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Cholinesterase (ChE) and Glutathione S-transferase (GST) Enzyme Activities of the Brackish Water Clam, *Meretrix Casta* Inhabiting Selected Estuaries in Sri Lanka

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A B S T R A C T

1. Environmental pollutants such as metals, pesticides, and other organics pose serious risks to many aquatic organisms.Bivalve are filter feeding bio accumulators that have been used as sentinel organisms in numerous monitoring programs. The activity of glutathione S-transferase (GST) and cholinesterase (ChE) was evaluated in the gills of the wild populations of *Meretrixcasta* inhabiting selected estuarine environments namely Mundalama in Mundal Lake, Marawala in Chilaw estuary, Pitipana and Pamunugama in Negombo estuary of Sri Lanka and to evaluate whether they are safe for human consumption. The clams were sampled from each site monthly during the period, September 2007 to May 2008.Therefore, the aim of this study was to investigate possible changes in two widely used biomarkers, the activity of the enzymes cholinesterases (ChE) and glutathione S-transferases (GST), of Meretrixcastafrom wild populations of the selected sites.The results showed that mean ChE activities of the gills of the clams collected from Mundalama, Marawala and Pamunugama were significantly higher than that of clams from Pitipana.When comparing the monthly variations during the study period, in months of April and May 2008, ChE activities of the gills of clams collected from Mundalama, Marawala and Pamunugama sites were higher in comparison to the ChE activities in the clams collected from the respective sites during the other sampling periods. No significant correlations were found between the ChE activities of the clams collected from the sites with individual environmental parameters, except in the site Pitipana where lead level in sediment samples was negatively correlated with the gill ChE activity of the clams. October 2007, clams collected from Pamunugama site showed the highest GST activity, but in the other months GST activity did not vary much site-wise. No significant correlations were found between the GST activities and theenvironmental parameters tested.Effects showed that gillChE and GST activities were infected by environmental factors, which indicate neurotoxic and detoxification capacities of the clams shows changing the patterns of the ChE and GST activities.Results of the present study revealed the clams in selected study sites of the three estuaries are safe for human consumption.Furthermore, the present study highlights the need of long-term monitoring with wild populations to assess effects of environmental pollution in the estuarine environmentfor sustainable exploitation of these resources as food and non-food sources.

Keywords: Brackish water Clam, Cholinesterase, Glutathione S- transferase, Physiological responses, Sri Lanka

1. Introduction

Physiological effects of environmental stress are numerous. While the ultimate end point of stress is mortality, sub-lethal stress may interfere with the normal physiological activities of the animals such as impairments in neurological functions, detoxification capacities and increased expenditure of energy

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reserves resulting in reduced growth, fecundity, and larval survival or an impaired defense system resulting in increased disease susceptibility (Volety, 2008).

In order to assess if and how organisms are affected by these environmental pollutants, several contaminant effect indicators have been developed and established during the past years (Vander Oost*et al.,* 2003).These early warning signals, or biomarkers, reflect adverse biological responses to anthropogenic environmental pollutants as well as temporal and spatial differences. They also provide information on sensitivity of organisms in terms of uptake, biotransformation and detoxification patterns. In this sense they allow an integrated measurement of bioavailable pollutants (Pfeifer *et al*., 2005).In recent years, the enzymes, cholinesterases and glutathione s-transferases have been widely used as environmental biomarkers, because they play important roles in two functions determinant for the survival and performance of organisms: neurotransmission and detoxification, respectively. In addition GSTs are also involved in anti-oxidative stress defenses (Cunha *et al*., 2007).

The cholinesterases (ChE) are a suitable biomarker for detecting environmental pollution caused by neurotoxic compounds, such as organophosphate and carbamate pesticides. ChEs are a family of enzymes, which includes acetyl cholinesterase (AChE) and Pseudo cholinesterase (PChE). AChE plays an important role in neurotransmission of both vertebrates and invertebrates, being responsible for the degradation of the neurotransmitter acetylcholine in cholinergic synapses. AChE inhibition disrupts nervous system function and may cause adverse effects on several functions including respiration, feeding and behavior. PChE degrades some xenobiotics and seems to have a protective role regarding AChE, by binding to anticholinergic agents and decreasing the amount that reach AChE, with the exception of nervous system, more than one ChE are generally present in tissues (Cunha *et al*.,2007).ChE inhibition has been measured in a variety of aquatic organisms, although the use of sessile molluscs is highly recommended within the framework of biosurveillanceprogrammes (UNEP, 1999). More recently, the responsiveness of AChEto many other chemical groups e.g. heavy metals, hydrocarbons and detergents (Zinkl*et al*., 1991; Payne *et al*., 1996; Guilhermino*et al*., 1998) and algal toxins (Lehtonen*et al*., 2003) have also been acknowledged. ChE may thus prove to be a useful biomarker for detecting general physiological stress in aquatic organisms caused by exposure to contaminants (Rank *et al*., 2007).

GST is an important enzymatic family of phase II of the biotransformation process of organic pollutants, catalyse the conjugation of reduced glutathione with a wide group of compounds bearing electrophillic centres, playing an important role in the detoxification and excretion of endogenous compounds, xenobiotics and products of oxidative stress (Moreira and Guilhermino, 2005). Since these enzymes are inducible by a wide range of chemicals, it has been suggested that the levels of GST in mussels might be useful index indicative of conjugating activities and exposure to chemical pollution (Fitzpatrick and Sheehan, 1993). According to the Fitzpatrick *et al*., (1997) the activity of GST in molluscs has been proposed as a potential organic pollution biomarker.

The aim of the present study was to assess the general health status, of wild populations of brackish water clam (*Meretrixcasta*) inhabiting selected estuarine environments in Sri Lankausing gross clinical observations and two physiological responses (AChE and GST of the gills) of the soft tissues.It was hypothesized that the general health of wild populations of the clam (*Meretrixcasta*) inhabiting the selected estuaries namely Mundal Lake, Chilaw estuary, and Negombo estuary (Pitipana and Pamunugama) is significantly affected by biological and environmental factors.

2. Materials and Methods

Sampling sites

Foursites (A, B, C and D) located in different estuarine environments in Sri Lanka were selected in the present study (Figure 1).These sampling sites contained wild populations of the clam, *Meretrixcasta*. Sampling site A is located at MundalLake in Mundalama (between longitude $79^0$49' 29.85"E and latitude 7^0 47' 19.51"N approximately). Sampling site B is located in Marawala in Chillaw estuary (between longitude $79^0$48'14.56"E and latitude 7^0 33' 11.49"N). These two sites are located in the NorthWesternProvince. The other two sampling sites (site C and site D) are located in Negombo estuary in the WesternProvince. Sampling site C is located in Pitipana area (between longitude $79^0$49'37.77"E and latitude 7^0 11' 45.71"N) and it is in the northern part of the Negombo estuary. Fourth sampling site (site D) is located in the southern part of the Negombo estuary (between $79^0$50' 50.56"E and 7^0 06'36.01"N), in the Pamunugama area. Samples of clams (*Meretrixcasta*) were obtained monthly from the study sites for nine months period starting from September 2007 to May 2008.

The four sampling sites were visited monthly for nine months from September 2007 to May 2008 and on each sampling day, physico-chemical parameters in the water were measured in situ in the sampling sites. Water pH, salinity, dissolved oxygen level, conductivity and temperature were recorded using water quality monitors (pH measured by pH meter-model PH315i, salinity measured by –Refractometer model-ATAGO S-28, dissolved oxygen measured by DO meter model-OXI315i, conductivity measured by conductivity meter-model-COND340i and temperature measured by mercury thermometer).

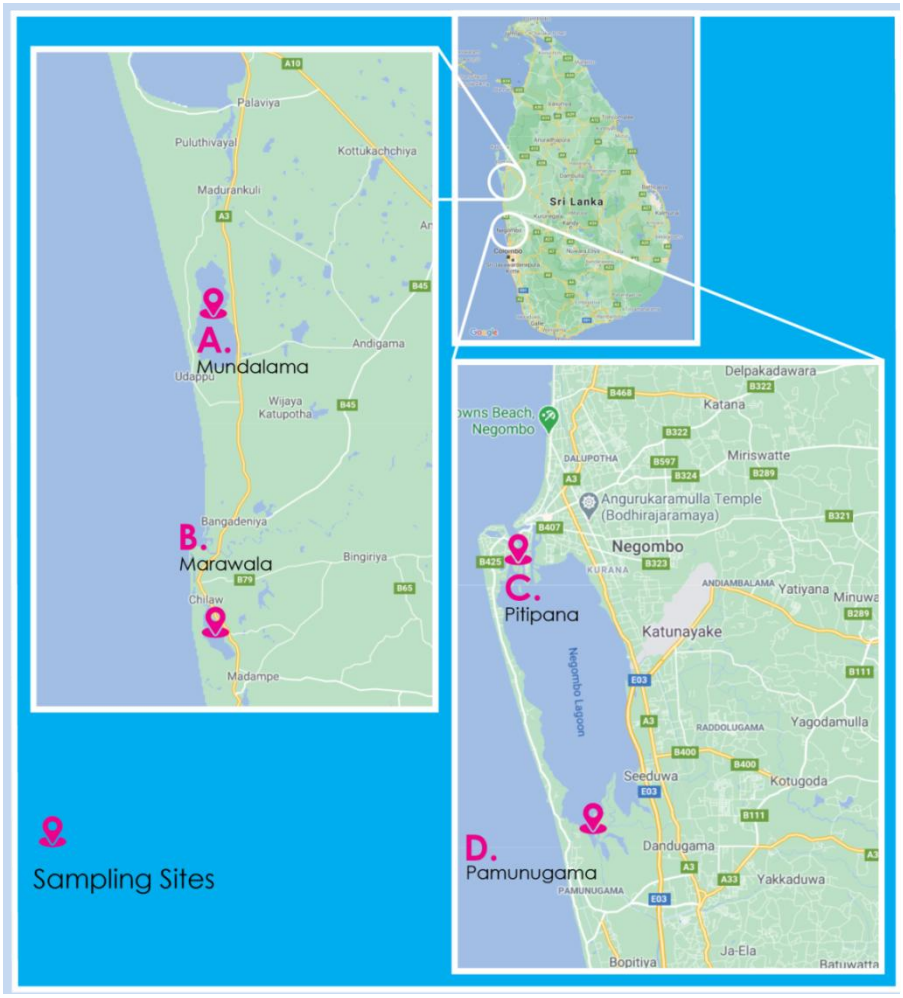

Figure 1- Location of the sampling sites A. Mundalama (MundalLake) B. Marawala (Chilaw estuary) C. Pitipana and D. Pamunugama (Negombo estuary)

Collection of clams and processing clams in the laboratory

The brackish water clams (*Meretrixcasta*) were collected from the four selected sampling sites on each visit. Thirty individuals of clams from each site were collected and transported to the laboratory in oxygenated polyethylene bags for further investigations.In the laboratory, clams were kept separately in plastic basins with the water collected from the same sampling sites, (not more than 24 hours) until further studies. The water in the basins was aerated continuously.

All the clams collected from each of the four sampling sites on each visit the shell length, shell width and whole weight of each clam were measured and their shells were opened and gross appearance of soft tissues and abnormalities (if any) in the internal side of the shell were recorded.Standard length and the width of the shell was measured using Vernier caliper before tissue removal. Then the weight of the soft tissue of each individual was recorded.Gills were removed and placed in polypropylene ependroff tubes which were placed on ice. Then the gill samples were frozen at -80 ^{0}C in an ultra low temperature freezer until further processing.

3. Determination of Enzyme Activities in the Gills of Clams

Preparation of enzyme source

Frozen gills were thawed on ice. Samples of gill tissues were homogenized in 0.1M phosphate buffer with 1% Triton, pH 8.00 (500mg gill tissue: 1ml buffer) using the Ultra- Turax T25 tissue homogenizer (IKA Labortechnik, Germany) at high speed. The homogenates were centrifuged at 10000g for 20 minutes at 4 ^{0}C (model-EBA12R HETTICH Zentrifugen). The supernatant was taken to another ependroff tube and was kept in crushed ice. All preparation steps of the enzyme source were carried out on ice.

Determination of Cholinesterase (ChE) activity in gill tissues of clams

ChE activities in the tissue homogenates were determined following the method of Ellman,*et al*. (1961)as a kinetic assay using a recording spectrophotometer (GBC Cintra 10e, Australia).20µl of supernatant was added to a cuvette containing 0.7ml of pH 8.0, 0.1M phosphate buffer. Then 25µl of 39.6mg 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) in 10ml of pH 7.0,0.1Mphosphate buffer was added to it and vortexed, The absorbance was read at 412nm until it stops increasing (for about 30 seconds).Then 20µl of (21.67mg dissolved in 2ml distilled water) acetylthiocholine iodidewas added to it and mixed. Then the change in absorbance at 412nm for 2 minutes was measured.Two samples were assayed for each supernatant.The ChE activity was calculated using the extinction coefficient of the 5-thio-2-nitrobenzoate ion (13600 M/ cm). ChE activity was expressed asnmoles /minute/ mg protein.

Determination of Glutathione S-transferase (GST) activity in gill tissues of clams

GST activity was measured in the gill tissues following the conjugation of glutathione at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate (Habig*et al*., 1974).Two ml 0.1M phosphate buffer (pH=6.75) was added to the tubes, containing 100µl of CDNB and the tubes were incubated in a water bath at 25 ^{0}C. Then 50µl of thawed supernatant were added to each tube and the mixture was vortexed. The absorbance of the mixture was recorded at 340nm for 20 seconds, and then 200µl of glutathione was added and again change in absorbance at 340nm measured for 2 minutes. The enzyme activity was expressed as nmoles /minute/mg protein.

Determination of protein content in the enzyme source

Protein content in 10000g gill supernatant was determined according to the method of Lowry *et al* (1951)with bovine serum albumin (BSA) as the standard. A 10 µL of the supernatant was added to a test tube with 490 µL of distilled water. 0.5ml of Lowry reagent (Sigma –Aldrich, USA) was added to the homogenate. The solution (500 µL) was mixed and left for 20 minutes at room temperature. Then 250 µL of Folin reagent (Sigma-Aldrich, USA) was added to the tube, mixed well and left for 30 minutes for colour development. The absorbance was read at 750 nm against the blank which contained all the chemical except the homogenate. A series of standard protein concentrations (0, 30, 60, 90, 120, 150 and 180 µg / ml) was prepared by using BSA and different volumes of distilled water. Final volume of the protein solution was 500 µL. To the standard protein solutions, Lowry reagent and Folin reagent were added as described above and the absorbance was measured. The protein content of the supernatant was determined using the standard curve made by known protein standards.

4. Results

Assessment of physiological responses using enzyme biomarkers: Cholinesterase (ChE) and Glutathione S transferase (GST) activities in the gill tissue of the clams

ChE activity

Table - 1Cholinesterase (ChE) activities in the gillsof*Meretrixcasta* sampled from four sites during nine months period

Sampling period	Enzyme activity (nmoles/min/mg protein)			
	Site A Mundalama	**Site B Marawala**	**Site C Pitipana**	**Site D Pamunugama**
Sep 2007	----	12.5 ± 2.42^{bc1}	14.22 ± 1.31^{c1}	3.67 ± 1.92^{a2}
Oct 2007	----	5.62 ± 1.64^{a1}	4.85 ± 2.40^{b1}	11.39 ± 2.56^{f2}
Nov 2007	4.79 ± 1.12^{a1}	5.17 ± 0.69^{a1}	1.78 ± 0.63^{a2}	6.13 ± 1.98^{b1}
Dec 2007	6.39 ± 1.24^{a1}	6.49 ± 1.28^{a1}	0.97 ± 0.15^{a2}	----
Jan 2008	3.90 ± 0.98^{a1}	2.57 ± 0.75^{a2}	0.96 ± 0.16^{a3}	2.76 ± 0.83^{a2}
Feb 2008	7.58 ± 2.23^{a12}	9.42 ± 1.64^{b1}	5.67 ± 1.85^{b2}	7.60 ± 0.73^{b12}
Mar 2008	5.53 ± 2.08^{a1}	4.29 ± 0.55^{a12}	1.95 ± 0.21^{a2}	5.62 ± 2.22^{c1}
Apr 2008	19.47 ± 3.32^{b2}	14.88 ± 3.70^{c3}	6.26 ± 1.25^{b4}	25.55 ± 0.39^{d1}
May 2008	30.82 ± 7.96^{c1}	15.84 ± 2.81^{c2}	7.58 ± 1.58^{b3}	21.62 ± 0.64^{e2}
Overall	11.21 ± 10.13^{1}	8.53 ± 4.84^{1}	4.92 ± 4.27^{2}	10.55 ± 8.52^{1}

*Data are presented as Mean ± SD values. In the columns data indicated with different superscript letters are significantly different from each other. In the rows, data indicated with different superscript numbers are significantly different from each other (ANOVA, Fisher's test,P<0.05). (In September 2007 at Mundalama and in December 2007 at Pamunugama clams were not sampled. In October 2007 at Mundalama replicates were not adequate for data analysis).

Cholinesterase activities in the gilltissue of the clamscollected fromfour sites are given in the Table1and Figure 2. The results showed that mean ChE activities of the gills of the clams collected from Mundalama, Marawala and Pamunugama were significantly higher than that of clams from Pitipana. When comparing the monthly variations during the study period, in months of April and May 2008, ChE activities of the gills of clams collected from Mundalama, Marawala and Pamunugama sites were higher in comparison to the ChE activities in the clams collected from the respective sites during the other sampling periods. Except in September, gill ChE activity in the clam collected from Pitipana was the lowest during the study period (Table 1, Figure2).

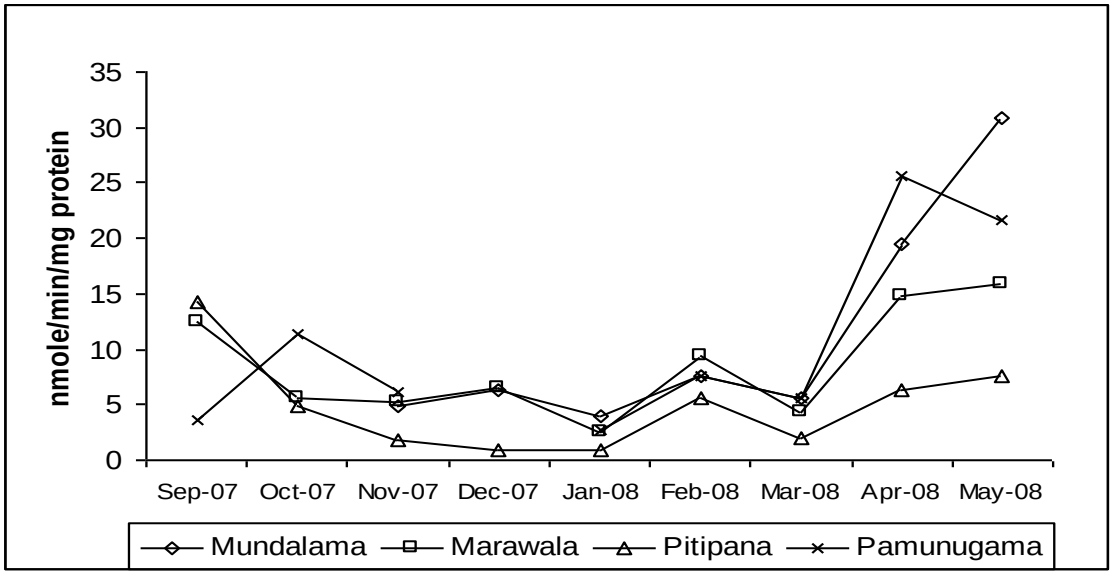

Figure 2 Temporal variationin mean gillChE activity of *Meretrixcasta* sampled from four sites during nine months period. In September 2007 at Mundalamaand in December 2007 at Pamunugama clams were not sampled.

Correlations between ChE activity and the environmental parameters are presented in Table2. No significant correlations were found between the ChE activities of the clams collected from the sites with individual environmental parameters,except in the site Pitipana where lead level in sediment samples was negatively correlated with the gill ChE activity of the clams.

Table 2Correlations (r) between ChEactivity in the gill tissue of the clams and environmental parameters

parameter	Mundalama Site A	Marawala Site B	Pitipana Site C	Pamunugama Site D
Rainfall	-0.512	-0.448	0.104	0.254
Conductivity	-0.732	-0.053	0.091	-0.113
Salinity	-0.643	-0.068	0.007	-0.664
DO	-0.413	-0.492	-0.255	-0.546
Temperature	-0.236	0.256	0.579	-0.509
pH	-0.094	-0.032	0.008	-0.264
Pb in water	-0.570	0.344	-0.259	-0.101
Pbin sediment	-0.165	0.163	**-0.978***	0.355
Cu in water	-0.125	0.247	-0.515	0.409
Cu in sediment	0.137	0.284	-0.316	0.395
Cd in water	0.534	-0.205	0.778	-0.318
Cd in sediment	-0.189	-0.138	-0.302	0.588

* Significant atP< 0.05 (Pearson's correlation test) n = 8-9

GST Activity

The GST activities in the gills of clams collected from four sites during the nine months period varied widely (Table 3 andFigure 3). During the month of October 2007, clams collected from Pamunugama site showed the highest GST activity than those of the other three sites. But in the other months GST activity did not vary much site-wise. In each site the lowest activities were observed during the period November 2007 to January 2008.

The overall means of the pooled GST activities in the gills of clams collected from the four sites were not significantly different from each other (Table 3).In the month of February, GST activities in the clams collected from all four sites were significantly higher than those in other months.

Table 3 Glutathione S transferase activity in the gills of *Meretrixcasta* sampled from four sites during study period

Sampling period	Enzyme activity (nmole/min/mg protein)			
	Site A Mundalama	**Site B Marawala**	**Site C Pitipana**	**Site D Pamunugama**
Sep 2007	-----	401.2 ± 227.70^{c1}	342.59 ± 94.90^{c1}	226.63 ± 63.2^{a1}
Oct 2007	83.34 ± 21.47^{a1}	185.88 ± 23.63^{a2}	316.18 ± 56.96^{c3}	708.77 ± 9.96^{cd4}
Nov 2007	55.4 ± 10.89^{a1}	35.34 ± 9.02^{a2}	30.72 ± 10.923^{a2}	29.55 ± 6.20^{a2}
Dec 2007	50.22 ± 22.19^{a1}	36.82 ± 7.38^{a1}	114.84 ± 94.58^{a2}	-----
Jan 2008	105.63 ± 27.71^{a1}	86.56 ± 24.08^{a1}	115.3 ± 28.88^{a1}	117.82 ± 8.80^{a1}
Feb 2008	680.01 ± 233.50^{b1}	625.67 ± 115.0^{b1}	991.5 ± 73.30^{b2}	963.42 ± 260.30^{b12}
Mar 2008	404.73 ± 74.13^{c1}	442.65 ± 37.24^{c1}	345.12 ± 67.19^{c2}	418.49 ± 49.10^{c12}
Apr 2008	333.43 ± 15.55^{c1}	349.54 ± 33.11^{c1}	353.76 ± 65.81^{c1}	476.29 ± 31.90^{c2}
May 2008	834.22 ± 102.52^{b1}	608.61 ± 62.08^{b2}	566.63 ± 91.00^{d2}	552.23 ± 83.46^{c2}
Overall	318.40 ± 303.80^{1}	308.10 ± 232.30^{1}	353.00 ± 289.30^{1}	436.70 ± 311.30^{1}

*Data are presented as Mean ± SD values. In the columns, data indicated with different superscript letters are significantly different from each other, In the rows, data indicated with different superscript numbers are significantly different from each other (ANOVA, Fisher's test,$P<0.05$). In September 2007 at Mundalama and in December 2007 at Pamunugama clams were not sampled.

GST activities of the clam collected from all four sites were correlated with the environmental parameters (Table 4). However, no significant correlations were found between the GST activities and theenvironmental parameters tested.

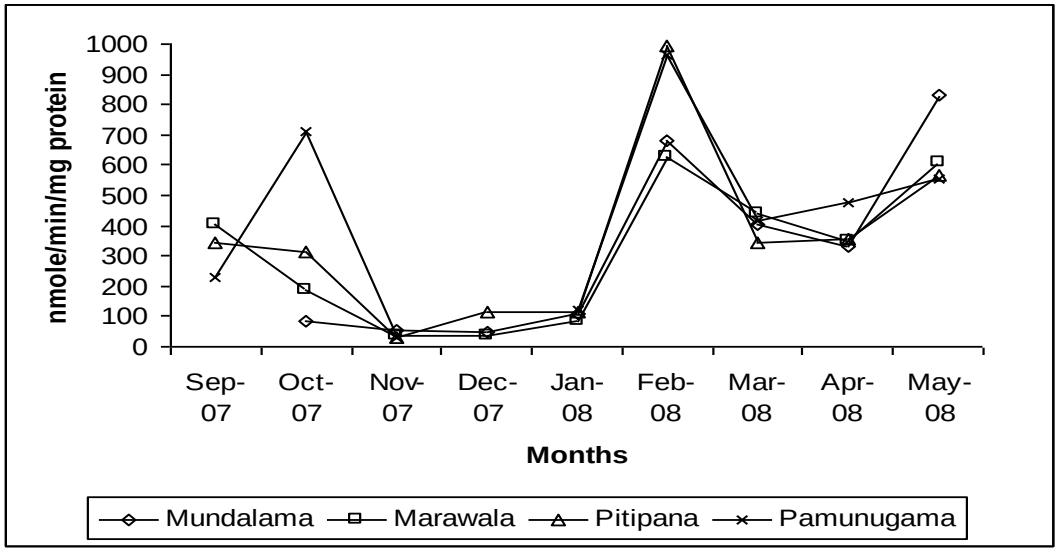

Figure 3Temporal variations in GST activity of *Meretrixcasta* collected from four study sites during nine months period. In September 2007 at Mundalama and in December 2007 at Pamunugama clams were not sampled.

Table 4 Correlations(r) between GST activity in the gill tissue of the clams and environmentalparameters

parameter	Mundalama Site A	Marawala Site B	Pitipana Site C	Pamunugama Site D
Rainfall	-0.519	-0.364	0.027	0.320
Conductivity	-0.282	-0.068	0.193	-0.124
Salinity	-0.116	0.085	0.121	-0.202
DO	-0.469	-0.427	-0.444	-0.328
Temperature	-0.230	0.122	-0.255	-0.348
pH	0.367	-0.192	-0.190	-0.432
Pb in water	-0.121	0.162	-0.142	0.161
Pbin sediment	-0.237	-0.255	0.143	0.128
Cu in water	0.077	-0.141	0.346	0.336
Cu in sediment	0.424	-0.155	0.421	-0.146
Cd in water	0.042	-0.253	0.602	0.576
Cd in sediment	0.148	-0.062	0.323	-0.057

Data are not significant (P>0.05)

5. Discussion

Exposure to environmental stressors can result in biochemical, physiological and histological (tissue) alterations in living organisms. Presence of these alterations may serve as signaling exposure to stressors or adverse effects; in the aquatic environment such stressors can constitute changes in physical parameters such as temperature, pH, salinity, conductivity, dissolved oxygen and rainfall, as well as toxic concentrations of chemical pollutants or any combination of these (Werner *et al* .,2003).Analyses of biomarkers in these bivalves have been also incorporated into biomonitoring studies to evaluate the effects of pollutants in areas contaminated with heavy metals, domestic sewage, pesticide residues and organic compounds. ChE and GST activities of different species have been used in several studies as biomarkers in biomonitoring studies carried on the estuaries and coastal areas of the NW coast of Portugal and a good base-line knowledge of the fluctuations of their activities in relation to abiotic changes and space already exists (e.g. Moreira et al., 2004; Cunha et al., 2005; Moreira and Guilhermino, 2005; Lima et al., 2007; Guimarães et al., 2009).Differences in some enzymatic biomarkers were found in gills *Meretrixcasta* in the selected sites.During the experiment, the selected sites were monitored for pollution using heavy metal assessments (e.g. water, soil and tissues of the clams, data not shown) and the analysis of trace elements in water, in which we recorded very low levels.

In this study temporal variations in general water quality parameters in selected sampling sites in three estuaries namely Mundalama in Mundal Lake, Marawala in Chilaw estuary, Pitipana and Pamunugama in Negombo estuary and rainfall pattern in the area was measured for nine months period starting from September 2007 to May 2008. In each site pH, water temperature in water did not vary much in the study period, but water pH level was comparatively low at the Pamunugama site. The salinity level, conductivity level and dissolved oxygen level varied widely during the study period. In the Mundalama site and Pitipana, salinity was higher than the other sites. These two sites Mundalama and Pitipana are closer to the sea area than the other two sites; Marawala and Pamunugama. This could be the reason for higher salinity level and conductivity level in these areas. Lowest level of salinity and conductivity were reported in the freshwater end of the Negombo estuary, Pamunugama. Marawala site also had lower salinity and conductivity levels due it location further to the sea.

Overall highest rainfall pattern was reported in the Negombo estuary area and Lower rainfall was observed in the Marawala site which is located at Chilaw estuary. Jayasiri and Rajapaksha (2000) showed that the salinity of Mundal lake depends on the flushing through the Dutch canal and on local evaporation, precipitation and runoff during periods of limited exchange of water, the MundalLake is likely to become strongly hypersaline. In this study also, the highest level of salinity was recorded in the site of Mundalama.

ChE and GST enzymes have been extensively employed as biomarkers in coastal monitoring programs, but the prediction of baseline levels at reference sites is difficult, and the possible alteration by factors other than pollution (e.g. environmental and biological) has been a matter of concern in field studies (Najimi et al., 1997; Bend and James, 1978; Fisher et al., 2000).

Cholinesterase (ChE) was inhibited in the clams on exposure to all pesticides (Devi, 2005). According to Hamza-Chaffai*et al*., (1998) clams when exposed to Xenobiotic compounds their ChE activity was inhibited. Inhibition of ChE activity by heavy metals was also suggested to explain the reduction of enzyme activity (Matozzo*et al*., 2005). Reduction of enzyme activity in the study sites may be related to high contamination levels, particularly in sediments and water. Inhibitions of ChE activity by neurotoxic substances such as Cd, Cu and Pb, organophosphorous, carbamate pesticides polyaromatic hydrocarbons have been well established (Matozzo*et al*., 2005). Highest ChE activity values indicate a lower level of contamination of environment by Organophosphorus compounds, carbamates and heavy metals (Dellali*et al*., 2001). ChE activity in gill tissues of clams collected from four study sites indicate that the ChE activity of clams collected from Mundalama, Marawala and Pamunugama were significantly higher than the clams from Pitipana site. Results indicate the occurrence of anticholinesterase contaminant in Pitipana site. Inhibition of ChE could affect neurological function of the clams.

Detoxification enzyme systems such as GST are useful in the animal response to toxins and xenobiotics because they are usually induced by the presence of these toxicants in the environment (Vasconcelos*et al*., 2007). GST inhibition may have occurred either through direct action of the metal on the enzyme. Metal accumulation in the cells can results in decreased availability of GSH (Glutathione) due to both binding and oxidation (Cunha *et al*., 2007). GST activity in the gills of the clams collected from four sites varied widely. During the month of October 2007, Pamunugama site GST activity was higher than the other sites. This indicates the clams have been exposed to higher organic pollutant loading during the study period.Dellali*et al*., 2001 found decrease in ChE activity as an indication of metal pollution. ChE activity correlated with the amount of lead in the sediments and due to that factor inhibition of ChE activity could be occurred.

6. Conclusions and Recommendations

In conclusion the use of biomarker responses in bivalves is sometimes controversial due to the variability in some enzyme activities related to gonad maturation stages, age, temperature and feeding stages of the specimens, making it difficult to clearly establish relationships between contaminants and biomarker changes (Najimi et al., 1997; Bend and James, 1978; Sheehan and Power, 1999; Fisher et al., 2000). The present study revealed GST and ChE activities of clam Meretrixcastawere found to be influenced by abiotic factors, mainly salinity and conductivity. The clams in selected study sites of the three estuaries are safe for human consumption in relation to the bio indicators, which measures the overall health; gill ChE and GST activities which indicate neurotoxic and detoxification capacities of the clams. Further studies on health aspects of bivalve resources in Sri Lanka are needed for sustainable exploitation of these resources as food and non-food sources.

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