Asian Pacific Journal of Tropical Disease

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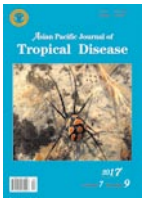

Original article https://doi.org/10.12980/apjtd.7.2017D6-346

Occurrence and consequence of the parasitic nematode *Philometra piscaria* (Moravec & Justine, 2014) on *Epinephelus coioides* (Hamilton, 1822) from the southeast coast of India

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ARTICLE INFO

Article history:
Received 8 Oct 2016
Received in revised form 12 Dec, 2nd revised form 19 Dec 2016, 3rd revised form 19 Jan, 4th revised form 26 Mar 2017
Accepted 25 May 2017
Available online 28 Aug 2017

Keywords:
Nematode
Philometra piscaria
Epinephelus coioides
Ovary infection
Histopathology analyze

ABSTRACT

Objective: To investigate the occurrence, seasonal prevalence and histopathological effect of the *Philometra piscaria* (*P. piscaria*) infestation in ovaries of orange-spotted grouper *Epinephelus coioides* (*E. coioides*) from the southeast coast of India.

Methods: The normal and infected gonads of orange-spotted grouper were collected from Parangipettai and Pazhayar landing center. Prevalence, mean intensity of nematode infection and percentage of gonadal somatic index (%GSI) of normal and infected gonads was examined. The histopathological abnormalities of nematode infected gonads were studied.

Results: In this study, among 452 fishes examined, 200 were infected with the nematode. The maximum prevalence (53% and 65%) of *P. piscaria* on *E. coioides* was observed in post-monsoon season 2015 from Parangipettai and Pazhayar landing center respectively. The mean intensity of nematode infection was found higher in post-monsoon season (4.62 ± 0.40) and average intensity of nematode infections was (2.42 ± 0.60). Values of %GSI showed increasing trend from April with a peak in June in both infected and uninfected fishes. The histopathological studies of infected fish gonads revealed tiny ovary wall with ruptured oocytes, necrosis of gonad cells and nuclei scattered in early and late yolk stage.

Conclusions: The varying degrees of gonadal damage caused by *P. piscaria* reveal a severe threat to the reproductive success of *E. coioides*.

1. Introduction

Nematodes of the family Philometridae Costa, 1845 has a large number of species parasitizing off the abdominal cavity of brackish water, freshwater, and marine fishes. Most species are known only from the large-sized gravid females[1]. During recent years increasing attention has been paid to studies on the Philometrids nematodes parasitizing fish ovary. High infections by these nematode parasites on both wild and cultured fishes have been recorded in many economically important species of Perciforms[2-4]. The large sized females of these nematodes are usually highly pathogenic, causing serious damages to the fish ovaries and affecting fish reproduction, which were recorded by Moravec and Bakenhaster[5], Moravec *et al*.[6], and Moravec and Justine[7]. Parasitic species in the fish ovary is lethargically distributed to our knowledge mainly in the subtropical and tropical regions of the Indo-Pacific and so far it has been reported from the Indian Ocean. Philometrids parasites in the ovary of fish hosts may cause serious damage by sucking blood, causing atrophy of developing ova in the ovary, fibrosis of ovarian tissue, increased granulocytes and hemorrhages, thus negatively affects the reproduction of some species of marine fishes[8].

Parasites of fish constitute one of the major problems confronting the modern fish culturist and pathological conditions arising from parasites infection, assuming a high magnitude especially under crowded conditions[9]. Parasites can affect various fish organs either directly or indirectly, depending on the target organ. Generally, the parasites negatively affect the reproductive effort with the decrease of its fecundity being proportional to the intensity of infection. The parasite absorbs energy and nutrients from the hosts, which are not destined to reproductive effort and the parasite induces physiological, immunological or pathological changes in the host and which also impair mating, gonad maturation or larval

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Foundation Project: Supported by Department of Science and Technology, Govt. of India (SB/SO/AS/063/2014).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

survival[10]. Nematode 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 nematode infestation in fishes may affect the host's reproductive potential and development[9]. But such studies are not conducted in *Epinephelus coioides* (*E. coioides*) fishes in Indian coastal environment. The present investigation was undertaken to report the occurrence, prevalence and intensity of gonad infecting parasitic nematode *Philometra piscaria* (*P. piscaria*) and the consequence of parasitic infection on reproductive capability of orange-spotted grouper *E. coioides* from the southeast coast of India.

2. Materials and methods

A total of 452 *E. coioides* specimens were collected from the Parangipettai (11°29' N, 79°46' E) and Pazhayar (11°21' N, 79°50' E) landing centre's in southeastern coast of India and were examined for nematode parasites during January 2015 to December 2015. All the fishes were subjected to morphometric analysis and infections were observed in the ovaries. In order to collect the parasites, the body cavity of fish was opened and the ovaries were dissected in the region of the anus. The ovaries of each fish were carefully removed from dissected fishes and the parasite infestation was analyzed adopting the method as described by Moravec and de Buron[1]. The nematodes were carefully removed and washed with a physiological saline solution. For light microscopy examination, the nematodes were cleaned with glycerin and fixed in a solution of 95% glacial acetic acid and 5% formalin. The parasites were identified according to the keys given by Moravec and Justine[7]. The percentage of gonadal somatic index (%GSI) was calculated as described by Ferrer-Maza *et al.*[11]. The gonads were removed from *E. coioides* and processed for histological examination using standard methods. Thin sections (3–5 μm) were obtained using a rotary microtome and stained with hematoxylin and eosin. Stained sections were analyzed under Olympus research microscope and photomicrographs were taken.

3. Results

In the present study, infected orange spotted grouper *E. coioides* collected from Parangipettai and Pazhaiyar fish landing centre's were examined for the ovarian nematode parasite infection. The prevalence and intensity of infection, %GSI and histopathological abnormalities due to an infestation of fish *P. piscaria* were investigated. Normal infected and uninfected fish ovaries were compared to find out the rate of infection (Figure 1). Table 1 shows that the average length and weight of fishes was (241 ± 67) mm and (613 ± 92) g, respectively and the average length of *P. piscaria* was (157 ± 26) mm recorded in the study.

3.1. Prevalence and intensity

A total of 452 *E. coioides* fishes were examined from the southeast coast of India, and 200 out of the 452 fishes were infected. The overall prevalence of *P. piscaria* parasite in *E. coioides* was 44%. In Parangipettai, the highest prevalence occurred in post-monsoon season (53%) and lowest in pre-monsoon (38%). Although, the prevalence in monsoon season (24%) was lower than in post-monsoon season (65%) at Pazhayar (Figure 2). A total number of 341 parasitic *P. piscaria* collected recovered from 200 infected *E. coioides* fishes, while parasitic intensity was higher in post-monsoon season (4.62 ± 0.40) and the intensity of infection was found lower (1.08 ± 0.40) in pre-monsoon at Pazhayar. The average intensity of nematode infection in both landing centres was (2.42 ± 0.60) (Figure 2). From the above data, it can be assumed that the prevalence of infection and intensity of the nematode infection is maximum in post-monsoon.

Table 1
Total length and body weight of *E. coioides* and the length of attached nematode *P. piscaria*.

S. No.	Total length (mm)	Body weight (g)	Worm length (mm)
1	252	485	136
2	336	506	142
3	452	708	154
4	398	555	185
5	299	490	166
6	468	776	148
7	324	600	156
8	372	636	166
9	412	712	143
10	266	478	181
11	252	458	176
12	304	672	132
13	405	796	160
14	392	696	171
15	368	612	182
16	292	518	154
17	390	699	139
18	367	596	148
19	408	701	156
20	303	577	148
Average	241.3	613.55	157.15

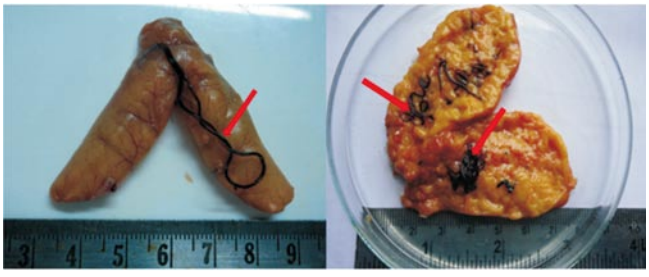

Figure 1. *E. coioides* ovary showing heavy infection of (*P. piscaria*) parasite.

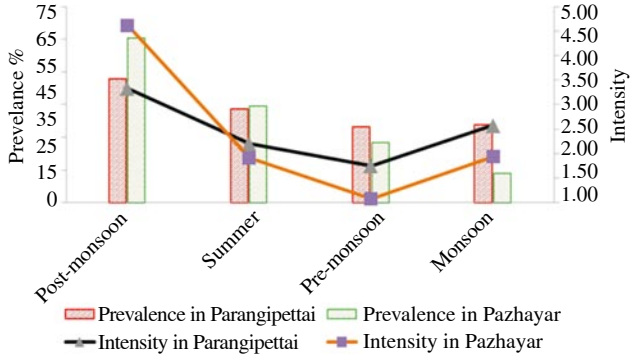

Figure 2. Prevalence and mean intensity of *P. piscaria* on *E. coioides* in relation to different season.

3.2. Percentage of GSI

The %GSI values of infected and uninfected females of *E. coioides* are plotted graphically in Figure 3. Values of %GSI showed an increasing trend from April with a peak in June. It declined from July onwards in both infected and uninfected females. The %GSI of uninfected fishes varied from 1.18 (September) to 6.14 (June) while the %GSI of infected fishes varied from 1.84 (January) to 5.97 (June). The higher %GSI values in June suggested that this species breeds once in a year with high spawning activity in that month. %GSI of infected fishes was considerably lower than the uninfected fishes.

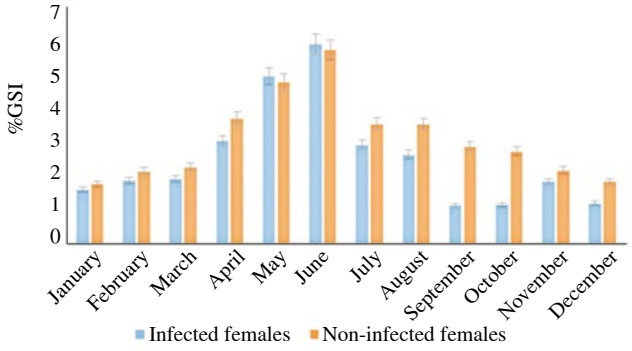

Figure 3. %GSI of infected and uninfected of *E. coioides*.

3.3. Histopathology

Ovaries of uninfected fishes showed a normal histological structure with a number of oocytes showing various stages of maturation. Histopathological studies of infected ovary showed that ovary wall became thin and ruptured oocytes, immature and degeneration of egg envelope were observed and oocytes also showed shrinkage, atretic follicles appeared, nuclei scattered in early and late yolk stage. Ovary revealed a severe reduction in size with the tiny wall; oocytes got detached to their envelope (Figure 4). Infected ovaries were also characterized by increased amount of necrosis and decreased in the formation of atretic follicles during the reproductive period.

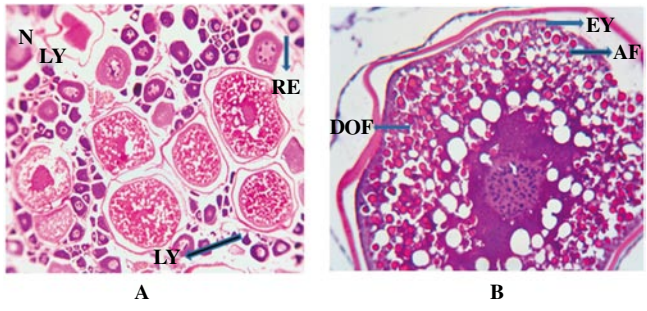

Figure 4. The cross section of *P. piscaris* infected overy.
(A): LY: Late Yolk stage; RE: Ruptured ovarian follicle; N: Necrosis; 100×. (B): EY: Early Yolk stage; AF: Atretic follicle; DOF: Disruptive ovarian follicle; 400×.

4. Discussion

Along the southeast coast of India, grouper fishes seem to be highly infected with the ovarian Philometrids nematodes[3]. Even though many studies provide descriptions of several Philometrids species, the very limited report exists about the occurrence and prevalence or effect of these nematodes. An earlier study shows that the average length and weight of *Epinephelus malabaricus* fishes (332 mm and 513 g) were reported. In earlier findings, the length of *Philometra* sp. was measured as 162.35 mm[3,4]. In this study there is a slight variation that is noted in the aspects of length and weight relationships of the host. The present study showed that the incidences increased mainly in post-monsoon season because this season has favorable environmental conditions. Likewise, the incidences were found decreased in summer season, which may be due to unfavorable environmental conditions. Qasim and Ayub[12] correlated the prevalence of nematode parasites to the summer season in various edible marine fishes of Karachi coast. Selvakumar *et al.*[8] stated maximum infection rate of nematodes (*Philometra* sp.) in August and minimum in June in the fish, *Otolithes ruber*. Seasonal variation in the prevalence of nematode parasitism has been recorded in two Serranidae species, *Epinephelus aeneus* and *Epinephelus marginatus* from Iskenderun Bay, Turkey[13]. Khanum *et al.*[14] reported highest in the rainy season (75%) and lowest infestation was observed in the winter season (31.81%). Seasonal variation of six species of nematode parasites *Porrocaecum trichiuri*, *Pseudoproleptus vestibules* and *Cucullanus cirratus* showed a remarkable fluctuation in *Macrognathus aculeatus*.

The result of the present investigation showed that the average prevalence and intensity of *P. piscaria* in *E. coioides* had 44.24% of infection 2–3 nematode per fish. Moravec *et al.*[6] reported a new gonad-infecting species of *Philometra genypteri* from the red cusk-eel *Genypterus chilensis* off Chile and *Philometra* sp. in *Johnius belengerii* having 48% of infection. The present investigation is similar to the findings of Poulin[15] who reported that the range of prevalence and intensity of *Philometra cylindracea* in *Perca flavescens* had 1%–7.5% of infection and 1 nematode per fish and *Philometroides huronensis* in *Catostomus catostomus* had 2%–10.2% of infection 3 nematodes per fish. Selvakumar *et al.*[8] considered Philometrids parasitizing in the gonads of their fish hosts may cause serious damage to these organs thus negatively affecting the reproduction of some species of marine fishes.

Accordingly, the %GSI analyses of the effects of parasitism on female orange spotted grouper as spawning condition and reproduction were carried out in relation to the status of gonadal maturation. The spawning condition of female bluefish as represented by %GSI did not show any temporal patterns in regards to non-infected versus infected fish. The infected fish had higher %GSI than non-infected fish; this difference is most likely not a functional one but a pathological one has been reported[16]. The present finding is in agreement with that of Heins and Baker[17] who reported that the average size of an egg in three-spined sticklebacks (*Gasterosteus aculeatus*) was smaller in fish infected with the cestode, *Schistocephalus solidus* than in uninfected fish. In the case of *Xenentodon cancila*, infestation with nematode parasite also showed a significant decrease in the %GSI and fecundity. The mass of infected ovaries have not increased by gonadal maturation. The increased gonadal mass is instead due to edema, hemorrhaging, and effect of pathological factors. Studies of the effects of *Philometra* on fish ovaries are uncommon, as are those addressing parasitism of fish gonads in general[16]. Bakenhaster *et al.*[18] reported that nematode

(*Philometra floridensis*) parasite infection resulted in reduced fecundity in *Sciaenops ocellatus* as a result of the effective volume of the ovaries was reduced. Infected ovaries of *Channa punctata* exhibited necrosis and decrease in the yolk formation due to the disappearance of vitellogenic oocytes. In the case of *Xenentodon cancila*, infestation with nematode parasite also showed a significant decrease in the %GSI and fecundity[9]. *Philometra* induced ovary damage has also been reported in various reef fishes[8,19].

Histopathological examination of ovaries of infected fishes collectively exhibited thickening and damage in the wall of the ovary and severe inflammatory reaction as also recorded and reported by Blazer[20]. The current histopathological studies of infected ovary and testes showed several alterations in normal histology of these tissues. Studies on the effect of helminths parasites on fish gonads are meager. The present finding is in agreement with those studies of Selvakumar *et al*.[8], Heins and Baker[17], and Hesp *et al*.[19]. The present observation attributes towards that nematode *P. piscaria* larvae resulted in a decrease in the formation of atretic follicles during reproductive period potential of infected host fish. Bakenhaster *et al*.[18] reported the histopathological changes caused by the *Philometra floridensis* in *Sciaenops ocellatus* which revealed damaged oocytes showing signs of necrosis, damaged and disrupted germinal epithelium, liquefaction of yolk globules and reduction in yolk formation in infected ovaries.

In the present study, the 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 the production of fry and their development. Further studies are needed to show whether certain ecological parameters, such as food or habitat, have an effect on parasitism of marine fishes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We thank Frantisek Moravec for their participation in the nematode identification. Thanks also go the Director of the Faculty of Marine Science, Annamalai University, Parangipettai for their encouragement and provided facilities. This work was financially supported by Department of Science and Technology, Govt. of India (SB/SO/AS/063/2014). We are also thankful to the fisherfolk of southeast coast for the supply of host fishes.

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