USE OF BROOK CHAR
(SALVELINUS FONTINALIS)
PHYSIOLOGICAL RESPONSES TO STRESS
AS A TEACHING EXERCISE

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Fish hematological changes during osmotic and cold stress are used to introduce the physiological reactions of the animal to an acute stress. Brook char (Salvelinus fontinalis) were subjected to 1 h of stress before being anesthetized and having blood taken from their caudal vein. Glucose, hemoglobin, hematocrit, and osmolarity were determined in the blood samples. Analyses showed that glucose concentration tends to increase and hematocrit tends to decrease in stressed fish. Changes in hemoglobin concentration occurred only in cold-stressed fish. A rise in blood glucose concentration is the result of cortisol secreted by the hypothalamic-pituitary-adrenal axis. The glucose produced is used as an osmolyte or energy source to resist or combat the stress. In stressed fish, changes in hematocrit could be the result of the osmotic concentration of the blood plasma, as shown by the increase in osmolarity for the same group. In cold-stressed fish, a decrease in hemoglobin concentration could be the result of hemodilution by body cell water.

Key words: fish; plasma glucose; hemoglobin; hematocrit; osmolarity

The undergraduate biology program of the Université du Québec à Rimouski has two major areas of interest, marine biology and wildlife management. We therefore adapted from research a number of experiments, dealing mainly with animal physiology and biochemistry, to emphasize our fields of interest (5, 13–16). The use of wild species of fish, birds, and mammals has allowed us to stimulate in students an additional interest in the teaching of physiology and biochemistry.

In this paper, we present an ecophysiological experiment that uses basic techniques encountered in animal physiology laboratories. We use the response of brook char (Salvelinus fontinalis) to a short-term exposure to saltwater or cold water to demonstrate acute physiological responses to an environmental stress. Temperature and salinity changes represent stresses that fish either have to cope with (short-term reaction) or acclimate to (long-term reaction). Thermal stress is a prime concern in ectotherm physiology when anadromous and catadromous fish are exposed to osmotic stress during migration.

Living organisms have developed many strategies to survive and cope with environmental changes to which they are exposed. These environmental constraints, known as stressor stimuli, induce short-term, predictable physiological changes, particularly in fishes that are ubiquitous inhabitants of the aquatic environment of all latitudes. Stressors can include many kinds...
of environmental changes: pH (8, 20), handling and transportation (17), osmotic stress (19), and high stocking-density stress (24). The typical primary physiological stress response is increased secretion of catecholamines (epinephrine) and corticosteroids (cortisol) by the hypothalamic-pituitary-adrenal axis (2). This response is generally independent of the type of stressor, but the quantitative aspect of the response depends on the intensity and duration of the stressor (9). The initial endocrine responses bring several metabolic adjustments including changes in plasma osmolyte, glucose, and lactate concentrations. Cortisol, the most important corticosteroid in teleosts, contributes to the regulation of gluconeogenesis and/or glycogenesis. The high concentration of cortisol favors glucose mobilization, providing a substrate for rapid threat response (23).

Hypersecretion of catecholamines and corticosteroids also induces hematological changes (9). Decreases in hematocrit and hemoglobin have been reported in fishes subjected to an acute cold stress (3). Elevated blood epinephrine increases gill permeability to water and sodium and leads to plasma electrolyte changes in a hyperosmotic or hyposmotic environment (2, 9).

The purpose of the laboratory is to initiate students to fish handling (anesthesia and blood sampling) and hematological analysis and to introduce them to the physiological responses of an animal to a stress (1, 4). We use cold temperature and saltwater to induce changes in blood glucose, hemoglobin, hematocrit, and plasma osmolarity of brook char. This species is an anadromous salmonid; however, the specimens used in this experiment, came from a freshwater pond. The first three variables are measured easily using simple and low-cost apparatuses that are found in almost any university biology department.

**MATERIAL AND METHODS**

**Fish and blood sampling.** Brook char (*Salvelinus fontinalis*) is widespread in Canada and the northern part of the United States. This species is anadromous but can spend its life in freshwater. The specimens used in this study (males and females) were purchased from a local freshwater fishing pond during the summer and brought to the laboratory with minimal stress. All fish were 2 yr old with a total mean length of 25 cm. The pond temperature was ~10–12°C. In the laboratory, they were immediately separated into three groups in 20-liter tanks: one control and two experimental tanks were subjected to an acute stress for 1 h. Osmotic stress was achieved by transferring fish to water of 10‰ salinity (one-third strength of sea water), and cold stress was achieved by transferring fish to a 4°C tank. The controls consisted of fish maintained at ~12°C. In all three groups, water was bubbled during experimentation for oxygen supply.

After the treatment, fish were anesthetized (5 min) in water containing 60 mg/l of 3-aminobenzoic acid ethyl ester (MS-222) and blood was sampled according to the technique described in Fig. 1. To perform blood sampling, the fish is held as shown in Fig. 1 and syringe introduced right at the back of the anal fin until the vertebral column is felt. When the syringe pierces the caudal vein lying below the vertebral column, blood will be withdrawn. Blood samples (1 ml) were placed in heparinized tubes. If such tubes are not available, regular glass tubes coated with heparin (100 units/ml) can also be used.

If one wants fish to survive after blood sampling, the anesthetizing bath temperature and pH must be adjusted to that of pond water. For a 100- to 150-g fish, 1 ml of blood can be withdrawn without compromising survival if the specimen is returned to the pond water.

**Glucose determination.** Blood glucose was determined with a blood glucose monitor, Accu-chek Easy (Boehringer Mannheim), that uses the glucose oxidase-ferrocyanide technique (11, 18). This monitor was chosen because of its low cost and high availability. However, any other glucose reflectometer may be used. The procedure for the use of this reflectometer is quite simple. A drop of blood is placed on the tagged area of the strip, which is immediately inserted into the reflectometer. Glucose concentration is displayed in millimoles per liter.

**Hematocrit and hemoglobin determinations.** Hematocrit and hemoglobin are two widely studied blood parameters in laboratory textbooks (6, 12, 21, 22). The blood sample is collected in a capillary tube and centrifuged for 5 min to separate the plasma from cells. Hematocrit, the percentage of packed cell volume to the total volume of a blood sample, was

The hemoglobin determination (in g/100 ml) of blood was performed using an Hb-meter (model 1010D, American Optical, Buffalo, NY). This simple and widely used apparatus compares the absorption of light by hemoglobin in a hemolyzed blood sample of known depth to that of a standardized glass plate (22). A drop of fish blood is introduced in the chamber surface and hemolyzed, and the results are then visually compared with a set of standards.

Osmolarity determination. We used a freezing point depression microosmometer (Advanced, model 3MO) for the measurement of the plasma osmolarity. The apparatus measures the freezing point of a 20-µl aqueous solution, which is a function of its osmolarity.

RESULTS

Changes in blood glucose, hemoglobin, hematocrit, and plasma osmolarity concentration for stressed and unstressed fish are presented in Fig. 2, A–D, respectively. Data shows that plasma glucose tends to increase in fish subjected to cold and osmotic stresses. Hemoglobin concentration fell only in cold-treated fish, and hematocrit tends to decreases in stressed fish. Stressed fish exhibit an increase in plasma osmolarity. To calculate the amount of hemoglobin per red blood cell (Hb/RBC), the total hemoglobin concentration was divided by the hematocrit value. The results obtained for Hb/RBC were $0.16 \pm 0.01$ for the control group, $0.16 \pm 0.02$ for the cold-stressed group, and $0.17 \pm 0.02$ for the osmotically stressed group. No difference in Hb/RBC was observed between the control and either experimental group.

DISCUSSION

Response to cold stress. As temperature decreases, the osmotic concentration and blood viscosity increase. Coping with low temperatures requires the ability to maintain homeostasis. Hematological changes are good indicators of this performance (3). The increase of blood glucose is the most studied response in this regard. It is the result of the activation of glycogenolysis that is under cortisol control (23). Hyperglycemia during cold exposure has been reported in many species (3, 19). In cold-treated fish, increased plasma glucose is used mainly as an osmolyte. Salmonid fish, including brook char, survive at low temperature by concentrating their plasma and cellular fluid electrolytes (Na$^+$ and Cl$^-$) and other osmolytes like glucose (7). The plasma osmolyte concentration is negatively correlated with the freezing point. Decreases in hematocrit and hemoglobin could be the result of blood osmoconcentration, as shown by an increase in plasma osmolarity. This osmoconcentration leads to hemodilution by body
cell water. The decrease in hemoglobin concentration in cold-treated fish could be also associated with a diminution of erythrocyte size (1). The increase in osmolyte concentration in the plasma leads to water loss from erythrocytes and thus to their shrinkage. Hemolysis has been reported in common carp (Cyprinus carpio) during acute water temperature changes from 8°C to 4°C (3). The observed increase in plasma osmolarity following cold stress is less important than that in osmotically stressed fish (Fig. 2D).

![Blood glucose (A), hemoglobin (B), hematocrit (C), and plasma osmolarity (D) of brook char (Salvelinus fontinalis) subjected to cold and osmotic stress for 1 h. Values are expressed as means ± SD.](http://advan.physiology.org.org/)

**FIG. 2.**
Responses to osmotic stress. Fish subjected to saltwater tend to increase their plasma glucose and osmolarity and lower their hematocrit (Fig. 2, A, B, and D). The transfer of salmonid species from freshwater to saltwater affects the water balance of the fish (10). A weight loss occurs in saltwater, whereas a weight gain occurs in freshwater. The adrenaline produced during stress increases gill water permeability in freshwater but decreases it in saltwater. These changes in gill function can be increased by 100% in a few minutes (9). Adrenaline has also been shown to affect the electrolyte balance in fish by causing a rise in gill sodium uptake in freshwater-acclimated rainbow trout (Onchorhyncus mykiss) (9). Combined effects of water loss, solute uptake, and an increase in plasma glucose contribute to the rise in plasma osmolarity. As a consequence of the increase in plasma osmolarity, water is withdrawn from erythrocytes or body cells and hematocrit decreases. Similar results have been reported in other species (19). Osmoregulatory processes are of great importance for the survival of anadromous species such as Salmonidae or catadromous species such as eels. Moving from saltwater to freshwater and vice versa requires performative osmoregulatory processes. Decreases in hemoglobin in osmotically stressed fish are less important than those in cold-stressed fish.

Suggestions. This experiment is easy to perform and is a good tool to introduce the physiological responses an animal to acute stress in an undergraduate biology course. It allows one to observe the physiological plasticity that allows animals to cope with changing environmental factors. The hematological responses are the results of increases in plasma corticosteroids and catecholamines.

Brook char was selected for this experiment because of its large availability and its economic importance in aquaculture and sport fishing. However, big goldfish or other salmonid species can also be used.

It is possible to perform this experiment by measuring the plasma cortisol and adrenaline. One can also test the effect of intraperitoneal injections of cortisol on temporal changes of blood parameters (1).

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