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A Comparative Microbiological Assessment of Five Types of Selected Fishes Collected from Two Different Market

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Abstract: The present study was conducted for microbiological assessment of five selected fishes in two different market conditions. For this, Total Viable Bacterial Counts (TVBC), Total *Pseudomonas* Counts (TPC), Total Coliform Counts (TCC), Total Fecal Coliform Counts (TFCC), Total Yeast Counts (TYC), Total Mold Counts and occurrence of *Salmonella* and *Vibrio* spp. were determined in Sarpunti (*Barbodes sarana*), Rui *(Labeo rohita)*, Tilapia (*Oreocromis niloticus),* Ayr (*Sperata seenghala*) and Kachki (*Corica soborna*) fish. In such investigation, highest TVBC (1.9 x10^8 cfu/g) was observed in Sarpunti collected from local market (LM-2) and lowest (2.0 x10^4 cfu/g) in Ayr fish collected from supershop (SS-1). Highest *Pseudomonas* count (4.80 x 10^7cfu/g) was found in Sarpunti of local market (LM-2) and lowest (1.35 x 10^3cfu/g) in both Rui and Ayr fish of supershop (SS-4 and SS-1 respectively). Highest total coliform (>240MPN/g) was found in a number of samples of local markets whereas lowest count (0.9 MPN/g) in Rui fish of supershop (SS-3). Similarly, highest count (110 MPN/g) of fecal coliform was found in many samples of local markets including Rui of SS-1 whereas absent in Kachki fish of SS-3. Pathogenic bacteria, *Salmonella* was mostly present in samples of local markets but usually absent in samples of super shop with a few exceptions. *Vibrio* spp. also showed similar type of results. Besides, highest yeast count (2.83 x 10^3cfu/g) was found in Kachki of local markets (LM-3) but absent in Ayr and Kachki fish of Supershop (SS-3 and SS-4, respectively). Similarly, highest count of molds (8.0×10^2 cfu/g) was found in local markets whereas absent in two super shop samples. In conclusion, five species of fish under study were more or less contaminated but comparatively highest load in local market samples indicated that the hygienic condition of these markets were very low.

Key words: TVBC · TPC · TCC · TFCC TYC · TMC

INTRODUCTION

Fish can be finfish, shellfish (molluscs and crustaceans), or any other form of marine or freshwater animal life that can be used for human or domestic animal consumption [1]. About 80% of animal protein in our diet comes from fish alone [2]. However, consumption of fish may sometimes cause disease due to infection or intoxication. It is believed to be the reflection of the general contamination in the aquatic environment. The true incidence of diseases transmitted by fish is usually unknown. It has been estimated that as few as 1% of the diseases are actual cases of food borne diseases [3]. Fish are conditioned by their environment and hence it is obvious that if the growing and harvesting environment of fish is polluted chemically or microbiologically, the fish are also polluted [4]. During transportation of these types of fish to landing center and wholesale market, these fish may also infect associate people by handling. When the consumers purchase these fishes, the associated microorganisms are transferred to the persons who carry those [5].

In Bangladesh, super shop has a special site for selling of fish where modern facilities like sufficient ice, display tray, uniform salesmen, electric balance and attractive packaging system are available. Local markets, on the other hand, have dirty, damp and unhealthy place, poor storage, display and packaging facilities, which

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encourage microbial contamination from different sources. But, collection of fish in both types of market may be same as super shop markets in Bangladesh, have no special site for production of fish. Thus, a comparative study of microbial load in fish of two types of market environments may give a clear idea about health concern. Although most of the retail fish markets and super shop sell freshwater fishes, it takes quite a while for transportation of fish from different catching sites to the far away markets. There is always a risk of deterioration of quality due to poor or unhygienic handling, transportation and storage. In Bangladesh, few studies have been carried out on fish microbiology. Hence, this study attempts to assess the microbiological quality and safety of fish in relation to environmental condition of fish markets.

MATERIALS AND METHODS

Sample Collection: Fish samples were collected from local market and super shop using a sterile aseptic container together with ice. For the analysis of microbial loads 5 species of fish were selected. These were - Sarpunti [*Barbodes sarana* (Hamilton, 1822)], Rui [*Labeo rohita* (Hamilton, 1822)], Tilapia [*Oreocromis niloticus* (Sykes, 1839)], Ayr [*Sperata seenghala* Hamilton, 1822] and Kachki [*Corica soborna* Linnaeus, 1758]. Identification of fish was done according to Rahman [6]. In the present study, eight microbiological parameters for examination of sample fishes were considered including- Total Viable Bacterial Counts (TVBC), Total Coliform Counts (TCC) and Total Fecal Coliform Counts (TFCC), Total *Pseudomonas* Counts (TPC), Total Yeast Counts (TYC), Total Molds Counts (TMC) and qualitative analysis of *Vibrio* spp and *Salmonalla* spp.

Preparation of Sample: About 20 g of fish muscle with skin, fin and head part was taken from each sample and suspended in 180 ml Ringer's solution and was homogenized by using a homogenizer. Each sample was serially diluted and aliquots of each diluted sample were plated for microbiological count. Microbiological analyses were carried out according to the APHA[7].

Microbial Count: An automatic colony counter counted the number of colonies from selected plates and the count was expressed as colony forming unit (cfu/g). The total and fecal coliform bacteria were enumerated by most probable number (MPN) procedure.

RESULTS AND DISCUSSION

Tables 1 and 2 show the individual results of microbiological analysis conducted on selected 5 sample fishes. Results show that bacterial count was always higher in every fish of local markets except Ayr fish.

Comparative analysis of TVBC, TPC, TCC, TFCC, TYC and TMC showed great variation from species to species as well as from market to market as shown in figures-1 to 3. Among local market samples, the highest count of TVBC was found in Sarpunti (1.9×10^8 cfu/g) collected from LM-2 and the lowest counts (3.3×0^5 cfu/g) was also found in Sarpunti fish collected from LM-3 (Table 1). The bacterial flora on newly caught fish depends on the environment in which it is caught rather than on the fish species [8]. Among supershop samples, the highest counts of TVBC was found in Kachki fish (1.72×10^7cfu/g) collected from SS-1 and the lowest count was found in Ayr fish (2.0×10^4 cfu/g) collected from SS-1 also (Table 2). This indicates that bacteriological quality of supershop fish was better than local markets. Besides, TVBC was beyond the acceptable limits (5×10^5 cfu/g) almost all the samples of local markets. By detecting the bacterial load on the fish surface it apparently gives an idea about the quality of the samples. When TVBC reaches to 10^6/g or more in processed food or food products, it is considered that these food items are spoiled [9]. The present findings agrees with Sewan [8], who said the total number of organisms vary enormously and a normal range of 10^2-10^7 cfu (colony forming units)/cm^2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g [8].

In samples collected from the local markets, the highest count of *Pseudomonas* spp. was found in Sarpunti fish (4.80×10^7 cfu/g) and the lowest count was in Kachki fish (1.31×10^4 cfu/g) (Table 1). Among the samples of supershop, the highest count was found in Tilapia (8.68×10^5 cfu/g) and lowest counts of *Pseudomonas* found in both Rui and Ayr fish (1.35×10^3 cfu/g) (Table 2). The results of TPC indicate that *Pseudomonas* was predominantly present in samples of local fish markets in comparing with super shop (Figure 1). The poor quality may be due to poor handling, improper storage system and sanitary condition at all the steps in the fish processing and selling. The isolation of *Pseudomonas spp* from the collected fish samples is of highly importance because this bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as spoilage

Table 1: Microbiological assessment of fish samples collected from local markets

		Microbial Count						Occurrence	
Sampling sites	Sample	TVBC cfu/g	TPC cfu/g	TCC MPN/g	TFCC MPN/g	TYC cfu/g	TMC cfu/g	*Salmonella* spp.	*Vibrio* spp.
LM-1	Sarpunti	2.64×10^{6}	1.49×10^{6}	>240	21	3.1×10^{2}	1.2×10^{2}	±	±
	Rui	6.60×10^{6}	3.75×10^{6}	>240	15	2.07×10^{3}	8.0×10^{2}	±	±
	Tilapia	1.64×10^{6}	1.61×10^{6}	>240	2.3	8.4×10^{2}	3.2×10^{2}	±	±
	Ayr	4.08×10^{6}	6.65×10^{5}	>240	110	2.22×10^{3}	3.0×10^{2}	±	±
	Kachki	7.95×10^{5}	1.3×10^{4}	>240	46	7.2×10^{2}	Nil	±	=
LM-2	Sarpunti	1.9×10^{8}	4.80×10^{7}	>240	110	9.3×10^{2}	2.0×10^{2}	±	±
	Rui	4.5×10^{6}	3.8×10^{6}	46	21	1.30×10^{3}	2.2×10^{2}	=	±
	Tilapia	4.3×10^{5}	3.04×10^{5}	>240	21	6.0×10^{2}	2.1×10^{2}	=	±
	Ayr	9.2×10^{6}	4.84×10^{5}	>240	15	8.4×10^{2}	4.5×10^{2}	±	±
	Kachki	4.16×10^{7}	2.0×10^{5}	110	110	1.21×10^{3}	5.1×10^{2}	=	=
LM-3	Sarpunti	3.3×10^{5}	1.16×10^{5}	2.3	0.4	2.55×10^{3}	7.4×10^{2}	±	±
	Rui	5.5×10^{7}	7.0×10^{5}	110	2.3	5.0×10^{2}	3.5×10^{2}	=	=
	Tilapia	5.84×10^{6}	1.3×10^{6}	21	2.8	8.0×10^{2}	3.2×10^{2}	±	±
	Ayr	7.0×10^{5}	1.76×10^{4}	>240	46	1.44×10^{3}	2.3×10^{2}	=	±
	Kachki	9.84×10^{6}	4.80×10^{5}	>240	110	2.83×10^{3}	4.0×10^{2}	±	±
LM-4	Sarpunti	6.40×10^{6}	5.84×10^{5}	110	24	4.2×10^{2}	4.8×10^{2}	=	±
	Rui	3.6×10^{5}	1.37×10^{4}	>240	46	1.05×10^{3}	2.5×10^{2}	±	±
	Tilapia	1.59×10^{6}	6.36×10^{5}	>240	21	6.4×10^{2}	1.2×10^{2}	±	±
	Ayr	1.7×10^{7}	3.35×10^{5}	28	20	3.8×10^{2}	4.1×10^{2}	=	±
	Kachki	4.84×10^{6}	2.72×10^{5}	110	15	1.25×10^{3}	2.1×10^{2}	±	±

** Total Viable Bacterial Counts (TVBC), Total Coliform Counts (TCC), Total Fecal Coliform Counts (TFCC), Total *Pseudomonas* Counts (TPC), Total Yeast Counts (TYC), Total Mold Counts (TMC).

Table 2: Microbiological assessment of fish samples collected from super shops

		Microbial Count						Occurrence	
Sampling sites	Sample	TVBC cfu/g	TPC cfu/g	TCC MPN/g	TFCC MPN/g	TYC cfu/g	TMC cfu/g	*Salmonella* spp.	*Vibrio* spp.
SS-1	Sarpunti	2.6×10^{5}	1.96×10^{5}	110	1.1	8.2×10^{2}	5.8×10^{2}	±	=
	Rui	4.8×10^{5}	2.6×10^{4}	>240	110	1.78×10^{3}	6.3×10^{2}	=	±
	Tilapia	2.75×10^{5}	4.0×10^{4}	110	15	4.6×10^{2}	1.5×10^{2}	=	±
	Ayr	2.0×10^{4}	1.35×10^{3}	110	21	2.4×10^{2}	1.8×10^{2}	=	=
	Kachki	1.72×10^{7}	1.12×10^{5}	>240	15	1.20×10^{3}	Nil	=	±
SS-2	Sarpunti	2.44×10^{6}	3.0×10^{5}	>240	46	1.51×10^{3}	1.39×10^{3}	=	=
	Rui	5.6×10^{6}	3.04×10^{5}	110	3.9	3.9×10^{2}	1.3×10^{2}	=	±
	Tilapia	1.75×10^{5}	5.2×10^{4}	110	21	1.5×10^{2}	0.8×10^{2}	=	=
	Ayr	3.1×10^{6}	0.6×10^{4}	9.3	3.9	5.5×10^{2}	Nil	=	±
	Kachki	4.4×10^{4}	3.10×10^{3}	46	3.9	4.9×10^{2}	1.4×10^{2}	=	=
SS-3	Sarpunti	4.02×10^{6}	3.20×10^{5}	110	12	1.82×10^{2}	4.0×10^{2}	±	±
	Rui	2.94×10^{4}	2.11×10^{4}	0.9	0.4	1.25×10^{3}	2.8×10^{2}	=	=
	Tilapia	5.9×10^{6}	6.65×10^{5}	>240	2.3	2.17×10^{3}	2.4×10^{2}	=	±
	Ayr	3.32×10^{5}	4.1×10^{4}	110	6.4	Nil	3.3×10^{2}	±	=
	Kachki	4.9×10^{5}	1.2×10^{4}	20	Nil	4.2×10^{2}	3.4×10^{2}	±	±
SS-4	Sarpunti	2.24×10^{6}	1.72×10^{4}	21	3.9	5.7×10^{2}	1.9×10^{2}	=	=
	Rui	1.6×10^{5}	1.35×10^{3}	21	1.5	6.3×10^{2}	1.0×10^{2}	±	=
	Tilapia	5.04×10^{6}	8.68×10^{5}	21	0.9	1.7×10^{2}	4.8×10^{2}	±	=
	Ayr	8.1×10^{5}	2.71×10^{3}	23	0.4	4.2×10^{2}	1.3×10^{2}	±	=
	Kachki	3.32×10^{5}	3.9×10^{4}	110	21	Nil	2.3×10^{2}	=	=

** Total Viable Bacterial Counts (TVBC), Total Coliform Counts (TCC), Total Fecal Coliform Counts (TFCC), Total *Pseudomonas* Counts (TPC), Total Yeast Counts (TYC), Total Mold Counts (TMC).

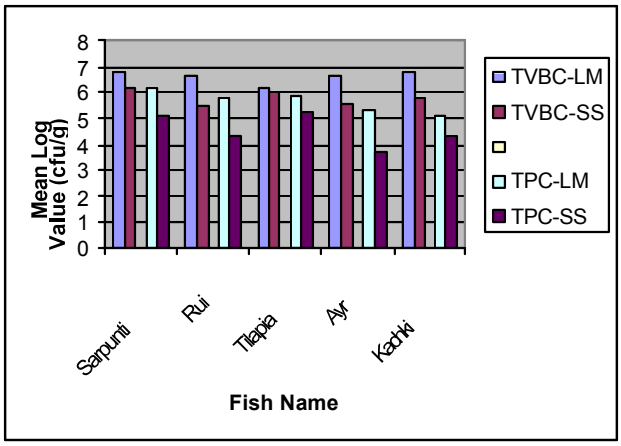

Fig. 1: Showing comparative analysis of TVBC and TPC in sample fishes collected from local markets and supershop

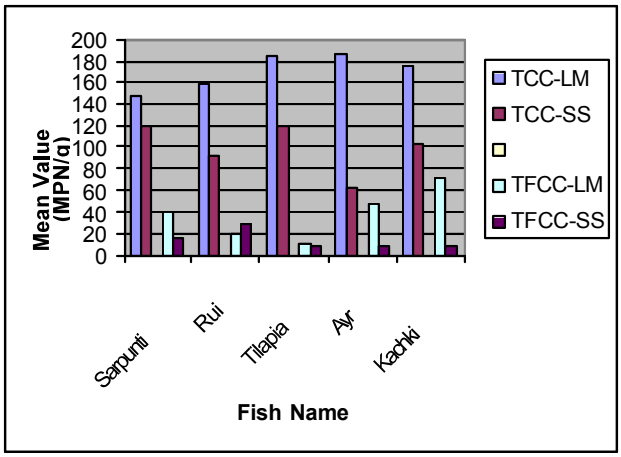

Fig. 2: Showing comparative analysis of TCC and TFCC in sample fishes collected from local markets and supershop

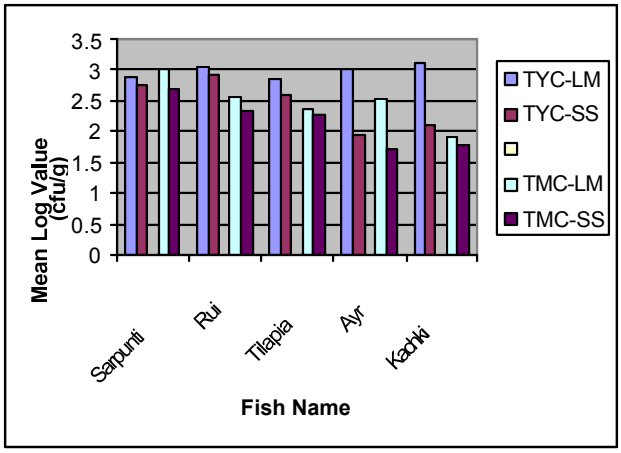

Fig. 3: Showing comparative analysis of TYC and TMC in sample fishes collected from local markets and supershop
**LM -Local Market, SS- Super Shop

organism. This is in accordance with previously mentioned by Jeyasekaran *et al*. [10] and Koutsoumanis and Nychas [11] who identified pseudomonad as a good spoilage index. Although *Pseudomonas spp* are not referred to as the cause of foodborne illnesses they are closely associated to food deterioration [12]. According to Tripathy *et al*. [13] *Pseudomonas* spp. are frequently associated to fish and have been isolated from skin, gills and intestine. Their load is explained by the population density in water. In an aquaculture, especially *P. aeruginosa* and *P. fluorescens* have been considered opportunistic pathogenic species [14].

Indicator organisms as the total coliform count (TCC) and total fecal coliform counts (TFCC) were found in almost all the samples of five fishes, collected from both local markets and super shop. The highest count of TCC (>240 MPN/g) found in any one of five fishes but lowest count was found in Sarpunti fish (2.3 MPN/g) in samples of local market and Rui fish (0.9 MPN/g) of super shop (Figures-2). TFCC count was always lower than TCC and found to absent in Kachki fish of super shop (Figure 2). Almost all values exceeded the IAMS [15] limits (100/g) for total coliform and (11/g) for fecal coliform that means the supply of low quality fish in most of our fish markets. The presence of coliform group (*E. coli*) in higher range, suggests contamination of the samples before or during handling, processing and marketing. But, lower load of TCC and TFCC in samples of super shop indicates low range of contamination than local markets. These results are also similar to the other works where they found the similar range of counts [5, 16, 17]. Thampuran *et al*. [18] also reported that the microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform were *Escherichia coli* is the most common contaminant and is often encountered in high numbers. The isolation of these groups of organisms indicated faecal and environmental pollution and these supported the findings of Yagoub *et al*. [19]. This also confirms the findings of Koutsoumanis and Nychas [11], Gonzalez [20] and Herrera *et al*. [21]; who isolated similar organisms from fish and fish products.

The present study showed the highest total yeast count (TYC) in Kachki fish (2.83×10^3 cfu/g) among local market samples and Tilapia fish (2.17×10^3 cfu/g) among super shop samples (Figure-3). TYC was absent in Ayr fish (SS-3) and Kachki fish (SS-4) of super shop. In local markets, total mold counts (TMC) were found highest in Rui fish (8.0×10^2 cfu/g) of LM-1 and was absent Ayr fish (LM-1) and Kachki fish (LM-1) (Table-1). Among 5 fishes collected from supershop, the highest count of TMC was recorded in Sarpunti fish (1.39×10^3 cfu/g) of SS-2 and absent in Ayr fish (SS-2) and Kachki fish (SS-1) samples (Table 2). Comparatively, highest TYC was found in local markets (2.83×10^3 cfu/g) like other microbial parameters under study but highest TMC was found in sample of super shop (1.39×10^3 cfu/g) exceptionally (Figure 3). Das *et al*. (2007) investigated TYC and TMC in 12 species of small indigenous fish and found highest count of TYC (1.03×10^4 cfu/g) in Batashi (*Clupesoma* sp.) and lowest (0.7×10^2 cfu/g) in Rui (*Labeo rohita*). TMC was found highest (7.5×10^2 cfu/g) in Shrimp and lowest (0.8×10^2 cfu/g) in Rui. The results of present investigation are similar to the work of Das *et al*. [5].

Salmonella is highly pathogenic and this is the major reason for isolation of such bacteria from sample fishes. In the present study, *Salmonella* was examined qualitatively and found almost in all sample fishes of local markets except Tilapia in LM-2, Sarpunti of LM-4, Rui of LM-2 and LM-3, Ayr fish of LM-3 and LM-4 and Kachki fish of LM-4 where it was absent. In super shop samples, *Salmonella* was almost absent in all sample of 5 fishes except Tilapia of SS-4, Sarpunti of SS-3 and SS-1, Rui fish of SS-4, Ayr fish of SS-3 and SS-4 and Kachki fish of SS-3 where it was present (Table- 1, 2). The results indicated that the processing, handling and storage condition of super shop markets is good and the quality of fish is better than the local markets. It also indicates better quality preservation, handling and hygiene and sanitary maintenance of super shop than local markets but not much satisfactory. It has been shown that *Escherichia coli* and *Salmonella* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment [22].

Presence of *Vibrio* spp. can be a cause of infection to the consumer. In the present investigation, *Vibrio* spp. was studied qualitatively and found almost in all samples of five fishes collected from local markets. It was absent in Rui fish of LM-3 and Kachki fish of LM-2 and LM-1. In supershop, *Vibrio* was absent in most samples of five fishes but present in Tilapia and Sarpunti of SS-3, Rui of SS-1 and SS-2, Ayr fish of SS-2 and Kachki fish of SS-1 and SS-3 as shown in Table 1 and 2. According to recommendation of International Association of Microbiological Societies [15], fresh and frozen fish should be free of *Vibrio* (0/gm). The present study revealed that microbial quality of local markets was not good due to presence of *Vibrio* in most samples. El Hadin *et al*. [23] detected the presence of eight potentially pathogenic *Vibrio* species,with overall incidence in

the samples as 4.6% for *V. cholerae*, 4.7% for *V. parahaemolyticus*, 6.0% for *V. vulnificus*, 11% for *Vibrio alginolyticus*, 9.9% for *Vibrio metschnikovii*, 1.3% for *Vibrio mimicus*, 13% for *Vibrio damsela*, 7.6% for *Vibrio fluvialis* and 52% for a combined population of all of the above from fish samples.

From the result it found that microbial condition of LM-1 and LM-2 (local market) were not so good in comparison with other markets as they showed higher counts in most of microbial parameters. The investigation also indicates that the fish of LM-1 and LM-2 were in high risk to transmit various types of pathogens to the consumers. The conditions of super shop markets were better than local markets. Although SS-1 and SS-4 showed higher counts in case of most fishes than SS-2 and SS-3, but not above the local markets (Figures-1, 2, 3). Shewan [8] also reported that freezing and cold storage reduced the bacterial load on fish. Though supreshop fishes have better cold storage facilities, so bacterial quality in super shop is better than local market.

This study gives a clear perspective on the variation of bacterial load comparatively in five fishes collected from 4 local markets and 4 super shops. The study also revealed that the microbiological state of super shop was less contaminated than local fish markets due to their airtight display tray with sufficient ice and careful handling. Among the scaly, scale less and small fish, scaly fish (Tilapia, Rui, Sarpunti) were more susceptible in bacterial contamination, which is another finding of present study. Besides this some collected samples from super shops were more contaminated. The fact is that, all frozen products such as meat, fish and shrimps are stored together in super shop; as a result cross contamination may occur. Not only that, but also the product is stored for relatively long period until sold and sometimes storage condition cannot be ensured properly due to technological disruption [18].

The bacterial ecology of fish products is connected to environmental factors such as water pollution, anthrop activities, fish feed quality, hygienic procedures of slaughter, handling, transport, commercialization and storage conditions. In freshwater aquaculture, the microbial load in the water used for cultivation is closely connected to several factors such as bacterial ecology of supply water, environment (air and contamination by animal excrements), fish feed, soil and water table (Figure 1). Hence, the microbial charge of fish gut and tegument depends on these factors too. Regarding the fresh fillets, the potential contamination in other steps of processing such as procedures of slaughter, handling, packing and storage need to be considered.

From the result, it can be concluded that the fish sold in the retail markets are not standard to consume since the observed microbial levels are always higher than the recommended levels. To overcome this situation, it is necessary to follow the code of practice concerning handling of the catch, icing, post-harvesting procedures and storage including depuration and hygienic measures. Proper hygienic condition should be maintained at every step of catching, landing and transportation, processing and marketing following HACCAP steps for good quality of fish and fishery products.

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