International Journal of Aquatic Science
ISSN: 2008-8019
Vol. 4, No. 2, 3-13, 2013

Alkaline Phosphatase in Shrimp *Artemesia longinaris*: Response to Feed

Nair de los Angeles Pereira[1], Analia Veronica Fernández Gimenez[1,2]*. Nora Selma Haran[1] and Jorge Lino Fenucci[1,2]

1) Instituto de Investigaciones Marinas y Costeras, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Argentina. Funes 3350, B7602AYL, Mar del Plata, Argentina
2) CONICET, Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

Received: 21 September 2012 **Accepted:** 13 November 2012 **Published:** 27 June 2013

Abstract: The purpose of this study was to evaluate the alkaline phosphatase activity in haemocytes and midgut gland of the penaeid shrimp *Artemesia longinaris* in relationship with different doses of vitamin D3 in feed and to estimate its potential use as biomarker for nutritional stress. A nine-week trial was carried out with juvenile shrimp in aquaria. Animals were fed semipurified feeds with increasing levels of vitamin D3 (0; 0.2; 0.375; 0.75 and 1 mg vitamin D3 kg^{-1} feed). Treatments without vitamin D revealed the significantly highest protein content in haemocytes ($P<0.05$), as the lowest values were recorded on 0.2 and 1 mg vitamin D kg^{-1} treatments. The analysis of protein content of midgut glands revealed a maximum content for diets containing 0.2 and 0.75 mg Vitamin D kg^{-1}. The optimum pH value for alkaline phosphatase in midgut gland was 9.5. The results show the occurrence of alkaline phosphatase activity in the tissues of shrimp *A. longinaris*, describing higher enzymatic activity values in haemocytes than in midgut glands. The highest enzyme activity in haemocytes was observed for shrimp fed without vitamin D (1.235 abs min^{-1} mg $protein^{-1}$), however, in midgut gland, the activity varied from 0.141 to 0.297 abs min^{-1} mg $protein^{-1}$, with the highest values on 0 and 1 mg vitamin D kg^{-1}. Histological analysis of the midgut gland confirmed a good health of the shrimp fed 0.375 and 0.750 mg vitamin D kg^{-1} and were used as optimal values for determining enzymatic activity. Shrimp fed diets lacking of vitamin D or 1 mg kg^{-1} showed signs of malnourishment. The results indicate that alkaline phosphatase activity in *A. longinaris* was influenced by dietary vitamin D and may be used as a biomonitor of nutritional stress.

Key Words: Alkaline phosphatase, Midgut gland, Shrimp, Vitamin D

(✉) fgimenez@mdp.edu.ar

Introduction

Many physiological processes in organisms are under regulation via formation (phosphorylation) or cleavage (dephosphorylation) of phosphate esters. Alkaline phosphatases (AP) (EC 3.1.3.1) are ubiquitous metalloenzymes located in several cell membranes, intestinal cells, kidney, placenta, osteoblasts and plasma and have been involved in several essential functions in mammals (Hessle *et al.,* 2002). AP activity determination is often used in clinical and ecotoxicological studies in vertebrates and it has been also studied in different invertebrate tissues. Vijayavel and Balasubramanian (2006) suggested that phosphatases play major roles in the molting physiology of many crustaceans. Although the involvement of vitamin D in calcium and phosphorus metabolism of various terrestrial vertebrates has been established, information in aquatic species is limited. In the green crab *Scylla serrata,* AP is important in the absorption of phosphate and calcium from seawater and for the integument formation (Park *et al.,* 2001); however the body calcium and phosphorus concentrations of the shrimp *Penaeus monodon* did not reflect the activity of the alkaline phosphatase (Shiau and Hwang, 1994).

Several authors determined that different fish species require a dietary source of vitamin D for normal growth and normal concentrations of ash, calcium and phosphorus in the whole body (Barnett *et al.,* 1979). Deficient dietary vitamin D causes poor growth, high mortality, reduced appetite and darkening of midgut gland in some penaeids such as *Marsupenaeus japonicus* and *Litopenaeus vannamei* (Kanazawa, 1985; He *et al.,* 1992). Shiau and Hwang (1994) observed a relationship between alkaline phosphatase activity and vitamin D in *P. monodon* with deficient growth; but there is scarce information to affirm that AP and vitamin D are related in other crustaceans.

The purpose of this study was to evaluate the alkaline phosphatase activity in haemocytes and midgut gland of the penaeid shrimp *Artemesia longinaris* in relationship with different doses of vitamin D3 in diet, and to estimate its potential use as biomarker for nutritional stress. This species is traded mainly in the Argentine market (Gavio and Boschi, 2004) and has seasonal and annual fluctuations in captures. Vitamin requirements were previously determined for *A. longinaris* in growth experiments under culture conditions (Fernández Gimenez *et al.,* 2008; Sarasa, 2010; Pereira, 2011).

Materials and Methods

Feed and feeding trials

Five semipurified formulated feeds were prepared with vitamin free casein, squid protein (as protein source) and a vitamin mixture according to Fernández Gimenez *et al.* (2008).

Cholecalciferol (Vitamin D3) was added separately to the isoproteic and isolipidic semipurified feeds at expense of carboxymethyl cellulose in order to obtain concentrations of 0; 0.2; 0.375; 0.75 and 1 mg vitamin D3 kg^{-1} feed. All the ingredients were mixed, cold pelletized (< 50ºC) by extrusion and oven-dried for 24 h at 50ºC and the chemical composition was confirmed according to AOAC (1990) (Table 1).

Previous studies in *A. longinaris* showed that the highest rates of growth occur in animals fed supplemented diets with 0.375 and 0.750 mg vitamin D kg^{-1} (Pereira, 2011). In the present work, these doses are used as control treatments for what histological studies of midgut gland were performed to corroborate they promote a good health.

Shrimp were obtained from a commercial fisherman in the coastal waters of Mar del Plata, Argentina (38°S). Individuals were kept in 150 L glass aquaria (33 ups salinity, 20°C, pH 7; 11h light- 13h dark photoperiod) with continuous aeration and the ammonium concentration never exceeded 0.2 mg L^{-1}. All groups were fed *ad libitum* once a day (09:30 h) during a nine-week period and the formulated feeds were tested in triplicate groups of eight animals *per* aquarium randomly chosen. At the beginning of the trial the mean weight of juvenile shrimp was 1.5 g ± 0.15.

Histological analysis

Midgut gland tissues were fixed in Davison fluid (Bell and Lightner, 1988) for 24h, dehydrated and included in butylparaffin and paraffin. Sections of 5 µm were stained with hematoxylin eosin and characterized according to Petriella and Fonalleras (1998) and Fernández Gimenez (2002).

Tab. 1: Percentual and Proximal Composition of feed

Ingredient	**g 100 g dry feed^{-1}**
Casein, vitamin-free	37.5
Squid protein	2.5
Gelatin	12
Manioc starch	22
Free Fatty acids [a]	7
Carboxymethyl cellulose	10
Lecithin	1
Cholesterol	2
Sodium alginate	3.7
Mineral Premix[b]	0.3
Vitamin Premix[c]	2
Proximal composition	**%**
Moisture	7.1 ± 0.77
Total Protein	44.5 ± 3.65
Total lipids	13.2 ± 2.63
Ash	4.16 ± 1.25

a) Polyunsaturated fatty acids ω-3 PUFAs. (Omega Sur, Mar del Plata, Argentina).

b) Mineral premix: calcium 1,000 mg; magnesium 500mg, potassium 99mg, zinc 30mg, 10mg iron, copper 2mg, iodine 150mcg, selenium 200mcg, molybdenum 500mcg (Twin Laboratories, Inc. USA).

c) Vitamin premix (mg kg-1): thiamin 163; rivoflavin 156; pyridoxine 213; calcium pantothenate 250; biotin 250; niacin 500; folic acid 25; B12HCl 20;; menadione 34; inositol 300; choline chloride 200 (Laboquímica, Argentina), L-ascorbic acid 2-phosphate 781 (A8960 Sigma), carboxymethyl cellulose was replaced by appropriate amounts of vitamin D3 (Parafarm, Argentina 4 x106 U g-1) in the premix to give different levels of vitamin D.

Haemocytes and Midgut gland samples

Molting stage was determined by microscopic examination of uropod setae (Petriella, 1984). At the end of the trials all specimens in intermolt stage were cryoanesthesized to reduce stress by manipulation, haemolymph was extracted and midgut glands were removed. Samples from eight individuals (n=8) of each treatment group were analyzed separately

Haemolymph was sampled (200-300 µL) through a prechilled 1 mL syringe needle inserted at the base of the fifth pereiopod, it was centrifuged at 800 *g* for 3 min at 4 °C, and haemocytes were stored for determinations while plasma was discarded. Haemocytes were washed with isotonic solution 100 mM $CaCl_2$ 0.45 M NaCl, re-suspended in 5mM CaCl2 0.45 M NaCl and newly centrifuged at 12,000 *g* a pulse. The supernatant (haemocytes lysate) was separated and stored at -20°C until analysis (Fernández Gimenez *et al.,* 2011).

Midgut glands were carefully dissected and immediately frozen at -20ºC. They were homogenized in cold distilled water, and the mixture was then centrifuged at 10,000 *g* for 30 min at 4 ºC. The supernatant was stored as protein extract, and the total soluble protein was evaluated in haemocytes lysate and protein extract of midgut gland, by Bradford (1976) using ovalbumin as standard (Sigma A7642).

Alkaline phosphatase activity

The alkaline phosphatase activity in haemocytes lysate and protein extract of midgut gland was determined using *p*-nitrophenylphosphate (*p*-NPP) (Sigma N4645) as substrate, according to Pinoni *et al.* (2005). Enzymatic activity was assayed in midgut gland at different pH (7.5; 8; 8.5; 9; 9.5 and 10) and substrate concentrations (0.1; 2.5; 3; 5; 9; 9.5 and 10 mM *p*-NPP) to obtain the optimal parameters.

The evaluation of alkaline phosphatase activity in relationship to dietary vitamin D was recorded in all samples, given pH and optimal substrate concentration. The procedure was developed following the hydrolysis of *p*-NPP in a medium containing 1 mM $MgSO_4$ in buffer 100 mM Tris-HCl at pH 9.5. An aliquot of protein extract (300 mg protein) was added to a medium containing 1 mM Tris-HCl pH 9.5 and preincubated 5 minutes at 37 ºC. The reaction was initiated with the addition of 95 mM *p*-NPP (final concentration 9.5 mM) incubating 30 minutes at 37 ºC and stopped by adding 0.1 M KOH. The released amount of *p*NP was determined by measuring absorbance at 405 nm. Each test was performed in triplicate and included a blank control. The alkaline phosphatase activity was expressed as change in absorbance min^{-1} mg $protein^{-1}$.

Statistical analysis of data

The enzyme activity was analyzed with ANOVA and Tukey-Kramer Multiple Comparison

at significance level of P<0.05 (Sokal and Rohlf, 1979). The value of the Michaelis-Menten constant (Km) was calculated using a Lineweaver-Burk plot of the program GraphPadPrism (Pinoni *et al.,* 2005). Analysis were made using NCSS 8 Software.

Tab. 2: Soluble protein content in haemocytes and midgut gland in response to feed

Treatments (mg Vitamin D kg^{-1} feed)	Soluble Protein (mg ml^{-1})	
	haemocytes	midgut gland
0	0.035 ± 0.0009 [a]	4.792 ± 0.3378 [a]
0.2	0.009 ± 0.0020 [b]	6.663 ± 0.2860 [b]
0.375	0.016 ± 0.0012 [c]	5.705 ± 0.2396 [a]
0.75	0.016 ± 0.0012 [c]	7.423 ± 0.0437 [b]
1	0.012 ± 0.0009 [bc]	5.323 ± 0.2989 [a]

Different letters in the same column indicate significant differences between treatments (P<0.05).

Results

Histological analysis of midgut glands corroborated previous results reported by Pereira (2011), in which animals given food supplemented 0.375 and 0.75 mg vitamin D kg^{-1} where healthy with normal morphology and cytology of the gland. The four typical cells were observed: E (embryonic); F (fibrillar), R (resorptive) and B (secretory), each one with its conspicuous brush border in the apical zone (Fig. 1A). Animals fed unsupplemented food and supplemented with 0.2 and 1 mg vitamin D kg^{-1} presented some histopathological signs of malnourishment, as wide tubular lumen with absence of brush border, shrinkage of cell, folding of the basal membrane, cellular peeling, foamy cells and abundant necrotic cells leading to glandular dysfunction and haemocyte infiltration was observed in some individuals (Fig. 1B and 1C).

Table 2 shows soluble protein content in haemocytes and midgut gland. Haemocytes from shrimp fed without vitamin D revealed the highest content of protein (P<0.05) as the lowest values were recorded on 0.2 and 1 mg vitamin D kg^{-1} diet. In midgut gland, the maximum content was registered for treatments 0.2 and 0.75 mg vitamin D kg^{-1}.

The optimum pH value for alkaline phosphatase in midgut gland of *A. longinaris* was 9.5 (Fig. 2). Enzymatic activity was therefore assessed at pH 9.5 in subsequent determinations.

The effect of substrate *p*-NPP concentrations

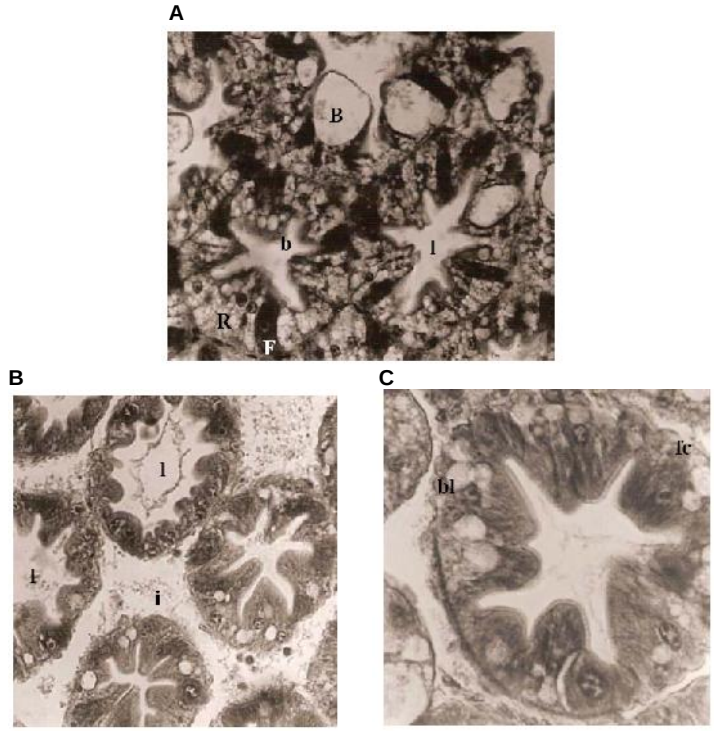

Fig. 1: Microphotographs of *Artemesia longinaris* midgut gland fed with semipurified diets with different levels of vitamin D3.

A: 0.375 mg vitamin D kg^{-1} feed, transverse section through tubules showing the arrangement of the normal organ and the cellular types similar to those wild shrimp. Note a conspicuous brush border in the apical zone of cells (X45)

B: 0 mg vitamin D kg^{-1} feed, transverse section through tubules revealing severe cytological alterations, showing the enlarged lumen, cellular retraction and the increased intertubular space (X45)

C: 1 mg vitamin D kg^{-1} feed, transverse section through a tubule in detail, showing folding of the basal lamina and foamy cells (X100)

b, brush border; bl, basal lamina; B, B cell; F, F cell; Fc, foamy cells; i, intertubular space ; l, lumen; R, R cell.

on alkaline phosphatase activity at pH 9.5 is shown in Figure 3. Enzyme activity exhibited Michaelis-Menten kinectics (Km = 4.33 mM). Since the maximum enzyme activity value was observed at a substrate concentration between 8 and 10 mM, a 9.5 mM was used.

The alkaline phosphatase activity in haemocytes and midgut gland in relationship with dietary vitamin D is shown in Figure 4. The highest alkaline phosphatase activity in haemocytes was observed in shrimps fed vitamin D free diets (1.235 abs min^{-1} mg $protein^{-1}$), however in midgut gland, the enzyme activity varied from 0.141 to 0.297 abs

min^{-1} mg $protein^{-1}$, with highest values for treatments 0 and 1 mg vitamin D kg^{-1}.

Discussion

In recent years, the knowledge of the structure and function of alkaline phosphatase has increased greatly. Alkaline phosphatase occurs widely in nature and it is found in many organisms from bacteria to men. AP is a homodimeric enzyme and each catalytic site contains three metal ions, i.e. two Zn and one Mg, necessary for enzymatic activity (Millán, 2006).

In the present work, we observed alkaline phosphatase activity in the studied tissues of shrimp, describing higher activity values for haemocytes than midgut gland. AP activity was demonstrated in invertebrates and was partially purified and characterized in crustaceans (Wojewodzic *et al.,* 2011). Saha *et al*. (2009) established AP activity in the haemocytes of *S. serrata*, Shiau and Hwang (1994) observed this in the midgut gland of *P. monodon* and Lovett *et al.* (1994) found it in the gills of the blue crab *Callinectes sapidus*.

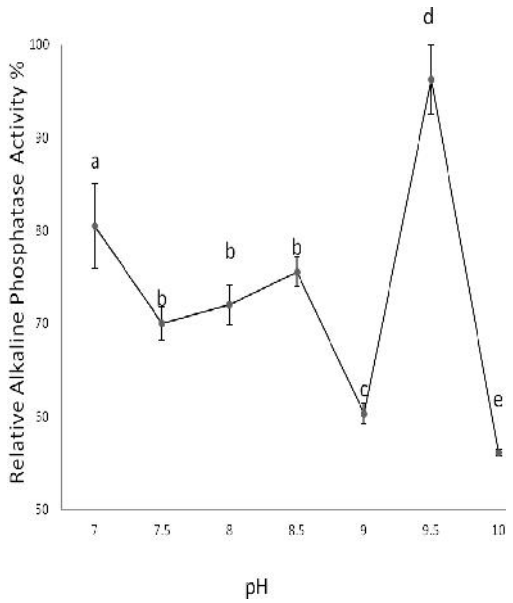

Fig. 2: Effect of pH on the alkaline phosphatase activity in midgut gland of *A. longinaris.* Enzyme activity values are expressed in relation to the activity at pH 9.5 (100%). Data are the mean ± SE for three individuals. Different letters indicate significant differences (P< 0.05).

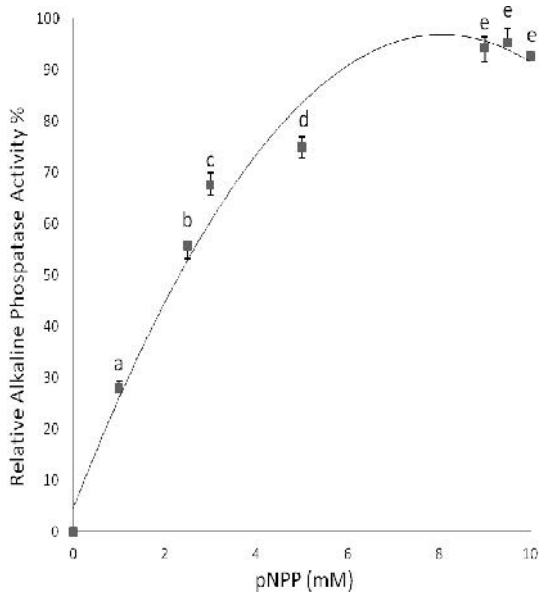

Fig. 3: Effect of *p*NPP on alkaline phosphatase activity in midgut gland of *A. longinaris*. The values of enzyme activity are expressed as a relation of the activity at 9.5 mM pNPP (100%). Data are the mean ± SE for three individuals. Different letters indicate significant differences (P < 0.05).

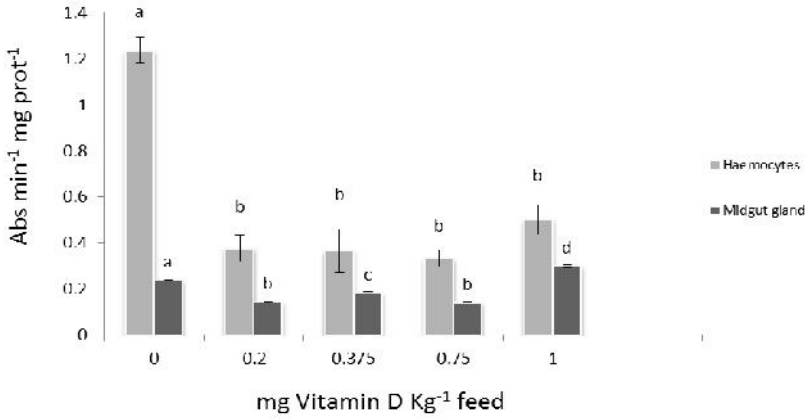

Fig. 4: Alkaline phosphatase activity in haemocytes and midgut gland of *Artemesia longinaris* fed with different levels of vitamin D in feed. Different letters in the same tissue indicate significant differences between treatments (P<0.05). Data are the mean ± standard error for three individuals.

In invertebrates, alkaline phosphatase activities exhibited a range of optimum pH values between 7.1 and 10.5 (Lovett *et al.,* 1994; Mazorra *et al.,* 2002). The highest AP activity in midgut gland of *A. longinaris* was obtained at pH 9.5, which is in agreement to the values described for *C. sapidus* (Lovett *et al.,* 1994) and *Pleoticus muelleri* (Sarasa, 2010; Pereira, 2011).

The Michaelis-Menten kinetics (Km) is related to the affinity of an enzyme for a given substrate and varies between the organs and species studied. The Km for alkaline phosphatase in *A. longinaris* was 4.33 which is similar to the described for *P. muelleri* (Pereira, 2011).

Zhang *et al.* (2007) investigated the interaction between vitamins A and D and alkaline phosphatase activity in abalone *Haliotis discus hannai*. The authors observed that both nutrients in diet increased the enzyme activity in viscera and found that the excessive supplementation of vitamin A decreased it. In crustaceans, Shiau and Hwang (1994) suggested that AP may be involved in the metabolism of calcium and phosphorus; they reported that the absence of vitamin D and levels above 0.1 mg kg^{-1} decreased the enzyme activity in *P. monodon.*

Saha *et al.* (2009) observed that chronic exposure of *S. serrata* to arsenate may lead to the decrease of the alkaline phosphatase activity in haemocytes, resulting on the impairment of immunological activity. In the present study, AP activity was evidenced in haemocytes for all treatments obtaining the highest activity for the one without vitamin D, nonetheless, Fernandez Gimenez *et al.* (2011)

found no relation between dietary vitamin D and immune response in *A. longinaris*. These results suggest that there is no relation between AP activity and immune response in haemocytes.

In *Litopenaeus setiferus* larvae it has been demonstrated that alkaline phosphatase activity is situated along the midgut gland, suggesting that absorption is widespread, however in juveniles the activity is restricted to the midgut gland and midgut region. Alkaline phosphatase activity in the digestive gland of decapods has been associated with metabolites transmembrane transport (Lovett and Felder, 1990). Using histochemical methods, Monin and Rangneker (1974) observed that midgut gland cells of crab *S. serrata*, showed a positive reaction for alkaline phosphatase only at the brush border. Meyran and Craf (1986) used cytochemical techniques and observed periodical changes in AP activity in the posterior caeca of the amphipod *Orchestia cavimana* during the molting cycle; suggesting its involvement in calcium transport.

In the present work, alkaline phosphatase activity in the midgut gland of *A. longinaris* recorded the maximum value in animals fed 0 and 1 mg of vitamin D Kg^{-1} in agreement with the ones obtained for *P. muelleri* (Pereira, 2011). Shrimp given diets vitamin D free or 1 mg kg^{-1} showed signs of malnourishment as increase of tubular lumen, folding of the basal membrane, loss of several cell types and brush border and foamy cells. Changes in the activity of alkaline phosphatase in midgut gland indicate disturbance in the structure and integrity of cells and physiology of organisms, causing deleterious consequences. Histological analysis is important to reflect the nutritional state of the cells of the midgut gland due to a diet and constitute practical means for the assessment of the nutritional value of diet in combination with statistical parameters such as growth and survival (Fernández Gimenez *et al.,* 2008). In this work, histological analysis of the midgut gland confirm the good health of the shrimp fed 0.375 and 0.750 mg vitamin D kg^{-1} for what they were used as optimal values for enzyme activity. Other vitamin had also a similar effect on alkaline phosphatase activity of midgut gland, Sarasa (2010) observed that *P. muelleri* responded to different levels of vitamin K in diet.

Different authors used the change of the rates of enzymatic activities to determine the effect of environmental and external factors. Among these, alkaline phosphatases are used in aquatic animals as stress bioindicators, due to their high sensitivity, less variability and high conservation among species (Vijayavel and Balasubramanian, 2006). Although several works studied AP as biomonitor in organisms (Sukhanova *et al.,* 1996; Saha *et al.,* 2009; McCarthy *et al.,* 2010) there are scarce studies that employed AP as monitor of nutritional stress.

The results of this work indicate that alkaline phosphatase activity in haemocytes and midgut gland of *Artemesia longinaris* were influenced by the addition of vitamin D in feed and may be used as biomonitor for determining nutritional stress.

Acknowledgements

This research was funded by CONICET Argentina grant, PIP Nº 11220080102585. We thanks to Lic. Maria Victoria Sarasa and Technical Scientific Translator Julieta Sapienza Vera, for correcting the English text.

References

- ✓ AOAC (1990) Association of Official Analytical Chemists. Official Methods of Analysis. Vol. I, 15th ed. Association of Official Analytical Chemist, Arlington, Virginia, USA. 957 pp.
- ✓ Barnett B.J., Cho C.Y. and Slinger S.J. (1979) The essentiality of cholecalciferol in the diets of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Phys., 63(A): 291-297.
- ✓ Bell T. and Lighter D.V. (1988) Male reproductive system. A Handbook of Normal Penaeid Shrimp Histology. Allen Press Kansas. 114 pp.
- ✓ Bradford M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.
- ✓ Fernández Gimenez A.V. (2002) Requerimiento de vitaminas liposolubles A y E en la dieta del camarón *Artemesia longinaris* Bate, 1888 y el langostino *Pleoticus muelleri* (Bate, 1888). Tesis Doctorado en Ciencias Biológicas, Universidad de Buenos Aires. 185pp.
- ✓ Fernández Gimenez A.V., Harán N.S., Pereira N.A., Sarasa M.V. and Fenucci J.L. (2011) Capacidad inmunoestimulante de la vitamina D en el camarón argentino *Artemesia longinaris*. Lira, Lodeiros, González, Rey Méndez, Guerra and Fernández (eds) Foro Iberoamericano Recursos Marinos y Acuicultura, III: 381-387.
- ✓ Fernández Gimenez A.V., Díaz A.C., Velurtas S.M., Petriella A.M. and Fenucci J.L. (2008) Effects of different dietary vitamin A levels in the red shrimp *Pleoticus muelleri* (Bate, 1888) (Decapoda, Solenoceridae). Rev. Biol. Mar. Oceanog., 43 (3): 483-490.
- ✓ Gavio M.A. and Boschi E.E (2004) Biology of the shrimp *Artemesia longinaris* Bate, 1888 (Decapoda:Penaeidae) from Mar del Plata coast. Nauplius, 12 (2): 83-94.
- ✓ He H., Lawrence A.L and Liu R. (1992) Evaluation of dietary essentiality of fat-soluble vitamins, A, D, E and K for penaeid shrimp (*Penaeus vannamei*). Aquaculture, 103: 177-185.
- ✓ Hessle L., Johnson K.A., Anderson H.C., Narisawa S., Sali A., Goding J.W., Terkeltaub R. and Millan J.L. (2002) Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. Proc Natl Acad Sci USA, 99: 9445-9449.
- ✓ Kanazawa A. (1985) Nutrition of penaeid prawn and shrimp. In: Taki, Primavera and Lobrera (eds) Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, Southeast Asian Fisheries: 123-130.
- ✓ Lovett D.L. and Felder D.L. (1990) Ontogenetic changes in enzyme distribution and midgut function in developmental stages of *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). Biol. Bull., 178: 160-174.
- ✓ Lovett D., Towle D. and Faris J. (1994) Salinity-sensitive alkaline phosphatase activity in gills of the blue crab, *Callinectes sapidus* Rathbun. Comp. Biochem. Phys., 109 (B): 163–173.
- ✓ Mazorra M.T., Rubio J.A. and Blasco J. (2002) Acid and alkaline phosphatase activities in the clam *Scrobicularia plana*: kinetic characteristics and effects of heavy metals. Comp. Biochem. Phys., 131 (B): 241–249.
- ✓ McCarthy S.D., Rafferty S.P., Frost P.C. (2010) Responses of alkaline phosphatase activity to phosphorus stress in *Daphnia magna*. J. Exp. Biol., 213: 256–261.
- ✓ Meyran J.C. and Craft F. (1986) Ultrahistochemical localization of Na^+K^+ ATPase, Ca^{2+} ATPase and alkaline

phosphatase activity in a calcium-transporting epithelium of a crustacean during moulting. Histochemistry, 85 (4): 313-320.

- ✓ Millán J.L. (2006) Alkaline Phosphatases. Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes. Purinerg. signal, 2: 335-341.
- ✓ Monin M.A. and Rangneker P.V. (1974) Histochemical localization of acid and alkaline phosphatases and glucose 6-Phosphatase of the hepatopancreas of the crab *Scylla serrata* (Forskål). J. Exp. Mar. Biol. Ecol., 14 (1): 1-16.
- ✓ Park Y-D., Yang Y., Chen Q-X., Lin H-N., Liu Q. and Zhou H-M. (2001) Kinetics of complexing activation by the magnesium ions on the activity of green crab (*Scylla serrata*) alkaline phosphatase. Biochem. Cell Biol., 79: 765–772.
- ✓ Pereira N.d.A. (2011) Vitamina D3 en la dieta del langostino *Pleoticus muelleri* y el camarón *Artemesia longinaris* en cultivo. Tesis de grado Licenciatura en Ciencias Biológicas, Universidad Nacional de Mar del Plata, Argentina, 52 pp.
- ✓ Petriella A.M. (1984) Estudio del ciclo de la muda del camarón *Artemesia longinaris* Bate. I. Setogénesis. Physis (Buenos Aires), 42 (103A): 93-100.
- ✓ Petriella A.M. and Fonalleras M.C. (1998) Citoarquitectura del hepatopáncreas del camarón *Artemesia longinaris* Bate (Crustacea, Decapoda, Penaeidae). Physis (Buenos Aires), 55 (128-129A): 25-30.
- ✓ Pinoni S.A., Goldemberg A.L. and López Mañanes A.A. (2005) Alkaline phosphatase activities in muscle of the euryhaline crab *Chasmagnathus granulatus:* Response to environmental salinity. J. Exp. Mar. Biol. Ecol., 326: 217-223.
- ✓ Saha S., Ray M. and Ray S. (2009) Activity of phosphatases in the hemocytes of estuarine edible mud crab, *Scylla serrata* exposed to arsenic. J. Environ. Biol., 30 (5): 655-658.
- ✓ Sarasa M.V. (2010) Vitamina K en la dieta del langostino *Pleoticus muelleri* y el camarón *Artemesia longinaris* en cultivo. Tesis de grado Licenciatura en Ciencias Biológicas, Universidad Nacional de Mar del Plata, Argentina, 50 pp.
- ✓ Sukhanova M.Z., Grenback L.G., Gruntenko N.E., Khlebodarova T.M. and Rauschenbach I.Y. (1996) Alkaline phosphatase in *Drosophila* under heat stress. J. Insect Physiol., 42 (2): 161–165.
- ✓ Shiau S.Y. and Hwang J.Y. (1994) The Dietary Requirement of Juvenile Grass Shrimp (*Penaeus monodon*) for Vitamin D. J. Nutr., 124: 2445-2450.
- ✓ Sokal. R and Rohlf J. (1979) Biometry. Freeman and Co., San Francisco, California.
- ✓ Vijayavel K. and Balasubramanian M.P. (2006) Fluctuations of biochemical constituent and marker enzymes as a consequence of naphthalene toxicity in the edible estuarine crab, *Scylla serrata*. Ecotox. Environ. Safe., 63: 141-147.
- ✓ Wojewodzic M.W., Kyle M., Elser J.J., Hessen D.O. and Andersen T. (2011) Join effect of phosphorus limitation and temperature on alkaline phosphatase activity and somatic growth in *Daphnia magna*. Oecologia, 165: 837-846.
- ✓ Zhang W., Mai K., Xu W., Tan B., Ai Q., Liufu Z., Ma H. and Wang X. (2007) Interaction between vitamins A and D on growth and metabolic responses of abalone *Haliotis discus hannai*, Ino. J. Shellfish Res., 26(1): 51-58.