Effect of experimental temperature fluctuations on some «oxidative stress» bio-indicators in the digestive gland of the shrimp *Litopenaeus stylirostris*.

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Introduction

Shrimp farming in New Caledonia, although small in size (2000 mT.year^{-1}) compared to the world's production (4'000'000 mT.year^{-1}), stands out as a success story among the small South Pacifi c islands countries. However this activity faces a bacterial disease outbreaks in cool season named "Syndrome 93" (Le Groumellec et al, 1996) (fi g1). Cool season temperatures, with decreases and fluctuations to the low side thermopreferendum, (LSTP) leads to osmoregulatory and respiratory disorders in the shrimp *L. Stylirostris* (Wabete et al., 2007). Moreover, the shrimps subject to thermal variations exhibit excessive reactive oxigen species (ROS) formation which subsequently alters cell's structures (lipid peroxidation) and functions. Those physiological perturbations may impair defence response and weaken the shrimps exposed to pathogens, leading to "syndrom 93". The main objective of this study was to investigate some aspects of the antioxidant defences and of the oxidative stress response of the shrimp upon exposure to temperature stress.

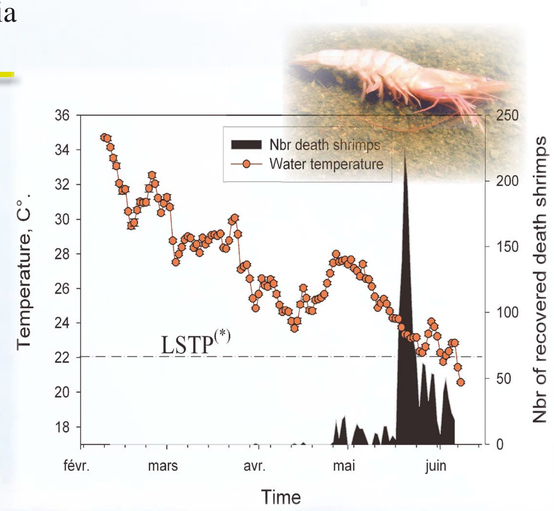


Fig1 : Evolution of pond temperature and shrimp mortality. (*) Low Side Thermopreferendum of *L.stylirostris*

Materials and method

Sub-adult shrimps (8.5g ± 1.3 SD) were split up into two groups (100 animals per treatment) and reared over 29 days in experimental tanks (300 liters each) (picture1) at two temperature regimes: within the thermopreferendum (ITP) and at lowside of the thermopreferendum (LSTP) (fig2a).

Twenty shrimps of each treatment were sampled at days 1, 10, 15 et 28 of the trial. Digestive glands were immediately dissected, plunged in liquid nitrogen and then kept in freezer (-80°C) until analysis. Measurements of oxidative stress parameters in the digestive gland were carried out only for shimps in the intermolt stage (Drach, 1939).

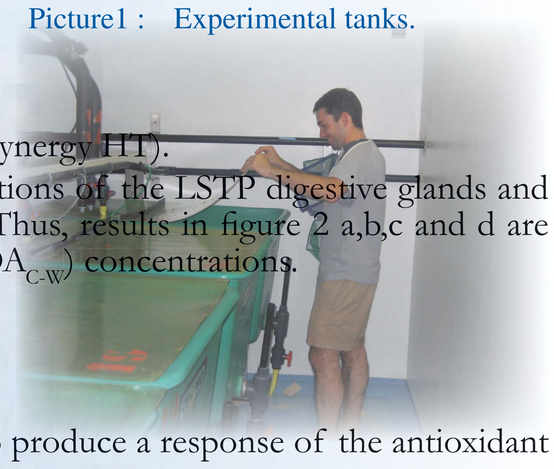
Measurements of the antioxidants and of the lipid peroxidation are based on methodologies described in the litterature:

- Superoxid Dismutase (SOD) (Marklund et Marklund, 1974)
- Glutathions (Akerboom etg Sies, 1981)
- Total Antioxidant Status (TAS) (Arnao et al., 1996)
- Malondialdehyde (MDA) (Richard et al., 1992).

Measurements of SOD, Glutathions, TAS have been adapted to microplate reader (Bioteck® Synergy HT).

The difference between SOD activity (unit.g^{-1} organ), Glutathiones, TAS and MDA concentrations of the LSTP digestive glands and the ITP digestive glands were used as an index for comparing the effects of the treatments. Thus, results in figure 2 a,b,c and d are expressed as delta SOD activity (SOD_{C-W}), Glutathion (GSH_{C-W}), TAS (TAS_{C-W}) and MDA (MDA_{C-W}) concentrations.

Picture1 : Experimental tanks.



Résults and discussion

Shrimps under LSTP treatment exibited an oxidative stress. Indeed, thermal drop was found to produce a response of the antioxidant defences system in the digestive gland and to increase lipid peroxidation following several steps:

- Day 10 the first antioxidant defence response is characterized by a drop of the concentration of the non-enzymatic antioxidant total and reduced glutathiones (table 1; fig2b) (p=0.11). This result is in accordance with previous statements reporting that low-molecular weight scavengers are often seen as the first line of defense, as they are rapidly reacting molecules and contribute to the enzyme protection from oxidation.
- Day 15, digestive gland exibited a second antioxidant response characterized by a reduced activity (-30%) of antioxidant enzyme SOD (p = 0.01) (table1; fig2c). The drop in SOD activity may be related to an excess of superoxid anion (O^{2-}) following the temperature stress leading to an hyperventilation and an subsequent increase of free oxygen in the shrimp blood to compensate its transient oxiphoric lower capacity (Wabete et al., 2007) .

  The same day, the digestive gland exibited a rise in the lipid peroxidation level, showed by a strong increase of the MDA concentration (+100%, p=0.0002) (table1; fig2e).
- Subsequently (from day 18 up to day 28) temperature stabilisation of the LSTP treatment could explain recovery from physiological stress and a come back to the control values of Glutathiones, SOD and MDA.

TAS in the digestive gland decreased during the course of the trial, but not significantly (p>0,05) (table1, fig2d). This parameter which measures non enzymatic antioxidant defenses provides integrative information on the susceptibility of the organism to oxidative stress. This decrease in TAS concentration may be explained by reduced feed intake of the shrimp reared in cool water, lowering the input of nutritional antioxidants.

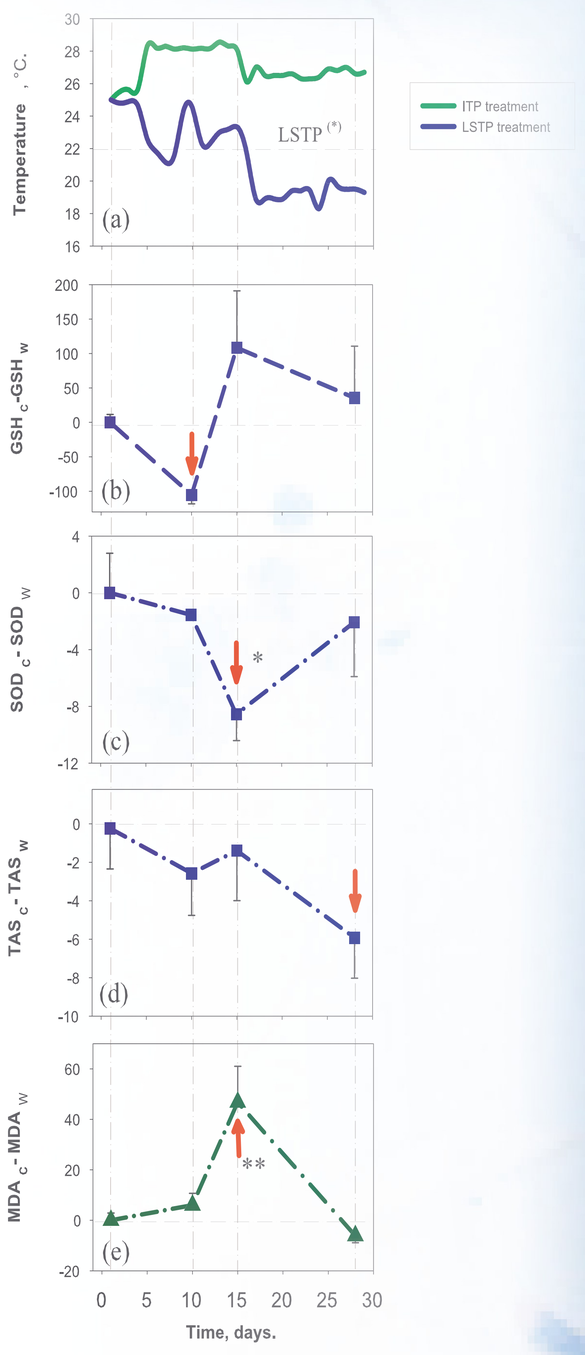


Fig2 : (a) Evolution of water temperature of the 2 treatments : Inside Thermopreferendum (ITP) and Low Side Thermopreferendum (LSTP).
Delta GSH (b), SOD (c), TAS(d) and MDA(e) according time.

Conclusions

Temperature decreases and fluctuations at the lowside thermopreferendum lead to an oxidative stress in the shrimp *Litopenaeus stylirostris*. Within their thermopreferendum, pro- and antioxidant processes are balanced whereas under the LSPT regime antioxidant molecules and enzyme equilibrum vary significantly.

On farm production, deterioration of antioxidant status of the shrimps upon exposure to temperature change in cool season may make the shrimps more vulnerable to *Vibrio penaeicida,* which has been identified as the pathogen of "syndrom 93". In the light of those results, enriching the shrimp's diet with nutritional antioxidants (vit C, vit E, carotenoids...), would be an interesting way to compensate deficiency of such compounds due to a decrease in the dietary intake in cool season. We hypothesize that better antioxidant status of the farmed shrimps may improve their resistance to oxidative stress, resulting in a higher resistance to opportunistic pathogens.

Table 1 : Mean values and standard deviation (SE) of SOD activity, GSHT, TAS and MDA concentrations in hepatopancreas of the shrimp exposed to the LSTP (Low Side Thermopreferendum) treatment according rearing days.

Day	SOD U.ml-1.g-1		GSHT nanoM.g-1		TAS µM.g-1		MDA nanoM.g-1	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	16.32	2.79	435	25	21.07	2.67	21.21	2.63
10	17.36	0	62	15	17.22	2.06	32.46	7.65
15	11.88	1.85	548	43	14.22	2.97	70.47	14.13
28	21.91	3.82	512	43	13.57	2.49	43.37	3.08

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