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Occurrence of *Vibrio* sp. in *Sardinella longiceps* during spawning season along the west coast of India.

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Present study consists the occurrence of potentially pathogenic Vibrio species in Sardinella longiceps during this season. Gut, gills and ovary of S. longiceps were studied for bacterial flora. Here we report presence of *Vibrio parahaemolyticus*, *Vibrio mimicus* and *Vibrio metschnikovii* in all the organs studied, except male testes where *Vibrio metschnikovii* was not reported. Vibrio isolated from S. longiceps are not fish pathogens but they are human pathogens so care should be taken to avoid vibrio food poisoning associated with the consumption of S. longiceps during this season.

[Key words: Fish**,** Sardine, *Vibrio*, Pathogens, Spawning]

Introduction

India with a coastline of over 7500 km and has a wide exclusive economic zone of 2.02 million km^2 with large potential for capture fisheries. Clupeids fishes, comprising oil sardine, white baits, and lesser sardines contribute about one third of marine fish catches of India. Oil sardine, *S. longiceps* ranks as a very valuable commercial fish owing to its food value and industrial usage for production of fishmeal and oil (Deshmukh et al., 2009).

As *S. longiceps* are filter feeders that live in areas with a dense microflora. They accumulate bacteria from the environment and may carry bacteria on their surfaces and in their tissues and body fluids. Majority of bacteria causing disease in marine fish are opportunistic pathogens that are present as part of the normal seawater microflora. Few are obligate pathogens, i.e., dependent on a living host for their propagation (Hansen and Olafsen 1999).
The disease syndrome termed vibriosis was one of the first diseases of marine fish to be described (Sindermann, 1970; Home, 1982). Vibriosis is one of the most prevalent fish diseases and is caused by bacteria belonging to the genus *Vibrio.* Vibrio species such as *V. anguillarum, V alginolyticus, V ordalii, V, salmonicida* and *V. Vulnificus* biotype 2 (serovar E) are pathogens for marine species of fish (Hjeltnes & Roberts 1993). These species cause haemorrhagic septicaemia in marine fish and may lead to substantial mortalities in cultured populations (Wong et al. 1990). While *V.cholerae, V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus*, V. metschnikovii, are among the most frequently isolated species in human diseases.

This study will help us to understand the occurrence of vibrio infections in *S. longiceps* during spawning season. Consumption of raw or improperly cooked infected fish may cause gastrointestinal disease thus having the knowledge of pathogens will help preventing their proliferation and spreading.

Methods and Materials

Bacteriological examinations were conducted as soon as possible after the fish were caught where the intestinal tracts, gills and ovary were immediately removed aseptically and examined immediately on return to this laboratory**.**

To enumerate bacteria from gut, gills and ovary, three successive washes in sterile seawater was given to each organ before being homogenized. Dilutions of filtered and sterile seawater obtained from the homogenate: total direct count (TDC), heterotrophic bacteria count (HBC) and total vibrio count (TVC). The results are expressed as cells /ml. For HBC, a 0.1 ml aliquot of each dilution was plated in triplicate on Zobell marine agar (ZMA). For TVC, a 0.1 ml aliquot of each dilution was plated in triplicate on thiosulfate citrate bile sucrose agar (TCBS). After 24 hours incubation at room temperature (27°C), the numbers of colonies on both media were counted and the average number of colony-forming units (CFU) was calculated.

2.3 Isolate characterization

Bacterial isolates were taken randomly from ZMA plates of each sampling organ. 11 colonies on the bases of phenotypic differences were isolated and

identification of isolates was determined. The isolates were characterized to genus level following the identification schemes of Bergey's Manual of Determinative Bacteriology (Sneath 1994), Routine identification included the following tests: colony pigmentation; Gram reaction, catalase and oxidase. Motility, fermentative or oxidative metabolism , growth on TCBS , 0% and 6% NaCl, and Thornley's decarboxylase for arginine , Moeller's decarboxylases for lysine and ornithine , Voges-Proskauer, methyl red and indole , production of L-galactosidase , acid production from L-arabinose, arbutin, myo-inositol, D-mannitol, salicin, sorbitol and sucrose, utilization as sole carbon sources of L-arabinose, citrate, D-glucose, D-glucosamine, lactose, K-ketoglutarate and D-melibiose, hydrolysis of urea, gelatine and esculin,

Result and Discussion

Total five samples, were analysed for checking length, weight, maturity stage as well as for isolating bacteria from different organs of *S. longiceps* (Table 1and Table 2). It was seen during sampling time *S. longiceps* was in its VI th stage of maturity.

Table 1 Average length weight and maturity stage of *S. longiceps* during sampling period.

Samples	Total length (cm)	Weight (gm)	Gonad weight (gm)	Gonad length (cm)	Maturity stage
Female Sardine	18.5 ± 0.4	60.8 ± 2.2	5.4 ± 0.3	6.5 ± 0.4	VI
Male Sardine	18.4 ± 0.6	61.5 ± 2.9	4.1± 0.4	5.6 ± 0.6	VI

Table 2 Bacterial counts obtained from gills, gut and ovary of *S. longiceps* during sampling period

Sampling organ	HBC (cfu/g)	TVC (cfu/g)
Gills	7.13 x 10^4	3.43 x 10^3
Gut	5.08 x 10^5	5.50 x 10^4
Ovary/ Testes	3.45 x 10^4	1.53 x 10^3

Gut, gills and ovary of *S. longiceps* were studied for bacterial flora. It was seen that maximum number of heterotrophic bacteria as well as TVC resided in gut that is 5.08 x 10^5 and 5.50 x 10^4 while least number was observed in ovary 3.45 x 10^4 and 1.53 x 10^3 respectively. Where as in gills 7.13 x 10^4 of hetrotrophic bacteria and 3.43 x 10^3 of TVC was observed.

A total of 11 isolates were isolated from TCBS plate according to its morphological appearance and undergone biochemical tests by referring to the identification schemes of Bergey's Manual of Determinative Bacteriology for the identification of Vibrio to the species level. Vibrio species identified were, *Vibrio parahaemolyticus, Vibrio mimicus and Vibrio metschnikovii* (Table 3). The type of vibrio sp. from each organ is given in Table 4. It was seen that all three types of vibrio's were observed in gills, gut and in the ovary of female while only two types of vibrio's are observed in male testes.

Table 3: Morphological, physiological and biochemical characteristics of Vibrio sp. Isolated from *S. longiceps*.

Characteristics	Biotype		
	Vibrio parahaemolyticus	*Vibrio metschnikovii*	*Vibrio mimicus*
Colour on TCBS	G	Y	G
Oxidase	+	-	+
Growth in 0% NaCl	-	+	+
Growth in 1% NaCl	+	-	+
Growth in 6 % NaCl	+	+	+
Voges-Proskauer reaction	-	-	-
Arginine utilization	-	-	-
ONPG	-	-	+
Citrate Utilization	-	-	-
Ornithine utilization	-	-	-
Mannitol Fermentation	+		+
Arabinose Fermentation	-	+	-
Sucrose Fermentation	-	+	-

Table 4: Presence of Vibrio sp.in different organs of *S.longiceps.*

Fish Organs	*Vibrio parahaemolyticus*	*Vibrio mimicus*	*Vibrio metschnikovii*
Gut	+	+	+
Gills	+	+	+
Female Ovary	+	+	+
Male Testes	+	+	

Discussion:

The present study was conducted during south west monsoon in the month of September. It was seen that during sampling time *S. longiceps* was in its VIth stage of maturity. Antony raja (1966) has reported that during July-September period *S. longiceps is* in various degrees of spent condition. Intense spawning usually takes place during August and September. The gonadal conditions of these spawners range from stage V to VI in the different shoals seen during these months.

The members of the genus vibrio have been frequently defined as opportunistic and potential pathogenic bacteria of the water bodies specially in warm climate zones (Feldhusen 2000). Therefore, from the public health perspective, the occurrences of these bacteria have caused concerns for authorities. Vibrio species which comes under biotype 2 (serovar E) are pathogens for marine species of fish (Hjeltnes & Roberts 1993). We have not found any of these vibrio species during the sampling period.

Vibrio species reported in this study were, *Vibrio parahaemolyticus, Vibrio mimicus and Vibrio metschnikovii.* All three types of vibrio's were seen in all three organs studied accept male testes which showed presence of only *Vibrio parahaemolyticus* and *Vibrio mimicus.* Total number of vibrio per gram of organ differed, gills had highest *Vibrio* as well as total bacterial count followed by gut and ovary.

V. Parahaemolyticus is a marine bacterium which causes acute gastroenteritis and food poisoning in humans who consume raw or improperly cooked seafood (Hlady & Montz 1996, Pan et al.1997*). Vibrio parahaemolyticus* is considered as a human pathogen more than a fish pathogen. There are few reports linking it to fish infections (Wong et al. 1990, Yii et al. 1997). In both reports this species is described as the causative agent of vibnosis in groupers *Epinephelus coioides* in Taiwan, while Alkaide et al., 1999 reported *V. parahaemolyticus* to be a bacterial pathogen in Iberian tooth carp *Aphanius iberus*.

Vibrio mimicus occurs in seawater and shellfish, and in humans, and has been associated with gastroenteritis following ingestion of seafood and with ear infection after exposure to sea water (Ciufecu et al.1983, Shandera et al. 1983). It has also been reported both in brackish and freshwater environments (Bockemiihl et al. 1986, Chowdhury et al. 1989), and was isolated from the gills and from under the carapace of freshwater prawns *Macrobrachium malcolmsonii* by Chowdhury et al. (1986*). V. mimicus* appear to be part of the normal bacterial flora of the aquatic environment in aquaculture ponds. But it can be an opportunistic pathogen causing a systemic infection following the stress of overcrowding or mismanagement and poor water quality (Eaves and Ketterer 1994).

Vibrio metschnikovii is a natural inhabitant of the aquatic environment and has been isolated from seafood, and associated with disease in humans. Matte et al., (2007) studied virulence factors produced by V. metschnikovii strains isolated from fish. It was seen that 38.5%, 92.3% and 100% of the strains were hemolytic on sheep, rabbit, and human blood agar, respectively. Verotoxin was produced by 100% of the strains tested and 76.9% were skin test positive. They concluded that potentially pathogenic Vibrio species, including V. metschnikovii, must be considered when investigating food borne diseases related to consumption of raw or undercooked seafood, mainly fish.

Presence of *V. Parahaemolyticus*, *V mimicus* and *V. Metschnikovii* in all three organs of *S. longiceps* studied during south west monsoon is not a serious problem for fish itself as these bacteria are not pathogens for them but the research has shown that these reported bacteria can affect human health. There are reports of gastrointestinal disease in humans caused by *V. Parahaemolyticus* and *V. mimicus* following consumption by improper cooking of contaminated fish and also *Vibrio metschnikovii* reported to be associated with human disease. Thus there is need to look into precautionary measures to avoid health hazards caused by them.

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