INVESTIGATING THE EFFECT OF ALGAL BLOOM IN TISSUE (HEPATOPANCREAS & GILL) OF WHITELEG SHRIMPS (LITOPENAEUS VANNAMEI) (A CASE STUDY: SHRIMP PONDS OF ABADAN)

Ali Teaghsazzadeh,* Mohammad Seddiq Mortazavi, Seyyedeh Laili Mohebbi Nozar and Keyvan Ejlali Khanghah

*Persian Gulf and Oman Sea Ecological Research Institute, BandarAbbas, Hormozgan, Iran

Abstract
In the late summer of 2009, some shrimp ponds of Abadan were faced with the phenomenon of algal bloom. To investigate the effects of this phenomenon on various organs of the shrimps’ body, and its effects on growth and survival of the shrimps, 100 contaminated shrimps which were subjected to harmful algal bloom, were collected, then, they were fixed in Davidson fixation solution after recording their apparent symptoms. The results of investigating apparent symptoms of the shrimps showed that, the shrimps exposed to the algal bloom showed lethargy and slowness of movement, breathing difficulty, coming on the water surface and sides of the pool and ultimately death. Also, the shrimps had no desire to eat, and their gills color became dark brown which shows that, the algae were deposited on shrimp gills. In pathologic studies, hepatopancreas, gills, digestive organs and lymph organs had been highly damaged and cells of these organs had hypertrophy. Also, the alga causing algal bloom was identified as Mesodinium rubrum which was observed in various parts of digestive system and gills of the shrimps.

Keywords: algal bloom, Mesodinium rubrum, white leg shrimp, Abadan.

1-1- Introduction
During the past two decades, the industry of shrimp farming has develop globally. By increasing the world population, this trend seems to be continued. Severe shrimp mortality in coastal farms causes millions of dollars of damage to this industry. In Mexico, almost 99% of ponds are constructed in coastal areas and use the sea water. Many damaged shrimp farms have pumped water contaminated by red tide which has caused high rate of shrimps’ mortality. In shallow areas such as shrimp ponds, high concentrations of phosphorus and nitrogen of the water around, can be another source of nutrition for phytoplankton bloom. Moreover,

* Corresponding author at: Department of Ecology, Persian Gulf and Oman Sea Ecological Research Institute, BandarAbbas, Hormozgan, Iran.
E-mail address: ali.teaghsaz@gmail.com

almost 80% of the added nitrogen to the pool as a part of shrimp food, is not kept in their biomass but also, it acts as nutrition for phytoplankton. Some scientists have mentioned that, some species make bloom even without thermal and light conditions when the ration of N/P goes less than Redfield ratio.

Sampling of the pools show that, various phytoplankton forms cause abundance of red tide in the pools dependent on farming environment. Consequently, it is important for shrimp farming industry to improve
its control on plankton population in the ponds and monitor the shrimp population since the first stage of life until harvesting. This task is more important particularly after algal bloom which is determinant of oxygen shortage potential and mortality issues. Therefore, it is needed to develop productive actions with environmental stability and biosafety.

In most of cases, the shrimps’ mortality is due to high concentration of algae. For instance, sever reduction of water oxygen, gels production and its deposition in the body particularly gills and \( \text{H}_2\text{O}_2 \) production, are the main causes for aquatic losses about Cochlodinium. Also, Cochlodinium with a density higher than 50 cells per liter causes death of 100% of the aquatics within 24 hours.

E. Galimany et al. (2008) made Mytilus edulis exposed to the cultivation of toxic species Prorocentrum minimum or a type of Rhodomonas sp. in a glass aquarium. After a short time of adaptation, the samples were collected at the zero day (before exposure) and after 3, 6 and 9 days. The results showed that, M.edulis has been contaminated by P.minimum. Anyways, the observed specific results in M.edulis bivalves were different to the other reports about bivalves. These findings make study of the effects of HABs on various bivalve species important and show that, the achieved immunity responses for exposure of a bivalve species cannot reflect them for all bivalve species.

A. Gago-Martinez et al. (2009) conducted a study on determination of toxicological effects in Litopenaeus vannamei shrimps exposed to various cell densities of Gymnodinium catenatum and Karenia brevis. Acute assessment showed an appropriate survival rate in the shrimps exposed to low density of Ginoflagellates while, abnormal behavior and mortality were observed in the shrimps exposed to high density of Ginoflagellates. Chronic assessment determined significant differences in survival rate, food percentage, and gained weight in the creatures exposed to Ginoflagellates compared to the control group. Also, textural damages were observed in heart, digestive gland and brain.

A. Anton et al. (2007) investigated the first bloom of species Cochlodinium in Sabah, Malaysia. This bloom was observed in January 2005 for the first time. This bloom of Cochlodinium polykrikoides was diagnosed simultaneous with mortality of farmed fishes in the cage. Determination of these cells’ concentration between June 2006 and January 2005 indicated two peaks in March–June 2005 and June 2006. Cellular abundance in the sampling station of farmed fishes’ caves (where qualitative parameter of water such as \( \text{NO}_3\text{--N} \) and \( \text{PO}_4\text{--P} \) were high) reached \( 6\times10^6 \) cells/l. This bloom remained stable in August 2005 too. Also, it was not diagnosed during monsoon season of the North East; but, it occurred again in 2006. Suitable temperature, salinity and concentrations of nutrition that is similar to the other blooms caused by Cochlodinium polykrikoides in the Asian region of Pacific Ocean, increased the growth of these species similarly.

I. Gárate-Lizárraga et al. (2009) investigated algal bloom of Scrippsiella trochoidea (Gonyaulacaceae) in farming shrimp pools in the Western South of California Gulf in Mexico. They finally concluded that, algal biomass bloom is caused by extra productions of shrimp ponds, and the effect of these productions has not been studied in Mexico properly. Notification of this task is more important for the farmers since, outflow from their pools can lead to high yielding in the near marine water.

Considering the mentioned items, the importance of this study is that, it determines the risks of Mesodinium rubrum alga for shrimp farming and if these risks are evaluated important, the need for their control will be proved and if the risks are low, the studies can be directed to the subjects such as health of the shrimp consumers.
The objective of the present study is to investigate clinical changes and apparent symptoms faced with algal bloom in ponds of Abadan.

1-2- Environmental effects of red tide
Many news have been spread about harmful effects of red tide on animals and even human; one of them was spread in 1987 about the death of 14 hump back whales in Cape Cod Bay, USA. An autopsy of the animals represented that, their death was due to food poisoning. Many reports also state poisoning and even death of the human caused by eating the aquatic.
The conducted investigations consider all these happenings directly or indirectly related to the poisons generated by some unicellular algae or plant plankton and through their intense bloom which is called red tide.
Also, environmental variations affect deeply the growth and accumulation trend of these plant plankton and their poisoning effect.
Some algae such as Pyrophyta cause aquatics’ mortality by consuming oxygen in addition to poison production; so that, Karenia btevis Pyrophyta frequently cause mortality of the fishes in Mexico Gulf. The conducted investigations in gills of the fishes showed Neurotoxin of such algae.
Damages of the algae causing red tide is higher in ponds since, closed environment of these pools provides better the context of high increase of these algae’ population. Annually, countless fishes are damaged by plant plankton and such cases represent harms of the harmful plankton on the aquatics.
Decomposition of pant plankton has proved Hydrogen sulfide in these algae that is so harmful for the creatures.

2- Materials and methods
2-1- Study area
The site of shrimp farm is located in the South East of Abadan city and Bahmanshir riverside and has 116 farms with 5000 ha area. The needed water for the farms is provided by tide method from Bahmanshir River of which water salinity is reduced due to increase of precipitation in the autumn and winter.

2-2- Collection of sample
In the late September 2009, algal bloom occurred in some pools which had been stored to determine appropriate density of Litopenaeus vannamei shrimps farming. 100 dying and lethargic shrimps were collected from these pools. Then, they were transported to Institute of Ecology Persian Gulf and Oman Sea for pathologic operations after recoding their apparent symptoms. By the way, a number of shrimps without algal bloom were collected, fixed and transported to the institute for comparison, after recording their apparent symptoms.

2-3- Pathologic operations
Here, Dr. Leitner’s method was used to conduct histopathology operations which the standard method for shrimp.

2-4- Samples fixation

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By fixation, the cells and tissues are maintained just like when they were alive. The shrimps are required to be fixed as alive immediately after exiting from water to prevent changes after death and damages to the tissues. Davidson fixator was used in this study to fix the samples.

2-5- tissues preparation and cutting
After fixation, the tissues were cut before being prepared for molding. Scissors or razor was used to avoid scratching or damaging the tissues. The shrimps smaller than 3 cm were cut longitudinally from the outer to the inner side.

2-6- Tissue preparation and dehydration
Putting the tissues in paraffin causes to makes appropriate cuts to study the tissues. But, when water exists in the tissues, paraffin does not enter the tissue. Dehydration of the tissues was conducted gently by putting them in low concentration alcohol and then, reaching a 100% concentration.

2-7- Placing the issues in paraffin or preparation of tissues block by paraffin
After tissues preparation including dehydration, paraffin penetration and air discharge were conducted completely in tissue processing device. The tissues were made as paraffin molds. For this task, the tissues were paced in a metal mold and was filled up by liquid paraffin. Tissues cutting was carried out using Microtome tissue slicer which was in Institute of Ecology Persian Gulf and Oman Sea. To produce pathology slides, the prepared slices were paced in warm bath device to open the tissues adequately. Then, the tissue slices were put on microscopic sides which were impregnated with a small amount of adhesive protein. The slides were fixed at temperature of 40-50 °C for 30 minutes.

2-8- Painting operation
For all cellular painting methods of slices generated by paraffin molds, first the slices must be cleaned from paraffin and dehydrated; so the colors are absorbed by the tissue. Hematoxylin and eosin / fluoxetine color was used to study shrimp’ tissues.

3- Results
3-1- Observation of shrimp apparent symptoms
The shrimps of contaminated pools suffered severe stress. They showed crazy behavior which was different to the healthy shrimps that had no sign of stress. Also, the affected shrimps came close to the water surface and swam with open mouth and gill. This swimming continued with shrimp activity reduction until reaching the pool sides, and then, mortality began.

Observation of pathologic symptoms: The profiles produced by hepatopancreas, gills and lymphoid organs were painted by common painting method of H&E and were observed under a microscope.
Figure 1. Gill of a vannamei shrimp affected by algal bloom. Accumulation of lymphoid cells in the gills represents the effect of alga on the gill which is confirmed by lymphoid cells accumulation in the location for defense. H&E/Phloxin. 10μm

Figure 2. Another part of gill that shows accumulation of lymphoid cells (the arrow)

Figure 3. Hepatopancreas tissue of the shrimp contaminated by alga Mesodinium rubrum which shows hepatopancreas cells hypertrophy (the arrow) H&E/Phloxin. 10μm
Figure 4. Hepatopancreas tissue of the shrimp contaminated by alga Mesodinium rubrum which shows both hepatopancreas cells hypertrophy (the blue arrow) and vacuolization of cells (the red arrow).

**4- Discussion and conclusion**

Hepatopancreas cells showed clearly hypertrophy and vacuolization and the presence of numerous vacuoles (Image 1). Star shape of the natural hepatopancreas tubules had been deformed as ellipsoid. Also, reduction the number of hepatopancreas lumen tubes, separation of the basal membrane of the tubules were observed and clear inclusions (Image 2).

In most of gill cells affected by bloom, accumulation of lymphoid cells in the prepared microscopic sections was observable. There are also, some other observations about these cells including enlarged nuclei, their hypertrophy, numerous specified blue inclusions in the cells affected by cell necrosis, and malformation of gill filaments (Images 3, 4).

Smith (1966) reported Cyanobacteria bloom in four different shrimp farms in the years 1992, 1994 and 1995 which was contemporaneous with shrimps’ mortality. Location of these farms was from Clarence River to Cairns. In these farms, the shrimps began to show symptoms at weight by 15-25 gr, and their growth had become slower and they molted with difficulty and at the next stage, the shrimps were found at a dying state on the pool edge.

In another experiment, Perez Linares (2009) showed that, toxic dinoflagellates causes severe damage to Gastric glands and esophageal nerve nodes of a shrimp. The results of this study were consistent with the results of our investigations as well as the results of pathologic operations of Abadan shrimps which were exposed to algal bloom.

**References**

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