ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 4, Issue, 7, pp. 1405-1410, July, 2014

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

ENVIRONMENTAL POLLUTION MONITORING: COMPARISON OF HISTOLOGICAL VARIATION OF TISSUES OF TWO DIFFERENT SAMPLING SITE PULICAT AND ENNORE

[1*]Saravanan, N., [3]Uma, T., [1]Madhu Magesh, K., [1]Ramachandraprabhu, S. and [2]Vinoth, A.

[1]Endocrinology Unit, Department of Zoology, Madras Christian College, Tambaram, Chennai – 600 059, Tamil Nadu, India
[2]Department of Marine Studies and Coastal Resource Management, Madras Christian College, Tambaram, Chennai – 600 059, Tamil Nadu, India
[3]Department of Zoology, Pachaiyappa's College for Women, Kanchipuram – 631 501

ARTICLE INFO

Article History:
Received 19th April, 2014
Received in revised form
08th May, 2014
Accepted 16th June, 2014
Published online 20th July, 2014

Key words:
Biomarkers,
Mullet, Pollution,
Pulicate,
Ennore,
Histology.

ABSTRACT

Estuaries are important sinks of pollutants derived from anthropogenic activities. Fish inhabiting these areas have been proposed as sentinels for pollution monitoring through assessment of sensitive biomarkers. Biomarkers can be defined as a change in biological response, ranging from molecular through behavioural changes, which can be related to exposure or effects of environmental contaminants. The present study was focused to examine the histological changes in the comparatively in polluted samples collected from two different sites. Tissue samples of Mullet, Oyster, Green mussels and Polychaete were collected from Pulicat lake and Ennore creek flow to the bay of bengal forming a common canal known as buckingham Canal. Samples were sectioned, stained and visualised by light microscopically the histological changes was observed and confirmed the environmental factors was involved in the structural, behavioural changes and also changing the biochemical activity of the organisms.

INTRODUCTION

Exposure biomarkers can be defined as any xenobiotic, its metabolite or the interaction product between the xenobiotic and a molecule or cell that is measured in organisms or in subunits of them (Depledge *et al*., 1995). Effect biomarkers represent any biochemical, physiological, behavioural and or other alteration that can modify the well-being of an organism. Several molecular and cellular components in different fish species have been used as exposure and effect biomarkers, including histological, biochemical, immunological and genetic parameters (van der Oost *et al*., 2003). Histology is necessary to describe the damage of cells in fish, reproductive events pertaining to gamete development, and quantitative estimates are important because they both eliminate the subjectivity and semantic problems associated with description and tend to provide ecologically meaningful information (Barber and Blake, 1991).

Corresponding author: Saravanan, N.
Endocrinology Unit, Department of Zoology, Madras Christian College, Tambaram, Chennai – 600 059, Tamil Nadu, India

Over the past two decades, the science of biomarkers has advanced considerably and there is a worldwide trend to supplement chemical and physical parameters with biomarkers in marine pollution monitoring. A variety of molecular, biochemical, physiological, histo-cytopathological, and population and community responses may be used to identify exposure to certain chemicals, provide information on spatial and temporal changes in the concentration of contaminants, and indicate environmental quality or occurrence of adverse ecological consequences. In general terms, responses at lower biological organization levels (molecular and biochemical responses) are more specific, sensitive, reproducible and easier to determine, but more difficult to relate to ecological changes and generally lack realism. On the other hand, responses at higher biological organization levels (population and community responses) are directly indicative of ecosystem health and hence, much more relevant to environmental management. However, they are more difficult to determine, less specific and only manifest at a late stage when environmental damages have already occurred (Connell *et al*., 1999). Histo-cytological responses are relatively easy to

determine, and can be related to health and fitness of individuals which, in turn, allows further extrapolation to population/community effects. A wide range of histo-cytological alterations in fish and bivalves have been developed and recommended as biomarkers for monitoring the effects of pollution. For instance, extensive studies in the USA (NOAA's National Status and Trends Program) and Europe (International Council for the Exploration of the Sea (ICES), and the North Sea Task Force Monitoring Master Plan) have established a causal relationship between fish pathology and levels of pollution in the marine environment. In these programs, externally visible diseases and histopathological lesions in fish served as primary indicators of exposure to contaminants, and certain diseases and lesion types have proven to be reliable biological indicators of toxic/ carcinogenic effects resulting from such exposure. Recently, bio monitoring surveys in the Mediterranean Sea (the United Nations Environment Program) recommended the use of certain cellular responses in molluscs and fish as cytological biomarkers to assess the impact of pollution in coastal environments.

Teleost liver is the primary organ for biotransformation of organic xenobiotics, and probably also for the excretion of harmful trace metals, food digestion and storage, and metabolism of sex hormones (Health, 1995). There have been numerous reports of histo-cytopathological changes in livers of fish exposed to a wide range of organic compounds and heavy metals (Hinton and Lauren, 1990). Livers of fish are sensitive to environmental contaminants because many contaminants tend to accumulate in the liver, making this organ exposed to a much higher levels (several orders of magnitude) than in the environment, or in other organs (Health, 1995). The US National Marine Fisheries Services (US NMFS) has conducted large scale surveys to determine the relationships between toxicopathic lesions in livers and exposure to chemical contaminants. A total of 18 marine bottom fish species collected from 45 sites on the Pacific Coast (Malins *et al*., 1988). In general, liver histopathological lesions are not specific to pollutants. Furthermore, not all hepatic lesions identified in feral fish can be used as biomarkers since certain liver lesions appear to be species specific. The objective of this study was to identify lesions in cells and identify cell damages in fish, mussel, oyster and polychaete as biomarkers of exposure to environmental contaminants from polluted estuarine water.

MATERIALS AND METHODS

Sampling Sites

Two sampling sites were selected in this area of the centre region of Ennore creek and northern region of Pulicat Lake. The Pulicat Lake is situated between 13°25' and 13°55' North, and 80°3' and 80°19' East. The lake is about 45 km north of Chennai and can be reached by bus from Chennai. Pulicat Lake has been a traditional fishing centre. This was a trading port for the Portugese and Dutch in the 16th and 17th centuries. The process of soil erosion and siltation is believed to have started with the Dutch over-exploiting the mangroves for commerce and trade. However, what is not so well known about this lake is the link of the livelihoods and traditions of the local people with the lagoon ecosystem, and people's struggles consequent efforts to save their livelihoods, and thus the lagoon, from over-exploitation, pollution and developmental pressures. Pulicat is an extensive brackish-to-saline lagoon with marshes and a brackish swamp on the north. This is the second largest saltwater lagoon in India and a Ramsar site (internationally recognized wetland under the Ramsar Convention). North Chennai Thermal Power Station (NCTPS) was set up by the Tamil Nadu Electricity Board (TNEB). This plant is located within CRZ-I; however clearance for this was granted prior to 1991 when CRZ came into existence. This plant draws 44 lakh litres of freshwater from Ennore creek and releases hot coolant water into Buckingham canal and discharges about 3000 tonnes of toxic fly-ash in the form of slurry every day. The release out of hot coolant water at temperatures of about 40°C leads to oxygen depletion and death of aquatic life. The combination of coolant water and fly-ash has had a serious impact on the livelihood of people by depleting fish populations. A fact-finding committee was set up to investigate the pollution caused by this plant. One of the basic Tamil nadu questions that concerned the fisher folk was whether they would be granted jobs if the pollution continued, resulting in loss of livelihood.

Kattupalli island is a narrow longitudinal island separated from the mainland by the backwaters extending from the Pulicat lake. The island is bordered by the Bay of Bengal on the east, Buckingham canal on the west, Pulicat Lake on the north and Ennore creek on the south. The total area of this island is about 18 sq km, and it supports a human population of about 2250 families. The island has a rich biodiversity of vegetation, especially mangroves, freshwater and brackish water flora and fauna, and medicinal plants. The Tamil Nadu Industrial Development Corporation (TIDCO) had planned to establish an Rs 6000 crore petrochemical complex on this island with the idea that the Ennore port would be used for transporting the products. TIDCO went ahead with the acquisition of land of 2,900 ha even before the public hearing under Environment Impact Assessment (EIA) rules was held. The first one was located near River mouth in front of the Ennore thermal power station: the second sampling site was located near pulicat lake in front of the pazhaverkadu lighthouse.

Sample Collection

Samples of mullet, green mussel, oysters and polychaete were collected at each sampling site the samples were dissected and samples of tissues of each organism were taken and stored in a small container with 10% formalin. Sample tissues were transported to the laboratory.

Histopathological Analysis

The sample tissues preserved in 10% formalin were exchanged to 70% ethanol, dehydrated and embedded in paraffin in the usual way. Microscopic sections were cut at 6 µm and stained with hematoxylin and eosin. All slides were histopathologically examined including histochemical and image analysis of Melano macrophage centers (MMC) in the liver. Basic hematoxylin (Mayer's) and eosin (H&E) stain was used to view cellular structures and combined with image analysis to estimate the relative area occupied by MMC. Another H&E stain was done in combination with a bleaching technique using potassium permanganate and oxalic acid.

Potassium permanganate is an oxidizing agent that removes melanin and allows for a confirmation of melanin pigments (Hartley, *et al*., 1996). Comparison of MMC in liver of mullet, ovary of mullet, polychaete, green mussels and oysters cells collected from Ennore creek and Pulicat Lake observed using light microscopy and image was taken.

RESULTS

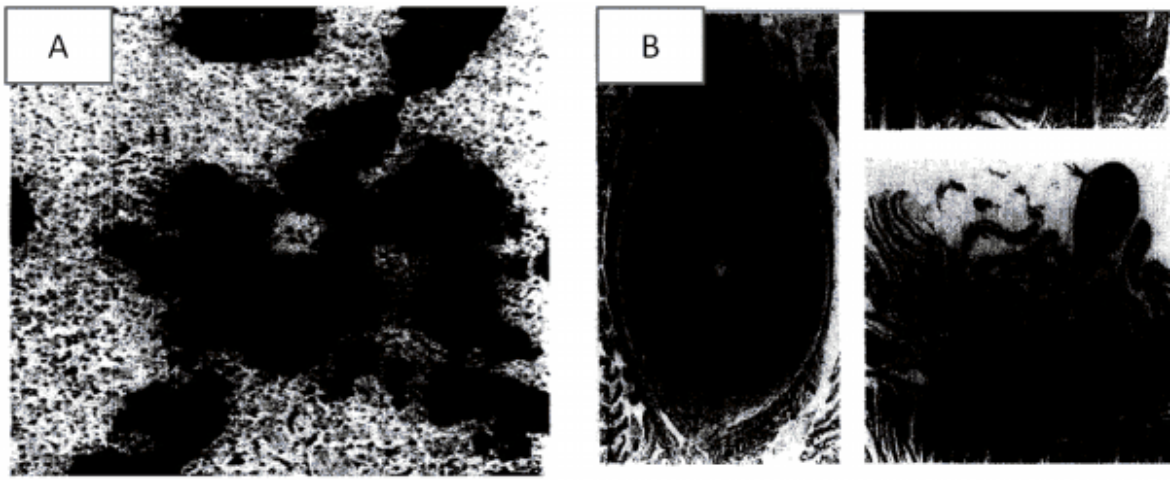
The tissue sections were scored for the abnormalities listed in Table 1. Possible scores for each lesion were: "**0**"- lesion absent, "+"- light, "++" - moderate and "+++" - severe. In the data analysis the scores for each lesion were summed for each tissue (ovary, muscle and liver of mullet, polychaete, green mussel and oyster). The maximum histopathological index scores in each individual species were severe for liver of mullet and moderate for ovary in mullet samples. Comparisons were made between species and sites based on the mean scores of individuals for each tissue. Inflammation was a consistent finding and included all inflammatory cell types except polymorphonuclear cells (neutrophils and eosinophilic granular leukocytes). Lymphocytes were concentrated in the epithelium and macrophages were concentrated in the interstitial tissues over the cartilage. Where the granulatomous inflammation was most severe, multinucleated syncytial giant cells were occasionally seen.

DISCUSSION

In these studies we examined a number of histological markers of exposure and effect in species of mullet from a natural oil seep and from reference areas in Ennore. All species like green mussel, polychaete and oyster from the seep environment had significantly elevated concentrations of pollutants. The lesser distinction effects from the Pulicat. The general surface flow of oil on the sea surface from the Coal Oil Point might be resulting in a greater exposure of biomarkers in the reference site at Ennore. In the estuary of Ennore and pulicat, the bivalve molluscs are a resource commonly exploited by fisherwomen. This resource represents an important socioeconomic activity, contributing to the livelihoods of communities that live around the estuary. Mangrove oysters (Crustaceae spp. Ostreidae), mussels (Mytella falcataand Mytella guyanensis, Mytilidae) and cockles. In the estuary of Pacoti River, located on the east coast of Ceará, the bivalve molluscs are a resource commonly exploited by fisherwomen. This resource represents an important socioeconomic activity, contributing to the livelihoods of communities that live around the estuary. The few studies on the occurrence of pathogens that affect natural populations of bivalve molluscs in Ceará State are limited to two bivalve species from the Jaguaribe River and Pacoti River estuaries (Sabry *et al*., 2007). Studies on diseases in molluscs are important because some pathogens can affect and can cause massive mortality among natural or cultivated populations. The digenetic nematodes of the genus Eucephalus (Digenea, ucephalidae) are important parasites of marine bivalves, sometimes causing significant pathological damage, mainly characterized by castration of the host. On the other hand, Tylocephalum larvae had already been recorded in a variety of shellfish without causing diseases (Cheng, 1988) but at high intensities of infection they could cause physiological stress and consequently affect the growth and reproduction. Mussels are inter-tidal filter-feeding invertebrates known to accumulate high levels of trace metals and organic compounds in their tissues, providing a time integrated indication of environmental contamination with observable cellular and physiological responses.

Table 1. Shows the abnormalities of lesion formation of different samples of two different sampling site collection and histochemically examined samples score

Samples	Ennore site samples				Pulicat site samples			
	Lesion absent	Light	Moderate	Severe	Lesion absent	Light	Moderate	Severe
Mullet Liver	0	0	0	+++	0	0	0	0
Mullet Ovary	0	0	++	0	0	0	0	0
Mullet Muscle	0	0	0	0	0	0	0	0
Green mussel	0	+	0	0	0	0	0	0
Oyster	0	+	0	0	0	0	0	0
Polychaete	0	+	0	0	0	0	0	0



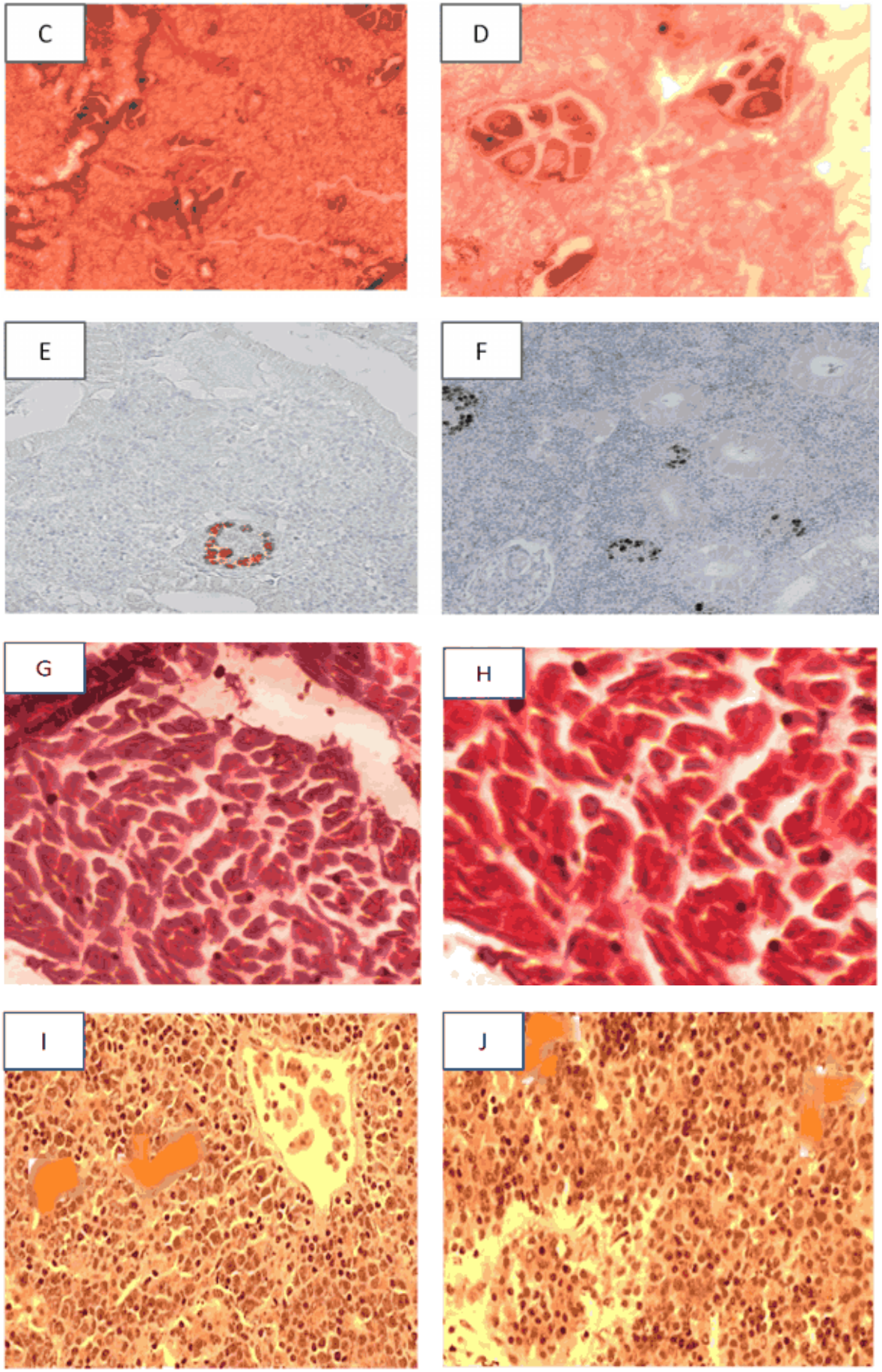


Fig.1. Shows the comparative analysis of two different site samples of histologically examined using basic haematoxylin (Mayer's) and eosin stained images (A and B shows the variations of Liver tissues of Mullet, C and D is muscle tissues of Mullet, E and F is Ovary tissues of Mullet collected from Ennore and Pulicat respectively. G and H show the variations of Green mussel of Ennore and Pulicat I and J plate showing the changes of Polychaete histology of Ennore and Pulicat site)

The inductive responses in mussels make them good candidates for environmental monitoring. Petroleum seep mussels are continuously exposed to relatively high hydrocarbon concentrations in their natural habitat and, thus, offer the opportunity to examine the relationship between parasitism, disease and contaminant exposure under natural conditions. Mussels commonly harbour parasites and diseases of a variety of types (Gee and Davey, 1986; Kent *et al*., 1989; Kim *et al*., 1998). The concept that reduced health, brought on by pollutants, limitations in food availability, and other stressors, results in increased susceptibility to a range of parasites and diseases in molluscs was initially propounded by Laird (1961). Recent work has focused on immune suppression by certain pollutants (Cheng, 1988; Anderson *et al*., 1992) the relationship of pollutant exposure to common parasite induced diseases (Sindermann, 1983; Winstead and Couch, 1988; Wilson-Ormond *et al*., 1992)

The influence of environmental changes in initiating and terminating disease processes. Modelling efforts (Hofmann *et al*., 1995) provide a theoretical underpinning for how small changes in environment can produce large changes in the prevalence and intensity of parasitism and disease (Wilson-Ormond *et al*., 1999) provide an example from the Gulf of Mexico where parasite infection intensity was significantly affected by nearness to oil and gas production activity, and analysis of NOAA National Status and Trends (Mussel Watch) data for the Gulf of Mexico has identified the influence of long-term changes in climate on organism health (Kim and Powell, 1998) and related parasite abundances to contaminant body burden in a number of parasite/contaminant pairs (Kim *et al*., 1998). Because of the close association of mussels with hydrocarbon seepage, parasites, diseases, and tissue pathologies may play an important role in population dynamics. The prevalence and intensity of parasites, diseases, and tissue pathologies may provide a valuable tool for comparing the health of populations with variations in site chemistry and may provide a useful early warning signal of long-term changes in the health of seep communities that may eventually result in local extinction. Evidence indicates that site chemistry varies on time scales as short as weekly to at least as long as decadal (Callender and Powell, 1999).

The parasite fauna was highly variable between populations and, potentially, population health varied significantly from one mussel bed to another. Whether this difference is environmentally driven or due to chance recruitment events remains unclear. Lysosomal alteration in marine mussels could represent nearly 80% of the variations of both integrated cellular and physiological responses in stressed state. The saline areas, especially in estuary the larger Nereis called polychaete which is a cosmopolitan dominant species of boreal and temperate regions in muddy sand of littoral and sub-littoral zones. Any reproductive impacts on this species may, therefore, have important consequences for the ecosystem as a whole. Most polychaetes (especially Nereis) spend the majority of their time in contact with sediment. Marine sediments are a complex form of many components to which metals have the ability to bind, so they act as a sink in aquatic environments. Understanding the effects of metal exposure via sediment is critical in relating the impact to the ecology of a species. Metals interact with sediment in many ways and to aid in the development of concentration-response relationships the use of spiked sediment has been used as an eco-toxicological tool. When trying to predict the responses of populations to a toxicant, reproductive outputs are more ecologically relevant than adult physiological or biochemical changes and can be linked directly to recruitment and modeled, yet studies in this area have been scarce. The present study results confirmed and it has been suggested that the cellular patterns of staining (induction) might reveal the routes of exposure and uptake, via gill. The staining of gill epithelia but not endothelia seen in the mullet fish. Suggests direct exposure of gills, liver and ovary are fully consistent with the presence of petroleum waste in the water there and in ingested sediments. Direct exposures of other organisms to make the lesions of the cells were slightly light scored. In this experiment the liver is relatively insensitive to pollutant water, or that the hydrocarbons in petroleum products in the seep experienced by these species are insufficient to cause severe liver histopathology. There are a limited number of studies of the long-term effects of petroleum on marine fauna. The seeps at Coal Oil Point are known to have been active for hundreds, if not thousands of years. The results of this present study indicate the potential for negative effects to fish living in seep environments. The question of whether the negative effects evident here are offset by potential for more food in this environment remains unanswered. The results here might also be applicable to oil spill environments with persistent petroleum hydrocarbon concentration in sediments. Present results confirmed and proved the changes of tissues collected from two different sampling sites of histological aspects of biomarkers caused by environmental pollutions.

REFERENCES

Anderson, R.S., Oliver, L.M., Jacobs, D., 1992. Immunotoxicity of cadmium for the eastern oyster (*Crassostrea virginica* [Gmelin, 1791]): effects of haemocyte chemiluminescence. *Journal of Shell fish Research* 11: 31-35.

Barber, B.J., Blake, N.J., 1991. Reproductive Physiology. In: Shumway, S.E. Ed.., Scallops: Biology, Ecology and Aquaculture, *Developments in Aquaculture and Fisheries Science Elsevier, Amsterdam*, 21: 377–428.

Callender, W.R., Powell, E.N., 1999. Why do shallow-water cold-seep assemblages not exist today? *Geologische Rundschau*.

Cheng, T.C., 1988. In vivo effects of heavy metals on cellular defence mechanisms of *Crassostrea virginica*: total and differential cell counts. *Journal of Invertebrate Pathology* 51: 207-214.

Connell, D.W., Lam, P.K.S., Richardson, B.R., Wu, R.S.S., 1999. Introduction to Eco toxicology. *Blackwell Science*, *Abingdon :* 170 pp.

Depledge, M.H., Aagaard, A., Gyo¨rko¨ s, R. 1995. Assessment of trace metal toxicity using molecular, physiological and behavioural biomarkers. *Marine Pollution Bulletin* 31: 19–27.

Gee, J.M., Davey, J.T., 1986. Stages in the life history of Mytilicola intestinalis Steuer, a copepod parasite of *Mytilus edulis* (L.), and the e!ect of temperature on their rates of development. *Journal du Conseil International pour l'Exploration de la Mer* 42: 254-264.

Hartley William, R., Arunthavarani Thiyagarajah. 1996. Liver Lesions in the Gar Fish (*Lepisosteidae*) as Biomarkers of Exposure. *Marine Environmental Research* 42(1-4): 217-221.

Health, A.G., 1995. Water Pollution and Fish Physiology, second ed. *CRC Lewis Publishers, Boca Raton, FL*: 125–140.

Hinton, D.E., Lauren, D.J., 1990. Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. In: McCarthy, J.F., Shugart, L.R. (Eds.), *Biomakers of Environmental Contamination*. *Lewis Publishers, Boca Raton, FL:* 17–57.

Hofmann, E.E., Powell, E.N., Klinck, J.M., Saunders, G., 1995. Modeling diseased oyster populations I. Modelling Perkinsus marinus infections in oysters. *Journal of Shell fish Research* 14: 121-151.

Kent, M.L., Elston, R.A., Wilkinson, M.T., Drum, A.S., 1989. Impaired defense mechanisms in bay mussels, Mytilus edulis, with hemic neoplasia. *Journal of Invertebrate Pathology* 53: 378-386.

Kim, Y., Powell, E.N., Wade, T.L., Presley, B.J., Sericano, J., 1998. Parasites of sentinel bivalves in the NOAA Status and Trends Program: Distribution and relationship to contaminant body burden. *Marine Pollution Bulletin* 37: 45-55.

Kim, Y., Powell, E.N., 1998. Influence of climate change on inter annual variation in population attributes of Gulf of Mexico oysters. *Journal of Shell fish Research* 17: 265-274.

Laird, M., 1961. Microecological factors in oyster epizootics. *Canadian Journal of Zoology* 39: 449-485.

Malins, D.C., McCain, B.B., Landahl, J.T., Myers, M.S., Krahn, M.M., Brown, D.W., Chan, S.L., Roubal, W.T., 1988. Neoplastic and other diseases in fish in relation to toxic chemicals: an overview. *Aquat. Toxicol.* 11: 43–67.

Sabry, R.C., Rosa, R.D., Magalhães, A.R., Barracco, M., Gesteira, T.C.V., da Silva, P.M. 2007. First report of *Perkinsus* sp. infecting mangrove oysters *Crassostrea rhizophorae* from the Brazilian coast. *Dis. Aquat. Org*. 88:13–23.

Sindermann, C.J., 1983. An examination of some relationships between pollution and disease. *Rapports et Proces-Verbaux des ReHunions Conseil International pourl' Exploration de la Mer* 182: 37- 43.

Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol.* 13: 57–149.

Wilson- Ormond, E.A., Powell, E.N., Wade, T.L., Taylor, R.J., Presley, B.J., Brooks, J.M., 1992. Spatial and temporal distributions of contaminant body burden and disease in Gulf of Mexico oyster populations: the role of local and large-scale climatic controls. *HelgolaKnder Meeresuntersuchungen* 46: 201-235.

Wilson-Ormond, E.A., Ellis, M.S., Powell, E.N., Kim, Y., Li, S., 1999. Effects of gas-producing platforms on continental shelf macro epi fauna in the north western Gulf of Mexico: reproductive status and health. *Internationale Revue des gesamten Hydrobiologie*.

Winstead, J.T., Couch, J.A., 1988. Enhancement of protozoan pathogen *Perkinsus marinus* infections in American oysters *Crassostrea virginica* exposed to the chemical carcinogen n-nitrosodiethylamine (DENA). *Diseases of Aquatic Organisms* 5: 205-213.

****