.05 - 1.11*(\text{Year} = 1999) + 1.43*(\text{Season} = \text{Summer})$$

No other independent factor explained significant variation in weight-change rate beyond that associated with “year” and “season.” Average daily mortality rate, transformed to its probit, tended to be greater at the downstream sites, less in summer than in winter, and more with decreasing absolute minimum DO ($R^2 = 0.27; P < 0.01$); the regression model was as follows:

$$\text{Probit of Average Daily Mortality Rate (\%·day}^{-1}, \text{per-fish basis}) = 4.09 + 0.73*(\text{Site} = \text{Lower}) - 1.71*(\text{Season} = \text{Summer}) - 0.27*(\text{AbsoluteMinDO})$$

Rates of routine metabolism and marginal metabolic scope (Neill and Bryan 1991) were higher during summer and in 1999. The temperature experienced by the fish while undergoing respirometry was the most significant predictor both of routine metabolism and marginal metabolic scope. The $Q_{10}$ of routine VO$_2$ was 2.02. On a per-treatment basis, the median of estimated metabolic scope—at DO = 3 mg L$^{-1}$, at median in-stream temperature, and logarithmically transformed—accounted for 51% of the variation in the median rate of bluegill weight-change (Figure 1).
Figure 1. Weight-change rate versus natural log transform of estimated metabolic scope at DO = 3 mg·L⁻¹, adjusted for median in-stream temperature. R² for regression = 0.51. The regression equation is %Wchng = 2.87 + 1.00*Ln(T-adj MS).

Leon and Salado creeks provided habitat of apparently equal quality for bluegill, during this study. Urbanization of these streams does not seem to have irrevocably compromised their water quality, in that bluegill performance, as measured by rate of weight-change, average daily mortality rate, and metabolic capacity, was comparable to that observed in “healthy” systems elsewhere (Gerking 1955; Lemke 1977; Springer and Neill 1988). Main differences observed in fish performance were associated with ephemeral differences in stream temperature and DO. The cage-study approach, combined with respirometric assay of metabolic status, provides a practical methodology for assessment of stream and watershed health.

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References


DIFFERENTIAL RESPONSE TO STRESS IN A POPULATION OF STRIPED BASS

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Stress is unavoidable in the aquaculture environment. Repeated or prolonged exposure of fish to common hatchery stressors such as handling and net confinement activates the hypothalamic-pituitary-interrenal axis leading to increased plasma cortisol, decreased growth rates and an increased incidence of disease (Pankhurst and Van Der Kraak, 1997). Because of the adverse effects of stress on performance, fish displaying a lower level of responsiveness to stress may perform better in a culture environment than those individuals that display a high level of responsiveness. Striped bass are an important component of the US aquaculture industry; however, the limited availability of wild broodstock and inconsistent reproduction in captivity hamper rapid expansion of the industry. If individuals with high and low responsiveness to stress could be identified, then a domestication program could be developed to select for lines of striped bass which are divergent for stress responsiveness. This would permit the evaluation to made of which trait is advantageous in a culture environment. With this goal in mind, we investigated the physiological response to stress in a
population of male striped bass maintained at the University of Maryland’s Crane Aquaculture Facility.

**Methods**

In separate experiments, we evaluated the response to: 1) repetitive semen collection (RP) during an 8-week spawning season and 2) a 1-minute net confinement provided monthly (MC) over a 6 month period to fish from 3 families (MD, MD36 and NC) of striped bass using plasma levels of cortisol as an index of the stress response. For the semen collection experiment, 16 male striped bass were removed from a 7 m diameter holding tank and placed into a 3.5 m diameter tank for easier access. The fish were handled once per week during an 8-week spawning season. Each week they were netted, anesthetized in MS-222, their abdomen was squeezed to express between 4 - 6 ml of milt, and 1.5 ml of blood was drawn from the caudal vessels. For the net confinement experiment, 36 male striped bass were maintained in a 3.5 m diameter-holding tank. At monthly intervals for 6 consecutive months, the fish were removed from their holding tank, held in a net out of water for 1 minute and then placed into a 2 m tank for easier access. After 1 hour, a time previously determined to be the peak for plasma cortisol levels in this population, groups of fish (n=6) were rapidly removed from the tank anesthetized and bled before being placed back into their permanent holding tank.

**Results and Discussion**

Circulating levels of cortisol in the RP population increased significantly (p <0.05) during the 8-week spawning season. Mean cortisol values determined for individuals during the same period ranged from 35-226 ng/ml indicating that males in this population are highly variable in their response to these stressors. Mean plasma cortisol in striped bass males exposed to the monthly net confinement were significantly lower (p <0.01) in NC family fish when compared to levels for MD or MD36 fish (see figure).

The cortisol response to the net challenge decreased significantly each month for fish sampled in July, August and September (p <0.05), leveled off in October and then decreased for the remainder of the study suggesting that the fish were adapting to the stressor. Within the MC group, there were fish that consistently demonstrated a high response and those that consistently demonstrated a low response to the net stressor. The differences in plasma cortisol levels between individuals and between the striped bass families compared in this study suggest
a heritable component to the response. Identifying individuals with a lower response to stress could enable the production of a stress-tolerant domestic broodstock with the ability to thrive in the artificial aquaculture environment.

**Literature Cited**

SHORT-TERM TRANSPORTATION STRESS AND RECOVERY
IN DELTA SMELT

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Water shortages and a growing population in southern California have led to the diversion of water from northern California to meet these demands. Many species of fish, including the threatened delta smelt (Hypomesus transpacificus) from the Sacramento-San Joaquin Delta are collected at pumping facilities, transported by truck, and returned upstream. Because delta smelt are delicate, mortalities from collection and transport stress can be high (Moyle, 2002). Swanson et. al. (1996) reported that the addition of salt and NovAqua, a commercial water conditioner, increased post-transport survival of captured delta smelt. However, transport and recovery stress responses were not assessed. To address these concerns, we subjected delta smelt (5-6 cm SL) to collection and short-term transport, and measured physiological stress parameters (plasma cortisol, pH, glucose, and lactate and hematocrit) before and after transport, and during recovery at 12°C.

Materials and Methods

Before fish were subjected to collection, resting samples were taken. We bled 3-4 fish (resting sample) by caudal transection from an undisturbed tank at the Center for Aquatic Biology and Aquaculture (CABA) on the University of California,
Blood was collected using hematocrit tubing and was spun down, hematocrit readings were taken, the plasma collected into a freezer vial, and the plasma pH measured. The plasma was frozen for plasma cortisol, glucose, and lactate analyses. Other fish (n= 22-42) were collected with nets and cups so that the fish were not exposed to the air. They were placed in a cooler with 0.1 ml/L NovAqua and 4-6 g/L NaCl in 50-60 L of aerated water. The cooler was placed on a polyurethane pad in a pick-up truck bed and transported (1.6 km, 10 min) to the UC Davis J. Amorocho Hydraulics Laboratory. Upon arrival, 3-4 fish were immediately sampled, and remaining fish were randomly selected to recover in either 250-L tanks (4-5 fish/tank) or, individually, in 4-L buckets in a 250-L water bath. Four fish from each recovery tank and buckets were sampled at 24 h intervals over 4 d.

**Results**

Data were analyzed using analysis of variance (ANOVA) and Bonferroni multiple comparison tests. No significant differences were found in any of the measured stress parameters during transport, indicating that our collection and short-term transport methods did not stress the delta smelt. During recovery in the buckets, there was a significant increase in plasma cortisol (a primary stress response) level at 24 h (compared with the resting samples’ mean level), with a trend of maintained elevated cortisol level continuing on at 72 h in the buckets. Hematocrit (a secondary response) was significantly decreased at 72 h, indicating possible hemodilution during that sampling time for fish held in the buckets. The other stress parameters (plasma pH, glucose, and lactate) displayed no significant differences for fish held in buckets and tanks. Post-recovery survival decreased significantly at 72 h for fish held in buckets when compared to those held in tanks against all other sampling times (sample size insufficient at 96 h). Our results indicate that transported delta smelt may not recover well in smaller, individual containers after collection and transport.

Our transport techniques do not stress this pelagic fish, however, recovery in small, isolated containers might.

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References


Grass carp, or *Ctenopharyngodon idella*, are eurythermal teleosts capable of tolerating large temperature fluctuations by maintaining physiological homeostasis utilizing inherited regulatory capabilities. Since temperature is one of the primary environmental factors influencing the behavioral and physiological responses of poikilothermal teleosts, we have investigated the physiological response of grass carp subjected to temperature shock, in order to understand the temperature adaptation mechanism in eurythermal teleosts.

Changes in blood glucose and cortisol content have been suggested as sensitive and reliable stress indicators. Hyperglycemia is also known as a typical sub-lethal response of aquatic animals to environmental stress, such as hypoxia and heavy metals. Lactate is the major anaerobic end product in fish under hypoxic and anoxic conditions. As a physiological energy indicator, adenylate energy charge (AEC) can also be used to quantify the level of high-energy phosphate in the adenosine storage system of organisms. The presented study focused on the metabolism of adenylate phosphate compounds and the energy distribution in grass carp under acute temperature shock, with respect to glycemica, lactacemia, and adenylate energy charge.
Before undergoing temperature shock, the grass carp were acclimated in fresh water at 25 °C and a 12L/12D photoperiod regime for 1 month. Next, they were directly transferred to the following temperatures: 5 °C, 10 °C, 12.5 °C, 15 °C and 35 °C. Six fish in each group were sampled at 0, 0.25, 0.5, 1, 3, 6 and 12 hours. Plasma glucose, plasma lactate and hepatic phosphate levels were quantified.

Grass carp exposed to extreme temperatures, 5 °C, 10 °C and 35 °C, showed symptoms of coma, and mortalities at 0.05, 0.25 and 7 h, respectively. The plasma glucose content of fish at 5 °C decreased from 41.80 ± 1.61 mg/dl to 32.21 ± 3.95 mg/dl after 3 min, while those at 10 °C increased from 46.15 ± 4.01mg/dl to 59.26 ± 8.70 mg/dl in 15 min. The trend in plasma lactate at these two temperatures was similar to that of the plasma glucose content. In addition, the plasma glucose content of fish exposed to temperatures varying from 12.5 to 35 °C increased continuously, however, plasma lactate content increased rapidly initially, and then gradually declined. Moreover, AMP, ADP, ATP and TA contents in fish at 5 °C and 10 °C showed similar trends of decreasing, but almost no changes in the AEC index were detected. All of the AMP, ADP, ATP, TA and AEC contents showed similar trends of increasing and decreasing for the fish at 12.5°C and 15 °C, however, they all decreased more significantly for the fish at 35 °C.

These observations suggested the urgent need for supplementary energy in order to maintain physiological homeostasis under thermal stress. Because the energy supply is prominently compensated by anaerobic metabolism, these changes suggest that the shift in the energy metabolic pathway occurred while in the stressed temperatures. Furthermore, the values of AEC, ATP/AMP and ATP/ADP of the fish all decreased throughout the experimental period at temperatures of 5 °C, 10 °C and 35 °C. This indicated that the fish were under the retarded states of energy metabolism. In summary, the plasma glucose content can be used as a stress indicator for grass carp, but the AEC value can only be used in imminent temperatures.

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HEPATIC CELLULAR RESPONSE IN GILTHEAD SEA BREAM
INDUCED BY SUDDEN WATER TEMPERATURE CHANGES

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EXTENDED ABSTRACT ONLY- DO NOT CITE

The gilthead seabream Sparus aurata reared in the Mediterranean sea, may suffer a metabolic disorder that happens after sudden water temperature changes, especially when at temperatures below 12°C. This syndrome, called winter syndrome (WS) often causes fish mortality. Other factors have an influence in WS, like bacterial infection, especially Pseudomonas anguilliseptica. Previous work on this disease has focused on a general organism viewpoint, including endocrine and immunological assays. In the present work, we intend to face a cellular point of view and hence we have designed an experiment to test the effect of the WS on the hepatic cell regulation.

Methods

Four groups of fish were used, three of these were acclimated for one month under laboratory conditions (17°C). The last one was obtained from the farm while WS was occurring (February at 11°C). In the first three subgroups, one was the control group (17°C) and the others were subjected to a thermal shock (down to either 12°C or 6°C) with or without bacterial challenge (P. anguilliseptica). Blood samples were taken from all animals to perform cortisol and glucose assays and liver was removed in order to determine heat shock protein (Hsp70) expression and DNA apoptosis.
Results and Discussion

A relationship between cortisol released and intracellular HSP has been previously described as playing a role in the recovery from metabolic imbalances. From our results, we have found a negative correlation between temperature, and both cortisol and HSP70 expression exists in all groups assayed. However the HSP values were only significant in fish exposed to thermal shock plus bacterial injection and in WS group. Although HSP production is known to inhibit the expression of many proteins, those involved in the hepatic gluconeogenesis pathway appear to remain functional, as glucose levels found in serum (also following cortisol increase) remained at normal or high values. However, the high levels of cortisol found in the WS fish (mean of 208.31 ng/mL, n= 15) and the high expression of HSP70, resulted in a drop in serum glucose levels compared with the other groups. These findings together with DNA assays showing apoptosis, confirm that temperature and bacterial infection are relevant factors in the generation of WS. The syndrome outbreaks, detected at metabolic level by the high levels of cortisol, would be related to an increase of HSP expression and to DNA apoptosis at the cellular level and perhaps following an initial drop of glucose.
CHEMICAL SIGNALS FROM THE SKIN OF RAINBOW TROUT

(ONCORHYNCHUS MYKISS) ELICIT PHYSIOLOGICAL AND CELLULAR STRESS RESPONSES

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Several aquatic organisms, including fish, release chemicals that may serve as alarm signals (Mirza et al, 2001). However, the function of the chemical signals is not clear. Several fish species utilize chemical alarm signals to warn conspecifics of a predation threat (anti-predator response) (Pfeiffer, 1982; Smith, 1992). The chemicals that elicit anti-predator responses, or “fright response”, in nearby conspecifics are generally produced, or contained in the epidermal cells (Smith, 1992). There are reports showing that the receivers (nearby fish) of the chemical alarm signals responded by changing behavioral (Brown and Goding, 1997; Smith, 1992) and physiological states (Rehnberg and Schreck, 1986). The goals of the present study were to determine whether a chemical signal, released by rainbow trout, would elicit a physiological (heamatocrit, plasma cortisol, and glucose levels) and cellular stress response (liver 70kDa heat shock protein), in their conspecifics.

We exposed rainbow trout for one hour to; 1) skin extract from a non-stressed fish, 2) skin extract from a stressed fish, 3) water from a stressed fish, and 4) head tank water as the control. There were 2 replicates per treatment and 5 fish
in each treatment. To prepare the skin extract from non-stressed fish, 5 grams of epithelium from a fish in the stock tank was collected from the dorsal section of the juvenile fish along the lateral line and placed in 100 ml of distilled water. Then the tissue was homogenized by Polytron homogenizer (Polytron, Switzerland), filtered, further diluted to 4L, and added to the test tanks. For preparation of stressed fish skin extract, a juvenile rainbow trout was first physically stressed by being chased in a bucket of water for one minute before the epithelium was sampled. For the water from a stressed fish, a juvenile rainbow trout was netted and placed in a 4L bucket of water, chased for one minute and the water was filtered and directly transferred into the test tanks. One-hour after adding the water to the test tanks, we sampled for blood and liver from each fish. Then we determined haematocrit (% red blood cells), plasma cortisol, and glucose levels as physiological stress responses, and liver Hsp70 levels as cellular stress response. Results are reported as mean ± SEM. Data was analyzed by ANOVA and to discern differences among treatments (P< 0.005), the Student-Newman-Keuls test was applied.

Plasma cortisol levels were significantly increased in fish exposed to water from a stressed fish and to the skin extract of the non-stressed fish (Fig. 1). All treated groups represented significantly higher haematocrit levels than the control group., and plasma glucose levels remained fairly constant in all treatments and there were no significant differences (data not shown). Hepatic Hsp70 levels were significantly higher in the group exposed to the water from a stressed fish (Fig. 2).

Our results demonstrated that juvenile rainbow trout elicited a stress response when exposed to conspecific chemical alarm signals. It has been shown that rainbow trout increased anti-predator behavior (fright response) when exposed to conspecifics skin extract (Brown and Smith, 1997). The authors suggested that this response would likely increase their chance of survival by reducing the risk of detection. In our study we did not examine the behavioral fright response but it is well known that stimuli that produce fright, discomfort, and pain generate a
stress response reaction in fish. Therefore, the increase in cortisol and hepatic Hsp70 levels we observed indicates that fish elicited physiological changes
probably in an attempt to compensate for the challenges imposed upon it. Further examination of the physiological, cellular and behavioral responses under the exposure to chemical alarm cues, as well as determination of the chemical component functioning as the alarm cue, may add new insights to understanding the functional importance of chemical alarm signals in fish under stress.

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EXTRAORDINARILY WARM SUMMER OCEAN TEMPERATURES
COULD ALTER THE CELLULAR AND ORGANISMAL STRESS
RESPONSES OF SUBTROPICAL FISH: EVIDENCE FROM FIELD
AND LABORATORY OBSERVATIONS OF THE INDO-PACIFIC

SERGEANT (*ABUDEFDUF VAIGIENSIS*)

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Recently, global warming has become a serious environmental issue. Increases in global temperatures by 2 to 4 °C are expected over the next half century (Somero and Hofmann, 1997). This scale of temperature change can affect physiological and ecological states of aquatic animals, especially when such changes occur near the upper thermal limits of animals. The El Niño/Southern Oscillation (ENSO) that occurred during 1997 to 1998 was unusually large, resulting in the warmest year in approximately 150 years (Wilkinson et al., 1999). Severe coral bleaching was observed during 1998 around the Andaman Islands in India (Ravindran et al., 1999) and in the Great Barrier Reef in Australia (Berkelmans and Oliver, 1999). The effects of unusually warm ocean temperatures on tropical fishes, however, have not been studied as extensively as they have been in corals although fish represent one of the most extensive groups of animals on the planet that may be directly affected by changes in the ocean temperature.
In the present study, we examined the effects of prolonged increased ocean temperatures during the summer of ENSO in 1998 on the physiological and cellular stress responses of the Indo-Pacific sergeant (*Abudefduf vaigiensis*), inhabiting the subtropical ocean of Okinawa, Japan. The peak surface ocean temperature at the tested area was more than 32 °C during the summer of 1998, the highest in 10 years (~1 °C higher). We compared Hsp70 (70-kDa heat shock protein) and ubiquitin levels in brain, gill, liver, and muscle tissues of fish in the field between the ENSO summer of 1998 and the summer of 1999 during which normal ocean temperatures were recorded. Hsp70 plays a critical role in the repair of damaged proteins denatured by stressors while ubiquitin assists degradation of denatured proteins. Therefore, Hsp70 is a useful indicator of cellular protein functions and stressed states, while ubiquitin is used as an indicator of cellular protein degradation processes. We also conducted heat shock experiments in the laboratory. Two test-temperatures were chosen to

![Figure 1. Muscle Hsp70 (A) and ubiquitin (B) levels in the Indo-Pacific sergeant, collected in the summer of 1998 and summer of 1999 in subtropical ocean of Okinawa, Japan. * indicates significant difference between the groups (p<0.05).](image)
represent normal summer (28 °C) and warm summer (32 °C) ocean temperatures. Thirty-one fish were collected from the ocean in the summer of 2001 (water temperature at the collection = 30 °C). Ten fish were sampled as controls, and the remaining were transferred to the tanks at 28 °C (n=11) and 32 °C (n=10), respectively, for 5 days. Plasma cortisol levels, and Hsp70 levels in the above mentioned tissues were measured. We also prepared otoliths from 26 fish for examination of the yealy growth rate.

The Indo-Pacific sergeant in the field had significantly higher Hsp70 and ubiquitin levels in muscle tissues in the summer of 1998 than in the summer of 1999 (Fig. 1). In the laboratory heat shock experiments, a 50% mortality occurred at 32 °C, while no mortality was observed at 28 °C. The plasma cortisol levels, as well as muscle Hsp70 levels, were significantly higher at 32 °C than at 28 °C (Fig. 2). There was no clear difference in levels of Hsp70 and ubiquitin in other tissues among test groups both in the field and laboratory. We observed checks (darker bands) in the summer of 1998 in the otoliths of 16 out of 26 fish, indicating a slower growth rate of these fish during the summer of 1998.
Our field observations indicated that the fish were exposed to a heat stress sufficient to elicit the cellular heat shock response in nature during the summer of 1998. Higher plasma cortisol and muscle Hsp70 levels at 32 °C than 28 °C in the laboratory supported the hypothesis that the highest surface ocean temperature of 32 °C in the summer of 1998 was stressful to the fish. Muscle tissue makes up a large portion of the body composition. Since synthesizing Hsps and maintaining these protein functions can represent a major energy demand for the cell (Martin et al., 1991), it is possible that whole-body energetics of the Indo-Pacific sergeant were altered in order to maintain high Hsp70 levels in muscle tissues during the summer of 1998. Reduction in the growth rate during the summer of 1998, indicated by the otolith check, may have been related to the whole-body energy imbalance, at least in part.

Overall, our data shows that: 1) the peak ocean temperature during a normal summer is relatively close to a stressful level for the Indo-Pacific sergeant, and thus 2) relatively small increases in ocean temperature during the summer could have major effects on the physiological states of the fish inhabiting subtropical areas. Further assessment both in the field and laboratory would be necessary for more realistic predictions of the effects of warm ocean temperatures on fish.

Acknowledgements

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References


The focus of stress research in fish has recently grown to encompass the cellular stress response in addition to that of the whole animal, broadening our understanding of the mechanisms allowing an animal to tolerate stress. Much of this expanded interest has focused on heat shock proteins and their role as molecular chaperones in the cellular response to stress (Iwama et al, 1998; Feder and Hofmann, 1999). Although HSPs have a relatively short half-life, their levels remain elevated in the whole organisms long after the stressor is removed, indicating a role in long term adaptation and increased stress tolerance (Parsell and Lindquist 1993; Morimoto and Santoro 1998).

By artificially enhancing a fish’s stress tolerance both in vivo and in vitro, through exposure to a mild heat shock, we have been able to explore some of the pathways involved in the protection against stress in fish hepatocytes, and to relate these to the physiological changes that occur in the whole animal. This phenomenon of cross protection, or the ability of a mild stressor to transiently increase the tolerance of an animal to a subsequent heterologous stressor, has not been well documented in fish. We have begun to define and describe cross protection in fish in vivo and more recently have used cross protection as a model to identify some of the critical pathways regulating the cellular stress response in fish in vitro.
Cross Protection in vivo

This study was designed to investigate the capacity of a mild heat shock to increase the tolerance of tidepool sculpins (*Oligocottus maculosus*) to a subsequent heterologous stressor, focusing on the functional role of heat shock proteins (Hsps). Survival of tidepool sculpins exposed to severe osmotic and hypoxic stressors increased from 68% to 96%, and from 47% to 76% respectively when 10°C acclimated fish were exposed to a 22°C heat shock. The magnitude of this heat shock was critical for protection (Fig.1). A 20°C heat shock was insufficient to confer cross protection while a 25°C heat shock impaired the ability of the sculpin to tolerate a second stressor. Further experiments demonstrated that cross protection was present in a defined temporal window between 12 and 48h following the 22°C heat shock. Western blot analysis revealed that hepatic Hsp70 levels were significantly elevated 12h following exposure to the mild heat shock, corresponding to the increase in stress tolerance.

In nature, a fish’s thermal history is important in structuring its cellular response to stress (Feder and Hofmann, 1999). In their natural environment there is approximately 12 hours of high tide between low tide cycles. The time frame of this cross protection may provide evidence of the tidepool sculpin’s ability to invoke a protective mechanism from one low tide period for the unpredictable nature of the next.

Cross Protection in vitro

Recently we have begun to investigate some of the underlying mechanisms that are responsible for the protection conferred by a mild heat shock in primary cultures of Rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Hepatocytes were
exposed to a 28°C heat shock (HS) for 2h, then allowed to recover for 14h at 13°C, and then exposed to a 24h oxidative shock (OxS, 2.0mM H₂O₂). Pretreatment with a 28°C heat shock significantly increased viability of cells exposed to an oxidative shock at 4h and 8h of exposure (Fig. 2A). However heat shock was unable to restore cell viability to that of controls and was unable to maintain protection against oxidative shock at 24h of exposure. Exposure to the oxidative shock alone significantly decreased Hsp70 levels at 4h and 8h of exposure. Pretreatment with heat shock eliminated this oxidative stress induced decrease in Hsp70 levels (Fig. 2B). Following 24h exposure to H₂O₂, heat shocked cells (HS only and HS/OxS groups) had significantly lower levels of

Figure 2. Viability (A) and Hsp70 (B) levels of hepatocytes exposed to a heat and/or oxidative shock. Different letters signifies significant difference (p<0.05). Cell viability of HS only group did not differ significantly from controls.
glucose in their media compared to the other groups, with HS/OxS cells having the lowest levels. This depletion of glucose suggests that cross protection may represent an energetic cost to the fish.

In summary, exposure to a mild heat shock increased the tolerance of hepatocytes exposed to a subsequent more severe stressor, and this tolerance persisted for a defined time period. Hsp70 levels correlated with the protection conferred by the heat shock; however, their exact involvement requires further investigation.

Conclusions

These results suggest that patterns of cross protection are similar whether studied in vivo or in vitro. In vivo experiments provide an appropriate model to investigate the relationship between the cellular and physiological stress responses, while in vitro studies will be invaluable in probing the mechanisms underlying the cellular stress response.

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References


GLUTATHIONE STATUS: A TRIGGER IN THE HEAT SHOCK PROTEIN RESPONSE IN FISH?

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Heat shock protein 70 (HSP70) is a ubiquitous intracellular molecule involved in the proper folding of proteins. HSP70 is upregulated in response to a wide variety of stressors to assist in the refolding of proteins damaged during stress. There has been much speculation on what may be the trigger for HSP70 upregulation during stress. One hypothesis is that decreased levels of cellular glutathione (GSH) may be involved in HSP70 regulation. GSH is a ubiquitous molecule responsible for the reduction of various oxidants, particularly lipid peroxides, through oxidation of GSH to oxidized glutathione (GSSG). Both GSH levels and GSH/GSSG ratios decrease during various stressors. Studies in mammalian systems have shown that a heat shock, a classical inducer of HSP70 upregulation, can also decrease GSH and GSH/GSSG, and increase lipid peroxidation (Lord-Fontaine and Averill-Bates, 2002; Ando et al., 1994; Ohtsuka et al., 1994). Although there has been an increase in knowledge about the role of HSP70 in fish, little is known about possible correlations between GSH and HSP70 in these organisms.

To determine if there is a correlation between GSH status and the HSP70 response in fish, we examined the HSP70 and GSH levels in cultured rainbow trout (Oncorhynchus mykiss) hepatocytes after a 30°C, one hour heat shock. Hepatocytes were isolated from a 300g rainbow trout and cultured in 6-well tissue culture plates at 1.5×10⁶ cells per well in 2mL L-15 media. The
hepatocytes were incubated at 15°C for 72 hours before experimentation, with a media change at 48 hours. Hepatocytes were sampled and then divided into two groups, a heat shock group and a control group. The heat shock group was placed in a 30°C incubator for 1 hour and then returned to 15°C, while the control group was moved concurrently with the heat shock group, but otherwise remained at 15°C. Hepatocytes were sampled at 0, 4, 8 and 24 hours post stress, and six wells were sampled per treatment and time period. Data were analyzed by two-way ANOVA followed by SNK test. All values are given in mean±SEM.

While HSP70 in the control group remained constant, HSP70 in the heat shock group increased significantly with time (p<0.001, Fig. 1). HSP70 levels were higher in the heat shock than the control group at times 4, 8 and 24 hours post stressor (p<0.001). Total GSH in the control group did not change significantly with time (p>0.05). However, in the heat shock group total GSH showed a significant increase at 4 hours, not only in time (p=0.021) but also between groups (p<0.001, Fig.2). There were no significant differences between control and heat shock groups in GSSG or GSH/GSSG levels at any time.

Unlike previous mammalian studies, rainbow trout hepatocytes showed a transient increase, rather than a decrease, in GSH levels after exposure to a heat shock at the times measured. This indicates that, in rainbow trout, a decrease in GSH or GSH/GSSG may not be involved in HSP70 upregulation after a heat shock. In a previous study, Harris et al. (1991) found that GSH levels increased one hour after a 30 minute heat shock in cultured rat embryos. However, Kurganova et al. (1999) found that pea plants exposed to a one hour heat shock...
had decreased GSH content and increased lipid peroxidation during the first 15 minutes, but 30-60 minutes into the heat shock had increased GSH and GSH/GSSG, as well as decreased lipid peroxide intermediates. The results of these studies as well as our own suggest that a 30-60 minute heat shock may upregulate GSH, and possibly confer a transient protection against lipid peroxidation. However, a transient decrease in GSH may also occur during the first 30 minutes of a heat shock. As GSH levels during the heat shock were not measured in this experiment, we cannot rule out the possibility that a transient decrease in GSH levels is involved in HSP70 regulation during a heat shock in cultured rainbow trout hepatocytes.

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References


INTRODUCTION

The trade of ornamental fish in Amazonas State is one of the most profitable and sustainable extractive activities in the region (Chao et al., 2001). The cardinal tetra (Paracheirodon axelrodi) is the Amazonian ornamental fish most requested in the world market, dominating the fish exports from Brazil, and representing 80% of the total fish exported annually from Amazon State. High rates of mortality had been reported by importers, contributing for market loss. Several factors cause fish mortality during commercialization, but water quality and the maintenance of the stability of water conditions are the most important factors that have been pointed out as one of the causes for the high fish mortality rates (Waichman et al., 2001). One parameter that suffers great variation mainly during exportation process is temperature. The objective of the present paper was to establish the lower and higher lethal temperatures (LC$_{50}$) for cardinal tetra Paracheirodon axelrodi.

MATERIAL AND METHODS

The specimens of cardinal tetras (0.08 ± 0.003 g) were collected at the forest streams (igarapés) on the middle Rio Negro basin in the municipality district
of Barcelos, Amazonas State. In the laboratory, the fish were maintained in a tank with aerated flow-through water supply, temperature of 24 ± 1°C and fed regularly with commercial ration for three weeks. Thermal tolerance tests were performed in four test chambers of 40 L equipped with an air compressor and a programmable thermostat bath, which controlled the gradual rising or lowering of the temperature to its desired point. Twenty-four hours prior to the experiment, four groups of ten fish were transferred to test chambers where the water quality was preserved. The tested temperatures, control (24 °C), high (25, 27, 29, 31, 33, 35 ± 1 °C) and low (21, 19, 17 and 15 ± 1 °C), were done with four replicates in a period of 96 h each. During that period, pH, dissolved O₂ and temperature were measured twice a day and the water samples were collected for total ammonia, nitrite and electric conductivity analysis.

High and low lethal temperatures (LC₅₀) values in bioassays were calculated using the Trimmed Spearman-Karber method (Hamilton et al., 1977).

Results and Discussion

We observed water quality uniformity among the replicates of each tested temperature, with no significant difference among physical and chemical parameter values. At lower temperatures, fish mortality increased after 19 °C and reached a total mortality index at 15 °C. In tests with high temperatures (25 to 35 °C), fish survival was 100% at 29 and 31 °C, being lethal above 35°C. These results corroborate those obtained by Waichman et al., (2001) on the evaluation of the water quality used for transportation of ornamental fish, whose capture and fishing camp temperatures fluctuated from 29 to 31 °C for the cardinal tetra.

According to the lethal temperatures found in this study cardinal tetra, a tropical fish has no tolerance to low temperatures. Therefore, the water temperature may be responsible for fish mortality, mainly when the cardinal tetra is exposed to temperatures exceeding the lethal limits (< 19 °C), which probably occurs during transportation to North American and European countries.

References


Acknowledgements

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**IN VITRO METABOLISM OF PREGNENOLONE BY RAINBOW TROUT EMBRYOS AND THE IDENTIFICATION OF ITS METABOLITE**

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

**Introduction**

In oviparous species, it is generally accepted that steroids are passively incorporated into oocytes during follicular growth. Since yolk is the sole source of nutrients for oviparous species prior to exogenous feeding, there is the possibility that salmonid embryos are exposed to these maternal yolk steroids,
which, if not metabolized, pose a potential hazard to the developing embryo. Some studies suggested that steroid hormones play specific functions during early development (Yeoh et al., 1996; Khan et al., 1997). However, studies related to gonadal steroid hormone metabolism in fish embryos have focused on the biologically potent androgens and estrogens (Yeoh et al., 1996; Khan et al., 1997). Much less is known about the metabolism and possible role of pregnenolone (P₅). Thus, the purpose of this study was to examine if P₅ metabolism occurs during embryogenesis, and if so, which metabolic pathways might be present at different developmental stages. The study is part of an ongoing investigation into the processes of steroid metabolism in fish embryos, and of the nature of intermediate steroid metabolites, and an evaluation of the toxicological implications if these metabolic pathways are influenced by xenobiotics.

Methods

Whole rainbow trout embryos (minus the yolk) were incubated in vitro in 24 well-tissue culture plates containing 2 ml of medium M199 at the presence of radiolabelled P₅ ([³H] P₅) at 8-10°C for 18 h. At termination, the media was collected and extracted using a solid-phase extraction, Sep-Pak C₁₈ cartridge. Free and conjugated fractions, following acid solvolysis and glucuronidase treatments, were subsequently separated using a reverse-phase High Performance Liquid Chromatography (HPLC), as previously described (Khan et al., 1997).

An identification of unknown compound(s) was carried out using a Gas Chromatography and Mass Spectrometry (GC-MS). The unknown metabolite from in vitro incubation, as previously explained, of rainbow trout embryonic tissue in the presence of non-radiolabelled P₅ was collected, derivatized, and subsequently identified using GC-MS (Condeca and Canario, 2001).

Results and Discussion

P₅ was converted mainly to an unknown metabolite in the free fraction (Figure 1) with much less conjugated metabolites.

Figure 1. A representative HPLC profile following the in vitro incubation of yolk-absorbed embryos (63 days post fertilization) in the presence of [³H] P₅ at 8-10°C for 18 h. The solid line represents radioactivity (count per minute), and the dotted line represents authentic standards (optical density).
The mass spectrum of the unknown compound eluted at 11.5 min was very similar to the authentic standard, 7α-hydroxyprogrenolone (7α-OHP₅). The similarity of the indices designated fit, reverse fit and purity were 977, 831 and 816 respectively, which was higher than the identification criteria of 900, 600 and 600 respectively (Condeca and Canario, 2001).

Although the conjugated steroids represented only a minute fraction in this experiment, there was a mark difference in that of the conjugated patterns. None of [³H]P₅ was found in the glucuronide fraction whereas [³H]P₅ and 7α-OH[³H]P₅ were present in the sulfate form. This suggests that sulfation may be preferred for [³H]P₅. However, the possibility exists that the amount of [³H]P₅ may be too low to provide sufficient substrate for the glucuronidation in competition with sulfoconjugation. The additional glucuronide of 7α-OH[³H]P₅ may imply that structural changes due to 7α-hydroxylation favor glucuronide conjugation. In addition to our result, 7α-hydroxylated steroids in fish have been reported earlier (Kime et al., 1991, Ponthier et al., 1998), but there is no strong evidence regarding its biological role. Taken together, the 7α-hydroxylation of [³H]P₅ and the additional glucuronide of 7α-OH[³H]P₅ may imply the excretion pathway of [³H]P₅ by rainbow trout embryos. Whether or not the 7α-OHP₅ has a physiological role in fish is not clear.
Conclusion

The study shows the production of 7α-OHP5, a novel steroid, by fish embryos. Based on the conjugation pattern in our study, we hypothesize that 7α-OHP5 is a possible route of excretion of P5. However, based on evidence of protective roles mammalian neurosteroids, a novel function of 7α-OHP5 cannot be ruled out.

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References


EXAMINING THE INTERACTION BETWEEN NEUROTRANSMITTERS AND THE CORTICOSTEROID RESPONSE FOLLOWING AN IMMUNE CHALLENGE IN YELLOW PERCH

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An interaction between the serotonergic systems and the corticosteroid response associated with stress has been observed in teleostean fishes that illustrate serotonin (5-HT) and serotonergic activity is increased following application of a variety of stressors (Winberg and Nilsson 1993). Additionally, the regulation of the cortisol response by serotonin has been suggested in light of the increase in the concentrations of cortisol in the plasma following activation of the 5-HT_{1A} receptors by 8-hydroxy-2-(di-n-propylamino)-tetralin (Winberg et al. 1997). Monoaminergic systems in the brains of mammals are also altered following lipopolysaccharide (LPS) challenge (Dunn et al. 1999) suggesting that the relationship between central monoamines and changes along the hypothalamo-pituitary-interrenal (HPI) axis may be consistent between a pro-inflammatory challenge and other types of stressors. Experiments performed at the University of South Dakota examined temporal neurophysiological changes in yellow perch following intraperitoneal challenge with LPS to correlate these observations with the corticosteroid response.

Brains from yellow perch, *Perca flavescens*, were sampled before and after fish were injected with LPS. Significant increases from pre-injection levels were observed in the 5-HIAA:5-HT ratios in both LPS- and saline-injected fish. At 12 h after injection the mean concentration of the dopamine catabolite, dihydroxyphenylacetic acid, in LPS-treated fish, 14 pg/mL, was significantly
higher than that observed in fish sampled 12 h after injection with saline. These data support a general model linking an increase in serotonin metabolism to handling stress but none of the monoaminergic indices measured correlate with the sustained elevations of plasma cortisol demonstrated following the LPS challenge.

References


PHYSIOLOGICAL DISTURBANCES ARISING FROM THE DIFFERENT SECTIONS OF A LIVE-RELEASE ANGLING TOURNAMENT: INSIGHTS FROM A SIMULATED TOURNAMENT

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Introduction

Live-release tournaments for black bass (Micropterus spp.) have grown in popularity over the last several decades, and it is currently estimated that there are over 30 000 of these events annually across North America (Wilde 1998). Mortality of bass following live-release tournaments can be highly variable (Wilde 1998) despite the fact that mortality resulting from the catch and immediate release of bass is typically quite low (Muoneke and Childress 1994). As a result, fisheries managers have questioned the potential impacts of angling tournaments on bass populations (Wilde 1998).
Recent investigations have suggested that mortality following tournaments likely results from the cumulative effects of numerous sub-lethal stressors incurred by fish throughout the tournament day (Schramm et al. 1987; Kwak and Henry 1995). Due to the variability in mortality rates, it has also been suggested that reductions in mortality should be possible if those sub-lethal stressors could be identified. In an attempt to better understand the relative contribution of different sections of a tournament to physiological disturbance, this study replicated a live-release angling tournament for largemouth bass (*Micropterus salmoides*), and fish were sampled for blood, white muscle, and cardiac parameters following each of the tournament sections.

**Methods**

Treatment groups in the study were designed to represent conditions observed at actual angling events in Southeastern Ontario. Briefly, the treatment groups were:

1. Control – fish were sampled while resting in blackened perspex boxes in the lab.
2. Exercise – fish were sampled following 1 minute of exercise to mimic angling.
3. Livewell – fish were exercised 1 minute, then confined for 6 hours in a livewell on a boat.
4. Weigh-in – fish were first subjected to exercise and livewell treatments. They were then confined in plastic transport bags for 5 min, then air exposed for 1 min.
5. Recovery – fish were subjected to exercise, livewell, and weigh-in treatments, then returned to blackened perspex boxes in the lab for 24 hours.

**Results and Discussion**

Under the conditions simulated, the most physiologically challenging portions of a live-release angling tournament were the exercise (angling) and weigh-in treatments. During these sections, largemouth bass experienced significant changes in muscle energy stores, lactate, osmolarity, cortisol and cardiac parameters. In contrast, largemouth bass were able to recover from exercise during livewell confinement as evidenced by clearance of lactate and replenishment of energy reserves. Elevated plasma cortisol, plasma glucose and heart rate during livewell confinement suggest that this portion of a tournament likely causes a disturbance for fish. During the 24 hour recovery period, all of
the parameters monitored returned to resting levels and no mortality was observed.

Results from this study can be used to minimize disturbance and maximize survival at live-release angling tournaments. Notably, the weigh-in imposes a relatively large physiological disturbance on largemouth bass, and efforts should therefore be made to reduce disturbance at this stage. A companion study from our group has shown that air exposure during the weigh-in may be a critical factor in this regard.

References


Acknowledgements

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THE PHYSIOLOGICAL EFFECTS OF HANDLING AND VARYING AMOUNTS OF AIR EXPOSURE ON WALLEYE DURING THE WEIGH-IN AT LIVE-RELEASE ANGLING EVENTS

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

In recent years, the popularity of live-release angling tournaments has increased dramatically (Schramm et al., 1991). Several authors have suggested procedural guidelines for reducing stress among tournament fish, but the importance of weigh-in handling and air exposure has been largely overlooked. During the weigh-in of live-release angling events, fish are handled and air exposed as they are weighed on a scale and held for photographs. In most fish species, air exposure causes a severe inhibition of gas exchange due to a collapse of the delicate gill lamellae and a reduction of functional gill surface area (Boutilier, 1990; Ferguson and Tufts, 1992). This may lead to a decrease or complete cessation of carbon dioxide excretion, as well as a reduced capacity to perform aerobic metabolism (Ferguson and Tufts, 1992). This study uses walleye (Stizostedion vitreum) to examine the impacts of weigh-in handling and air exposure from a physiological perspective.
Methods

We subjected adult walleye to a weigh-in simulation with varying amounts of air exposure. Individual walleye were carefully netted from a common holding tank and transferred (three fish per trial) into a portable livewell (35 x 35 x 87 cm). The livewell was continuously supplied with flowing water (11-12°C), which was spray aerated by passing through a section of perforated plastic tubing located approximately 5 cm above the surface of the water. Fish remained undisturbed for 4 hours, at which time individual fish were removed from the livewell and held for 5 min in a clear plastic bag containing 15 L of lake water (one fish per bag). The plastic bag was then emptied into a laundry basket that allowed the drainage of water. Fish remained in the basket and were air exposed for either 30 s or 90 s. Another set of fish received the same livewell and plastic bag treatment but were not air exposed during the weigh-in simulation. Instead of being placed into the laundry basket, these fish were transferred into a plastic container full of water for 90 s.

Immediately after either of the three weigh-in treatments (no air exposure, 30 s of air exposure, or 90 s of air exposure), individual fish were anaesthetized in a buffered solution of MS-222 (250 mg / L) and sampled for blood and white muscle. The blood samples were later analyzed for plasma lactate, and the white muscle samples were analyzed for phosphocreatine (PCr), adenosine triphosphate (ATP), glycogen, lactate, and intracellular pH. Data collected for the three air exposure treatments were also compared to control fish that were sampled after 48 hours of quiet rest in darkened Perspex boxes. Immediately before sampling, water flow into the boxes was stopped, and anaesthetic was added. Following full anesthesia, fish were sampled for blood and white muscle as was previously described for the air exposure groups.

Results and Conclusions

The handling associated with plastic bag confinement caused a physiological disturbance that was intensified when followed by air exposure. Most walleye struggled during bag confinement, and even the no air exposure treatment showed substantial decreases in white muscle PCr and glycogen when compared to resting values. However, increasing amounts of air exposure after the period of bag confinement caused a further physiological disturbance, with significant white muscle ATP depletions after 90 s of air exposure. Furthermore, white muscle lactate after 90 s of air exposure was about 12 times higher than resting
values, and about 3 times higher than in fish receiving the no air exposure weigh-in treatment. There was also an intracellular acidosis that progressively increased with handling and air exposure. Plasma lactate showed a gradual increase with longer duration of air exposure, but the differences between weigh-in treatments were not statistically significant.

Together, these results indicate that handling during the weigh-in causes a significant physiological disturbance in walleye, and that this disturbance is exacerbated by air exposure. We recommend that tournament organizers focus on reducing the duration of air exposure during the weigh-in at competitive live-release angling events.

References


MONITORING CAPTURE PROCEDURES AND TRANSPORT OF
CARDINAL TETRA, AN AMAZONIAN ORNAMENTAL FISH, FROM
ITS NATURAL HABITATS TO EXPORTERS IN MANAUS - BRAZIL

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Despite their particular chemical and physical properties (acidity, low transparency, primary production and low buffer capacity), distinct environments like flooded forest (igapós), forest streams (igarapês), lakes and tributaries of Rio Negro basin, share an huge fish diversity (Goulding et al., 1988), specially those represented by small sized ones commonly known as “piabas”. Local riverine populations have exploited them as ornamental fish since the 60s, when Dr. H. Axelrod discovered the cardinal tetra (*Paracheirodon axelrodi*), a new and very promising Amazonian ornamental fish. Nowadays, Amazonas State exports approximately 23 million ornamental fish every year (Chao, 2001) most being cardinal tetra. However, high mortality rate, which have been reported so far are contributing for the decreasing participation of Amazonian ornamental fish on international trade. The present study aims to evaluate capture procedures and transporting conditions of cardinal tetra from Barcelos to exporters in Manaus, in order to assess the critical events affecting water quality and fish survival.
We followed the capture of cardinal tetra at three fishing camps: Igarapé do Cuiuni (August/2001), Ig. do Zamula (October/2001) and Ig. do Téia (April/2002). At the Ig. do Cuiuni, only water quality parameters (temperature, dissolved oxygen, conductivity, pH and total ammonia) were monitored in six plastic tubs. At the other igarapés, a number of fish (n=10, ten replicates) was also sampled in the different steps of capture and transport, for further stress assessment.

Cardinal tetras are mostly captured during the low water season (August-February) and, in some areas, it continues during rising water (April-May). Fishermen (or so called piabeiros) use different fish gears, such as a special kind of dip net (rapiché) and a baited fixed trap (cacuri) in shallow waters of iigapós and igarapés, where schools of cardinal tetra are found. After capture, fish were kept in plastic tubs (n=500-800 according to size) and sometimes stored in nylon screen made tanks for a day or week intervals. During capture at the fishing camps, fish were gently handled and kept in covered plastic tubs, where water was regularly (about 20-30 minutes) renewed by piabeiros. As a result, water quality parameters were very similar to those recorded for waters from the fishing camps. However, as the fish were stored in plastic tubs and transported (6 to 15 hours) to the exporter’s facilities in Barcelos, water quality lowered gradually: oxygen levels dropped steadily to values slightly above 1 mg/L, while pH and total ammonia levels increased, the latter reaching values around 5 mg/L. Despite the lowering water quality cardinal’s mortality was negligible unlike the previous studies in which high mortality rates were attributed to exported Amazonian ornamental fish (Chapman et al., 1997).

At the exporter’s facilities in Barcelos, the water in plastic tubs was totally renewed with water from Rio Negro River, with comparable quality to that of igarapés. After that, table salt (NaCl) and tetracycline were added to the water to prevent opportunistic infection. They were kept in this condition during the all period of transport to Manaus (20-30 hours). During the trip, the water was not replaced. This procedure resulted in lowering oxygen levels, and concomitant increasing water pH, conductivity and total ammonia levels. When cardinals arrived at the exporter’s facilities in Manaus, oxygen levels were near 0.5 mg/L in almost all the monitored plastic tubs. Hence, cardinal’s mortality was negligible.

According to these findings, and the ones from a previous study (Waichman et al, 2001), we may assume that transportation from fishing camps to Barcelos,
storage and their subsequent transportation to exporters’ facilities in Manaus, are
the critical stages in this phase of marketing process of cardinal tetra. However,
this did not have any effect on the negligible mortality recorded for this fish
species. Physiological investigation is under way and will make it possible to
evaluate the extent of the stress fish must experience from capture and
transportation procedures.

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PHYSIOLOGICAL CONSEQUENCES OF LOW DOSE ANAESTHESIA IN RESTED AND EXERCISED CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA: SALMONIDAE) AND SNAPPER (PAGRUS AURATUS: SPARIDAE).

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EXTENDED ABSTRACT ONLY – DO NOT CITE

This study examined the physiological consequences of anaesthesia for chinook salmon (Oncorhynchus tshawytscha) and snapper (Pagrus auratus) during simulated rested and exercised harvesting. Tank-rested fish were anaesthetised in their home tank, were not handled prior to anaesthesia and were exposed to low (non-irritant) concentrations of the anaesthetic agent (AQUI-S™; 20 mg/L for salmon; 17 mg/L for snapper). For exercised harvesting fish were forced to exercise prior to anaesthesia. Mixed venous blood samples were taken by ventricular stab during exposure to the anaesthetic. Sampling was initiated from when the first fish was insensitive to the stab. Plasma adrenaline (ADR) and nor-adrenaline (NOR) were measured to indicate primary responses to any acute stress during exposure to the anaesthetic. Plasma glucose was monitored as a secondary, indirect indicator of stress. Blood pH, and lactate levels were monitored as secondary indicators of oxygen debt caused by ventilatory arrest or by swimming activity. Antero-dorsal “white” muscle (WM) samples were taken for each treatment. Cut-surface pH and [lactate] measurements were made to assess the extent of WM fatigue during anaesthesia. WM pH and WM lactate content confirmed the rested state of the fish in that both rested salmon and snapper had high WM pHs and low WM lactate. The exercised treatment produced fish with low and variable WM pHs and high WM lactates.

In rested salmon, blood pH was high (~7.6), and blood glucose, lactate, plasma adrenaline (ADR) and noradrenaline (NOR) levels were low until
~150 min exposure to the anaesthetic. After ~150 min mixed venous blood pH dropped rapidly with concomitant rises in plasma NOR, ADR, blood glucose and lactate. In rested snapper, plasma NOR and ADR increased after ~70 min exposure but did not coincide with decreased blood pH or sharp increases in blood lactate and glucose. Blood glucose levels in rested snapper were still high after 120 min even when catecholamine levels were consistently low, possibly indicating a post-ADR release state. In contrast to rested salmon, rested snapper showed a progressive respiratory acidosis of the blood shown by the steady rise in [lactate] and drop in blood pH, rather than a sudden change that coincided with catecholamine release.

In salmon, pre-anaesthesia exercise reduced the time fish became insensitive to ventricular stab by ~60%. Blood pHs of exercised salmon were lower than rested salmon. After ~30-40 min exposure there were concomitant rises in plasma NOR, ADR, blood glucose and lactate. Release of ADR and NOR did not appear to occur during the exercise protocol. Blood pH of exercised snapper was not different to rested snapper, even though blood lactate and blood glucose levels were high. High blood glucose levels suggested that catecholamines had been released during pre-anaesthesia exercise. In both rested and exercised salmon release of catecholamines only occurred when ventilation was depressed by anaesthesia, producing mixed venous blood pH values below ~7.4 (a common blood pH reported for exhaustively exercised salmonids). In rested snapper catecholamine release occurred prior to any decline in blood pH or sharp increase in blood lactate and glucose.

It is suggested that the mechanisms triggering catecholamine release in salmon (e.g. blood pH, hypoxia) may be less sensitive than in snapper due to the differences in activity of the two species. Because salmon burst exercise frequently they may only release catecholamines in extreme cases. Snapper burst exercise less frequently than salmon, but the severity of each burst may be greater, requiring catecholamine release for recovery purposes. These results highlight significant species differences in physiological response to a stressor. It is an excellent reminder that we should not assume that all species react in a similar way to the same treatment.
RESPIRATORY AND CIRCULATORY EFFECTS OF HYPOXIA IN LARGEMOUTH BASS AND SMALLMOUTH BASS: AN APPLICATION TO "LIVE-RELEASE" COMPETITIVE ANGLING EVENTS

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Introduction

Live-release angling tournaments are a growing industry in North America, and every year thousands of largemouth bass (Micropterus salmoides) and smallmouth bass (M. dolomieu) are caught at these events. Largemouth bass and smallmouth bass are closely related species, but display different habitat preferences with largemouth preferring shallower, weedy areas, and smallmouth bass preferring deeper and colder waters. As a potential consequence of physiological adaptation to their specific habitats, largemouth bass appear to be less affected by stresses incurred during tournaments, and exhibit lower mortality than smallmouth angled in the same competitions (Hartley and Moring, 1995).
The purpose of this study was to compare the effects of hypoxia on arterial blood variables, ventilation rates and cardiac output in the two bass species. Results of this study are meant to be considered by anglers and tournament organizers when handling the two bass.

Methods

In vivo experiments – Bass were fitted with cannulae in either their dorsal aorta or caudal artery, and blood samples were collected over the course of a graded hypoxia exposure, and a subsequent return to normoxia. Whole blood variables (PaO$_2$, hematocrit, pH) were measured immediately, while plasma samples were frozen in liquid N$_2$ and later analysed for lactate and catecholamines. Cardiac output variables were measured in fish fitted with ventral aortic flow probes, and ventilation rates were measured using video analysis.

In vitro experiments – Blood was collected by caudal puncture, washed 3 X with physiological saline, and allowed to incubate in a refrigerator over night. O$_2$ dissociation curves were constructed using the method of Tucker (1967).

Results

During the in vivo hypoxia study, PwO$_2$ decreased from approximately 160 mm Hg to 45 mm Hg. For both species, PaO$_2$ decreased with decreasing water oxygen concentrations, however, the blood of largemouth bass consistently held greater quantities of oxygen than the blood of smallmouth bass during the experiment. These changes resulted despite the fact that hematocrit concentrations for smallmouth bass were greater than that of largemouth bass throughout the study. Additionally, largemouth bass arterial blood showed no significant change in pH throughout the experiment, whereas the blood of smallmouth bass became acidotic at the lowest levels of hypoxia, and remained acidotic following the 12 hour recovery period. Oxygen dissociation curves obtained during in vitro experiments were consistent with these results and showed that the hemoglobin:oxygen affinity is greater in largemouth bass.

Cardiac output decreased in smallmouth bass during moderate hypoxia (PwO$_2$ = 90 mm Hg) and remained disrupted following 12 hour recovery, while the cardiac output of largemouth bass changed little throughout the in vivo study. Similarly, ventilation rates for smallmouth bass immediately instantly following the onset of hypoxia, but ventilation rates for largemouth bass increased only after PwO$_2$ reached 90 mm Hg. Both species showed increased plasma lactate.
concentrations and increased plasma catecholamine concentrations at the lowest levels of hypoxia. The only mortality observed in the study occurred in smallmouth bass during the 12 hour recovery in normoxic water.

**Discussion**

Smallmouth bass displayed higher sensitivity to hypoxia as compared to largemouth bass. The PaO$_2$ of both bass species decreased similarly with the decrease in PwO$_2$, but largemouth bass were able to maintain a higher blood O$_2$ content at lower oxygen tensions, indicating that the blood of largemouth bass has a higher affinity for O$_2$ (lower P$_{50}$) compared to smallmouth bass blood. Even following exposure to hypoxia, smallmouth bass were unable to recover from the resulting physiological disturbances. Based on these experiments, it is recommended that smallmouth bass receive extra attention to reduce hypoxic stress during live-release angling tournaments.

**References**


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TIME AND DOSE-RELATED EFFECT OF CORTISOL ON
TESTICULAR AND FOLLICULAR APOPTOSIS IN
GOLDFISH (Carrassius auratus)

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Apoptotic cell death plays a critical role during development of organism tissues including testis and ovary during spermatogenesis and oogenesis. Apoptosis is also involved in modulating pathogenesis of a variety of diseases via regulated genetically and biochemically suicide program. Its disregulation has been shown to be associated with infertility. In the present study we investigated the effect of cortisol which is the main stress hormone in fishes on the goldfish testicular and follicular apoptosis. Although apoptosis occurs in the normal conditions in testis and ovary, but stress is known to cause problems with fertility in fish. However no information is available on the effect of cortisol on gonadal apoptosis in fish. The results demonstrate that cortisol treatment was found to stimulate caspase 3 activity which was used as an indicator of apoptosis in the immature goldfish testis (GSI less than 2.4). No significant change in caspase 3 activity was observed in mature testis (GSI > 2.4) following treatment with cortisol in goldfish. Cortisol was also found to stimulate caspase 3 activity in the goldfish ovary in a stage-dependent manner. Follicles in early vitellogenesis (less than 0.6 mm in diameter) did not respond to cortisol treatment. However, treatment of 0.8-0.9 mm goldfish follicles with cortisol significantly stimulated caspase 3 activity in a dose-related manner. Cortisol treatment in fully mature goldfish follicles (> 1.0 mm) was without effect.

The results provides evidence that cortisol effects caspase 3 activity in goldfish ovary and testis in a stage-dependent manner. The findings supports the hypothesis that stress may cause lower level of fertility in fish due to the production of cortisol leading to onset of apoptosis in the ovary and testis.
Keywords: Apoptosis; Cortisol; Caspase 3; Testis; Follicles; Goldfish; in-vitro; Dose and time-response; Mature; Immature