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Evaluation of sublethal biomarkers in *Litopenaeus vannamei* on foodborne exposure to methyl parathion

L. Comoglio[a,b,*], O. Amin[a,b], A. Roque[b,c], M. Betancourt-Lozano[b], D. Anguas[b], B.M. Haro[b]

[a]*Centro Austral de Investigaciones Científicas, 9410 Ushuaia, Tierra del Fuego, Argentina*
[b]*CIAD, Av Sabalo Cerritos s/n Estero del Yugo 82010 Mazatlan Sin, Mexico*
[c]*IRTA, Carr al Poblenou Km 5.5, 43540 Sant Carles de la Rapita, Spain*

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Abstract

Sublethal effects of foodborne exposure to methyl parathion (0.62 and 1.31 μg methyl parathion*g^{-1} dry weight of food) on juveniles of *Litopenaeus vannamei* using integrated biochemical (acetylcholinesterase (AChE) and ATPases) and physiological (feeding rate (FR), egestion rate (ER), and hepatosomatic index (HI)) biomarkers were evaluated. The HI was significantly higher in controls than in pesticide treatments. The FR was significantly lower in controls than in pesticide treatments while no significant differences were detected in the ER. AChE activity was significantly higher in controls than in pesticide treatments (control = 0.11 ± 0.02; solvent control = 0.11 ± 0.03; 0.62 = 0.07 ± 0.01; 1.31 = 0.08 ± 0.02 μM*min^{-1}*mg $protein^{-1}$). The total-ATPase activity was significantly lower in controls than in pesticide treatments (control = 77.90 ± 12.41; solvent control = 83.69 ± 22.05; 0.62 = 110.03 ± 22.17; 1.31 = 121.54 ± 19.84 μM P_i*h^{-1}*mg $protein^{-1}$). The Mg^{2+}-ATPase activity was significantly higher in treatments than in controls (control = 65.14 ± 10.76; solvent control = 75.12 ± 21.10; 0.62 = 100.53 ± 20.97; 1.31 = 108.94 ± 17.26 μM P_i*h^{-1}*mg $protein^{-1}$). Finally, the results obtained for the Na^+/K^+-ATPase activity were significantly higher in control and in 1.31 than in solvent control and in 0.62 (control = 14.06 ± 2.63; solvent control = 7.30 ± 4.13; 0.62 = 7.60 ± 3.81; 1.31 = 13.42 ± 2.88 μM P_i*h^{-1}*mg $protein^{-1}$). The results in this study showed that pulse exposures to methyl parathion via food could elicit measurable effects on the marine shrimp *L. vannamei*, indicating that foodborne exposure can be a reliable toxicological procedure and, if combined with pulse exposures, could also simulate more realistic exposure scenarios.

Keywords: Foodborne; *Litopenaeus vannamei*; Methyl parathion; Biomarkers; ATPase; AChEase; Feeding rate

1. Introduction

The Pacific white shrimp *Litopenaeus vannamei* is cultured in extensive, intensive, and semiintensive systems and is, together with *Litopenaeus stylirostris*, the most popular shrimp for aquaculture in Mexico and Central and South American countries. Mexico is ranked as one of the top 10 shrimp producers (FAO, 2002) but lacks well-documented studies on the effects of coastal environments on shrimp aquaculture.

The state of Sinaloa located on the northwest coast of Mexico, considered the most important agricultural producer in the country, has had an important increase of aquaculture farms in recent years, from approximately 100 ha of ponds in 1984–1850 ha in 1998, with a growth rate of about 125 ha/year. Nowadays, Sinaloa has more than 200 shrimp farms (about 75% of the national total) and produces around 10,000 tons yearly (65% of the national total) (Hernández Cornejo and Ruiz Luna, 2000). These farms are close to the margins of estuaries and coastal lagoons and use the same

*Corresponding author. Centro Austral de Investigaciones Científicas, 9410 Ushuaia, Tierra del Fuego, Argentina. Fax: 54-2901-430644.
E-mail address: lcomoglio@hotmail.com (L. Comoglio).

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waters that receive drainage from extensive agricultural areas.

Agrochemicals can be introduced in coastal lagoons through agricultural runoffs, soil drainage, aerial overspray, precipitation, and accidental spills (Edwards, 1978; Eidt et al., 1989) and through direct use for pest control in aquaculture (Baird, 1994). Research in Sinaloa has mainly addressed levels, dynamics, and fate of the most used pesticides such as organochlorines, organophosphates, carbamates, and pyrethroids (Rosales and Escalona, 1983; Mee et al., 1992; Carvalho et al., 1996; Galindo Reyes et al., 1997; González-Farias et al., 1997), but few studies have focussed on the potential, yet real, effects that such toxicants pose on local species (Galindo Reyes et al., 2000, 2002).

During the past decade, interest in the use of biomarkers in environmental assessment has increased. Biomarkers, or stress indices, can be defined as biochemical, cellular, and physiological changes caused by adverse environmental conditions (including xenobiotic exposure) that are measurable in a biological system and can be evaluated at different levels of organization, on the assumption that low toxicant levels cause biochemical responses within individual organisms before those effects are observed at higher levels of biological organization (Schlenk, 1999; Mc Loughlin et al., 2000). In this sense, the utilization of integrated responses to evaluate the effects of pollutants has been suggested as a novel way to assess an organism's survival potential and to allow a better understanding of possible effects at higher levels of biological organization (Hebel et al., 1997).

Acetylcholinesterase (AChE) is recognized to be one of the oldest environmental biomarkers. This enzyme is involved in the deactivation of acetylcholine at nerve endings, preventing continuous nerve firings, which is vital for normal functioning of sensory neuromuscular systems (Murphy, 1986). Many organophosphorous and carbamate pesticides are effective AChE inhibitors (Coppage and Braidech, 1976; Hassall, 1990; Galgani et al., 1992; Kumar and Chapman, 1998), therefore AChE has been used as biomarker to assess the nature and extent of the exposure of wildlife and humans to agricultural and forestry sprays (Payne et al., 1996).

On the other hand, ATPases play important roles in intracellular functions and in all types of physiological activities. Satyavathi and Prabhakara Rao (2000) have reported four different ATPase activities in the plasma membrane/mitochondrial fractions of *Penaeus indicus*, Mg^{2+}-ATPase, Na^+/K^+-ATPase, Na^+-ATPase and K^+-ATPase, although the stimulation caused by Na^+ and/or K^+ could be due to the effect of each cation acting on a single ATPase. The sodium pump or Na^+/K^+-ATPase is a membrane bound enzyme found in animal cells, with its most important feature being the coupling of the free energy stored within the ATP molecule to the translocation of Na^+ ions outward and K^+ ions inward across the plasma membrane (Skou, 1957, 1965). These ionic gradients are essential to many cellular functions such as the maintenance of the osmotic stability of the cell despite large variations in salt and water intake.

Often, ATPase activity is used as a sensitive indicator of heavy metal toxicity (Haya and Waiwood, 1983), although there is evidence that organic pollutants can inhibit ATPase activity in concentration-based experiments (Reddy et al., 1992). The measurement of feeding rate (FR) has been proposed as a general, but sensitive, physiological indicator of toxic stress in both freshwater and marine species (Naylor et al., 1989; Roddie et al., 1996). Furthermore, a toxicant-induced reduction in FR is relevant from the ecological point of view as it could be related to reductions in an organism's energy assimilation, which, in turn, could lead to a reduction in resource allocation to growth, reproduction, and survival and finally translate into effects at the population level (Maltby and Naylor, 1990; Matlby, 1994; Maltby et al., 2001; Irving et al., 2003). Calow and Sibly (1990) suggested that this commonly used index of environmental stress could lead to differences in the intrinsic rate of population growth, depending on whether the reduction in reproduction was due to a reduction in food intake or to an increase in metabolic cost. Barber et al. (1990) referred to these modes as supply sided effects and demand sided effects, respectively. Once the dominant mode of action of a toxicant has been determined it should be possible to estimate potential effects to the population (Gurney et al., 1996; Sibly, 1996), taking into account other parameters related to feeding behavior, such as assimilation efficiency, hepatosomatic index (HI), or egestion rate (ER), which can indicate particular metabolic strategies when facing contaminant exposure (Mc William and Baird, 2002; Irving et al., 2003).

In aquatic ecotoxicology, most studies on the effects of pesticides have been carried out using waterborne exposures. Nevertheless, foodborne (= dietary) exposure represents another ecologically important uptake route that can provide useful insights on the ecotoxicological responses of an organism to a contaminant (Irving et al., 2003). The aim of this study was to evaluate the sublethal effects of foodborne exposure to the organophosphorous insecticide methyl parathion on juveniles of *L. vannamei* using integrated biochemical (AChE and ATPase) and physiological (FR, ER, and HI) biomarkers.

2. Materials and methods

2.1. Test organisms

Juveniles of *L. vannamei* (0.73 ± 0.13 g) were obtained from a commercial hatchery in Sinaloa (Mexico). The

shrimp were fed daily with a commercial diet (Cenzone; Aquature, Mexico; 35% protein) and maintained in a 1000-L raceway recirculation system (24 ± 0.5 °C, 34‰, and 12-h dark/12-h light photoperiod) with constant aeration until they reached the experimental size of 0.79 ± 0.10 g wet weight.

2.2. Food preparation and chemical analysis

Pellets of commercial food (Cenzone) were sieved through 3.3- and 1.7-mm-diameter stainless steel sieves to obtain a homogenous pellet diameter appropriate to the shrimp size and then lyophilized (Labconco; Freeze zone plus 6) (average dry weight of each pellet was 15.14 ± 2.28 mg). The proximal composition of the food was 35% protein, 3.5% lipids, 30% fiber, 16% ash, and 12% humidity.

Exposure of *L. vannamei* to methyl parathion was achieved by incorporating methyl parathion (technical grade; Tekchem, Mexico) into food pellets using acetonitrile (Sigma, Mexico) as carrier. The pellets were subsequently dried for at least 5 h until the initial weight was achieved at room temperature and dark conditions to allow the complete evaporation of the acetonitrile. To determine the concentration of pesticide, pellet samples (2 g) were extracted using ethyl acetate and hexane (7:3) and petroleum ether was used to remove fat (IAEA, 1997). Samples were centrifuged and the top layer containing the fat and the petroleum was removed. Methyl parathion (99% purity; ChemService, USA) was used as the internal standard. All solvents used were HPLC grade from Fisher Scientific (USA) except acetone, which was from Merck (Germany). Methyl parathion was determined using a gas chromatograph (Agilent 6890N; USA) equipped with a nitrogen phosphorous detector and an HP-5 phenyl methyl siloxane column (30 m × 320 μm × 0.25 μm; Model 19091J-413). Helium was used as carrier at 18 psi. The injector was held at 200 °C and the detector at 320 °C. The temperature was programmed from 60 to 200 °C starting with 1 min at 60 °C and then with increments of 20 °C/min until 150 °C where it was held for 12 min; then temperature was increased at 10 °C/min, maintaining the final temperature for 10 min. Recoveries were 96.3%. All methyl parathion concentrations are hereinafter expressed as actual concentrations (μg methyl parathion $*g^{-1}$ dry weight of food).

2.3. General experimental conditions

The experimental system was semistatic, consisting of 8-L glass aquaria with individual aeration. The system was installed in a constant-temperature room (24 ± 1 °C), with a 12-h dark/12-h light photoperiod. The seawater used in this study was pumped from a nearby beach (Cerritos beach, Sinaloa, Mexico) at 34 ± 1‰, filtered through a cartridge filter of 5 μm and then through a UV light (Aquaplus; Mexico), and kept with aeration (Siemmens; Germany, 1HP) in a 600-L dark tank until use.

Two concentrations of methyl parathion were tested: 0.62 and 1.31 $\mu g*g^{-1}$ dry weight of food. A control without pesticide and a solvent control were also included (solvent control was prepared with acetonitrile levels equivalent to the highest concentration tested). During the experiments, all experimental pellets were stored in a freezer at −20 °C and used on demand.

For the experiment, groups of eight shrimp were randomly placed in 8-L glass aquaria. Three aquaria were used per treatment (total $N = 96$). The organisms were acclimated for 48 h in the experimental aquaria (no mortalities were recorded during this period). The exposure period lasted 7 days. During each exposure day, shrimp were offered one contaminated or uncontaminated pellet (depending on the treatment) until it was completely eaten. After this, uncontaminated pellets were added ad libitum for 150 min, followed by a 90% water renewal that removed all feces and pellet leftovers. Daily molts and dead organisms were recorded and removed as soon as they were detected. Shrimp recently molted were discarded from further analyses. After exposure, two separate sets of organisms were randomly selected to evaluate the effects of methyl parathion on *L. vannamei* using different responses. The first experimental set was used to determine feeding (FR) and egestion (ER) rates, while the second was used to assess AChE activity, ATPase activity, and HI.

2.4. Feeding rate and egestion rate

After the exposure period, nine organisms were individually placed in 1-L chambers containing clean and aerated water for 2 h. After that, individual FRs were determined by offering a known amount of previously freeze-dried pellets (Free Zone 6L; Labconco) to each organism. Nonconsumed food was collected after 2.5 h, freeze-dried, weighed, and used to calculate the individual FR (= amount of dry food consumed in 2.5 h*100/shrimp wet weight). Feces were also collected and freeze-dried in order to calculate the ER (= feces production dry weight * 100/shrimp wet weight).

2.5. AChE activity, ATPase activity, and hepatosomatic index

At the end of the exposure period, eight shrimps were collected from each treatment and sacrificed in ice-cold water. Shrimp were then dissected to obtain the eyes and gills to determine AChE and ATPase activity, respectively, and to obtain the hepatopancreas weight to estimate the HI. To measure AChE activity, the eyes of each shrimp were weighed and homogenized in

phosphate buffer (0.1 M, pH 7.2, in 1:10 w/v) and then centrifuged (Beckman; GS-15R) at 4 °C for 30 min at 8500*g*. Supernatants were then used to determine AChE activity and total protein content, according to Ellman et al. (1961) and Bradford (1976), respectively. The specific AChE activity is expressed in $\mu M*min^{-1}*mg\ protein^{-1}$. ATPase activity was measured following the procedure described by Tentes and Stratakis (1991) with some modifications. Gills were separated, weighed, and homogenized in 10 volumes of homogenization buffer (0.25 M sucrose, 6 mM EDTA, 30 mM imidazole, 0.1 mM dithiothreitol, 10 μM phenylmethylsulfonyl fluoride, pH 6.8). Homogenates were centrifuged at 4 °C for 30 min at 10,000*g*. Supernatants were separated and the pellets formed were resuspended in 400 μL of homogenization buffer and centrifuged again under the same conditions. The supernatants from the first and the second centrifugation were combined. To determine Mg^{2+}-ATPase and total-ATPase activities the reaction was run with and without, respectively, a final concentration of 1 mM ouabain for 15 min at 30 °C. The reaction was stopped by adding 0.9 mL of trichloracetic acid (8% w/v) and absorbance was measured at 820 nm using a spectrophotometer. The activity of Na^+/K^+-ATPase was calculated as the difference between samples incubated in the absence and in the presence of ouabain, a specific inhibitor of Na^+/K^+-ATPase (Sola et al., 1995). Total protein content was determined according to Bradford (1976). The specific ATPase activity is expressed in $\mu M\ P_i*h^{-1}*mg\ protein^{-1}$. The hepatopancreas was wet weighed and used to calculate the HI (= hepatopancreas wet weight*100/shrimp wet weight).

2.6. Statistical analysis

To determine significant differences among treatments, normally distributed data were analyzed by one-way ANOVA and LSD range test. Data not normally distributed were evaluated through the Kruskal–Wallis test and Dunn's multiple comparisons test (Daniels, 1978). The proportion of molting was estimated on the initial number of shrimp and compared between each treatment and control by means of the Fisher's test (Sokal and Rohlf, 1981). For all analyses, a 0.05 probability level was used.

3. Results

3.1. Weight, molt, and survival

The final wet weight was significantly higher in controls than in treated groups (ANOVA, $p<0.05$). The average increment was around 20% in controls, while in treatments increments of 1.56% and 4.62% in low and high concentrations of methyl parathion, respectively, were observed. No mortality on control and solvent control was observed, while mortality in the pesticide treatments reached 12.5% and 4.16% in 0.62 and $1.31 \mu g*g^{-1}$, respectively (Table 1). No significant differences in the cumulative molts were observed (Fisher's exact test, $p<0.05$), molts ranged between 37.5% and 58.3% (Fig. 1).

3.2. Hepatosomatic index, feeding rate, and egestion rate

The HI was significantly different among controls and treatments (Kruskal–Wallis, $H = 11.29$, $p = 0.01$): the HIs in the control and solvent control (mean values: 5.0% and 5.33%, respectively) were higher than those in the treatments (mean values: 4.39% and 4.45% in 0.62 and $1.31 \mu g*g^{-1}$, respectively) (Fig. 2).

The FR was significantly lower in the control than in the pesticide treatments (Fig. 3A). The FR in the control group was 5.29%, while in the treatments the FRs were 7.04 and 6.94% for 0.62 and $1.31 \mu g*g^{-1}$, respectively (Kruskal–Wallis, $H = 11.53$, $p = 0.01$). The ER was also lower in the control than in the pesticide treatments, although no significant differences were detected (Kruskal–Wallis, $H = 5.94$, $p = 0.12$) (Fig. 3B). Mean values were 1.25% in the control group, while in the treatments they were 1.48% and 1.70% for 0.62 and $1.31 \mu g*g^{-1}$, respectively.

3.3. AChE and ATPase activity

The results obtained are shown in Fig. 4. No significant differences in AChE activity in control and

Table 1
Weight and mortality of *L. vannamei* exposed to methyl parathion via food

	n	Final wet weight (mean ± SD in g)	Increment weight (%)	Total mortality (%)
Control	24	0.949 ± 0.092^a	20.73	0
Solvent control	24	0.955 ± 0.086^a	21.32	0
$0.62 \mu g\ g^{-1}$	21	0.799 ± 0.086^b	1.56	12.5
$1.31 \mu g\ g^{-1}$	23	0.823 ± 0.086^b	4.62	4.16

Values in the same column that share the same superscript letter do not differ significantly ($P>0.05$).

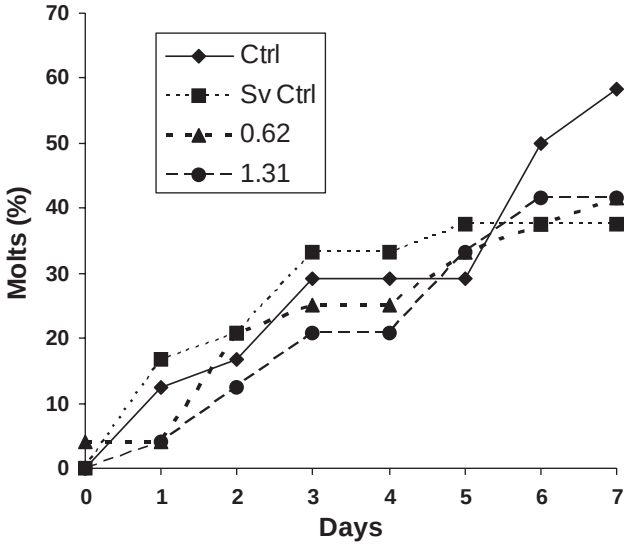

Fig. 1. Time course for molting of *L. vannamei* exposed to methyl parathion via food. (Ctrl, control group; Sv Ctrl, solvent control; 0.62 and 1.31 expressed as μg methyl parathion*g^{-1} dry weight of food). $n = 24$ in all groups.

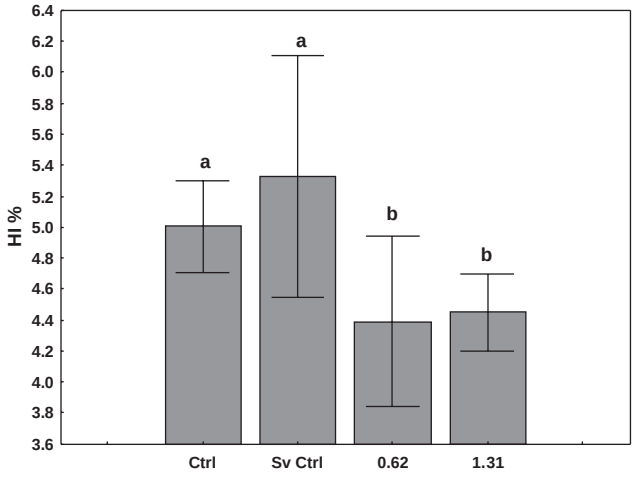

Fig. 2. Hepatosomatic Index (HI) in *L. vannamei* exposed to methyl parathion via food. (Ctrl, control group; Sv Ctrl, solvent control; 0.62 and 1.31 expressed as μg methyl parathion*g^{-1} dry weight of food). Mean ± SD; $n = 8$ in all groups. Treatments sharing the same lower case letters are not significantly different ($P > 0.05$).

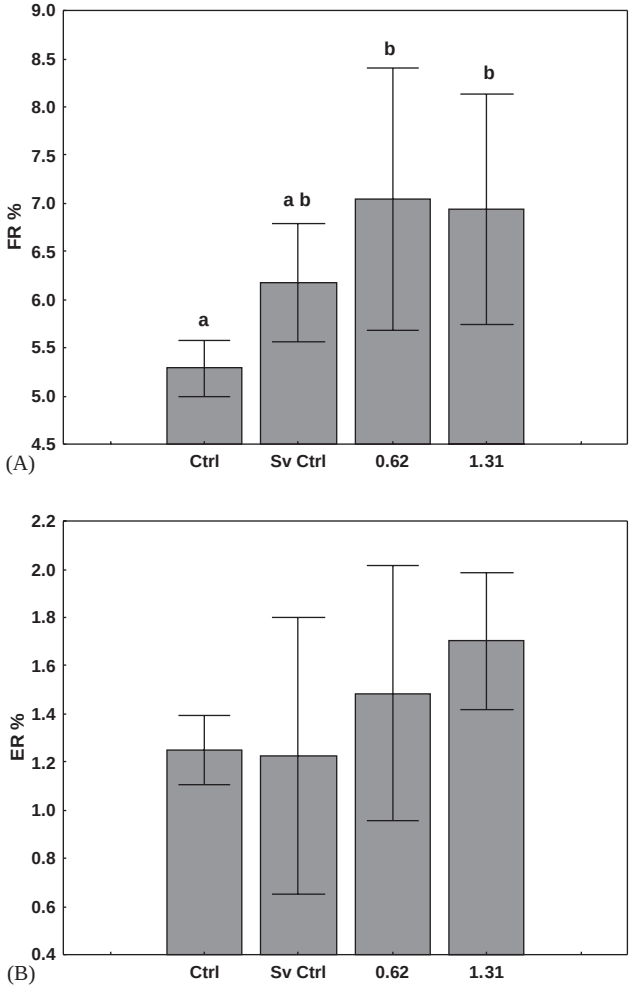

Fig. 3. Feeding rate (FR) (A) and Egestion rate (ER) (B) in *L. vannamei* exposed to methyl parathion via food. (Ctrl, control group; Sv Ctrl, solvent control; 0.62 and 1.31 expressed as μg methyl parathion*g^{-1} dry weight of food.) Mean ± SD, $n = 9$ in all groups. Treatments sharing the same lower case letters are not significantly different ($P > 0.05$).

solvent groups were observed. AChE activity of controls was significantly higher than that in the pesticide treatments (ANOVA, $p < 0.05$).

The total-ATPase activity was significantly different among controls and pesticide treatments (ANOVA, $p < 0.05$) (Fig. 5A): it was higher in the pesticide treatments than in the controls. Mg^{2+}-ATPase activity was significantly higher in the pesticide treatments than in the controls (ANOVA, $p < 0.05$) (Fig. 5B). Finally, the results obtained for the Na^{+}/K^{+}-ATPase activity are shown in Fig. 5C. In this case, control and 1.31 μg*g^{-1} were significantly higher than solvent control and 0.62 μg*g^{-1} (ANOVA, $p < 0.05$).

4. Discussion

Most experimental aquatic toxicology studies on the effects of contaminants in marine environments are carried out through aqueous exposures (Newman and Jagoe, 1996). However, relatively few studies to evaluate the effects of contaminants incorporated through food have been conducted. The results in this study showed that pulse exposures to methyl parathion via food could elicit measurable effects on the marine shrimp *L. vannamei*, while it is possible to appreciate an increment on particular responses depending on the concentration of the pesticide. This indicates that foodborne exposure can be a reliable toxicological

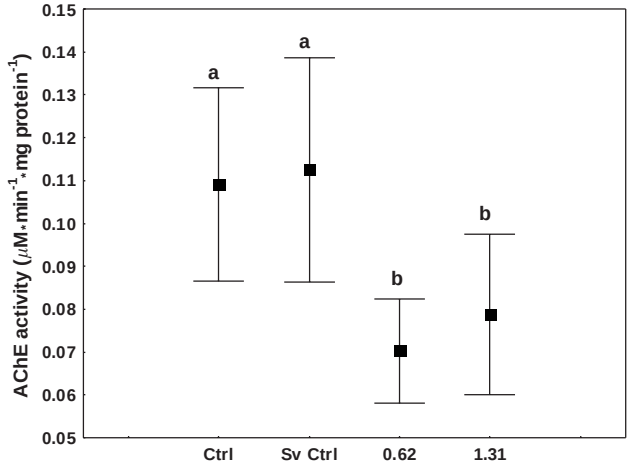

Fig. 4. AChEase activity in *L. vannamei* exposed to methyl parathion via food. (Ctrl, control group, Sv Ctrl, solvent control; 0.62 and 1.31 expressed as μg methyl parathion*g^{-1} dry weight of food.) Mean ± SD; $n = 8$ in all groups. Treatments sharing the same lower case letters are not significantly different ($P > 0.05$).

procedure and, if combined with pulse exposures, could also simulate more realistic exposure scenarios.

In addition to organophosphate and carbamate insecticides, other classes of environmental contaminants and heavy metals have shown the potential to decrease AChE in exposed organisms. However, in aquatic organisms there is considerable diversity in the biochemical properties and distribution of AChE and in their sensitivity to anticholinesterase agents (Habig et al., 1988; Bocquené et al., 1990; Pascal Mora and Narbonne, 1999). Several authors have tried to correlate inhibition of ChE activity with mortality to establish levels that could be dangerous to species survival, but this relationship changes according to the species, the compound, and the exposure conditions (Monserrat et al., 1997). The results in this study showed an inhibitory effect on AChE when the toxicant was administered via food, but no differences between the concentrations tested were observed.

Previous studies reported no changes in Na^+/K^+-ATPase activity in organisms exposed to heavy metals (Vanegas Pérez, 1996; Torreblanca et al., 1989; Rodríguez Moreno et al., 1998; Canli and Stagg, 1996). In contrast, other authors have demonstrated that Na^+/K^+-ATPase activity generally decreases after chemical stress, although the effects could vary depending on the pollutant (Jowett et al., 1981; Dhavale et al., 1988; Reddy et al., 1992; Lignot et al., 2000). However, in a few cases, the Na^+/K^+-ATPase activity increased in stress-exposed animals, probably as a compensating mechanism for the impairment of ionic regulation (e.g., from an increased surface permeability) (Jowett and Rhead, 1978; Lin et al., 1992, 1993). The latter reflects the results of this study, where the Na^+/K^+-ATPase activity decreased in the lower concentration of methyl

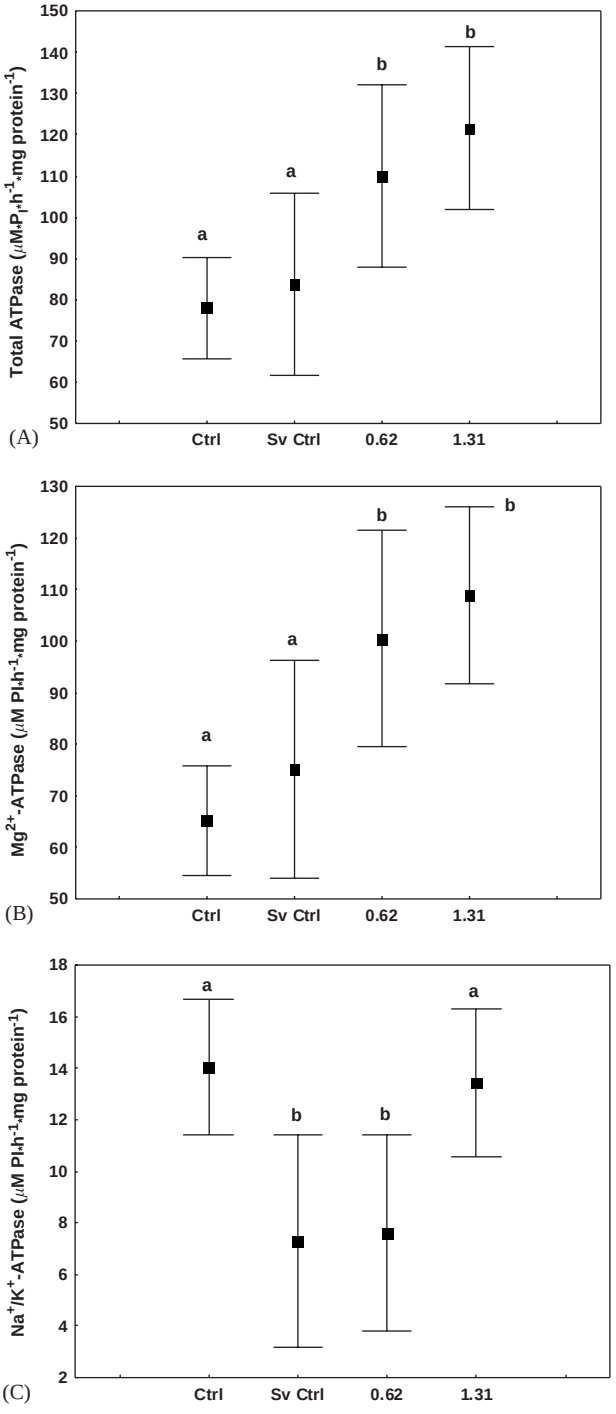

Fig. 5. Total-ATPase (A), Mg^{2+}-ATPase (B), and Na^+/K^+-ATPase (C) activities in *L. vannamei* exposed to methyl parathion via food. (Ctrl, control group; Sv Ctrl, solvent control; 0.62 and 1.31 expressed as μg methyl parathion*g^{-1} dry weight of food). Mean ± SD; $n = 8$ in all groups. Treatments sharing the same lower case letters are not significantly different ($P > 0.05$).

parathion tested. In addition, it was observed in the present study that the total ATPase and Mg^{2+}-ATPase activities increased significantly in both pesticide concentrations. These results indicate that, as has been observed by Torreblanca et al. (1989) in *Procambarus*

clarkii exposed to heavy metals, Mg^{2+}-ATPase is more sensitive than the Na^{+}/K^{+}-ATPase in *L. vannamei* gills. Nevertheless, ATPase activity does not seem a valuable parameter in the determination of physiological damage at sublethal concentrations, mainly when this enzymatic activity is the only indicator used. In this sense, Giesy et al. (1983) and Haya and Waiwood (1983) have proposed a nonspecific multitest assay to study toxic stress produced by pollutants. These procedures could include the ATPase activity modification as complement of other biochemical, histological, and metabolical tests (Torreblanca et al., 1989).

The nutritional alterations produced by stress can have critical consequences on most vital processes. In this sense, the hepatopancreas in decapod crustaceans has an important role in the processes of digestion and absorption, and it has been indicated as a major storage depot of organic and mineral reserves (Huggins and Munday, 1968; Allen, 1971). However, there is evidence that variations in the levels of digestive enzymes could be related to developmental cycles and external factors (Van Wormhoudt, 1974). Therefore, the significant detriment observed in the HI due to exposure to methyl parathion could be explained by a possible alteration in the concentrations of digestive enzymes or changes in the rate of utilization of reserves. It has been demonstrated that several shrimp species, such as *L. vannamei*, *Penaeus japonicus*, and *Litopenaeus adspersus,* adopt reductions in their rates of oxygen consumption as survival strategies under exposure to some organophosphorous pesticides. This reduction ultimately implies a physiological cost that can be translated into alterations in growth and reproduction (Galindo Reyes et al., 2002), although further studies to determine the different energetic changes related to digestive processes that the organisms could undertake in response to toxic stress are necessary. For instance, in exposed organisms, the FR after exposure increased significantly but the increment in weight and the HI were lower, which suggests that compensating mechanisms are involved.

Other strategies depending on the toxicant type, concentration, and mode of action could be adopted (Orchand et al., 2002), including changes in feeding strategies. In this regard, some authors have indicated that FR is a sensitive endpoint that allows rapid assessments, while it is physiologically and ecologically relevant (Taylor et al., 1998; Barata and Baird, 2000). Some studies suggest that exposure to a contaminant can increase the FR, but this response may be due not to a stimulatory effect but rather to the result of the need for increased energy to ameliorate metabolic damage (Bodar et al. 1988; Roux et al., 1993). In this sense, Orchand et al. (2002) determined that the feeding of *Moinodaphnia macleayi* increased slightly at the lowest treatment concentration of copper in water ($10\,\mu g\,L^{-1}$), suggesting that the increase in feeding may have been a requirement to meet the demand for higher maintenance costs.

One important objective of toxicity assessment is the identification of biomarkers that can potentially be useful as early indicators of sublethal effects of pollutants in aquatic animals.

Whereas sublethal tests are generally considered improvements over traditional lethal test methods, arguments over the most appropriate sublethal endpoints to measure will continue if responses across multiple levels of biological complexity are not examined (Mc Loughlin et al., 2000).

Hebel et al. (1997) pointed out the importance of an integrated approach in gaining an understanding of the effects of toxicants on the biology (in particular, the ecology) of crustaceans and highlighted the importance of including multiple measurements of variables in the experimental design which signal the functional status of each key physiological system to assess the organism's integrated responses to the toxic substance. In this sense, *L. vanammei* proved to be a test organism appropriate for tropical marine ecotoxicity tests.

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References

Allen, W.Y., 1971. Amino acid and fatty acid composition of tissues of the Dungeness crab (*Cancer magister*). J. Fish. Res. Board Can. 28, 1191–1195.

Baird, D.J., 1994. Pest control in tropical aquaculture: An ecological hazard assessment of natural and synthetic control agents. Mitt. Int. Ver. Limnol. 24, 285–292.

Barata, C., Baird, D.J., 2000. Determining the ecotoxocological mode of action of chemicals from measurements made on individuals: results from instar-based tests with *Daphnia magna* Straus. Aquat. Toxicol. 48, 195–209.

Barber, I., Baird, D.J., Calow, P., 1990. Clonal variation in general responses of *Daphnia magna* Straus to toxic stress. II. Physiological effects. Funct. Ecol. 4, 409–411.

Bocquené, G., Galgani, F., Truquet, P., 1990. Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. Mar. Environ. Res. 30, 75–89.

Bodar, C.W.M., Van Leeuwen, C.J., Voogt, P.A., Zandee, D.I., 1988. Effect of cadmium on the reproductive strategy of *Daphnia magna*. Aquat. Toxicol. 12, 301–310.

Bradford, M., 1976. A rapid sensitive method for the quantitation of microgram quanties of protein utilizing the principle of the protein dye binding. Anal. Biochem. 7, 248–254.

Calow, P., Sibly, R.M., 1990. A physiological basis of populates processes. Ecotoxicological implication. Funct. Ecol. 4, 283–288.

Canli, M., Stagg, R.M., 1996. The effects of in vivo exposure to cadmium, copper and zinc on the activities of gill ATPases in the Norway lobster, *Nephrops norvegicus*. Arch. Environ. Contam. Toxicol. 31, 494–501.

Carvalho, F.P., Fowler, S.W., Gonzalez-Farías, F., Mee, L.D., Readman, J.W., 1996. Agrochemical residues in the Alta-Ensenada del Pabellon coastal lagoons (Sinaloa, Mexico): a need of integrated coastal zone management. Int. J. Environ. Health Res. 6, 209–220.

Coppage, D.L., Braidech, T.E., 1976. River pollution anticholinesterase agents. Water Res. 10, 19–24.

Daniels, W.W., 1978. Applied Nonparametric Statistics. Ed. Houghton Mifflin Company, Boston, USA 503 pp.

Dhavale, D.M., Masurekar, V.B., Giridhar, B.A., 1988. Cadmium induced inhibition of Na + K + ATPase activity in tissue of crab *Scylla serrata* (Forskal). Bull. Environ. Contam. Toxicol. 40, 759–763.

Edwards, R.R.C., 1978. Ecology of a coastal lagoon complex in Mexico. Estuarine Coastal Mar. Sci. 6, 75–92.

Eidt, D.C., Hollebone, J.E., Lockhart, W.L., Kingsbury, P.D., Gadsby, M.C., Ernst, W.R., 1989. Pesticides in forestry and agriculture: Effects on aquatic habitats. In: Nriagu, J.O., Lakshminarayana, J.S.S. (Eds.). Aquatic Toxicology and Water Quality Management, vol. 22. Wiley Series in Advances in Environmental Science and Technology, 292pp.

Ellman, G., Courtney, K.D., Andress, V., Featherston, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmac. 7, 88–95.

FAO, 2002. FAO Fisheries Department, Fishery information, Data and Statistics Unit. FISHSTAT Plus: Universal software for fishery statistical time series. Version 2.3. 2000. Datasets: Aquaculture production: quantities 1970–2000, Aquaculture production: values 1984–2000, Capture production 1970–2000.

Galgani, F., Bocquene, G., Cardiou, Y., 1992. Evidence of variation in cholinesterase activity in fish along a pollution gradient in the North Sea. Mar. Ecol. Prog. Ser. 13, 77–82.

Galindo Reyes, J.G., Medina, J.A., Villagrana, C., Ibarra, C.L., 1997. Environmental and pollution condition of the Huizache-Caimanero lagoon, in the North-west of Mexico. Mar. Pollut. Bull. 12, 1072–1077.

Galindo Reyes, J.H.G., Dalla-Venezia, L., Lazcano Alvarez, M.G., Rivas-Mendoza, H., 2000. Enzymatic and osmoregulative alterations in white shrimp *Litopenaeus vannamei* exposed to pesticides. Chemosphere 40, 233–237.

Galindo Reyes, J.H.G., Dalla-Venezia, L., Lazcano Alvarez, M.G., 2002. Effect of some organophosphorus pesticides on oxygen consumption of Shrimp, *Litopenaeus vannamei*. Ecotoxicol. Environ. Saf. 52, 134–136.

Giesy, J.P., Duke, C.S., Bimgham, R.D., Dicson, G.W., 1983. Changes in phospoadenylate concentrations and adenylate charge as an integrated biochemical measure of stress in vertebrates: the effects of cadmium on the freshwater clam *Corbicula fluminea*. Toxicol. Environ. Chem. 6, 259–295.

González-Farias, F., Carvalho, F.P., Fowler, S.W., Mee, L.D., 1997. A tropical coastal lagoon affected by agricultural activities. The importance of radiolabeled pesticide studies. In: Environmental Behavior of Crop Protection Chemicals. Proceedings Series of International Atomic Energy Agency-Food and Agriculture Organization. Vienna, Austria, pp. 289–299.

Gurney, W.S.C., Middleton, D.A.J., Nisbet, R.M., Mc Cauley, E., Muroch, W.W., De Roos, A., 1996. Individual energetic and the equilibrium demography of structured population. Theor. Popul. Biol. 49, 344–368.

Habig, C., DiGiulio, R.T., Abou-Donia, M.B., 1988. Comparative properties of channel catfish *Ictalurus punctatus* and blue crab *Callinectes sapidus* acetylcholinesterases. Comp. Biochem. Physiol. 91, 293–300.

Hassall, K.A., 1990. The Biochemistry and Uses of Pesticides, Second ed. Macmillam, London, UK.

Haya, K., Waiwood, B.A., 1983. Adenylate energy charge and ATPase activity: Potential biochemical indicators of sublethal effects caused by pollutants in aquatic animals. In: Nrigau, J.O. (Ed.), Aquatic Toxicology, vol. 13. Wiley, New York, p. 525.

Hebel, D.K., Jones, M.B., Depledge, M.H., 1997. Responses of crustaceans to contaminant exposure: a holistic approach. Estuarine Coastal Shelf Sci. 44, 177–184.

Hernández Cornejo, R., Ruiz Luna, A., 2000. Development of shrimp farming in the coastal zone of southern Sinaloa (Mexico): operating characteristics, environmental issues, and perspectives. Ocean Coastal Manage. 43, 597–607.

Huggins, A.K., Munday, K.A., 1968. Crustacean metabolism. In: Lowenstein, O. (Ed.), Advances in Comparative Physiology and Biochemistry, vol. 3. Academic Press, New York, pp. 271–378.

Irving, E.C., Baird, D.J., Culp, J.M., 2003. Ecotoxicological responses of the mayfly *Baetis tricauadatus* to dietary and waterborne cadmium: implications for toxicity testing. Environ. Toxicol. Chem. 22, 1058–1064.

Jowett, P.E., Rhead, M.M., 1978. In vitro changes in the activity of ATPases in the gills of *Carcinus maenas* exposed to various concentrations of p, pX-DDT. Environ. Pollut. 17, 1–6.

Jowett, P.E., Rhead, M.M., Bayne, B.L., 1981. In vivo changes in the activity of gill ATPases and haemolymph ions of *Carcinus maenas* exposed to p, pX-DDT and reduced salinities. Comp. Biochem. Physiol. C 69, 399–402.

Kumar, A., Chapman, J.C., 1998. Profenofos toxicity to the rainbow fish (*Melanotaenia duboulayi*). Environ. Toxicol. Chem. 17, 1799–1806.

Lignot, J.H., Spanings-Pierrot, C., Charmantier, C., 2000. Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. Aquaculture 19, 209–245.

Lin, H.P., Charmantier, G., Thuet, P., Trilles, J.P., 1992. Effects of turbidity on survival, osmoregulation and gill Naq–Kq ATPase in the shrimp *Penaeus japonicus*. Mar. Ecol. Prog. Ser. 90, 31–37.

Lin, H.P., Thuet, P., Trilles, J.P., Mounet-Guillaume, R., Charmantier, G., 1993. Effects of ammonia on survival and osmoregulation at various developmental stages of the shrimp *Penaeus japonicus*. Mar. Biol. 117, 591–598.

Matlby, L., 1994. Stress, shredders and streams: using *Gammarus* energetics to assess water quality. In: Sutcliffe, D.W. (Ed.), Water Quality and Stress Indicator in Marine and Freshwater Ecosystems. Linking Levels Organizations Individuals, Populations and Communities. Freshwater Biological Association, Windemere, UK, pp. 98–110.

Maltby, L., Kedwards, T.J., Forbes, V.E., Grasman, K., Kammenga, J.E., Munns Jr., W.R., Ringwood, A.H., Weis, J.S., Wood, S.N., 2001. Linking individual-level response and population-level consequences. In: Baird, D.J., Burton, Jr., G.A. (Eds.), Ecological Variability: Separating Natural and Anthropogenic Causes of Ecosystem Impairment. SETAC, Pensacola, FL, USA, pp. 27–82.

Maltby, L., Naylor, C., 1990. Preliminary observations on the ecological relevance of the *Gammarus* "scope of growth" assay: Effect of zinc on reproduction. Funct. Ecol. 4, 393–397.

Mc Loughlin, N., Daquiang, Y., Maltby, L., Wood, R.M., Hongxia, Y., 2000. Evaluation of sensitivity a specificity of two crustacean biochemical biomarkers. Environ. Toxicol. Chem. 19, 2085–2092.

Mc William, R., Baird, D., 2002. Postexposure feeding depression: a new toxicity end point for use in laboratory studies with *Daphnia magna*. Environ. Toxicol. Chem. 21, 1198–1205.

Mee, L.D., Readman, J.W., Carranza-Fraser, J., Gonzalez-Farias, F., 1992. Fate of Agrochemicals in Tropical Coastal Lagoon

Ecosystems. International Scientific Cooperation, Consolidated Report of Activities 1986–90, EC-Mexico. Published by Commission of the European Communities, Brussels 111–115.

Monserrat, J.M., Bianchini, A., Rebelo, M., 1997. Toxicity and Anticholinesterase effect of formulated Methyl Parathion to the Estuarine Crab *Chasmagnathus granulata* (Decapoda, Grapsidae) pre-exposed to sesamol. Comp. Biochem. Physiol. C 3, 329–334.

Murphy, S.D., 1986. Pesticides. In: Doul, J., Klassen Anderse, C.D., Anders, M.O. (Eds.). The Basic Science of Poisons. Macmillan, NY, pp. 519–581.

Naylor, C., Malby, L., Callow, P., 1989. Scope for growth in *Gammarus pulex*, a freshwater detritivore. Hydrobiologia 188/189, 517–523.

Newman, C., Jagoe, C.H., 1996. Ecotoxicology. A hierarchical treatment. Edit. CRC Press, USA.

Orchand, S.J., Holdway, D.A., Barata, C., Van Dam, R.A., 2002. A rapid response toxicity test based on the feeding rate of the tropical cladoceran *Moinodaphnia macleayi*. Ecotoxicol. Environ. Saf. 53, 12–19.

Pascal Mora, X.M., Narbonne, J.F., 1999. Cholinesterase activity as potential biomarker in two bivalves. Environ. Toxicol. Pharmacol. 7, 253–260.

Payne, J.F., Mathieu, A., Melvin, W., Fancey, L., 1996. Acetylcholinesterase, an old biomarker with a new future? Field Trials in association with two urban rivers and paper mill in newfound-land. Mar. Pollut. Bull. 32, 225–231.

Reddy, A.N., Venugopal, N.B.R.K., Reddy, S.L.N., 1992. Effect of endosulfan 35 EC on ATPases in the tissues of a freshwater field crab *Barytelphusa guerini*. Bull. Environ. Contam. Toxicol. 48, 216–222.

Roddie, B.D., Redshaw, C.J., Nixon, S., 1996. Sublethal biological effects of monitoring using the common mussel, *Mytilus edulis*. Comparison of laboratory and in situ effects of an industrial effluent discharge. In: Tapp, J.F., Hunt, S.M., Wharfe, J.R. (Eds.), Toxic Impacts of Wastes on the Aquatic Environment. Royal Society of Chemistry, Cambridge, UK, pp. 125–137.

Rodríguez Moreno, P.A., Schuwarzbaun, P.J., Rodríguez, F.M., 1998. Effects of cadmium on Gill Na,K-ATPase of the estuarine crab *Chasmagnathus granulata* (Decapoda, Grapside) during postmolt: In vivo and in vitro studies. Bull. Environ. Contam. Toxicol. 61, 629–636.

Rosales, M.T.L., Escalona, R.L., 1983. Organochlorine residues in organisms of two different lagoons of northwest Mexico. Bull. Environ. Contam. Toxicol. 30, 456–463.

Roux, D.J., Kempster, P.L., Truter, E., Van der Merwe, L., 1993. Effects of cadmium and copper on survival and reproduction of *Daphnia pulex*. Water SA 19, 169–274.

Satyavathi, C., Prabhakara Rao, Y., 2000. Inhibition of Na+, K+ ATPase in *Penaeus indicus* postlarvae by lead. Comp. Biochem. Physiol. C 127, 11–22.

Schlenk, D., 1999. Necessity of defining biomarkers for use in ecological risk assessments. Mar. Pollut. Bull. 39, 48–53.

Sibly, R.M., 1996. Effects of pollutants on individual life stories and population growth rates. In: Newman, M.C., Jagoe, C.H. (Eds.), Ecotoxicology: A Hierarchical Treatment. Lewis, Boca Raton, FL, USA, pp. 197–223.

Skou, J.C., 1957. The influence of some cations on the adenosine triphosphatase from peripheral nerves. Biochim. Biophys. Acta 23, 394–401.

Skou, J.C., 1965. Enzymatic basis of active transport of Na^+ and K^+ across cell membrane. Physiol. Rev. 45, 596–617.

Sokal, R.R., Rohlf, R.J., 1981. Biometry. W.H. Freeman and Company, New York, USA, pp. 859.

Sola, F., Isaia, J., Mansoni, A., 1995. Effects of copper on gill structure and transport function in the rainbow trout, *Oncorhynchus mykiss*. J. Appl. Toxicol. 15, 391–398.

Taylor, G., Baird, D.J., Soares, A.M.V.M., 1998. Surface binding of contaminants by algae: consequences for lethal toxicity and feeding to *Daphnia magna*. Environ. Toxicol. Chem. 17, 412–419.

Tentes, I., Stratakis, E., 1991. Partial purification and properties of $(Na^+ + K^+)$-ATPase from *Potamon potamios* Comp. Biochem. Physiol. 3, 619–624.

Torreblanca, A., Del Ramo, J., Diaz-Mayars, J., 1989. Gill ATPase activity in *Procambrus clarkii* as an indicator of heavy metal pollution. Bull. Environ. Contam. Toxicol. 42, 829–834.

Vanegas Pérez, R.C., 1996. Efectos Subletales del Cadmio y Zinc en el Camarón Blanco *Penaeus setiferus*. Tesis de Doctorado en Ciencias (Biología). Facultad de Ciencias, UNAM, México, pp. 118.

Van Wormhoudt, A., 1974. Variations of the level of the digestive enzymes during the intermolt cycle of Palaemon serratus: influence of the season and effect of the eyestalk ablation. Comp. Biochem. Physiol. A 49, 707–715.