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Note

Suppression of ovarian development in freshwater fishes due to endo-helminthic infection

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ABSTRACT

A study was undertaken to investigate suppression of ovarian development caused by endohelminthic parasites in three species of freshwater fishes *viz*., *Channa punctata, Nandus nandus* and *Xenentodon cancila*. Ovaries of host fishes were primarily found infected with encapsulated larvae, indirectly affecting ovarian development. Lower gonadosomatic index and fecundity were recorded in infected fishes compared to uninfected ones. The study also assessed histopathological changes caused by the parasites, which revealed damaged and disrupted germinal epithelium, damaged oocytes showing signs of necrosis, liquification of yolk globules and reduction in yolk formation in infected ovaries.

Keywords: Endo-helminth infection, Fecundity, Fishes, Histopathology, Ovarian development, Parasites

Fish parasites form one of the major problems confronting fish culturist, and pathological conditions arising from parasite infection assume high magnitude of losses especially, under crowded conditions and in intensive fish farming systems (van den Broek, 1979). Parasites can act as one of the factors regulating the host populations by affecting their survival and reproduction. According to Snieszko (1975) and Daniel (1978), under natural conditions, 50-90% of freshwater fishes harbour at least one species of parasite. Parasitism is much more common and diversified in the wild than in the farms, ponds and hatcheries. Parasites cause deleterious effects on fish health because they occur in vital organs such as liver, kidney, and gonads affecting their function. Parasitic infestation tends to decrease growth, resulting in stunting of fish (Cross, 1933). Generally, the intensity of parasite infection negatively and proportionally affects the reproductive potential by decreasing the fecundity and gonadosomatic index (GSI) of the host fish. Parasites extract energy and nutrients from the hosts, and induces physiological, immunological as well as ethological changes in the host, which may impair mating, gonadal maturation and fry survival.

A heavy worm burden may reduce the host's reproductive potential or may delay sexual maturity in the fish, and both these factors could limit the host population size (van Duijn, 1956). Worm infections differ radically from bacterial or protozoan infections, as in most cases, the worms do not multiply in the host body and the infections are chronic in nature. Chronicity of helminths infestation in fishes may affect the host's reproductive potential and development. The present investigation was undertaken to study ovarian development in freshwater fishes naturally infected with endo-helminth parasites.

Live fishes were collected during the period from April, 2010 to March, 2011 from the Lower Lake, Bhopal and also from a local fish market in Bhopal and brought to the laboratory. The fishes were examined for occurrence of helminth parasites adopting the methods employed by Mayer and Olsen (1975) and Madhavi *et al*. (2007). The fish specimens were dissected out in physiological saline (0.75% NaCl solution) for collecting endo-helminth parasites including digenetic trematodes and nematodes. Encysted trematodes were collected from the gonads and body cavity, fixed in hot alcohol-formol-acetate (AFA) solution, and stained with aceto-carmine to prepare permanent mounts. The parasites were identified according to the keys given by Yamaguti (1958) and Gibson *et al*. (2002). The collected nematodes were washed thoroughly in normal saline, killed and fixed

in hot 70% alcohol, stored in glycerin alcohol (1:3), and studied as wet mounts or temporary mounts in glycerin (Meyer and Olsen, 1975). Taxonomical identification of nematode parasites was done by adopting the method outlined by Yamaguti (1961) and Anderson *et al.* (1974-1983).

Gonad weight of infected and uninfected females were measured and GSI was calculated according to the formula given by Dahlgren (1979) as:

GSI (%) = Weight of gonad/Total body weight ×100

Fecundity was estimated by mature ova counting method adopting the formula outlined by Sinha (1975):

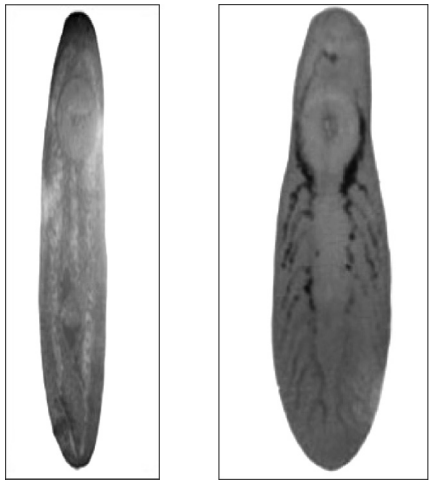
Fecundity (F) = W × n/w

where, W = total weight of ovary and w and n are the mean weight and mean ova counts of the subsamples respectively.

The infected ovaries of host fishes were taken out and fixed in alcoholic Bouin's fluid for 24 h. After the complete removal of picric acid, the tissues were dehydrated, cleared in xylene and processed for preparation of paraffin wax blocks. The blocks were cut at 4 - 5 µm thickness using a rotatory microtome and stained with haematoxylin and eosin (H & E) for histopathological examination (Luna, 1968). Stained histopathological sections were examined under Olympus research microscope and photomicrographs were taken.

The prevalence and intensity of parasite infestation was analysed adopting the method as described by Margolis *et al.* (1982) as given below:

Prevalence = Total no. of hosts infected/Total no. of hosts examined ×100



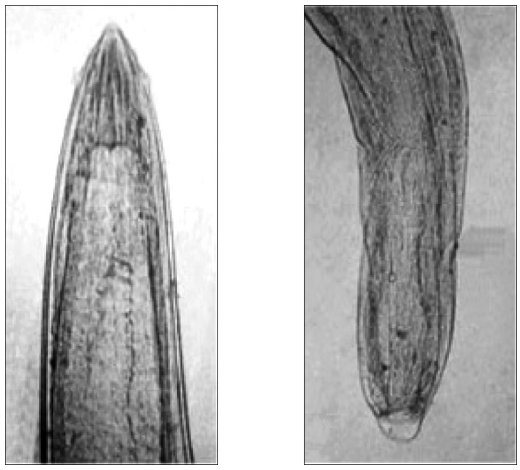
(a) (b)

Fig. 1. Metacercariae of (a): *Clinostomum complanatum,* (b): *Euclinostomum heterostomum* (X40)

Mean intensity = Total no. of parasites/Total no. of infected hosts examined

The data were subjected to one way ANOVA using SPSS program (version 11.5).

During the present investigation, 175 specimens of *Nandus nandus* were examined out of which, 75 were found infected by metacercariae of the digenetic trematode *Clinostomum complanatum* (Fig. 1a). The body of the fluke was elongated with rounded ends measuring 5.13-8.40 mm. The prevalence rate recorded was 42.8% with a mean intensity of 2.13. A total of 115 specimens of *Channa punctata*, out of 175 examined) were found infected by metacercariae of *Euclinostomum heterostomum* (Fig. 1b). The encysted metacercariae of *E. heterostomum* was linguiform having both ends bluntly rounded with distinct number of intestinal caecae and measured 3.92–8.24 mm. The total prevalence of metacercarial



(a) (b)

Fig. 2. *Eustrongylides* sp. larvae. (a): Anterior end, (b): Posterior end (X40)

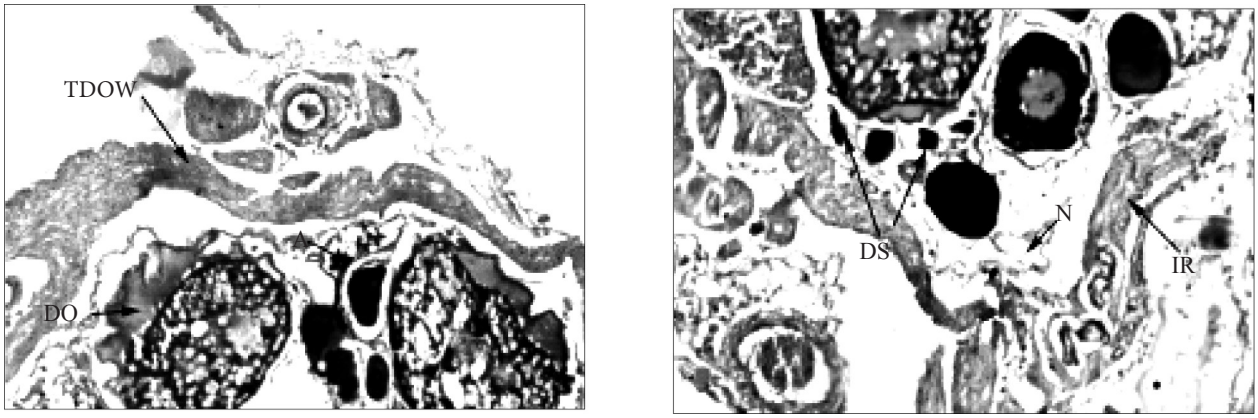
infection was calculated to be 65.7% with a mean intensity of 15.4. One hundred and three specimens of *Xenentodon cancila* were examined, out of which 42 were found infected with *Eustrongylides* sp. larvae (Fig. 2a and b). The larvae were bright red colored, measuring 30–55.36 mm and showed a total prevalence of 40.7% with a mean intensity of 2.33

Histopathologically the ovaries of *N. nandus* exhibited thickening and damage in the walls due to presence of *C. complanatum* metacercariae. Pathological effects included irregular shape of oocytes, necrosis and severe inflammatory reaction (Fig. 3a and b). Quantitative analysis showed a significant ($p<0.05$) change in the gonadosomatic index (GSI = 0.36±0.16)

and fecundity (726.4±17.4) of infected ovaries of *N. nandus* as compared to uninfected ovaries of the same fish (GSI = 7.26±3.31 and fecundity = 1485.8±348.2). Ovaries of infected *C. punctata,* exhibited a strong inflammatory effect histologically, due to *E. heterostomum* metacercaria. The ovigerous lamellae and follicular lining were freely floating near the oocytes. The developing oocytes were represented only by primary oocytes. Pre-vitellogenic and vitellogenic oocytes were completely absent, which may lead to decrease in the vitellogenic activity and yolk formation (Fig. 4a, b and c). Quantitative analysis showed a significant ($p<0.05$) change in the GSI (1.14±0.39) and fecundity (1250.98±2.65) of infected ovaries as compared to the uninfected ovaries (GSI = 2.31±0.265 and fecundity 3305.0±4.63)

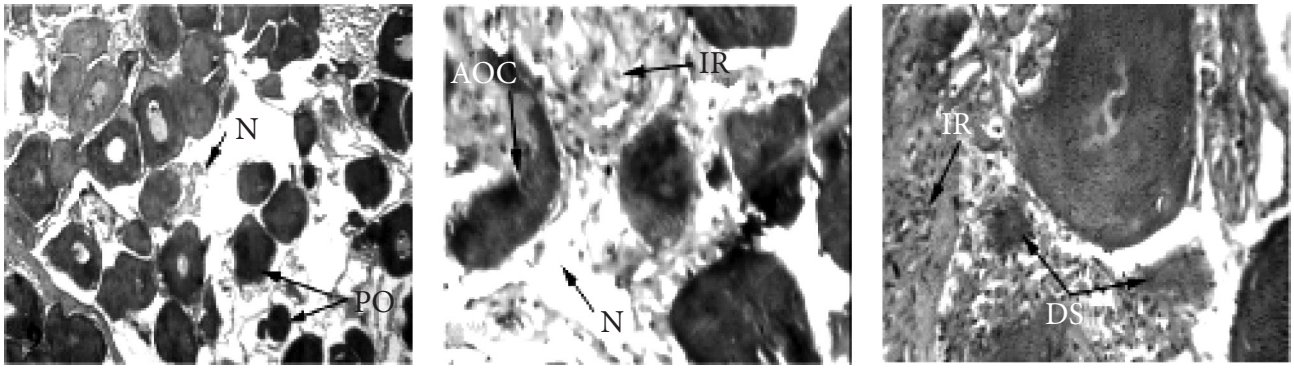
In cross section of the ovary of infected *X. cancila,* most of the oocytes were found irregularly shaped and showed necrosis (Fig. 5a). Lumen of the ovary was filled with sections of parasite showing strong inflammatory reaction and liquification of yolk globules resulting in reduction of ova formation (Fig. 5b). Quantitative analysis showed a significant ($p<0.05$) change in the GSI (6.52±0.87) and fecundity (146.36±29.58 of infected ovaries as compared to uninfected ovaries (GSI = 11.84±1.53 and fecundity = 209.6±23.68).

Histopathological examination of ovaries of infected fishes collectively exhibited thickening and damage in the wall of ovary and severe inflammatory reaction as also recorded and reported by Blazer (2002). It was also observed that the gonadosomatic index and fecundity of infected *N. nandus* decreased significantly as compared to uninfected fishes. The present finding is in agreement with those of Ramachandran (1975), Oliva *et al*. (1992), Hesp *et al.* (2002), Moravec *et al*. (1997; 2002) and Heins and Baker (2003). Clarke *et al.* (2006) studied prevalence, intensity and the effect of the nematode parasite, *Philometra saltatrix* in the ovaries of *Pomatomus saltatrix* and stated that infection was associated with a range



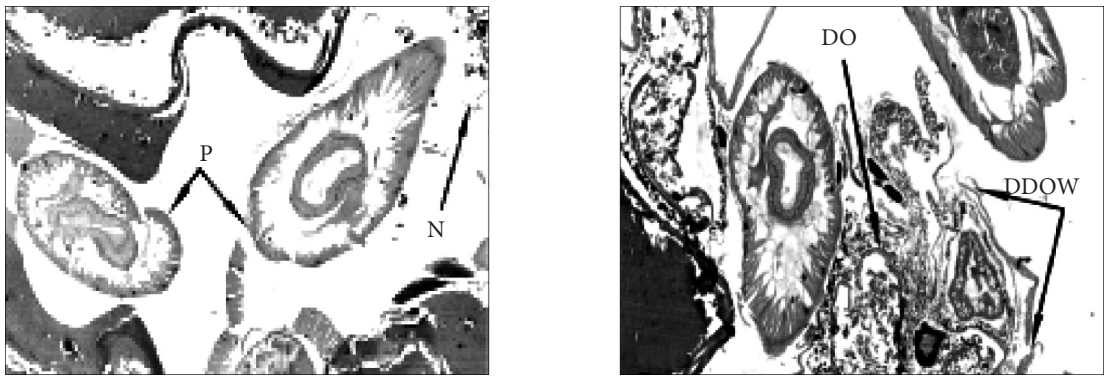
(a) (b)

Fig. 3. Photomicrograph of the cross section of ovary of *Nandus nandus* infected by *C. complanatum*. (a): Thickened and disrupted ovarian wall (TDOW), deshaped oocytes (DO) and atrophy (A) (H&E; X100). (b): Necrosis (N), inflammatory reaction (IR) and damaged stroma (DS) (H&E; X100)



(a) (b) (c)

Fig. 4. Cross section of ovary of *C. punctata* infected by *E. heterostomum*. (a): Necrosis (N) and primary oocyte (PO, (H&E;X40). (b): Inflammatory response (IR), necrosis (N) and atrophied oocytes (AOC) (H&E; X100). (c): Damaged stroma (DS) and inflammatory reaction (IR) (H&E; X400)



(a) (b)

Fig. 5. Histological section of infected ovary of *X. cancila.* (a): Necrosis (N) and several transverse sections of parasite (b): Damaged and disrupted ovary wall (DDOW) and damaged oocytes (DO) (H&E; X100).

of disorders *viz*., hemorrhage, inflammation, edema, pre-necrotic and necrotic changes and follicular atresia, which resulted in poor development of oocytes and affected fecundity as also observed under present investigation. During the current study, infected ovaries of *C. punctata* exhibited necrosis and decrease in the yolk formation due to disappearance of vitellogenic oocytes. It was observed that the gonado-somatic index and fecundity of infected *C. punctata* decreased significantly as compared to uninfected ones. The present finding is in agreement with that of Heins and Baker (2003) who found that mean egg size in three spined stickleback (*Gasterosteus aculeatus*) were smaller in fish infected with the cestode, *Schistocephalus solidus* than in uninfected fish. In case of *X. cancila,* infestation with nematode parasite also showed a significant decrease in the gonadosomatic index and fecundity. Oliva *et al.* (1992) suggested that philometrid infection resulted in reduced fecundity in Chilean seabass (*Paralabrax humeralis*), as the effective volume of the ovaries was reduced. Necrosis was described in the ovaries of striped mullet (*Mugil cephalus*) due to infection with *Philometra cephalus* (Ramachandran, 1975). Atrophy in the ovaries of New Zealand snapper (*Chrysophrys auratus*) due to infection with *Philometra* sp. was described by Hine and Anderson (1981). *Philometra* induced ovary damage have also been reported in various reef fishes (Moravec *et al*., 1997; Hesp *et al*., 2002; Moravec *et al.,* 2002).

Prevalence of parasitic loads causes chronic infection in fishes which may lead to suppression of reproductive potential, immunity and health of brood fishes which in turn can affect production of fry and their development. The information reported in the present study may help control parasitic infections in fish.

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References

Anderson, R. C., Chabaud, A. G. and Willmott, S. 1974-1983. CIH Keys to the Nematode Parasites of Vertebrates, Nos. 1-10. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, UK.

Blazer, V. S. 2002. Histopathological assessment of gonadal tissue in wild fishes. *Fish Physiol. Biochem.*, 26: 85-101.

Clarke, L. M., Dove, A. D. M. and Conover, D. O. 2006. Prevalence, intensity, and effect of a nematode (*Philometra saltatrix)* in the ovaries of bluefish (*Pomatomus saltatrix)*. *Fish. Bull.,* 104: 118-124.

Cross, S. X. 1933. Some host parasite relationship of the Trout lake region of Northern Wisconsin. *J. Parasit.,* 20: 132-133.

Dahlgren, B. T. 1979. Effects of population density on fecundity and fertility in the guppy, *Poecilia reticulata* (Peters). *J. Fish Biol.,* 15: 71-91.

Daniel, W. C. 1978. Sublethal effects of three ectoparasites on fish. *J. Fish Biol.,* 7(3): 283-294.

Gibson, D. I., Jones, A. and Bray, R. A. 2002. *Keys to the trematoda,* vol. 1. CABI publishing and Natural History Museum, London, UK, 521 pp.

Heins, D. C. and Baker, J. A. 2003. Reduction of egg size in natural populations of three spined stickleback infected with a cestode macroparasite. *J. Parasitol.*, 89: 1-6.

Hesp, S. A., Hobbs, R. P. and Potter, I. C. 2002. Infection of the gonads of *Glaucosoma hebraicum* by the nematode *Philometra lateolabracis*: occurrence and host response. *J. Fish Biol.,* 60: 663- 673.

Hine, P. M. and Anderson, C. D. 1981. Diseases of the gonads and kidneys of New Zealand snapper, *Chrysophrys auratus* Forster (F. Sparidae), In: Fowler, M. E. (Ed.), *Wildlife diseases of the Pacific Basin and other countries*, Academic Press, London, p. 166-170.

Luna, L. G. 1968. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*, 3rd edn. McGraw-Hill, New York, 258 pp.

Madhavi, R., Vijayalakshmi, C. and Shyamasundari, K. 2007. *Collection, staining and identification of different helminth parasites: A manual of the Workshop on fish parasites - taxonomy capacity building*. Andhra University Press, India, p. 65-74.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M. and Shad, G. A. 1982. The use of ecological terms in parasitology. Report of an Ad Hoc Committee of American Society of Parasitology. *J. Parasitol.*, 68: 131-133.

Meyer, M. C. and Olsen, O. W. 1975. *Essentials of parasitology*, 2nd edn. Wm. C. Brown Co., USA, 303 pp.

Moravec, F., Ogawa, K., Suzuki, M., Miyazaki, K. and Donai, H. 2002. On two species of *Philometra* (Nematoda: Philometridae) from the serranid fish *Epinephelus septemfasciatus* in Japan. *Acta Parasitologica*, 47: 34-40.

Moravec, F., Vidal-Martinez, V. M., Vargas-Vazquez, J., Vivas-Rodriguez, C., Gonzalez-Solis, D., Mendoza-Franco, E., Sima-Alvarez, R. and Guemez-Ricalde, J. 1997. Helminth parasites of *Epinephelus morio* (Pisces: Serranidae) of the Yucatan peninsula, South-eastern Mexico. *Folia parasitologica*, 44: 255-266.

Oliva, M. E., Borqueez, A. S. and Olivares, A. N. 1992. Sexual status of *Paralabrax humeralis* (Serranidae) and infection by *Philometra* sp. (Nematoda: Dracunculoidea). *J. Fish Biol.*, 40: 979-980.

Ramachandran, P. 1975. *Philometra cephalus* sp. infecting the gonads of the striped mullet, *Mugil cephalus* L. from the Arabian coast of Kerala, India, with a note on its pathology. *Zool. Anz.,* 19: 140-144.

Snieszko, S. F. 1974. Effects of environmental stress on outbreak of infectious diseases of fishes**.** *J. Fish Biol.,* 6: 197-208.

Sinha, M. 1975. Observation on the biology of *Puntius sarana* (Ham.) of Loni Reservoir (MP). *J. Inland Fish. Soc.*,7: 49-57.

Van Duijn, C. 1956. *Diseases of fishes*. Water life, Dorset House, London, 174 pp.

Van den Broek, W. L. F. 1979. Copepod ectoparasites of *Merlangus merlangus* (L) and *Platichthys flesus* (L). *J. Fish Biol.,* 14(4): 371-380.

Yamaguti, S. 1958. *Systema helminthum*, *vol. I: the digenetic trematodes of vertebrates*. Inter Science Publishers, New York and London, 1575 pp.

Yamaguti, S. 1961. *Systema helminthum III; The nematodes of vertebrates*. Inter Science Publishers, New York and London, 1261 pp.

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