Evolution of the antioxidant defences and «oxidative stress» bio-indicators in the shrimp *Litopenaeus stylirostris* after handling stress.

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Abstract

Defences response and oxidative stress have been studied in the shrimp L. stylirostris *after handling during transfer from the rearing pond into the hatchery. Antioxidant defenses measured in gills, digestive gland and muscle were : Superoxide dismutase (SOD) and the glutathiones : total glutathiones (GSHT) and oxidized glutathiones (GSSG). Malondialdehyde (MDA) considered as an indicator of "oxidative stress" was also measured in the three tissues.*

A significant evolution of antioxidant defences and "oxidative stress" occurred after handling mainly in the shrimps in premolt stage. Evolution trend was similar for the oxidative defenses parameters in the three tissues tested. MDA evolution was different according to the molting stage and tissue of the animal : at intermolt stage, MDA increased significantly at 24 and 48 hours after handling in gills and muscle respectively and at premolt stage MDA raised in the 3 tissues 144 h after handling.

Introduction

The farming of the tropical species *Litopenaeus stylirostris* in subtropical regions has shown an excessive fragility of prawns when the temperature falls below 20-22 °C, which appears to be the lower edge of the animals' thermopreferendum range (Wabete et al., 2008). Under these low-temperature conditions, *L. stylirostris* is extremely sensitive to heavy stressful events like net-fishing followed by handling associated with a rapid transfer from large outdoor rearing earthen ponds into indoor hatchery facilities, as practiced for breeders. Our preliminary studies had shown that the high mortality of animals, occurring 48 h after handling, is explained by the impairment of haemolymph oxygen transport – probably induced by the ionoregulatory disturbance – when the aerobic metabolism has returned to steady-state low levels. In relation to this previous work, we were interested in the consequences of this handling stress, and subsequent increase arterial oxygen partial pressure, on antioxidant defences and the oxidative stress.

Materials and method

Experiment was performed in winter (average water temperature 22.4 ± 1.6°C) periods on 97 penaeid shrimps (mean weight 36.6 ± 0.5 g) in the intermolt and premolt stages (Drach, 1939). Shrimps were harvested from the earthen pond using a castnet and transferred to the laboratory in 50 l plastic bins filled with pond seawater within about 10mn. Shrimps were then held in a 15 m^3 concrete tank previously filled with filtered seawater (~35 ‰) and maintained at the temperature of the earthen pond.

Twelve shrimps were sampled 2, 24, 48 and 144 hours after harvest. Gills, digestive gland and muscle were immediately dissected, plunged in liquid nitrogen and then kept in freezer (-80°C) until analysis.

The 2 antioxydants and the product of lipid oxidation were measured according the methodologies described in the litterature and adapted to microplate reader (Bioteck® Synergy HT) : Superoxide Dismutase (SOD) (Marklund et Marklund, 1974), Total (GSHT) and oxidised (GSSG) Glutathiones (Akerboom and Sies, 1981) and Malondialdehyde (MDA) (Richard et al., 1992).

All data are presented as means ± 1SE. Statistical analyses were performed with the computing program StatView® and differences were considered significant when $p < 0.05$.

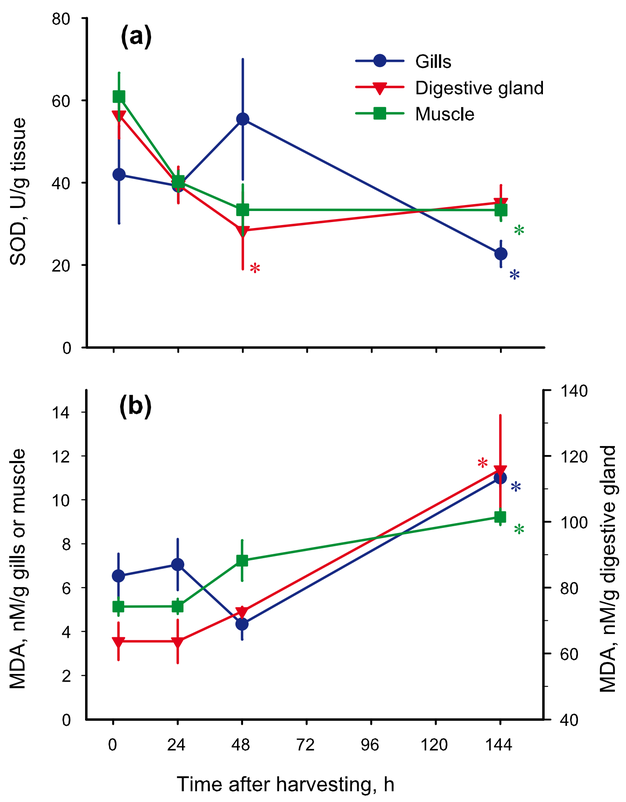

Figure 1 : Evolution of SOD (a) and MDA (b) in the 3 tissues of shrimps in premolt stage according time after harvesting.

Results and discussion

A significant evolution of antioxidant defences and "oxidative stress" after handling stress was observed in the 3 tissues tested.

Shrimps in premolt stage have shown the most significant evolution with a similar fall of the SOD activity and total glutathiones in the digestive gland and muscle (Fig. 1a) starting in the first hours after handling stress. In the mean time, the ratio of oxidized glutathiones on total glutathiones (GSSG/GSHT) rose slightly during the first 48h after handling (Fig. 2a). In the gills, SOD increased ($p>0.05$) between 24 and 48h and then decreased afterwards. The drop in SOD activity may be related to an excess of superoxyde anion (O_2^-) following stress. This fall of antioxydant defences was concomitant with a rise of lipids peroxidation in the 3 tissues studied (Fig. 1b).

In intermolt stage, the evolution of tested indicators was moderate compared to animals in premolt stage. Shrimps exhibited 48 h after handling an increase of the GSSG/GSHT ratio in digestive gland and muscle (Fig. 2b). Besides, MDA increased sharply at 24 h and 48 h after stress in gills and muscle respectively (Fig. 3).

The observed phenomenons highlight an oxidative stress of the prawn in both molt stages probably resulting from increase production of reactive oxidative species (ROS) caused by the handling stress. Previous work had shown that this stress has a considerable and rapid effect (in the first minutes) on respiratory function (Wabete et al., 2008).

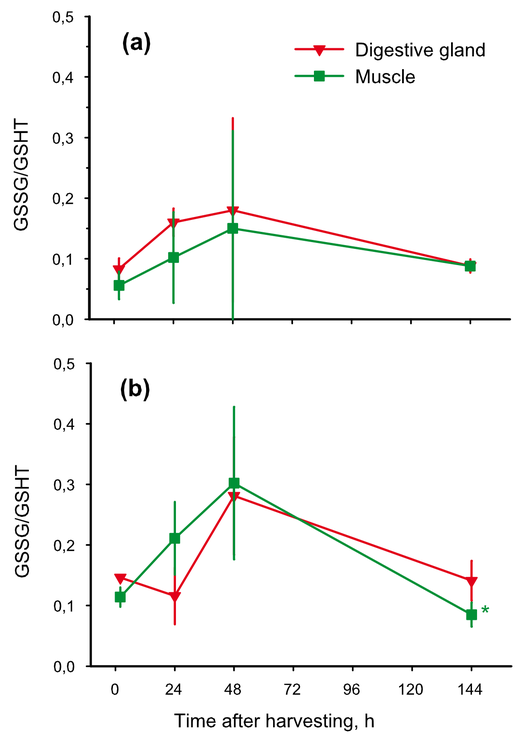

Figure 2 : Ratio of oxidized and total glutathiones of shrimps in premolt (a) and intermolt (b) stage.

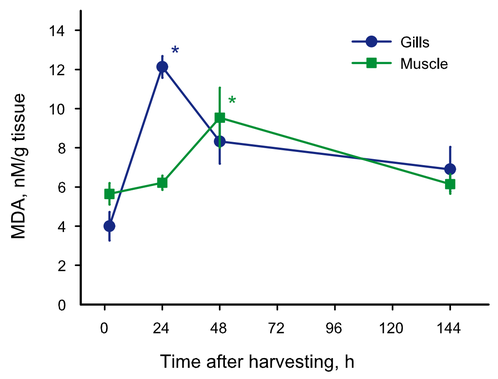

Figure 3 : Evolution of MDA in Gills and Muscle of shrimps in intermolt stage.

Conclusion

These first results show that a simple handling of the animals during their transfer into hatchery causes a response of antioxidant defences in the 24 hours following stress and the development of a durable oxidative stress. This oxidative stress could - by the tissues damage, particularly in the gills - contribute to the osmoregulatory and respiratory disorders leading to the weakening and finally to the death of shrimp (Wabete et al., 2008).

References

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