PHYSIOLOGICAL RESPONSES OF RAINBOW TROUT

TO DIFFERENT TYPES OF STRESSORS

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Introduction

The homeostatic system of fish is continuously challenged by environmental variables that may induce stress reaction in organism. Majority of studies on stress responses have been performed for identification or characterization of several of the components of the stress response " but it must be recognized that the response is an integrative reaction with behavioral, neural, hormonal and physiological elements all combining to give the fish the best possible chance of survival" (Pickering, 1993). It was determined that magnitude of stress response (as concerned catecholamine release) may be directly correlated with increasing intensity and type of stress in elasmobranchs (Butler et al., 1979) and teleosts (Ristory et al., 1979). Corticoid stress responses to various forms of stressors had been studied by Strange et al. (1977), and Strange and Schreck (1978). However comparative assessments of physiological, biochemical stress responses of fish to different stressors had been performed occasionally.

Behavioral and physiological reactions of juvenile coho salmon (*Oncorhynchus kisutch*) were examined before and after the chemodetection of alarm substance and chemical stimuli released from predators. It was found that studied reactions to chemical stimuli from predators do not necessary co-occur (Rehnberg and Schreck, 1986). Meanwhile, substances released from the skin predators (pike, pike-perch) induced in silver carp behavioral, physiological responses and changes in composition of fish mucus similar to observed after exposure of fish to physiological disturbance (Lebedeva, Golovkina, 1987, 1993).

The objective of this study was to determine the changes of physiological responses of rainbow trout (*Oncorhynchus mykiss*) to various stressors (exposure to electric current, physical disturbance, hypoxia, social interaction) and to establish whether the exposure will lead to release by the trout of alarm substances - "stress pheromones" capable of inducing stress responses in fish-recipients.

Materials and methods

All the experimental work was performed in the Laboratory of Physiology and Ecology of Hydrobionts of the Institute of Ecology, Vilnius. The rainbow trout used in research was obtained from Zeimena hatchery. Fish were kept in holding tanks of about 3000-L capacity supplied with aerated water, approximately 200 mg/L HCO_3 and pH 7,4. The fish were transferred from holding

tanks to 100L capacity aquaria for experiments and acclimated for two weeks before experiments. Water temperature during experiments was 9-10^0 C and dissolved oxygen was 8-10 mg/L.

The fish under study was rainbow trout (*Oncorhynchus mykiss*) total length 24-29 cm in experiments with physical stressors and 15-17 cm - with social interactions, the weight ranges were 210 -300g and 45-55g, respectively.

Experimental fish were subjected to different types of stressors : a. Direct exposure to different stimuli; b.Exposure to water after subjected fish; c. Exposure to mucus of fish (dominant and subdominant individuals).

a. Direct exposure to different stressors. Four groups of fish were exposed to: the first group - electric current (E=40 V/m, f=100 Hz) for 30 s (EC); the second group was disturbed for 2 min imitating the capture of fish (Ph. dist.); the third group - hypoxia for 2 min. All the fish studied in these groups were taken for analysis 1h after the stress was applied; the fourth group was exposed to social stress (separately keeping fish were placed together into experimental aquarium). This group of fish was observed for 1,5h just after placing.

b. Four groups of fish were exposed for 1h to water after fish-donors subjected to different stimuli. A half of water in aquarium was gently changed by the water of fish-donors. Water of control fish was cahanged by pure water.

c. Two groups of fish were exposed for 1 h to skin mucus of dominant and subdominant individuals.

Physiological responses. Ventilation frequency (N/min) was observed during 3 min at 5 min and 1 h after exposure to different stressors. Blood samples were taken from caudal vessels and blood glucose concentration mmol/L was detected by electrochemical method with "EKSAN-G", haematocrit by centrifuging in haematocrit capillaries. Fish mucus from the skin of fish was gently taken 1 h after exposure of fish to physical stressors. Mucus sodium and potassium concentrations (mekv/g of dry weight) were determined by flame photometer IL-943. Mucus collection and preparation for analysis were performed according to Lebedeva et al. (1988).

All data were calculated as the means ±s.e. from 8-12 individuals. The changes of ventilation frequency, glucose concentration, sodium, potassium concentrations in fish mucus were calculated in %. 100% - mean value of ventilation frequency of fish before stressor was applied, as concerned other parameters studied, 100% - mean value of control fish.

Results

a. The effects of EC, physical disturbance and hypoxia on ventilation frequency, blood glucose concentration, electrolyte concentrations and their ratio in skin mucus of rainbow trout are shown in Fig. 1.

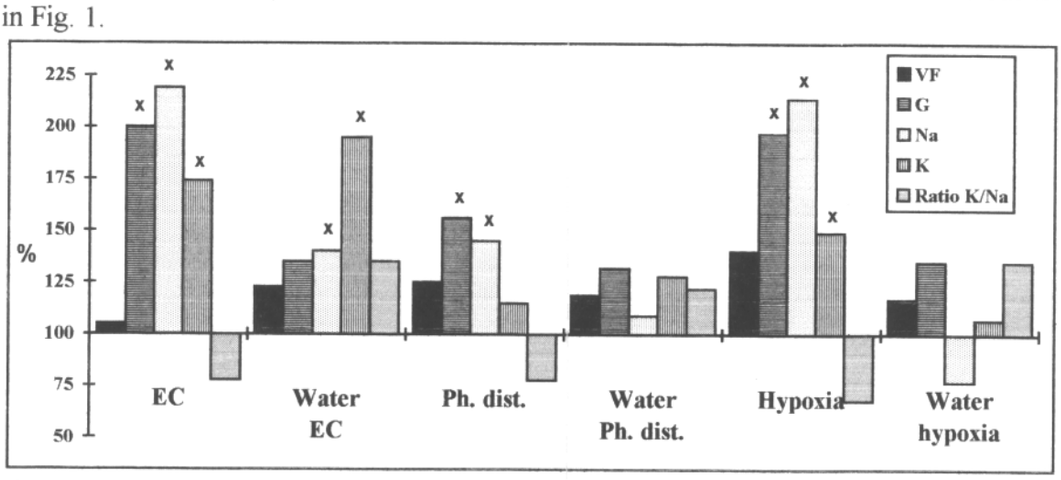

Figure 1. Changes (in %) of physiological responses in rainbow trout exposed to different stressors (EC - electric current, ph. dist. - physical disturbance, hypoxia) and water after fish exposed to these stressors (100% - mean value of control fish; x - value significantly different from controls $p<0.05$).

Significant increase ($p < 0,05$) in ventilation frequency of 40% was recorded 1 h after exposure of fish to hypoxia. There was a statistically significant increase ($p<0,05$) in blood glucose levels 1 h after subjecting of fish to all stressors applied. Exposure of fish to EC and hypoxia significantly increased ($p<0,05$) sodium and potassium concentration in fish mucus, however, ratio of K/Na did not change significantly. It was observed slight decrease ratio of electrolytes after exposure of fish to all stressors applied.

Statistically significant elevation of sodium and potassium concentrations was found in mucus of fish exposed to water after EC subjected fish. Slight increase in blood glucose concentration and in electrolytes' ratio in fish mucus was found.

b.Physiological responses of trout to social stress are indicated in Fig. 2. Ventilation frequency of subdominant fish had been slightly, however, significantly increased ($p<0,05$) 1,5 h after placing fish together as compared to control fish and dominant individuals. A noticeable increase in blood glucose concentration was observed in blood of dominant and subdominant fish ($p<0,05$) as compared to controls.

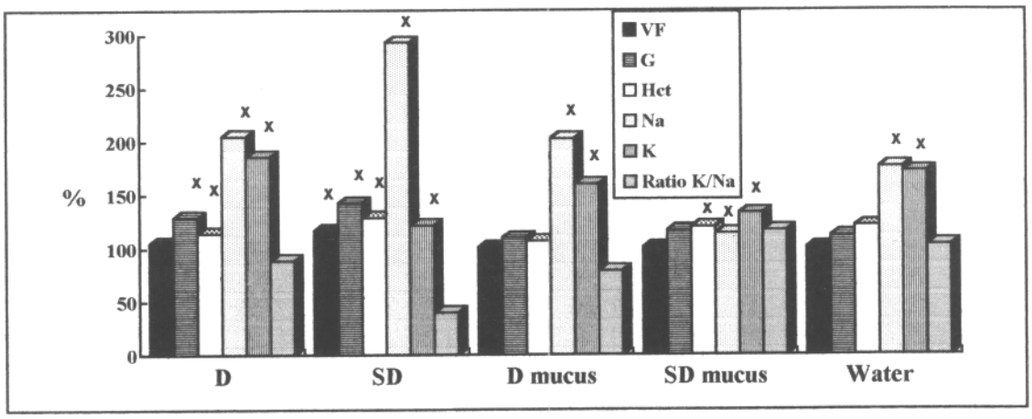

Figure 2. Change (in %) of physiological responses in rainbow trout exposed to social stress in fish-donors (dominant (D) and subdominant(SD) individuals) and in fish-recipients (checked on mucus from dominant and subdominant fish, and on water after fish social interaction)(x - value significantly different from controls $p<0.05$).

Haematocrit levels of dominant and subdominant individuals exhibited similar to glucose level alterations ($p<0,05$). Mucus of fish-donors (dominant and subdominant) did not induce changes in fish ventilation frequency, however, induced slight hyperglycemia in fish-recipients ($p<0,05$) as compared to controls, more significant in fish exposed to mucus of subdominant individuals. Fish water after social interaction caused no statistically significant increase in glucose concentration and haematocrit levels. The significant increase in sodium concentration was recorded in dominant and subdominant individuals and potassium in dominant fish ($p<0,05$) . However the ratio of electrolytes in mucus of dominant fish did not differ significantly from controls and significantly decreased electrolytes' ratio was recorded in mucus of subdominant individuals. Mucus of dominant and subdominant fish induced no significant contrary alterations of electrolytes' ratio in fish -recipients as well as water after social interaction fish did not change this parameter in fish.

Discussion

The results indicated that respiratory system, blood glucose level, osmotic balance in trout mucus were significantly affected by different types of stressors. Changes of parameters studied confirmed an activation of sympatho-chromaffin system of trout induced by exposure of fish to stressors. This system stimulates immediate responses in the cardiovascular system, respiratory and osmoregulatory functions (Mazeaud et al., 1977). Changes in indices of this system are

generally short-lived (i.e. from minutes to hours) (Colombo et al., 1990). In our experiments statistically increased ventilation frequency had been registered 5 min after exposure to physical stressors: 99,3±4,05 (N/min) (EC), 104,4±3,7 (N/min), (phys. dist.), 106,2±3,2 (N/min) (hypoxia). This elevation of ventilation frequency of stressed fish declined during the first hour of recovery. Ventilation frequency of rainbow trout exposed to EC did not differ from the control value 2 h after stress initiation (Vosyliene, 1990).

It was considered that the magnitude of stress response of trout is related to intensity of stressors applied (Mazeaud, Mazeaud, 1981). Changes of several parameters studied confirmed this suggestion. The exposure of fish to EC and hypoxia induced more significant elevations of blood glucose, electrolytes concentrations as compared with that observed in physical disturbed fish. Sodium and potassium concentrations in mucus of fish subjected to stressors slightly increased, however, the K/Na ratio did not differ among the fish stressed by different stimuli. Similarly to data obtained with rainbow trout decrease of K/Na ratio had been observed in mucus of cyprinids subjected to various types of stressors (Lebedeva et al., 1988).

The water after fish stressed did not induce significant changes in parameters studied. Physical stressors had caused an elevation of glucose concentration till 6,0 -6,5 mmol/L (control value - 2,9±0,14 mmol/L), water after fish stressed had been induced an increase till 4,5±0,26 mmol/L. We would consider this glucose level "physiological hyperglycemia" i.e. concentration which is in the ranges of the homeostatic alterations. Sodium and potassium concentrations in skin mucus of fish subjected to water had been changed differently, however, slight increase was observed in all experiments studied. During social interaction and formation of hierarchy in rainbow trout pairs both individuals (dominant and subdominant fish) had been stressed. In our study subdominant fish was under stronger stress than dominant one. It was indicated by alterations in behavior, body color and changes in parameters studied. Physiological evidence for psychological stress in subordinate individuals had been found by Schreck (1981). Changes in physiological parameters dominant and subdominant individuals had been observed in trout at various stages of social stress (Vosyliene et al., 1993) and the most significant changes of glucose, lymphocyte counts were observed in subdominant fish. Changes of electrolyte ratio in dominant and subdominant individuals confirmed that dominant individuals were less stressed as compared to subdominant ones. The K/Na ratio of dominant fish 0,73 was very similar to control - 0,80, meanwhile that of subdominant individuals had been - 0,33. Changes of parameters studied in fish-recipients suggested about stress of physiological responses induced by mucus of fish and possibility of rainbow trout to detect alarm substances (alarm pheromones) released from stressed fish. Alarm substances from mucus of subdominant individuals induced more significant changes of parameters tested as compared to those from dominant fish. Changes of biochemical parameters in muscle of ide (*Leuciscus idus*) were observed 10 min after exposure fish to its own alarm chemicals (Gronow, 1974). The effect of conspecific alarm substances induced elevation of plasma potassium concentration and decrease of sodium in bream (*Abramis brama*) (Malyukina et al., 1982). Our data suggest that rainbow trout may detect its own chemical alarm signals released from the skin mucus of other fish.

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