PARASITE DIVERSITY AND HISTOPATHOLOGICAL CHANGES DUE TO INFECTION ON THREE CROAKERS (*PENNAHIA ANEA, PANNA MICRODON* AND *JOHNIUS ARGENTATUS*) OF SAINT MARTIN'S ISLAND, BAY OF BENGAL

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ABSTRACT: This study investigated the parasites of three species of croakers (*Pennahia anea*, *Panna microdon*, and *Johnius argentatus*) and their effect on host's tissues. Fish samples were collected directly from the local fishermen and the fish landing station of Saint Martin's Island, Bay of Bengal on a regular monthly basis. A total of 448 individuals of parasites were collected of which nine species of parasites were identified including three crustaceans, four nematodes, one pentastomida, and one acanthocephala. The nematode *Procamallanus longus* had the highest prevalence (50%) and intensity (10.33) compared with the other parasites. The prevalence and intensity of parasites were slightly higher in female fish (55%, 35) than in male fish (50%, 20). The stomach and liver were the most favorable infection sites (53.33%). Furthermore, parasites with the largest length and weight showed the highest prevalence and intensity (66.66% and 38.4 as well as 80% and 72.5, respectively). In histopathological investigations, the most important alterations in various tissues of fish were hemorrhage, necrosis, fatty droplets, edema, vacuolar degenerations, missing villi, haemosiderotic nodules, ovarian follicle disruption, and parasitic cell distribution.

KEYWORDS: Croakers, parasites, prevalence, intensity, histopathology.

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Introduction

Croakers are carnivorous fishes and many species are of considerable commercial value. These fishes are important fisheries resources in the shallow warm seas and estuaries of the world (Holt *et al*., 1985). Croaker is one of the most desirable and highly priced large fishes that used as food in the coastal belt of Bangladesh. That's why, assessment of the health status of fish species forms an important approach and such approaches use combined assessment of externally visible diseases and internal evaluation of pathology (Lang *et al*., 2006).

Parasites influence host survival and reproduction because they can alter fish behavior and migration patterns, regulate fish populations, and affect fish community structure (Barber and Poulin, 2002). Copepods were found to be parasitic on fishes at least approximately 110 to 120 million years ago. Approximately 30 parasitic copepod families exclusively infest fishes. Endoparasitic helminths often induce inflammation and modify the structure and function of local tissues (Castro, 1992). In fish, one of the major factors of parasite species richness is host body size (Bell and Burt, 1991). *Philometra* (Philometridae, Dracunculoidea) infects the gonad of the marine fishes in the Atlantic, Indian, and Pacific Oceans; this species is severely pathogenic and affects reproduction (Gaglio *et al.*, 2009; Moravec and Buron, 2009 and Perez *et al.*, 2009). Parasitic disease is the most common problem of wild and farmed fishes in Bangladesh and causes histological alterations. Histological technique is one of the important procedures that are widely and successfully used to diagnose fish diseases. Histological studies have revealed the effect of parasitic infection on fish because it provides direct translation of toxic effects on vital anatomical functions. Histopathology is a sensitive parameter for determining possible cellular changes in target organs, such as the stomach, intestine, liver and gonad (Dutta, 1996). The histology of fish liver serves as a model for evaluating the interactions among stress factors, including biotoxins, parasites, infectious germs, pollutants, and physicochemical parameters (Brusle and Anadon, 1996). Parasites cause certain pathological changes in fish tissues, such as liver necrosis, kidney tubular damage, and stomach and intestinal abnormalities (Ramalingam, 1985). Therefore, histological studies are necessary to evaluate potential lesions in fish exposed to various parasitic infections.

Croakers have been poorly explored in Bangladesh because they are difficult to collect due to their muddy bottom habitats; hence, they are rarely studied. Some taxonomic studies on

parasites in croakers have been conducted in different countries worldwide (Kritsky and Boeger, 2002) but not in Bangladesh. The main purpose of present investigation was to identify associated parasites in relation to host length, weight, different organs, and sex in three croakers species (*Pennahia anea*, *Panna microdon* and *Johnius argentatus*) and determine the effect of such parasites on the histology (stomach, intestine, liver, and ovary) of the species in St. Martin's Island, Bangladesh.

Materials and Methods

The experiment was conducted from October 2015 to September 2016 in St. Martin's Island, Bangladesh. Fish samples were collected directly from local fishermen and fish landing stations. The samples were brought directly to the laboratory and preserved in a deep freezer (-18 °C) for further analysis. In parasitic and histological studies, 30 host fish of three croaker species [*P. anea* (10), *P. microdon* (10), and *J. argentatus* (10)] were collected on a monthly basis at regular intervals. At the laboratory, the fishes were identified, classified according to sex, and divided into three major length groups (**Group-1**: 9-14cm, **Group-2**: 14.1-19cm, and **Group-3**:19.1-24cm) and weight groups (**Group-1**: 12-72g, **Group-2**: 72.1-132g, and **Group-3**:132.1-192g).

Parasite preservation and mounting

For ectoparasites, the external body surface and gills were examined by a magnifying glass. The fishes were dissected to remove the stomach, intestine, rectum, pyloric caeca, gall bladder, liver, kidney, and gonad from the body. Each organ was placed into separate petri dish containing (0.85%) saline water. Both ectoparasites and endoparasites were collected and preserved in 70% ethyl alcohol. The parasites were permanently mounted on slides by following the methods suggested by Cable (1977). The parasites were identified using a compound microscope through the methods of Yamaguti (1961 and 1963) and Arthur (2002). Prevalence and intensity were calculated with the equations reported by Margolis *et al.* (1982) as follows:

$$\text{Prevalence} = \frac{\text{Number of host infected}}{\text{Number of host exmined}} \times 100$$

$$\text{Prevalence} = \frac{\text{Total number of parasites}}{\text{Number of infected host}}$$

Histopathological study

For histological study, the tissues (stomach, intestine, liver, and ovary) of infected fish were collected and fixed in alcoholic Bouin's fluid for 24 h. The tissues were preserved in 70% alcohol and sectioned using a microtome. The samples were stained and mounted in accordance with the method of Luna (1968). The tissues mounted on the slides were viewed under a digital compound microscope. Photomicrographs were obtained after the examination of the histological condition of each slide.

Results and Discussion

Parasite investigation

During the study period, 30 individuals of three species of croakers (*P. anea*, *P. microdon* and *J. argentatus*) were examined; of which, only 16 were infected with parasites. The overall prevalence and intensity were 53.3% and 28, respectively **(Table 3)**. This finding is similar to the reports of Morenikeji and Adepeju (2009). A total of 448 ectoparasites and endoparasites were detected, and they belong to nine species; of which, four species were ectoparasites and five were endoparasites. In the present study, four species of ectoparasites were recorded under two groups; namely, Crustacea (Copepoda: Caligidae) and Pentastomida. Figure 1 showed the ectoparasites attachment on gill and operculum. Fifty ectoparasites were collected of which *Lernanthropus rathbuni* had the highest prevalence (26.67%), and *Sebekia okavangoensis* had the highest intensity (4) **(Table 1).** The five species of endoparasites included four nematoda and one acanthocephalan (*Floridosentis mugilis*). *Procamallanus longus* had the highest prevalence (50%) and intensity (10.33) **(Table 1).**

Table 1. Prevalence and intensity of both ectoparasite and endoparasite species

	Group	Name of Species	No. of host infected (n=30)	Prevalence (%)	Total no. of parasites	Intensity
Ectoparasites	Pentastomida	*Sebekia okavangoensis*	5	16.67	20	4
	Copepoda	*Lernanthropus giganteus*	3	10	18	3
		Lernanthropus rathbuni	8	26.67	8	2
		Caligus irritans	2	6.67	4	2
Endoparasites	Achanthocephala	*Floridosentis mugilis*	6	20	30	5
	Nematoda	*Philometra cephalopholidis*	3	10	18	3
		Camallanu scarangis	12	40	115	9.58
		Procamallanus longus	15	50	155	10.33
		Pseudoterranova decipiens	9	30	80	8.88

Prevalence and intensity are dependent on parasite species and their biology, host and its feeding habits, physical factors and hygiene of the water body, and presence of intermediate hosts where necessary (Shukerova *et al*., 2010; Hussen *et al*., 2012). Croakers are commercially important and so much handling occurred by fishermen and consumer though also ectoparasites were found. Caligid ectoparasites are known to damage young fish (Wootten *et al.*, 1982).The majority of the host fish are parasitized by nematoda. Gonad infection of the *Philometra* nematode was recorded in the case of *Micropogonias furnieri* croaker from the coast of Brazil (Moravec and Barton, 2015). This nematode was newly discovered from the coast of the Bay of Bengal, Bangladesh.

Ectoparasites were collected from the skin and gill, where the latter had higher ectoparasite prevalence (20%) and intensity of infection (7.5). The stomach and liver showed the highest prevalence (53.33%) of endoparasites, and the body cavity had the highest intensity (16.6) **(Table 2).** These findings are attributed to the fact that the stomach and liver are privileged and nutrient-rich sites.

Table 2. Prevalence and intensity of different infected organs

Name of organs	No. of host examined	No. of host infected	Prevalence (%)	Total no. of parasites	Intensity
Skin		4	13.33	40	10
Gill		6	20	30	5
Body cavity		6	20	168	28
Stomach	30	16	53.33	40	2.5
Intestine		10	33.33	30	3
Liver		16	53.33	140	8.75
Ovary		3	10	18	3

Female fish had higher prevalence (55%) and intensity (35) than male (50% and 20, respectively) **(Table 3).** In the present study, no significant differences were observed between male and female fish, although the former had lower levels of parasitism in general. The sex of the host can potentially influence the levels of parasitism due to behavioral and physiological differences (Esch *et al*., 1988). Susceptibility to parasites is greater for some fish species during the reproductive period, as in the case of *Cichlamon oculus* (Machado *et al*., 2000). Omeji *et al.* (2013) reported that the physiological state of female fish during gonad development could have reduced resistance to parasitic infestation. The higher intensity in female fish may be related to their involvement in reproduction is more costly than that in males; thus, females are more susceptible to parasitic infection (Shukerova *et al*., 2005).

Table 3. Sex wise Prevalence and intensity of three host species

Host Sex	No. of examined fish	No. of infected fish	Prevalence (%)	No. of parasites	Intensity
Female	20	11	55	350	35
Male	10	5	50	98	20
Total	30	16	53.3	448	28

In the three length groups, the prevalence (66.66%) and intensity (38.4) rates were the highest in group 3. In the weight groups, the prevalence (80%) and intensity (72.5) rates were the highest in group C **(Table 4)** because of higher feeding habit and frequency in large hosts.

Table 4. Length and weight wise prevalence and intensity of three host species

	Group	No. of Examined fish	No. of infected fish	Prevalence (%)	No. of parasites	Intensity
Length	1: 9-14 cm	7	3	42.85%	38	12.66
	2: 14.1-19 cm	8	3	37.5%	26	8.66
	3: 19.1-24 cm	15	10	66.66%	384	38.4
Weight	A: 12-72 gm	20	9	45%	128	15.5
	B: 72.1-132gm	5	3	60%	30	10
	C:132.1-192 gm	5	4	80%	290	72.5

In general, the standard length of fish is directly related to its age and body size (Shotter, 1973). Parasite prevalence and intensity are positively associated with fish age (Chandler *et al.*, 1995). The present data are similar to those obtained in a previous work by Mohammed *et al*. (2008), who found that prevalence increases as the fish grows due to the longer time of

exposure to the environment. Additionally, the highest percentage of infection observed in large-weight groups indicated that the number of parasites increases with increasing weight. This finding might be attributed to the fact that large fish take more food and travel wider areas in searching for food and thus are more susceptible to parasitic infestation. This finding is consistent with the report of Omeji *et al*. (2013) but contradicts that of Tasawar *et al*. (2007), who stated that smaller fish have higher parasite load than large ones.

Parasitic effects on tissues determined by histopathology analysis

Three infected host tissues (stomach, intestine, liver, and ovary) were subjected to histopathological study to investigate the effects of parasites on the host tissue. Figure 7 showed the normal view of the stomach, intestine, liver, and ovary, in which all layers are clearly observed (Begum *et al*., 2013). Parasites attachment showed on Figure 2. Parasites can affect various fish organs either directly or indirectly, depending on the target organ. Parasites induce physiological, immunological, or ethological changes in the host and impair mating, gonad maturation, or larva survival (Razia *et al*., 2012). Histopathological alteration in the stomach of the three host fishes showed submucosal layer edema, damaged mucosal layer, mucosa layer necrosis, and longitudinal muscle layer hemorrhage and degeneration (P1-J1 of Figure 3). In *P. anea*, parasites in the serosa layer, blood vessel congestion, and parasitic cell distribution were observed (P1 and P2).In *J. argentatus*, submucosa layer vacuolation was detected (J1).

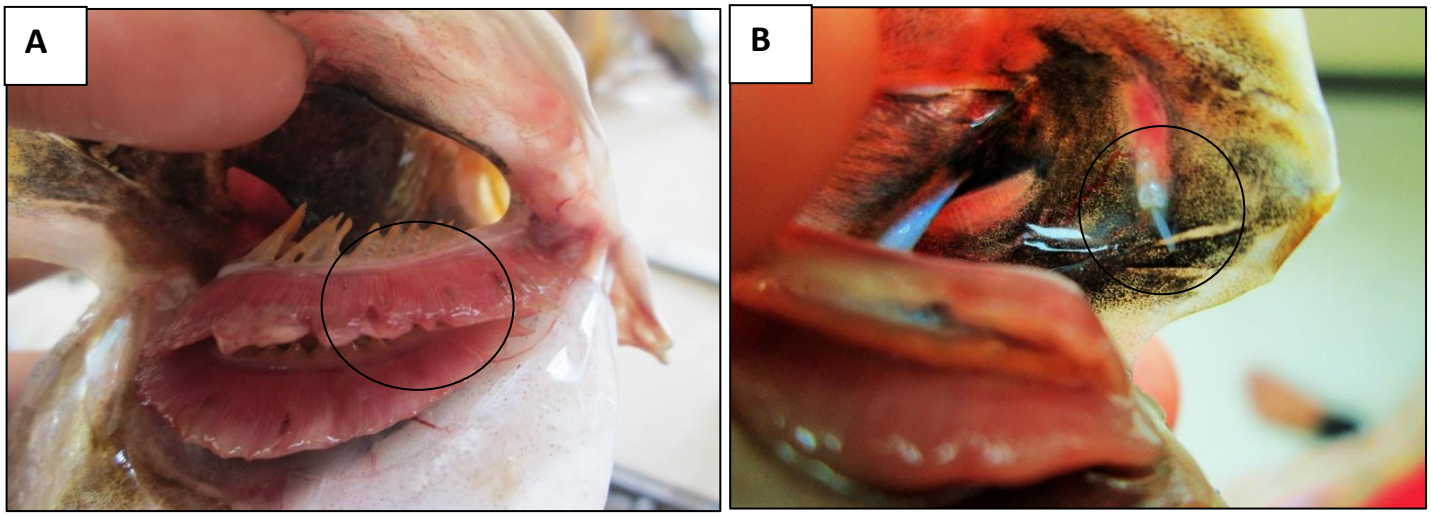

Figure 1. Infected gill with (*Lernanthropus giganteus)* (A) Infected operculum with *Caligusirritans* (B)

The intestine of *P. anea* showed missing villous process, necrosis in villous process, and degeneration of the submucosa layer (P1). The intestine of *P. microdon* showed blood vessel congestion, submucosa layer hemorrhage, parasitic cell distribution, submucosa layer degeneration, and necrosis (M1). The intestine of *J. argentatus* showed missing villi, missing epithelial layer, massive atrophy in the villi region, and longitudinal and circular muscle layer degeneration (J1) (Figure 4). The present finding agrees with those of Ramachandran, (1975) and Oliva *et al*. (1992). Orecka-Grabda, (1991) reported that nematode larvae are more harmful than adults and can penetrate into the tissues of digestive organs, causing severe tissue damage and destruction of the organ cells. Sabur (2006) observed missing villous process and necrosis in villous process in intestinal tissues. The mucosa of the infected intestine appeared congested and edematous. The deformation of mucosa and submucosa resulted in the separation of muscle fibers, and the shapes of villi changed. The parasite also caused blood vessel dilation in the submucosa, resulting in intestinal fold degeneration and villi shrinkage (Esch *et al.*, 1988).

Hepatocyte necrosis in the liver, hepatocyte hemorrhage, hepatocyte degeneration, hemosiderotic nodules, vacuolation, extensive hepatocyte vacuolar degeneration, blood vessel congestion, hepatocyte edema, hepatocyte focal necrosis, and parasitic cell distribution were observed in present investigation (P1-J1 of Figure 5). Fish liver isconsidered a target organ of microorganisms; as such, investigating the altered liver structure of fish would be significant in health studies (Myers *et al*., 1985). Moreover, the liver plays a crucial role in complex enzymatic reactions that are responsible for various vital functions, such as accumulation and biotransformation of xenobiotics in fish (Gaglio *et al*. 2009). Doreen *et al.* (2009) investigated the Indian major carp, *Catla*, and showed some structural abnormalities, such as disruption of the normal chordal arrangement of the liver cells and heavy deposition of hemosiderin pigments.

The ovary of *P. anea* was subjected to histopathological study because it was the only organ infected with parasites. The ovary had the following abnormalities: ovarian follicle disruption, oocyte necrosis, ovarian follicle rupture, vitelline envelope began to break ovarian follicle degeneration, atretic follicle, and yellowish-brown pigment accumulation surrounded by fibroblast-like cells (P1 of Figure 6). Razia *et al.* (2012) performed histopathological studies and reported that the infected *Clarias gariepinus* ovarian wall became thin, oocytes were slightly ruptured, the egg envelope degenerated, and oocytes shrank. The size of the ovary with thin wall drastically decreased, and oocytes were detached from their envelope. The infected ovaries also showed increased necrosis, decreased formation of atretic follicles during reproductive period,

degeneration and necrosis, and damaged blood vessels in the ovary. The present findings also agree with those of Moravec and Barton (2015).

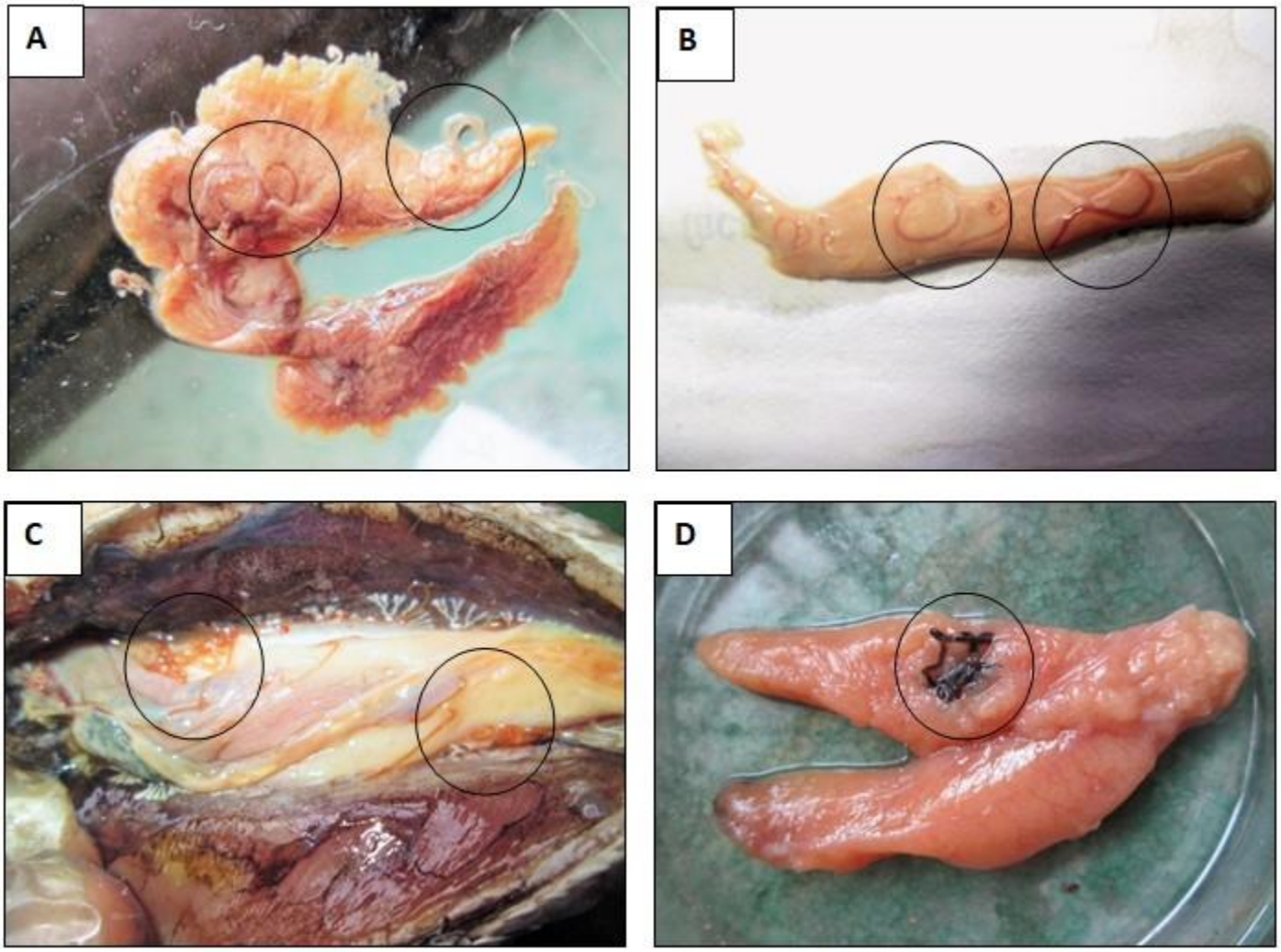

Figure 2. Helminth parasites attached with liver (A); Stomach and Intestine (B); Body cavity (C); and Ovary (D) of infected fish

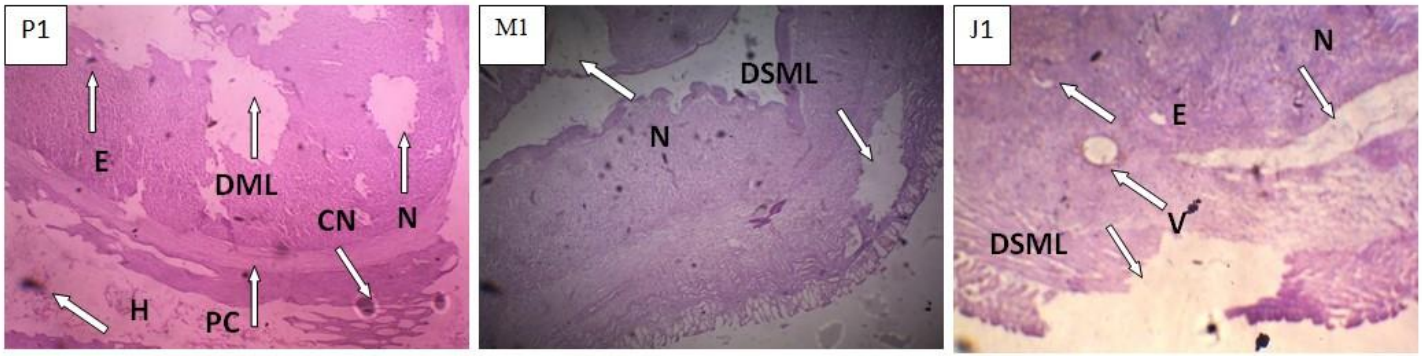

Figure 3. (P1-J1): Histological Changes in three species of Croakers fish stomach. All section Stained with H&E. (X100). In *Pennahia anea* (P1) shows Edema in submucosa layer (E) ; damaged mucosa layer (DML) ; necrosis in mucosa layer (N) ; Congestion of blood vessel (CB) ; hemorrhage (H); distribution of parasitic cell (PC). In *Panna microdon* (M1) shows Necrosis (N); damaged submucosa layer (DSML). In *Johnius argentatus* shows damaged submucosa layer (DSML); and vacuolation in the submucosa layer (V).

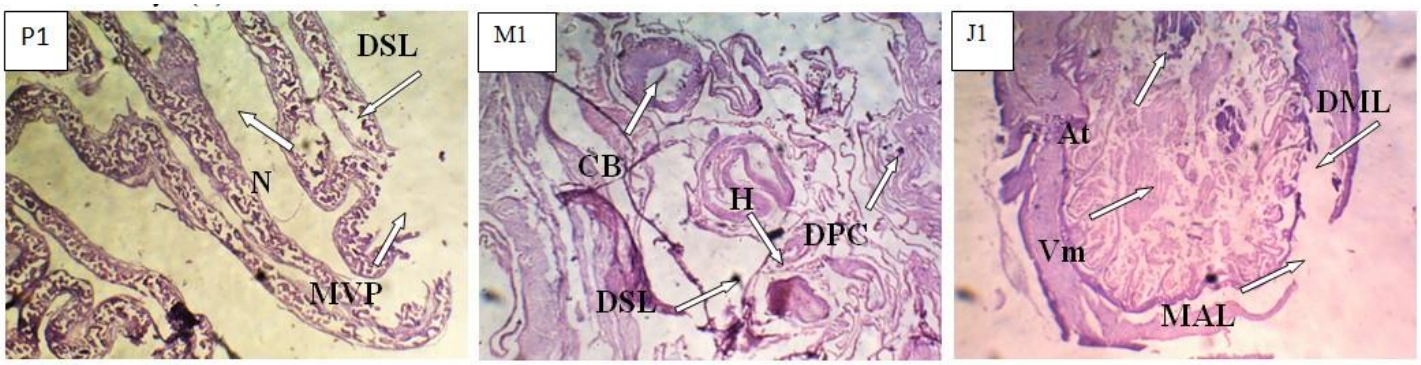

Figure 4. (P1-J1): Histological Changes in three species of Croakers fish Intestine. All section Stained with H&E. (X100). In *Pennahia anea* (P1) observed following alteration Missing villous process (MVP); necrosis in villous process (N); degeneration of submucosa layer (DSL). In *Panna microdon* (M1) species observed Congestion of blood vessel (CB); hemorrhage in submucosa layer (H); distribution of parasitic cell (DPC); degeneration of submucosa layer (DSL). In *Johnius argentatus* (J1) shows Missing epithelial layer (MAL), villi missing (Vm), massive atrophy in the villi region (At), degeneration of longitudinal and circular muscle layer (DML).

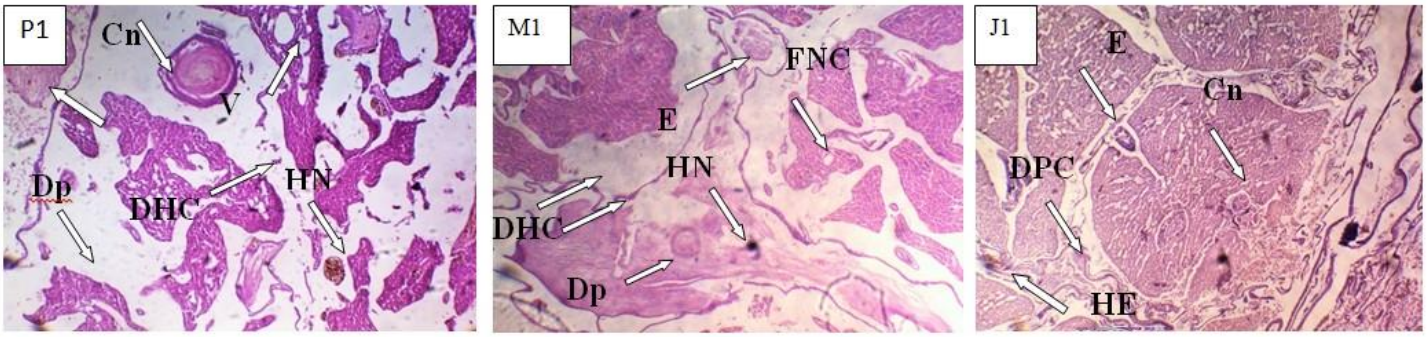

Figure 5. (P1-J1): Histological Changes in three species of Croakers fish Liver. All section Stained with H&E. (X100). In *Pennahia anea* (P1) observed following alteration Congestion of blood vessel (Cn), degeneration of hepatocytes (DHC), haemosiderotic nodules (HN), extensive vacuolar degeneration of the hepatocytes (Dp). In *Panna microdon* (M1) found Extensive vacuolar degeneration of the hepatocytes (Dp), Focal necrosis (FNC). In *Johnius argentatus* (J1) shows Congestion of blood vessel (Cn), edema (E) in the hepatocytes, hemorrhage (HE) between the hepatocytes, distribution of parasitic cell (DPC).

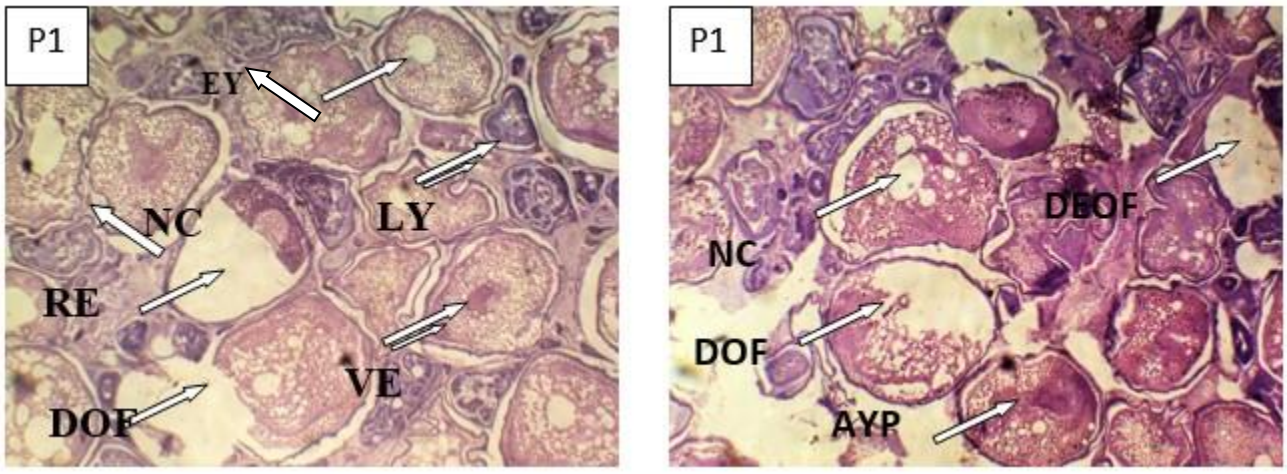

Figure 6. P1: Histological alteration in *Pennahia anea* (P1) species of Croakers fish Ovary. All section Stained with H&E. (X100). Following alterations were observed during study period. Early Yolk Stage (EY), disruptive ovarian follicle (DOF), late yolk stage (LY), necrosis in the oocyte (NC), ruptured ovarian follicle (RE), the vitelline envelope (arrow) has begun to break down (VE). Necrosis in the oocyte (NC), degeneration of ovarian follicle (DEOF), disruptive ovarian follicle (DOF), accumulation of yellowish-brown pigment surrounded by fibroblast-like cells (arrow)(AYP).

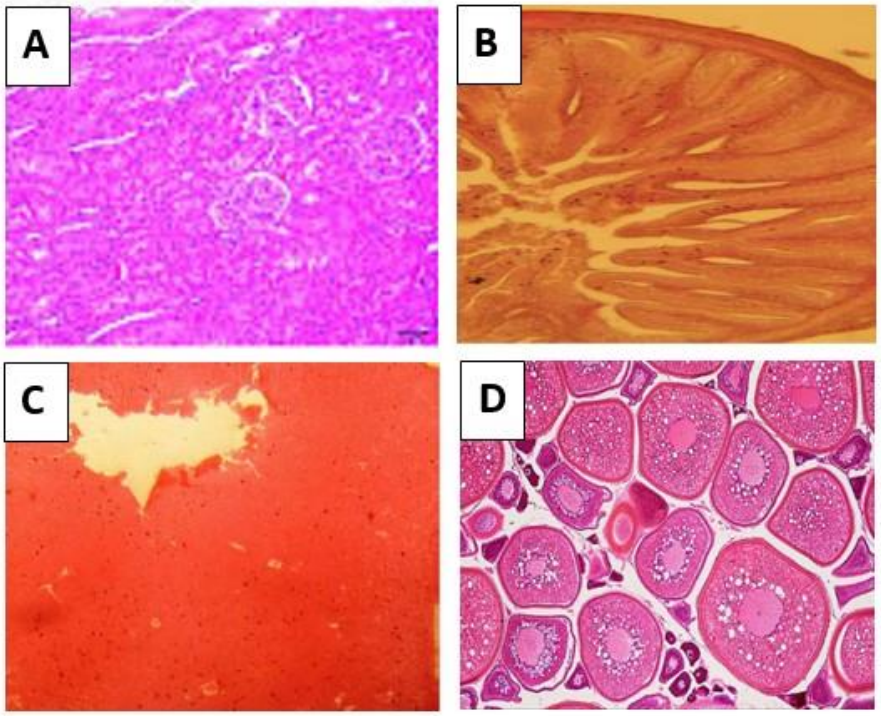

Figure 7. Normal view of Stomach (a); Intestine (b); Liver(c); and Ovary (e) (Begum *et al.*, 2013)

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