The Effect of Water pH on Ventilation Frequency and Oxygen Consumption in Mullet (*Mugil cephalus*)

M. K. Kanagaraj[1], Lakshmipathi M. T[2], Asta Zerue[3], Samuel Berhane[4], Yemane Mobaetsion[5], T.J. Ramesha[6], A. T. Ramachandra Naik[7]

[1]Associate Dean for Research and Post graduate Program, College of Marine Science and Technology, Massawa, Eritrea., N.E.Africa

[2]Colleges of Fisheries, Mangaluru, Karnataka, India

Abstract: ***Estuarine and coastal marine environments in particular are affected by a wide range of pollutants owing to increased anthropogenic activity from a burgeoning population in the coastal zone. The marine environment has long served as major repositories for disposal of industrial and municipal wastes, sewages, sluges, and dredged material. Ventilation frequency of control fish did not show significant (at 5% significance level and p<0.001) variation throughout the period of study. Mugil cephalus exposed to pH4 survived only for six days of the ten days period of study, whereas the fish exposed to pH5 and pH6 survived till the end of the period of study. Increased ventilation frequency was observed in all the treatments immediately after the fish were exposed to low pH.***

Keywords: pollutants, ph, ventaliation, fish

1. Introduction

Estuarine and coastal marine environments in particular are affected by a wide range of pollutants owing to increased anthropogenic activity from a burgeoning population in the coastal zone. The marine environment has long served as major repositories for disposal of industrial and municipal wastes, sewages, sluges, and dredged material. Contaminants associated with these waters have impacted biotic communities and sensitive habitat areas. Pollutants primarily enter the near shore oceanic waters via pipeline discharges, disposal from vessels, riverine input, atmospheric deposition and non-point source running off from land (Kennish, 1994). Ocean dumping of anthropogenic wastes has been gradually reduced since 1972, because of enactment of national, regional and international regulations designed to minimize environmental impacts on aquatic ecosystems and increased public awareness, however most of the regulations do not have an enforcement body, as a result they are all being infringed by offenders and pollutants are entering the marine environment.Despite its vastness, its unique physical characteristic and its importance as a primary dilutor of deposited waste, the ocean has a limited assimilative capacity for pollutants. Given some unassimilated materials, such as long-life radioactive wastes and synthetic toxic organic compounds will accumulate and remain essentially unaltered in the marine environment. The persistence of these hazardous wastes in oceanic environments may pose a long term danger to marine food webs.

The toxic effect of chemicals can be influenced by various physico-chemical factors including temperature. Exposure to sub-lethal concentrations of chemicals can cause stresses, which limit an organism’s ability to survive or ability to tolerate changes in various environmental factors, such as temperature (Carrier and Beitinger, 1988).

The pH of marine waters is usually quite stable (between 7.5 and 8.5 worldwide) and is similar to that of estuarine waters because of the buffering capacity provided by the abundance of strong basic cations such as sodium, potassium, and calcium and of weak acid anions such as carbonates and borates (Wetzel 1983). Higher pH are usually found in near-surface waters because of solar radiation. The effect of solar radiation on pH is twofold, it promotes photosynthesis and increases surface temperatures, both of which decrease the amount of free carbonic acid and consequently raise the pH (Wetzel 1983). Such effects can be the result of both diurnal and seasonal fluctuations.

In the marine environment, pH changes can also significantly affect the chemical forms and toxicity of other substances. In water, ammonia exists in two forms: a non-ionic species, NH_3, and an ionic species, NH_4^+. The toxicity of ammonia to marine species is largely determined by the concentration of the non-ionic species. The relative concentration of NH_3 in seawater is influenced in part by pH; with a reduction of one pH unit, the amount of NH_3 in water diminishes by a factor of 10 (Miller *et. al.*, 1990). Responses of marine organisms to alterations in ambient NH_3 concentrations as a result of pH fluctuations will vary between species. The speciation of metals and the solubility of some organic chemicals are also strongly influenced by pH. Environmental health is generally determine by monitoring changes in physical and chemical characteristics, but discharge of complex mixtures of contaminants into aquatic environments also necessitates biological assessment to determine damage to aquatic organisms. Ventilator behavior has been successfully uses as a tool for indicating environmental stress (Diamond *et. al.*, 1990), and a variety of techniques have been developed and utilized for such monitoring, ranging from simple visual observation to non-invasive sensing electrodes.

Ventilation rate (VR) is a good tool for assessing both alertness and stress in fish. (Barreto *et. al.*, 2010). VR can be measured by direct observation of the fish and counting; thus, there is no requirement for sophisticated equipment, and invasive painful or stress-including techniques can be avoided (Barreto *et. al.,* 2010).Ventilation rate and similar responses of fish can be further amplified by exposure to chemicals discharged in these environments, resulting in damage to their gills and heart, and affecting their metabolic and cardiac rates (Ford *et. al.,* 2004).

Ventilator responses of fish are considered to be more sensitive indicators of sub-lethal effects because gills are a vital interface between their external and internal environment and are the major organs for respiratory and ionic exchange. Respiration may also be important in the process of toxicant accumulation by fish and other aquatic biota (Black *et. al.,* 1991). Ventilation frequency is a sensitive behavioral indicator for distinguishing stress responses in fish among different stressors. Hence, it is a particular interest to examine changes in the pattern of ventilatory responses caused by exposure to sub-lethal concentration of a toxicant at different temperature regimes.

Measure of oxygen consumption has been used as an indicator of stress in organisms. But most of the work done on changes in oxygen consumption is on the effect of temperature, metal and pesticide toxicity. Kieffer and Wakefield (2009) studies the effect of temperature on oxygen consumption and ammonia excretion in juvenile Atlantic salmon, *Salmo salar*. Leonard and Malcom (2010) observed changes in oxygen consumption and ventilation frequency of blue cod *Parapercis colias* exposed to hydrogen sulphide. Review of literature suggests that few long term or life cycle studies have been conducted to find out the effect of water pH on the oxygen consumption and ventilation frequency of commercially important fish species *Mugil cephalus*. As pH may influence sea water chemistry in several important ways notably the carbonic acid equilibrium, chemical form of metals, and the characteristics of dissolved substances, it was decided that pH tolerance deserves more interest from biologists than it has received hitherto. The insight purpose of this paper is to serve as an introduction and possibly stimulate further research. The complex physiological aspects involved require a detailed analysis for each type of processes that may be affected.

2. Materials and Methods

Mullet (*Mugil cephalus)* were used in the present study. Fish were caught from the Gurgussum area of Red Sea and were transported immediately to the laboratory. Fish were kept in glass tanks and acclimatized to the laboratory condition for ten days. Water was changed twice a day in the initial stage of five days and later on once in a day. Fish were fed with artificial fish feed supplied by the animal and fish meal company Massawa, Eritrea.

Sea water was collected from Hirgigo bay and transported to the laboratory and stored in the laboratory condition before being used for filling the fish tanks. The physicochemical property of sea water used in the present study was as follows: temperature 30± 0.196°C, salinity 39± 0.133 ppt, pH 7.8± 0.113, Dissolved oxygen 11.6± 0.083 mg/L (the values are average ± S.E for the period of study).

Healthy fish of 9-10cm were selected from the stock. Fish of both sexes were used for the experiment. Four glass tanks of 40L capacity were filled with 20L water each and twenty fish were introduced in each tank. One tank served as the control and the three were kept as experimental.

Hydrochloric acid and Sodium hydroxide were used to adjust the pH of the water to attain the desired pH for the experiment. Three different pH (pH 6, pH 5, and pH 4) were selected for the present study. Tests of pH effects are difficult to perform because the organisms tend to modify the incipient conditions by their metabolic activities. In some cases this has resulted in pH variations (mostly rising pH) during the experiment as reported by Knutzen (1981). So pH of the water was checked and was adjusted at regular intervals to maintain constant pH. This is because the laboratory does not contain facilities for conducting experiment using continuous flow method.Water was changed every day and observation made for a period of ten days. Ventilation frequency and oxygen consumptions of control and the experimental fish were recorded on alternate days for a period of ten days.

Ventilation frequency

Fish from the control and experimental tanks were transferred to a plastic trough containing one liter of sea water with the desired pH. Ventilation frequency was recorded according to the method of Barreto *et al.* (2003) and Alvarenga and Volpato (1995). Twenty successive opercular movements were counted by visual inspection the time elapsed was registered with the aid of a chronometer and the frequency per minute was calculated Care was taken to exert minimum stress to the fish and time was given for the fish to settle down before the ventilation frequency was recorded. Six individual observations were made and the mean was taken similarly six fish were used from each tank and the mean value was recorded.

Oxygen consumption

Dissolved oxygen was estimated using dissolved oxygen meter, transparent bottles of one liter capacity were used. The bottle was filled with sea water and one fish from the experimental tank was selected, weighed and then introduced into the trough. The trough was covered by a polythene sheet to avoid contact with air. Care was taken to avoid any air bubbles in the container. A small hole was made on the polythene sheet to insert the electrode of the oxygen meter. Another polythene sheet was used to cover the electrode and the trough to avoid any air that can interact with water in the trough. The trough was kept undisturbed and the dissolved oxygen reading was recorded after one hour. Oxygen consumption per gram body weight of fish per hour was calculated by using the formula suggested by Barreto and Volpato (2004).

$$\text{Oxygen consumption (mg/ g body weight/ hour)} = \frac{\text{Initial DO} - \text{Final DO}}{\text{Weight of Fish}}$$

Ventilation frequency and oxygen consumption values were tabulated and statistical analysis was done using‘t’- test.

Correlation between ventilation frequency and oxygen consumption was determined using Karl Pearson's correlation co-efficient method.

3. Results and Discussion

The Changes in frequency and oxygen consumption for control and fish exposed to low pH are given in Table 1 and Fig 1.

Ventilation frequency of control fish did not show significant (at 5% significance level and p<0.001) variation throughout the period of study. *Mugil cephalus* exposed to pH4 survived only for six days of the ten days period of study, whereas the fish exposed to pH5 and pH6 survived till the end of the period of study.Increased ventilation frequency was observed in all the treatments immediately after the fish were exposed to low pH. In fish exposed to pH4, maximum ventilation frequency (287.50 ± 10.83) was observed on the second day of exposure. There after the ventilation frequency declined and reached the minimum of 71.66 ± 10.32 on the sixth day.Fish exposed to pH5 exhibited a gradual increase in ventilation frequency throughout the experimental period. A value of 195.00 ± 4.47 beats/ min was recorded immediately after exposure to low pH and a maximum of 226.66 ± 4.08 beats/ min. was recorded on the tenth day.In pH6, fish showed an increase in ventilation frequency which recorded the maximum of 190.00 ± 4.47 beats/ min on the second day. Ventilation frequency declined there after reaching 172.50 ± 2.73 beats/ min on the tenth day.

Statistical analysis revealed that the value of ventilation frequency recorded on eight and tenth day of fish exposed to pH6 were not significant when compared to that of control.

Table 1 and Fig.2 shows the values of oxygen consumption in fish *Mugil cephalus* exposed to three different pH levels of water. Control fish did not show any significant change in its oxygen consumption throughout the period of study. Fish exposed to pH4 water exhibited a sudden increase in oxygen consumption up to second day. Oxygen consumption declined thereafter and recorded a minimum of 0.25 ± 0.023 mg/g body weight/hour on the sixth day before the fish died.Oxygen consumption of fish exposed to pH5 recorded a maximum on second day of exposure (0.91 ± 0.020) and then exhibited a steady decline till the tenth day of exposure recording a minimum of 0.62 ±0.032.

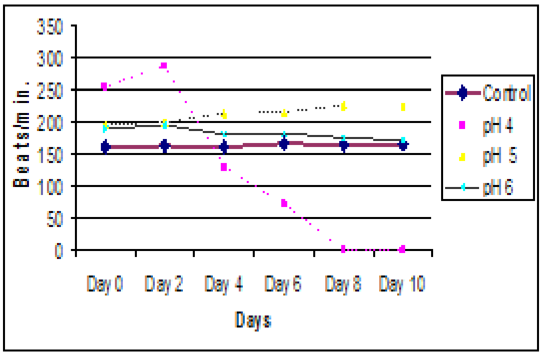

Figure 1: Changes in Ventilation Frequency (VF) of fish *Mugil cephalus* exposed to different pH levels of water.

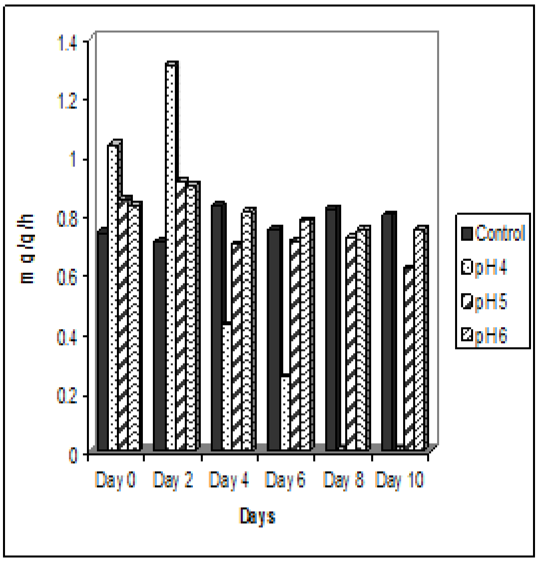

Figure 2: Changes in oxygen consumption of fish *Mugil cephalus* exposed to different pH levels of water.

Fish exposed to pH6 also showed an increase in the ventilation frequency on the day of exposure (0.83 ± 0.063) and on the second day (0.90±0.030). On the subsequent days the oxygen consumption showed a slight decreasing trend.

Statistical analysis revealed that the oxygen consumption of fish exposed to pH4 and pH5 were statistically significant when compared to that of the control. But in fish exposed to pH6, the oxygen consumption did not show significant variation from that of the control on the fourth, sixth and tenth day of exposure (at 5% of level of significance and p<0.001).

An attempt was made to correlate the ventilation frequency and oxygen consumption in *Mugil cepahlus* exposed to different pH levels of water. A low positive correlation (correlation co-efficient 0.0257) between ventilation frequency and oxygen consumption was recorded for the control fish. For fish exposed to pH4 and pH6 a high positive correlation (correlation coefficient 0.992 and 0.943, respectively) was obtained. A negative correlation (-0.900) was obtained for fish exposed to pH 5.Research and technological advancements have significantly changed some of our views towards marine pollution. Recently the importance of the effects of pollutants on behavior of marine organisms has been recognized that pollutant may not cause the death of an individual, it may cause serious impairment to behavioral responses.A broad spectrum of marine and estuarine organisms has been shown to be adversely affected by pH fluctuations (Wickins, 1984). Many of these effects are physiological. An increase in acidity can affect physical process in organism. Barreto *et. al.* (2010) reported that ventilation rate of fish increased after the introduction of alarm cue. The above authors suggested that ventilation frequency can be regarded as a reliable indicator of alarm reaction. According to Fernandes and Rantin (1994), hyperventilation increases the uptake of oxygen and this automatic adjustment may prepare the fish for escape from a predation threat.

Ventilatory frequency (VF) responses represent a good alternative for alert or stressful condition assessment in fish, because it has several advantages. Investigations have shown that ventilation frequency is changed quickly in responses to disturbances imposes, as demonstrated in fish subjected to social stress (Volpato *et. al.*, 1989; Alvarenga and Volpato, 1995), strobe light (Sager et.al, 2000), predation risk (Baretto *et. al.*, 2003; Hawkins *et. al.*,2004 a.b; Queiroz and Magurran, 2005) and confinement (Baretto and Volpato, 2004; Baretto *et. al.*, 2003). These studies, however, have not corroborated ventilation frequency as an unequivocal indicator of acute stress in fish. Therefore, in the present study, we evaluated the adequacy of this non-invasive and inexpensive technique as an indicator of stress in Mullet.

In the present study the initial increase in ventilation frequency of fish exposed to low pH is supported by the finding of Lang *et. al.* (1998) who suggested that the first clearly recognizable reactions of fish to pollutant were detected immediately after the start of the experiment. The increase in ventilation frequency of fish exposed to pH 4 on the second day and a steady drop in ventilation frequency up to day six and then the death of fish after sixth day is supported by the findings of Lang *et. al.*(1987).

The decrease in the ventilation frequency after an initial increase in more acidic water (pH4) can be attributed to the fact that the stress induced in the fish due to low pH at the end of sixth day of exposure was so high that the stress induced in the fish due to acidic water could not be tolerated any longer and they died. Vutukuru *et. al.* (2005) recorded similar observations in fish exposed to copper sulphate toxicity. Decreased ventilation frequency in higher concentrations may be due to the failure of the homeostatic mechanism to cope up with the increasing stress due to acidic water.The reaction and survival of aquatic animals depend on not only the biological state of the animals and physiochemical characteristics of water, but also on kind, toxicity, type and time of exposure to the toxicant. Nielsen and King (1995) and Gehrke (1988) found a large innate variation in ventilation pattern in fish exposed to high temperature and suggested that an increase in temperature in the aquatic environment can influence the respiratory functions of fish in relation to their rate of heart beat, ventilation frequency, oxygen consumption and uptake of water borne compounds. Similar observations were also made by Moffit and Crawshaw (1983); Berschick (1987) and Ford *et. al.*(2004).

The changes in oxygen consumption in fish exposed to low pH may be due to respiratory distress as a consequence of impairment in oxidative metabolism. A similar observation was made by Vineetkumar and Davidl (2009) in fish exposed to malathion toxicity. Tilak *et. al.*(2007) reported that the rate of oxygen consumption decreased progressively with the increase of ammonia, nitrite and nitrate in the water. Ketpadung and Tangkrok-Olan (2006) observed that copper toxicity affects the oxygen consumption of crab *Portunus pelagicus*.

Simic and Brancelj (2006) found that crustacean metabolism is affected by acidic waters. The above authors suggested that the lowered respiration ratios recorded in crustaceans exposed to strongly acid water may be due to the fact that acidic water affects metabolism of organisms. The above authors further reported that metabolic potential is lower in strongly acidic water. The abnormal rise in the oxygen consumption in fish exposed to pH 4 may be due to the fact that the fish was trying to cope up with the stress and then it exhibited a low oxygen consumption rate as suggested by the above authors. The low oxygen consumption recorded after the second day of exposure and subsequent death of the fish may be due to accumulation of mucous on the gill and alteration of osmo-ionic balance. Increased oxygen consumption in strongly acidic water indicates an acid stress that leads to the collapse of metabolism and subsequent death of the organisms, as suggested by the above authors.

A link between the ventilation frequency and oxygen consumption was observed in the present study. Low pH can cause excess secretion of mucus on the gills as a protective mechanism to prevent further damage to the gills. This may also disrupt the oxygen uptake by gills and disruption of osmo-ionic balance which may cause stress to the fish. Sornaraj *et. al.* (1995) and Rupa-Mathew *et. al.* (1997) suggested that as a result of gill damage oxygen consumption decreases which will cause further stress in fish. In order to make more oxygen available from the water, the fish increase their ventilation frequency; similar observations were also made in present study when fish were exposed to pH4 and pH5.In fish exposed to pH6, after the initial increase of the ventilation frequency, a steady decrease occurred. This may be due to the fact that the fish were able to acclimatize to the less acidic water to a certain degree. This gets support form similar observations made by Lang *et. al.* (1987) in trout exposed to ammonia toxicity.

The results of the present study reveal that under the given experimental conditions, the fish exposed to pH six were apparently capable of a relatively rapid adaptation to low pH when compared to that of fish exposed to pH5 and pH4. Never the less, they must be considered stressed as revealed by the elevated ventilation frequency and alteration in the oxygen consumption.

4. Conclusion

It can be observed that, with the current trend of global warming and pH altering dumping of industrial and domestic wastes in the oceans combined together has a synergistic effect and highly pronounced negative impacts on the marine environment and its biota. The final results of the present study show that lowering of the pH of sea water affects fish ventilation frequency and oxygen consumption. At more acidic levels (lower pH) fish mortality occurs. More importantly as Eritrea is found in the tropical region which exhibits the highest average annual temperature and the Red sea owing a limited water exchange, pH alteration in any case would have times fold acute and catastrophic effect. Thus, based on our results of the current study we strongly recommend that studies on the pH alteration of the marine environment should be given a due regard.

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