JFLS | 2020 | Vol 5(1) | Pp 30-35

Journal of Fisheries and Life Sciences
International Peer-Reviewed Biannual Journal
www.fishlifesciencejournal.com

Research Article

Occurrence and pathological studies on acanthocephalan (*Neoechinorhynchus agili*) infestation in fishes from Tamil Nadu, South-east coast of India

Alagarsamy Sakthivel, Ayyaru Gopalakrishnan*, Periyasamy Selvakumar

Centre of Advanced Study in Maine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai- 608 502, Tamilnadu, India

ISSN: 2456-6268

ARTICLE INFO
Received: 15 June 2019
Accepted: 28 June 2020
Available online: 30 June 2020

KEYWORDS
Neoechinorhynchus agili
Histology
Histochemical

***CORRESPONDENCE**
aquagopal@gmail.com

ABSTRACT

The present study was undertaken to analysis the occurrence, pathology and histochemical investigation of acanthocephalan infested fishes. The adult worm proboscis was globular and the length and width ranging between 2.3–6.1 mm and 0.6–1.1 mm, respectively. *Thunnus albacares* was the major host in terms of prevalence and intensity among the other fish species examined for infestation and seasonal variation of *Neoechinorhynchus agili* over two year study period. The higher prevalence of infested fishes *T. albacares* (43.7 %±1.1) and *Coryphaena hippurus* (41.6%±1.5) were observed. Mean intensities ranged between 5.8 and 10.8 worms per fish. Histopathologically, the infested intestinal mucosal epithelium, stratum granulosum, lamina propria, muscular and serosa layers were highly degraded. The lesions were infiltrating with basophil-like inflammatory cells. The parasite-affected lesions were histochemically positive for alcian blue, azo dye, toluidine blue and oil red O. The histological findings revealed that the pathogenicity was depends on the intensity of the parasitic burden and depth of the length of proboscis penetration into the host intestine tissues. Long live parasite increases the cellular infiltration, necrosis and it may leads to the tumorous conditions of the infested hosts.

INTRODUCTION

Parasitic infestations in fishes are very common, particularly in marine fish population were highly infected with parasites since, the ecological requirements and parasite high transmission of intermediate host to fishes (Feist and Longshaw, 2008). The parasites are an important group of pathogen causes infection and diseases to both fresh water and marine fishes. However, the marine fishes infested by many parasites such as; isopod, copepod, nematode, trematode and acanthocephala. Among, the parasitic infestation the acanthocephala infestation is severe, but these reports are meagerly available in worldwide. Even though, Sakthivel *et al.* (2014; 2015a; 2015 b and 2016) has been reported the prevalence and pathological investigation of acanthocephala infestation in marine finfishes of Tamilnadu coastal waters.

Individual parasites belonging to different populations are expected to differ in the way they exploit their hosts, because of local adaptation phenomena (Lambrechts *et al.*, 2006). Parasites can influence the health of their hosts either directly or indirectly making them less resistant to environmental stresses. Some are capable of regulating host populations and they can influence community structure of the host fishes. Parasites also can be used as biological tags in population studies of fish (Lambrechts *et al.*, 2006).

The ecological parameters such as prevalence, mean intensity and abundance were calculated to determine abundance of the parasitic species (Margolis, 2004). Parasitic disease, either alone or in conjunction with other environmental stresses, may influence weight or reproduction of the host, alter its population characteristics and affect its economic importance (Rohade, 1967).

The attachment of the armed proboscis affects the nature of the intestinal tissues leading to pathological changes (Janovy, 2002). It is now generally accepted that gut inhabiting acanthocephala actively select the part of gut, which is most suitable for survival and reproduction, resulting in ordered to distribute within the tract that is consistent between individuals of a host species. These adaptations totally decrease the muscular, nervous, circulatory and the excretory system and also complete loss of the digestive system (Janovy, 2002).

The infected fish intestines suffer irreversible mechanical damage due to the attachment of the acanthocephala armed proboscis which also affects the architecture of the intestinal tissues leading to pathological changes. The direct effect of acanthocephala mainly depends on their attachment organs. The endoparasitic helminths often induce the inflammation and modification of the structure and function of infected tissues. According to Sharkey (1992), the inflammation consists of a complex series of homeostatic mechanisms involving the immune, nervous and circulatory systems in response to tissue injury or infection. There are several published records on the essential role of enteric immune cells in inflammatory processes caused by parasitic helminths (Dezfuli *et al.*, 2002a, b, 2003b; Bosi *et al.*, 2005).

Therefore, the present study was aimed to evaluate the occurrence, pathology and histochemical investigation of acanthocephala infested fishes of Tamil Nadu coastal waters. On the basis of histological and histochemical findings, the lesions in the organs were highly damaged due to the parasitic infestation. The high density of the parasite and severe penetration of proboscis into the host intestinal tissues is the main reason for the pathogenicity of the host

MATERIALS AND METHODS

Sample collection

The present study was carried out, for two years from January 2014 to December 2015. The host fishes *Thunnus albacares* and *Coryphaena hippurus* were collected from the two different landing centers of Tamilnadu. Station I: Pazhayar (N 11° 21' 16.8408", E 79° 49' 38.6508"); Station II. Parangipettai (11° 29′ 24″ N, 79° 45′ 36″ E). The parasites were carefully removed from the host intestine, fishes using fine forceps and the biopsies were immediately stored in 70% cold ethanol for further study. The site of the attachment, orientation of parasites on the host and the numbers of parasites in each fishes were recorded. Further the parasites were studied using different types of microscope; Stereo microscope, Shapes-contrast microscope and the morphology was drawn with the help of Camera Lucida. The collected parasites were identified by the method of Yamaguti 1935, Omer Amin 1985, 2013 and Bhattacharya 2007. Parasitic taxonomy followed by WoRMS (2011).

Scanning electron microscopic study

For scanning electron microscopic studies, Lee, 1992 method was followed. The parasites were fixed in 2.5 % glutaraldehyde followed by 1% osmium tetroxide, dehydrated in acetone series, critical point dried, gold coated and observed under a SEM (JEOC JMS 5610LV, Annamalai University) at an acceleration voltage of 8kv and electron micrographs were taken.

Pathology

The infected intestinal lesions were excised then preserved in 10% neutral phosphate buffer formalin. The biopsies were dehydrated with graded series of ethanol, processed and embedded with paraffin wax. The thin section of 4 µm was incised with rotary microtome and stained with Harris Haematoxylin and Eosin (Kienan, 2002). Alcian blue staining method of Bancroft and Stevens (Weijie Dong 2012) was followed. The mast cells analysis was through the toluidine blue staining method of Migliaccio *et al.* Lipid accumulation of the infected intestine was studied by Oil Red O stain (Young and Heath 2002). The sections were treated with Azodye as per the method of Malaty, Hoda (2007) for detection of the activity of acid and alkaline phosphatases. The stained sections were observed under a phase contrast microscope (10 and 20× magnification).

RESULTS

Body 1.2-6.8 cm length; width 0.8-2.4 mm (Fig 1A and 2A); body elongated covert with double walled layer, Posterior field of trunk spines continues down ventral side nearly too anterior tip; Proboscis globular longer than broad narrowing backwards, Anterior division of proboscis hooks longer than posterior division (Fig. 1B and 2B); Posterior end of the proboscis inwards join the neck, proboscis 16-18 longitudinal rows evenly curved hooks, in each row 10-12 hooks; arrangement of the unarmed hooks in circular, first 7 rows of hooks increase in size to 50-55µm length. Hooks in each row different in size and shape, all rooted. Reproductive system longer than broader; 1.6 mm length, 655 width. The male acanthocephalan have 2 bursal pockets round and oval shape cement glands (Fig. 1 C and 2C). Round and oval shape cement gland reservoir leading to 4 cement glands arranged regularly 245-675. Genital opening of female closed vaginal lips showed slightly curved slit-shaped opening. Genital pore terminal and absent of genital spines (Fig. 1D and 2D)

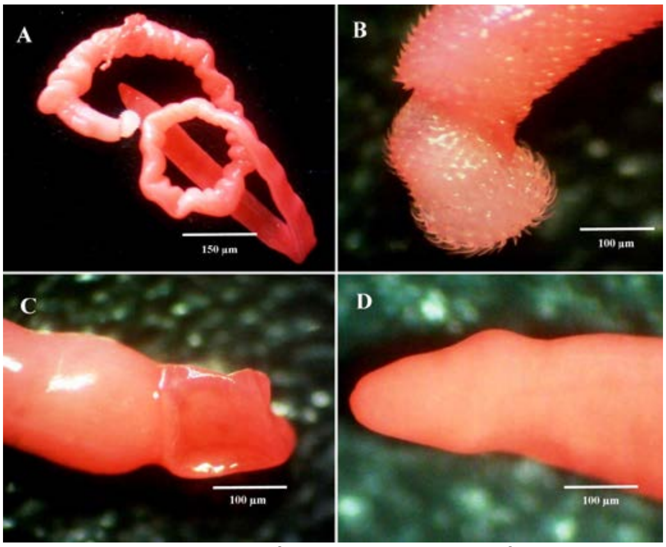

Fig. 1: Dorsoventral view of *the N. agili* (A); Pyriform proboscis with anterior end of the proboscis B); Dorsoventral view of (bursa) male reproductive system (C); Dorsoventral view of (genital opening) female reproductive system (D)

In *T. albacares* the higher prevalence of *N. agili* infestation 43.7% ±1.1 was observed in post-monsoon season 2017 at Pazhayar, whereas, minimum infestation 11.9% ±1.2 was noticed in pre-monsoon season 2016 at Parangipettai. The higher mean intensity 11.9±1.2 of *N. agili* infestation was noticed from Pazhayar during post-monsoon 2017, whereas the lower 7.3±1.2 was observed from Parangipettai during summer 2016 (Table 1). In *C.*

hippurus the higher prevalence of *N. agili* infestation 41.6% ±1.5 was observed in post-monsoon season 2017 at Pazhayr, whereas, minimum infestation 14.3%±1.7 was noticed in pre-monsoon season 2016 at Parangipettai. The higher mean intensity 9.3 ±1.5 of *N. agili* infestation was noticed from Pazhayar during post-monsoon 2017, whereas the lower 6.11±1.7 was observed from Parangipettai during summer 2016 (Table 2).

Table 1: Seasonal variation of Acanthocephala infestations in *T. albacares*

	Prevalence (%)			
	Parangipettai		Pazhayar	
Seasons	**2016**	**2017**	**2016**	**2017**
Post-monsoon	35.8 ±1.8	40.6 ±1.8	36.3 ±1.8	43.7 ±1.1
Summer	13.6 ±1.5	17.3 ±1.6	15.7 ±1.3	23.8 ±1.4
Pre-monsoon	11.9 ±1.2	13.8 ±1.2	14.5 ±1.6	17.3 ±1.3
Monsoon	20.3 ±1.4	24.4 ±1.4	26.6 ±1.3	31.5 ±1.2
	Intensity (%)			
Post-monsoon	8.7 ±1.2	9.8 ±1.4	9.7 ±1.1	9.9 ±1.4
Summer	7.3 ±1.6	8.4 ±1.2	8.8 ±1.3	9.2 ±1.1
Pre-monsoon	8.3 ±1.3	9.1 ±1.7	9.2 ±1.1	9.5 ±1.6
Monsoon	8.5 ±1.5	9.5 ±1.1	9.5±1.84	9.7 ±1.2

Table 2: Seasonal variation of Acanthocephala infestations in *C. hippurus*

	Prevalence (%)			
	Parangipettai		Pazhayar	
Seasons	**2016**	**2017**	**2016**	**2017**
Post-monsoon	28.6 ±1.3	33.8 ±1.5	38.8 ±1.8	41.6 ±1.5
Summer	14.3 ±1.7	16.7 ±1.2	18.7 ±1.4	22.4 ±1.3
Pre-monsoon	10.4 ±1.2	14.2 ±1.4	15.3 ±1.6	16.9 ±1.4
Monsoon	17.8 ±1.1	25.8 ±1.6	26.9 ±1.3	24.7 ±1.6
	Intensity (%)			
Post-monsoon	7.8 ±1.7	8.5 ±1.6	8.6 ±1.7	9.3 ±1.8
Summer	6.1 ±1.4	7.2 ±1.5	7.1 ±1.6	7.4 ±1.3
Pre-monsoon	7.3 ±1.2	7.8 ±1.3	7.8 ±1.4	8.2 ±1.4
Monsoon	7.6 ±1.3	8.1 ±1.2	8.2 ±1.3	8.6 ±1.7

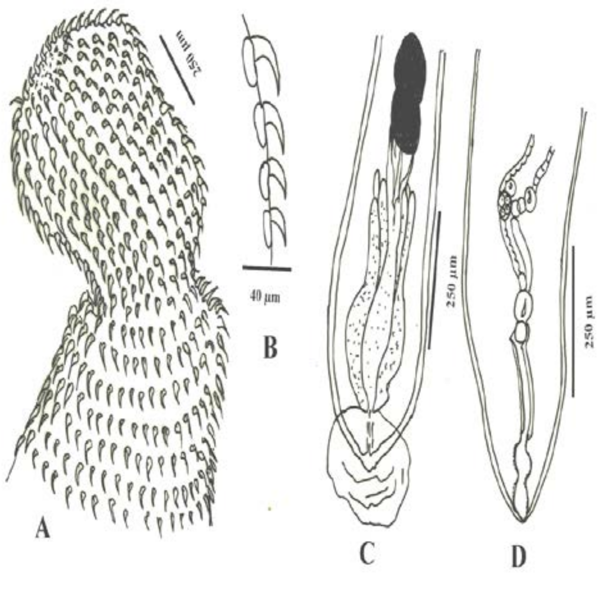

Fig. 2: Armature of globular proboscis with anterior end (lateral view of *N. agili* (A); Detail of curved hook row showed roots (A); Dorsoventral view of reproductive system and posterior end bursa of a male (C); Dorsoventral view of reproductive system and posterior end genital opening of a female (D).

The transverse section of normal muscle fibers of *T. albacares* (Fig. 3A). The infected host intestine obstructs and damages the absorbing and affected the nutritional potential of the mucosal lining (Fig. 3B). The necrotic cells and connective tissue are commonly near the armed proboscis. *N. agili* migrates through the outer mucosa and sub-mucosal layers into the outer muscular layers. Smooth muscle fibers are visible surrounding the proboscis with numerous hooks disorganizing the host muscle layers (Fig. 3C and D). The general host responses to the initial entry of these acanthocephala were manifested as hemorrhaging, tissue necrosis, and occlusion of the absorptive surfaces. All sections for the Acanthocephala invasions were extensive hemorrhaging and subsequent necrotic infested tissue (Fig. 3E and F).

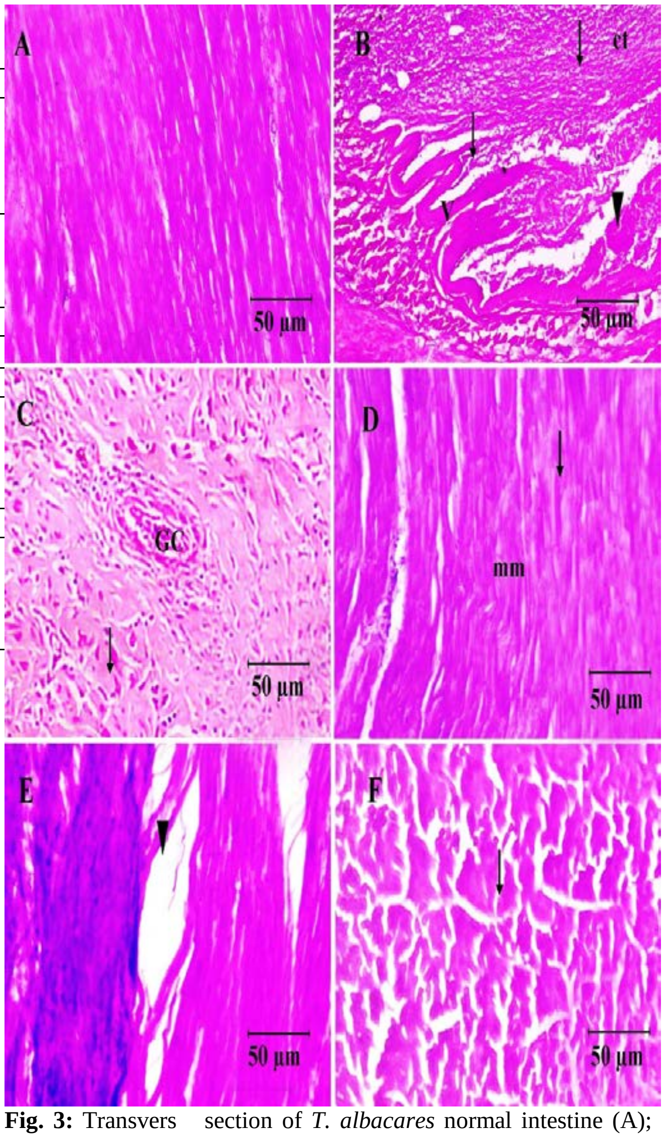

Fig. 3: Transvers section of *T. albacares* normal intestine (A); The proboscis Acanthocephala invading host mucosa Ct-Connective tissue (head arrow) (B); Hooks are visible with extensive hemorrhaging surrounding the proboscis Gc-Goblet Cells (arrow) (C); Migrating granulocytes, Mm-Muclaris mucosa (arrow) (D); Damaged intestinal wall (head arrow) (E); Cellular necrosis of the infested intestinal wall (arrow) (F).

The transvers section of normal *C. hippurus* intestinal tissue (Fig. 4A). The *N. agili* was surrounded in a fibrous capsule of host origin, the integument of the parasite appeared to be unaffected by the inflammatory responses mounted by the host's immune system. Dilation and congestion of blood vessels in stromal connective tissue between the muscles bundles detected (Fig. 4B). The large, last row of spines on the proboscis were seen anchored firmly in the surrounding connective tissue capsule and posterior region of the intestine intensity was very heavy (Fig. 4C). The neck region of the parasite was particularly long, in many cases. Intestine tissue appeared swollen with the anterior ends of some Acanthocephala projecting into the peritoneal cavity enclosed in a connective capsule (Fig. 4D). The proboscis was seen traversing the entire breadth of the intestinal wall reached the peritoneal cavity, enclosed in a fibrous connective tissue jacket of host origin. Spines of the proboscis anchored firmly in the surrounded connective tissue capsule (Fig. 4E and F).

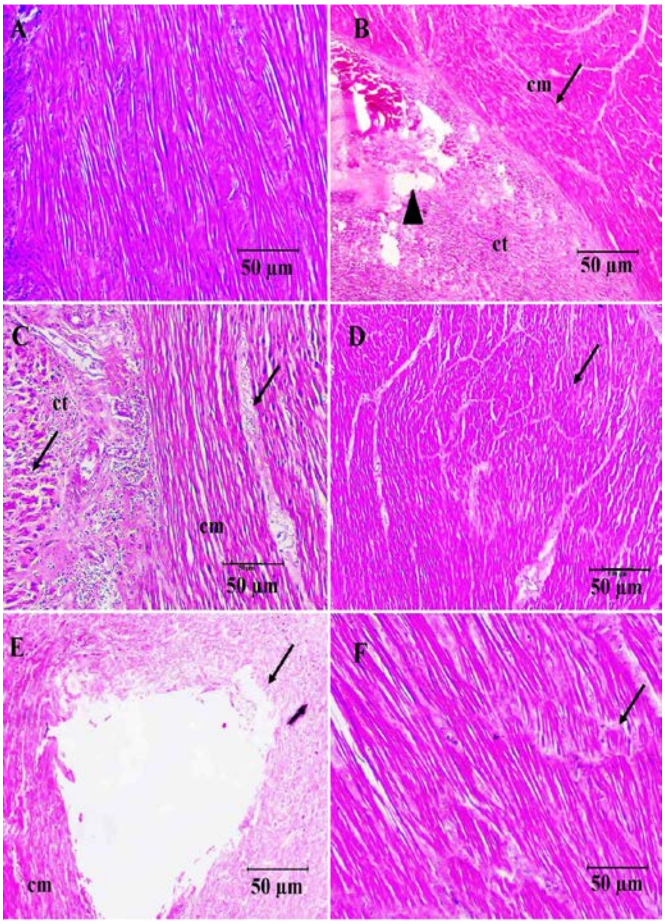

Fig.4: Transvers section of the *C. hippurus* normal intestinal muscle fibers (A); Dilation and congestion of blood vessels in stromal connective tissue between the muscles bundles detected (head arrow) Ct- Connective tissue; Cm- Circular muscle, (B); Section of connective tissue capsule Ct- Connective tissue; Cm- Circular muscle (arrow) (C); Intestine posterior region was intensity of infection was very heavy (arrow) (D); Infected posterior region of the intestine appeared swollen with the anterior ends of some worms projecting into the peritoneal cavity enclosed in a connective capsule (E); Spines on the proboscis anchored firmly in the surrounding connective tissue capsule (arrow) (F).

Alcian blue stain positive for sulfated and carboxylated acid muco-polysaccharides and sialomucins, the acanthocephala infested region contain abundant acid muco-polysaccharides (Fig. 5A). The stain toluidine blue positive for the metachromatic mast cells were observed from the infested host lesions (Fig. 5B). Oil-Red-O stain was used to observe in lipid droplets of infested host intestinal region (Fig. 5C). The red granule of the Azodye deposits indicates the acid phosphatase sites and bluish granules indicate the alkaline phosphatase sites. The lesion exhibits the mild and moderate expression of acid and alkaline phosphatases, which indicates the intestinal cellular proliferation (Fig. 5D).

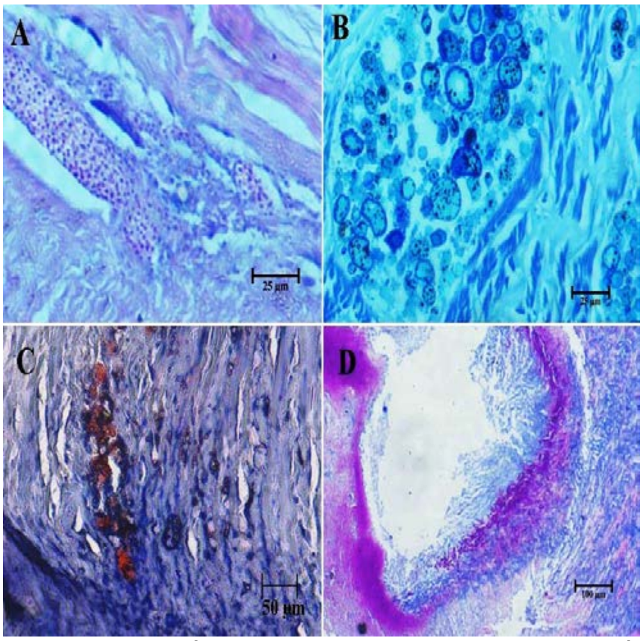

Fig. 5: *N. agili* infested intestine; Stain alcian blue positive for acid mucopolysaccharide (a). Stain toludine blue positive for matachromatic mast celles (b). Stain oil red-O positive for oil droplet (c); Stain azodye positive for acid and alkaline phosphatase (d).

DISCUSSION

The results of the present study clearly showed that, there was no much more variation found in the prevalence and mean intensity of parasitic infestation between the two stations but there was a significant variation found in all the four seasons. Among the four seasons, the higher acanthocephala infestation was recorded during post-monsoon season (Wang *et al.*, 2001; Poulin 2002).

Infections are highly reported in tropical seas than in temperate regions. However, the environmental conditions of the tropical waters are quite favorable in pre-monsoon for the transmission of intimidated host to the final host (Shulman, 1950). The present study also supported the role of temperature was controlling the acanthocephala infestation directly or indirectly in the different study areas.

Formerly, more or less similar prevalence of parasitic infestations was reported by many authors. Amin (1977) reported that 38 % of prevalence in *C. cognatus by Acanthocephalus dirus* in Lake Michigan. Laskowski *et al.* (2009) recorded only one Echinorhynchid species, *Heterosentisheter acanthus*, in *Platycleistes sellata* (prevalence 15 %). Brattey (2012) who has been studied the prevalence of acanthocephala was recorded in summer (80%) followed by spring (68%), autumn (64%) and winter (44%). The highest prevalence of acanthocephala 12% in autumn respectively (Heba *et al.*, 2012).

Seasonal variations of marine ecosystems, affect the proportion of suspended and dissolved material and the physical-chemical characteristics of the water bodies (Affonso *et al.*, 2011). Therefore, it was important to determine the roles of different parasite species within a community, if their infestations levels were constant and if environmental variations caused by the seasonal dry or rainy cycle influences the availability of intermediate host and can lead to consequent alterations in the recruitment processes of parasites species. This cycle also affects the feeding behavior of hosts modified the exposure to certain parasite species, and it may lead to changes in the community structure of parasites from the host fishes (Violante- Gonzalez *et al.*, 2008).

In this pathological study revealed that, the structural abnormalities such as disruption of infected intestine cells, degeneration, necrosis and also damage of blood vessels observed in the infested region. The longer praesoma of acanthocephala penetrate the wall and often enter the peritoneal cavity where it is encapsulated by proliferated connective tissue. The deposition of hemosiderin pigments in both healthy and diseased specimens of *Catla* may probably have some relevance with the poor gonadal maturation of the fish. Haemosiderosis occurs in the form of brown intracellular granules which gives an intensely positive Prussian blue reaction (Bamidele, 2007). Similarly, in the present study also exhibited, the numbers of goblet cells were found to be increased in affected region, and increased mucus secretion was observed on the intestine surface. Accumulation of lymphocytes and the presence of large number of granulocytes as well as fibroblasts suggest inflammatory responses.

Acid phosphatase is a lysosomal enzyme that plays an important role in the physiology of the intestine. The increase of the activity of this enzyme may be related to autolysis of any foreign substances and microbial agents. Similar observations have been reported in the intestine of birds and rodents with a trematode infection (Bassiouni *et al.*, 1985 and Abo-Shafey, 1992). The role of alkaline phosphatase in transport of glucose 6- phosphate from the intestinal lumen, the moderate decrease in the level of this enzyme in the infected ileum, especially in the villi, may be explained by the destruction of the intestinal epithelium and altering of its absorption power. Similar observations have been reported in the intestine of mice with a trematode infection (Hamdy and Saleh 1983).

CONCLUSION

The prevalence and intensity of acanthocephala infestation were observed in marine fishes in consonance with the various findings and can be linked with many ecological factors. Many acanthocephala were responsible for high mortality rate, particularly in younger stages. The severe pathological changes caused by the acanthocephalan have totally destroyed the architecture of the intestinal tissues. The damage was based on the parasitic proboscis' length, hooks' length and parasitizing durations. Therefore, in recent years there has been recognition that sub-lethal physiological effects on hosts which may lead to alterations in the behavior of infected fishes may also play an important role in regulating populations through demographic effects of marine fishes in Tamil Nadu coast.

ACKNOWLEDGMENT

Authors would like to acknowledge the Ministry of Human Resource Development. Government of India, New Delhi for the financial assist (Grant No. G4 (1)/1630/2013). We are also thankful to the fisherwomen and fishermen on the Tamil Nadu coast for the supply of host fishes both on landing and also in the market.

REFERENCE

Abo-Shafey A.E. Masoud A.A**.** Kelada E.F**.** Khalil A.I**.** Tosson E.M. 1992. Histological changes in the small intestine of rats and mice experimentally infected with *Cynodiplostomum azimi* (Trematoda). Bull. Fac. Sci. Zagazig Univ. 14: 375-393.

Affonso A.G. Barbosa C. Novo E.M.L.M. 2011. Water quality changes in floodplain lakes due to the Amazonas River flood pulse: Lago Grande de Curuaí (Para). Brazilian Journal of Biology. 71: 601–610.

Akinsanya Bamidele. 2007. Histopathological study on the parasitised visceral organs of some fishes of Lekki Lagon, Lagos, Nigeria. Department of Zoology, University of Lagos, Akoka, Yaba, Lagos, Nigeria.

Amin O.M. 2013. Classification of the Acanthocephala. Fol. Parasitol. 60: 273-305.

Amin. O.M. 198. Classification of the Acanthocephala. Cambridge University Press, London and New York. 27–72.

Bassionui G.A. Nada S. M El-Boulagi H.A Ali A.A El Naggar S.M. 1985. Histochemical studies in experimental Heterophyasis. *J. Egpt. Soc. Parasitol.* 15: 109-117.

Chaicharn A. Bullock W. L. 1967. The histopathology of acanthocephala infections in suckers with observations on the intestinal histology of two species of catostomid fishes, *Acta zoological* 48: 19-42.

Dezfuli B.S. Giari L. E.Simoni G. Bosi Manera M. 2002b. Histopathology, immunohistochemistry and ultrastructure of the intestine of *Leuciscuscephalus* (L.) naturally infected with *Pomphorhynchus laevis* (Acanthocephala). Journal of Fish Diseases. 25: 7-14.

Dezfuli B.S. Giari L. S. Arrighi C, Domeneghini Bosi G. 2003b. Influence of enteric helminths on the distribution of intestinal endocrine cells belonging to the diffuse endocrine system in Salmotrutta (L.). *Journal of Fish Diseases*. 26: 155-166.

Dezfuli B.S. Pironi F. Giari L. Domeneghini C. Bosi G. 2002a. Effect of *Pomphorhynchus laevis* (Acanthocephala) on putative neuromodulators in the intestine of naturally infected Salmotrutta. *Diseases of Aquatic Organisms*. 51: 27-35.

Feist S.W. Longsha M. 2008 Histopathology of fish parasite infections importance for populations. *J. Fish Biol.* 73: 2143–2160.

Hamdy B.H. Salh N.A. 1983. Histochmical studies of alkaline phosphatase in different hamster tissues with *Schistosoma mansoni. J. Egypt. Soc. Parasitol*. 13: 419-498.

Heba I. Abdel-Mawla Walaa T. El-Ekiaby. 2012. Some studies on parasitic infection among Morone labrax (Seabass fish) as bio-indicator of environmental conditions. *Egyptian Journal for Aquaculture.* 3: 28-36.

Janovy J. 2002: Concurrent infections and the community ecology of helminth parasites. *Journal of Parasitology.* 88: 440–445.

Kienan J. A. 2002. Histological and histochemical methods, theory and practice. Churchill Livingston, Edinburgh, U.K.

Lambrechts L. Chavatte J.M. Snounou G. Koella J.C. 2006. Environ- mental influence on the genetic basis of mosquito resistance to malaria parasites. *Proceedings of the Royal Society of London. Series B, Biological Sciences.* 273: 1501–1506.

Malaty Hoda M. 2007. Epidemiology of Helicobacter pylori infection." Best Practice & Research Clinical Gastroenterology. 2: 205-214.

Margolis K. L. Bonds D. E. Rodabough, R. J. Tinker L. Phillips L. S. Allen C. Howard B. V. 2004: Effect of estrogen plus progestin on the incidence of diabetes in postmenopausal women: results from the Women’s Health Initiative Hormone Trial. *Diabetologia*. 47:1175-1187.

Migliaccio A. R. Rana. R. A. Sanchez M. Lorenzini R. Centurione L. Bianchi L. 2003. GATA-1 as a regulator of mast cell differentiation revealed by the phenotype of the GATA1 low mouse mutant. *J Exp Med*. 197: 281–96.

Rohade K. 1967. Ecology of marine parasite, an introduction to marine parasitology. 2nd ed. Trowbridge, UK: Redwood Books.

Sakthivel A. Selvakumar P. Gopalakrishnan A. 2014. Acanthocephalan (*Echinorhynchus* sp.) infection of Yellowfin tuna (*Thunnus albacares*) from Nagapattinam, south east coast of India. *Journal of Coastal Life Medicine*. 2: 596-600.

Sakthivel A. Selvakumar P. Gopalakrishnan A. 2016. Acanthocephala (*Acanthocephalus lucii*) infection on *Caranx ignobilis* from Nagapattinam, south east coast of India. *Indian Journal of Geo Marine Sciences*. 45: 448-452.

Sakthivel A. Selvakumar P. Gopalakrishnan A. 2016. Morphological and histophathological studies of *Acanthocephalus dirus* infection in internal organ of *Caranx ignobilis*. *Indian Journal of Geo Marine Sciences*. 45: 453-458.

Sakthivel A. Selvakumar P. Gopalakrishnan A. 2016. Pathological manifestation of the *Acanthocephalus dirus* in *Thunnus albacores*. *Asian Pac J Trop Dis*. 6, 350-353.

Sharkey K.A 1992: Substance and calcitonin gene-related peptide (CGRP) in gastrointestinal inflammation. Annals of the New York Academy of Sciences. 664: 425–442.

Shulman S.S 1950. Parasites of fishes in the aquatories of the catvian republic. (abstract of thesis) *Trud. gelmint. Lab.* 110: 127-135.

Violante-González J. Rojas-Herrera A. Aguirre-Macedo, M.L. 2008. Seasonal patterns in metazoan parasite community of the “fat sleeper” *Dormitator latifrons* (Pisces: Eleotridae) from Tres Palos Lagoon, Guerrero, Mexico. *Revistade Biologia Tropical*. 56: 1419-1427.

Wang G. T. Yao W. J. Nie P 2001. Seasonal occurrence of *Dollfustrema vaneyi* (Digenea: Bucephalidae) metaceraria in the bull head cat fish *Pseudobagnus fluvidraeo* in a reservoir in china. *Diseases of Aquatic Organisms.* 44: 127-131.

Weijie Dong. Yu-ki Matsuno. Akihiko Kameyama. 2012. A Procedure for Alcian Blue Staining of Mucins on Polyvinylidene Difluoride Membranes. *Anal. Chem.* 84, 8461–8466.

World Register of Marine Species (WoRMS) 2011. http://www.marinespecies.org

Young B. Heath J. W. 2000. Wheater’s functional histology: a text and colour atlas, 4th edn. Churchill Livingstone, Philadelphia.

Zdzislaw Laskowski Z. Zdzitowieck K. 2009. Occurrence of acanthocephalans in notothenioid fishes in the Beagle Channel (Magellanic subregion, sub Antarctic). *Polish Polar Research*. 30: 179–186.