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Perilous prevalence of helminth parasites in *Nandus nandus* at Tamluk, Purba Medinipur, West Bengal, India

Biplab Mandal[1], Antara Mahapatra[2*], Priti Ranjan Pahari[2]
[1] Department of Zoology, Vidyasagar University, Midnapore, West Bengal, India
[2] Department of Zoology, Tamralipta Mahavidyalaya, Tamluk, Purba Medinipur, West Bengal, India

Abstract
Helminth parasites like Acanthocephala and Nematode causes a series of problems of health diseases in Gangetic Leaffish (*Nandus nandus*) which invites the reasons of the extinction from the environment. Study revealed that these two helminth parasites, Acanthocephala and Nematode are scattered through out the gut, liver, stomach and intestine of the Nados fishes. Owing to the severe attacks, these two parasites occur the reduction of the weight of each fishes. These fishes showed the appearance of pale, weak and sick which causes the results of decreasing of the reproductive ability. Female fishes are more susceptible to these parasites than the males. These dangerous worms are mostly infectious in summer season followed by rainy and winter. The larger fishes are most susceptible followed by the medium and smaller. Ultimately these two notorious parasites occur the damage of the production quantity as well as the quality of this food fish which welcomes the economic loss of the fish farm.

Keywords: Acanthocephala, Nematode, Infection, Prevalence, Intensity, *Nandus nandus*

Introduction

Thirty thousand fish species reported in the world. Out of this 800 belongs to the Ornamental fishes including the Gangetic Leaffish (*Nandus nandus*). This fish is called in India as a Nados, Nayana, Royna, Abro (Froese and Pauly, 2016) [10]. This fish has a good demand in the aquarium market due to its attractive cryptic colour pattern. This colourful fish is called the "living jewels" of the environment which are enlightented in various parks, temples, corporate houses (Mitra *et al*., 2007) [20]. This Nados fish is highly nutritious. It is a good source of protein (52.5%), fat (2%), carbohydrate (0.21%) and minerals (5.7%) (Ray and Dhar, 2012) [28]. Due to its high nutritive value, the physicians prescribed as a diet to the convalescent patients, pregnant and milching mothers. This fish have high market value (Talwar and Jhingram, 1991 [32], Chakraborty, 2020) [8] and has a great scope for culture to earn more money for the unemployed youths (Panda, 2016 [23]; Prakash *et al*., 2018) [24].

Nodos fish lives in the wetland, paddy field, swamps, pools, tanks, rivers, lakes, canals, streams and reservoir (Rainboth, 1996) [26]. Natural population of this fish is decreasing deadly due to the reckless fishing by drying out and poisoning the water reservoir, habitat destruction, housing for the people by filling up the tanks, wetlands, pollution and other ecological factors. Besides these reasons, there is another threatening by the disease problem of this fish. Day by day, the Nados fish is going to be extinguished from this universe (Mukherjee *et al*., 2002 [21], Hossain, 2014) [12]. Global population is assumed to reach 9.6 billion by the year 2050. To provide the quality protein to the next generations, animal protein is not sufficient. In this situation, fish protein is the supplement to the animal protein. So, the fish protein is required inevitably. Now the demand of the day is to produce more fishes to make up the shortfalls of this world. Nados fishes control the mosquitoes by eating the eggs, larvae, pupae, etc. So, the people get relief from the attack of dangerous fatal diseases like Dengue, Chikungunia, Encephalitis, etc. So, Nados fish is a friend of the human being since long (Oldalin *et al*., 2017) [25].

Parasitic infection causes the harmful effects on the health of this fish which inhibits their normal growth that causes the high mortality (Akhter *et al*., 2018) and welcomes the extinction from the nature (IUCN, 2017) [13]. Helminth parasites acts as an endoparasites of this fishes (Gautam *et al*, 2018 [11]; Marma *et al*., 2007) [19]. The present study was aimed to investigate the role of helminth parasites in Nados fishes.

Materials and Methods

Fifty fishes were collected from the local fish markets of Tamluk, Purba Medinipur, West Bengal from July 2021 to June 2022 and studied in the PG Laboratory, Department of Zoology, Tamralipta Mahavidyalaya, Tamluk in alive condition. The collected fishes were examined thoroughly to study the parasitic abundance and the prevalence of infestation in relation to sex and length. The fishes were divided into three groups in lengthwise i.e., Small (5.0-10.0 cm), Medium (10.1-15.0 cm) and Large (15.1-20.0 cm).

The sample fishes were dissected along the mid ventral line by a fine scissor and knife. Gut were carefully isolated by the forcep and placed in the normal saline (0.9%) water solution. The active living parasites were found and collected mainly from liver, stomach and intestine by using forceps and were placed in the normal saline water solution (0.9%). The collected specimens were fixed in AFA [Alcohol 50%: Formalin: Acetic acid (100: 6: 2.5)], stained with acetocarmine, dehydrated in ascending grades of ethanol, cleared in xylene and then mounted in DPX.

The fixed parasites in the slides were studied meticulously under the microscope Magnus MSZ- Bi. Parasites were identified upto the genus level following the literature of Yamaguti, S. Vol III (1961) [34], Bhattacharya, 2007 [7] and Naidu, 2012 [22]. The Prevalence and Intensity of the Parasites were estimated following the formula suggested by Margolis *et al*., 1982 [18].

$$\text{Prevalence (\%)} = \frac{\text{Number of infected host}}{\text{Total number of host examined}} \times 100$$

$$\text{Intensity } (\pm) = \frac{\text{Number of parasites}}{\text{Total number of infected host}}$$

Fig 1: Study site of Purba Medinipur district.

Fig 2: Satellite image of the study site (22.2858° N, 87.9189° E).

Fig 3: *Nandus nandus*

Fig 4: Collected parasites from *Nandus nandus.*

Fig 5: Microscopic image of Acanthocephala parasite.

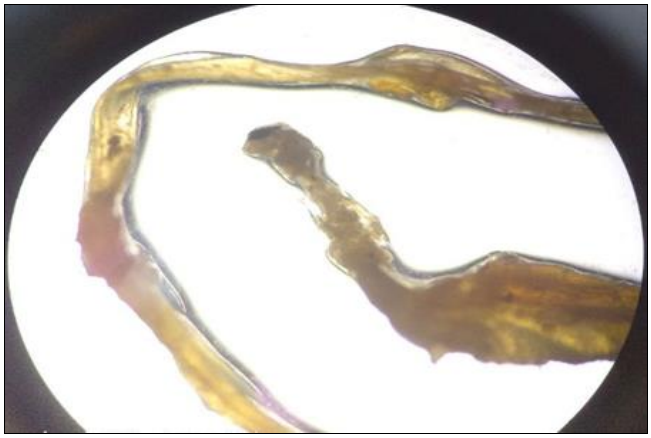

Fig 6: Microscopic image of Nematode parasite.

Results and Discussion

Table 1: Study on the distribution of the infective helminth parasites in the body organs of Nados fishes (*N. nandus*).

Parasites detected In	Total no. of the parasites found				Total
	Acanthocephala		Nematode		
	Pallisentis nandai	*Neoechinorhynchus sp.*	*Porrocaecum sp.*	*Contracaecum sp.*	

Intestine	23	06	06	49	84
Stomach	05	02	26	28	61
Liver	05	08	07	05	25
Total	33	16	39	82	170

The results in Table no. I revealed that the total number of helminth parasites (84) is found in the intestine of the Nados fishes (*N. Nandus*). But in the stomach it is 61 and in liver 25 only. The parasites, *Contracaecum sp* is the highest (49) followed by *Pallisentis nandai (23), Neiochinorhynchus sp. (6) and Porrocaecum sp.* (6). In case of stomach, *Contracaecum sp*. is the highest in number (28) followed by *Porrocaecum sp.* (26), *Pallisentis nandai* (5), and *Neiochinorhynchus sp. (2)* in liver, is the highest (8) in number of *Neiochinorhynchus sp*. followed by *Porrocaecum* sp. (7). The least number (5) is noticed in *Contracaecum sp.* and *P nandai*. This experiment demonstrated an interesting result. Out of four parasites, two prefer to attack more in the intestine organ of the Nados fishes. They are *Contracaecum sp.* and *P. nandai.* The stomach option is the second option followed by Liver. That means, intestine is the most succeptible place followed by stomach and liver. The parasites like to attack intestine most suitably. The second choice is stomach followed by the liver.

Table 2: Study on the intensity and prevalence of various groups of helminth parasites in Nados fishes (*N. nandus*).

Parasites	No. of fish examined	No. of fish infected	No. of Worm collected	Prevalence (%)	Intensity (±)
Nematode	50	27	138	54	5.11
Acanthocephala	50	11	32	22	2.90

The results in Table II demonstrated that the 38 Nados fishes are infected out of 100 i.e., 38% fishes are infected. It is recorded that the total number of Nematode affected fishes are 27 out of 50 whereas only 11 fishes are affected by Acanthocephala. The total number (170) parasites are collected from the 38 infected fishes. The data reveals that between the two parasites, Nematode is more infective where prevalence is 54%, intensity of infection is 5.11 and the 138 parasites are collected. But in case of Acanthocephala, it is lesser infective comparatively to Nematode as the prevalence is 22% and intensity is 2.90 and 32 parasites are collected. So after thorough study, it might be concluded that Nematode parasites are more dangerous than the Acanthocephala.

Table 3: Study on the severity of infection by helminth parasites in Nados fishes (*N. nandus).*

Helminth Parasites	Parasites Species	No. of fish examined	No. of Fish ninfected	No. of Worm collected	Intensity (±)	Prevalence (%)
Nematode	*Porrocaecum sp.*	50	10	39	3.90	20
	Contracaecum sp.	50	15	82	5.46	30
Acanthocephala	*Pallisentis nandai*	50	08	33	4.12	16
	Neoechinorhynchus sp.	50	05	16	3.20	10

The results in Table III showed that out of 4 parasites, *Contracaecum sp*. is the most virulent pathogen where number of parasites collected is 82, prevalence is 30% and intensity of infection is 5.46. The second virulent is *Porrocecum sp.* where number of parasites collected is 39, prevalence is 20% and intensity of infection is 3.90. The third parasite is *Pallisentis nandai* where number of parasites collected is 33, prevalence is 16% and intensity of infection is 4.12. The fourth parasites is *Neoechinorhynchus* sp. where number of parasites are collected is the least (16), prevalence is 10% and intensity is 3.20. In case of infected fish, 15 fishes were infected by *Contracaecum sp.* out of 50 followed by *Porrocaecum sp.* (10), *P. nandai* (8) and *Neoechinorhynchus sp.* which is the least (5). Here, *Contracecum sp.* is the most virulent, followed by *Porrocaecum sp., P. nandai* and *Neoechinorhynchus sp*. After meticulously studied of the data of this experiment, it might be conclude that out of 4 parasites *Contracaecum sp.* is the highest infective, followed by *Porrocaecum sp., P. nandai and Neoechinorhynchus sp.*

Table 4: Study on the intensity of infection of helminth parasites of Nados fishes (*N. nandus*) as per length of fishes.

Length of fishes (cm)	Groups	No. of fish examined	No. of fish infected	No. of Parasites collected	Prevalence (%)	Intensity (±)
5.0 – 10.0	Small	12	07	23	58.33	3.28
10.1 - 15.0	Medium	24	19	101	79.16	5.31
15.1 - 20.0	Large	14	12	46	85.71	3.83

The results in Table no. IV showed that the larger size (15.1-20.0 cm) fishes of Nados (*N. nandus*) is the most susceptible where prevalence is 85.71% which is followed by the medium group (10.1-15.0 cm) where prevalence is 79.16%. But the small group of the fish (5.1-10.0 cm) is the least susceptible where the prevalence is 58.33%. In case of intensity of infection, medium group is the highest (5.31) followed by large (3.83) and small (3.28). Same result is observed in the parasites collection where medium group is the highest (101) followed by the large (46) and small (23). One remarkable findings is observed i.e., in the early age of the fishes, the small group is the most resistant to the parasites followed by the medium and large group. As the age is increasing of the fishes, the resistance power is decreasing conversely which is a vice versa process.

Table 5: Study on the severity of infection of helminth parasites of Nados fishes (*N. nandus*) as per sex of the fishes.

Sex of fishes	No. of fish examined	No. of fish infected	No. of Parasites collected	Prevalence (%)	Intensity (±)
Female	32	26	128	81.25	4.92
Male	18	12	42	66.66	3.50
Total	50	38	170	76	4.47

The results in the Table no. V demonstrated that the female Nados fishes (*N. nandus*) are more susceptible to the helminth parasites where prevalence is 81.25% and intensity of infection is 4.92. But in case of male fishes, it is lesser susceptible to the parasites where prevalence is 66.66% and intensity is 3.50. Female Nados fishes are more infected that is 26 out of 32 and 128 helminth parasites are collected but in case of male fishes, 12 fishes are infected out of 18 and 42 parasites only are collected. It is observed that female Nados fishes are more vulnerable to the parasites in comparison to the male fishes.

Table 6: Study on the intensity of infection of helminth parasites of Nados fishes (*N. nandus*) in relation to season.

Name of Season	No. of fishes examined	No. of fish infected	No. of Parasites collected	Prevalence (%)	Intensity (±)
Pre-monsoon (Summer)	20	18	103	90	5.72
Monsoon (Rainy)	20	15	54	75	3.60
Post-Monsoon (Winter)	10	05	13	50	2.60

The results in Table no. VI showed that intensity of infection by helminth parasites of the Nados fishes (*N. nandus*) is the highest in the summer, prevalence is 90% and intensity is 5.72 followed by rainy and winter where prevalence is 75% and 50% and the intensity is 3.60 and 2.60 respectively. Parasites are collected highest in number in summer season (103) followed by rainy (54) and winter (13) season. In summer, the 18 Nados fishes are infected out of 20 species. Whereas, in rainy season 15 fishes are infected out of 20. But in winter only 5 fishes are infected out of 10. The data reveals that the Nados fishes are mostly attacked by the helminth parasites during the summer season followed by rainy and winter season. It might be due to the aquatic pollution. In the summer season, when the water level of the fishery decreases, then profusely increase the pollutants of the water reversely. The polluted water enhances the profuse multiplication of the parasites.

Out of 4 parasites, it is found that *Contracaecum sp*. and *P. nandai* prefer to choose to attack at the site of intestine organ followed by stomach and liver which is the most vulnerable and choiced striking place of invasion by this two parasites. *Porrocaecum sp*. prefers to attack the stomach but *Neoechinorhynchus sp.* prefer liver (Khanum *et al*., 2011 [16]; Sarma 2012 [30]; Barua *et al*., 1989 [6]; Koski and Scott, 2003) [17]. During the study of the intensity and prevalence of infection by different groups of helminth parasites, it is observed that the Nematode parasites are more virulent than the Acanthocephala (Khanum *et al*., 2011 [16]; Sultana and Parveen, 2014 [31]; William and Jones, 1994).

To measure the severity of infection of the helminth parasites of Nados fishes, it is revealed that out of four parasites, *Contracaecum sp.* is the highest infective followed by *Porrocaecum sp*, *P. nandai*, and *Neoechinorhynchus sp*. (Sarkar, 1953 [29]; Akhter *et al*., 2006; Ahmed *et al*., 2004) [1]. To assess the intensity of infection by helminth parasites of Nados fishes as per length of the fishes, it is noticed that small group (5.1-10.0cm) is the most resistant to the helminth parasites followed by medium and large. When the age is enhanced the resistance ability is reduced reciprocally (Fig.8) (Khanum and Parveen, 1997 [14]; Sultana and Parveen, 2014) [31]. On the other hand, the keeness of infection of the helminth parasites of Nados fishes enhances according to length of the fishes, small (58.33%), Medium (79.16%), large (85.71%).

During the study of the severity of infection of helminth parasites of Nados fishes as per the sex, it is noticed that the female Nados fishes are more vulnerable to these parasites with comparison to the males (Dobson, 1961 [9]; Khanum *et al*., 2008 [15], Marma *et al*., 2007) [19]. It might be due to the hormonal effects or genetic aspects or other factors. But in case of seasonal effects on the intensity of the infection by helminth parasites to the Nados fishes, it is remarkably observed that the Nados fishes are mostly attacked by the helminth parasites during the summer followed by rainy and winter season. Probably it might be the reason of pollution and temperature. During the summer, the water level of the fisheries diminished and increase the pollutants which encourages the enhancement of the population of the parasites (Fig.7) (Austin, 1998 [5]; Arizo *et al*., 2022 [4]; Gautam *et al*., 2018 [11]; Khanum *et al*., 2011 [16]; Sarma, 2012 [30]; Ranibala *et al*., 2013) [27], which might be the causes of the extinction of the Nados fishes from the fishery sector (IUCN, 2017) [13]. Ultimately it invites the financial loss of the fishery business.

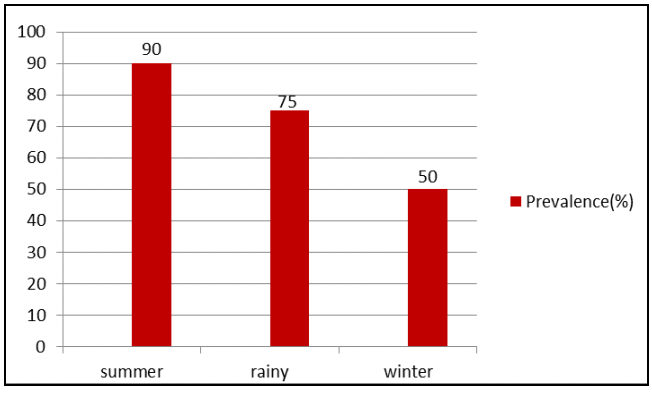

Fig 7: Severity of infection of helminth parasites to as per season in Nados fish (*N. nandus*).

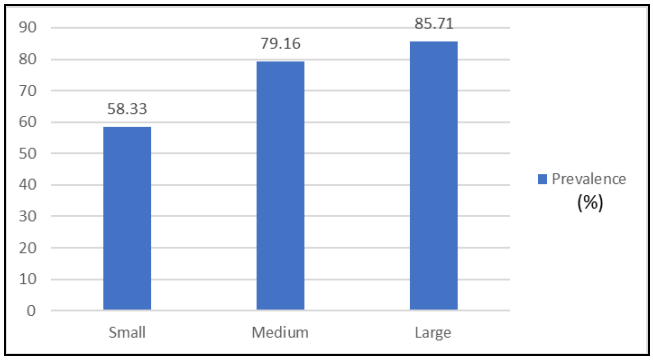

Fig 8: Prevalence of infection of helminth parasites as per length of the Nados fish (*N. nandus).*

Conclusion

Thirty nine (39) species of indigenous fishes including Nados which might be extinguished shortly from the water areas of West Bengal, India. There are so many reasons like over-exploitation, pesticide and aquatic pollution, uncontrollable introduction of exotic fishes, change of habitat due to housing, industrialisation, etc. But the main factor is the spread of the diseases in Nados fishes. Helminth parasites are the endoparasites of Nados fishes. It is revealed that Nematode parasites are the most aggressive and dangerous parasites which are *Contracaecum sp*. and *Porrocaecum sp.*, mostly attack in the intestine organ followed by stomach and liver. The other Acanthocephala parasites such as *P. nandai* and *Neoechinorhynchus sp*. are lesser infective. The female Nados fishes are more susceptible to these parasites in comparison to the males. The prevalence and intensity of these parasites are higher in the summer season followed by rainy and winter. The larger fishes are mostly susceptible followed by the middle and smaller groups. Now it is the high time to take the proper action plan to save the Nados fishes from their extinction from this universe.

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