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Resistance to stress and Hc functional modulation in *Liocarcinus* sp.

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Abstract

This study is included in a project aimed to study the alterations on the structure of the Northern Adriatic Sea ecosystem produced by fishing activity. The indirect or secondary effects of fishery such as the changes of the structure and trophic relationships of the ecosystem are under investigation and we have particularly considered the effects on species such as *Liocarcinus depurator* that are captured and then rejected because devoid of commercial value.

The objective of this study is the *Liocarcinus* sp. adaptative resistance to stress and the effects of biochemical parameters (allosteric effectors) on Hc functional modulation.

This study is included in a project about the alterations on the structure of the Northern Adriatic Sea ecosystem produced by fishing activity (Hall, 1998). Commercial fishing produces a deep impact on the biodiversity of the marine ecosystem through direct and indirect effects that change the structure and the trophic relationship (Jennings and Kaiser, 1998). The interest is focused on the analysis of the indirect effects of this activity and in particular we have analysed the problems of species devoid of commercial value and for this reason rejected and returned to the sea after the capture, with the resulting exposure to hypoxic and hyperthermal shocks (Pauly, 1998). These animals also undergo mutilation and physical damage of several types, resulting for the most sensitive species in alteration of the fish stock. In contrast, ‘scavenger’ species can benefit, in a trophic point of view, from the animals just damaged or killed (Pranovi et al., 2001). Among the species that are suitable for an integrated eco-physiological study we have considered *Liocarcinus depurator*, because it shows high recovery capability and adaptative resistance to the stresses resulting from trawling followed by re-immersion in the sea.

The object of the present study is the analysis of the physiological basis that give to the *Liocarcinus* sp. adaptative resistance to the stresses and the determination of precise functional parameters to estimate the effects of fishing activity on the ecology of the ‘*reject*’ animals (rejected after capture because they are devoid of commercial value).

Individuals were collected along six stations of the North Adriatic Sea, from the catch of commercial vessels (‘rapido’ trawling) and trap-cages, in March 2002; these organisms had been exposed to air and subsequently reintroduced in sea water for the recovery. Hemolymph samples were removed from these animals, rapidly frozen in liquid N_2 and immediately stored at −20 °C. The pH of the hemolymph was measured using a microelectrode pH meter, at the in situ temperature (15 °C). In parallel we used respirometric chambers equipped with a Clark’s electrode to measure the oxygen consumption curve and the oxyregulatory capability. Finally we have analysed the effects of alteration of biochemical parameters on the hemocyanin oxygen binding.

The aim of quantitative analysis was to determine whether stress (hypoxic and hyperthermal shocks) can be revealed by an alteration of the values of metabolites. We have firstly considered the alteration of some biochemical parameters of the hemolymph (lactate, glucose and ammonia concentrations) and we have analysed the effects of such parameters on the oxygen-binding properties of the hemolymph hemocyanin, through oxygen saturation curves (tonometric method) in Tris/HCl (50 mM), $CaCl_2$ (20 mM) at different pHs. The metabolites considered in this study are allosteric effectors of hemocyanin, capable of modifying the oxygen-binding functional parameters of the protein, increasing the oxygen capacity of the hemocyanin (Trouchot, 1992). Exposition to air induces a pronounce increase

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Table 1
Average (standard deviation) values of biochemical parameters of three groups of *Liocarcinus depurator*

Condition	pH	NH_4^+	L-lactate	D-glucose
A: absence of stress $N = 6$	7.52 ± 0.04	0.107 ± 0.007	0.026 ± 0.004	0.167 ± 0.017
B: mechanical stress $N = 37$	7.44 ± 0.04	0.145 ± 0.009	2.123 ± 0.5676	0.150 ± 0.0436
C: air exposure (1 h) $N = 6$	7.25 ± 0.03	0.399 ± 0.088	6.88 ± 0.745	0.469 ± 0.097
D: recovery (24 h) $N = 16$	7.59 ± 0.01	0.123 ± 0.011	0.469 ± 0.011	0.175 ± 0.016

'Absence of stress' is defined by a group of individuals (A) collected without mechanical stress (trap cage); 'Mechanical stress' is defined by a group (B) collected by rapido trawling; 'Air exposure' by animals (C) were collected and exposed to air for 1 h; 'recovery' as sample (D) after reimmersion in sea-water for 24 h.

of lactate, glucose and ammonia concentrations as documented in Table 1.

The increase of L-lactate and D-glucose can be interpreted as a consequence of an increased metabolic activity of the animals in low tissue oxygen, following capture under conditions that do not allow for a correct oxygen supply through gills. The two parameters are correlated since in crustacea a hyperglycemic hormone increases glucose concentration (Santos and Keller 1993), thus supporting the glucose consumption under low oxygen, which is responsible for lactate accumulation. Loss of gill functionality due to removal from water is also responsible for the increase of ammonia, since an impairment of the epithelial NH_3 diffusion and Na^+/NH_4^+ counter transport can be expected. The metabolic effects lead to hemolymph acidosis. It is worth noting that the biochemical alterations are almost completely reversed after 24 h recovery period in sea water.

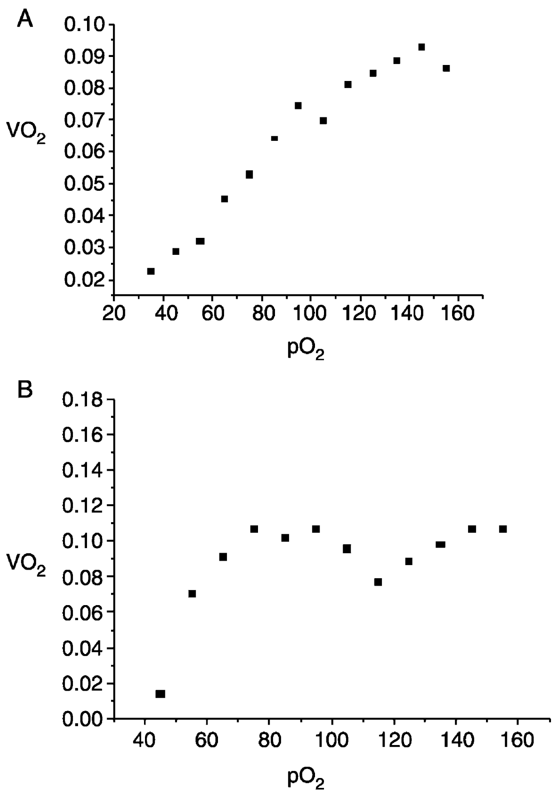

Fig. 1. Oxygen consumption velocity as a function of partial pressure in control (A) and 1 h air exposed crabs (B).

The study of the respiratory physiology shows that upon exposure to air, the organisms, once re-immersed in sea water, shift from an oxyconformer (Fig. 1A) to an oxyregulative behaviour (Fig. 1B). Foran oxyconformer behaviour, as exhibited by the control animals, the oxygen consumption velocity (VO_2) changes as a function of oxygen partial pressure (pO_2), thus the recovery of oxygen demand is efficient only with well-oxygenated water. In contrast, the oxyregulatory behaviour, exhibited by the air exposed animals that maintain an elevated VO_2 as pO_2 decreases, ensures the ability to efficiently recover from oxygen demand by in increase of respiration rate, within a rather broad (down to the critical pressure of 80 mmHg) pO_2 range.

The described shift of respiratory physiology can, at least in part, be correlated with a change in the efficiency of oxygen supply to the hemolymph, due to allosteric modulation of Hc oxygen binding properties. The Hc of *Liocarcinus depurator* is characterized by a low affinity and cooperativity, under control conditions. From the data of Table 2, it is shown that the oxygen-binding properties of Hc are modulated by the alteration of biochemical parameters. In particular, the presence of metabolites in the buffer solution compensate for the Bohr effect due to acidosis and decreases p_{50} to 50 mmHg. Hence, an improved oxygen affinity of Hc could be important for the recovery of the animal from stress. The improved capability of Hc to efficiently supply oxygen under declining pO_2 may represent an important molecular adaptation for the oxyregulative behaviour in the presence of stress.

Table 2
Oxygen binding parameters obtained by Hill' plot

Condition	P_{50} (mm Hg)	n
pH 7.6	91	2.1
pH 7.4	100	2.6
pH 7.2	110	2.5
pH 7.2, L-lactate, D-glucose and ammonium as Table 1, C	50	2.8

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