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M Tanvir Hossain
Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh

M Rafiqur Rahman
Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

M Kamruzzaman Hossain
Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh

M Arif Hossain
Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh

Al-Minan Noor
Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh

M Mezanur Rahman
Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh

Salma Begum
Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh

Correspondence
M Arif Hossain
Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh

Impact of epizootic ulcerative syndrome (EUS) on the biodiversity of *Nandus nandus* and *Puntius sophore* in Beel fisheries of Bangladesh

M Tanvir Hossain, M Rafiqur Rahman, M Kamruzzaman Hossain, M Arif Hossain, Al-Minan Noor, M Mezanur Rahman and Salma Begum

Abstract
An experiment was carried out for a period of eleven months from March 2012 to January 2013 in order to observe the effect of epizootic ulcerative syndrome (EUS) on the biodiversity status of *Nandus nandus* and *Puntius sophore* in two beels of Kailla and Dighli are situated in Ishwargonj upazilla of Mymensingh district. All the water quality parameters were within the suitable range during the experimental period except temperature and hardness. Fishes were examined clinically and histopathologically. By clinically examined, it was observed that fishes were more affected from October to January having red spots, ulcer, scale loss and fin rot. Almost normal in appearance was observed during the months from March to October in fishes of both the *beels* under clinical and histopathological observations. Marked pathological changes like fungal granuloma, necrosis, haemorrhages, vacuums, fat droplets, pyknosis and hyperplasia were observed in the months of November, December and January. Fungal granuloma was observed in skin, muscle and kidney of *N. nandus* and only in skin and muscle of *P. sophore.* In FGD, fishermen of both the beels mentioned that EUS was the most significant disease especially during the colder months i.e., November, December and January. Fishermen could easily recognize EUS clinically and according to them the disease accounts about 80-90% in Kailla beel and 75-80% in Dighli beel. Moreover, in histopathological observation, it could be mentioned from farmers opinion (FGD) that diseases especially EUS has significant effect in the reduction of SIS (small indigenous species) from open water bodies of both the beels. According to fishermen opinion, some SIS were reduced to almost half in comparison to a decade ago in both the beels.

Keywords: EUS, Biodiversity, *Nandus nandus*, *Puntius sophore*, Beel

1. Introduction
Bangladesh has an aquatic area in the form of rivers, reservoirs, lakes, floodplains, canals, haor, baor and beels. Beels are the most suitable environment for large and small indigenous fishes which are commercially important and major carp, large catfishes and small indigenous fishes are dominating species of the beels in Mymensingh regions [1]. There are 40-50 small indigenous species (SIS) in our country which identified maximum length of about 25 cm [2]. Some SIS have high nutritional value in terms of protein, vitamins and minerals and these vitamins and minerals are not commonly available in other foods. But it is a matter of great regret that natural fishery resources in our country, especially small indigenous fishes have been gradually declining due to deteriorate of water quality of beels, baors, rivers and canals. As a result, fish species that bred and reared in natural waters are reducing quickly. On the other hand, indiscriminate use of insecticides, pesticides and inorganic fertilizers, natural water body becomes polluted. Man made bridge and culverts on the natural water resources disturbs the aquatic ecosystem and it imbalances the biodiversity. Thus fishes of open water bodies are facing continuous stress, which leads to infection and disease.
Disease has become a major problem in fish production both in culture system and wild condition in Bangladesh [3]. Common diseases of freshwater fishes of Bangladesh are EUS, tail and fin rot, nutritional diseases, red spot and gill rot [4]. Of those diseases, epizootic ulcerative syndrome (EUS) is a very serious disease of freshwater and brackish water fish which has extended rapidly across Southeast and South Asia over the past few decades. The epizootic ulcerative syndrome is an ulcerative condition of wild and cultured freshwater fishes in

Asia and the Indo Pacific region [5, 6]. In Bangladesh EUS affected at least 38 species of fishes among which snakeheads (Channa sp.) and Puntius sp. were most severely affected. The lowering of water temperatures together with heavy rainfall, low alkalinity and pH fluctuations was recognized as predisposing factors for EUS [6, 7].

Clinical signs are important diagnostic procedure which describes behavioral, external, physical or gross pathological changes. In fish, the most obvious external clinical signs are inflammation, erythema and hemorrhage of fins, skin or head, frayed or eroded fins, hemorrhaged opaque eye, open necrotic and ulcerative lesions at any location on the body, lepidorthosis of scales and excessive mucus production [8]. The clinical signs, combined with parasitic investigation and histopathology may very helpful in diagnosing fish diseases which has been successfully used throughout the world. Scott [9] worked on the extensive histopathology in channel catfish that had been hypoxically stressed and found hypertrophy, hyperplasia, hemorrhage, oedema, hyperaemia and necrosis in the gill, liver, spleen, trunk kidney and anterior kidney. Roy *et al.* [10] observed marked pathological changes like necrosis, pyknosis, inflammation, haemorrhage, hypertrophy and hyperplasia, missing of primary and secondary gill lamellae in *Puntius sophore*, *Mastacembelus pancalus* and *Nandus nandus* in the months of November, December and January in Ailee beel, Mymensingh. It is thus important to know the cause of reduction of SIS especially from disease point of view. The present investigation is aimed at the observation of the impact of EUS on the biodiversity of small indigenous species like *Puntius sophore* and *Nandus nandus* from two beels of the region through clinical, histopathological and participatory rural appraisal (PRA) techniques.

2. Materials and Methods

The present study was conducted for a period of eleven months from March 2012 to January 2013. Fishes were collected from Kailla beel and Dighli beel situated in Ishwargonj Upazilla of Mymensingh district in Bangladesh. Fish samples were collected from the selected fishermen of the nearest markets (Bharati Bazar and Shakoa Konapara bazaar) of Kailla beel and Dighli beel. To collect the data, the participatory rural appraisal (PRA) tool such as focus group discussion (FGD) was conducted with the fishermen of both the beels. Two small indigenous fishes such as *Nandus nandus* and *Puntius sophore* were collected from the mentioned fish markets of both the beels and studied once in a month during experimental period. Fishes were collected and immediately carried to the Fish Disease Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Each species of fish was transported by separate plastic buckets filled with water. Four fishes from each species were randomly selected for investigation. Water quality parameters like water temperature, pH, dissolved oxygen, ammoniun and hardness were recorded from both the beels once in a month between 09.00 to 10.00 hrs.

The sampled fishes were examined just after taking out of the container to observe external symptoms and record any injury, infection and other abnormal conditions of fish body. For histopathological observations, samples of fish from various organs such as skin, muscle, liver, gill and kidney were collected by a sharp scalpel and forceps and fixed in 10% neutral buffered formalin. After 8 hours of fixation, the fixed samples were taken out, trimmed and placed separately in a perforated plastic holder, which was covered by perforated steel plates. The samples were then dehydrated, cleared and infiltrated in an automatic tissue processor (SHANDON, CITADEL 1000) through alcoholic series of higher concentrations, xylene and paraffin wax. The samples were then embedded with melted wax, steel mold and perforated plastic holder. The blocks were then kept in the deep freeze for 30 minutes. Sections were taken from the blocks at a thickness of 5 micrometers by using a microtome (Leica JUNG RM 2035). Suitable sections were selected and placed over glass slides. The glass slides were then labeled with a diamond marker and kept on a plate (photox-dishwarmer 2) at 40 °C for 8 hours to fix the sections properly with the slides. The sections were then stained with haematoxylin and eosin stains proceeding through various chemicals of different concentrations and time schedule. After staining the sections were mounted with Canada balsam and covered by cover slips. The prepared sections were examined under a compound microscope (Olympus). Photomicrograph of the stained sections was obtained by using a photomicroscope (OLYMPUS, Model CHS, Japan). Pathology and diseases will be identified through microscopic observation, photographic view and presence of pathogen.

3. Results and Discussion

Water quality of the Kailla and Dighli beels was studied from March 2012 to January 2013. Data regarding water temperature, pH, dissolved oxygen (DO) and ammonia analyzed and presented in Table 1. Environmental parameters play an important role in the occurrence of diseases in fish. In the present investigation, water quality parameters did not show variation between the beels and among the months except water temperature and hardness. The lowest value of water temperature was 15 ºC in December and January from both the beels which was significantly lower from the optimum temperature of tropical fish culture in South East Asia. In Ailee beel of Mymensingh, *M. cuchia* and *M. aculeatus* were more affected (clinically and histologically) in December and January when water temperature were at minimum level [11].

Table 1: Monthly variations of water quality parameters of the kailla beel and Dighli beel

Parameters	Water body	March-13	April-13	May-13	June-13	July-13	Aug-13	Sep-13	Oct-13	Nov-13	Dec-13	Jan-14
Temperature (^{0}C)	Kailla beel	29	28.5	28	28.8	28.6	28	26.5	23.0	21.0	16.5	15.0
	Dighli beel	29	27.8	28.7	28.5	28.2	28.0	26.0	24.0	21.0	16.5	15.0
pH	Kailla beel	6.5	7.4	7.0	7.2	6.8	6.5	7.5	7.0	7.3	6.9	7.0
	Dighli beel	6.5	7.0	7.2	6.8	7.5	6.3	7.5	6.8	7.1	7.0	7.2
Dissolved Oxygen (mgl^{-1})	Kailla beel	5.2	8.0	8.2	8.0	7.1	5.5	6.5	7.8	8.0	7.0	8.2
	Dighli beel	5.1	7.1	8.1	7.5	8.1	8.0	7.0	7.4	7.8	7.2	8.1
Ammonium (mgl-1)	Kailla beel	0.8	0.5	0.5	0.1	0.5	0.5	0.5	0.4	0.6	0.45	0.75
	Dighli beel	0.5	0.5	0.5	0	0.5	0.5	0.5	0.25	0.5	0.5	0.7
General Hardness (mgl-1)	Kailla beel	80	60	100	60	60	70	60	60	70	50	40
	Dighli beel	80	80	100	60	70	50	90	50	60	70	40

Mondal [12] recorded water temperature were ranged from 17 to 31 ^{0}C in BAU fish farm. The water temperature recorded in decreasing value during winter season in Kailla beel of Mymensingh [13]. These result more or less similar with the present findings. In the present experiment, the pH values were ranged from 6.5 to 7.4 in Kailla beel and 6.3 to 7.5 in Dighli beel. The pH values were ranged from 7.0 to 7.7 in BAU fish farm and Swopon fish farm throughout the experimental period [12]. Most natural water has pH values of 6.5 to 9 [14]. Similar result was also reported by Swingle **[15]**. In the present experiment, the values of dissolved oxygen were ranged from 5.2 to 8.2 mgl^{-1} in Kailla beel and 5.1 to 8.1 mgl^{-1} in Dighli beel. The dissolved oxygen ranged from 5.5 to 3.0 in four beels of Mymensingh [16]. The dissolved oxygen values of fish ponds ranging from 3.8 to 6.9 mgl^{-1} and 2.04 to 7.5 mgl^{-1} respectively at Mymensingh region [17, 18] which was agreed with the present findings. In the present study, the values of ammonia were very much nearer that ranged from 0.0 to 0.8 mgl^{-1} in both the water bodies. Wahab *et al.* [19] reported that ammonia content of 0.19 to 0.28 mgl^{-1} in their experimental areas. Akter *et al.* [13] observed increased level of ammonia during winter period. The highest value of general hardness was 100 mgl^{-1} in May from both the beels, where lower value was 40 mgl^{-1} in January from both the beels. Similar results were also found in the studies of FRI [20]. Reduced level of general hardness would not cause of death of fishes but would help in the development of pathologies and diseases.

Clinically it was observed that, during the months from March to October all the fish species were more or less normal in their appearance. But different clinical symptoms like scale loss, dermal lesion, loss of caudal fin were seen in November to January in the fishes of both the beels. These findings were almost similar to the work by Ruksana [21], Khatun [22] and Moniruzzaman [23]. Rough skin and scale loss in some places, lesion in tail region, ulcer, red spots in ventral and caudal region and loss of caudal fin were observed in *P. sophore* in December and January from Dighli beel (Fig. 5). Red spots in ventral region, ulcer, hemorrhage and necrosis in gill, weak body with scale loss and skin lesion were observed in *N. nandus* during experimental period from both the beels. Parveen [24] examined through clinical observation of three small indigenous fishes like *P. ticto, N. nandus* and *C. punctatus* from four beels of Mymensingh district and found that all fishes were severely affected during the months of December and January. From the result of the present experiment it was observed that severity of clinical signs was increased in November, December and January. Almost similar clinical signs have also been reported by Barua *et al.* [25].

Histolopathologically, it was observed that, skin and muscle of *P. sophore* were normal from March to October of both the beels. Epidermis were lost and fungal granuloma, necrosis, hemorrhage and vacuums were observed in muscle from both the beels in December and January (Fig.1) whereas, in case of *N. nandus*, muscles had fungal granuloma, melanomacrophage, necrosis, pyknosis and vacuums during the months of December and January from both the beels (Fig. 7). Marma [26] mentioned that in *N. nandus*, epidermis and dermis were lost; hemorrhage, necrosis, vacuums and fungal granuloma were observed in the skin and muscle during December and January. Moniruzzaman [23] also found almost similar results in skin and muscle of major carps in winter season. Roy *et al.* [10] described fungal hyphae and many granulomas in the skin and muscle of *P. sophore* and *N. nandus* during the months of December and January. Almost similar observations were also made by Ahmed *et al*. [27] in small indigenous fishes from Kailla beel of Mymensingh.

Appearance of fungal granuloma and fungal hyphae in both the fishes indicated that the fishes were suffered from EUS in the months of December and January. Hatai *et al.* [28] reported fungal hyphae and many granulomas in the internal organs and musculature of EUS affected *Colisa lalia* in Japan. Mohan and Sankar [29] described that in EUS affected fishes of fresh and brackish water, numerous granulomas were found as a result of chronic inflammatory response with fungal hyphae. Ahmed and Hoque [30] reported that, in case of EUS positive fish a massive necrosis of the epithelium and muscle tissue was in the affected area surrounding granuloma. According to the authors, the granuloma due to fungal infection consisted of mononuclear epithelioid cells and fibrillar structure.

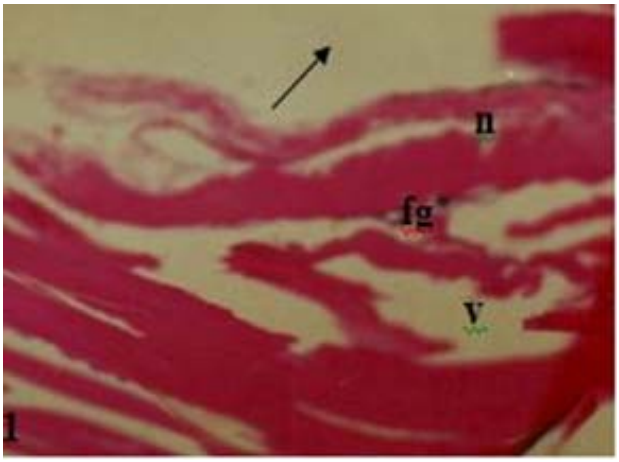

Fig 1: Section of skin and muscle of *P. sophore* in December and January from Kailla beel. Epidermis lost (↗), muscle had vacuums (v), necrosis (n) and fungal granuloma (fg). H and E × 125.

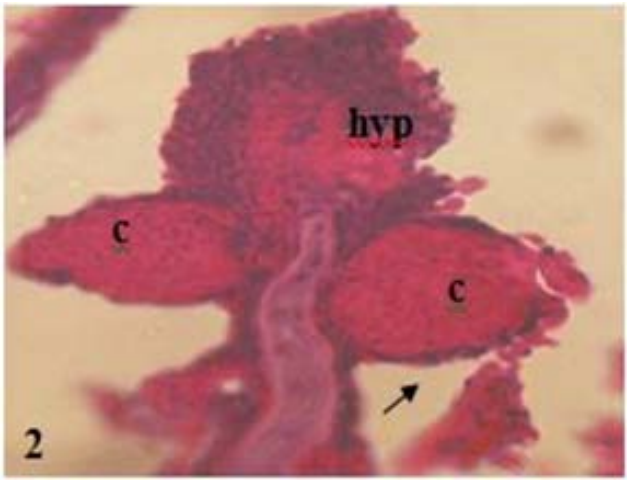

Fig 2: Section of gill of *P. sophore* in December and January from Kailla beel. Secondary gill lamellae lost (↗), hypertrophied (hyp) and cyst (c) present. H and E × 425.

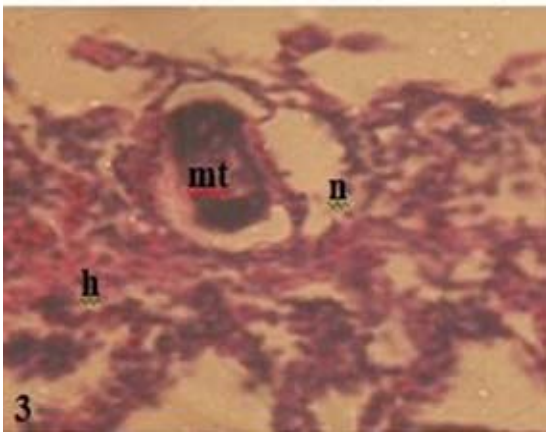

Fig 3: Section of gill of *P. sophore* in December and January from Dighli beel. Monogenetic trematode (mt), necrosis (n) and hemorrhage (h) were found with gill lamellae. H and E × 425.

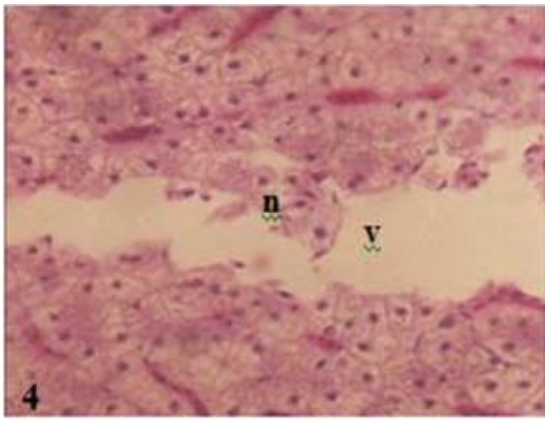

Fig 4: Section of liver of *P. sophore* in December and January from Dighli beel. In hepatocytes, necrosis (n) and vacuums (v) were seen. H and E × 425.

Fig 5: P. sophore with dark red ulcer (↖) in December and January from Dighli beel

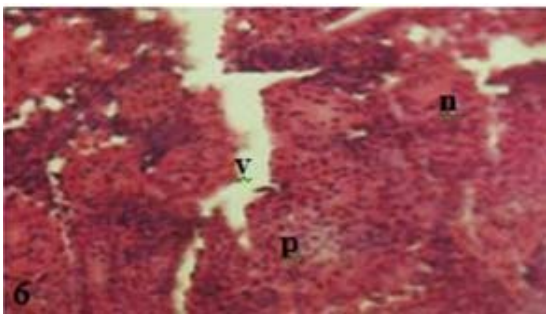

Fig 6: Section of kidney of P. sophore in December and January from Kailla beel. Necrosis (n), pyknosis (p) and vacuums (v) were observed in kidney tubules. H and E × 425

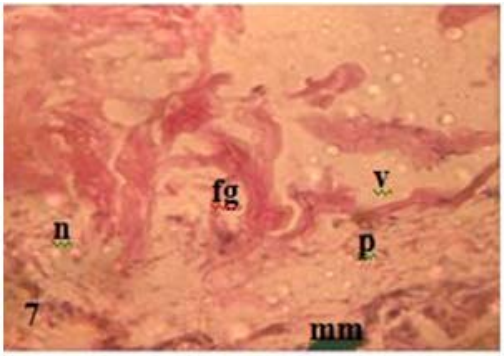

Fig 7: Section of skin and muscle of N. nandus in December and January from Kailla beel. Fungal granuloma (fg), necrosis (n), pyknosis (p), melanomacrophage (mm) and huge vacuums (v) were found in muscle. H and E × 125

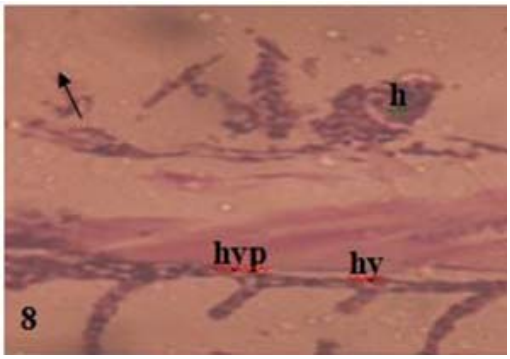

Fig 8: Section of gill of N. nandus in December and January from Kailla beel. Secondary gill lamellae were lost (↖), hemorrhage (h), hypertrophy (hyp) and hyperplasia (hy) were observed. H and E × 425

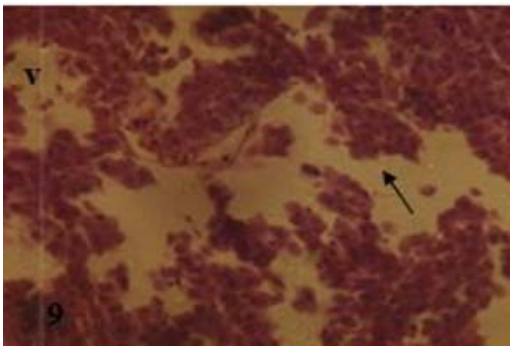

Fig 9: Section of liver N. nandus in December and January from Dighli beel. Fungal hyphae (fh), necrosis (n), vacuums (v) and hemorrhage (h) were seen in hepatocytes. H and E × 425

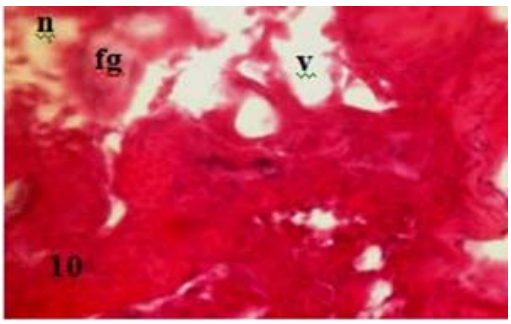

Fig 10: Section of kidney of N. nandus in December and January from Dighli beel. Fungal anuloma (fg), vacuums (v) and necrosis (n) were observed. H and E × 425

Necrosis, hemorrhage, protozoan cysts, hypertrophy, monogenetic trematode and missing of secondary gill lamellae were observed in December and January in *P. sophore* and *N. nandus* of both the beels (Figs. 2, 3 and 8). Parveen *et al*. [31] mentioned that in December and January marked hypertrophy and hyperplasia were observed in gill lamellae of *C. punctatus* and *N. nandus*. Secondary gill lamellae were missing in some places of *P. ticto*. Marked hypertrophy, missing of gill lamellae and appearance of parasitic cyst were observed in the gill of *C. punctatus* in December and January [32]. Ahmed *et al.* [27] found necrosis, pyknosis, hemorrhage, hypertrophy, hyperplasia and missing of primary and secondary gill lamellae in *C. punctatus, P. ticto* and *N. nandus.* It has been observed that, gill is one of the most affected organs. This is due to the fact that gill is rich in nutrient where the pathogens can easily settle and obtain their nourishment. From the research findings of Roy *et al.* [10], it was clear that gills were more affected than the other organs, both the gill lamellae were hypertrophied, hyperplasid and missing of some portion having many monogenetic trematodes in *P. sophopre* and *N. nandus* during the months of December and January in open water fishes of Bangladesh.

An internal organ such as liver was also affected in the fishes of Kailla beel and Dighli beel in December and January. In case of *P. sophore*, necrosis, pyknosis, hemorrhage and vacuums were found in liver from both the beels during November, December and January (Fig.4). Whereas, in liver of *N. nandus*, necrosis, fungal hyphae, pyknosis, hemorrhage and vacuums were observed in the same period from both the beels (Fig. 9). Moniruzzaman [23] and Chakma [33] observed fungal hyphae and vacuums in the liver of *A. testudineus* from Mymensingh district. Ahmed *et al.* [27] found marked necrosis with blood cells, pyknotic cells and many fungal granulomas from the liver cells of *C. punctatus* in the months of December and January. Roy *et al.* [10] reported that internal organs like as liver had highly necrotic hepatocytes, pyknotic cells and inflammatory cells during the months of December and January.

On the otherhand, the kidney tubules were severely necrotic having wide vacuums, fungal granuloma, pyknosis and hemorrhages in both the fishes during the months of December and January from both the beels (Figs. 6 and 10). Kidney tubules of *P. ticto* were ruptured having wide empty spaces, hemorrhage and pyknotic cells [27]. Similar pathological symptoms of kidney of major carp were observed by other authors like Ahmed and Haque [30] and Islam *et al*. [34]. Ahmed and Haque [**30**] reported that histopathologically the internal organs like kidney and liver were more affected and disease like EUS occurred during the months of December and January. Moniruzzaman [23] observed almost similar results in kidney of major carps from the farms of Mymensingh area, Bangladesh.

Results of the present study indicated that, apparently normal appearance were observed in all the organs of fishes from March to October and reduced level of pathological changes were found during November, whereas, severe pathological changes were recorded during December and January. The observed disease in the investigated fish species from Kailla beel was EUS and some pathology such as necrosis, hemorrhages and vacuums were found along with the diseases. Almost similar findings recorded by Ahmed *et al.* [35]. The authors observed through clinical and histopathological techniques and reported that more pathological symptoms were seen towards the months of December and January, when compared with the other months. So it could be mentioned that, prevalence of diseases and pathologies in freshwater fishes of Bangladesh might be related to seasonal variations and environmental factors. It was further observed that clinically and histopathologically fishes of Kailla beel were more affected than those from Dighli beel. This is due to the fact that Kailla beel is linked with many tributaries and agricultural land. So surface runoff from these sources may carry harmful pollutants and pathogens, which might be responsible for the occurrence of diseases.

From focus group discussion, clinical and histopathological findings, *N. nandus* was the most severely affected species followed by *P. sophore*. Marma [26] also found that *N. nandus* was severely affected species than *Puntius ticto* and *Mystus vittatus* from August to February obtained from K-R market of Bangladesh Agricultural University, Mymensingh, Bangladesh.

Fishermen also revealed that mentioned small indigenous species were more affected and diseased in the months of December and January. According to fishermen, among diseases, EUS was a dominating one (80% and 75%) in both the beels. This has been proved from the appearance of EUS in both the investigated fishes during colder months of both the beels. Fishermen further mentioned that before the occurrence of EUS in Bangladesh, there were abundance of SIS especially *N. nandus* and *P. sophore* in those two beels. Here EUS appeared as a serious threat through which SIS are becoming endangered. Thus EUS played a significant role in the survivality of SIS especially *N. nandus* and *P. sophore* in beels. On the other hand, it could be mentioned here that EUS had a serious impact on the reduction and extinction of SIS especially *N. nandus* and *P. sophore* in beels i. e., on the biodiversity of other small species too (Table 2).

Table 2: Availability (%) of some SIS compared with a decade ago in both the beels on the basis of focus group discussion

Sl. No.	Name of fish	Availability (%) compared with a decade ago	
		1994-1995	2006-2007
1	Taki (*Channa punctatus*)	100 %	80%
2	Koi (*Anabas testudineus*)	100 %	55%
3	Shing (*Heteropneustes fossilis*)	95 %	65%
4	Shol (*Channa striatus*)	100 %	45%
5	Gozar (*Channa marulius*)	85 %	40%
6	Magur (*Clarias batrachus*)	100 %	65%
7	Meni (*Nandus nandus*)	90 %	45%
8	Puti (*Puntius sophore*)	95 %	75%
9	Baila (*Awaous guamensis*)	90 %	50%
10	Darkina (*Esomus danricus*)	90 %	40%
11	Baim (*Mastacembelus armatus*)	85 %	40%
12	Tengra (*Mystus vittatus*)	90 %	45%

Thus preventive measures should be taken in order to reduce or stop occurrence of such diseases. Surface runoff from agricultural lands should not allow directly to the beels. Introduction of pathogens and infected fish to the waterbodies from other sources should be prevented as far as possible. Steps should be taken to prevent the occurrence of pollution and habitats destruction. However, till now very few steps have been taken to prevent and control the diseases of open water fishes. In such situation there is a need to formulate a sound management technique for open water habitat. It is expected that from such attempts, fish production from these unmanaged or under managed open water bodies of Bangladesh would be increased through prevention of disease like EUS, which played a significant role on the reduction of *N. nandus* and *P. sophore* of beels i.e. on their biodiversity.

4. Conclusion

Aquaculture activities in Bangladesh are predominantly pond based. But the present study was conducted to know the impact of EUS on small species i.e. *N. nandus* and *P. sophore* of beel fisheries in Bangladesh. According to the farmer's opinion, indigenous small species of beel areas are the most susceptible to EUS in winter season. Among them, *N. nandus* and *P. sophore* were found having severe clinical signs. In addition, clinical and histopathological studies were carried out to examine disease status of sampled fish. Massive pathological changes were found in the organs of them compared to the other species. As a result, small indigenous species gradually decreased from the open water bodies by affecting the EUS. So it can be concluded that *N. nandus* and *P. sophore* were more affected by EUS than other species in beel areas of Bangladesh.

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