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THE AVAILABILITY OF *Mytilus galloprovincialis* FOR MONITORING ENTERIC BACTERIA

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Abstrac: *In this study, the usage of Mediterranean Mussel (Mytilus galloprovincialis Lamarck, 1819) as monitoring organism on enteric bacteria concentrations in heavily polluted marine environments and its use possibilities as water quality improving tool were investigated.*

The ability of the Mediterranean Mussel to accumulate and purge fecal coliform bacteria investigated in laboratory experiments. First, increase on bacteria concentration was observed on 1,5th hour and sharp decrease rate lasted until 10th hours after that period slow but steady declining bacteria concentration rate was observed and beginning bacteria concentration rate was reached within next 30- 50 hours. Time dependent bacteria concentration reduction has found statistically significant at $p<0.001$ (r-sq = 0.81).

The investigation has also revealed that mussel farming could be established in the over polluted area which is the case only in the different discharge points in the sea.

Keywords: Mediterranean Mussel, *Mytilus galloprovincialis*, enteric bacteria

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I. INTRODUCTION

Total and fecal coliforms are used as indicator organisms to evaluate the degree of fecal pollution in aquatic environment [1]. The presence of *Escherichia coli* or other coliform bacteria in streams, rivers and marine ecosystems has been used as an indicator of the possible presence of human pathogens [2]. Federal, state and local regulations limit the allowable concentrations of bacteria in the aquatic environment, requiring frequent monitoring and remedial actions when allowable concentrations are exceeded. High bacterial concentrations and resultant remedial actions, such as closing beaches and changing water treatment methods, have important human health and economic implications. Therefore, it is important to locate, evaluate and if it is possible to eliminate sources of bacterial contamination. A key component of this process is developing efficient monitoring methods for locating bacterial sources [3]. The most important biofilter is the sum total of filter-feeding aquatic animals. Among them are bivalves, crustaceans, rotifers, some protests, bryozoans, tunicates and other organisms [4]. Filter-feeding organisms are very important to regulate a variety of parameters and processes in aquatic ecosystems. Bacterial adsorption and sedimentation may also pose an indirect public-health risk as a result of the consumption of contaminated sea-food due to settlement of the particles carrying pathogens to the bottom layers of the water [5]. For example, oysters, mussels, clams and cockles are known as efficient filter-feeders, concentrating particulate matter and microorganisms [6]; therefore bacteria adsorbed by particulate matter reaching the bottom layers of the water appear to have a considerable change of being taken up by these shellfish [7].

In marine environment, high efficiency of the filter-feeders in removing phytoplankton (and others) from the water column was shown in different studies in South Eastern Black Sea and the Marmara Sea.

The Mediterranean mussel (Figure 1), a bivalve mollusc, two shells are equal, nearly quadrangular, outside black-violet coloured and made of mother-of-pearl inside; at one side the rim of the shell ends with a pointed and slightly bent *umbo* while the other side is rounded. The maximum size reaches nearly 15 cm but the most common one is about 5-8 cm.

The mussel's feed is made of microscopic algae and organic particles detained by gills, which absorb in the same time the oxygen, necessary for breathing. For the survival of the species the temperature of the water has to be between a few degrees above the 0°C and 35°C. Usually the seeds settlement takes place within the first 3 m in depth. The juveniles of 1 mm length are already fixed at the substratum and have the same aspect as the adult mussels and after one year they'll reach the commercial size of 5 cm. Once they've reached the sexual maturity, 2 or 3 seminal cycles (corresponding to autumn, winter ad spring) will succeed every year, with activity peaking in the wintertime (URL 1).

Fig. 1. *Mytilus galloprovincialis* (Mediterranean mussel) (URL 2)

Many of the bacteria algae and zooplankton captured by this filtration, sorting and selection process may still be viable even after passing completely through the gut, indicating that digestion of captured organisms is often incomplete in mussel. Since bivalves do not normally contain fecal coliform bacteria in their gut, the presence of such bacteria in a mussel is hypothesized to indicate a recent environmental exposure of the mussel to bacteria rather than to reflect their endogenous presence in the mussel ([8]; URL 1).

The clearance rate of bacteria by zebra mussels recalculated from Silverman was 86.4 ml/mussel/hr for *E.coli* and 40-50 ml/mussel/hr for other fecal coliform bacteria, such as Citrobacter and Enterobacter [9]. Moreover, these bacteria were filtered at a much faster rate when compared with other unionids. Low bacterial concentrations combined with wastewater conditions might play an important role in a decreased filtration rate by mussels.

The study design was employed a static system that determined the filtering capability of Mediterranean mussels for enteric bacteria in marine water under laboratory conditions.

II. MATERIAL AND METHODS

Adult (> 50 mm shell length) Mediterranean mussels (*Mytilus galloprovincialis*) used in the study were collected from fish cage mooring lines in Yomra harbour, near province of Trabzon (41º 01’ 29” N – 39º 36’ 35” E). Mussels were carefully detached from settlement surfaces and transported to the laboratory in a cooling box. On arriving the laboratory mussels were cleaned off fouling organisms and other unwanted periphyton matter on shell surface using a clean blade and hard brush. Around 30 mussels were stocked in a clean aquarium containing sterile seawater at 16-18ºC for 3 days for acclimation and purification before the filtration trial.

A static experimental system consisting of 4 jars of 1 L volume, three replicas and one control was set up to determine filtration and consumption rates. Purified selected mussels were placed in experimental jars containing designated concentrations of laboratory cultured coliform bacteria. Mussels with shell length of 61.8 -70.9 mm were used in the experiment. Starting from the experiment water samples of 10 ml from each jar were taken every 1 hr. Before water samples were taken water in jars were mix thoroughly without disturbing the mussels. On the base of previous studies [3], the experiment has lasted 70 hours.

Fecal coliform (cfu/100 ml) concentrations were determined by the membrane filter technique according to the Standard Methods procedure [10]. Membrane filter technique was applied for coliform analysis. An appropriate volume of sample was immediately vacuum filtered through a sterile mixed cellulose esters membrane filter, 0.45 µm pore size and 47 mm diameter. The filter papers were placed in Petri dishes having special chromogenic agars and incubated at 24 h at 37±2 C°. After 24 h incubation period, the blue colonies were counted *E.coli* and the pink colonies as fecal coliforms. All analyses were performed in double controls as recommended in methods.

Consumption was estimated from reductions in cell numbers, while filtration rate (FR), the volume of water filtered of particles (cells) per hour, was estimated by following the exponential decline in bacteria cell numbers [11]:

$$FR\ (l/h) = V \times \log_e (Co/Ct) / t;$$

where V is volume suspension in experimental chamber, Co and Ct are cell concentrations at successive samplings at time *t*.

A descriptive statistical analysis was applied to determine mean and standard deviations of shell length, live weight and dry tissue weight of mussels, consumption and filtration rates of enteric bacteria.

III. RESULTS

Changes in average coliform bacteria concentrations in water and filtration rates during trial period are presented in Figure 2. Within three hours, the mussels dramatically decreased the fecal coliform (FC) bacteria from seawater: they removed %90 of the bacteria in 1.5 hours. This % 90 removal period was consistent with the value obtained in a study of other similar marine bivalve, *Mytilus edulis* [3].

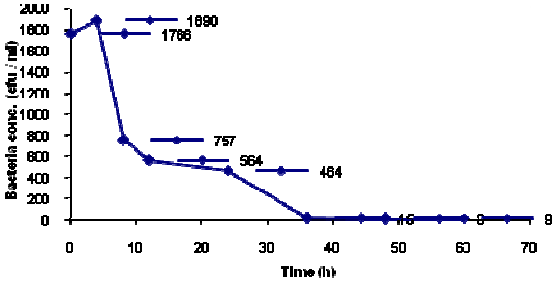

Fig. 2. Uptake of coliform bacteria from water by Mediterranean mussels as a function of time.

The concentration of bacteria in the water remained in an elevated range for about 4 hours and then sharp decline observed until 10th hour. Then, declined slowly to lower concentrations 20-24 h after that again strong decline trend was observed until 36th hour, then concentration rate was almost stabilized around 9 cfu/ml through end of the experiment. Time dependent bacteria concentration reduction has found statistically significant at $p<0.001$ (r-sq = 0.81).

IV. CONCLUSION

This study shows that, mussels are able to accumulate enteric bacteria rapidly. Mediterranean mussels are able to concentrate the filtered bacteria, that increasing the sensitivity for detecting them. Rosley et all., investigated the relationship between the enterococci concentrations in seawater and the accumulation in *M.edulis* in their study [12]. In this laboratory experiments showed that maximum concentrations of enterocci in *M.edulis* was reached after 4–6 h of exposure, and target bacteria accumulated by a factor 27–330 relative to concentrations in the surrounding seawater. Solic et all., found that FC concentrations in mussels were in high correlation with FC concentration in the shellfish growing waters [13]. At low initial concentrations of FC in bivalves, the rate of FC concentration increased with the concentration of FC in seawater and with changes of temperature toward optimum. As the concentration of FC in bivalves increased, the rate of FC concentration decreased more rapidly as the concentration of FC in seawater increased and as the temperature was closer to optimum.

Filtration rate generally increases with temperature [14]; however, temperatures above 20°C may also cause a decrease in filtration rates [15]. During experiment, water temperature was between 16-18ºC. Solic et all., showed that at the same concentration FC in seawater, *M.galloprovincialis* concentrated more FC during winter than during the summer period, that is at lower temperature [13]. Although the Mediterranean mussel is most efficient at filtering particles ranging in size from 15 to 40 µm in diameter , they have also been observed to filter particles as small as 0.7 to 1 µm. Typical *E.coli* size is approximately 2 µm long [1]. While this size is near the lower end of the mussel's filtering ability, these bacteria are often found attached onto particles, such as algae and detritus. This size is also within the optimum size range for zebra mussel consumption. Lei et all. demonstrated nearly 100 % removal of the 2 µm size class, while Silverman et all., showed excellent clearance of *E.coli*.[16; 9]

This study confirmed, characterized and utilized this capability of Mediterranean mussels at filtering and concentrating fecal coliforms. However, some practical problems include:

Laboratory conditions may greatly differ from actual environment. For example; fecal coliform concentration may change with local current direction in sea water. Although, we know the mussel's internal bacteria concentration rate is significantly greater than surrounding water. This is supported, who concluded the concentration of *E. faecalis* in *M.edulis* corresponded to a two-fold greater concentration of *E. faecalis* in the mussels compared to the surrounding seawater [12]. But in our study we did not measure the mussel's internal bacteria concentration rate. Mussels may not filter at the same rate or for the same amount of time at different locations.

Temperature, presence of noxious chemicals and concentrations of particulates in the water are all known to affect filtration rates and could account for differences in bacterial uptake at different sites. Seawater temperatures affect the survival of the microorganisms and then their clearance from mussels. *E.coli* survives better at 14°C. On the other hand, *E. coli* elimination may be enhanced at 21 °C. Marino et all., investigated that the uptake of *E.coli, Vibrio cholerae* and *E.durans* by *Mytilus galloprovincialis* at temperatures of 14 and 21 °C [17;18].

The study showed that in water contaminated with either single or mixed organisms, the bacteria accumulated rapidly in the mussels reaching high concentrations after 1 h. With both single and mixed organisms, the maximum numbers of *E. coli* in mussels were 6.6 $\log_{10}$ CFU/g at 14 °C and 5.4 $\log_{10}$ CFU/g at 21 °C.

This study have shown that the uptake and persistence of enterococci are less influenced by temperature making them potentially better indicators in mussels. In spite of above stated problems use of Mediterranean mussel to monitor coliform bacteria and water quality improving tool looks quite promising.

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