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Bacterial pollution indicators associated in the tissues of an estuarine fish *mugil cephalus* from Ashtamudi lake, a RAMSAR site (Kerala, India)

Indicadores de contaminación bacteriana asociados en los tejidos de un pez estuarino *Mugil cephalus* del lago Ashtamudi, un sitio RAMSAR (Kerala, India)

Jyothylakshmi. K[1*], S. Nandakumar[2] and M.G. Sanal Kumar[3]

[1, 2 &3]Post Graduate and Research Department of Zoology, NSS College, Pandalam, Kerala, India

*Email: jyothylakshmik@gmail.com

ABSTRACT

Fishes are continuously exposed to the microorganisms present in water and in the sediment. The present study was attempted to screen the pollution indicator bacteria in the tissues of an estuarine fish *Mugil cephalus* from Kureepuzha Backwater, (Ashtamudi Lake). Total heterotrophic bacteria, total coliform bacteria, *Escherichia coli* and fecal *streptococci* were enumerated from the fish tissues using standard microbiological procedures. Comparison of bacterial count in the skin, gill and gut of *Mugil cephalus* revealed that highest count of indicator microbes were found in the skin followed by the gill and gut of fish from all four sites except site 4. Fishes from site 4 (Panamukkam Kadavu) they show bacteria predominantly in the gut. Two way ANOVA showed a significant difference in microbial count between sites and between tissues except Total heterotrophic bacteria and fecal *streptococci* between sites. Results of physico chemical parameters also supports that the anthropogenic activities accumulated in the study area, causing the disruption of water quality of Lake and thus increases the load of detrimental microorganisms. Hence it is recommended that good processing of fish such as washing, scraping scales, removal of gills and gut contents and proper cooking help to reduce microbial pathogen in fish body and make it safe for consumption.

Keywords: *Escherichia coli,* fecal *streptococci,* Fish, Total coliforms, Total heterotrophic bacteria

RESUMEN

Los peces están expuestos continuamente a los microorganismos presentes en el agua y en el sedimento. El presente estudio intentó detectar las bacterias indicadoras de contaminación en los tejidos de un pez estuario *Mugilcephalus*Kureepuzha. Las bacterias heterotróficas totales, las bacterias coliformes totales, *Escherichiacoli* y los estreptococos fecales se cuantificaron en los peces utilizando procedimientos microbiológicos estándar. La comparación del recuento bacteriano en la piel, las branquias y el intestino de *Mugilcephalus* reveló que se encontró el mayor recuento de microbios indicadores en la piel seguido de las branquias y intestino de los peces todos los sitios, excepto el sitio 4, que muestran bacterias predominantemente en el intestino. ANOVA mostró una diferencia significativa en el recuento microbiano entre sitios y entre tejidos, excepto bacterias heterotróficas totales y estreptococos fecales entre sitios. Los resultados de los parámetros fisicoquímicos también respaldan que las actividades antropogénica impactan en el área de estudio, causando un detero ro de la calidad del agua del lago y, por lo tanto, aumenta la carga de microorganismos perjudiciales. Por lo tanto, se recomienda la palicaicon de buenas prácticas en el manjeo del pescado el lavado, la limpieza de escamas, la eliminación de vísceras (branquias, intestinos, etc) y la cocción adecuada, para ayuden a reducir la presencia de patógenos microbianos en los peces él cuerpo del pescado y permitir el consumo de manera segura.

Palabras clave: *Escherichiacoli*, estreptococos fecales, peces, coliformes totales, bacterias heterotróficas totales.

INTRODUCTION

Fishes are one of the main sources of protein for humans in many parts of the world; especially in developing countries. Fish industry has declined due to many of factors which include overfishing, loss of fish habits and environmental pollution (Soura*et al*., 1996). Good water quality and the health of the aquatic ecosystem are indicated by fishes because they are the species which determine the distribution and diversity of other organisms (Moyle and Leidys, 1992). Numerous studies have shown that fish possess bacterial population on their skin, gills, gut and light emitting organs (Olafsen, 2001). The numbers and diversity of the bacterial population often reflect those of the surrounding water. Microbial pathogens on fishes have a strong correlation with pathogens in water. The type of bacteria enumerated from the skin, gill and gut, of fish depends on the habitat, physical and biological parameters of water such as salinity and bacteria composition (Olafsen, 2001). Bacterial load in fish body is not determined by the size of fish, it is actually determined by the rate of pollution and nature of fish environment (Cahill, 1990).

Grey mullets have a significant role in maintaining the fishery of tropical and subtropical regions of the world (Wijeyaratne and Costa, 1986; Koutrakis*et al.,* 1994; Callico*et al.,* 2014; 2017a, b, c Thompson *et al.,* 2015). They are one of the major sources of protein to humans. The study of *Mugil cephalus*has greater importance because they constitute an important group of fishes in commercial fishery. Ashtamudi Lake has become a major receiver of contaminants generated through coconut husk retting, ceramic paper, palm oil industries, tourism activities, cashew factories and hospitals. Fishing is the major economic activity in Ashtamudi Lake,

having rich and varied fishery resources and an annual production of 23000 t of fish (Kurup and Thomas, 2001). Kureepuzha backwater, a part of Ashtamudi Lake that envelops the sub islands of Kureepuzha was taken in to consideration for this study. The present study aims to screen the fish samples from study sites for microbial contamination and to assess the pollution indicator bacteria in specific tissues *Mugil cephalus* quantitatively.

MATERIALS AND METHODS

Study area:The present study was conducted along Kureepuzha Backwater, a part of Ashtamudi Lake, Kerala. Thestudysiteswere site1- Thoppilkadavu (8° 53' 42.52"N; 76°34' 20.37"E), site 2-Sambranikodi (8°55' 44.12"N; 76° 34' 12.22"E), site- 3 MukkadPallikadavu (8°55 '48.57"N; 76° 33' 40.77"E) and site- 4 PanamukkamKadavu (8° 54' 28.53"N; 76°34' 29.85"E) Fig 1.

Sample Collection and Microbial analysis: The fish *Mugil cephalus* was collected from four selected sites of Kureepuzha Backwaters with the help of local fishermen using different types of nets, six samples were collected from each site with an average length of (12.5 – 18.7 cm) and were immediately transported to the laboratory in sterilized bottles and the samples were processed for bacteriological analysis within 2-4 hours of sampling following aseptic conditions. The fishes were aseptically dissected with the aid of dissecting kits and one gram of tissue samples were collected from the skin, gill, and gut for the enumeration of total heterotrophic bacteria, total coliforms, *Escherichia coli*, and fecal *Streptococc*i. Samples collected from skin, gill, and gut were blended with 99 ml sterilized distilled water and macerated using sterile glass rods to obtain a dilution factor of 10^{-2}. The samples were further diluted up to 10^{-3}.

Bacteria culture and identification: The bacteria were grown on specific agar medium by adopting pour plate method. To detect the total heterotrophic bacterial count, 1ml of serially diluted solutions were spread on separate nutrient agar plates and incubated at 37°C for 24hrs. Enumeration of total coliforms were done by transferring 1ml of serially diluted solutions of skin, gill and gut and were spread on separate Tergitiol-7 agar plates and incubated at 37°C for 24hrs. Lactose fermentors were identified as yellow colonies and non fermentors as blue colonies. TTC (Triphenyl Tetrazolium Chloride) is reduced in the bacterial cell to form a red coloured insoluble complex there by producing red colonies. To detect *E.coli*, 1ml of serially diluted solutions of skin, gill and gut were spread on MacConkey agar plates and incubated at 44°Cfor 24 hrs. Pink colonies surrounded by a zone of acid precipitated bile were identified as *E.coli*. For the enumeration of fecal s*treptococci*, 1ml of serially diluted solutions of skin, gill, and gut were spread on KF- *streptococcal* agar plates and incubated at 37°C for 24hrs and brown colonies were identified as fecal streptococci. All the procedures during the preparations

of culture plates were carried out in laminar flow work station to avoid contamination through air.

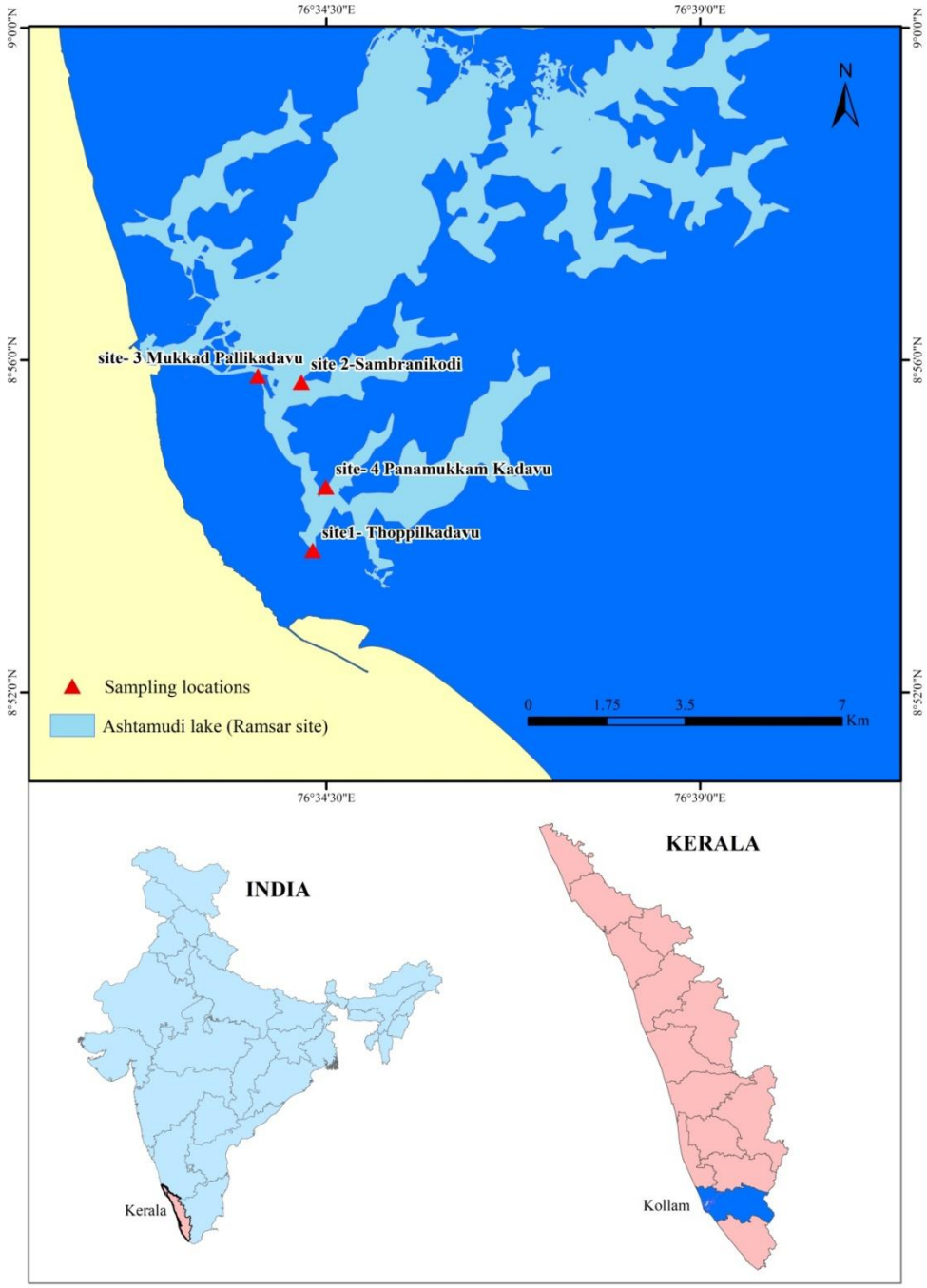

Fig 1. Location map of the study area with sampling sites

Bacteria colonies were counted with the help of Digital Colony Counter. Colony Forming Units (CFU) were estimated using the formula,

$$CFU = \frac{\text{Number of colonies}}{\text{Volume of water sample}} \times \text{dilution factor}$$

Physico-chemical analysis: Water samples were collected from selected sites and standard analysis of water quality parameters such as pH (°C), water temperature (°C), salinity (ppt), Dissolved oxygen (mg/L), BOD (Biological Oxygen Demand mg/L), and free carbon dioxide (mg/L) were measured. The pH of water sample was measured by electronic portable pH meter (Electrometric method). Water Temperature was measured using Mercury thermometer and noted in °C. The salinity was determined by Chlorosity titration method. Dissolved oxygen was determined using Winkler's method. BOD checked was 5day BOD and free carbon dioxide determined using Titration method by following the methodologies of APHA (2012).

Statistical Analysis: The data obtained were subjected to descriptive statistics such as Mean and Standard Deviation. For assessing significant difference in bacterial count between different tissues of fish (Skin, gill and gut) and to check significant difference in bacterial population on fishes from different sites (Between sites) the two-way ANOVA was carried out. Analysis performed using the Analysis Tool pack in Microsoft Excel (version 7.0).

RESULTS AND DISCUSSION

Comparison of bacterial count in the skin, gill and gut of *Mugil cephalus* from Kureepuzha backwater revealed that, in general the bacterial load of skin was higher than all other parts of the fish. Among the tissues analyzed highest count of total heterotrophic bacteria*,* total coliform, *Escherichia coli* and fecal *streptococci* found in the skin followed by the gill and gut of fish from all sites except site 4, they show bacteria predominantly in gut. The intensity of microbial contamination between the same fish from different sites revealed that maximum number of pollution indicators were observed in the tissues of fishes collected from site 1 followed by site 3 and 2. Site 4 shows comparatively minimum contamination and the fecal *streptococcal* strains were completely absent on the tissues of fishes collected from this site. Two way ANOVA showed a significant difference in microbial count between sites and between tissues except total heterotrophic bacteria and fecal *streptococci* between sites (Table 1 - 4). Absence of fecal *streptococcal* strains on tissues of fishes from site 4 is a clear evidence of minimum fecal contamination hence this site is recognized as unpolluted compared to other sites. Fecal *streptococci* are indicators of immediate fecal contamination and rainfall has an important effect on fecal coliforms and *streptococcal* densities (Jenelia*et al.,* 2011). The differences in the rate of pollution in different sites may responsible for these changes in microbial count. Fishes are continuously exposed to the microorganisms present in water and in the sediment. Skin is directly exposed to polluted water and gill serves as a respiratory organ for fishes and these may contribute high microbial load in skin and gill than gut (Olafsen, 2001).

The skin microbial composition depends on the way of handling and pre-capture environment of fish (Colwell, 1962).

Physico chemical parameters revealed that among the four sites, site 1 is highly polluted having low dissolved oxygen (mg/L), high BOD (mg/L), high carbon dioxide(mg/L), neutral pH (°C), moderate temperature (°C) and salinity(ppt) followed by site 3 and 2. Site 4 is recognized as least polluted with high dissolved oxygen (mg/L), low BOD value (mg/L), less biogenic carbon dioxide (mg/L), alkaline pH (°C), low temperature (°C), and moderate salinity(ppt) (Table 5). High BOD value may be an indication of the presence of aerobic bacteria (Otokunefor and Obiukwu, 2005) (Table 5).

Table 1. Total heterotrophic bacteria count in the tissues of *Mugil cephalus* from four sites at dilution 10^{-3} CFU/g (Mean ± SD)

Site/ Tissue	Skin	Gill	Gut
Site 1	75.2 ± 0.53	54.4 ± 0.63	13.6 ± 0.60
Site 2	54.1 ± 1.15	36 ± 0.96	23.1 ± 0.70
Site 3	64.1 ± 0.15	20.2 ± 0.22	8.8 ± 0.21
Site 4	6.9 ± 0.12	2.8 ± 0.09	9.4 ± 0.08

Between three tissues *P< 0.05; (significant) Between sites P> 0.05; N.S (Not significant)

Table 2. Total coliform bacteria count in the tissues of *Mugil cephalus* from four sites at dilution 10^{-3} CFU/g (Mean ± SD)

Site/ Tissue	Skin	Gill	Gut
Site 1	44.7 ± 0.74	26.8 ± 1.10	13.4 ± 0.51
Site 2	15.5 ± 0.68	8 ± 0.96	2.5 ± 1.30
Site 3	34.3 ± 0.26	9.7 ± 0.22	6.4 ± 0.05
Site 4	0.5 ± 0.08	0.3 ± 0.05	0.8 ± 0.05

Between three tissues *P< 0.05; (significant) Between sites P> 0.05; N.S (Not significant)

Table 3. *Escherichia coli* count in the tissues of *Mugil cephalus* from four sites at dilution 10^{-3} CFU/g (Mean ± SD)

Site/ Tissue	Skin	Gill	Gut
Site 1	12.4 ± 0.70	6.5 ± 0.57	3.9 ± 0.78
Site 2	7.8 ± 0.12	1.5 ± 0.05	1.0 ± 0.09
Site 3	9.3 ± 0.05	3.2 ± 0.17	1.4 ± 0.14
Site 4	0.3 ± 0.08	0.2 ± 0.08	0.4 ± 1.00

Between three tissues *P< 0.05; (significant) Between sites *P< 0.05; (significant)

Table 4. Fecal *streptococci* count in the tissues of *Mugil cephalus* from three sites at dilution 10^{-3} CFU/g (Mean ± SD)

Site/ Tissue	Skin	Gill	Gut
Site 1	7.2 ± 3.00	3.5 ± 1.29	1.2 ± 0.50
Site 2	2.7 ± 0.50	1.5 ± 1.00	1 ± 0
Site 3	4.5 ± 1.30	1.5 ± 0.57	1.2 ± 0.5

Between three tissues *P< 0.05; (significant) Between sites P> 0.05; N.S (Not significant)

Table 5. Variation in physico-chemical parameters of water samples collected from four study sites (Mean ± SD).

Parameters	Site 1	Site 2	Site 3	Site 4
pH (°C)	7.60 ± 0.42	8.20 ± 0.29	7.80 ± 0.53	8.40 ± 0.65
Temperature (°C)	28.00 ± 0.50	28.00 ± 1.41	29.00 ± 0.5	24.00 ± 1.63
Dissolved Oxygen (mg/L)	2.40 ± 0.40	6.40 ± 0.34	4.00 ± 0.81	7.20 ± 0.22
Dissolved Carbondioxide (mg/L)	31.60 ± 1.50	21.10 ±5.90	29.90 ± 3.40	14.08 ± 2.94
BOD (mg/L)	2.20 ± 1.25	1.00± 0.70	1.20 ± 0.40	0.80 ± 0.08
Salinity(ppt)	0.70 ± 0.05	0.70 ± 0.00	0.80 ± 0.05	0.80 ± 0.00

Estuarine fishes are one among the most edible fishes which are of great demand (Wijeyaratne and Costa, 1986). People believe that freshly caught fishes are less harmful, but the microbial contamination on fishes from its environment plays a crucial role and it may contain pathogenic bacteria. It get transferred to human body by many ways on contact with

contaminated fishes, enters to human body through wounds in the skin and improper cooking may also lead to bacterial infections from fishes. Bacteria, virus and protozoan's can be introduced in to water bodies in various ways, including leaking septic tanks, sewage malfunction, contaminated storm drains, run off from animal feed lots, human fecal discharge and other sources (Yilmaz *et al*., 2004). There has been some Link between high levels of indicator bacteria and spread of diseases (Chou *et al*., 2004). Lake receives domestic sewage, it carriers a number of microorganisms which are pathogenic may enter in to humans through the consumption of contaminated fish flesh (Niewolak and Tucholski, 2000). Dysentery, typhoid fever, bacterial gastroenteritis and many other water borne diseases associated with fecal coliform contamination (Doyle and Ericson, 2006). Mammalian microflora, including the presence of *E.coli* and fecal coliform, on processed sea food is a clear evidence of contamination (ICMF, 1986). The presence of fecal coliform may affect both humans and aquatic organisms (Doyle and Ericson, 2006).

As conclusion, Estuarine fishes are one of the most edible fishes which are of great demand. The present study attempted to screen the pollution indicator bacterial contamination on *Mugil cephalus*. When comparing the intensity of microbial contamination on the tissues of fishes from different sites, maximum contamination was observed on fishes from site 1 followed by site 3 and 2. Site 4 shows comparatively minimum contamination and the complete absence of fecal *streptococcal* strains. The differences in the rate of pollution in these sites may contribute these changes in bacterial count. House boat tourism affects the environment and ecosystem of the Ashtamudi Lake. The pollution from the house boats including sewage from toilets, oil from engines, plastic wastes and food wastes enhances the number of coliform bacteria in water as well as in fishes. Dysentery and bacterial gastroenteritis may occur due to the ingestion of contaminated fish as food. Hence it is recommended that good processing of fish such as washing, scraping scales, removal of gills and gut contents and proper cooking help to reduce microbial pathogen in fish body and make it safe for consumption. Further, the sources of pollution are to be ceased immediately in order to secure a healthy biotic system in the lake.

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