Evolution of the antioxidant defenses and «oxidative stress» bio-indicators during larval ontogenesis of the shrimp *Litopenaeus stylirostris*.

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Introduction

Shrimp industry in New Caledonia entirely depends on *Litopenaeus stylirostris*, imported from Mexico Gulf in 1980. More than 40 generations of captive broodstock have been reared since to date. Hatchery production is closely linked to the larvae capacity to withstand stressful events and particularly, oxidative stress.
Oxidative stress occurs when there is an imbalance in the generation and removal of radical species within an organism (Sies, 1986). These radicals involve mostly oxygen and reactive oxygen species (ROS). These small molecules can cause widespread damage, particular in membrane lipids, but also in proteins and nucleic acids with negative consequences in cellular structures and associated physiological functions. Our laboratory has recently focused on shrimp larval susceptibility to oxidative stress according to antioxidant defences evolution during ontogenesis.

Materials and method

Larvae in different stages (nauplius, zoea 2, mysis 2 and 10 days old postlarvae) were plunged in liquid nitrogen and then kept in freezer (-80°C) until analysis. The antioxidants and the products of lipid and protein oxidation were measured according to methodologies described in the literature:
4 endogeneous antioxidants:

- Superoxide Dismutase (SOD) (Marklund and Marklund, 1974);
- Catalase (CAT) (Clairbone, 1985);
- Glutathione peroxydase (GPx) (adapted from Gunzler et al.,1974);
- Total (GSHT) and oxidized (GSSH) Glutathiones (Akerboom and Sies, 1981).

2 secondary oxidation products from lipids and proteins respectively:

- Malondialdehyde (MDA) (Richard et al., 1992);
- Protein carbonyl (Rodney et al., 1990).

Measurements of SOD, CAT, GPx and Glutathiones, have been adapted to microplate reader (Bioteck® Synergy HT).

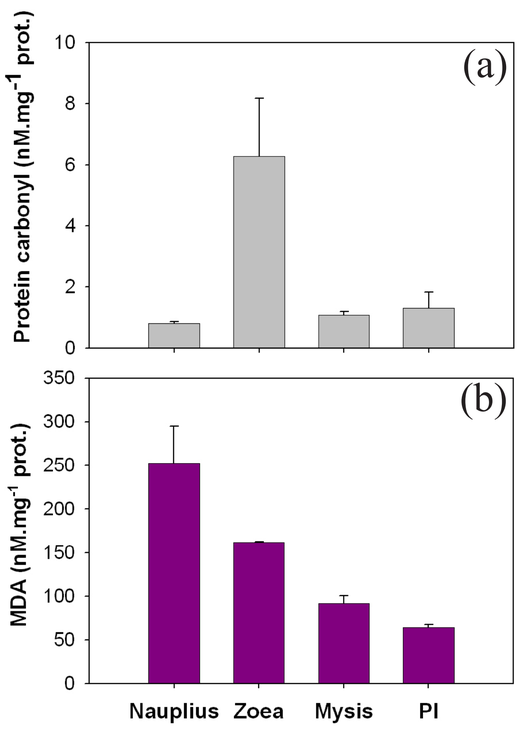

Fig 3 : Evolution of protein carbonyl (a) and MDA (b) according to larval stage.

Results and discussion

At the antioxydant defences level, nauplius stage is caracterized by no SOD and low GPx activity (fig 1a & 1c). Zoea stage exhibits highest level of all measured antioxydants ($p<0.05$): SOD, CAT, GPx and GSHT (fig 1a,b,c et fig 2a). Beside, the ratio GSSH/GSHT is higher in zoea compared to other larval stages ($p<0.001$) (fig 2b). These changes in antioxydant defences and "oxidative stress status" could be related with the shift in feeding behaviour, the progressive implementation of the antioxidant defences and/or the ontogenetic variation in metabolism of the shrimps.
At the level of the oxidative stress damages, lipids peroxydation (MDA level) in nauplius and zoea are much higher than in mysis and postlarvae stages ($p<0.01$) (fig 3b). MDA level is especially high in nauplii which obtain most of their energy from the catabolism of lipoprotein yolk reserves (Agard, 1999), rich in lipids particularly in polyunsaturated fatty prone to peroxidation. With a higher metabolism compared to the other stages (Lemos and Phan, 2001), nauplii could be more sensitive to the free radicals from the respiratory chain. The MDA accumulated in the nauplius stage are slowly eliminated and remained high in zoea stage to decrease in mysis and PL afterward (fig 3b). Beside, carbonyl concentration is six times as high in zoea compared to other larval stage (fig 3a). This could be explained by the drastic modification in feeding regime and the different levels of lipid storage when the nauplius turns into zoea (Lemos and Phan, 2001).

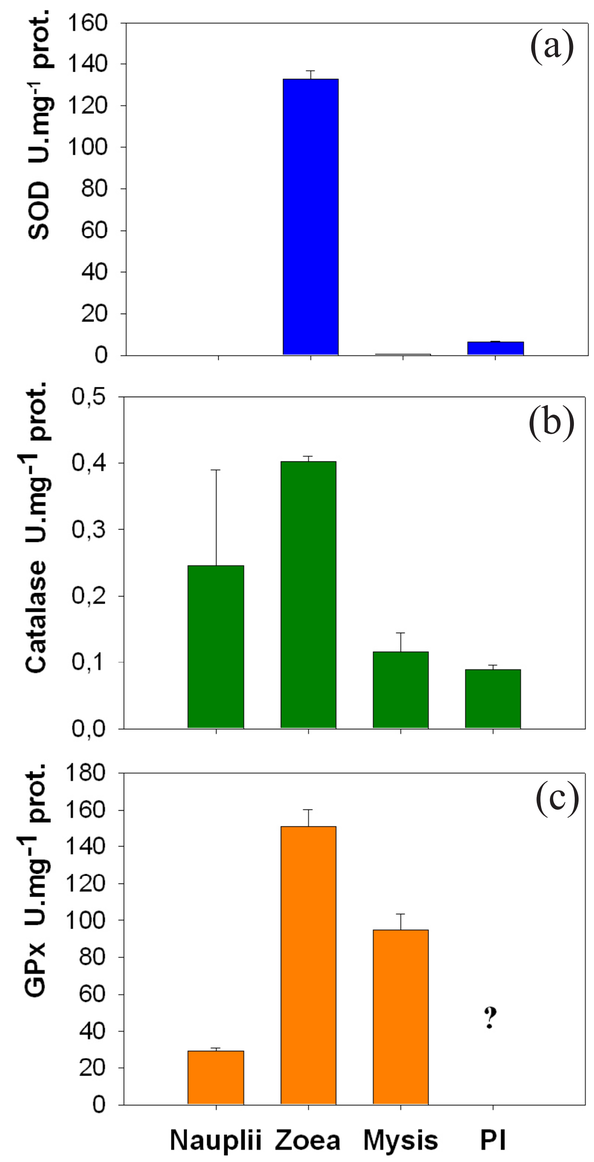

Fig 1 : Antioxidant enzymes activity SOD (a), CAT (b) and GPx (c)

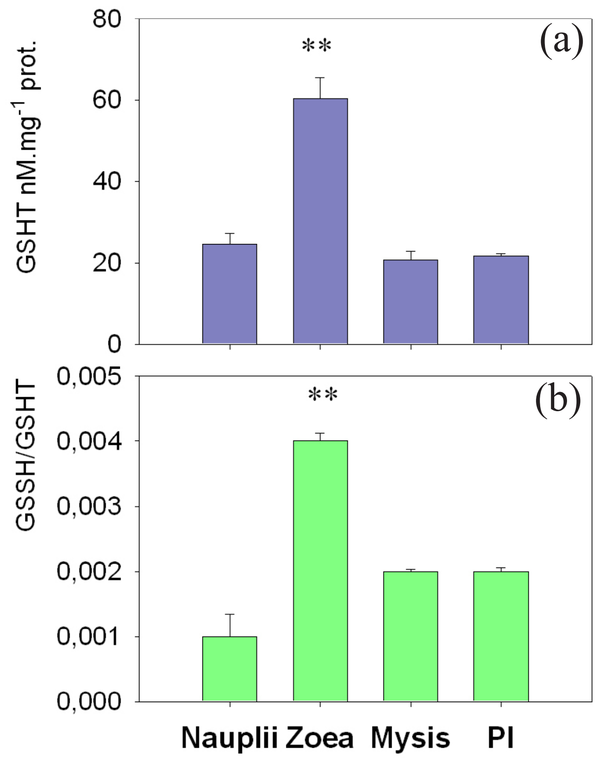

Fig 2 : Total Glutathiones (a) and ratio of oxidized and total Glutathiones (b)

Conclusion

These first results show that the antioxidant defences and the response of the shrimp to an "oxidative stress" evolve during the embryonic development. These preliminary observations indicate that nauplius and zoea stages could be more subject to oxidative stress. Further analysis must be assessed to confirm these results.
This research should allow to identify the main risk factors and thus prevent from oxidative stress in larval commercial production. From a practical angle, some zootechnical or nutritional recommendations could be suggested to improve the antioxidant status of the shrimp during its larval ontogenesis.

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