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Note

Polymicrobial skin lesions in the red spot emperor, *Lethrinus lentjan* (Lacepede 1802) during mass incursion towards shore along Kanyakumari coast, south India

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ABSTRACT

Mass incursion of fishes with polymicrobial skin lesions, fin erosions and scale loss was recorded in the red spot emperor *Lethrinus lentjan* (Lacepede 1802) along the Kanyakumari coast, south India during August 2009. An estimated 2.5 t of fish, mostly the red spot emperors were found to migrate in live condition to the shore areas in a stressful state. Microbiological analyses of tissue from sampled fishes revealed three distinct types of bacterial colonies forming 5.2 x 10^5 CFU g^{-1} of the infected tissues. The predominant bacterial colonies were characterized as *Aeromonas* sp. (70.0%) followed by *Flavobacterium* sp. (20%) and *Vibrio* sp. (10%). The *Aeromonas* isolate was highly susceptible to norfloxacin while the *Flavobacterium* and *Vibrio* isolates were susceptible to chloramphenicol. The *Aeromonas* and *Vibrio* isolates exhibited protease and amylase enzyme activities *in vitro,* suggesting their possible role in the progression of skin lesions and scale loss. The possibilities of ambient unknown stressors weakening the fish and subsequent infections by these bacterial isolates are discussed.

Keywords: *Aeromonas*, *Flavobacterium*, Polymicrobial skin lesion, Red spot emperor, *Vibrio*

Instances of mass mortality of fish and washing ashore of large numbers of fishes are reported during algal bloom (Subramanian and Purushothaman, 1985), drastic environmental changes (Pinheiro *et al*., 2010) including seasonal upwelling events of anoxic or hypoxic water conditions (Martill e*t al.,* 2008). The crowded conditions predispose fishes to bacterial infections as in the case of typical aeromonad infections (Hiney and Oliver, 1999). The surface of marine fishes is populated by a wide variety of bacterial genera like *Vibrio* spp., *Pseudomonas* spp., *Photobacterium* spp., *Alcaligenes faecalis*, *Acinetobacter calcoaceticus* and *Flexibacter* spp. (Austin, 1983). Vibrios cause characteristic haemorrhagic septicaemia, external lesions and discharge of blood leading to mortality (Thompson *et al.,* 2004). *Vibrio anguillarum* was predominantly reported from coastal environments, causing septicemia and death in both wild and cultured fish throughout the world (Larsen *et al*., 1991). Among the pseudomonads, *P. anguilliseptica* is an important pathogen of saltwater fish (Austin and Austin, 1999). *Aeromonas* sp., affects a variety of non-salmonid fish (Bricknell *et al*., 1999). These are ubiquitous and opportunistic ones that take advantage of stressed fishes. However, mass migratory behavior towards coast with polymicrobial skin lesions is rare and reported sparingly. Microbiological analyses of fishes exhibiting polymicrobial lesions were carried out from affected fish, *Lethrinus lentjan* (Lacepede 1802) and the salient findings are presented in this paper.

Areas of observation and sample collection

On 28-8-2009, stressful movement of numerous fishes towards the coast and washing ashore of them in live condition along a stretch of coast in Kanayakumari (Tamil Nadu) extending from Rajakkamangalam thurai to Manakudi was noticed. Investigations were conducted by a team from the Vizhinjam Research Centre of the Central Marine Fisheries Research Institute. The areas of observations along the Kanyakumari coast are indicated in the map (Fig. 1). The areas included:

Manakudi (thurai)	- N: 8º 05’ 39"; E: 77º 38’ 53"
Pallam thurai	- N: 8º 05’ 93"; E: 77º 25’ 84"
Chothavilai	- N: 8º 05’ 60"; E: 77º 25’ 84"
Sangu thurai	- N: 8º 05’ 98"; E: 77º 25’ 54"
Periakadu	- N: 8º 05’ 98"; E: 77º 25’ 54"
Rajakkamangalam thurai	- N: 8º 06’ 90"; E: 77º 22’ 35"

Although the instances of washing ashore of fishes in live conditions were confirmed upon visiting the coastal sites, 10 numbers of just dead fish could be recovered from

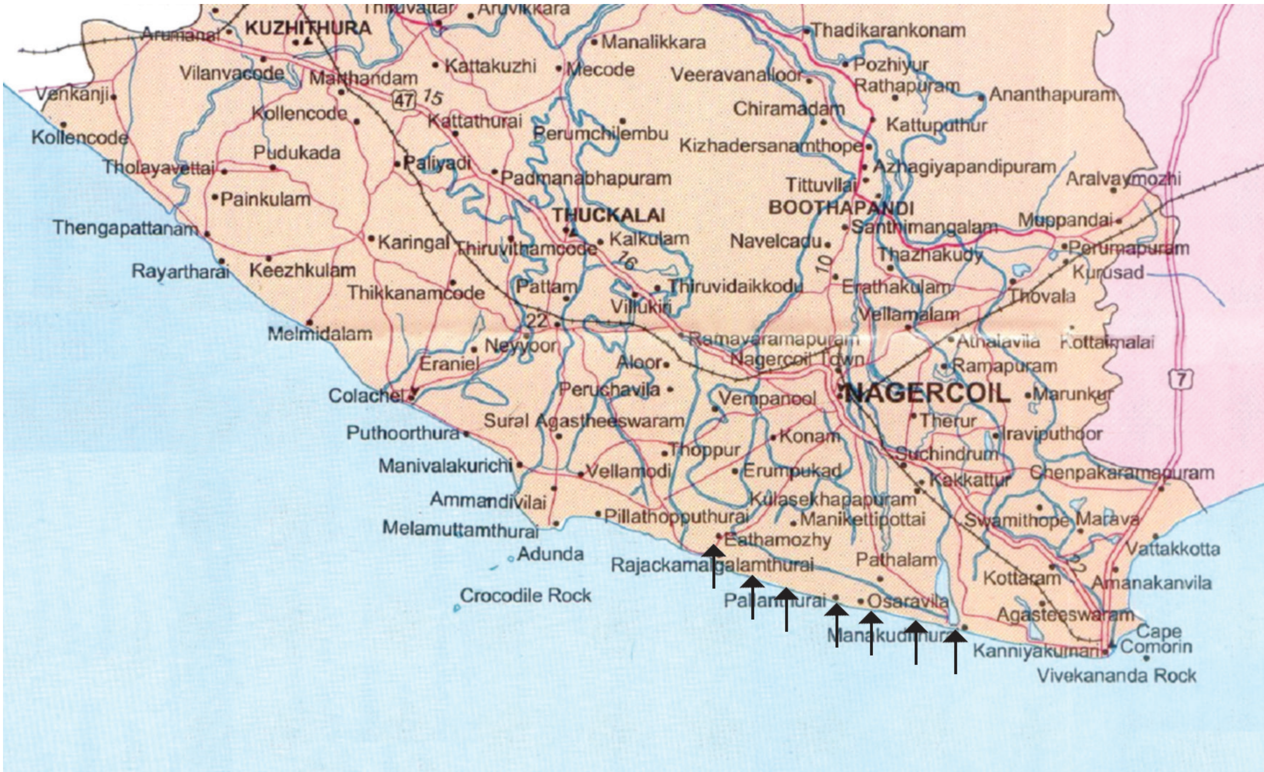

Fig. 1. Location map of Kanyakumari coast indicating the migration of fish in the sampling area (arrows)

the sea shore. Enquiries revealed that all the fishes though weak, were in live condition when they emerged towards the shore. These were immediately hand picked by the nearby residents, fisher folk and fish vendors and were sent to nearby markets for sale. .

All the ten fishes collected from Manakudi area (N: 8° 05' 39"; E: 77° 38' 53") and were identified as red spot emperor, *Lethrinus lentjan* (Lacepede 1802). The fishes exhibited skin erosions and scale loss along the body surfaces on lateral and ventral sides (Fig. 2). An estimated 2.5 t of fish, mostly red spot emperor in live and stressful condition emerged from the sea towards the shore area with similar symptoms.

Fig. 2. Red spot emperor, *Lethrinus lentjan* with scale erosions and sloughing of scales along the body surface

From the affected fishes, microbiological samples were collected as per the standard procedures (Austin and Austin, 1999). The tissue samples from the lesions were aseptically excised, after surface sterilization, weighed, ground in sterilized mortar with pestle, serial dilutions were made and then plated on Zobell Marine Agar. The plates were incubated at room temperature (30.5±2.5 °C). After 18 h of incubation, the countable colonies noted in 10^{-3}dilution were taken into account and from this, the total bacterial load was estimated as Colony Forming Units (CFU) per g of tissue. Three distinct types of bacterial colonies were observed. The isolates were characterized to genus level by standard methods (Austin and Austin, 1999) using selective media such as Aeromonas agar, Pseudomonas agar and Thiosulphate Citrate Bilesalt (TCBS) agar. The sensitivity of the isolates towards common antibiotics was evaluated by the standard disc diffusion assay (Bauer *et al* ., 1966).

The mean bacterial load recorded was 5.2×10^5 CFU g^{-1} of tissue in the infected area. Three types of isolates were noted with distinct morphological, cultural and biochemical characteristics. The results of different characterization tests are summarized in Table 1.

All the isolates retrieved from *L. lentjan* were Gram negative rods. The isolate 1 exhibited spreading and moist nature in the Zobell Marine Agar (ZMA) plates. The same

Table 1. Characteristics of the three distinct types of bacterial isolates retrieved from the skin lesions of *Lethrinus lentjan*

Characterization tests	Isolate- 1*	Isolate- 2*	Isolate- 3*
Colony morphology on Zobell marine agar	Low, spreading and moist colonies	Beige, shiny moist appearing, low crenate colonies	Dirty white and mild yellow irregular colonies
Gram staining	Gram negative short rods	Gram negative, comma shaped rods	Gram negative spiral rods
Biochemical tests			
Indole test	+	+	-
VP test	-	-	-
Citrate utilization test	-	+	-
Catalase test	+	+	+
Arginine dihydrolase	+	-	+
Ornithine decarboxylase test	-	-	-
Growth in selective media			
Mannitol salt agar (MSA)	No growth Pinkish purple colonies	No growth	Yellow coloured colonies
Eosin methylene blue (EMB) agar	No growth	No growth	Pinkish purple colonies
Xylose lysine deoxycholate agar (XLD)	Lactose fermenting (LF) colonies	No growth	Pink colonies
Mac Conkey agar	No growth	No growth	No growth
Thiosuphate citrate bile salt sucrose agar (TCBS)	Profuse growth	Greenish yellow colonies	No growth
Aeromonas isolation medium	No growth	No growth	No growth
Pseudomonas isolation agar	No growth	No growth	No growth
de Mann Rogosa Sharpe (MRS) agar		No growth	No growth
Protease production	+	+	-
Gelatinase	-	-	-
Amylase	+	-	-
Percentage of occurrence	70%	10%	20%

+ = positive; - = Negative

**Identity of the isolates*:

Isolate -1: *Aeromonas* (70%); Isolate -2: *Vibrio* (10%); Isolate -3: *Flavobacterium* (20%)

isolate formed a pinkish purple colour in the eosin methylene blue (EMB) agar. Profuse growth of greenish colonies, the typical characteristic feature of *Aeromonas* species in Aeromonas isolation medium (Hi Media) was noted. The isolate was Indole and Arginine dihydrolase positive. Considering these characteristic features, the isolate was identified and grouped under *Aeromonas* sp. This *Aeromonas* isolate from *L. lentjan* formed 70.0% of the total bacterial load from the infected tissues. The bacterial isolate produced exocellular protease and amylase. The least predominant bacteria, the isolate 2 forming about 10.0% exhibited greenish yellow colony formation, characteristic of *Vibrio* species in the TCBS agar. The isolate was susceptible to the vibriostatic agents. Considering these characteristics, the isolate 2 was grouped as *Vibrio* species. The *Vibrio* strain produced protease in its active growth phase. The isolate 3 identified as *Flavobacterium* sp., formed irregular and translucent mild yellow colonies in ZMA plates. The isolate was catalase positive and citrate negative. Yellow and pink coloration of colonies of the isolate were noted in mannitol salt agar and in xylose lysine deoxycholate agar respectively. The isolate, however, has not produced any exocellular enzymes such as protease, amylase and gelatinase.

The *Aeromonas* isolate was highly susceptible to cephotaxime (30 mm dia), norfoxacin (32 mm dia), co-trimoxazole (26 mm dia) followed by nalidixic acid (25 mm dia). *Flavobacterium* sp., was comparatively more susceptible to chloramphenicol (27 mm), furazolidone (25 mm) and norfloxacin (25 mm) while the *Vibrio* isolate was susceptible to cephotaxime (32 mm), chloramphenicol (32 mm), co-trimoxazole (32 mm), cephalexin (25 mm) and tetracycline (23 mm). The results of antibiotic susceptibility pattern of the three isolates are presented in Table 2.

Table 2. Antibiotic susceptibility pattern of the bacterial isolates from *L. lentjan* [diameter of zone of inhibition (mm)]

Antibiotic	*Aeromonas* sp.	*Vibrio* sp.	*Flavobacterium* sp.
Ampicillin	Nil	Nil	Nil
Cephalexin	Nil	25	20
Cephotaxime	30	32	12
Chloramphenicol	18	32	27
Co-Trimoxazole	26	32	Nil
Erythromycin	15	16	20
Furazolidone	16	Nil	25
Gentamycin	23	Nil	12
Nalidixic acid	25	12	23
Nitrofurantoin	13	14	18
Norfloxacin	32	11	25
Oxytetracycline	12	20	14
Tetracycline	21	23	Nil
Vancomycin	Nil	15	Nil

Nil: Not susceptible (resistant)

Instances of mass mortality of marine fish have been noticed to occur all over the world mainly in the coastal waters as occurrences in deep water areas go unnoticed. A few of these are reported as scientific publications (Pinheiro *et al.*, 2010). In India, Durve and Alagarswami (1964) while reporting the incidence of fish mortality in Athankarai estuary near Mandapam inferred that abnormal environmental factors including rise in salinity levels could have caused such mass mortality of fishes. Blooms of plankton population, largely dinoflagellates have been implicated as reasons for wide spread mortality of many species of fishes. Mortality of fishes and invertebrates associated with a bloom of *Hemidiscus hardmannianus* (Bacillariophyceae) present at a density of 9 to 49 $x10^3$ l^{-1} in Parankipettai (South India) was reported by Subramanian and Purushothaman (1985). High concentration of dinoflagellates causing red water and fish mortality were correlated along the Cape Town (Grindley *et al.*, 1964). In all these instances, the possibility of intake of biotoxins through preferential diet items could have triggered the susceptibility pattern of the fishes. The biological toxins could be ingested by fish either through direct consumption of macroalgae such as *Caulerpa s*p. or indirect consumption of toxic epiphytic dinoflagellates by the reef fishes belonging to Pomacentridae, Acanthuridae and Lutjanidae as indicated by Landsberg (1995). Considering the less mortality of fishes and other invertebrates, the biotoxins as a trigger for mass migration could not be rated high though it cannot be fully ruled out.

Another possibility is the alterations in ambient environment brought about by upwelling events during monsoon. Off Kanyakumari coast, upwelling is effected by the south-west monsoon winds (Smitha *et al.*, 2008). With the onset of the south-west monsoon in May, weak to moderate upwelling occurs off the Kanyakumari coast and spreads northwards along the coast as the monsoon advances, reaching up to Goa coast during peak monsoon season (July to August). Maheswaran *et al.* (2000) reported strong signals of upwelling observed off Kanyakumari and the concomitant reduction in sea surface temperature near the coast by 1.7 ^{o}C off Kanyakumari. Oceanographic changes due to monsoon along the coast have been also published by Rao *et al,* (1992).

In the present instance, predominantly *L. lentjan* was affected and the phenomenon of migration towards the shore areas was noted only on one day and was not repeated. Sloughing or withering of scales were noticed in affected fishes. It has been reported earlier that scale loss could be attributed to the stress and resulting dieresis in salmonid fishes (Smith, 1993). Thus, the incursion of *L. lentjan* with polymicrobial lesions on body surface could be attributed to the ambient stressful conditions as well as secondary invasion by opportunistic pathogenic microbes. Similar trend of multiple pathogens infecting tropical reef fishes along the Florida coast was reported by Landsberg (1995). According to the author, no primary pathogen could be detected from the reef fishes affected by the infections. It is possible that unknown stressors might have weakened the fish, which could have subsequently become susceptible to a number of microbial pathogens including secondary or opportunistic bacteria. The necrosis and erosions in fin and skin could have been caused by the exocellular enzyme activities of the bacteria lodged on the skin of the weakened fish. In the *in vitro* tests, protease activity was noted in *Aeromonas* and *Vibrio* isolates. The *Aeromonas* spp. have been isolated from naturally infected fish in brackish water fishes such as mullets and sea breams (Manal I. El-Barbary, 2010). *Vibrio damsela* causing skin ulcer in damsel fish was reported by Lov *et al.* (1981). Vibrios producing exocellular enzymes are known to degrade hardy body surface areas of fish such as the sea horse, *Hippocampus kuda* (Thampiraj *et al*., 2010).

In the natural marine environment, whenever chronic stressors or disease affect the marine fish population, the moribund fish or recently dead fish will be quickly removed from the affected area by predation, wind or prevailing water currents. Detection of such outbreaks is rare unless they occur closer to the shore areas as in the present reported incidence.

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