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Application of sensory and microbial analysis to assess quality of fish in Siliguri city of West Bengal, India

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Abstract: *Sensory and certain microbial analyses were applied to assess the quality of raw fish sold at a market in Siliguri city of West Bengal, India. In regular surveys undertaken during June to August 2008, a particular fish species was randomly selected, its source was noted and a sensory analysis, the quality index method (QIM) was applied to assess its quality. Raw fish samples were also collected and a small quantity (about 1 g) of scales or an upper layer of the skin surface (for scale-less fish samples), gill, liver and a portion of gut with gut-contents were aseptically removed for enumeration of the total aerobic heterotrophic bacteria, Aeromonas spp., Pseudomonas spp., Salmonella spp. and coliform counts. Oreochromis mossambicus and Tenulosa ilisha recorded significantly higher QIM scores, compared to other species ($p<0.05$). Riverine fish, Lepidocephalichthys guntea and Channa punctatus scored the lowest QIM scores (0) while scores for Puntius ticto and Mystus vittatus and pond cultured species like Cirrhinus mrigala, Catla catla, Labeo rohita, Labeo bata and Cyprinus carpio were very marginal ($p<0.05$). Aeromonas spp., Salmonella spp. and total coliforms were recorded from all the studied species while Pseudomonas spp. was isolated from only seven species. Among the tissues examined, the lowest counts of total heterotrophic bacteria, Aeromonas spp., Pseudomonas spp., Salmonella spp. and total coliforms were recorded from the skin in every fish species. Highest counts of pathogenic bacteria (except Pseudomonas spp.) were recorded in Tenulosa ilisha for all the tissues except liver. Since fish are properly cooked in Bengali households, the risk of disease from fish consumption is relatively less. However, some tribes residing in the region are known to consume undercooked fish and proper cooking methods should be followed in view of the present findings to avoid health risks. Besides, utmost care should be taken while handling fish.*

Key words: *Fish quality, Sensory, Microbial analysis, Siliguri fish market*
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Introduction

In the world, 80 to 90 million more people have to be fed each year and the most reliable source of protein for many is fish (World Fish Centre, 2002). Although there is an argument regarding the concept of "fish for all" in India initiated by the World Fish Centre, since 30 to 50% of the Indian population is predominantly vegetarian, there is no dispute that fish is very popular diet among the people of the states of West Bengal, Goa and Kerala (Sakthivel, 2003). The monthly per capita consumption of fish in the rural and urban areas of West Bengal is 0.54 and 0.72 kg, respectively, the only states with a higher per capita consumption being Kerala, Goa, Tripura and the urban centers of Orissa (Anon, 2001). The union territories like Andaman and Nikobar Islands, Lakshwadeep, Daman and Diu and Pondichery also scored higher than West Bengal (Anon, 2001). Among all these states and union territories, people of West Bengal, Orissa and Tripura preferred predominantly freshwater fish, while others preferred predominantly marine fish.

The quality of the raw fish (fresh or iced) at markets in the consumer centres varies so widely that there is an obvious need for developing quality standards. During the last 50 years many schemes have been developed for sensory analysis of raw fish. The quality index method (QIM), originally developed by the Tasmanian Food Research Unit (Bremner *et al*., 1987), is now used by many research institutions in the developed world (Olafsson and Ingthorsson, 1992; Larsen *et al*., 1992; Huss, 1995). Besides, bacteriological standards for quality assessment of fish have long been used by various researchers (Silverman *et al*., 1964; Watanabe, 1968; Watanabe and Ulstrup, 1973; Raccach and Baker, 1978; El-Zanfaly and Ibrahim, 1980; Ashenafi, 1989; Vishwanath *et al*., 1998; Achinewhu and Oboh, 2002; Ola and Oladipo, 2004; Basti *et al*., 2006; Hernandez-Herrero *et al*., 2006; Baygar *et al*., 2008).

Bacteria present on the fish are normally associated with those found in their natural environment and influenced by the season and the harvesting conditions. The proportion of the initial population can easily be changed after the harvesting process depending on the ability of those bacteria to adapt to the new conditions (ICMSF, 1998). Spoilage bacteria are predominant on newly caught fish, but some pathogenic bacteria could also be present in the skin, gills or guts. According to Vanderzant and Spilttstoesser (1992), and Huss (1995), the type and number of pathogenic bacteria found in fish can be divided in two groups: (1) indigenous pathogenic bacteria, which are commonly found in the aquatic environment, they are present on the live fish and their presence in the final product is predictable (*e.g. Clostridium botulinum*, *Listeria monocytogenes*, *Aeromonas hydrophila* and

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Vibrio sp.) and (2) nonindigenous pathogenic bacteria, which are normally associated with human or warm-blood animals and their faeces, and not naturally present in fish or seafood products (*e.g. Salmonella*, *Escherichia coli* and *Stapylococcus aureus*). When found they are a consequence of process contamination or mishandling. The presence of indigenous pathogenic bacteria does not represent a hazard in itself, because they are normally at very low levels. The problems can arise when handling, processing or storing conditions provide favourable conditions for the growth of the pathogenic bacteria.

In the present study, we applied sensory and certain microbial analysis to assess quality of raw fish sold at a market in Siliguri, the largest city in the northern region of West Bengal, India.

Materials and Methods

Regular surveys were undertaken in a fish market at Shivmandir (near North Bengal University campus) of Siliguri city during June to August 2008. During each survey, a particular fish species was randomly selected, its source was noted, *i.e.*, whether (a) freshly caught from river, (b) pond-cultured or (c) iced fish supplied from far off places (southern districts of West Bengal or other states like Bihar and Andhra Pradesh, or imported from Bangladesh) and a sensory analysis, *i.e.*, quality index method (QIM) was applied to assess its quality (Table 1). The scores for all the characteristics were then summed to give an overall sensory score. QIM gives scores of zero for very fresh fish while increasingly larger totals result as fish deteriorate (Huss, 1995). Most of the parameters choosen are equal to many other schemes. However, some alterations were carried out to the original scheme developed by the Tasmanian Food Research unit to suit freshwater species sold in indian markets. The QIM scores of the different fish species were examined using one-way analysis of variance (ANOVA) and further subjected to Duncan's Multiple Range Test to determine significant differences at $p<0.05$. The statistical analyses were performed using MS Excel and Mstat programmes.

For microbiological analysis, raw fish samples were collected from the market during 10:00 to 11:00 AM in the morning and transferred to the laboratory in polythene bags. Samples were processed within an hour of collection. A small quantity (about 1 g) of scales or an upper layer of the skin surface (for scale-less fish samples) were aseptically removed by a forceps. Other organs like gill, liver and a portion of gut with gut-contents were also aseptically removed and transferred to a 0.85% NaCl solution. The organs were then macerated and serial dilution was made from the washing. The total aerobic heterotrophic bacteria were enumerated in nutrient agar by the conventional spread plate method (Chen and Kueh, 1976; Cappuccino and Sherman, 1992). *Aeromonas* spp. and *Pseudomonas* spp. were similarly enumerated on *Aeromonas* Isolation Medium Base (along with *Aeromonas* selective supplement) and *Pseudomonas* Isolation Agar, respectively. *Salmonella* spp. was enumerated on Bismuth Sulphite Agar, while for the coliform count, MacConkey Agar was used. All the bacteriological media were obtained from Himedia Laboratories Ltd., Mumbai, India. After inoculation, the petridishes containing the culture media were incubated at 37°C for 24 hr. The populations of bacteria were expressed in terms of cfus. g^{-1} (colony forming units).

Results and Discussion

The source and QIM score of different fish species recorded in the present experiment are presented in Table 2. *Tenulosa ilisha* and *Oreochromis mossambicus* recorded significantly higher QIM scores, compared to other species ($p<0.05$). They were followed by species like *Ompok pabda* and *Eutropiichthys vacha*, which recorded QIM scores, lower than *Oreochromis mossambicus* and *Tenulosa ilisha*, but higher than the other species studied. Riverine fish, *Lepidocephalichthys guntea* and *Channa punctatus* scored the lowest QIM scores (0) while scores for *Puntius ticto* and *Mystus vittatus* were very marginal. Pond cultured species like *Cirrhinus mrigala*, *Catla catla*, *Labeo rohita*, *Labeo bata* and *Cyprinus carpio* also scored trivial QIM scores ($p<0.05$).

Counts of total heterotrophic bacteria, *Aeromonas* spp., *Pseudomonas* spp., *Salmonella* spp. and total coliforms recorded from the tissues of different fish species are presented in Tables 3 (skin), 4 (gill), 5 (gut), and 6 (liver). The number of colonies of heterotrophic bacteria were highest in liver (14 species) and gut (11 species). In only two of the studied species, the total heterotrophic bacteria counts were found to be highest in the gill, with respect to other tissues. Highest counts of *Aeromonas* spp. were recorded

Table - 1: Quality Index Method (QIM) as applied to assess quality of raw fish

Quality parameter	Character	Observation	Score
General appearance	Skin	Bright	0
		Dull	1
	Bloodspot on gill cover	None	0
		Small (10-30%)	1
		Big (30% or more)	2
	Stiffness	Stiff, in *rigor mortis*	0
		Elastic	1
		Soft	2
	Belly	Firm	0
		Soft	1
		Belly burst	2
	Smell	Fresh	0
		Neutral	1
		Musty/sour	2
		Stale meat/rancid	3
Eyes	Clarity	Clear	0
		Cloudy	1
	Shape	Normal	0
		Sunken	1
Gills	Colour	Red	0
		Faded	1
	Smell	Fresh	0
		Neutral	1
		Rancid	2
Sum of scores			**min 0; max 15**

Table - 2: Source and Quality Index Method (QIM) score of different fish species

Species	n	Source	QIM score
Family: Bagridae			
Aorichthyes seenghala	4	iced fish supplied from far off places	3.25[d]
Mystus vittatus	5	freshly caught from river	0.20[g]
Mystus cavasius	5	iced fish supplied from far off places	4.40[c]
Family: Cyprinidae			
Puntius gonionotus	4	pond-cultured fish	1.75[f]
Puntius ticto	8	freshly caught from river	0.13[g]
Cirrhinus reba	3	freshly caught from river	1.33[f]
Cirrhinus mrigala	5	pond-cultured fish	0.40[g]
Catla catla	5	pond-cultured fish	0.40[g]
Barilius shacra	4	freshly caught from river	2.25[e]
Amblypharyngodon mola	8	freshly caught from river	2.13[e]
Labeo rohita	5	pond-cultured fish	0.60[g]
Labeo bata	5	pond-cultured fish	0.40[g]
Labeo calbasu	3	freshly caught from river	3.33[d]
Hypophthalmichthys molitrix	4	pond-cultured fish	2.00[e]
Cyprinus carpio	4	pond-cultured fish	0.50[g]
Family: Cobitidae			
Lepidocephalichthys guntea	8	freshly caught from river	0[h]
Family: Xenentodontidae			
Xenentodon cancila	3	freshly caught from river	3.67[d]
Family: Clupeidae			
Tenulosa ilisha	3	iced fish supplied from far off places	7.00[a]
Family: Notopteridae			
Notopterus notopterus	4	freshly caught from river	3.25[d]
Family: Siluridae			
Ompok pabda	3	iced fish supplied from far off places	5.33[b]
Family: Schilbeidae			
Eutropiichthys vacha	3	iced fish supplied from far off places	5.00[b]
Family: Cichlidae			
Oreochromis mossambicus	3	iced fish supplied from far off places	6.33[a]
Family: Channidae			
Channa punctatus	4	freshly caught from river	0[h]
Family: Mastacembelidae			
Mastacembelus armatus	3	freshly caught from river	3.00[d]
Macrognathus aculeatum	4	freshly caught from river	2.75[e]
Family: Gobiidae			
Glossogobius giuris	4	freshly caught from river	2.33[e]
Family: Stromateidae			
Pampus argenteus	3	iced fish supplied from far off places	4.33[c]

Different superscripts in a column indicate statistically significant differences at $p<0.05$

from gut in 13 species, liver in 13 species and gill in one species. *Pseudomonas* spp. was isolated from only seven species, among them, highest counts were recorded from liver in four species, gut in two species and gill in one species. Interestingly, four among the seven species in which *Pseudomonas* was isolated are exotic in nature (*Puntius gonionotus*, *Hypophthalmichthys molitrix, Cyprinus carpio* and *Oreochromis mossambicus*), but are increasingly being cultured in India.

Highest counts of *Salmonella* spp. were recorded from liver in 14 species, gut in 10 species and gill in three species. Highest counts of coliform organisms were found in liver of 12 species, gut of 11 species and gill of four species. Among the tissues examined, the lowest counts of total heterotrophic bacteria, *Aeromonas* spp., *Pseudomonas* spp., *Salmonella* spp. and total coliforms were recorded from the skin in every fish species. Interestingly, similar to the QIM score, the highest counts of pathogenic bacteria (except

Table - 3: Total heterotrophic bacteria, *Aeromonas* spp., *Pseudomonas* spp., *Salmonella* spp. and total coliform count recorded from the skin of different fish species

Species	Total plate count (cfus × 10^3 g^{-1})	*Aeromonas* plate count (cfus × 10^2 g^{-1})	*Pseudomonas* plate count (cfus × 10^2 g^{-1})	*Salmonella* plate count (cfus × 10^2 g^{-1})	Total coliforms (cfus × 10^2 g^{-1})
Family: Bagridae					
Aorichthyes seenghala	66	46	40	55	61
Mystus vittatus	59	32	00	29	55
Mystus cavasius	81	53	00	25	52
Family: Cyprinidae					
Puntius gonionotus	51	35	20	40	48
Puntius ticto	49	44	00	35	45
Cirrhinus reba	52	42	00	32	61
Cirrhinus mrigala	87	61	00	25	60
Catla catla	77	60	00	58	72
Barilius shacra	98	31	00	39	78
Amblypharyngodon mola	66	29	00	42	93
Labeo rohita	78	66	00	76	71
Labeo bata	61	60	00	45	66
Labeo calbasu	57	46	00	35	50
Hypophthalmichthys molitrix	77	79	00	65	82
Cyprinus carpio	72	67	60	53	49
Family: Cobitidae					
Lepidocephalichthys guntea	88	51	00	33	70
Family: Xenentodontidae					
Xenentodon cancila	91	75	00	64	81
Family: Clupeidae					
Tenulosa ilisha	92	115	00	107	112
Family: Notopteridae					
Notopterus notopterus	73	73	00	52	91
Family: Siluridae					
Ompok pabda	78	46	00	64	79
Family: Schilbeidae					
Eutropiichthys vacha	96	40	00	85	93
Family: Cichlidae					
Oreochromis mossambicus	83	66	32	55	75
Family: Channidae					
Channa punctatus	69	62	37	51	55
Family: Mastacembelidae					
Mastacembelus armatus	109	51	00	55	76
Macrognathus aculeatum	96	72	00	31	71
Family: Gobiidae					
Glossogobius giuris	62	42	00	33	39
Family: Stromateidae					
Pampus argenteus	92	77	41	69	77

Pseudomonas spp.) were recorded in *Tenulosa ilisha* for all the tissues except the liver. Due to degeneration of inner body organs, the liver could not be dissected out from the samples of *Tenulosa ilisha.*

The artisanal fisherman, fishing for a few hours and returning to sell his catch while the fish is still alive or very fresh, does not need a complicated quality assurance system. The customers know very well the quality of the fish, and most often the fish are caught, sold and consumed within the same day (Huss, 1995). For fish culturists, netting out fish for sale depends on the demand of the market, and higher demand invariably results in greater harvest. Both, freshly caught fish from a river or fish netted out from a pond were of good quality, as indicated from the QIM scores. In our study, species like *Oreochromis mossambicus*, *Tenulosa ilisha*, *Ompok pabda* and *Eutropiichthys vacha* recorded significantly higher QIM scores, indicating a decline in quality. The problem of quality generally arises with

Table - 4: Total heterotrophic bacteria, *Aeromonas* spp., *Pseudomonas* spp., *Salmonella* spp. and total coliform count recorded from the gill of different fish species

Species	Total plate count (cfus × 10^3 g^{-1})	*Aeromonas* plate count (cfus × 10^2 g^{-1})	*Pseudomonas* plate count (cfus × 10^2 g^{-1})	*Salmonella* plate count (cfus × 10^2 g^{-1})	Total coliforms (cfus × 10^2 g^{-1})
Family: Bagridae					
Aorichthyes seenghala	98	76	69	70	79
Mystus vittatus	98	61	00	56	77
Mystus cavasius	115	67	00	72	98
Family: Cyprinidae					
Puntius gonionotus	80	67	39	73	92
Puntius ticto	93	69	00	55	78
Cirrhinus reba	98	61	00	63	109
Cirrhinus mrigala	108	75	00	72	65
Catla catla	109	88	00	60	75
Barilius shacra	117	59	00	42	83
Amblypharyngodon mola	110	42	00	55	121
Labeo rohita	112	67	00	79	88
Labeo bata	99	70	00	82	88
Labeo calbasu	73	73	00	56	66
Hypophthalmichthys molitrix	127	115	00	68	82
Cyprinus carpio	122	70	91	61	88
Family: Cobitidae					
Lepidocephalichthys guntea	122	77	00	81	98
Family: Xenentodontidae					
Xenentodon cancila	118	101	00	98	108
Family: Clupeidae					
Tenulosa ilisha	122	130	00	132	130
Family: Notopteridae					
Notopterus notopterus	117	88	00	92	129
Family: Siluridae					
Ompok pabda	121	51	00	64	79
Family: Schilbeidae					
Eutropiichthys vacha	118	53	00	90	100
Family: Cichlidae					
Oreochromis mossambicus	107	91	51	55	75
Family: Channidae					
Channa punctatus	95	62	46	67	79
Family: Mastacembelidae					
Mastacembelus armatus	120	66	00	59	79
Macrognathus aculeatum	117	85	00	47	110
Family: Gobiidae					
Glossogobius giuris	105	78	00	76	72
Family: Stromateidae					
Pampus argenteus	125	97	52	80	106

iced fish supplied from far off places since it is very difficult to adjudge the duration of storage.

In Bengali households, after the removal of scales and digestive tract, pieces of fish are rubbed with salt and powder of turmeric, *Curcuma longa*. Then, pieces of fish are either fried in oil or, the oil is heated and fish pieces, along with ingredients necessary for preparation of a fish curry are placed in the oil and pressure-cooked (Anon, 1990). Such procedure ensures that the cooked food is bacteria-free. However, some communities and tribes residing in the northern region of West Bengal, particularly, the Rabha, Dhimal, Mech and Toto tribes consume undercooked fish (personal observations).

Ashenafi (1989) observed that it was not possible to obtain food items with low levels of contamination in local markets in Ethiopia because they were unhygienically handled by various possible customers before they are finally purchased. In addition, according

Table - 5: Total heterotrophic bacteria, *Aeromonas* spp., *Pseudomonas* spp., *Salmonella* spp. and total coliform count recorded from the gut of different fish species

Species	Total plate count (cfus × 10^3 g^{-1})	*Aeromonas* plate count (cfus × 10^2 g^{-1})	*Pseudomonas* plate count (cfus × 10^2 g^{-1})	*Salmonella* plate count (cfus × 10^2 g^{-1})	Total coliforms (cfus × 10^2 g^{-1})
Family: Bagridae					
Aorichthyes seenghala	122	102	110	95	125
Mystus vittatus	90	66	00	70	77
Mystus cavasius	117	86	00	77	91
Family: Cyprinidae					
Puntius gonionotus	118	89	77	85	119
Puntius ticto	110	69	00	79	93
Cirrhinus reba	91	79	00	57	102
Cirrhinus mrigala	109	79	00	38	80
Catla catla	119	63	00	92	95
Barilius shacra	139	98	00	43	119
Amblypharyngodon mola	129	43	00	93	101
Labeo rohita	111	83	00	89	71
Labeo bata	125	65	00	70	86
Labeo calbasu	103	81	00	80	85
Hypophthalmichthys molitrix	118	129	52	80	130
Cyprinus carpio	133	95	103	75	98
Family: Cobitidae					
Lepidocephalichthys guntea	115	83	00	47	96
Family: Xenentodontidae					
Xenentodon cancila	130	95	00	129	117
Family: Clupeidae					
Tenulosa ilisha	137	133	00	149	140
Family: Notopteridae					
Notopterus notopterus	126	123	00	67	113
Family: Siluridae					
Ompok pabda	130	98	00	95	115
Family: Schilbeidae					
Eutropiichthys vacha	135	57	00	108	105
Family: Cichlidae					
Oreochromis mossambicus	115	90	57	69	103
Family: Channidae					
Channa punctatus	107	87	45	81	105
Family: Mastacembelidae					
Mastacembelus armatus	123	80	00	84	86
Macrognathus aculeatum	96	93	00	53	109
Family: Gobiidae					
Glossogobius giuris	121	85	00	68	85
Family: Stromateidae					
Pampus argenteus	128	106	66	95	122

to Ashenafi (1989), the hygienic condition of fishing boats and fishermen in Ethiopia was very unsatisfactory. Lakshmanan *et al.* (1984) reported high coliform counts in fish landed in Cochin harbour in southern India. Most of the species in the present study were either captured from rivers or harvested from ponds in relatively smaller quantities, compared to the massive harvest by fishing vessels operating in open sea. Besides, since most fish sellers dealt with small amounts of fish carefully, the hygienic conditions can be said to be relatively satisfactory. In spite of attaining better quality through lower QIM scores (Table 2), higher counts of pathogenic bacteria in the internal organs of some pond cultured species, particularly, *Cirrhinus mrigala*, *Catla catla*, *Labeo rohita* and *Labeo bata*, compared to some of the river-captured species (which scored higher QIM scores), appear bit confusing. The higher counts could be related to manuring in the fish culture ponds. In an earlier investigation, we found high counts of *Aeromonas* spp. and *Pseudomonas* spp. from fish ponds manured with cow dung and poultry excreta (Jha *et al*., 2008). According to Apun *et al.* (1999),

Table - 6: Total heterotrophic bacteria, *Aeromonas* spp., *Pseudomonas* spp., *Salmonella* spp. and total coliform count recorded from the liver of different fish species

Species	Total plate count (cfus × 10^3 g^{-1})	*Aeromonas* plate count (cfus × 10^2 g^{-1})	*Pseudomonas* plate count (cfus × 10^2 g^{-1})	*Salmonella* plate count (cfus × 10^2 g^{-1})	Total coliforms (cfus × 10^2 g^{-1})
Family: Bagridae					
Aorichthyes seenghala	123	90	82	88	118
Mystus vittatus	102	70	00	78	89
Mystus cavasius	112	81	00	51	95
Family: Cyprinidae					
Puntius gonionotus	95	80	45	77	105
Puntius ticto	106	80	00	83	96
Cirrhinus reba	79	44	00	33	91
Cirrhinus mrigala	114	62	00	69	69
Catla catla	115	60	00	74	75
Barilius shacra	151	138	00	52	116
Amblypharyngodon mola	125	38	00	82	132
Labeo rohita	118	93	00	90	79
Labeo bata	101	85	00	100	68
Labeo calbasu	111	88	00	81	91
Hypophthalmichthys molitrix	139	128	77	93	142
Cyprinus carpio	145	93	111	90	115
Family: Cobitidae					
Lepidocephalichthys guntea	110	75	00	50	101
Family: Xenentodontidae					
Xenentodon cancila	120	102	00	125	112
Family: Clupeidae					
Tenulosa ilisha	N.D.	N.D.	N.D.	N.D.	N.D.
Family: Notopteridae					
Notopterus notopterus	120	130	00	67	140
Family: Siluridae					
Ompok pabda	123	49	00	89	108
Family: Schilbeidae					
Eutropiichthys vacha	142	61	00	112	115
Family: Cichlidae					
Oreochromis mossambicus	120	97	60	88	110
Family: Channidae					
Channa punctatus	112	96	40	92	115
Family: Mastacembelidae					
Mastacembelus armatus	124	84	00	88	85
Macrognathus aculeatum	121	90	00	51	111
Family: Gobiidae					
Glossogobius giuris	117	109	00	78	73
Family: Stromateidae					
Pampus argenteus	131	120	68	99	120

N.D. = Not dissected / damaged

there is a strong correlation between the bacterial species present in the pond water and the fish cultured. The presence of coliform bacteria and species such as *Salmonella*, known to be the cause of enteric and other infectious disease, on the flesh of fish indicated that they have the potential of causing human diseases when consumed or handled (Pal and Dasgupta, 1992).

In the present study, different types of pathogenic bacteria were encountered in the raw fish sold in a market in Siliguri city. Since fish are properly cooked in Bengali households, the risk of disease from fish consumption is relatively less. However, some communities and tribes (other than the Bengali populace) residing in the region are known to consume undercooked fish and proper cooking methods should be followed in view of the present findings

to avoid health risks. Besides, utmost care should be taken while handling fish, particularly the iced fish.

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