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Stress effect of different temperatures and air exposure during transport on physiological profiles in the American lobster *Homarus americanus*

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Abstract

Homarus americanus is an important commercial species that can survive 2–3 days out of water if kept cool and humid. Once caught for commercial purpose and shipped around the world, a lobster is likely to be subjected to a number of stressors, including emersion and air exposure, hypoxia, temperature changes and handling. This study focused on the effect of transport stress and specifically at different animal body temperature (6 and 15 °C) and air exposure during commercial transport and recovery process in water. Animals were monitored, by hemolymph bleeding, at different times: 0 h (arrival time at plant) 3 h, 12 h, 24 h and 96 h after immersion in the stocking tank with a water temperature of 6.5±1.5 °C. We analysed the effects by testing some physiological variables of the hemolymph: glucose, cHH, lactate, total protein, cholesterol, triglycerides, chloride and calcium concentration, pH and density. All these variables appeared to be influenced negatively by high temperature both in average of alteration from the physiological value and in recovering time. Blood glucose, lactate, total protein, cholesterol were significantly higher in the group with high body temperature compared to those with low temperature until 96 h after immersion in the recovery tank.

Keywords: Crustacea; cHH; Hemolymph parameters; Hypoxia

1. Introduction

The American lobster, *Homarus americanus*, is a fully aquatic species but it is well known that this species can survive 2–3 days out of water if kept cool and humid. Once caught for commercial purpose and shipped around the world, a lobster is likely to be subjected to a number of stressors, including emersion and air exposure, hypoxia and temperature changes, handling and interactions with other lobsters. All of these stressors will adversely affect the intrinsic quality of the lobsters (Danford et al., 2001). 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 (behaviour, vigour) or expressed quantitatively by measuring changes in physiological variables such as oxygen uptake, blood composition, pH, hormones, ions and hemocytes (Paterson and Spanoghe, 1997; Taylor et al., 1997).

Glucose concentration in crustaceans hemolymph rises in response to a number of stress such as handling (Paterson et al., 1997; Bergmann et al., 2001), emersion (Spicer et al., 1990; Morris et al., 1986; Morris and Olivier, 1999; Speed et al., 2001), salinity (Spaargaren and Haefner, 1987), disease and pollutants (Lorenzon et al., 1997, 2000). Various peptides, amines and hormones circulate in the hemolymph and regulate the physiological status of crustaceans (Fingerman, 1995). In particular the crustacean Hyperglycemic Hormone (cHH), that regulates mainly the release of glucose, is involved in the mediation of stress responses (Keller and Orth, 1990; Webster, 1996; Chang et al., 1998; Lorenzon et al., 2004).

Lactate can accumulate in the hemolymph during emersion and hypoxia (Spicer et al., 1990; Paterson et al., 1997; Durand et al., 2000). Other hemato-immunological parameters involved in the stress response following handling, transport and emersion are total hemocytes count (THC) and total protein

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concentration (Jussila et al., 1999; Lorenzon et al., 1999; Gomez-Jimenez et al., 2000; Perazzolo et al., 2002).

In this work we focused on the effect of transport stress and specifically at different animal body temperature during commercial transport shipping and recovery process in water on physiological profiles in the American lobster *H. americanus*. We analysed the effects by testing some physiological variables of the hemolymph: glucose, cHH, lactate, total protein, cholesterol, triglycerides, chloride and calcium concentration, pH, and density. An increased understanding of the mechanism of stress and physiological modification induced in lobsters by capture, handling, transport and storage may lead to a better commercial practice for processing with consequent better survival, quality and animals' well being.

2. Material and methods

2.1. Animals and experimental design

This study, carried out at Fiorital srl plant (Venice, Italy) in the period between September 2003 and December 2004, focused on the effect of transport stress and specifically at two different animal body temperature (6 and 15 °C) as recorded upon arrival in different seasons on air-shipped *H. americanus*. The lobsters used were chosen randomly among the animals that arrived and each lobster was tagged, measured, weighed and inspected for damage. The animals were intermolt (to minimize intrinsic metabolic variations), both sexes, 531±75 g live mass (mean±S.D.), 22.7± 1.5 cm total body length (n=35).

Animals were monitored, by hemolymph bleeding, at different times. 0 h (arrival time at plant in polystyrene boxes refrigerated with frozen gel pack and seawater-soaked paper, after a travel of about 48 h), 3 h, 12 h, 24 h and 96 h after immersion in the stocking tank with a water temperature of 6.5± 1.5 °C and salinity of 34–35 psu. Groups of 10 animals were tested for each temperature and kept unfed throughout the monitoring period.

For each parameter, values recorded in animals maintained after the 96 h in the stocking tanks were considered as control since no further change was detected later on (2 weeks).

Animals were blotted dry and hemolymph was withdrawn from the pericardial sinus with a sterile 1-mL syringe fitted with a 25 G needle. Animals were bled 1 mL hemolymph each time. Hemolymph was centrifuged for 1 min at 10,300×g and 4 °C and the plasma fraction was quickly frozen at −20 °C and stored until required for study.

Eyestalks for the quantification of cHH were removed from 5 animals at time 0 h and at 12 h, Animals were anaesthetized for 1 min on ice before ablation. The eyestalk was quickly frozen and eyecup cut off for removing visual and screening pigment. Eyestalk homogenate was prepared from 2 eyestalks (from the same animal) sonicated in 200 μL cold phosphate-buffered saline (PBS; Sigma) pH 8.0 and then centrifuged for 1 h at 930×g and 4 °C. The clear homogenate was quickly frozen at −20 °C.

2.2. Determination of hemolymph parameters

Glucose content was quantified by using One Touch® II Meter (Lifescan, Milpitas, CA, USA) and commercial kit test strips (precision strips±3% coefficient of variation in the tested range).

Lactate concentration of the hemolymph was obtained using CDR-Lact photometer (CDR-Mediared, Florence, Italy) with reagents in prefilled cuvettes.

Total protein, cholesterol, triglycerides, chloride and calcium concentration, were determined using Screen Point (Hospitex Diagnostics srl., Florence, Italy) a multi-channel (420–590 nm), photometric reading system, clinical chemistry analyser with reagents in prefilled cuvettes. Standard solution calibrations for each parameter were used between samples.

pH of the hemolymph was measured with pH meter GPL 21 (Crison, Italy) using a micro-electrode (5 mm diameter; Crison). Standard pH calibrations were checked between samples.

The density of the hemolymph was obtained by the use of a density–salinity refractometer (Scubla s.n.c., Udine, Italy) with automatic temperature compensation.

2.3. Direct enzyme-linked immunosorbent assay (ELISA) of cHH

ELISA was performed as described by Lorenzon et al. (2004). 6xHis-*Nen*cHHwt (MW=11 kDa; Mettulio et al., 2004) recombinant protein was used as standard (y=1.49514−0.37828x and the correlation coefficient of the fitted curves is R=0.9937, S.D.=0.44, P<0.001; Lorenzon et al., 2004).

Briefly, 100 μL of the samples of eyestalk homogenate (n=10 eyestalk) from the different treatments as well as standards were loaded onto a 96-microwell plate (Costar) and incubated in duplicate overnight at 4 °C. Then 100 μL of the biotinylated anti-*Nen*cHH (anti *Nephrops norvegicus* cHH; interspecific cross-reactivity tested by Giulianini et al., 2002) antibody (1 μg μL^{-1}) diluted 1:1000 was added to each well and the plate was incubated for 3 h at 36 °C. After removal of the biotinylated antibody, plates were washed extensively followed by the addition of 100 μL of streptavidin–peroxidase (Sigma) solution diluted 1:5000 and incubated for 1 h at RT. The plates were once again washed and developed with 2,2′-azino-bis 3-ethylbenz-thiazoline-6-sulphonic acid solution (Sigma, liquid substrate ready for use) in darkness for 1 h at RT (100 μL per well).

The absorbance was measured in a multiwell plate reader (Anthos 2020 version 1.1; Anthos, Krefeld, Germany) at 405 nm.

2.4. Statistical analysis

All statistics were performed by using a SPSS 9 (R) for Windows package and data are given as arithmetic means± standard deviations. Student's t test were used to test the null hypotheses that all treatment means were equal and then all the data were tested by LSD and Dunnett's *post hoc* tests. The levels of significance were then calculated by Student's t test for