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Studies on Seasonal Variations of Total Glycogen, Protein and Lipids in Estuarine Clam, *Meretrix Meretrix* (Linnaeus) after Chronic Exposure to Cadmium Chloride

Sanjay Kumbhar

Department of Zoology, Fergusson College (Autonomous), Pune, Maharashtra, India

ABSTRACT

In the present investigation, estuarine clam, *M.meretrix* was employed to study effect of cadmium chloride stress on biochemical components of different soft tissues. During summer, monsoon and winter clams were subjected to chronic exposure (96 hours) to predetermined sub lethal concentrations of cadmium chloride. During the experimental period water samples were analyzed for temperature, pH, salinity, dissolved oxygen and cadmium content. It was observed that rainfall, salinity, oxygen saturation and temperature of estuarine water has profound effect on seasonal changes in biochemical content of *M.meretrix*. During summer cadmium content in estuarine water was below detectable level, while in monsoon and winter it was 0.005 and 0.001ug/ml respectively. Clams from control group showed low glycogen content in gills and high in gonad and foot . Clams from chronic sub lethal concentration showed increase in glycogen content in gill in monsoon, while hepatopancreas showed significant increase during winter. Clams from control and chronic group showed higher protein content in gonad and foot for three different seasons. In chronic group, cadmium induced toxicity caused significant depletion in gonad and foot, while gill and hepatopancreas showed significant elevation. Chronic exposure of cadmium chloride depleted lipids in all soft tissues, but depletion was significant ($p<0.001$) in hepatopancreas and mantle. Hepatopancreas showed 30.85 and 28.55% decrease in monsoon and winter respectively. During summer, mantle showed (39.77%) considerable depletion than hepatopancreas. In the present study, it was found that, alterations in total glycogen, proteins and lipids from control group were attributed to highly labile estuarine water parameters and breeding season of clams, while, alterations in clams from chronic group are moreover attributed to cadmium chloride stress.

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KEYWORDS: *Meretrix meretrix, Bhatye estuary, total glycogen, Proteins, Lipids, Chronic toxicity*

INTRODUCTION

Studies of the seasonal alterations in different biochemical components and enzymes in marine pelecypods have been studied extensively (Rao, K.V. 1969[1] Nagbhushnam and Mane, 1982[2]; Peter and Albert, 1984[3]; K.K. Appukuttan and C.M. Arvindan, 1995[4]; Patil, S.S. and U.H. Mane, 1997[5]; Tendulkar S. P. and B.G.Kulkarni,1998[6]), no study to date, however, has compared heavy metal induced seasonal changes in total glycogen, proteins and lipids in gill, mantle, hepatopancreas, foot and gonads of marine bivalves. These soft tissues and organs are locationaly, structurally and functionally different and hence their energy requirement, biochemical composition and physiological processes are different. Zandee and Klutman (1971)[7] first time recorded seasonal changes in biochemical composition in *Mytilis eudilis.* Of all these studies, impact of cadmium on marine biota is least studied subject .Geochemistry of cadmium has been discussed by Eaton (1979)[8] it is well established that although concentration of cadmium in surface water is negligible it increases many fold in sediments and still higher in marine biota. Recent understanding of biochemical processes has proved useful in determining the mechanism of toxicity. Sanjay Kumbhar (2001)[9] studied accumulation of cadmium in different soft tissues of *M.meretrix* and observed great varience in concentration of cadmium in different soft tissues during summer, monsoon and winter. There must be metabolic strategies to utilize or sequester these metals depending upon the toxic potential and it may be operated by transport, conversion and utilization of biochemical components like glycogen, proteins and lipids. The present work is an attempt to correlate seasonal estuarine water parameters and biochemical constituents of soft tissues like gill, mantle, hepatopancreas, foot and gonads under cadmium chloride stress.

Material and Methods:

Physio-chemical parameters like temperature, salinity, pH and dissolved oxygen were determined for each season. Temperature of the aerated estuarine water used for holding the clams during experimentation was recorded at regular intervals. Salinity was recorded with the help of hand salinometer (Hanna instruments, Italy). pH of the estuarine water was measured by digital pH meter. Dissolved oxygen was measured by Winkler's titrimetric method. Cadmium in estuarine water was analyzed on Atomic Adsorption

Spectrophotometer (Perkin Elmer Model 3030.USA). Clams of medium size measuring 4.0-4.8cms were selected for experiment. These clams were exposed to predetermined sub lethal concentrations of cadmium chloride. After chronic exposure, various tissues (gill, mantle, hepatopancreas, foot and gonad of live clams were removed from the control and experimental groups in different seasons. Pooled tissues were weighed and dried in an oven at 100°C (+ 5°C) until a constant weight was obtained. Oven dried tissue powder was used for biochemical analyzed for total glycogen (De Zwan and Zandee, 1972), proteins (Gornal et al, 1949) and lipids (Barnes and Blackstock, 1973).

Result and discussion:

Literature survey revealed that, the independent variables like tides, river discharge, density, differences between water masses in the estuary caused by differences in salinity and temperature makes estuarine hydronomics very complex. Quasim,(1980) [12] reported that, magnitude of variations in the environment depends on a large extent on the time of the year and the place of the observation In the present study it was decided to focus on few estuarine parameters and unveil their probable relationship in intensifying or decreasing toxicity of cadmium chloride. It was observed that, average water temperature was minimum in winter (25.7 ^{0}c). The maximum average water temperature was recorded in summer (26.5^{0}c). Maximum average salinity was recorded in summer (35.1). It was 30.36mg/l in winter. Minimum average salinity was recorded in monsoon season (5.2mg/l). In the present study, it was observed that, salinity changes according to season. Differences in seasonal variations in salinity were due rainfall, heavy influx of fresh water and temperature, pH fluctuations was observed in monsoon than summer and winter. The average pH was 8.2, 7.1 and 8.3 in summer, monsoon and winter respectively. The average dissolved oxygen was 3.9, 5.6 and 4.7 ml/l in summer, monsoon and winter respectively. During monsoon estuarine water showed maximum saturation of oxygen (5.6ml/l). It was minimum (3.9ml/l) in months of summer. Average pH during summer, monsoon and winter was 8.2, 7.1 and 8.3 respectively. Variations in pH did not show significant pattern, however, it was low in monsoon due to heavy influx of fresh water. Similar observations were recorded by Chandran and Ramamorthy (1984) [14] while studying hydronomics of Vellar estuary. The average rainfall during monsoon was 29.15mm. During winter scanty rainfall was recorded. Summer received no rainfall. In tropical countries rainfall is regular cyclic phenomenon. It brings profound changes in hydronomics of the estuarine environment (Quasim, 1980) [13]. Heavy rainfall in monsoon reduced salinity, water temperature, pH of estuarine water and increases turbidity and oxygen saturation. The hydronomics of an estuary in general is very complex. It was decided to analyze preexistence of cadmium before conducting chronic static bioassay. It was observed that, during summer, cadmium concentration in water was below detectable level. In monsoon and winter average concentration of cadmium was 0.005 and 0.001 µg/l respectively. Maximum accumulation of cadmium was observed in monsoon. Unfortunately data is not available stating cadmium concentration in Bhatye estuary. At Ratnagiri, heavy rainfall was recorded in monsoon (34.39 mm/day).Rainy freshets brought heavy load of soil that impregnated with heavy metals like cadmium. It resulted in increase in concentration of cadmium in monsoon. It also showed rapid loss of cadmium during summer and winter. Fowler and Oregioni (1976) [15] suggested that seasonal maximum concentration appeared in the spring was due to high water run-off which increased the amount of available metals. Philips (1973) [16] proposed similar conclusion with zinc, cadmium, lead and copper. In the present investigation concentration of cadmium in estuarine water was considered while quantifying sub lethal and lethal concentrations of cadmium chloride.

Table1. Cadmium chloride induced alterations in total glycogen content of *M.meretrix* after chronic exposure (Results are expressed in mg/100mg. dry wt.basis).

TISSUE	SEASON	CONTROL	CHRONIC GROUP
GILL	SUMMER	6.872 ± 0.071	6.039 ± 0.052 (12.12)
	MONSOON	4.045 ± 0.106	5.455 ± 0.081 (34.85)* *
	WINTER	8.121 ± 0.046	6.247 ± 0.162 (23.07)* * *
MANTLE	SUMMER	14.785 ± 0.089	19.367 ± 0.229 (30.99)* * *
	MONSOON	15.410 ± 0.025	18.950 ± 0.158 (22.97)* * *
	WINTER	15.202 ± 0.096	19.792 ± 0.624 (30.19)* * *
FOOT	SUMMER	42.063 ± 0.012	26.239 ± 0.123 (37.61)
	MONSOON	4.145 ± 0.328	24.365 ± 0.095 (44.80)* *
	WINTER	52.062 ± 0.061	29.155 ± 0.012 (43.99)* * *
GONAD	SUMMER	38.802 ± 0.920	31.091 ± 1.024 (19.87)*
	MONSOON	29.969 ± 1.041	26.117 ± 0.162 (12.85)
	WINTER	32.015 ± 1.134	27.980 ± 0.524 (12.60)

Values in parenthesis are percent change, ± =S.D. of five animals.
(*=p<0.05; **=p<0.01; ***=p<0.001).

The studies on biochemical changes enable to define the dose response relationship, threshold limit value, revercible and irrevercible nature of pollutant effect. In the present study significant alterations in total glycogen, proteins and lipids were observed both in control and chronic group of clams. In summer, the glycogen content in the control group showed ascending order of gill< hepatopancreas< mantle< gonad< foot, with 6.872 ± 0.071, 13.536 ± 1.083, 14.785 ± 0.089, 29.155 ± 0.015, 38.802 ± 0.920 mg/100 mg dry tissue respectively. In chronic group, glycogen content was present in increasing order of gill< hepatopancreas< mantle< foot< gonad, with 6.036 ± 0.052, 12.286 ± 0.864, 19.367 ± 0.229, and 31.091 ± 1.024 mg/100mg dry tissue respectively. As compared to controls, there was 30.99% (p<0.001) increase in mantle, while 9.23 (P<0.05), 37.61(p<0.001) and 19.87% (P<0.05) significant decrease in hepatopancreas, foot and gonad respectively During monsoon, control group showed glycogen content in ascending order of gill< hepatopancreas< mantle< gonad< foot, with 4.045 ± 0.106, 14.369 ± 0.427, 15.410 ± 0.025, 29.969 ± 1.041, and 44.145 ± 0.328 mg/100mg dry tissue respectively. In chronic group, glycogen content was low in gill, followed by hepatopancreas, mantle, foot and gonad, with 5.455 ± 0.081, 12.078 ± 0.080, 18.950 ± 0.158, 24.365 ± 0.095 and 26.117 ± 0.162 mg/100mg dry tissue respectively. Based on percent change, there was 34.85 (p<0.001) and 22.97 % (p<0.001) increase in gill and mantle, while 15.94 (P<0.05), 20.47 (p<0.001), 44.80% (p<0.001) significant decrease in hepatopancreas and foot respectively. During winter, control group exhibited high glycogen content in gill, followed by hepatopancreas, mantle, gonad, foot, with 8.121 ± 0046, 14.216 ± 0.035, 37.485 ± 1.050 and 52.062 ± 0.061 mg/ 100mg dry tissue respectively. In chronic group, glycogen content was present in ascending order of gill< hepatopancreas< mantle< gonad<foot, with 6.247 ± 0.162, 17.662 ± 0.0061, 20.616 ± 1.603, 27.980 ± 0.521 and 29.155 ± 0.012 mg in 100mg dry tissue. As compared to control group, there was 24.24 and 30.19 % significant (p< 0.001) increase in hepatopancreas and mantle while 23.07 and 43.99 % significant (p<0.001) decrease in gill, and foot respectively Clams from control group showed less glycogen content in gills and high glycogen content in gonad and foot .Glycogen content was at its peak during gametogenesis and it was low during the spawning period. Similar observations were noted by Nagbhushnam and Talikhedkar (1977)[17] in wedge clam, *Danus cunaeutus*. The result obtained are in good agreement with earlier workers (Masumoto and Hibino,1973[18] ;Dhavale and Masurekar,1985[19] ;George and Mathews,1996[20]) Clams those were subjected to chronic sub lethal concentration showed increase in glycogen content in gill in monsoon, while hepatopancreas showed significant increase during winter. Hepatopancreas and gills are more modified in their biochemical composition than muscles and hence accumulate higher level of cadmium and to reduce toxicity they require more glycogen. Similar observation was noted by Diaz Mayans et al (1986) [21]. In rest of soft tissues there was significant decrease in glycogen content. Decline in glycogen content was may be due to stress resulting in breakdown of tissue glycogen to meet energy demand due to toxic stress of cadmium chloride.

Table 2: Cadmium chloride induced alterations in total protein content of *M.meretrix* after chronic exposure (Results are expressed in mg/100mg. dry wt.basis).

TISSUE	SEASON	CONTROL	CHRONIC GROUP
GILL	SUMMER	10.620 ± 1.013	11.427 ± 0.907 (7.49)
	MONSOON	12.206 ± 0.077	16.524 ± 0.012 (35.37)* *
	WINTER	10.376 ± 0.520	16.600 ± 0.039 (59.93)* * *
MANTLE	SUMMER	20.400 ± 1.047	17.942 ± 0.213 (-12.04)* * *
	MONSOON	21.025 ± 0.093	20.102 ± 1.054 (-4.39)*
	WINTER	23.552 ± 0.034	20.763 ± 0.615 (-11.84)* *
HEPATOPANCREAS	SUMMER	6.526 ± 1.022	7.902 ± 0.091 (21.85)
	MONSOON	7.102 ± 0.316	10.022 ± 0.097 (41.11)* *
	WINTER	7.230 ± 1.069	7.500 ± 0.016 (3.73)
FOOT	SUMMER	25.059 ± 0.085	21.709 ± 0.007 (13.33)
	MONSOON	27.500 ± 0.104	22.406 ± 0.025(18.52)* *
	WINTER	27.560 ± 0.458	22.970 ± 0.044 (16.65)* * *
GONAD	SUMMER	27.521 ± 0.015	21.164 ± 0.033 (23.09)***
	MONSOON	23.746 ± 0.705	16.047 ± 1.091 (32.42)**
	WINTER	24.854 ± 1.802	20.803 ± 0.196(16.29)

Values in parenthesis are percent change, ± =S.D. of five animals.
(*=p<0.05; **=p<0.01; ***=p<0.001)

Proteins play vital role in the spawning and other metabolic activities. Alteration in protein content after chronic exposure to cadmium chloride are shown in Table 2. During summer, control group showed protein content in different organs, in increasing order of hepatopancreas < gill< mantle< foot< gonad with 6.526 ± 1.022, 10.620 ± 1.013, 20.400 ± 1.047.8, 25.059 ± 0.085 and Chronic (0.18 ppm) group, exhibited low protein content in hepatopancreas, followed by gill, mantle, gonads, foot; with 7.902 ± 0.091, 11.427 ± 0.907, 17.942 ± 0.051, 21.164 ± 0.033 and 21.709 ± 0.015 mg/ 100mg dry tissue in respective organs. As compared to control group, there was 12.04, 23.09 and 13.33 % significant (p<0.001) decrease in mantle, gonad and foot respectively .In monsoon, control group exhibited protein content in increasing order of, hepatopancreas< gill<mantle < foot < gonad, with 7.102 ± 0.316, 12.206 ± 0.077, 21.025 ± 0.093, 23.746 ± 0.705 and 38.640 ± 0.070mg proteins in 100mg dry tissue respectively. Chronic (0.20 ppm) group, showed protein content in the ascending order of hepatopancreas < gill< mantle< gonad< foot, with 10.022 ± 0.097, 16.047 ± 1.091, 19.944 ± 0.074, 20.102 ± 1.054 and 22.406 ± 0.025 mg/ 100 mg dry tissue in respective organs. As compared to control, there was 41.11 (p<0.01), 35.37% (p<0.001) increase in hepatopancreas and gill and 32.42 (p<0.01), 18.52% (p<0.001) increase in gonad and foot respectively. During winter, control group exhibited protein content in ascending order of hepatopancreas < gill< mantle< female gonad< foot, with 7.230 ± 1.069, 10.376 ± 0.520, 23.552 ± 0.034, 24.854 ± 1.802, 27.560 + 0.458mg proteins in 100mg dry tissue respectively. Chronic (0.21 ppm) ground, showed proteins in ascending order of hepatopancreass< gill< mantle< female gonad< foot, exhibiting 7.500 ± 0.016, 16.600 ± 0.039, 003, 20.763 ± 0.615, 20.803 ± 0.196, 22.970 + 0.044mg proteins in dry tissue. As compared to control, there was 59.98% significant (p<0.001) increase in gill, while 11.84 (p<0.01), and 16.65% (p<0.01) decrease in protein content in mantle and foot respectively. Clams from control group showed marked variations which were attributed to season. During winter protein content was better in most of the soft tissues. It may be due to abundance of food material, increased shell valve opening period and less energy expenditure. Protein content was low in summer and monsoon. It may be due to utilization of proteins for spawning Mane,U.H et al (1987)[22] observed that spawning period of *K.opima* and *M. meretrix* from Kalbadevi estuary was in months of monsoon and summer. Durve and Bal (1961) [23] analyzed *Crossostrea gryphoides* and reported that low protein values coinsides with spawning season. Clams from chronic group showed significant depletion during three different seasons. Marked depletion was observed in gonad and foot, while gill and hepatopancreas showed considerable elevation in protein level. It may be due to mobilization of proteins from depositories and conversion in to glycogen. Gills and hepatopancreas were primary target organs for cadmium chloride stress. These tissues and organs might have utilized and demanded more glycogen to reduce cadmium chloride stress.

Table 3: Cadmium chloride induced alterations in total lipid content of *M.meretrix* after chronic exposure (Results are expressed in mg/100mg. dry wt. basis).

TISSUE	SEASON	CONTROL	CHRONIC GROUP
GILL	SUMMER	5.500 ± 1.008	4.330 ± 0.203 (21.27)
	MONSOON	6.102 ± 0.065	5.702 ± 0.041 (6.55)* *
	WINTER	6.795 ± 0.092	5.967 ± 0.396 (12.18)* * *
MANTLE	SUMMER	5.187 ± 0.086	3.124 ± 0.008 (39.77)* * *
	MONSOON	4.715 ± 1.013	4.225 ± 0.013 (10.39)* * *
	WINTER	4.870 ± 0.318	4.020 ± 0.015 (17.45)* * *
HEPATOPANCREAS	SUMMER	5.127 ± 0.205	4.925 ± 0.142 (3.93)*
	MONSOON	6.080 ± 0.516	4.204 ± 0.057 (30.85)*
	WINTER	4.970 ± 1.061	3.551 ± 0.016 (28.55)* * *
FOOT	SUMMER	42.063 ± 0.012	26.239 ± 0.123 (37.61)
	MONSOON	44.145 ± 0.328	24.365 ± 0.095 (44.80)* *
	WINTER	52.062 ± 0.061	29.155 ± 0.012 (43.99)* * *
GONAD	SUMMER	38.802 ± 0.920	31.091 ± 1.024 (19.87)*
	MONSOON	29.969 ± 1.041	26.117 ± 0.162 (12.85)
	WINTER	32.015 ± 1.134	27.980 ± 0.524 (12.60)

Values in parenthesis are percent change, ± =S.D. of five animals.
(*=p<0.05; **=p<0.01; ***=p<0.001).

Alterations in lipid content after chronic exposure to cadmium are shown in Table 3. In summer, control group of clams showed variations in lipid content in different organs in ascending order of hepatopancreas< mantle< gill< gonad< foot, with 5.127 ± 0.205, 5.187 ± 0.086, 5.500 ± 1.008 and 9.946 ± 0.509 mg/ 100mg dry tissue respectively. Chronic (1.18 ppm) group exhibited lipid content with increasing trend of mantle, gill, hepatopancreas, gonad, foot, with 3.124 ± 0.008, 4.330 ± 0.203, 4.925 ± 0.142, 5.210 ± 0.081, and 7.630 ± 0.091 mg/ 100mg dry tissue respectively. As compared to control group, there was 39.77 (p<0.001), 21.27, 3.93, 36.15 (p<0.001), and 23.28% (p<0.01) decrease in respective organs. During monsoon, control group showed lipid content in ascending order of mantle, followed by hepatopancreas, gill, gonad, foot, with 4.715 ± 1.013, 6.080 ± 0.516, 6.102 ± 0.065, 7.020 ± 0.816, and 10.421 ± 0.012 mg lipids in 100mg dry weight respectively. Chronic group (0.20 ppm) exhibited lipids in increasing order if hepatopancreas <mantle< gill< gonad< foot, with 4.204 ± 0.057, 4.225 ± 0.013, 5.702 ± 0.041, 6.820 ± 0.105, and 9.400 ± 1.091 mg/100mg dry tissue respectively. As compared to control group, there was considerable decrease in lipid content of all organs, but decrease was significant in gill only. During winter, control group, showed lipid content, in increasing order of mantle< hepatopancreas< gill< gonad < foot. The lipid content was 4.870 ± 0.318, 4.970 ± 1.061, 6.795 ± 0.092, 8.205 ± 0.032, and 12.072 ± 0.076, mg in 100mg dry tissue respectively. In chronic group, (0.21ppm) the lipid content in different organs was present in ascending order of hepatopancreas< mantle< gill< gonad< foot, with 3.551 ± 0.016, 4.020 ± 0.015, 5.967 ± 0.392, 7.010 ± 1.030.As compared to control group, there was 12.18 (p<0.001), 16.85 (p<0.01), and 25.22% (p<0.001) decrease in lipid content in gill, gonad and foot respectively. In general, alterations in lipid content of chronic group showed considerable depletion in all soft tissues, but depletion was significant (p<0.001) in hepatopancreas and mantle. Hepatopancreas showed 30.85 and 28.55% decrease in monsoon and winter respectively. During summer, mantle showed (39.77%) considerable depletion than hepatopancreas. Ansari (1983) [24] reported copper induced decrease in lipid content of *Crossostrea punctatus* .The decrease in lipid content of clams from chronic sub lethal exposure of cadmium chloride is in good agreement with observations of few workers.(Jana and Bandopadhya,1987[25]; Builter and Wilson, 1998)[26.]

Conclusion:

The present study gives a detailed account of influence of cadmium chloride stress and seasonal water parameters on biochemical alterations in soft tissues like gill, mantle, hepatopancreas, gonad and foot of *M.meretrix*. Alterations in glycogen, protein and lipids were attributed to various factors like environmental stress, physiological status of clams and concentration of cadmium chloride. Alterations in glycogen content in clams from control group was attributed to seasonal water parameters and spawning season. In chronic group of clams, there was significant increase in glycogen content of gill and hepatopancreas, while in rest of organs there was marked decrease during summer, monsoon and winter. The significant increase in glycogen content in gill and hepatopancreas indicated that these soft tissues were prominent target organs for cadmium chloride toxicity. In control group, protein content was better during winter. It was attributed to favourable environmental parameters and abundance of food. Clams from chronic group showed significant elevation in gill and hepatopancreas. It may be due to mobilization or induction of new proteins to combat against cadmium stress. Lipid content was better during winter and gametogenesis period, Clams from chronic group showed significant decrease in almost all soft tissues during summer, monsoon and winter.

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