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Impact of coastal pollution on biological, biochemical and nutritional status of edible oyster in Phoenix Bay Jetty and North Wandoor of Andaman

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ABSTRACT

Edible oyster, *Crassostrea rivularis* were collected from North Wandoor (NW) and Phoenix Bay Jetty (PJ) of Andaman and their biological, biochemical and nutritional status were assessed. Water quality parameters were estimated from both places and recorded higher levels of nitrite, nitrate and phosphate in PJ samples compared to NW. There was significant difference in length and weight of oysters collected from the 2 sites. Oysters from NW samples were bigger in size compared to PJ samples. All biochemical parameters showed reduction in the *C. rivularis* collected from PJ compared to NW. The extent of reduction in liver glycogen and ascorbic acid, acetyl choline esterase and alkaline phosphatase activities in gill tissue were 65, 42, 17.07 and 46.01% respectively. Overall results suggested that, oysters dwelling at PJ were not healthy and under stress due to continuous exposure to organic discharge which in turn might have caused lower growth rate of the oyster and depression in the biochemical and enzyme activities compared to NW oysters.

Key words: Acetyl choline esterase, Alkaline phosphatase, Ascorbic acid, Glycogen, Oyster

Oysters filter up to 1,500 litres of sea water per day looking for food. This may lead to >100 times higher concentrations of pollutants in the oysters than surrounding waters. Oysters have wide geographical distribution. They are sensitive to environmental pollutants and accumulate anthropogenically derived chemicals at a high rate (Roesijadi 1994a, 1994b, Binelli *et al.* 2006). Biochemical and molecular responses of fishes to aquatic stressors are the earliest indicators of environmental perturbations to animal which can be useful as early signs of organismal alteration (Pal and Ayyappan 2002). Biochemical alteration of oysters and mussels are widely used in pollution monitoring studies (Cajaraville *et al.* 2000, Lau and Wong 2003, Roméo *et al.* 2003, Silva *et al.* 2003, Zanette *et al.* 2006, Bernal-Hernández *et al.* 2010). Many biomarkers are extensively used globally for pollution monitoring (Livingstone 1993, Kaaya *et al.* 1999). Biochemical parameters are extensively used to characterize anthropogenic pollution (Burgeot *et al.* 1996, Cajaraville *et al.* 2000, Zanette *et al.* 2006).

In Andaman 3 dominant species of edible oysters *Saccostrea cucculata*, *Crassostrea rivularis* and *Crassostrea gryphoides* (Ahlawat *et al.* 2002) are available. The present study was undertaken to study biological parameters, biochemical indices and nutritional status of oyster, *Crassostrea rivularis* collected from two different ecological regimes. The sites selected for the present study included an unpolluted area and a sewage discharge area.

MATERIALS AND METHODS

Study area: Andaman & Nicobar Islands (ANI) is the largest archipelago in Bay of Bengal. Port Blair, the capital city is with an estimated population of 1.33 lakh (2011 census). The sewage and other effluents generated in the city are released to the sea at different points through small drainage channels. PJ is a harbour area where interisland ships are stationed. It is also a discharge point of sewage water. The organisms, especially sessile oysters living in these areas are constantly under stress due to ship passages and municipal discharge.

Two locations were selected for the present study — PJ (latitude-11° 40'27.63" N, longitude 92° 44'12.96"E) is close

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to the northern part of Andaman harbour and NW is located in the Mahatma Gandhi Marine National Park (latitude-11° 36'43.24"N, longitude 92° 36'59.53"E). NW is a pollution-free and undisturbed area with no industrial development and less habitation. The distance between these 2 locations is about 30 km.

Sample collection: Oysters were collected randomly during high tide from 2 different sites for analysis — 25 oysters were collected from each site on monthly basis for 3 consecutive months (January, February and March 2010). They were removed slowly using a shovel and scupper without breaking the oyster. After collection, oysters were brought to the laboratory in aerated condition. In the laboratory shell length, shell weight, meat yield were recorded. Liver and mantle tissues were dissected out and used for biochemical parameters. Temperature, pH, salinity, alkalinity, turbidity and CO_2 of the water samples were analyzed *in situ* using a thermometer, pH meter and Model 611 Intelligent Water Quality Analyzer. Dissolved oxygen, phosphate, nitrite and nitrate were estimated using standard methods (Clesceri *et al.* 1998).

Proximate composition of the oyster: Proximate analysis of the oyster for moisture, crude protein, lipid and ash was done following Malik and Sirohi (2004). The total carbohydrate (TC) was calculated by subtracting the percentage of other nutrients from 100 (Hasting 1969). In the present study carbohydrate value was calculated by summing up the average values of protein, fat and ash and then subtracting from 100.

TC (%) =100-crude protein (%) -ether extract (%) -ash (%)

Biochemical parameters: Biochemical parameters like glycogen, ascorbic acid and acetyl choline esterase (AChE) and alkaline phosphatase (ALP) in gill tissues were studied. Glycogen was estimated from liver and mantle calorimetrically by treating with anthrone reagent as described by Hassid and Abraham (1957). Ascorbic acid was estimated in gill tissue by the method of Roe and Keuther (1943). The OD was recorded at 540 nm against standard ascorbic acid. Quantification of protein was carried out using Lowry's method (Lowry 1951).

Enzyme assay: For enzyme activity, gill tissue was used and 5% homogenate was prepared on wet weight basis. Organs were weighed and homogenized with chilled sucrose (0.25 M) solution in a Teflon coated mechanical homogenizer. Centrifugation was done at 5,000 rpm for 10 min at 4°C. The supernatant was transferred to sample vials and used for estimation of enzyme activity. The whole procedure was done in ice cold condition. The AChE activity was assayed following Hestrin (1949) modified by Augustinasson (1957) and ALP activity was estimated following Garen and Levinthal (1960).

Data analysis: The data obtained in the present experiment were subjected to student "t" test using Statistical Package, SPSS version 11 to find out the differences in treatment means at $P<0.05$.

RESULTS AND DISCUSSION

Nitrate, nitrite and phosphate level was higher in the water samples collected from PJ compared to NW whereas dissolved oxygen level was low in PJ than NW (Table 1). Other water quality parameters were not significantly different ($P<0.05$).

Data pertaining to the length and weight of *Crassostrea rivularis* collected from NW and PJ (Table 2) revealed significant difference ($P<0.05$) in the length and weight parameters of oysters collected. The oysters from PJ were smaller in terms of length and weight than those from NW. Minimum and maximum range in length and weight of the oysters collected from NW was 9.5 to 13.3 cm and 113 to 331 g and for PJ it was 5.88 to 9.1 cm and 42 to 92 g respectively. Moreover, oysters from NW were very clean and shining white, whereas the oysters from the PJ were dark in colour and dirty with debris. Average meat yield of each oyster from 2 areas were also significantly different. The average shell yield and meat yield of samples were 95.61% and 4.39% at NW and 94.07% and 5.93% at PJ respectively. However, while comparing shell weight vis-a-vis meat yield, PJ samples have higher meat yield in comparison to NW

Table 1. Average variations in the water quality parameters from two different ecosystems

Parameters	Jan 2010		Feb 2010		Mar 2010	
	North Wandoor	Phoenix Jetty	North Wandoor	Phoenix Jetty	North Wandoor	Phoenix Jetty
Temperature (°C)	29.0±0.0	31.1±0.6	31±0.67	30.9±0.03	31.3±0.17	31.5±0.29
Dissolved oxygen (mg/l)	4.8±0.40	0.6±0.35	5.2±0.35	2.4±0.19	4.4±0.37	2.2±0.20
pH	8.4±0.11	8.44±0.01	8.35±0.03	8.39±0.00	8.35±0.08	8.48±0.01
Salinity (g/l)	33.71±0.21	31.24±0.44	32.99±0.36	31.45±0.19	33.35±0.41	31.915±0.20
Turbidity (NTU)	20.4±2.01	21.52±0.02	18.2±2.1	21.4±0.13	20.8±1.59	24.37±1.37
Alkalinity (mg/l)	116±3.06	145±1.76	108±0.33	142±1.11	130±3.46	142±1.15
Carbon dioxide (mg/l)	nil	4.0±1.33	nil	nil	nil	Nil
Nitrite (mg/l)	0.0060±0.00	0.014±0.001	0.0075±0.001	0.0127±0.004	0.0063±0.00	0.0115±0.001
Nitrate (mg/l)	0.17±0.014	1.00±0.15	0.42±0.03	0.58±0.09	0.33±0.03	0.67±0.13
Phosphate (mg/l)	0.03±0.007	0.20±0.01	0.06±0.007	0.01	0.03±0.006	0.008

Table 2. Biological data and proximate composition of oyster *Crassostrea rivularis* collected from two different ecosystems

Parameters	North Wandoor	Phoenix Jetty
Biological parameters		
Length (cm)	11.757±0.31[a] (9.5 - 13.3)	7.213±0.28[b] (5.88 –9.1)
Weight (g)	205.16±12.91[a] (113.89 - 331.48)	62.8452±3.03[b] (42.101– 92.715)
Average meat yield from each oyster	9.846±1.11	3.85±0.30
Meat yield per shell weight (%)	4.80	6.13
Average shell percentage	95.612±0.28	94.065±0.41
Average meat percentage	4.388±0.28	5.935±0.41
Proximate composition		
Moisture (%)	80.25±1.25	77.81±1.92
Fat (%) *	15.38±0.56	13.53±0.57
Protein (%) *	57.73±0.39	58.41±1.55
Ash (%) *	11.72±0.68	10.69±0.35
Carbohydrate (%)	15.17±0.74	17.37±0.84

* on dry weight basis;proximate composition: data are expressed as mean±SE, n=5.

Different superscripts of a parameter differ significantly (P<0.05). Parenthesis in the bracket is the range.

samples. This is mainly due to bigger shell size (expressed in weight) of the NW sample in comparison to meat weight. This indicated that calcium carbonate deposition/mal formation was much faster in NW oysters in comparison to PJ sample. Data pertaining to proximate composition in *C. rivularis* collected from 2 sites, viz. NW and PJ are also presented in Table 2. It was observed that there was no significant (P> 0.05) variation in the moisture, fat, protein and ash content of the oyster samples collected from NW and PJ.

At PJ, apart from the discharge point of city sewage, there is contamination from dockyard of inter-island ships. Hence, the oysters settled at PJ are constantly under stress and reduced growth due to combined effect of different types of pollutants. Similarly Gifford *et al.* (2006) recorded significant reductions in total growth in oysters when exposed to high concentrations of either zinc or lead.

Data pertaining to glycogen, ascorbic acid, AChE activity and ALP activity in different tissues of *C. rivularis* collected from 2 sites are given in Table 3. It was observed that glycogen content was more in NW samples than oysters collected from PJ. There was significant (P<0.05) difference in glycogen content in the liver and mantle of oysters collected from different sites. The stored glycogen in respective organs was 65 and 51.6% respectively. Persistent low level glycogen content in PJ sample may be due to high rate of utilization of carbohydrate by the organism to mitigate pollution induced stress. Glycogenolysis and/or hyperglycaemia appeared to be a response of pollution stress

Table 3. Glycogen, ascorbic acid, acetylcholine esterase (AChE) and alkaline phosphatase (ALP) content in gill tissues of *Crassostrea rivularis* collected from 2 different ecosystems

	North Wandoor	Phoenix Bay
Gross parameters		
Glycogen in mantle tissue	0.40±0.03[a]	0.19±0.04[b]
Glycogen in liver tissue	0.51±0.02[a]	0.14±0.01[b]
Ascorbic acid	159.44±16.84[a]	92.56±10.01[b]
Enzymes		
AChE in gill tissue	0.023±0.003[a]	0.019±0.002[a]
ALP in gill tissue	25.609±2.86[a]	13.827±2.041[b]

Units: Glycogen and ascorbic acid : mg/g of wet tissue, enzyme, AChE: μ moles of acetyl choline hydrolyzed/mg protein/minute at 37°C, ALP: n moles of paranitrophenol released/mg protein/minute at 37°C. Different superscripts of a parameter differ significantly (P<0.05). Values are mean±standard error (n= 4).

(Dange 1986). Nakano and Tomlinson (1967) observed that all types of stresses stimulate the secretion of catecholamine which in turn increased the breakdown of glycogen and enhanced blood sugar level. Encomio and Chu (2000) reported that decline in glycogen content in the adductor muscle of *Crassostrea virginica* with increasing polychlorinated biphenyls exposure and opined that decline in Chesapeake Bay oyster populations might be due to diseases and reduced water quality from pollution. Similarly Liu *et al.* (2000) also observed decrease in glycogen content in the mantle, adductor muscle and gonad of starved *Crassostrea gigas*.

Similarly, ascorbic acid level was 42% lower in PJ in comparison to NW. Ascorbic acid popularly known as vitamin C is well known for its major anti-stress activity (Misra *et al.* 2007). Ascorbic acid and α-tocopherol are the most widely cited antioxidants that prevent oxidative damage to the cell membrane induced by radicals (Lee and Dabrowski 2003, Sealey and Gatlin 2002). Vitamin C (ascorbate) is rapidly depleted in fish subjected to sub-lethal levels of organic and inorganic substances (Heath 1995). In the present study vitamin C in mantle of oyster *C. rivularis* collected from PJ was relatively less compared to NW (Table 3). Phoenix Bay Jetty is discharge area of city sewage and coastal waters might contain many harmful pollutants along with heavy organic load. These resultant stress effects might have forced the oyster to spend more vitamin C for detoxification process or for prevention of cell peroxidation (Mauck *et al.* 1978, Winston and Diguila 1991, Sarma *et al.* 2009).

AChE activity in the gill tissues of *C. rivularis* collected from PJ also recorded 17.07% less (Table 3) in comparison to NW. AChE is one of the most widely used enzymes as a biomarker for environmental pollution. This enzyme is associated with neuronal synapses and the concentration is proportional to the extent of innervations of the tissue (Rao and Rao 1984). Several studies have demonstrated that AChE

enzyme gets inhibited by various toxicants (Shaw and Panigrahi 1990, Assis *et al.* 2007, Da- Fonseca *et al.* 2008) and also by combination of various stressors like pesticide and pathogen (Eder *et al.* 2007). In the present study (Table 3), though there was no significant inhibition (P>0.05) of acetyl choline esterase (AChE) activity in the gill tissues of *C. rivularis* but there was a reduction in PJ samples compared to NW. AChE is a sensitive biomarker and inhibit its activity in presence of any aquatic stresses like pesticide, heavy metal etc. Bernal-Hernandez *et al*. (2010) reported about 65% reduction in AChE activity in oyster, *C. corteziensis* collected from Boca de Camichin due to pesticide pollution. In the present study, reduction in AChE activity in the gill tissue of oyster collected from PJ might be due to combined effect of several different types of inhibitors persistently present in the effluents discharged at PJ.

ALP activity in the gill tissues of *C. rivularis* collected from PJ also recorded 46.01% less in comparison to NW. Alkaline phosphatase is a zinc containing metallo-enzyme and plays an important role in metabolism of phosphorus in the body. This enzyme catalyses the hydrolysis of various phosphate containing compound and acts as a transphophorylase at alkaline pH (Mazorra *et al.* 2002). In ecotoxicology, ALP can be used as an indicator of intoxication because of its sensitivity to metallic salts (Boge *et al.* 1988). Liver ALP activity changes in fishes in response to water born metal (Lan *et al.* 1995) making it useful as indicator of heavy metal exposure. In the present study, ALP activity was also lower in PJ samples compared to NW. Similar to present study, Mazorra *et al.* (2002) also observed significant inhibition in ALP activity in digestive gland and in gill when exposed to mercury. A dose-dependent inhibition of alkaline phosphatase activity was reported in the liver of zebra fish *(Brachydanio rerio*) exposed to Malathion (Kumar and Ansari 1986). Sharma (1990) also showed consistent inhibition of ALP activity in liver, kidney and muscle of *Channa gachua* exposed to endosulfan for a longer period (15–30 days).

Overall results suggested that, the environment and oysters living in PJ are not very healthy in comparison to NW. This might be due to combined effect of several stressors present in PJ. Effort has to be made to treat the effluents discharged in the PJ before releasing to the sea. More systematic and laboratory oriented studies can be initiated to identify the prevailing stressors in discharged waters globally to elucidate the exact impact of sewages on organism level.

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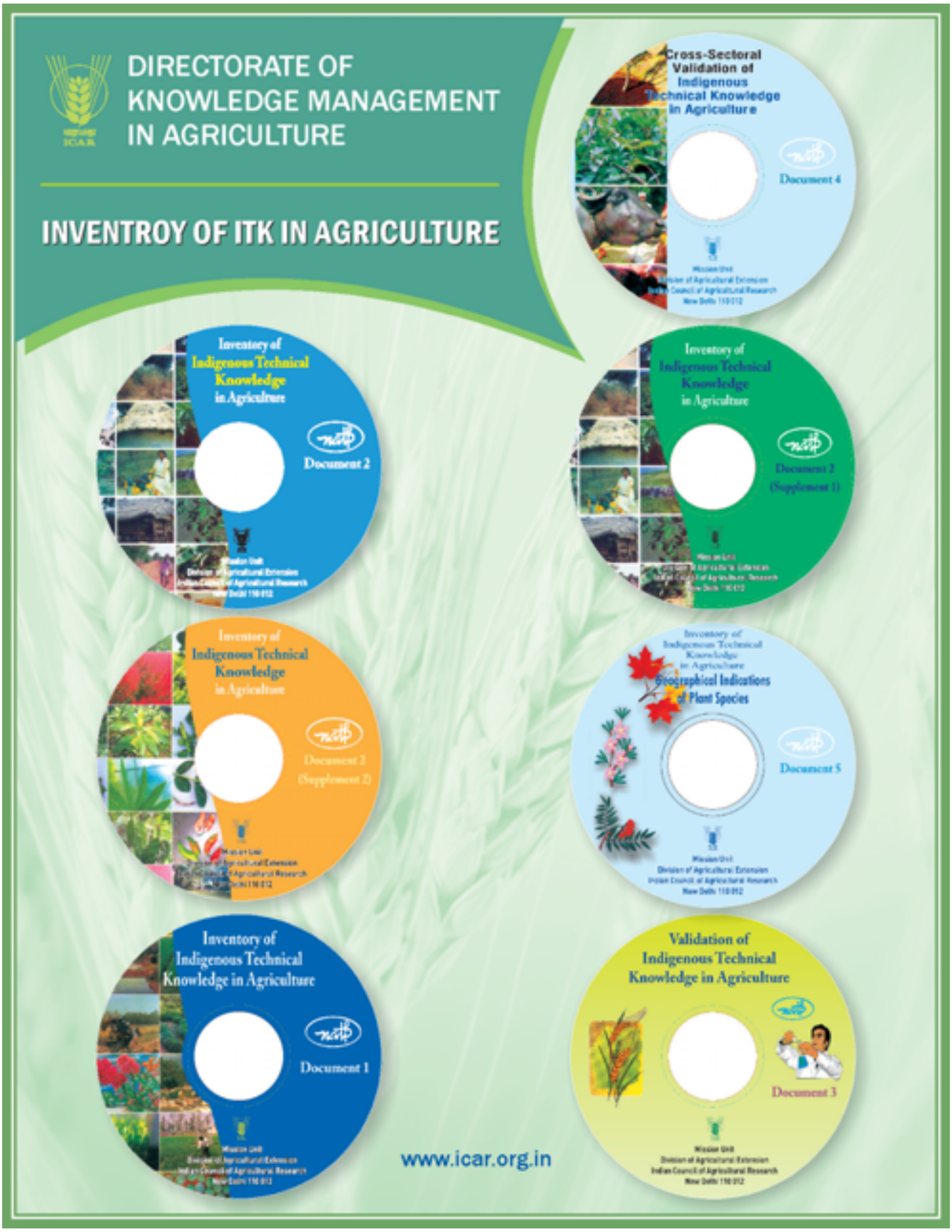
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