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Effects of Handling on Physiological Profiles in Turkish Crayfish, *Astacus leptodactylus*

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Abstract: The aim of this research was to investigate influence of an environmental stressor, such as handling on haemolymph parameters in freshwater crayfish, *Astacus leptodatylgrus*, as a bioindicator species. Crayfish haemolymph was subsequently sampled in 1 hour after exposure handling stress. Crayfish (n=30) were exposed to handling stress (disturbed by net during 15 min.). After the experimental period, haemolymph (approximately 0.4 ml) of each crayfish was taken. Physiological alterations were measured, indicated as stress response in crayfish exposed stress. In the stress condition (handling stress) used in this research, the alterations in level of haemolymph triglyceride were different. It was reported that the mean triglyceride level decreased during exposure to handling stress, while the total protein ($p>0.05$) and glucose levels ($P<0.05$) increased.

Key words: Stress % Handling % Haemolymph % *Astacus leptodactylus* % Crayfish

INTRODUCTION

Stress induced by changes in environmental parameters: air exposure, food deprivation etc. requires homeostatic regulation that brings behavioral and physiological alterations in aquatic animals [1, 2]. The stress effects tend to be cumulative and measurements of physiological changes can give useful information as to what is stressful and used as indicators of the animals' well being. Stress responses may be evaluated subjectively (behavior and vigor) or expressed quantitatively by measuring changes in physiological variables such as oxygen uptake, blood composition, pH, hormones, ions and hemocytes [3, 4]. In particularly, different types of environmental stress produce enhanced haemolymph crustacean hyperglycemic hormone (CHH) levels [5, 6]. The CHH levels have a major play in regulate of glucose concentration. The glucose concentration in crustacean's haemolymph rises in response to a number of stress such as handling [7, 8], disease and pollutants [9, 10]. Other hemato immunological parameters involved in the stress response following handling, transport and emersion, are total hemocytes counts (THC) and total protein concentration [11-14] and however, triglyceride content [15].

Crayfish that is a very high of economic value have a key role organism in protecting of the ecological balance of freshwater resources [16-18]. The economic value of freshwater crayfish (*A. leptodactylus*) consumed in many countries, rises with everyday. Unconscious fishing, water pollution, some stress factors and some research subjects like its bio-indicator facilities are so important.

Crayfish is one of the crustacean groups that have successfully adapted to a wide range of environmental conditions. So that, we chosed *A. leptodatylgrus*, as model lives in our research, which is widespread throughout the waterways of Turkey and can be obtained in large numbers throughout the years. One of the further advantages in using crayfish is easily maintenance in the laboratory for many months or even years. This makes it an ideal model live in cellular and biochemical studies.

In this research, we focused on some physiological effects of handling. We determined its effects by analyzing of total protein and glucose levels that are some of physiological variables in haemolymph. Understanding of the stress mechanism and physiological modification in handling of crayfish may ever-increasingly, provide to some commercially better practices to ensure a better survival rate, meat quality and animals' welfare.

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MATERIALS AND METHODS

Study Area and Animals: Crayfish (*A. leptodactylus*) were obtained from Agin Region in the Keban Dam Lake (Elazig, Turkey) (Figure 1). After capture, crayfish were transported to the Fish Production Farm of Firat Fishing Faculty (CIP). They transported with containers in water from sampling area to CIP. In the CIP, crayfishes were stocked in tanks (40 l) and were maintained at 14±1°C and under a controlled photoperiod (12 h lightness / 12 h darkness). They fed with a formulated diet during 2 weeks before the experimental period. The experiments were carried out in intermolt specimens of *A. leptodactylus*. Apparently healthy thirty male crayfish (37.65±1.49 g live mass, 52.60±0.82 mm carapace length) were selected randomly for the experiments.

Handling Stress: After acclimatization, 30 crayfish (male) were exposed to handling stress (disturbed by net for 15 min.) and control individuals (30 crayfish) were maintained in water (14±1°C) in the tank throughout the experiment and sampled in the same intervals as the exposed ones after the stress period, animals were monitored, by haemolymph bleeding at 1 hour of stress.

Determination of Haemolymph Parameters: Before the haemolymph samples to obtain plasma, crayfish and syringe were packed in ice for 10 to 15 minutes. Approximately 0.4 ml of haemolymph was taken from each crayfish by inserting a syringe fitted with a 20-gauge needle through the membrane at the base of the fifth walking leg. 0.4 ml haemolymph was centrifuged for 1 min at 10300 × *g* and the plasma fraction was quickly frozen at -20°C and stored until required for measurements of physiological parameters. The samples required for measurements of physiological parameters were transported to the laboratory (In vitro Laboratory, Elaz—, Turkey) in insulated containers with ice (4°C).

Physiological Parameters: Plasma glucose levels were measured colorimetrically according to Trinder's glucose oxidase method [19]. Triglyceride levels and total protein contents were measured by the colorimetric enzymatic test using glycerol-3-phosphate-oxidase (GPO). Result of the glucose and triglyceride levels are expressed in mg dL^{-1}. Result of the total protein contents are expressed in g dL^{-1}.

Statistical Analysis: The data are expressed as mean ± Standard Error (SE). All of the results were analyzed by one sample T-test and at 0.05 significance value (P=0.05). Statistical analyses were carried out using the Statistical Program of SPSS (version, 10.0) for Window.

RESULTS

To determine the effects of handling on crayfish, haemolymph samples were taken after the period of stress and analyzed for the total protein, glucose and triglyceride contents.

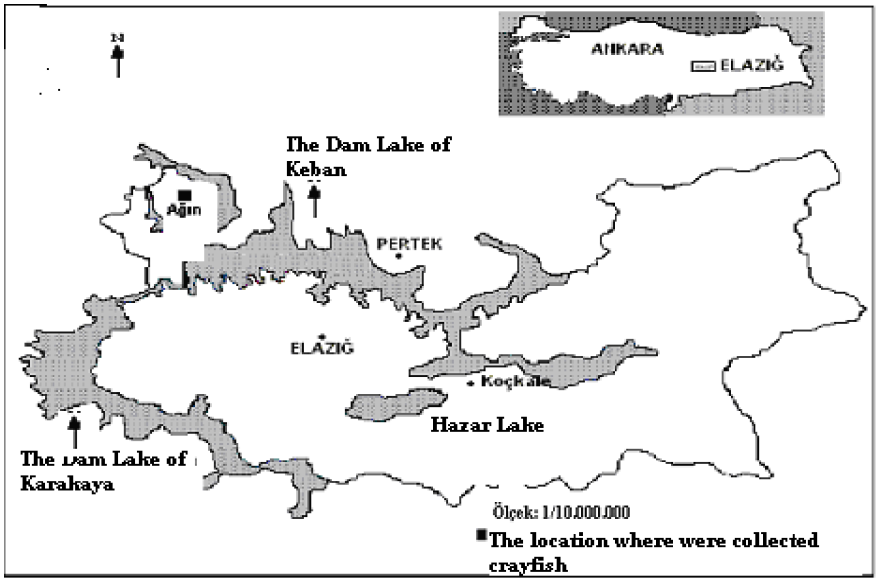

Fig. 1: Geographic location of the study

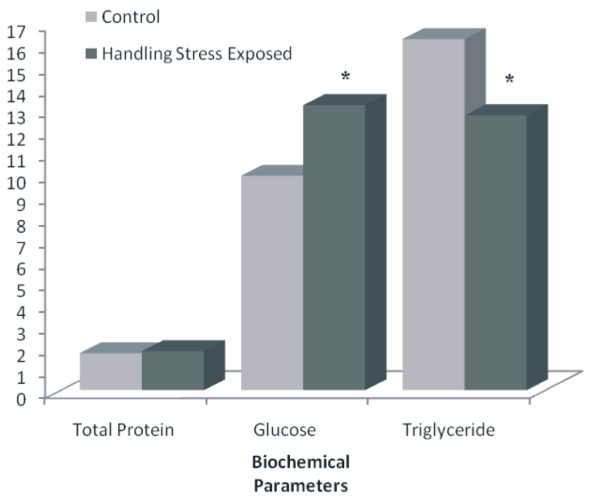

Fig. 2: Time course of total protein (g dLG^{1}), glucose (mg dLG^{1}) and triglyceride (mg dLG^{1}) values in the haemolymph of *A. leptodactylus* exposed handling stress by haemolymph bleeding at 1 hour of stress. Values are expressed as means ± SE (*p<0.05; n=30 for all groups)

Table 1: Total protein, glucose and triglyceride levels in experimental groups

B?ochem?cal Parameters	Handl?ng Stress Group	Control Group
Total Protein (g dLG^{1})	1.81±0.11	1.71±0.12
Glucose (mg dLG^{1})	13.20±0.93*	9.93±1.31
Triglyceride (mg LG^{1})	12.73±0.35*	16.26±1.62

*Significantly different at 0.05 Significance value, n=15.

The total protein levels in the haemolymph in during the stress period were shown in Figure 2. The total protein contents (1.81±0.11 g dLG^{1}) were higher than that of the animals in the control group (1.32±0.80 g dLG^{1}). No significant difference between the groups in total protein content was noted (p>0.05).

In the stressful crayfish (handling stress during 15 min.), the glucose levels were significantly different from values after the stress and was in the higher range of the values obtained from the control (P<0.05). (Table 1). The glucose levels in haemolymph were a peak of 13.20±0.93 mg dLG^{1} within 1 h. (Figure 2).

The concentrations of triglycerides of the control group (16.26±1.62 mg LG^{1}) in the haemolymph (Figure 2) significantly showed lower values than these obtained after exposure to handling stress (P<0.05). In all of the values during the stress period, the triglyceride level decreased of the mean values while the total protein and glucose contents caused their increase.

DISCUSSION

Handling stress is one of the important environmental stress conditions which are caused to changes in physiological variables such as oxygen uptake, haemolymph characteristics and composition (glucose, lactate and total protein), hormones and ions [3, 20-22]. One of the most interesting aspects of crayfish is the hyperglycemic response to these conditions. Crustacean hyperglycemic hormone family (CHH) hormone which is important in the stress response as an effecter of a homeostatic control system has a major play in the hyperglycemic response [6, 23, 24]. Different types of environmental stress produce enhanced haemolymph CHH levels [6, 23-4]. Some investigator reported that hypoxia, thermal changes and salinity result in CHH secretion to the haemolymph in *Homarus americanus* [6, 25].

Lorenzon *et al.* [10, 26] stated that the variation in amount and time course of blood glucose supports the homeostatic regulation of glycemia when CHH levels haemolymph of *Palaemon elegans* following exposure to various types of stress (heavy metals and lipopolysaccharide). Bergmann *et al.* [8] determined that glucose concentration in crustaceans' haemolymph increases in response to a number of stresses such as handling. The results obtained in the present study, indicated that glucose concentration in the haemolymph of *A. leptodactylus* increased after the stress period, a similar response has also been observed in other crustaceans [20, 27].

It has been found that within a species, several environmental stressors are changes in the total haemocyte count (THC) and total protein concentration [11, 14, 28 -30]. Malev *et al.* [31] found alterations in the total protein concentration in *A. leptodactylus* during the exposure to higher water temperatures. Our results indicated that total protein concentration in the haemolymph of *A. leptodactylus* increased after the stress period a similar response. In contrast, it was found that in the mean triglyceride levels, there was a decrease during exposure to handling stress.

In conclusion, the results obtained in the present study showed that an array of biomarker (total protein and glucose) that could be used to evaluate *A. leptodactylus* stress condition was measured and handling stress affect physiological condition of *A. leptodactylus*. We remain ignorant of the genetic features and species differences, age and size. Certainly, further studies are needed to be better defined to the interactions and effect on the physiological variables.

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