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Combined effects of deltamethrin, temperature and salinity on oxidative stress biomarkers and acetylcholinesterase activity in the black tiger shrimp (*Penaeus monodon*)

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ABSTRACT

This study aimed to investigate the interactions of two abiotic factors (temperature and salinity) and deltamethrin (pyrethroid pesticide) exposure on some oxidative stress biomarkers as well as on acetylcholinesterase activity (AChE) in hepatopancreas, gills and muscle of black tiger shrimp (*Penaeus monodon*). A combination of three temperatures (24, 29 and 34 °C), two salinities (15 and 25 ppt), and the absence or presence of 0.1 μg L^{-1} deltamethrin was applied on shrimp during 4 d under laboratory conditions. Lipid peroxidation level (LPO) and glutathione S-transferase activity (GST) were not affected by combined effect of temperature, salinity and deltamethrin in any of the studied tissues. Deltamethrin impaired other tested oxidative stress biomarkers, i.e. total glutathione (*t*GSH), catalase (CAT), glutathione peroxidase (GPx). *t*GSH level significantly increased in hepatopancreas due to deltamethrin exposure mainly at 34 °C, while pesticide effects on *t*GSH and CAT activity in gills were influenced by both temperature and salinity. In addition, GPx activity in hepatopancreas decreased after deltamethrin treatment mainly at 24 °C. Finally, AChE in muscle was strongly inhibited by deltamethrin at all tested temperatures and salinities. These novel findings demonstrate that interactions between abiotic factors and a commonly used pesticide exposure should be taken into account when analyzing some widespread biomarkers in black tiger shrimp.

1. Introduction

Marine crustaceans are under the influence of numerous environmental factors such as natural environmental changes according to daily or seasonal rhythms, environmental stress from contaminants, or physico-chemical changes. Most existing studies were directed towards the effects of a single environmental factor, such as temperature or salinity, on some biochemical markers (Leinio and Lehtonen, 2005; Menezes et al., 2006; Cailleaud et al., 2007). However, this is rarely the case in reality where aquatic animals are exposed to a variety of environmental stressors. In Mekong River Delta (MRD), Viet Nam, water temperature in shrimp ponds, ranged from 23.9 to 34 °C, fluctuating diurnally and seasonally as it depends on air temperature, water depth, pond design and water management (Phuong, 2005). In a same way, the salinity in MRD is higher during the dry season, ranging from 21.5 to 25.0 ppt, but decreasing to 15.0 ppt after heavy rainfall (Phuong, 2005). Sub-optimal temperature, or unsuitable salinity level in water may interact in an antagonistic, additive or synergistic manner with toxicants thereby causing changes in the tolerance capacity of aquatic animals. Thus, toxicological researches have recently emphasized the need for testing the interaction between multiple factors.

The long-term ecological hazard associated with the use of organochlorine, organophosphate, and carbamate compounds propelled the introduction of a new generation of pesticides with a lesser degree of persistence. As a consequence, the use of pyrethroids as insecticidal and anti-parasitic formulations has markedly increased as a viable substitute and currently accounts for over 30% of insecticides used globally (Prasanthi et al., 2005). Deltamethrin is a pyrethroid, that kills insects on contact and through ingestion. The exact mode of action for deltamethrin is currently not known. It is generally assumed that pyrethroids affect neuroactivity by delaying the closing of sodium channels (Corbett et al., 1984). This affects action potentials and often results in

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repetitive activity or blockage of nerve conduction. The main reaction involved in the metabolism of deltamethrin in mice and rats is ester cleavage mainly due to the action of carboxyesterase. Metabolism in fish is largely oxidative and deficient in esterases metabolization (Demoute, 1989). Acute toxicity data for deltamethrin in fish have been summarized in a report of the WHO (1990). Moreover, crustaceans are generally even more sensitive to pyrethroids than fish (Clark et al. 1989), and deltamethrin is often the most toxic to crustaceans in comparative tests (Haya 1989). Smith and Stratton (1986) showed that lobster and shrimp are susceptible to all pyrethroids. L'Hotellier and Vincent (1986) showed that the 96 h LC50 value of deltamethrin (25% purity) in pink shrimp (*Penaeus duorarum*) was 0.35 $\mu g\ L^{-1}$. As in case of fish, a high rate of absorption of deltamethrin through gills could also make shrimp a vulnerable target of its toxicity. Study of deltamethrin-induced oxidative stress and its influence on various antioxidants could provide useful information on the ecotoxicological consequences of deltamethrin use.

In MRD, deltamethrin is extensively used in black tiger shrimp farms because of its high activity against a broad spectrum of insect pests (Tu et al., 2006). The widespread use of this pesticide consequently leads to the exposure of manufacturing workers, field applicators, the ecosystem, and finally the public to the possible toxic effects of this pesticide. Both the World Health Organization (WHO) and the United States Environmental Protection Agency list deltamethrin as moderately hazardous, with the WHO labeling the compound as a Type II Acute Hazard. As a widely used broad spectrum insecticide the uses of deltamethrin require careful evaluation of the potential impact on organisms in the environment. The impacts of pesticide contaminations on aquatic ecosystems have been well studied in North America, Japan and many parts of Europe. In Viet Nam, pesticide concentrations in the environment are not documented in the yearly environmental reports and information on this type of contamination is generally lacking.

Comprehension of the mechanisms related to the sublethal effects caused by chemicals upon shrimp metabolism would help to develop sensitive and precise diagnostic tools with a predictive capability in assessing the toxic effects, thus contributing to better pond management (Bainy, 2000). It is documented that pyrethroids may trigger the generation of reactive oxygen species (ROS) (Sayeed et al., 2003; El-Demerdash et al., 2004; Prasanthi et al., 2005) leading to a situation of oxidative stress, which can be monitored by the level of lipid peroxidation (LPO) (Bhattacharya et al., 2007). Antioxidant defences such as total glutathione content (*t*GSH), glutathione S-transferase (GST), catalase (CAT) and glutathione peroxidase (GPx) are involved to counteract the toxicity of ROS (Ketterer et al., 1983; Halliwell and Gutteridge, 1999; Sies, 1999; Ventura et al., 2002). Under normal conditions, these antioxidants protect the cells and tissues from oxidative damage. On the other hand, the measurement of acetylcholinesterase activity (AChE) has been widely used as a specific biomarker of organophosphorus and carbamate pesticides (Fulton and Key, 2001). However, several studies have indicated that AChE is also sensitive to other types of environmental contaminants, such as pyrethroids, metals, detergents, and complex mixtures of pollutants (Gill et al., 1990; Payne et al., 1996; Guilhermino et al., 1998, 2000; Badiou and Belzunces, 2008).

Biomarkers are "early-warning" signals reflecting the adverse biological responses toward environmental contaminants that are commonly employed in environmental quality and/or risk assessment (Van de Oost et al., 2003). However, the interpretation of biomarker data is challenging because natural variation in environmental factors probably influences enzyme activity (Power and Sheehan, 1996). Thus, it is important to know and understand their effects to avoid misinterpretation of results in environmental studies. There is a scarcity of data to support the assumption that environmental changes including contaminants induce a modification of oxidative stress responses in shrimp, leading to an enhanced susceptibility to infectious disease. Thus, as the first comprehensive report, the present study was carried out to investigate the combined effects of temperature, salinity and deltamethrin on some biomarkers in the black tiger shrimp (*Penaeus monodon*) under laboratory conditions.

2. Materials and methods

2.1. Chemicals

Deltamethrin (98% purity, Merck) was dissolved daily in acetone to prepare a stock solution of 0.1 $mg\ mL^{-1}$.

Potassium phosphate dibasic (K_2HPO_4), potassium phosphate monobasic (KH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4), acetylthiocholine iodide (ATCH), 5,5′-dithio-2-bis-nitrobenzoate (DTNB), bovine serum albumin (BSA), trichloroacetic acid (TCA), thiobarbituric acid (TBA), malondialdehyde (MDA), ethylene diamine tetraacetate (EDTA), sodium azide (NaN_3), glutathione reductase (GR), reduced glutathione (GSH), nicotinamide adenine dinucleotide reduced (NADPH), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1-chloro-2,4-dinitrobenzene (CDNB), imidazol, titanyl sulfate ($TiOSO_4$), triton X-100, hydrogen peroxide (H_2O_2), 5-sulphosalicylic acid (SSA) were purchased from Sigma–Aldrich Chemicals (Germany).

2.2. Experimental animals

The experiment was carried out at the College of Aquaculture and Fisheries, the University of Cantho, Viet Nam. Shrimp, average weight of 12.2 ± 1.7 g, were brought from extensive shrimp farms in Camau province. Shrimp were transported to the laboratory in polystyrene boxes filled with natural seawater (21 ppt). Seawater in each box was aerated during transportation by a battery-powered aeration pump (Blue Ribbon Vibra-Flo, USA). Upon arrival, shrimp were acclimated to laboratory conditions in 2 m^3 composite tanks filled with 21 ppt natural aerated water at 30–32 °C for 2 weeks. During the acclimation period, shrimp were fed with commercial shrimp feed (35% crude protein), four times a day, but were starved 1 d before starting the experiment.

2.3. Shrimp acclimation

After 2 weeks of acclimation, shrimp were placed in experimental tanks (capacity 100 L) filled with 70 L of 21 ppt natural water. Stocking density was 12 shrimp per tank. The salinity was determined using a hand refractometer (Westover™ Model RHS10ATC, USA). The water salinity in experimental tanks was slowly increased or decreased by 1.5 ppt every hour. Salinities higher and lower than 21 ppt were obtained by adding natural sea water of 30 ppt or dechlorinated freshwater. After then, the water temperature was lowered or elevated by 0.5 °C per hour till the target value. The set-up for 24 °C was in an air conditioned room, the set-up for 29 and 34 °C was maintained with submersible thermostat-controlled heaters (Ruud, Montgomery, AL, USA).

2.4. Experimental design

Thirty-six experimental tanks were obtained by combining three temperatures (24, 29 and 34 °C), two salinities (15 and 25 ppt) and the absence or presence of 0.1 $\mu g\ L^{-1}$ deltamethrin in triplicate. A concentration of 0.1 $\mu g\ L^{-1}$ deltamethrin was chosen

according to our previous investigations showing a significant pesticide effect on some enzymatic activity of black tiger shrimp (Dorts et al., 2009). All deltamethrin treatments and controls received the same acetone concentration (0.01%). This acetone concentration was below the no observed effect concentration (NOEC) of 0.1% reported by Mayer (1987). Shrimp were sampled before exposure (day 0) and after 4 d of exposure (4 d exposure).

During the exposure period, water was gently siphoned out daily and replaced by freshly prepared water under the same conditions. Shrimp were starved during exposure and were submitted to a natural light cycle during the experiment. Water samples in each experimental tank were collected daily, 10 min and 24 h after the water renewal process for analysis of nitrates and nitrites. At the same time, water samples were collected and stored at −20 °C for further deltamethrin analysis. Temperature, dissolved oxygen and pH were checked twice a day using a water quality meter (YSI Environmental 556, USA).

2.5. Sample preparation

At each sampling time, hepatopancreas, gills and muscle from 5 shrimp per tank were collected on ice, pooled per tank and kept at −80 °C until assay. Tissues were homogenized in ice-cold buffer containing 50 mM KH_2PO_4/K_2HPO_4, pH 7.5. Post-mitochondrial fractions (PMF) were obtained after centrifugation at 10000*g* for 10 min at 4 °C.

2.6. Biochemical investigations

2.6.1. LPO measurement

LPO was measured following the procedure of Fatima et al. (2000). Tissue homogenate was precipitated with TCA and reacted with TBA in a boiling water bath for 15 min. After cooling the sample, the absorbance was read at 535 nm with a spectrophotometer (Model Biomate 5, Thermo Electro, UK). A calibration curve with increasing MDA concentration (0–100 μM) allowed the calculation of LPO level which was expressed in nmol MDA equivalents/g w.w. tissue.

2.6.2. Determination of tGSH

*t*GSH was determined by the method of Akerboom and Sies (1981). Briefly, we used the reaction of reduced GSH with DTNB that can be read spectrophotometrically at 412 nm for 5 min. The oxidized form of glutathione (GSSG) was reduced back to GSH by GR in presence of NADPH. The sample, after homogenization, was diluted in SSA 5% in a ratio 1:1 and centrifuged at 1000*g* for 10 min. tGSH content was expressed as nmol GSH/g w.w tissue.

2.6.3. Antioxidant enzyme measurements

GPx activity was assayed using the method of Mohandas et al. (1984). Hundred microliter PMF was added to the reaction mixture containing 50 mM KH_2PO_4/K_2HPO_4, 1 mM EDTA, 1 mM NaN_3 buffer (pH 7.4) and 1U mL^{-1} GR, 1 mM GSH, 0.2 mM NADPH. After 10 min incubation at 37 °C, the overall reaction was started by adding 100 μL of 0.25 mM H_2O_2. The decrease of absorbance at 340 nm was monitored during 5 min with a spectrophotometer. The enzyme activity was expressed as μmol NADPH oxidized/min/mg protein.

GST activity was performed by the method of Habig et al. (1974) by following the conjugation of GSH with CDNB at 340 nm. The activity was expressed as nmol CDNB/min/mg protein.

CAT activity was estimated by the method of Baudhuin et al. (1964) with some modifications. First, a stock solution of blank mixture (BC) was prepared containing 1 g BSA, 100 mL Imidazol buffer 0.2 M, pH 7.0, 900 mL L^{-1} H_2O. The quantity of H_2O_2 to be added to BC was determined by adding 40 μL of H_2O_2 30% to 250 mL of BC (solution called sample mixture or SC). 0.75 mL of $TiOSO_4$ was added to 1.3 mL of SC, then the absorbance was read at 420 nm and must be comprised between 0.75 and 0.95 (the maximum possible absorbance). The reaction mixture consisted of 1250 μL SC, 25 μL Triton X-100 0.02%, 25 μL PMF. After 6 min incubation at 0 °C, 750 μL of $TiOSO_4$ was added to stop the reaction. The absorbance was recorded at 420 nm. One unit of activity is defined as the amount of enzyme causing the destruction of 90% of the substrate in 1 min under the assay condition.

2.6.4. AChE measurement

AChE activity was measured using a modified version of a colorimetric technique described by Ellman et al. (1961). The reaction mixture was prepared in 50 mM of sodium phosphate buffer pH 7.4 containing ATCH and DTNB at a final concentration of 1.5 mM and 150 mM, respectively. Fifty microliter of PMF was added to start the reaction. AChE activity was recorded at 412 nm for 10 min. AChE activity was expressed in nmol of hydrolysed acetylthiocholine iodide/min/mg protein.

2.6.5. Protein estimation

Protein concentration was determined following the method of Lowry et al. (1951), using BSA as a standard.

2.7. Determination of deltamethrin in water and shrimp muscle

Deltamethrin was extracted from water samples by solid phase extraction according to the method describes by De la Colina et al. (1996) using a Supelco Supelclean™ Envi-18 SPE tubes 6 mL (1 g) (Supelco, Bellefonte, PA, USA). Deltamethrin retained on the column was eluted with 5 mL of an isooctane:ethyl acetate solution (85:15, v:v). Deltamethrin was extracted from shrimp muscle by means of 4 mL of acetonitrile:water solution (2.5:1.5, v:v). For water samples, the final elution was evaporated under a gentle stream of nitrogen to a volume of 50 μL using a Supelco Visidry™ 24. For shrimp samples, the final elution was evaporated with a Zymark TurboVap® LV evaporator just to dryness. The purified extract was analysed by high resolution gas chromatography using a Thermo Quest Trace 2000 gas chromatograph equipped with a Ni^{63} ECD detector (Thermo Quest, Milan, Italy) and an autosampler for liquid samples Thermo Quest AS 2000 (Thermo Quest, Milan, Italy). Two microliter of the solution was injected by means of a cold "on column" injector. The chemicals were separated on a 30 m × 0.25 mm (0.25 μm film thickness) Restek Rxi-5 ms capillary column (Restek, Bellefonte, PA, USA). Identification and quantification of deltamethrin were performed using the Chromcard 2.2 (Fisons Instruments, Thermo Quest, Milan, Italy) software for Windows. Quantification was performed using the internal standard method. The linear calibration curve for which concentrations ranged from 5 to 100 pg μL^{-1} was prepared with certified calibration standards. The recovery efficiency was 64.2%. The limit of quantification of deltamethrin was 1.7 ng L^{-1} (ppt) of water samples and 0.5 ng g^{-1} (ppb) of muscle fresh weight.

2.8. Statistical analysis

Data were expressed as mean ± standard deviation (SD). The experiment was designed for analysis using a repeated measures analysis of variance (three-way ANOVA) with a significant level of 0.05 to determine the effects of time, temperature, salinity and deltamethrin as well as their interaction. In case of significant interaction detected with the ANOVA test, a multiple comparison test (Fisher LSD test) was performed to examine differences between groups. This model, summarized by Paine (1996), was chosen because of the repetitive sampling within the same tanks, meaning the non-independence of the groups. The variation of bio-

marker levels was assessed as a result of combined factors: temperatures (24, 29 and 34 °C), salinities (15 and 25 ppt), deltamethrin (0 and 0.1 μg L^{-1}), replicate tanks (within treatments), sampling times within replicates (day 0 and 4 d exposure). Data were first checked for normality (Shapiro–Wilk's test) and homogeneity of variance (Levene test). All tests were performed using the software Statistica 5.5 (Statsoft, Inc., 2000).

3. Results

3.1. Water quality

Throughout experiment, water quality parameters ranged from 6.8 to 7.0 mg L^{-1} for dissolved oxygen, and from 8.1 to 8.2 for pH. Salinity was comprised between 14.9 and 15.1 (nominal 15 ppt), and between 24.9 and 25.1 (nominal 25 ppt). Temperature ranged from 23.7 to 24.1 °C (nominal 24 °C), from 28.8 to 29.0 °C (nominal 29 °C), and from 33.8 to 34.0 °C (nominal 34 °C). Nitrates and nitrites ranged from 1.6 to 2.0 mg L^{-1} and from 0.03 to 0.05 mg L^{-1}, respectively. Mean values of these parameters were not significantly different among treatments at any sampling time ($p > 0.05$).

3.2. Deltamethrin concentration in water and in shrimp muscle

Deltamethrin concentration in water was 0.12 ± 0.02 μg L^{-1} after 10 min, but dropped significantly after 24 h when it was only 70%, 28% and 16% of the concentration measured after 10 min at 24 °C, 29 °C and 34 °C, respectively. The concentrations of this pesticide in muscle of shrimp were under the limit of quantification (LOQ = 0.5 ng g^{-1} for deltamethrin) in all treatment groups at all sampling times.

3.3. Oxidative stress

LPO level in hepatopancreas was significantly affected (general increase pattern) during the 4 d experiment ($p = 0.001$) in all treatments, while there was no effect in gills. Repeated measures ANOVA revealed that the interaction between time, temperature, salinity and deltamethrin was not significant either in gills or in hepatopancreas (Table 1).

GST activity was weakly affected by time in gills ($p = 0.04$) while a highly significant decrease was observed over time in hepatopancreas ($p = 0.0004$). None of those time effects was dependent on salinity, temperature or deltamethrin (Table 1).

In hepatopancreas, GPx activity was affected by time ($p = 0.00002$) and weakly affected by the combined effects of time, temperature and deltamethrin exposure ($p = 0.04$) (Table 1). For all treatment groups but the unexposed shrimp at 24 °C, GPx activity was significantly lower after 4 d exposure compared to day 0 (Fig. 1), suggesting that temperatures of 29 and 34 °C can alter GPx activity independently of chemical. The fact that this effect was not observed in unexposed shrimp at 24 °C while it was observed after deltamethrin exposure, indicates that, at this temperature, GPx activity was maintained at a normal level but can be impaired by pesticide exposure.

A significant interaction among time, temperature, salinity and pesticide ($p = 0.018$) was observed for gill *t*GSH (Table 1). In this organ, we reported an increase of *t*GSH after 4 d exposure for all treatments. However, at 34 °C, the increase was higher for shrimp exposed to deltamethrin compared to the unexposed shrimp, indicating a pesticide effect (Fig. 2a). In the same way, salinity interfered with deltamethrin after 4 d exposure since the *t*GSH increase in deltamethrin treated shrimp was significantly higher at 25 ppt than at 15 ppt (Fig. 2b). In hepatopancreas, *t*GSH also increased during the 4 d exposure in all conditions ($p = 0.00008$) and a slight but significant interaction between time, deltamethrin and temperature ($p = 0.048$) was observed. In Fig. 2c we observed that the *t*GSH level was higher after 4 d exposure than in day 0, but that this level was even significantly higher for shrimp maintained at 34 °C and exposed to deltamethrin than for shrimp exposed at 29 and 24 °C, suggesting, as in gills, that deltamethrin increased the *t*GSH level in hepatopancreas at 34 °C.

CAT activity in gills was slightly significant affected by the combined effects of time, temperature, salinity and deltamethrin ($p = 0.04$) (Table 1). Interactions among time, temperature and deltamethrin ($p = 0.02$) and among time, salinity and deltamethrin ($p = 0.04$) are shown in Fig. 3a and b. Despite a high variability, the repeated measures ANOVA detected that deltamethrin treated shrimp presented after 4 d exposure a significant lower CAT activity at 34 °C only, and at 25 ppt only, compared to day 0. In hepatopancreas, CAT activity was affected by the combined effects of time, salinity and deltamethrin ($p = 0.01$). Its activity decreased with deltamethrin exposure at 15 ppt only (Fig. 4).

3.4. AChE activity

The combination of time and deltamethrin induced highly significant effects on AChE in muscle ($p = 0.00008$). Its activity was decreased over time in both control and exposed shrimp (Fig. 5). However, the activity depletion was more pronounced for the shrimp exposed to deltamethrin, indicating an inhibition of AChE activity. Moreover, we could not report any significant effect of temperature and salinity, neither any interaction of time or deltamethrin exposure with these both parameters. This indicates that the effect of the pesticide was independent of temperature and salinity.

Table 1
The interaction of temperature, salinity and deltamethrin (0.1 μg L^{-1}) on biomarker responses.

	LPO		GST		GPx	*t*GSH		CAT		AChE	
	Gills	Hp.	Gills	Hp.	Hp.	Gills	Hp.	Gills	Hp.	Gills	Muscle
Pest.	ns	ns	ns	ns	ns	$p = 0.0049$	$p = 0.0003$	ns	$p = 0.04$	ns	$p = 0.001$
Temp. × Pest.	ns	ns	ns	ns	ns	$p = 0.00001$	$p = 0.002$	ns	ns	ns	ns
Sal. × Pest.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Temp. × Sal. × Pest.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Time	ns	$p = 0.001$	$p = 0.04$	$p = 0.0004$	$p = 0.00002$	$p = 0.00002$	$p = 0.00008$	ns	$p = 0.04$	$p = 0.00001$	$p = 0.00005$
Time × Pest.	ns	ns	ns	ns	ns	ns	$p = 0.03$	ns	ns	ns	$p = 0.00008$
Time × Temp. × Pest.	ns	ns	ns	ns	$p = 0.04$	$p = 0.0005$	$p = 0.048$	$p = 0.02$	ns	ns	ns
Time × Sal. × Pest.	ns	ns	ns	ns	ns	ns	ns	$p = 0.04$	$p = 0.01$	ns	ns
Time × Temp. × Sal. × Pest.	ns	ns	ns	ns	ns	$p = 0.018$	ns	$p = 0.04$	ns	ns	ns

ns: non-significant effects or interaction.
Pest.: pesticide; Temp.: temperature; Sal.: salinity; Hp.: hepatopancreas.
LPO: lipid peroxidation; GST: glutathione S-transferase; GPx: glutathione peroxidase;
*t*GSH: total glutathione; CAT: catalase; AChE: acetylcholinesterase.

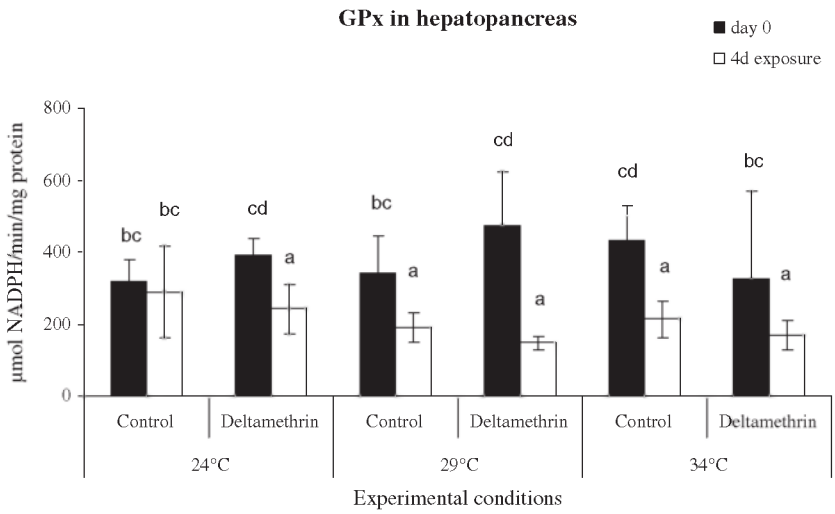

Fig. 1. GPx activity in black tiger shrimp hepatopancreas as affected by the interaction of time, temperature and deltamethrin. Data are reported as mean ± SD ($n = 3$). Different letters above bars indicate significant differences ($p < 0.05$).

In gills, there was no interaction effect among these factors whereas a significant time effect was reported (Table 1). AChE activity was decreased over time in both control and exposed shrimp.

4. Discussion

The combined effects of temperature, salinity and toxicant were studied in the grass shrimp (*Palaemonetes pugio*) (Howard and Hacker, 1990), in zooplankton (Heugens et al., 2001), and recently in the rotifer (*Brachionus rotundiformis*) (Gama-Flores et al., 2005).

In the present study, we tested the interaction effects of realistic concentration of deltamethrin with temperature and salinity that can be encountered in shrimp pond during a normal annual cycle. Our results suggest that deltamethrin affects some biomarkers in a temperature and/or salinity dependent manner. *t*GSH level and CAT activity are the most evident biomarkers jointly affected by the studied pyrethroid and abiotic factors. Higher *t*GSH levels in gills and hepatopancreas were reported in shrimp exposed to deltamethrin at the highest tested temperature, i.e. 34 °C, but not at 29 °C and 24 °C. This could be due in part to the necessity of this tissue to cope with an oxidative stress, by using GSH as a co-factor for antioxidant enzymes or alternatively to bind ROS, suggesting that deltamethrin can induce an oxidative stress at 34 °C. In parallel, CAT activity was impaired in gills by deltamethrin exposure at 34 °C only. This result strengthens the possibility of an oxidative stress induction due to the combined effect of deltamethrin and high temperature. The reason could be an enhanced ROS production explained by the intensification of respiration at high temperature, resulting in a higher oxidative stress (Rajagopal et al., 2005). Moreover, Laskowski (2002) showed that temperature has a direct impact on the degradation of deltamethrin as well. In contrast to biomarker responses in shrimp at elevated temperature, the actual concentration of deltamethrin in water decreased rapidly 24 h after the water renewal under our experimental conditions. This decrease was correlated to the temperature and was more pronounced at 34 °C (16% of the concentration measured at 10 min). Results have also been reported by Shi et al. (2007), who indicated that deltamethrin has a short half-life and is fairly rapidly degraded in water. Haug and Hoffman (1990) showed that deltamethrin has a high toxicity to aquatic animals under laboratory conditions. However, in field condition, due partly to rapid adsorption of deltamethrin onto sediment and evaporation, it does not exhibit the same level of toxicity. Moreover, we found that deltamethrin residue in shrimp tissue were under the limit of quantification at 4 d of exposure. These results are in accordance with Spehar et al.(1983) and Morolli et al. (2006) who indicated that deltamethrin was rapidly eliminated from red swamp crayfish (*Procambarus clarkii*) and common carp (*Cyprinus carpio*) tissues after the exposure ended.

We showed that pesticide exposure decreased GPx activity in hepatopancreas at 24 °C, the lowest tested temperature, while 4 d exposure at 29 and 34 °C seemed to decrease its activity independently of the pesticide. This finding is, in part, supported by the work of Kaushik and Kaur (2003), who found a decrease in GPx in the heart and liver of the cold-stressed rats. A reduced GPx activity in the present study indicates the possible decreased capacity to scavenge hydrogen peroxide produced in this tissue under combined effects of deltamethrin and low temperature of 24 °C. However, this result could also be due to the strong degradation of deltamethrin with increasing temperature, which could result in an indirect impact of temperature on deltamethrin toxicity. Therefore, the temperature-dependent toxicity of deltamethrin seems to be related to the biomarker tested. In the same way, Chiu et al. (1990) indicated that, in some cases, the toxicity of pyrethroid may change with increasing temperature, in the range of 30–38 °C, partly due to an increased stress of the animals at high temperature.

CAT activity in gills was modulated not only by temperature but also by salinity. Its activity was found to be significantly decreased in gills of shrimp exposed to deltamethrin, but only at 34 °C and at 25 ppt. This could be due to the flow of superoxide radicals, which have been reported to inhibit CAT activity (Kono and Fridovich, 1982). The relationship between salinity and toxicity of xenobiotics is still relatively unclear. Brecken-Folse et al.(1994) indicated that several pesticides seem to have an increased toxicity for grass shrimp (*Palaemonetes spp.*) at high salinity. While Cynthia and Carl (1990) reported that a decreased salinity resulted in an increase in net uptake of cadmium in grass shrimp, therefore boosting its toxicity. O'Hara (1973) reported that animals must increase salt uptake in order to maintain a hyperosmotic balance under low salinities. In the present study, the decreased CAT activity in shrimp hepatopancreas was observed at 15 ppt, possibly associated with energy cost of osmoregulation in conjunction with deltamethrin contamination. The isosmotic point of this species is 750 mosM kg^{-1}, equivalent to 25 ppt (Cheng and Liao, 1986). In the present study, we also found that at 25 ppt CAT activity de-

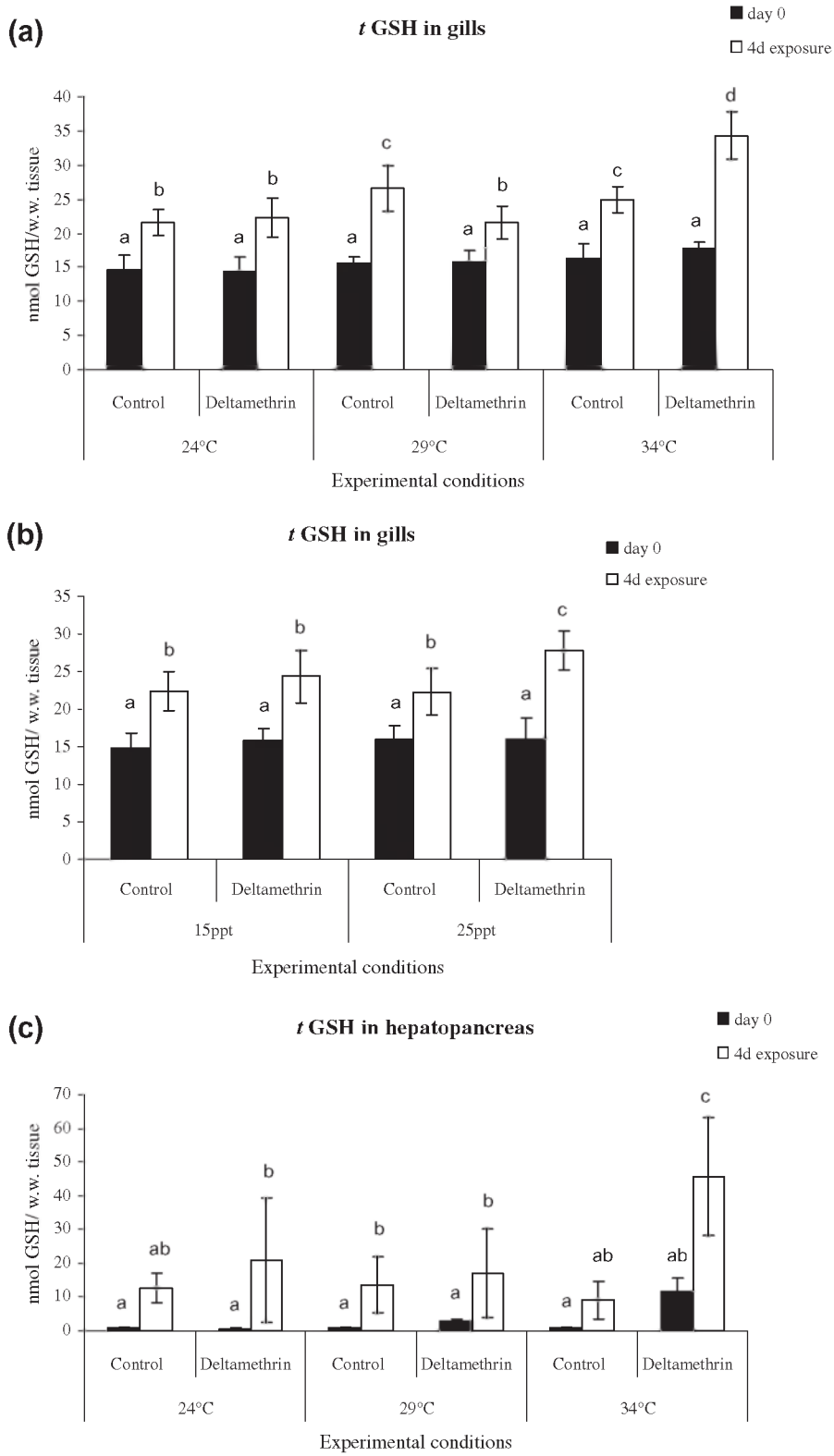

Fig. 2. *t*GSH in black tiger shrimp gills as affected by the interaction of time, temperature and deltamethrin (a) and by the interaction of time, salinity and deltamethrin (b). *t*GSH in black tiger shrimp hepatopancreas as affected by the interaction of time, temperature and deltamethrin (c). Data are reported as mean ± SD ($n = 3$). Different letters above bars indicate significant differences ($p < 0.05$).

creased significantly but the *t*GSH increased in gills of shrimp exposed to deltamethrin, suggesting that the salinity could induce oxidative stress.

For the other tested biomarkers, LPO, GST and AChE, we could not point out any significant interaction between deltamethrin exposure and temperature and/or salinity. Moreover, for all biomarkers except LPO and CAT in gills, we observed significant changes of the responses in almost all treatments between day 0 and day 4, most importantly including the control treatment. This is particularly striking in the case of *t*GSH where the evaluation

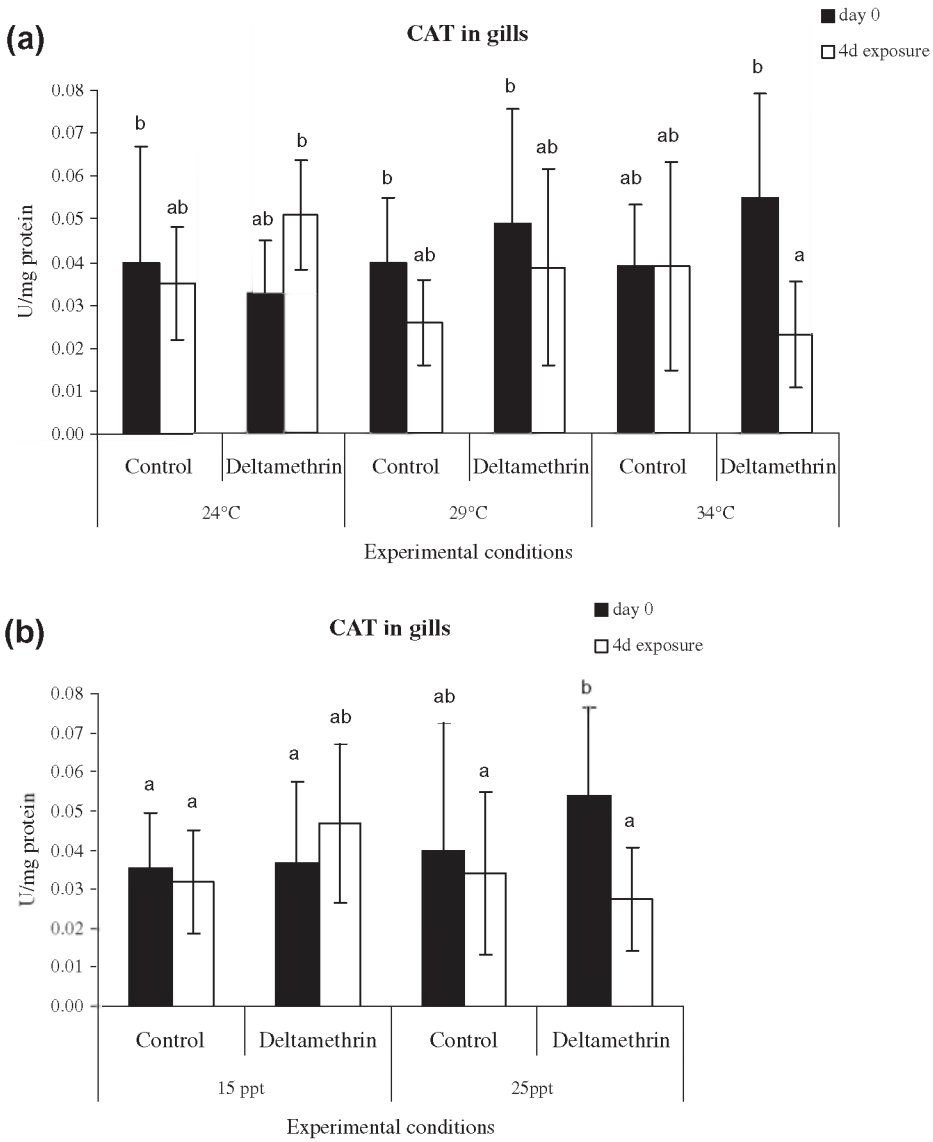

Fig. 3. CAT activity in black tiger shrimp gills as affected by the interaction of time, temperature and deltamethrin (a) and by the interaction of time, salinity and deltamethrin (b). Data are reported as mean ± SD ($n = 3$). Different letters above bars indicate significant differences ($p < 0.05$).

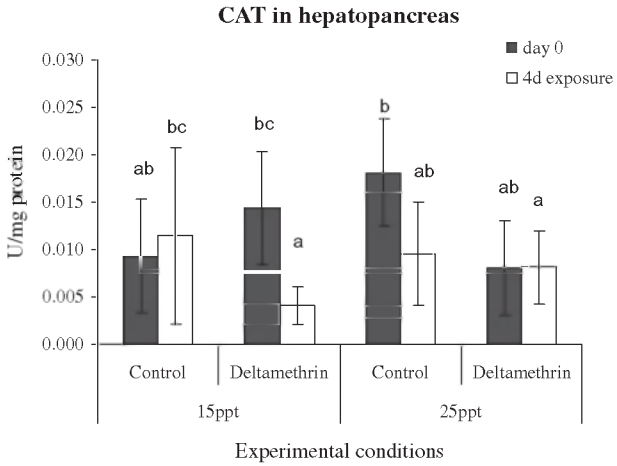

Fig. 4. CAT activity in black tiger shrimp hepatopancreas as affected by the interaction of time, salinity and deltamethrin. Data are reported as mean ± SD ($n = 3$). Different letters above bars indicate significant differences ($p < 0.05$).

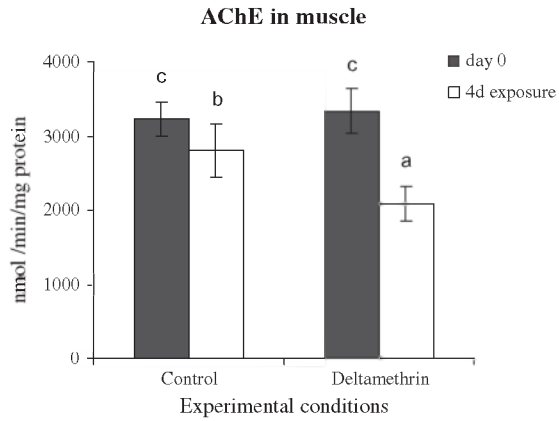

Fig. 5. AChE activity in black tiger shrimp muscle as affected by the interaction of time and deltamethrin. Data are reported as mean ± SD ($n = 3$). Different letters above bars indicate significant differences ($p < 0.05$).

was up to an order of magnitude higher at day 4 compared to the start of the experiment. The control treatments were set-up to determine the effects of temperature and salinity and handling stressors on biomarkers over the experimental period so deltamethrin induced effects could be distinguished from non-toxic stressors. In the present study, no significant differences in biomarker responses were found at the start of experiment (day 0) within all treatments, while significant differences were found between day 0 and day 4 in controls. It is also possible that other factors such as starvation and/or handling affected biomarkers. Therefore, the true magnitude of deltamethrin induced effects could be masked because the biomarker had already been stimulated by temperature, salinity and handling stress.

AChE activity inhibition is well known as a biomarker indicating the effect of neurotoxic substances (Talesa et al., 1992). Only few studies have been reported regarding the effect of pyrethroids on AChE. Generally a reduction of activity was observed after intoxication at sublethal concentrations. For example, Reddy et al. (1991), after administration of sublethal concentrations of cypermethrin, observed a decrease of 29% of AChE activity in the brain tissue of freshwater teleost (*Tilapia mossambica*), while Szegletes et al. (1995) showed a 20% decrease in the plasma of carp (*C. carpio* L.) exposed to deltamethrin. Moreover, Balint et al. (1995) also found a 70–90% decrease in AChE activity in brain, heart, blood, liver and skeletal muscle of carp (*Cyprinus carpio* L.) after 5 d exposure to deltamethrin. Badiou and Belzunces (2008) explained that the effect of pyrethroids on AChE activity could result from a modulation of AChE biosynthesis by increasing the level of the secreted soluble form and decreasing the AChE addressed to cellular membrane. Temperature and salinity have been shown to influence AChE activity in some species such as bluegills (*Lepomis macrochirus*), roach (*Rutilus rutilus* L.) and Japanese medaka (*Oryzias latipes*) (Hogan, 1970; Chuiko et al., 1997; El-Alfy and Schlenk, 1998). In the present study, AChE in muscle was strongly inhibited by deltamethrin at all tested temperatures and salinities, suggesting that the assessment of AChE activity in shrimp muscle might be advantageous as a biomarker because its activity seems not to depend upon temperature and salinity.

5. Conclusions

Deltamethrin, because of its beneficial features, has attracted shrimp farmers to use it for pest control. The present study emphasizes the fact that this pesticide can affect some biomarkers of oxidative stress as well as AChE in shrimp exposed under laboratory conditions at realistic level, suggesting an increased general stress level in animals treated with this pyrethroid. Moreover, strong interactions between deltamethrin exposure and temperature and/or salinity, mostly on GPx, *t*GSH and CAT, highlight the necessity to monitor those biomarkers jointly with abiotic parameters. In this way, deltamethrin, even if it is degraded at 34 °C, seems to induce an oxidative stress at this temperature, at least via CAT inhibition. It comes out of this study that AChE activity in muscle could be the most appropriate biomarker of deltamethrin exposure since we observed an inhibition due to the pesticide independently of temperature and salinity. To avoid misinterpretation of biomarker responses, the study presented here is therefore an essential preliminary step before using biomarkers to assess contaminant exposure in the black tiger shrimp.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.09.022.

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