International Journal of Fisheries and Aquatic Studies 2015; 2(4): 178-183

ISSN: 2347-5129
IJFAS 2015; 2(4): 178-183

www.fisheriesjournal.com
Received: 01-01-2015
Accepted: 05-02-2015

C.Muthu Ramakrishnan
Centre for Aquaculture Research and Extension (CARE), St. Xavier's College (Autonomous), Palayamkottai – 627002, Tamil Nadu, India.

M.A.Haniffa
Centre for Aquaculture Research and Extension (CARE), St. Xavier's College (Autonomous), Palayamkottai – 627002, Tamil Nadu, India.

P.Jeya Sheela
Centre for Aquaculture Research and Extension (CARE), St. Xavier's College (Autonomous), Palayamkottai – 627002, Tamil Nadu, India.

Correspondence
M.A.Haniffa
Centre for Aquaculture Research and Extension (CARE), St. Xavier's College (Autonomous), Palayamkottai – 627002, Tamil Nadu, India.

Isolation and identification of microbial flora from EUS infected singhi *Heteropneustes fossilis*

C.Muthu Ramakrishnan, M.A.Haniffa, P.Jeya Sheela

Abstract
Heteropneustes fossilis, commonly called singhi is an obligatory air- breathing fish occurring shallow inland waters of India. For the past two decades this species is declining due to habitat loss, high fishing pressure and easy prone to Epizootic Ulcerative Syndrome (EUS). The infected individuals showing external symptoms like unresponsiveness, wounds, superficial lesions, swelling, discoloration, and deep ulcerative hemorrhages were subjected to bacteriological examination and the pathogens associated with EUS were isolated. The highest microbial load $6.3 \pm 0.4 \times 10^7$ cfu g^{-1} was observed in muscle followed by 5.7 ± 0.6 10^6 cfu g^{-1} in gills and $7.2 \pm 0.9 \times 10^5$ cfu g^{-1} in liver whereas the lowest load $4.3 \pm 0.7 \times 10^4$ cfu g^{-1} was found in intestine. Similarly, a higher percentage of *A. invadans* (15%) and *A. hydrophila* (12.5%) was determined from the muscle of the diseased *H. fossilis* whereas the same was found to be low in the intestine (6% and 5.5% respectively). One fungal species, *Aphanomyces invadans* and nineteen bacterial species were isolated and identified. Among the 20 isolates, *A. invadans* was the only fungus and *A. hydrophila* was dominant among the bacterial isolates from muscle, gill, liver and intestine.

Keywords: *Heteropneustes fossilis*, EUS, *A. invadans*, *A. hydrophila*

1. Introduction
Among the 142 species of catfish found in the Indian subcontinent, the freshwater catfish *Heteropnuestes fossilis*, commonly called singhi is an obligatory air- breathing fish occurring shallow inland waters of India [1]. *H. fossilis* has been identified as vulnerable among the 327 threatened freshwater fish species in India [2]. It constitutes a major bulk of the production in India. *H. fossilis* is an omnivore, highly tolerant to oxygen depleted waters. It commands good consumer preference due to fewer intra muscular spines, tender flesh with delicious taste, high protein, iron and low fat contents [3] and is often recommended to convalescent people [4]. For the past two decades this species is declining due to habitat loss, high fishing pressure and easy prone to EUS disease [5]. At this moment, *H. fossilis* has to be conserved for the future population, since alien species viz: African catfish *Clarias gariepinus* and Thai catfish *Pangasius sutchi* pose a heavy threat to biodiversity of freshwater fishes of our country.
Environmental factors and poor water quality resulting from increased pollution due to effluents discharge and pathogen transfer appear to be as important underlying cause of epizootics. EUS is one of the most destructive diseases among freshwater as well as brackish water fish species in the Asian Pacific region [6, 7]. It is very common in both northern and southern India and has spread through rivers, reservoirs and paddy fields to neighboring states, causing considerable loss to fish farmers. A diverse group of biotic agents such as viruses, bacteria and cutaneous ectoparasites may initiate skin lesions which are subsequently colonized by *Aphanomyces invadans* and ultimately lead to EUS [8].
A. invadans, a highly invasive, specific slow growing fungus causes Epizootic Ulcerative Syndrome [9]. Different pathogenic organisms including bacteria [10, 11], fungi [12, 13] and virus [14] have been reported from naturally infected fish. Roberts *et al.* [15] have reported natural outbreaks of EUS in more than 100 fish species especially in air-breathing fishes. According to Das [7], studies of affected fishes carried out in different countries recorded a wide range of pathogenic fungi including *A. invadans* and the bacterium *A. hydrophila* as the most prevalent.
A critical problem in the study of microbial pathogens of the fish is the correct identification of the causative agents. To facilitate the precise identification of microbes involved in diseases, the usual practice is to undertake biochemical tests and thus identifying the isolates

by their reaction to standard tests following the Bergey's manual [16]. Hence, the present study deals with the isolation and identification of pathogenic microbes from the EUS infected *H. fossilis.*

2. Materials and Methods

2.1 Sample collection

A total of 167 infected *H. fossilis* of average mean length 20 ± 3 cm and average weight 65 ± 2.5 g were collected from Thamiraparani River (8.44^0 N, 77.44^0E) fed systems and also purchased from local fish market at Melapalayam, Tirunelveli, Tamil Nadu, India in the month of April 2006 (Figure 1). They were transported to CARE Aquafarm and acclimatized in stocking ponds for further analysis. All the infected individuals showed external symptoms like unresponsiveness, external wounds, superficial lesions, swelling, discoloration, and deep ulcer hemorrhages.

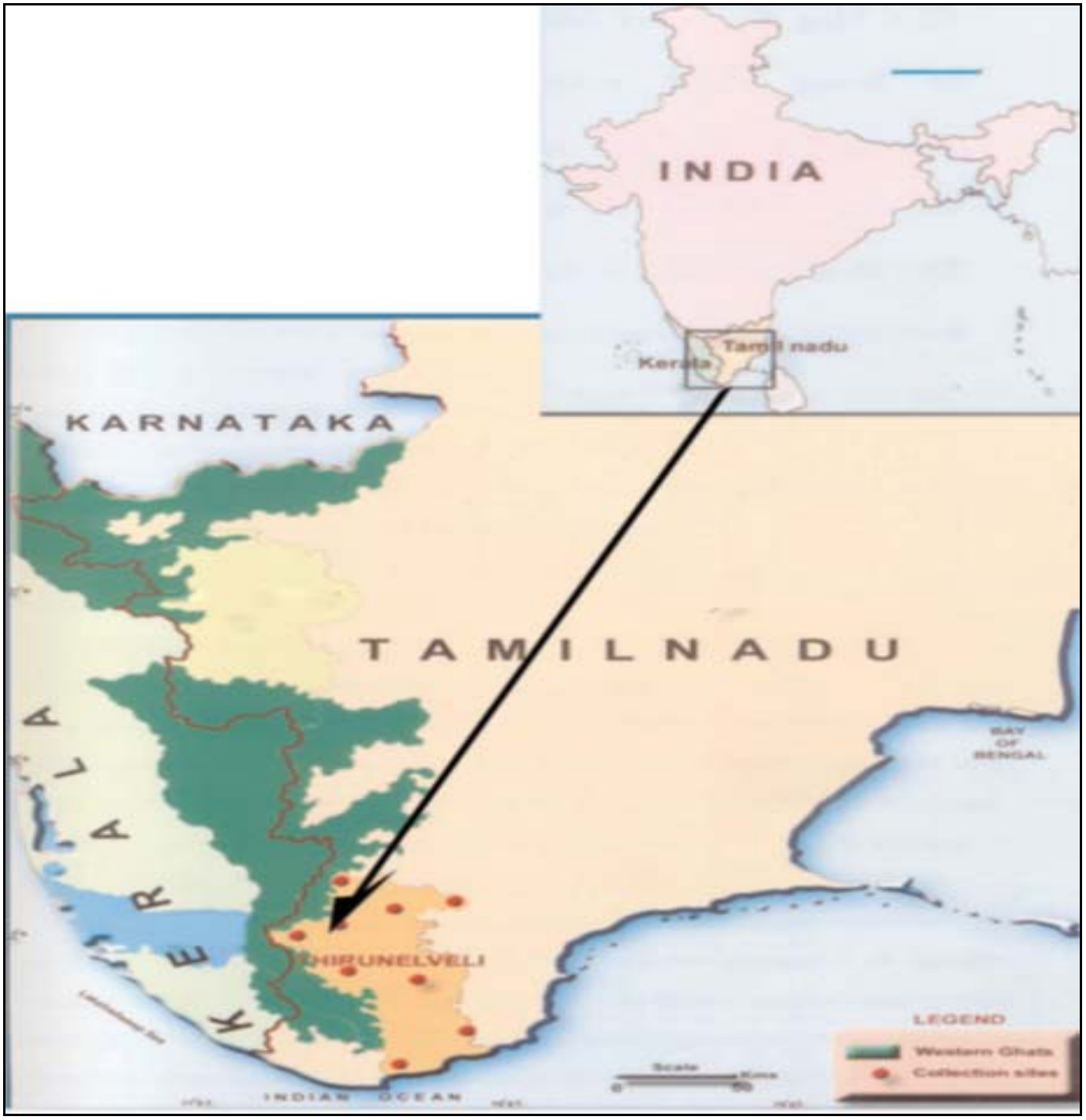

Fig 1: Fish collection site-Tirunelveli, Tamil Nadu, India [8.44°N, 77.44°E]

2.2 Enumeration of Total Heterotrophic Count (THC)

One gram of each samples from muscle, gills, liver and intestine were taken from the diseased fish with moderate lesions, wound and septicemia using a sterile scalpel under aseptic condition. A small piece of muscle (2 mm^3) beneath the margin of the ulcer was scrapped thoroughly and fixed in Czapek Dox agar with Penicillin G (100 units/ml) and oxolinic acid (100 mg/ml) to observe fungal growth [17]. Remaining muscle was homogenized with sterile distilled water and centrifuged at 1000 rpm for 10 minutes. One ml of the supernatant was serially diluted up to 10^{-9} dilution. 1ml was taken from each dilution and pour plate technique was carried

out for the enumeration of total heterotrophic bacterial count using sterile nutrient agar for bacterial and Czapek Dox agar for fungal growth. The bacterial plates were incubated at 37 ^{0}C for 24 - 48 h and fungal plates were incubated at 24 ^{0}C for 7 days. After incubation, the bacterial colonies and fungal hyphae were observed. After the quantitative analysis, the isolated fish pathogens were subjected to standard microbial techniques for identification.

2.3 Identification of bacterial pathogens

Colonies obtained from different selective plates were isolated and streaked on nutrient agar slants and incubated overnight at 37 ^{0}C. The following biochemical tests were conducted to identify the fish pathogens following Bergey's manual of Bacteriological classification [16] and Surendran and Gopakumar [18]. Biochemical tests include Gram's staining, Motility tests (Hanging drop method and Soft agar stabbing method), Kovacs oxidase test, Catalase test, Acid production test (Huge and Leifson synthetic medium), Starch hydrolysis, Gelatin hydrolysis, NaCl tolerance (0%, 5% and 7%), Methyl Red test, Voges-proskauer test, Citrate test, Urease test, Triple sugar iron test, ONPG test, 0/129 Sensitivity test and Amino acid decarboxylase test.

2.4 Isolation of fungus

For fungal isolation, the suspected colonies were inoculated in basic GP broth and one loop of colony was streaked on Czapek Dox agar and incubated at 27 ^{0}C for 72 hrs. After incubation, the fungal colonies were identified based on their morphology and the results were recorded. The identification of the fungus *A. invadans* was made on the basis of attachment to the surface, hyphae and sporangial morphology [19].

3. Results and Discussion

The isolation of microorganisms was based on the infected fish species, their disease status, clinical signs and biochemical diagnosis. The results of the quantitative estimation of microbial count in muscle, gill, liver and intestine of diseased *H. fossilis* are given in Table 1. The highest microbial load of $6.3 \pm 0.4 \times 10^7$ cfu g^{-1} was observed in muscle followed by 5.7 ± 0.6 10^6 cfu g^{-1} in gills and $7.2 \pm 0.9 \times 10^5$ cfu g^{-1} in liver whereas the lowest load of $4.3 \pm 0.7 \times 10^4$ cfu g^{-1} was found in intestine. The percentage distributions of mycotic and bacterial isolates are shown in Table 2. A higher percentage of *A. invadans* (15%) and *A. hydrophila* (12.5%) was determined from the muscle of the diseased *H. fossilis* whereas the same was found to be low in the intestine (6% and 5.5% respectively). One fungal species and nineteen bacterial species were isolated and identified. Among the 20 isolates, *Aphanomyces invadans* was the only fungus and *A. hydrophila* was dominant among the bacterial isolates. Other bacteria isolated from muscle, gill, liver and intestine include *Pseudomonas* sp, *Vibrio* sp, *Serratia* sp, *Enterobacter* sp, *Edwardsiella* sp, *Flavobacterium* sp, *Yersinia* sp, *Klebsiella* sp, *Haemophilus* sp, *Staphylococcus* sp, *Alcaligenes* sp and *V. parahaemolyticus*.

Table 1: Total heterotrophic bacterial count in muscle, gill, liver and intestine of diseased *H. fossilis* (values are mean ± SD)

S.No	Sample (n= 167)	Colony forming unit g^{-1} (cfu g^{-1})
1	Muscle	$6.3 \pm 0.4 \times 10^7$
2	Gill	$5.7 \pm 0.6 \times 10^6$
3	Liver	$7.2 \pm 0.9 \times 10^5$
4	Intestine	$4.3 \pm 0.7 \times 10^4$

Table 2: Microbial load (percentage) in muscle, gill, liver and intestine, and of diseased *H. fossilis*

1	Genera	Number of colonies (%)				
		Muscle	Gill	Liver	Intestine	Total
1	*Aphanomyces invadans*	15	13.5	6.5	6	41
2	*Aeromonas hydrophila*	12.5	9.2	7.8	5.5	35.8
3	*Pseudomonas sp*	2	1	-	0.1	3.1
4	*Vibrio sp*	0.5	0.6	0.5	1.2	2.8
5	*Serratia sp*	0.9	0.5	0.1	0.6	2.1
6	*Enterobacteria sp*	0.6	0.4	0.4	-	1.4
7	*Edwardsiella sp*	0,7	0.2	0.4	0.4	1.7
8	Flavobacteria *sp*	0.4	0.1	0.3	0.1	0.9
9	*Yersinia sp*	0.2	-	0.3	-	0.5
10	*Klebsiella sp*	0.1	0.1	0.1	-	0.3
11	*Haemophilus sp*	0.2	0.1	-	0.2	0.5
12	*Staphylococcus sp*	0.3	0.1	0.2	0.1	0.7
13	*Alcaligenes sp*	0.5	0.5	0.3	0.4	1.7
14	*V. parahaemolyticus*	1.2	0.9	0.3	0.4	2.8
15	*A. salmonicida*	0.9	0.5	0.6	-	2..2
16	*Salmonella sp*	0.7	0.6	0.4	0.1	1.8.
17	*Escherichia coli*	0.6	0.1	0.1	0.4	1.2
18	*Micrococcus*	0.2	-	-	0.3	0.5
19	*Proteus rettgeri*	0.6	0.3	0.2	0.2	1.3
20	*Vibrio alginolyticus*	0.4	0.4	0.5	0.4	1.7

A. invadans was found in the ulcerative tissue as macroscopic lesions in the muscles of the diseased *H. fossilis*. *A. invadans* was observed in czapek dox agar incubated at 27 ^{0}C for 72 h.

A. hydrophila is a motile gram negative bacterium, oxidase positive, catalase positive and produce H_2S. It never grows in NaCl and showed negative growth in 5% and 7% NaCl tolerance test. *A. hydrophila* was isolated and cultured using aeromonas isolation agar and the results are recorded (Table 3). The present study showed a high prevalence of motile aeromonad bacteria (35.8%) next to *A. invadans* (41%) in all lesions (n= 167) from internal organs of muscle, gills, liver and intestine in ulcerated fish indicating systemic invasion.

A good number of reports on fish diseases of temperate regions are available[7]. Among the fish diseases Epizootic

Ulcerative Syndrome is a dreadful disease predominantly affecting the fishes with *A. invadans* and *A. hydrophila* as the main pathogenic organisms. In the present study, the mean bacterial load was observed to be higher than fungal load. The mean bacterial load was found to be more in muscle ($6.3 \pm 0.4 \times 10^7$ cfu/ml) followed by gills ($5.7 \pm 0.6 \times 10^{[6]}$ cfu/ml). Similarly, Al-Harbi and Uddin [20] reported higher bacterial load in gills ($8.7 \pm 1.1x10^{[6]}$ cfu $g^{-[1]}$) followed by intestine ($5.8 \pm 0.4 \times 10^{[7]}$ cfu $g^{-[1]}$) of hybrid tilapia. Hazen *et al*. [21] and Duenci and Candan [22] have stated that *Aeromonas* sp was the predominant microorganism isolated from the skin, gills and intestine of some diseased freshwater fishes. In the present study, 19 bacteria and one fungus were isolated from infected *H. fossilis*. Our results are supported by Katoch *et al*. [23] who have reported 25 bacterial and fungal species isolated and identified in freshwater carp at Himachal Pradesh, India and by Al-Harbi and Uddin [20] who have recorded 15 isolates of bacteria in hybrid tilapia from Saudi Arabia. Similarly, a total of 17 bacterial and mycotic species were isolated and identified in *C. striatus* in India with most of the isolates from muscle and gills [24].

The fungal species *A. invadans* was found in all the lesions of infected individuals in the present investigation. *Aphanomyces* attributed lesions in fish have been reported in the southeastern US, Australia, and Indo-Pacific region of Asia [25]. Atlantic menhaden are the estuarine fish species severely affected by ulcerative lesions characterized by solitary, typically perianal, focal, deep, granulomatous lesions containing oomycete hyphae, primarily those of *Aphanomyces* [26]. *A. invadans*, a highly invasive, specific, slow growing fungus, causes epizootic ulcerative syndrome [27]. This fungus *A. invadans* was identified by the attachment to the surface, hyphae and sporangial morphology. Hatai [28] identified the fungus based on morphological characteristics of the hyphae, zoosporangia, primary zoospore cysts and asexual reproduction. *A. invadans*, *Aspergillus flavus* and *A. fumigates* were the main fungi isolated from the Nigerian freshwater fishes [29].

The finding of the present study, *A. hydrophila* dominating the bacterial isolates in infected *H. fossilis* is supported by Thampuran *et al*. [30] who have reported dominance of *A. hydrophila* in the EUS affected *C. striatus*. Similarly, Manohar[31] has also reported the dominance of *A. hydrophila* in the infected *C. carpio*. Motile aeromonads have been associated from the surface of lesions in EUS affected fishes [32, 33]. The predominance of *A. hydrophila* in EUS affected fish has also been reported previously by Kumar *et al*. [34] in India, Tonguthai [35] in Thailand, Wong and Leong [36] in Malaysia, Dana [37] in Indonesia, Roberts *et al*. [38] in Myanmar and Balasurya [39] in Srilanka. Lio-Po *et al*. [32] reported that several species of bacteria and fungi were found to be associated with EUS affected snakehead *C. striatus* and that 89% of the total isolates were *A. hydrophila*.

Outbreaks of EUS and hemorrhagic septicemia caused by *A. hydrophila* infection occur in both captured and cultured fishes such as snakehead (Channa striatus), walking catfish (*Clarias batrachus*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*Oncorhynchus tshawytscha*), Japanese eel (*Anguilla japonica*), American eel (*Anguilla rostrata*), goldfish (*Carassius auratus*), golden shiner (*Notemigonus crysoleucas*) and tilapia (*Oreochromis mossambicus*) [40, 41, 8].

Meanwhile, *Aeromonas* sp., *Pseudomonas fluorescens*, *Vibrio anguillarum*, *Flavobacterium columnare*, *Edwardsiella tarda*, *Streptococcus* sp. and *Enterococcus* sp. are also the common pathogens infecting fish species [42]. Under predisposing factors such as poor water quality, for instance high ammonia due to high stocking density and careless handling, stressful conditions and ectoparasites, the microorganism find a portal of entry into the fish host [43]. There are several studies on fish bacteria identification, infection and/or disease resistance [44, 20, 45]. Isolation and bacteriological examination from the ulcerated area of *C. punctatus*, *Puntius* sp. and *Mystus* sp. revealed the presence of 16 strains of bacteria belonging to the genus *Pseudomona*s sp, *Aeromona*s sp, *Micrococcus* sp, *Bacillus* sp, *Vibrio* sp and *Moraxella* sp., among which *Pseudomonas* sp and *Aeromonas* sp were the most common [46]. Reantaso [47] and Roberts *et al*. [12] reported that fungal and bacterial pathogens play an important role in EUS and *A. hydrophila* is the causative agent of motile aeromonad septicemia, found in a wide variety of freshwater fish species [48]. *Aeromona*s sp are considerd as autochronous inhabitant of aquatic environment [49]. They are opportunistic pathogens of many immune compromised poikilotherms and homeotherms [21, 49].

In the present investigation, Gram negative bacteria were found comprising about nearly 60%. Similarly Kumar *et al*. [34] have reported that Gram negative bacteria comprised major part (75%) among the isolated microorganisms. *A. hydrophila* can often be isolated from ulcers or internal organs of EUS-affected fish [10]. Some of these *A. hydrophila* strains have been characterized as virulent [50] or cytotoxic [51]. Yesmin *et al*. [52] have reported that *A. hydrophila* is one of the important pathogens of fish in freshwater and brackish water. In the present investigation, *Pseudomonas* sp, *Flavobacterium* sp, *Alcaligenes* sp and *Vibrio* sp. were found in addition to *A. hydrophila* and *A. invadans*. Thampuran *et al*. [30] have also reported the presence of *Pseudomonas* sp, *Alcaligenes* sp *Micrococcus* sp and *E. coli* in infected *C. striatus*. This study revealed the dominance of *A. hydrophila* over other microbial species by the frequency of isolation from the samples. The role of *A. hydrophila* bacterium at the ulcerative stage of the disease cannot be ignored. Although the phenotypic diversity and variations in virulence suggest that the bacterial infection is secondary in nature, they are the main reason for the dreadful infections and ultimate mortality.

The introduction of alien fishes like African catfish, *Clarias gariepinus* and Thai catfish, *Pangasius sutchi* is a major cause of biodiversity decline in freshwater ecosystems. The impacts associated with the introduction of alien fishes are many, including; competition, habitat alteration, parasitism, predation, alteration of habitat quality and/or ecosystem function, host of pests or parasites. The alien species pose a severe threat to the already existing biota and in addition to this, if the native catfishes are allowed to succumb due to diseases like EUS, then there is no doubt that the native fishes will become extinct. The conservation of native catfishes is essential at this juncture, as in almost all ponds and lakes of our country, the alien catfishes we dominant.

4. Conflict of interest

We declare that there is no conflict of interest.

5. Acknowledgement

We acknowledge the financial assistance received from UGC (F6-6/2012-2013/Emeritus.2012-2013-OBC-675/(SA-II) dt.25.04.2013) to carry out this study. We are grateful to the Principal, St. Xavier' College (Autonomous), Palayamkottai, for providing necessary facilities.

6. References

1. Haniffa MA, Sethuramalingam TA, Dhanaraj M, Muthu RC, Ananthkumar Y, Arunsingh SV. Breeding and larval rearing of the native Indian catfish *Heteropneustes fossilis*. Infofish International, 2007, 16 -19.
2. CAMP. Conservation assessment and management plant workshop report by Zoo. Outreach organization, CBSG, Indian Edn. Sanjay Molur and Sally Walker.1998, 100.
3. Haniffa MA, Sridhar S, Nagarajan M. Introduction of triploidy and tetraploidy in stinging catfish, *Heteropneustes fossilis* (Bloch) using heat shock. Aquaculture Research 2004; 35:937-942.
4. Pondey AK, Nayak PK, Singh BN, Das RC and Saha C. Brood stock management, Induced breeding and larval rearing of the Indian catfish, *H. fossilis* (Bloch). Fishing chimes 2001; 21:80-81.
5. Haniffa MA, Dhanraj M, Muthu RC, Arunsingh SV, Ananth KY, Arthi MR. Threatened fishes of the world: *Heteropneustes fossilis* (Bloch, 1794). Environmental Biology of Fishes 2008; 82:203-204.
6. Bondad-Reantaso MG, Lumanlan SC, Natividad JM, Phillips MJ. Environmental monitoring of the epizootic ulcerative syndrome (EUS) in fish from Munoz, Nueva Ecija in the Philippines. In: Shariff, M, Subasinghe, R.P., Arthur, J.R. Eds., Disease in Asian Aquaculture I. Fish Health Section, Asian Fishery Society, Manila, The Philippines 1992; 475-490.
7. Das MK. Epizootic Ulcerative Syndrome in fishes–its present status in India. Bulletin of the Central Inland Captive Fisheries Research Institute, Barrackpore, West Bengal. 1997.
8. Lilley JH, Phillips MJ, Tonguthai K. A review of epizootic ulcerative syndrome (EUS) in Asia. Aquatic Animal Health Research Institute and Network of Aquaculture Centres in Asia-Pacific, Bangkok.1992.
9. Lilley JH, Roberts RJ. Pathogenicity and culture studies comparing the *Aphanomyces* involved in epizootic ulcerative syndrome (EUS) with other similar fungi. Journal of Fish Diseases 1997; 20:135-144.
10. Pal J, Pradhan K. Bacterial involvement in ulcerative condition of air breathing fish from India. Journal of Fish Biology 1990; 36:833-839.
11. Chakrabarty AN, Dastidar SG. Repeated isolation of chemoautotrophic nocardioform bacteria from fish epizootic ulcerative syndrome. Indian Journal of Experimental Biology 1991; 29:623-627.
12. Roberts RJ, Willoughby LG, Chinabut S. Mycotic aspects of epizootic ulcerative syndrome (EUS) of Asian fishes. Journal of Fish Diseases 1993; 16:169-183.
13. Chinabut S, Roberts RJ, Willoughby LG, Pearson MD. Histopathology of snakehead, *Channa striatus* (Bloch) experimentally infected with the specific *Aphanomyces* fungus associated with epizootic ulcerative syndrome (EUS) at different temperatures. Journal of Fish Diseases 1995; 18:41-47.
14. Frerichs GN. Viruses associated with the epizootic ulcerative syndrome (EUS) of fish in south-east Asia. Veterinary Research 1995; 26:449-454.
15. Roberts RJ, Frerichs GN, Tonguthai K, Chinabut S. Epizootic Ulcerative Syndrome of Farmed and Wild Fishes. In: Muir, J.F. and Roberts, R.J. (Eds). Chapter 4. Recent Advances in Aquaculture V. Blackwell Science, 1994, 207-239.
16. Bergey's Manual of Determination Bacteriology. John G. Holt- Noel. R. Krieg Peter, H.A, Sheath James, T, Stanely and Stanely T. Williams (Eds) 9th Edn. Lippincott Williams and Wilkins Publication, Philadelphia, PA.19106. USA.1998.
17. Fraser GC, Callinan RB, Calder LM. *Aphanomyces* species associated with red spot disease: an ulcerative disease of estuarine fish from eastern Australia. Journal of Fish Diseases 1992; 15:173-181.
18. Surendran PK, Gopakumar K. Selection of bacterial flora in the ice chlortetracycline treated oil sardine (*Sardinella longiceps*) Indian Mackerel (*Rasirelliger kanagurata*) and prawn (*Metapenaeus dobsoni*) during ice storage. Fishery Technology 1981; 18:133-141.
19. Anon. Notes on mycological procedure. Workshop on Mycological Aspects of Fish and Shellfish Disease, Bangkok, Thailand, January 1992. Aquatic Animal Health Research Institute, 1992.
20. Al-Harbi AH, Uddin N. Quantitative and qualitative studies on bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) cultured in earthen ponds in Saudi Arabia. Aquaculture Research 2003; 34:43-48.
21. Hazen TC, Filermans CB, Hirsch RP, Esch GW. Prevalence and distribution of *Aeromonas hydrophila* in the United States. Journal of Applied Environmental Biology 1978; 36:731-738.
22. Duenci S, Candan A. Isolation of *Aeromonas* strains from the intestinal flora of Atlantic salmon (*Salmo salar* L. 1758). Turkish Journal of Veterinary and Animal Sciences 2003; 27:1071-1075.
23. Katoch REC, Mandeep S, Pathania D, Subhash V, Rajesh C, Arvind ML. Recovery of bacterial and mycotic fish pathogen from carp and other fish in Himachal Pradesh. Indian Journal of Microbiology 2003; 43:65-66.
24. Dhanaraj M, Haniffa MA, Muthu Ramakrishnan C and Arun Singh SV. Microbial flora from the Epizootic Ulcerative Syndrome (EUS) in Tirunelveli region. Turkish Journal of Veterinary and Animal Sciences 2008; 32(3):221 -224.
25. Callinan RB. Pathogenesis of red spot disease (epizootic ulcerative syndrome) in estuarine fish in eastern Australia and the Philippines. Ph.D. Thesis, University of Queensland, 1997, 232.
26. Lilley JH, Callinan RB, Chinabut S, Kanchanakhan S, MacRae IH, Phillips MJ. Epizootic Ulcerative Syndrome (EUS) Technical Handbook. The Aquatic Animal Health Research Institute, Bangkok, 1998, 88.
27. Lilley JH, Hart D, Richards RH, Roberts RJ, Cerenius L, Soderhall K. Pan-Asian spread of single fungal clone results in large scale fish-kills. Veterinary Records 1997; 140:653-654.
28. Hatai K. Studies on pathogenic agents of saprolegniasis in fresh water fishes. Special Report of Nagasaki Prefectural Institute of Fisheries No.8, Matsugae-cho, Nagasaki, Japan, In Japanese, 1980.
29. Opkonasili GC, Ogbulie JN, Opkonasili NP. Fungal flora of sample from Nigerian fresh water fish culture. Journal of Aquaculture in Tropics 1998; 13:269-276.
30. Thampuran N, Surendar PK, Mukundan MK, Gopakumar K. Bacteriological studies on fish affected by Epizootic Ulcerative Syndrome (EUS) in Kerala, India. Asian fisheries Sciences 1995; 8:103-111
31. Manohar M. Probiotic, *Spirulina* as a Source of Immunostimulants and Growth in Common Carp. Ph.D. thesis, Manonmaniam Sundaranar Univ., Tamilnadu,

India. 2005.
32. Lio-Po GD, Albright LJ, Alapide-Tendencia EV. *Aeromonas hydrophila* in the epizootic ulcerative syndrome (EUS) of snakehead, *Ophicephalus striatus*, and catfish, *Clarias batrachus*: Quantitative estimation in natural infection and experimental induction of dermonecrotic lesion. *In* M. Shariff, R.P. Subasinghe and J.R. Arthur (eds.) Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila. The Philippines, 1992, 461-474.
33. Karunasagar, Karunasagar I. Bacteriological studies on ulcerative syndrome in India. *In* R.J. Roberts, B. Campbell and I.H. Macrae (eds.) Proceedings of the ODA Regional Seminar on Epizootic Ulcerative Syndrome. Aquatic Animal Health Research Institute, Bangkok, 25-27 January, 1994, 158-170.
34. Kumar D, Dey RK, Sinha A. Outbreak of epizootic ulcerative syndrome of fish in India. In: Aquaculture and productivity (eds. V.R.P Sinha and H.C. Srivastava), Oxford and IBH Publishing Co. Pvt. Ltd, Bombay, 1990, 345–357
35. Tonguthai K. A preliminary account of ulcerative fish diseases in the Indo-Pacific region (a comprehensive study based on Thai experiences). National Inland Fisheries Institute, Bangkok, 1985, 39.
36. Wong SY and Leong TS. Current fish problems in Malaysia. In: Fish quarantine and fish disease in South and Southeast Asia: 1986 update (ed. J.R. Arthur), Special Publication No. 1. Asian Fisheries Society, Manila, 1987, 12-21.
37. Dana P. Current fish problem in Indonesia. In: Fish quarantine and fish disease in south and Southeast Asia: 1986 update (ed. J.R. Arthur), Special publication No. 1. Asia fisheries society, Manila, 1987, 9-11.
38. Roberts RJ, Macintosh DJ, Tonguthai K, Boonyaratpalin S, Tayaputch N, Phillips MJ *et al*. Field and laboratory investigations into ulcerative fish diseases in the Asia-Pacific region. Technical Report of FAO Project TCP/RAS/4508. Bangkok, 1986, 214
39. Balasurya LKSW. Current fish disease problems in Srilanka in fish quarine and fish disease in south and southeast Asia: 1986 update (ed. J.R. Arthur), Special publication No .1. Asian fisheries society, Manila, 1987, 36-40.
40. Aoki T. Motile Aeromonads (*Aeromonas hydrophila*). *In Fish Diseases and Disorders*. (eds. Woo, P.T.K. and D. W. Burno). CABI Publishing. USA. 1999.
41. Bullock GL, Mac Laughin JJA. Advance in symposium on disease of fishes and shellfish (ed. By S.F. Snieszko) publication No. 5. *American fisheries soci.* Washington D.C, 1970, 231-242.
42. Plumb JA. Health Maintenance and Principal Microbial Diseases of Cultured Fishes. Ames, IA Iowa State University, 1999.
43. Moraes FR, Martins ML. Favourable conditions and principal teleostean diseases in intensive fish farming. In Cyrino, J.E.P, Urbinati, E. C, Fracalossi, D. M. and N. Castgnolli (Eds.). *Especial topics in tropical intensive freshwater fish farming*. Sao Paulo: *Tec Art*, 2004, 343-383.
44. Azad IS, Rajendran KV, Rajan JJS, Vijayan KK, Santiago TC. Virulence and histopathology of *Aeromonas hydrophila* (Sah 93) in experimentally infected tilapia *Oreochromis mossambicus* (L.). Journal of Aquaculture in Tropics 2001; 16:265-275.
45. Cai WQ, Li SF, Ma JY. Diseases resistance of Nile tilapia (*Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*) and their hybrid (female Nile tilapia x male blue tilapia) to *Aeromonas sobria*. Aquaculture 2004; 229(1-4):79-87.
46. Saha D, Pal J. Bacterial studies on fishes affected with epizootic ulcerative syndrome. Asian Fisheries Science 2000*;* 13:343-355.
47. Reantaso MB. EUS in brackish waters of the Philippines. Fish Health Section Newsletter 2(1), 8-9. Asian Fisheries Society, Manila, 1991.
48. Thune RL, Stanley LA, Cooper RK. Pathogenesis of Gram negative bacterial infections in warm water fish. Annual Review of Fish Diseases 1993; 3:17-68.
49. Kaper JB, Lockman H, Colwell R, Joseph SW. *Aeromonas hydrophila* Ecology and Toxigenicity of isolates from an estury. Journal of Applied Bacteriology 1981; 50:359-377.
50. Suthi G. Pathogenicity of motile *Aeromonads* for *Puntius schwanfeldi* and *Oreochromis niloticus* with particular reference to the ulcerative disease syndrome (EUS). M.Sc. Thesis, University of Stirling, Scotland, 1991, 71.
51. Yadav M, Indira G, Ansary A. Cytotoxin elaboration by *Aeromonas hydrophila* isolated from fish with epizootic ulcerative syndrome. Journal of Fish Diseases 1992; 15(2):183-189.
52. Sabina YMH, Rahman M, Afzal HAR, Khan FP, Hussain MA. *Aeromonas hydrophila* infection in fish of swamps of Bangladesh. Pakistan Journal of Biological Sciences 2004; 7(3):409–411.