Original Research Article

Population dynamics of metazoan parasites of *Rhizoprionodon acutus* from Nellore Coast off Bay of Bengal.

Short running title: Population dynamics of metazoan parasites of *Rhizoprionodon acutus*

Abstract:

Rhizoprionodon acutus Rüppell 1837, commonly known as 'Milk shark' is the frequently available elasmobranch from the Nellore (14.43°N 79.97°E.) coast off Bay of Bengal. A total of 152 *R. acutus* were collected from this coast during January, 2014- December, 2015, of which 89 hosts were infected with one or more parasites. A total of eleven species were collected, comprising of 6 cestodes, 2 nematodes, one monogenic, one copepod and one isopod. Various ecological parameters such as prevalence, mean intensity, mean abundance and index of infection were calculated to determine the monthly population dynamics and the seasonal dynamics of the parasites in *R.acutus*. The study was carried out for both overall and groupwise parasitization. Endoparasitic infection predominated the ectoparasitic infection in the host. The study reveals the role of the temperature and season in the recruitment of parasite fauna in the hosts. The present study would be a great contribution to the knowledge of the metazoan parasite fauna of elasmobranch fishes to the future helminthologists from this Nellore Coast, Bay of Bengal.

KEYWORDS: Population dynamics, metazoan parasites, *Rhizoprionodon acutus*, prevalence, mean intensity, mean abundance.

Introduction:

Parasites are an imperative part of a distinct operating ecosystem [1]. Just as every other organism has a part within the ecosystem; parasites also have an ecological niche. Their niche includes the resources and space of the host organism's body and the abiotic conditions they survive in while completing their life cycle. These aquatic parasites are acquiring potential attention in ecological point of view due to their interrelationship with their hosts. Further, their role as 'biological tags' has attracted many scientists to use them as sensitive probes to monitor changes in the environmental factors. But on the other hand, parasites affect fish health, growth, behavior, fecundity and mortality and also regulate host population dynamics and their community structure [1].As a parasite develops an ecological association with a particular host, there may be host specificity but also an immune reaction by the host. Site specificity within the host indicates parasitic adaptation to its environment. Elasmobranchs serve as a very good host for all types of metazoan parasites especially cestodes. *Rhizoprionodon acutus* commonly known as 'Milk Shark' and vernacularly as 'Kukkasorrah' and 'Pala sorrah' in Southern India especially, Andhra Pradesh is believed to improve the milk production of a human mother in some localities of Southern India. It is a coastal species observed in a broad range of artisanal, survival, profitable fisheries and recurrently spotted in fish markets but it is assessed as 'Least Concern' due to their extensive distribution and moderately productive life history [2]. Data on the population dynamics of helminth parasites of sharks are very scarce. Seasonal variations in the parasitic infections are very frequent in tropical waters. The occurrence of some species is throughout the year while some other species are restricted to particular season. [3] Opined that seasonal changes of water such as temperature, pH and conductivity severely influence the incidence of parasites in aquatic hosts. Several eminent authors like [4-18] also discussed the role of certain environmental factors on the incidence of parasitization. The present study was focused on the population dynamics of both ectoparasitic and endoparasitic helminth parasites. Even today there is dearth in our knowledge in this field, mainly the contributions in the ecology of elasmobranch fish parasites are very meager and there is a big lacuna in this field and many more studies are very much required to fill this gap. Hence, the present study is a genuine attempt to interpret the status of parasitic communities of *R.acutus* in terms of prevalence, mean intensity, mean abundance, standard deviation and index of infection and also to study the seasonal impact on the incidence of parasitization.

Materials and Methods:

In the present study, various ecto and endoparasites of *Rhizoprionodon acutus* were collected from Nellore (14.43°N 79.97°E.) coast Bay of Bengal, India (Plate-1; Fig-1). The collections were made for a period of 2 years i.e., from January, 2014-December, 2015. A total of 152 *R.acutus* were obtained from fishing landing centers and local fish markets along the coast (Fig-1). Monthly an average of 5-10 fishes were collected and brought to the laboratory.

Before dissecting the fish, various morphometric characters such as sex, weight and length were noted and thoroughly examined for ectoparasites from various possible organs like eyes, buccal cavity, skin and gills. The collected ectoparasites like Copepods and isopod parasites were fixed in 10% formalin. For the identification purpose, these parasites were cleared in a few drops of lactic acid for 12– 24 hours. Lactic acid acts as a good clearing solution and other monogenetic trematodes were collected with the help of small pipettes worms are kept in the center of the slide in a small drop of water and a coverslip is placed and then water is removed using filter paper and glycerine is added underneath the coverslip and all the four corners are sealed with a nail enamel. The visceral organs like heart, stomach, liver, intestine and air bladder were also examined thoroughly for the endoparasites. The spiral intestine were kept in petri-dishes filled with physiological saline and were dissected with a longitudinal incision and the intestinal mucosal spirals were unrolled as a flat sheet. Then they were shaken thoroughly in order to dessipate the gut contents. The gut contents were decanted many times to remove the excess mucous and observed under stereozoom microscope (LM-52-3621 Elegant) for parasites. The collected cestode parasites were kept between two slides or with a cover glass for proper pressing. During this process, proper care was taken to avoid any damage to the parasite. Properly pressed parasites were preserved in FAA (Formalin-10ml, Alcohol-85ml, and Acetic acid-5ml). Later, these parasites were washed thoroughly and stained with alum caramine. After proper dehydration in alcoholic grades (70%, 90%, 95% and absolute alcohol), the parasites were cleared in xylene and mounted in Canada balsam by the conventional techniques adopted from[19-20]. Nematodes collected from different locations such as liver, intestine and coelom were preserved, in 70% ethanol or less fluid (9 parts 70% ethanol and 1 part glycerol). These parasites uncoil and killed instantaneously and were finally transferred into bottles containing a mixture of 70% ethanol and glycerol (3:1). Standard biostatistical books by [21-23] were followed. Ecological terminologies were adapted from [24-26].

Prevalence: Prevalence is the number of individuals of the hosts infected with particular parasite species (or) with total parasites divided by the number of hosts examined. Prevalence is expressed in terms of percentage (%).

Number of individuals of a host species infected with particular parasite species
(or)
Number of individuals of a host species infected with total parasites

Prevalence (usually expressed as %) = ————————————————————

Number of hosts examined

Mean Intensity: Mean intensity is the average intensity of total number of individuals of particular parasite species in a sample of host species or total number of individuals of all parasites found in a sample of host species divided by the number of hosts infected with that parasite or the total number of parasites.

$$\textbf{Mean intensity} = \frac{\text{Total number of individuals of a particular parasite species in a sample of a host species (or) Total number of individuals of all parasites in a sample of a host species}}{\text{Number of infected individuals of the host species in the sample}}$$

Mean Abundance: Mean abundance is the total number of individuals of a particular parasite species in a sample of particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts).

$$\textbf{Mean abundance} = \frac{\text{Number of individuals of a parasite in a sample of host}}{\text{Total number of individuals of the host species (infected + uninfected)}}$$

Index of infection: Index of infection is the product of number of individuals of total parasitic species and the number of infected fish in a sample of host species divided by the square of total number of fish examined in the sample (infected and uninfected).

$$\textbf{Index of infection} = \frac{\text{Total number of infected hosts (b) X Total number of parasites in a sample of host species (c)}}{\text{square of total number of hosts examined } (a^2)}$$

To determine the seasonal influence on the parasitic infection, aach annual cycle was catalogued into three seasons: **Summer (**March to June), **rainy** (June to September) and **winter** (October to January) for expediency of calculation, though there is no significant division between one season and the other. A chi-square test was performed to test the significance between the season and the incidence of infection (Prevalence) (www.socscistatistics.com).

Results:

Total 152 fishes were examined, out of which 89 were found to be infected.5 species of metazoan parasites were collected, comprising 6 cestodes, 2 nematodes and each 1 of Monogenean, copepod and isopod (Table-1).

Monthly population dynamics of overall parasites in *Rhizoprionodon acutus*

The month-wise prevalence, mean intensity, mean abundance and index of infection of metazoan parasites in *Rhizoprionodon acutus* were presented graphically.

Prevalence of parasitization with total parasites during the years 2014-15 (Plate-2, Fig. 1a)

The prevalence of infection was nil in the first six months and raised in July and reached to the peak in the months of August, September, October and November (100 %) and slightly declined in the month of December. However, for the 2015 cycle, first 3 months showed highest prevalence (100 %) and after that in the months of May and December it shows highest prevalence and it was moderate during the months of July to September and shows lowest in the Months of April, June, October and November.

Mean intensity, mean abundance and index of infection of parasitization with total parasites during the years 2014-15 (Plate-2, Fig. 1b, 1c & 1d)

Mean intensity was highest in the months of in the months of August, October to November, and reaches to a peak in the month of December (5.9) and shows lowest in the month of July for 2014 cycle. While in 2015 cycle Mean intensity was moderate during the months of January to April, shows lowest in the month of May and reaches to highest in the months of August (7.7) and somewhat declined during September to December. Mean abundance was highest in the month of December (5.1) in the year 2014 and after that it shows highest values in the month of August and November and lowest in the month of July. In the year 2015 it shows highest mean abundance in the month of August (6.8) and it was lowest in the month of April. Index of infection was high in the month of August (4.87), and showed highest during the months of October to December for 2014 cycle and lowest in the month of July. However, for the 2015 cycle, Index of infection was highest in the month of August (5.90) and lowest in the month of April.

Prevalence, Mean intensity, Mean abundance and Index of Infection of parasitization with Monogenean parasites (Plate-3, Fig. 1a, 1b, 1c & 1d)

Prevalence of Monogenean parasite is highest in the month of December in the year 2015, and it shows zero prevalence from February to July in both the years. In the month of August in both the years and in December 2014 it shows moderate values. Mean intensity of monogenean parasites reaches to a peak in the month of august in the year 2015 and its mean intensity is zero from February to July in both the years. When compared to 2014 in the year 2015 there is slight increase in the mean intensity values. Same like mean intensity, mean abundance also shows highest abundance in the august of 2015 and zero abundance from February to July in both the years. Infection of monogenean parasite is almost zero except for august and December of the year 2014. In the year 2015 its infection reaches to a peak in the month of august and shows moderate infection in the months of January and November and observed zero infection in the remaining months.

Prevalence, Mean intensity, Mean abundance, Index of Infection of parasitization with cestode parasites (Plate-4, Fig. 1a, 1b, 1c & 1d):

Prevalence of parasitization with cestodes showed some variations in both the years. Zero infection was recorded during Jan to June in 2014 and was higher during the months of July to December in both the years 2014 and 2015. Besides this, there is a coincidence in both the years during the months of July to December. Mean intensity of cestodes reaches to peak in the month of August in the year 2015 and it was higher from November to December and moderate during September and October in both the years and it shows zero intensity from January to July in the year 2014. Mean abundance of cestodes shows zero abundance from January to June in the year 2014 and reaches to peak in the month of August in the year 2015 and it was higher from November to December and modest during July to October in both the years and there is a coincidence in the month of September in both the years. Index of infection of cestode parasites is zero from January to June in the year 2014 and slowly raises and reaches to a highest value in the months of November and December. In the year 2015 it shows moderate from January to July and reaches to a peak in the month of August.

Prevalence, Mean intensity, Mean abundance, Index of Infection of parasitization with Nematodes (Plate-5, Fig. 1a, 1b, 1c & 1d):

Nematodes showed zero prevalence in the months of April to August in both the years and reaches to higher in the months of September and October and moderate during the months of November and December in both the years. Mean intensity of nematodes is higher in the months of January and March in the year 2015 and however it also shows higher mean intensity values in the months of November and December in both the years 2014 and 2015. Zero intensity was observed from April to September in both the years. Mean abundance reaches to a peak in the month of October during the first year and it shows higher in the months of January and November in the year 2015 and shows moderate in the month of December in both the years and mean abundance was zero from April to September in both the years. Infection of nematode parasites is zero from January to September of the year 2014 and where as it reaches to a peak in the October month and shows moderate in the months of November and December, where as in the year 2015 it reaches to higher in the months of January and November and observed moderate in march and December and almost shows zero infection in the remaining months.

Prevalence, Mean intensity, Mean abundance and Index of Infection of parasitization with copepod (Plate-6, Fig. 1a, 1b, 1c & 1d):

Prevalence of copepod reaches to a maximum in the month of December in the year 2014 and shows higher in the month of October and November in the year 2015 it was moderate in the months of august and December in the year 2015.And almost observed zero prevalence from April to July in both the years. Zero Intensity of parasitization is observed from April to July in both the years, and reaches to a peak in the month of March & December and it was highest in the month of august and shows moderate intensity from September to November in the year 2015. Except, December it almost shows zero abundance in the year 2014 whereas it reaches to a peak in the month of august and shows moderate from September to November in the year 2015. However it is observed slight difference in abundance in the month of December in both the years. Except, December remaining all months of the year 2014 showed zero infection of copepod parasites. In the year 2015, 5 months shows zero infection of copepod parasites and remaining 7 shows moderate infection of copepod parasite.

Prevalence, Mean intensity, Mean abundance and index of infection of Isopods (Plate-7, Fig. 1a, 1b, 1c & 1d):

Isopods were obtained only once in the month of August, 2014 and in rest of the months, the infection was nil. Hence, prevalence, mean intensity, mean abundance and index of infection of Isopod parasites were shown only in the month of August, 2014 and remaining rest of the months it showed zero value. There was no infection of isopods in the year 2015.

Seasonal dynamics of parasites in *Rhizoprionodon acutus*

Influence of Seasons on the parasitic infection in *Rhizoprionodon acutus:*

The impact of seasons on the incidence of parasitization showed mixed results as the first the chi-square value (χ^2 = 19.63, p=0.000055) for annual cycle 2014 at 5% level of significance and 2 degrees of freedom shows that there might be a significant impact of seasons on the parasitization. However, the chi-square value (χ^2 = 0.1700, p=0.918) for annual cycle 2015 at 5% level of significance and 2 degrees of freedom shows that there is no influence of seasons on the parasitization. Prevalence of infection was high during rainy season for the year 2014 and lowest during the summer season whereas, during the year 2015 prevalence was high in the winter season. (Table-2; Plate-8, Fig. 1a & 1b). Recruitment of the parasites may take place after summer and reach their peak periods in the winter months.

Discussion:

The overall prevalence, mean intensity, mean abundance and index of infection showed very less similarity during the two annual cycles with significant deviations. First six months of the of the first annual cycle showed no infection and remaining six months showed moderate to high infection rate while the second annual cycle showed seasonal changes with highest infection rates in rainy and winter seasons and least in summer season. These disparities may be due to host density and its feeding behavior. Elasmobranchs were infected with large number of parasites during the winter months; parasitization being more in winter months than other seasonal months. Temperature is considered as one of the crucial factors in determining the seasonal periodicity of parasitic infection[13,16,][27-35]. The environmental conditions of tropical waters are quite favorable in winter months where the waters are warm but not too cold. [29] also expressed the same view that infections are more in warm seas than in colder ones. At moderate temperatures the zooplankton fauna may be rich when compared to high temperatures of summer months in tropical areas. The sea is remains quiet and calm with very few disturbances during the winter months; hence the recruitment of infection may take place after summer and reach to its peak in winter months. Thus, temperature and season plays a crucial role in the recruitment of parasite fauna. The present study comes closer to the views of the [27-29] where the prevalence of *R. acutus* was relatively high in the rainy and winter season than the summer season. In the present study, endoparasitic infection dominated the parasitic communities than the ectoparasites. The present study showed highest cestode infection which was correlated with the experimental studies of [36] who illustrated that the cestode parasites in a fish can survive for longer period at low temperature, but with the increase in water temperature, parasitic infections gets eliminated and thus, the temperature is a major controlling factor of seasonal periodicity of infection. Thus, parasites recruitment within the host is totally dependent on the temperature which might influence the seasonality of parasitic infections either directly or indirectly [37-38].Thus it can be concluded that the present work showed an evident variations in the population dynamics statistics

Conclusion

Marine fishes are very significant in commercial fisheries in various parts of the world including India. The study has been conducted for two consecutive years to depict the nature of occurrence of parasites within *R .acutus.* This study has put forward very fine results since the overall parasitization and groupwise parasitic infestation within the host showed less uniformity for both the consecutive years. The temperature and season plays a crucial role in the recruitment of parasite fauna. In the present study, the prevalence of infection, mean intensity, mean abundance, index of infection were high during the months of July to December due to low temperature and low during the months of January-June because of high temperature.

Significance Statement: This study discovers the fact that the abiotic factors such as temperature play a significant role in determining the parasitic infestation within the hosts.

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PLATE-1

Fig-1: Fish landing centers of Nellore Coast

PLATE-2

Monthly population dynamics of overall parasites in *Rhizoprionodon acutus*

Fig.1a-Prevalence (%), Fig.1b- Mean intensity, Fig.1c- Mean abundance, Fig.1d- Index of infection

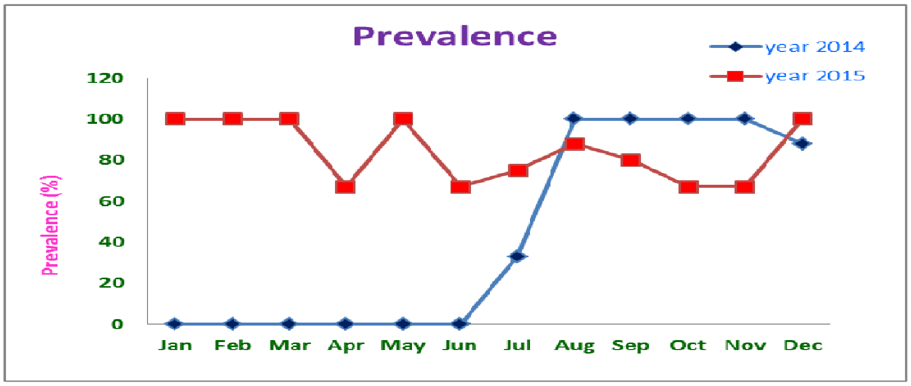

Fig.1a

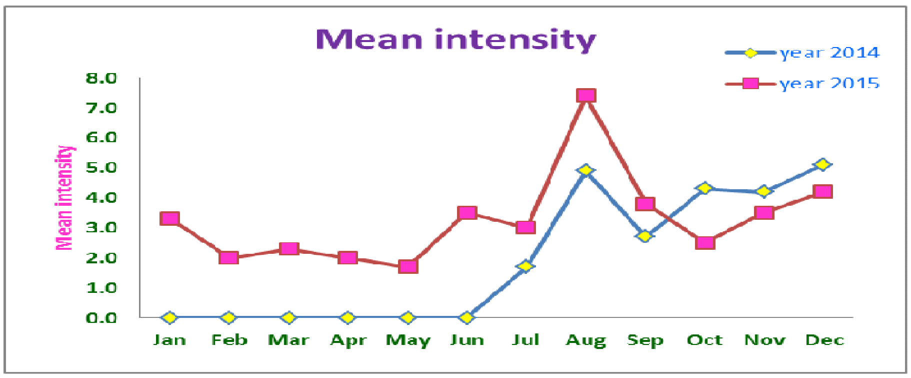

Fig.1b

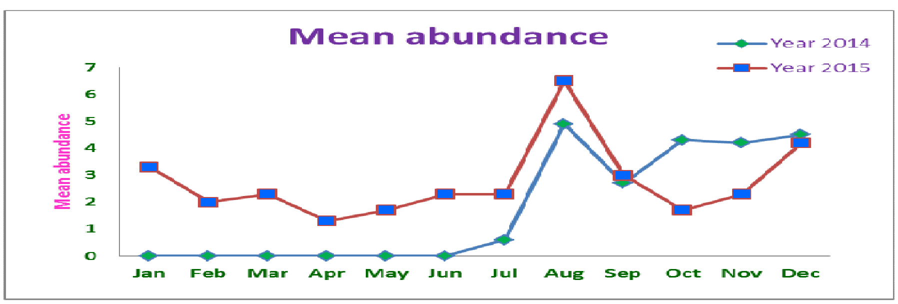

Fig.1c

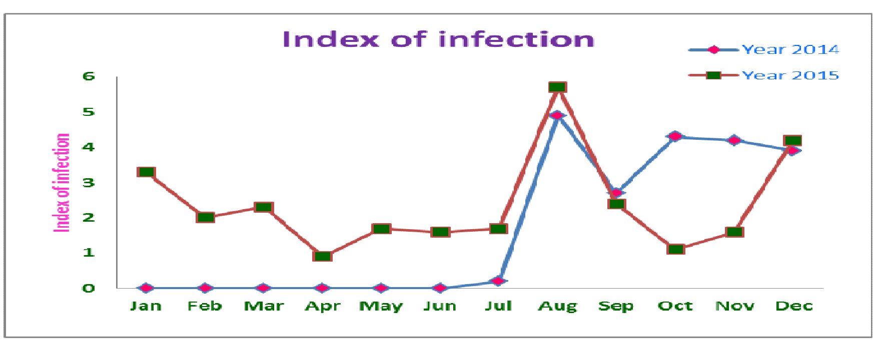

Fig.1d

Plate-3

Monthly population dynamics of monogenean parasites in *Rhizoprionodon acutus*

Fig.1a- Prevalence (%), Fig.1b- Mean intensity, Fig.1c- Mean abundance, Fig.1d- Index of infection

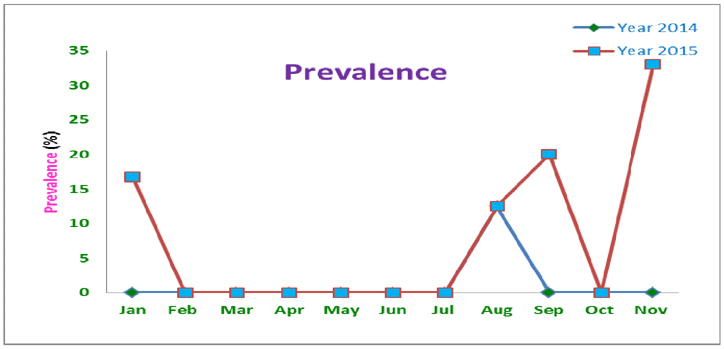

Fig.1a

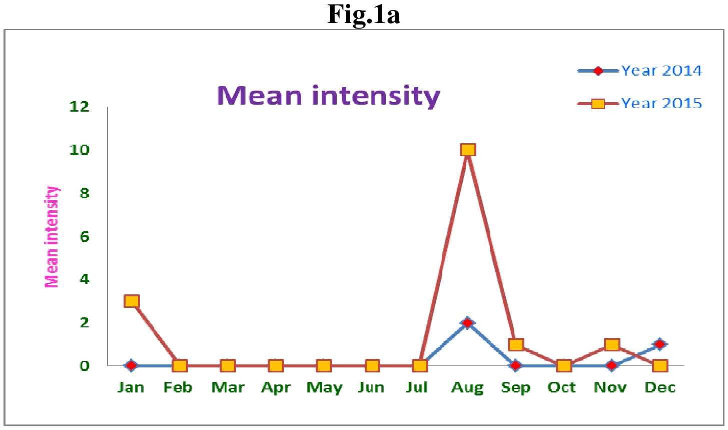

Fig.1b

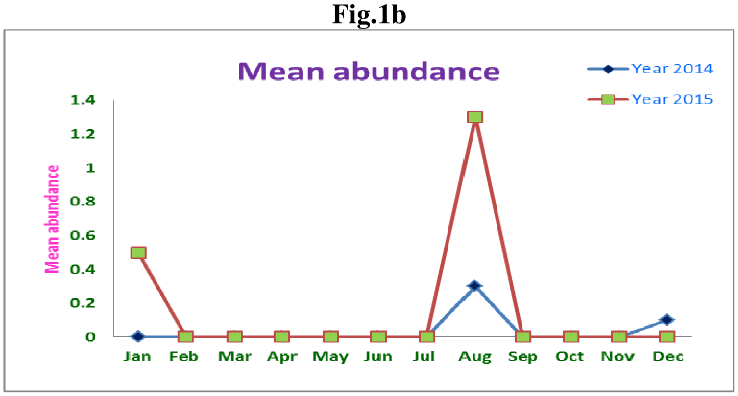

Fig.1c

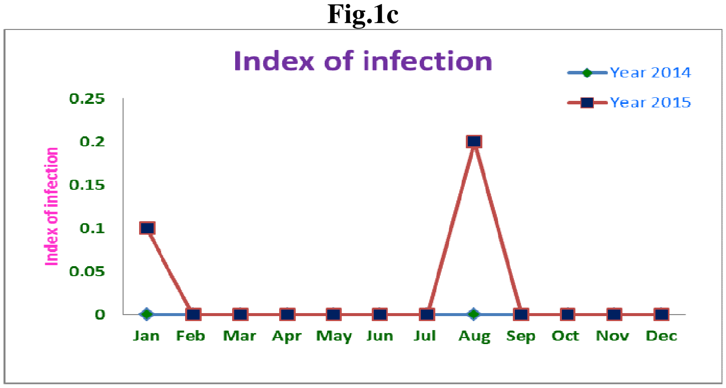

Fig.1d

Plate-4

Monthly population dynamics of cestode parasites in *Rhizoprionodon acutus*
Fig.1a- Prevalence, Fig.1b- Mean intensity Fig.1c- Mean abundance, Fig.1d- Index of infection

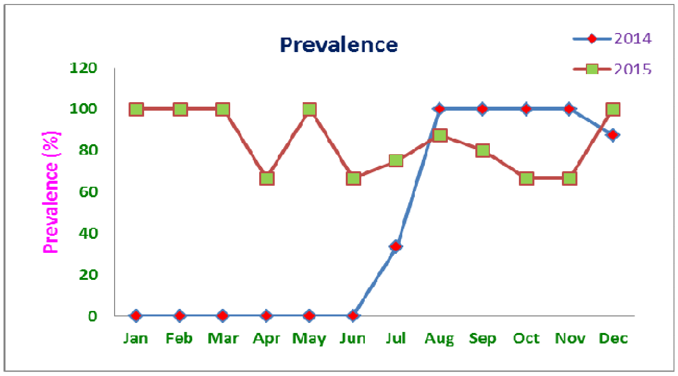

Fig.1a

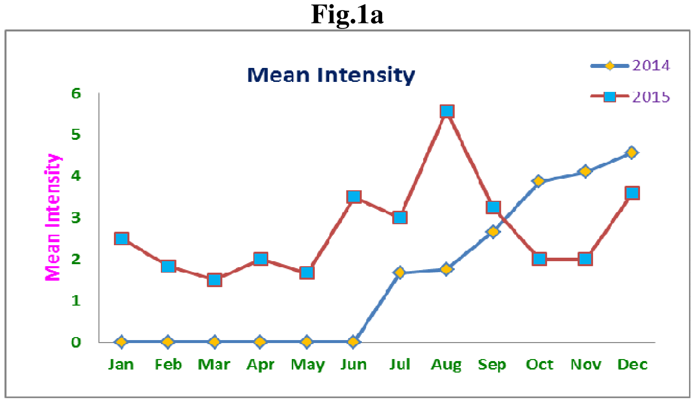

Fig.1b

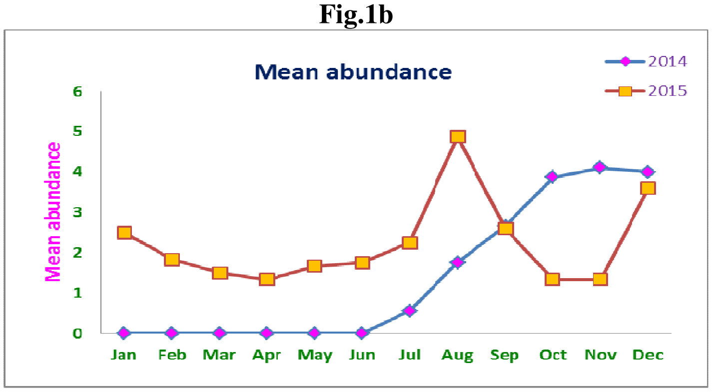

Fig.1c

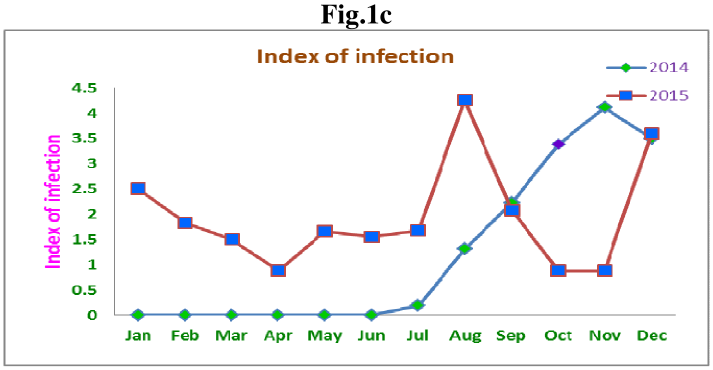

Fig.1d

Plate-5

Monthly population dynamics of nematode parasites in *Rhizoprionodon acutus*

Fig.1a- Prevalence, Fig.1b- Mean intensity, Fig.1c- Mean abundance, Fig.1d- Index of infection

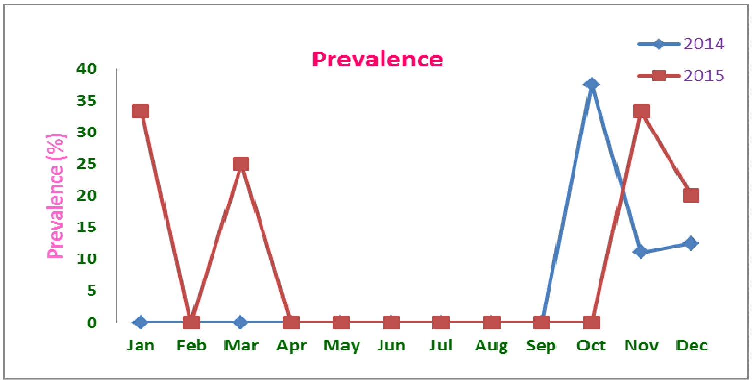

Fig.1a

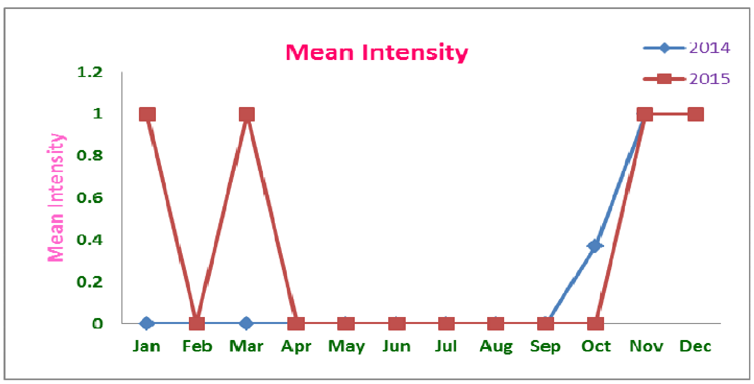

Fig.1b

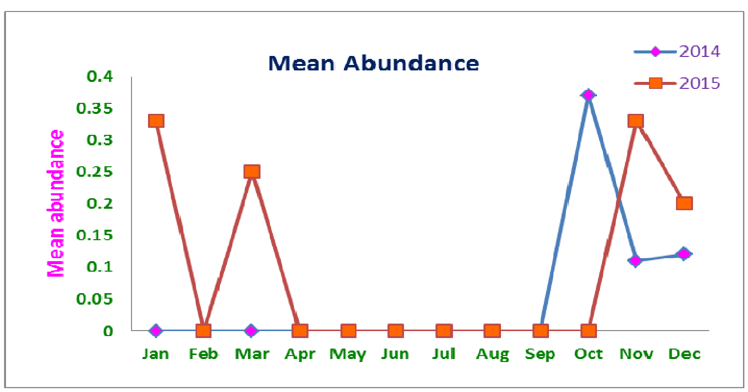

Fig.1c

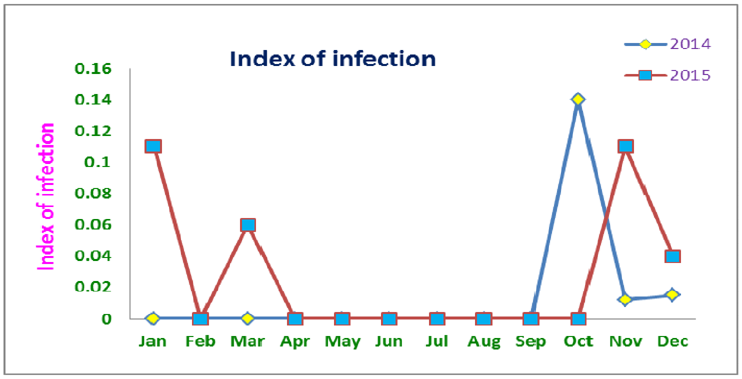

Fig.1d

Plate-6

Monthly population dynamics of copepod parasites in *Rhizoprionodon acutus*

Fig.1a- Prevalence, Fig.1b- Mean intensity, Fig.1c- Mean abundance, Fig.1d- Index of infection

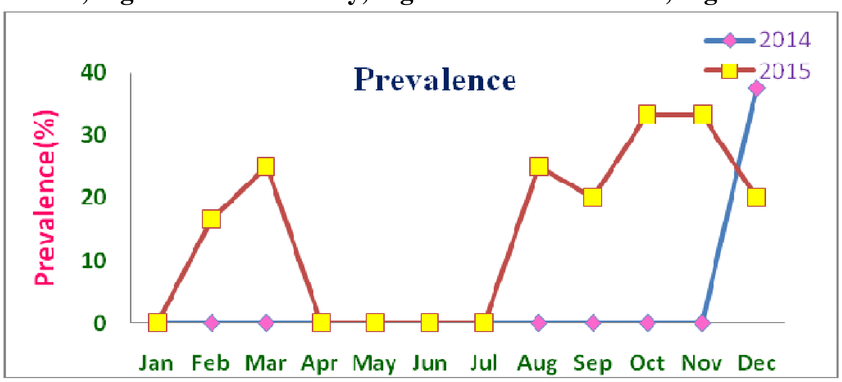

Fig.1a

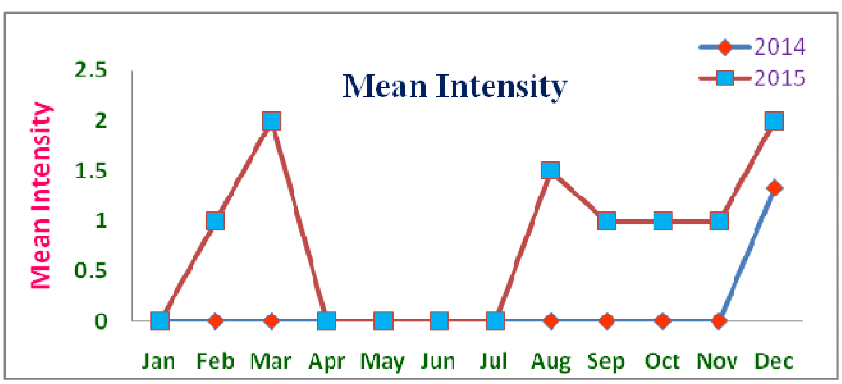

Fig.1b

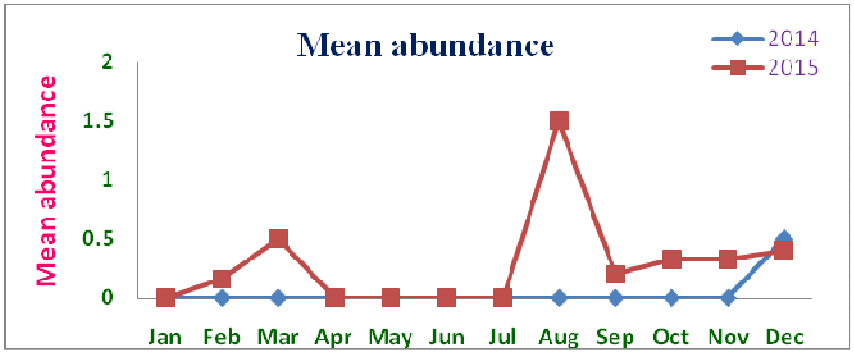

Fig.1c

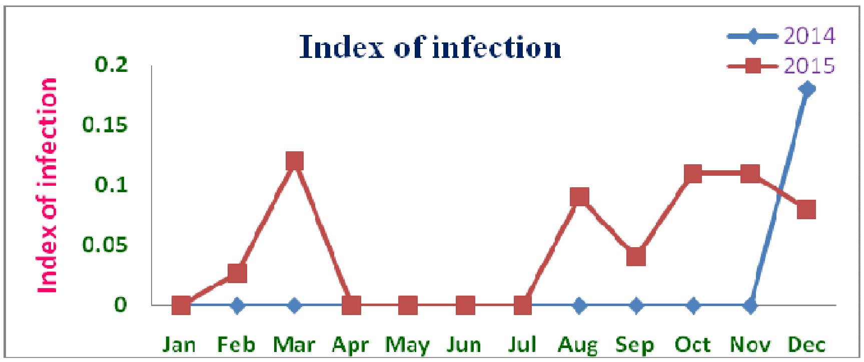

Fig.1d

Plate-7

Monthly population dynamics of isopod parasites in *Rhizoprionodon acutus*

Fig.1a- Prevalence, Fig.1b- Mean intensity, Fig.1c- Mean abundance, Fig.1d- Index of infection

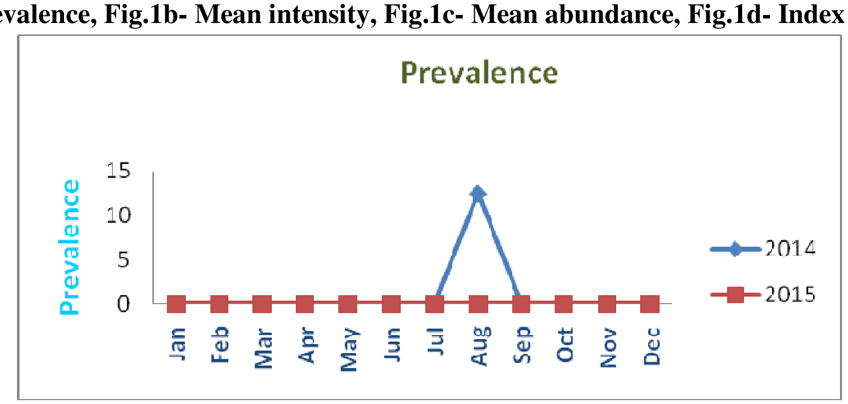

Fig.1a

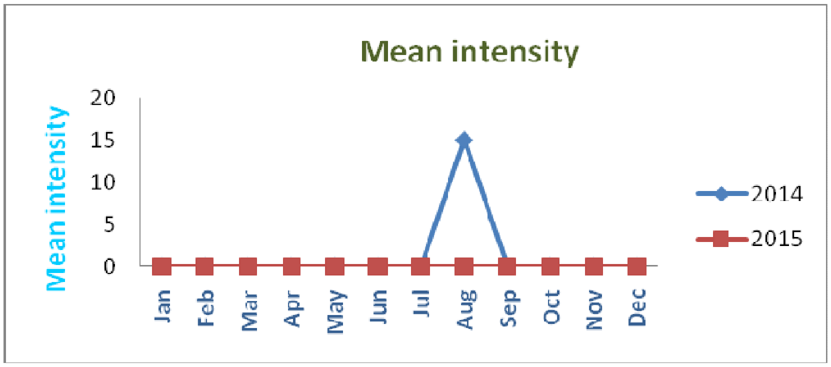

Fig.1b

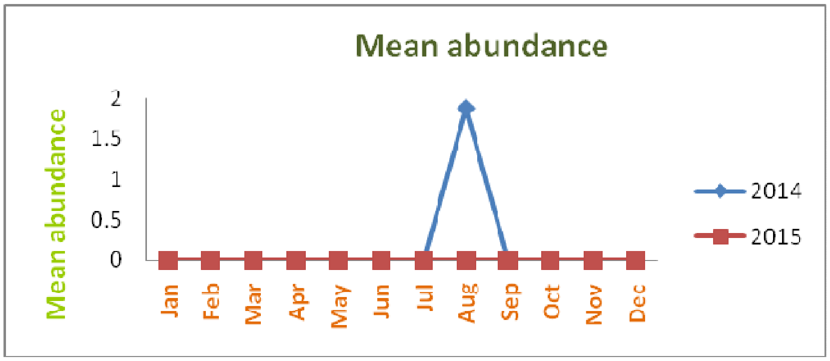

Fig.1c

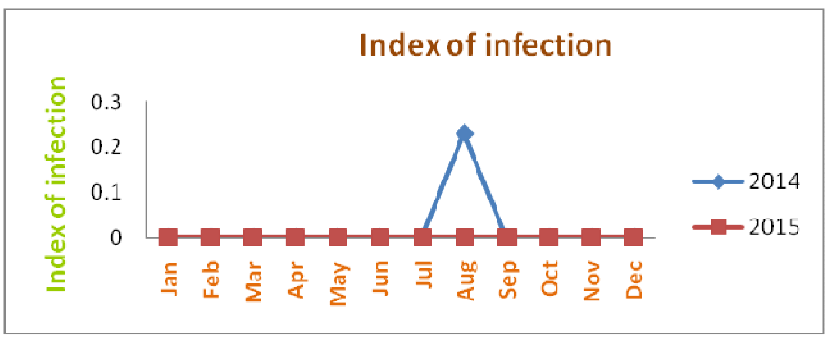

Fig.1d

Plate-8

Fig.1: Seasonal dynamics of overall parasitization in *R.acutus*

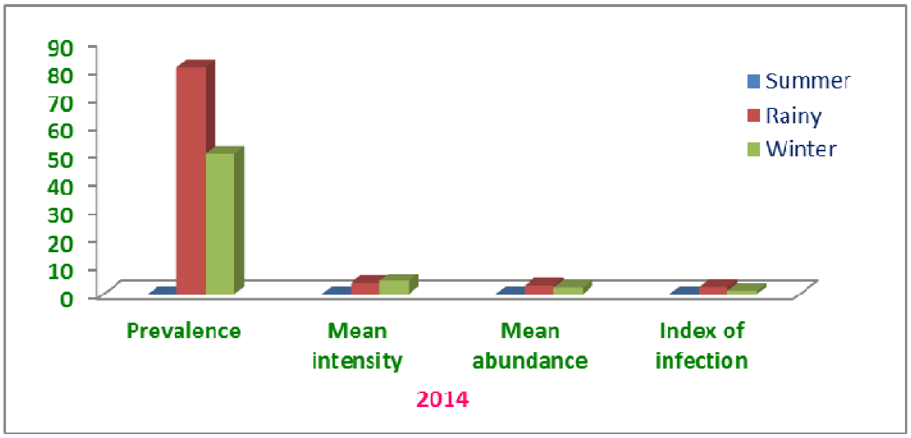

Fig. 1a

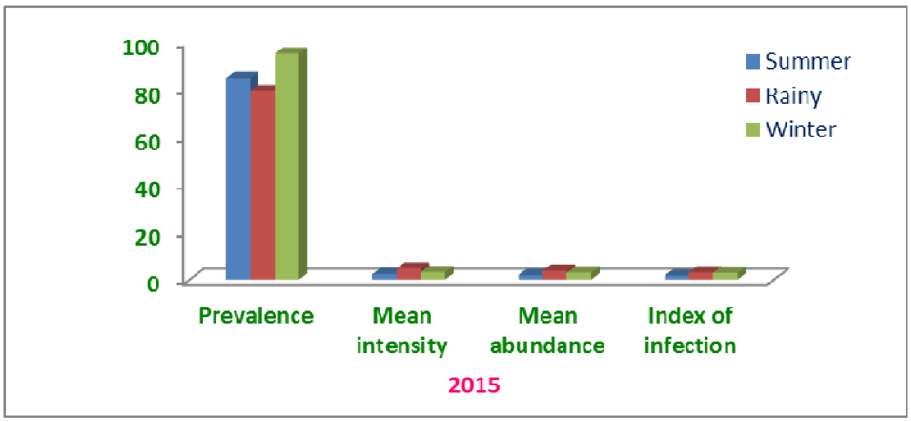

Fig. 1b

Table-1

Metazoan parasites of ***Rhizoprionodon acutus*** **Rüppell, 1937**

Sl. No.	**Name of the host**	**Name of the parasite**	**Number of parasites collected**
1.	***Rhizoprionodon acutus*** **Rüppell,1937**	**Monogenean**: 1. *Loimos secundus* Chauhan and Bhalerao, 1945	18
		Cestodes: 2. *Paraorygmatobothrium floraformis* (Southwell, 1912) Ruhnke, 2011	149
		3. *Nybelinia lingualis* (Cuvier, 1817) Dollfus,1927	24
		4.*Heteronybelinia peridareus* Shipley et Hornell, 1906	58
		5. *Nybelinia indica* Chandra, 1986	6
		6. *Phoreobothrium sp.*	3
		7. *Poecilancistrum ilishae,* Southwell et	

		Prashad,1918	45
		Nematodes: 8. Larva of *Anisakis* sp. 9. Larva of *Hysterothylacium* sp.	 6 4
		Copepod: 10. *Kroyeria minuta* Pillai, 1968	 15
		Isopod/Amphipod: 11. *Lafystius sturionis* Kroyer, 1842	 15

Table-2

Seasonal changes in overall prevalence, mean intensity, mean abundance and Index of infection of parasites of *R. acutus*

Seasons	No. of examined fishes (a)	No. of infected fishes (b)	No. of parasites (c)	Prevalence = b/a*100	MI c/b	MA c/a	Index of infection $b*c/a^2$	χ_2 Value (at 5% level of significance and 2 degrees of freedom)
R.acutus (Jan, 2014-Dec, 2014)								
Summer	32	0	0	0	0	0	0	χ_2 = 19.63; p=0.000055 (The result is significant)
Rainy	31	25	94	80.6	3.8	3	2.4	
Winter	32	16	74	50	4.6	2.3	1.2	
R.acutus (Jan, 2015-Dec, 2015)								
Summer	13	11	25	84.6	2.3	1.9	1.62	χ_2 = 0.170; p=0.918 (The result is not significant)
Rainy	24	19	90	79.2	4.9	3.9	3.06	
Winter	20	19	60	95	3.3	3.1	2.94	