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Research Article

# The Effects of Acute Stress on Rainbow Trout (*Oncorhynchus mykiss*)

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**Abstract:** The physiological effects of acute stressors (transport, handling, netting and confinement) on rainbow trout in an aquaculture system were investigated. Serum cortisol level, serum glucose and lysozyme activity were determined in rainbow trout stressed by acute stressors, and compared with those of unstressed (control) fish. Serum cortisol, glucose levels and lysozyme activity were significantly higher($P<0.05$) in stressed rainbow trout. Cortisol levels in the unstressed fish (average 31.50 µg/dL) were lower than those in stressed fish (average 45.16 µg/dL). In stressed fish, serum glucose levels were an average at 58.53 mg/dL while in unstressed fish were 26.23 mg/dL. Lysozyme activity was typically elevated in rainbow trout upon acute stress (minimum 140- maximum 900 unit/ml). These physiological parameters indicated that rainbow trout are sensitive to disruption by acute stressors in an aquaculture system.

Key Words: Rainbow trout (*Oncorhynchus mykiss*), acute stress, serum, cortisol, glucose, lysozyme activity

## Gökkuşağı Alabalıkları (*Oncorhynchus mykiss*) Üzerine Akut Stresin Etkileri

**Özet:** Bu çalışmada kültüre alınan gökkuşağı alabalıklarında akut stresin (taşımacılık, balıkların yakalanması, sıkıştırılması) balıklar üzerinde oluşturduğu fizyolojik etkiler araştırılmıştır. Stres uygulanmayan (kontrol) ve akut stresorler ile stres uygulanan gökkuşağı alabalıklarının serum kortizol, serum glukoz düzeyleri ve lizozim aktiviteleri belirlenerek karşılaştırılmıştır. Stres uygulanan balıklarda serum kortizol, glukoz ve lizozim aktiviteleri belirgin bir artış göstermiştir ($P<0.05$). Kortizol düzeyleri stres uygulanmayan balıklarda (ortalama 31,50 µg/dL) akut stres uygulanan balıklardan (ortalama 45,16 µg/dL) daha düşük düzeylerde saptanmıştır. Stressiz balık grubunda serum glukoz düzeyi ortalama 26,23 mg/dL bulunurken, stresli balıklarda ise ortalama 58,53 mg/dL olmuştur. Akut stres uygulanan balıklarda lizozim aktivitesi stres uygulanmayanlara göre artış göstermiştir (min. 140- max. 900 unit/ml). Bu fizyolojik parametreler, kültür ortamında akut stresörlerin etkisine bırakılan gökkuşağı alabalıklarının strese karşı duyarlılığını göstermiştir.

Anahtar Sözcükler: Gökkuşağı alabalığı (*Oncorhynchus mykiss*), akut stres, serum, kortizol, glukoz, lizozim aktivitesi

## Introduction

In fish culture, stress associated with the numerous naturally occurring changes in chemical, biological and physical disturbances in the aquatic environment results in increased susceptibility to disease (1,2). Fish under aquaculture conditions are often subject to environmental changes or stressors, such as handling, crowding, transporting, and changing water quality (3).

For vertebrate animals, stress is commonly defined as a state or condition in which the homeostasis of an individual is disturbed as a result of the actions of external stimuli, termed stressors. Stressors elicit changes in the animal's physiological state, which is interpreted as the stress response (4).

Fish stressors such as handling, confinement, and transport have been associated with increases in plasma cortisol concentration. Plasma cortisol level is widely used as a general indicator of stressful conditions in vertebrates, particularly in fish (5). In response to a stressful event, the hypothalamic portion of the brain stimulates the release of adrenocorticotropic hormone (ACTH). ACTH is circulated into the anterior kidney, where it stimulates the interrenal cells to produce cortisol and other corticosteroid hormones. Cortisol has been shown to suppress immunoglobulin production by lymphocytes and reduce the production of various intercellular mediators, such as prostaglandin. Reduced disease resistance resulting from stressful

environmental conditions is of particular importance in fish culture because of the high economic costs of epizootics (1,4).

Serum cortisol, which usually operates as a hyperglycemic hormone, and serum glucose levels have been proven to be reliable endocrine and physiological indicators of the relative severity of many different acute stressors to fish (6,7).

Stressor increases in blood glucose levels have also been used as monitors; glucose levels are easy to measure, and relatively inexpensive, and are the most commonly measured indicator of stress response in fish (8,4). Since these increases result from cortisol induced gluconeogenesis, blood glucose changes have sometimes been used as indirect measures of altered cortisol secretion (4).

Lysozyme has been found in the blood, mucus and organs of various fishes. Reports on modulation of the lysozyme activity in fish are rare. It is known that an increase in the lysozyme concentration in fish blood is caused by infections or injections of foreign materials (3). In this paper, the lysozyme activity in fish that suffered from physical disturbances in culture was investigated.

To date, there have been several studies on the effects of stress on a variety of fish species in aquaculture. The sum of the physiological changes that occur in fish as react to physical, chemical, or biological challenges and the attempt to compensate are commonly referred as stress response (1,8). The responses to stressors have been classified as an acute (primary) response, a chronic (secondary) response and a prolonged chronic (tertiary) response (4). The acute response involves the activation of the neuroendocrine system, resulting in an increase in the level of catecholamine and corticosteroid hormones. The chronic response includes physiological responses to these hormones (e.g., increased oxygen uptake). The prolonged chronic response is identified at the level of the whole organism and includes the inhibition of growth, impaired reproductive success and impaired immune response (4).

Fish stressors have been studied in many fish species by several researchers; for example, handling (6,9-12), stocking density (9), transportation (3), water pollution (3), applying anesthesia (6), and confinement (2).

The aim of this study was to determine the serum cortisol, serum glucose levels, and lysozyme activity in the blood of immature rainbow trout that suffered from typically encountered physical disturbances in fish culture. Fish were exposed to a combination of stressors, namely, transport, handling, confinement and netting.

## Materials and Methods

### Experimental fish

Sixty immature rainbow trout (*Oncorhynchus mykiss*) were used. The fish were obtained from the fish culture facility of the Fisheries Faculty at Süleyman Demirel University (S.D.U.). Fish length and weight averaged at 24 cm and 147 g, respectively. The fish were kept under normal environmental conditions prior to experiment in concrete ponds. Each pond was supplied with a constant aerated flow of underground water. The water temperature was 10-12ºC, pH was 8.2 and dissolved oxygen was 7-8 mg/L. Fish were fed three times a day with commercial pellets.

### Applying stressors to the fish

Two groups containing a total of 60 fish were studied in July 2000. In the first group, 30 unstressed (control) rainbow trout were randomly removed from the pond. In the second group, varieties of acute stressors were applied to the 30 fish in the pond. The fish were confined into a small area of pond and then netted, and half of them were transported in a small bucket (10 L) into a circular tank in ten minutes and then the fish were chased and caught in the same tank with a net.

### Blood collection and sample preparation

Thirty unstressed fish and 30 stressed fish were anesthetized in MS-222 (Tricaine methanesulfanate). Blood was collected from each fish by cutting the caudal peduncle. The blood samples were left at room temperature for one hour and then stored in a fridge overnight. The blood was centrifuged at 3000 rpm over 10 min for the collection of serum. The serum was divided in two portions from the test tube. The aliquots of serum were used for cortisol and glucose analysis and the other parts of the aliquots were used for lysozyme assays. The serum samples were frozen and stored (-20ºC) until required for assays.

### Cortisol, Glucose and Lysozyme Assays

Cortisol levels were determined by an Automated Chemiluminescence System (ACS: 180) with a Chiron

Diagnostics kit (Cat. No. 672304), and serum glucose levels were obtained using a hexokinase method in Enzymatic UV Test for Clinical Chemistry Analyzers (Olympus System Reagent for Glucose) at the medical school biochemistry laboratory at S.D.U.

Lysozyme activity was measured with the turbidimetric method described by Engstad et al. (13), using 0.2 mg/ml lyophilized *Micrococcus lysodeikticus* (Sigma M 3770) as the substrate in phosphate buffer adjusted to pH 5.75. Serum was added to 3 ml of the suspension and the reduction in absorbency at 540 nm was measured by a Shimadzu spectrophotometer after 0.5 min and 4.5 min at room temperature. One unit of lysozyme activity was defined as a reduction in absorbency of 0.001/min (13).

### Data Analysis

The data of unstressed and stressed fishes were analyzed using Student's *t* test, where significance ($P<0.01$ and $P<0.05$) was indicated.

## Results

In contrast to the controls, the fish to which stressors were applied such as transport and handling proved to be unequivocally stressed after 10 min. Indicators of stress, the cortisol level (Fig. 1) and glucose level in the serum (Fig. 2) were significantly increased. The lowest serum cortisol level was 0.06 µg/dL, the highest was 3.02 µg/dL in the unstressed group. In comparison with the unstressed group, a significant elevation in cortisol levels was observed in fish to which acute stressors were applied ($P< 0.05$) (Table 1). In contrast to the stressed fish had a minimum serum cortisol level of 3.01µg/dL and a maximum level of 12.27 µg/dL. The serum cortisol

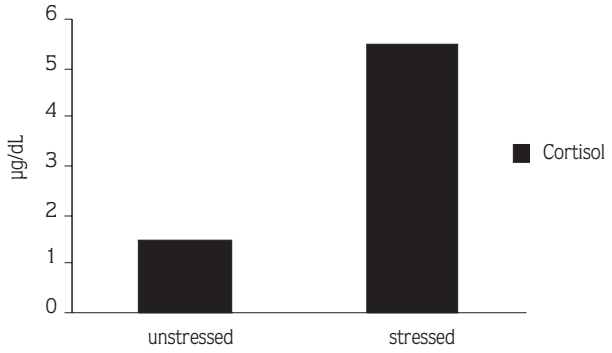


Figure 1. The mean values of serum cortisol levels (µg/dL) in unstressed and stressed rainbow trout.

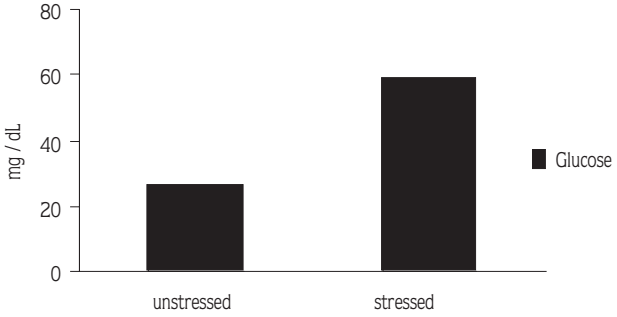


Figure 2. The mean values of serum glucose levels (mg/dL) in unstressed and stressed rainbow trout.

levels of the transported and handled rainbow trout were higher, averaging 5.50 µg/dL and 1.447 µg/dL, than those of the unstressed fish (Table 1).

There was also a significant increase in the serum lysozyme activity of the stressed group. The lowest serum lysozyme activity in the unstressed group was 140 units/mL and the highest 480 units/mL. In contrast, the stressed fish had a minimum serum lysozyme activity of 320 units/mL, and a maximum 980 units/mL (Table 2). The lysozyme activity was typically elevated in rainbow trout upon exposure acute stressors (Fig. 3).


Figure 3. The mean values of lysozyme activity (unit/mL) in unstressed and stressed rainbow trout.

## Discussion

Stress is an unavoidable component in intensive aquaculture during transport, handling, netting and catching. Stress in fish can cause immunosuppression and results in an increased susceptibility to diseases. Therefore, understanding of the physiology of fish and the careful management of the environment is required in intensive aquaculture. In intensive aquaculture, the fish population is routinely chased, netted, handled, graded,

Table 1. Serum cortisol and glucose levels in stressed group versus unstressed (control) group of rainbow trout.

| Fish Number (Stressed) | Serum Cortisol (µg/dL) | Serum Glucose (mg/dL) | Fish Number (Unstressed) | Serum Cortisol (µg/dL) | Serum Glucose (mg/dL) |
|---|---|---|---|---|---|
| 1 | 6.23 | 41 | 1 | 1.41 | 16 |
| 2 | 12.27 | 50 | 2 | 2.06 | 19 |
| 3 | 4.01 | 61 | 3 | 0.30 | 27 |
| 4 | 5.12 | 41 | 4 | 0.45 | 23 |
| 5 | 5.14 | 75 | 5 | 0.71 | 10 |
| 6 | 3.68 | 53 | 6 | 2.75 | 22 |
| 7 | 10.67 | 61 | 7 | 1.63 | 31 |
| 8 | 5.67 | 48 | 8 | 1.87 | 21 |
| 9 | 6.45 | 60 | 9 | 1.87 | 20 |
| 10 | 4.66 | 55 | 10 | 0.97 | 30 |
| 11 | 4.32 | 57 | 11 | 0.30 | 30 |
| 12 | 5.29 | 40 | 12 | 2.46 | 25 |
| 13 | 5.38 | 53 | 13 | 2.00 | 33 |
| 14 | 5.52 | 49 | 14 | 0.99 | 33 |
| 15 | 5.08 | 58 | 15 | 0.06 | 32 |
| 16 | 6.80 | 73 | 16 | 2.01 | 30 |
| 17 | 3.01 | 70 | 17 | 0.55 | 17 |
| 18 | 3.57 | 63 | 18 | 0.32 | 29 |
| 19 | 3.40 | 51 | 19 | 1.22 | 32 |
| 20 | 5.84 | 68 | 20 | 1.77 | 22 |
| 21 | 4.98 | 40 | 21 | 2.00 | 23 |
| 22 | 6.24 | 66 | 22 | 0.54 | 35 |
| 23 | 6.25 | 68 | 23 | 1.87 | 26 |
| 24 | 5.77 | 71 | 24 | 2.14 | 31 |
| 25 | 4.11 | 48 | 25 | 1.97 | 39 |
| 26 | 6.50 | 63 | 26 | 2.06 | 37 |
| 27 | 4.15 | 84 | 27 | 3.02 | 12 |
| 28 | 3.20 | 51 | 28 | 1.04 | 33 |
| 29 | 6.91 | 47 | 29 | 1.59 | 24 |
| 30 | 4.82 | 91 | 30 | 1.48 | 25 |

transported and treated with drugs. Since the present fish were exposed to acute stressors such as transporting (10 min), handling and netting, it was impossible to attain standardized conditions between the various stress factors. Stressors cause a set of physiological reactions in fish as well as in other vertebrates (3). Physiological parameters can be used as indicators of stress in fish. In most of the studies on stress, serum cortisol levels have been studied as the main physiological parameter (1,4,14,15). In addition, the levels of serum glucose and lysozyme activity were used as indicators in fish exposed to stress (3,16). In the present study, the serum cortisol levels, serum glucose levels, and lysozyme activities were determined in rainbow trout.

While the unstressed fish exhibited a low mean serum cortisol level (1.447 µg/dL), the stressed fish showed a high mean serum cortisol level (5.50 µg/dL) after a short period of exposure to acute stressors. The significant increase in serum cortisol concentration seen in the stressed fish after 10 min handling is consistent with the findings of Mazur and Iwama, 1993 (9).

The result of the present study show that rainbow trout exposed to stressors appear to experience acute stress as indicated by the significant elevation in serum cortisol, serum glucose and lysozyme activity.

Post-stressor increases in blood glucose levels have also been used as monitors; glucose levels are easy to measure and relatively inexpensive, and are the most commonly measured indicator of the secondary phase stress response in fish (8). Since this increased result, in part, is from cortisol-induced gluconeogenesis, blood glucose changes have sometimes been used as indirect measures of altered cortisol secretion.

All stressors employed in this study affected lysozyme activity. Möck and Peters (3) reported lysozyme activity in rainbow trout, *Oncorhynchus mykiss*, stressed by handling, transport and water pollution. In their findings,

Table 2. Serum lysozyme activity in both unstressed (control) and stressed groups of rainbow trout.

| Fish Number (Unstressed) | Lysozyme reduction in absorbance | Lysozyme activity Units /ml | Fish Number (Stressed) | Lysozyme reduction in absorbance | Lysozyme activity Units/ml |
|---|---|---|---|---|---|
| 1 | 0.022 | 440 | 1 | 0.045 | 900 |
| 2 | 0.007 | 140 | 2 | 0.018 | 360 |
| 3 | 0.023 | 460 | 3 | 0.026 | 520 |
| 4 | 0.015 | 300 | 4 | 0.041 | 820 |
| 5 | 0.016 | 320 | 5 | 0.018 | 360 |
| 6 | 0.024 | 480 | 6 | 0.024 | 480 |
| 7 | 0.008 | 160 | 7 | 0.039 | 780 |
| 8 | 0.007 | 140 | 8 | 0.018 | 360 |
| 9 | 0.008 | 160 | 9 | 0.026 | 520 |
| 10 | 0.008 | 160 | 10 | 0.023 | 460 |
| 11 | 0.023 | 460 | 11 | 0.018 | 360 |
| 12 | 0.024 | 480 | 12 | 0.021 | 420 |
| 13 | 0.007 | 140 | 13 | 0.022 | 440 |
| 14 | 0.017 | 340 | 14 | 0.023 | 460 |
| 15 | 0.023 | 460 | 15 | 0.049 | 980 |
| 16 | 0.022 | 440 | 16 | 0.049 | 980 |
| 17 | 0.007 | 140 | 17 | 0.021 | 420 |
| 18 | 0.015 | 300 | 18 | 0.027 | 540 |
| 19 | 0.023 | 460 | 19 | 0.021 | 420 |
| 20 | 0.024 | 480 | 20 | 0.016 | 320 |
| 21 | 0.015 | 300 | 21 | 0.038 | 760 |
| 22 | 0.022 | 440 | 22 | 0.032 | 640 |
| 23 | 0.024 | 480 | 23 | 0.024 | 480 |
| 24 | 0.008 | 160 | 24 | 0.027 | 540 |
| 25 | 0.024 | 480 | 25 | 0.024 | 480 |
| 26 | 0.015 | 300 | 26 | 0.024 | 480 |
| 27 | 0.009 | 180 | 27 | 0.026 | 520 |
| 28 | 0.009 | 180 | 28 | 0.016 | 320 |
| 29 | 0.017 | 340 | 29 | 0.038 | 760 |
| 30 | 0.007 | 140 | 30 | 0.038 | 760 |

lysozyme activity showed a significant decrease after handling in addition to the usual increase in plasma glucose levels. In the present study, all stressors were used at the same time and therefore the lysozyme activity increased after applying the stressors. Demers and Bayne (17) determined that lysozyme activity was significantly increased after acute stressors, like in this study.

Plasma cortisol levels rise rapidly following acute exposure to physical stressors (4). It has been considered that elevated plasma cortisol levels increase the susceptibility of fish to fungal, bacterial, and parasitic infections (1).

In conclusion, the immature rainbow trout is under a more severe physiological stress, which causes changes in some parameters, during 10 minute periods of transporting, handling, confinement and netting, which are widely used in fish farms. In this study, it was observed that physiological parameters could be used as monitors in fish culture systems. It would probably be wise to avoid any operations that would cause additional stress in aquaculture. More detailed experimental research is required to provide a better understanding of the effects of acute stressors on fish in fish farms.

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