Population Dynamics of Metazoan Parasites of *Rhizoprionodon acutus* from the Nellore Coast off Bay of Bengal

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Original Research Article

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, 1 monogenic, 1 copepod and 1 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: Prevalence; mean intensity; mean abundance; index of infection; seasonal changes.

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1. 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. The 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, behaviour, 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* (Rüppell) 1837, frequently well-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. Dogiel et al. opined that seasonal changes of water such as temperature, pH and conductivity severely influence the incidence of parasites in aquatic hosts [3]. Several eminent authors also discussed the role of certain environmental factors like season and temperature on the incidence of parasitisation [4-18]. 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 parasitisation.

2. MATERIALS AND METHODS

In the present study, various ecto and endoparasites of *R. 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. An average of 5-10 fishes were collected and brought to the laboratory for monthly sampling. Before dissecting the fish, individuals were sexed, weighed and the length was measured 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. The monogenetic trematodes were collected with the help of small pipettes, worms were kept in the centre of the slide with a small drop of water and a coverslip was placed. Then water was removed using filter paper and glycerine was added underneath the coverslip and all the four corners were sealed with nail enamel. The visceral organs like heart, stomach, liver, intestine and air bladder were also examined thoroughly for the endoparasites. The spiral intestine was kept in petri-dishes filled with physiological saline solution and was dissected with a longitudinal incision and the intestinal mucosal spirals were unrolled as a flat sheet. Then they were shaken thoroughly to dissipate 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-10 ml, Alcohol-85 ml, 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 Hiware et al. and Madhavi et al. [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 were followed from Sundar Rao & Richard, Daniel and Sokal & Rohlf [21-23]. Ecological terminologies were adapted from Margolis et al. Grabda-kazubski et al. [24-26].

Plate 1. Fig-Study area showing Nellore coast, Bay of Bengal

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 (%).

Prevalence (usually expressed as %) = {(Number of individuals of a host species infected with particular parasite species) (or) (Number of individuals of a host species infected with total parasites) / (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.

Mean intensity = {(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) / (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).

Mean abundance = (Number of individuals of a parasite in a sample of host / Total number of individuals of the host species (infected + uninfected))

Please check these highlighted equations

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).

Index of infection = (Total number of infected hosts (b) X Total number of parasites in a sample of host species (c) / square of total number of hosts examined (a^2))

To determine the seasonal influence on the parasitic infection, each 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).

3. RESULTS

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

3.1 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.

I. 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 slowly 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, the 2015 cycle showed the highest prevalence (100%) during the first 3 months (January-March) and in the months of May and December, whereas prevalence was moderate during the months of July to September and least in the Months of April, June, October and November respectively.

II. 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 August, October to November, and reached to peak in December (5.9) and least in July for 2014 cycle. However, 2015 cycle showed a zig-zag pattern with moderate mean intensity during the months of January to April, lowest values in May, inclination to higher value in August (7.7) and declination from September to December correspondingly. Mean abundance was highest in December (5.1) and lowest in July for the 2014 cycle and the remaining months showed moderate to higher values. The 2015 cycle showed highest mean abundance in August (6.8) and least MA in April. Index of infection was high in August (4.87) and low in July for 2014 cycle. However, for the 2015 cycle, index of infection was highest in August (5.90) and lowest in April.

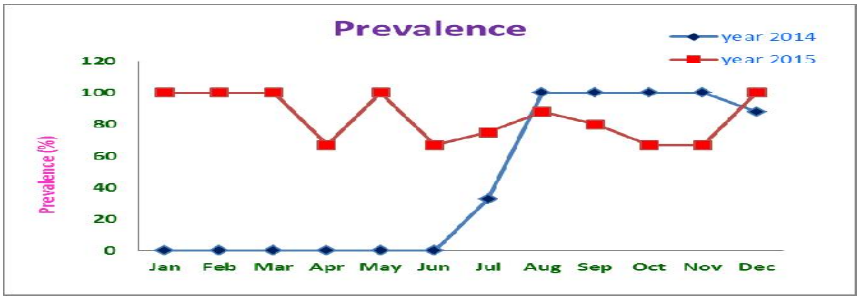


Fig.1a

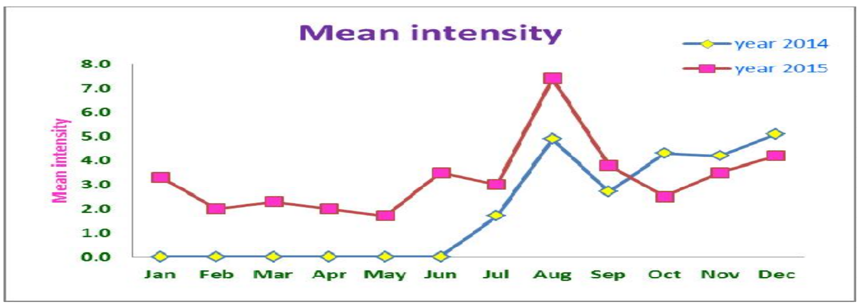


Fig.1b

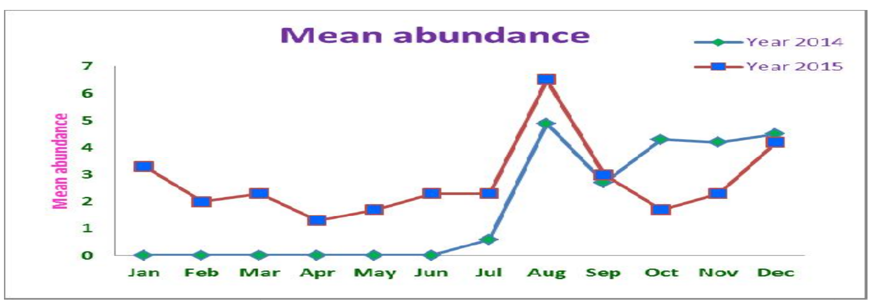


Fig.1c

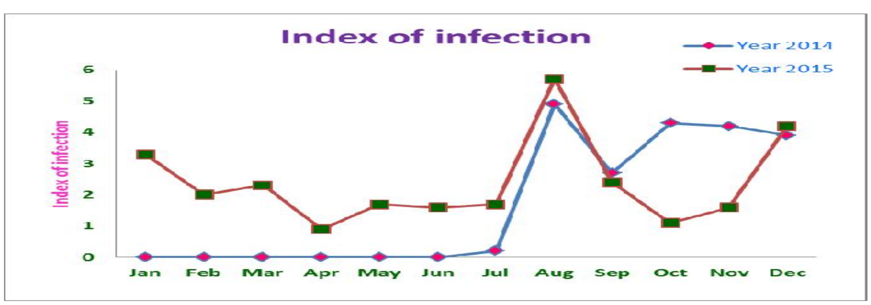


Fig.1d

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

Plate 2. Monthly population dynamics of overall parasites in *Rhizoprionodon acutus*

Table 1. Metazoan parasites of *Rhizoprionodon acutus* Rüppell, 1937 collected from Nellore coast, Bay of Bengal

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.	6
		9. Larva of *Hysterothylacium* sp.	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	Average temperature	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	38-40ºC	32	0	0	0	0	0	0	χ_2 = 19.63; p=0.000055 (The result is significant)
Rainy	24-26ºC	31	25	94	80.6	3.8	3	2.4	
Winter	20-22ºC	32	16	74	50	4.6	2.3	1.2	
					R. acutus (Jan, 2015-Dec, 2015)				
Summer	39-42ºC	13	11	25	84.6	2.3	1.9	1.62	χ_2 = 0.170; p=0.918 (The result is not significant)
Rainy	23-26ºC	24	19	90	79.2	4.9	3.9	3.06	
Winter	21-22ºC	20	19	60	95	3.3	3.1	2.94	

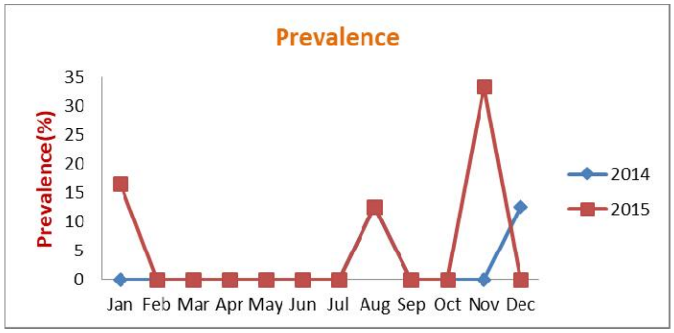


Fig.1a

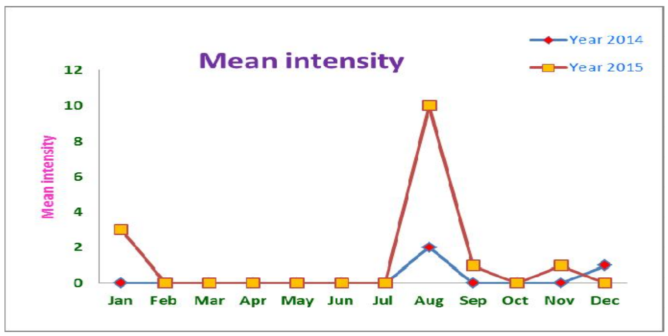


Fig.1b

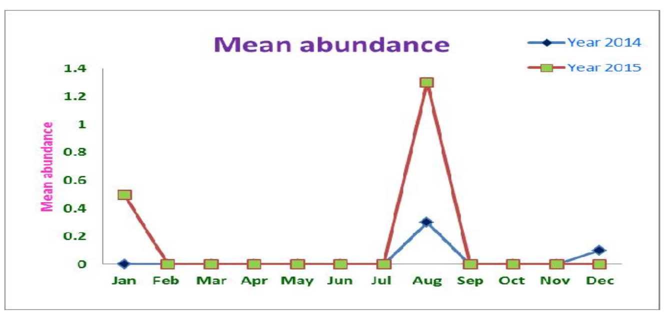


Fig.1c

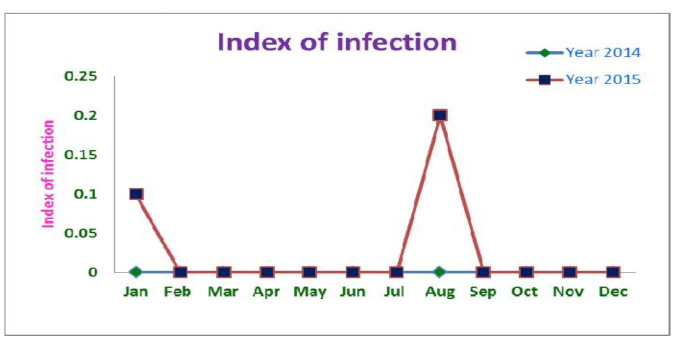


Fig.1d

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

Plate 3. Monthly population dynamics of monogenean parasites in *Rhizoprionodon acutus*

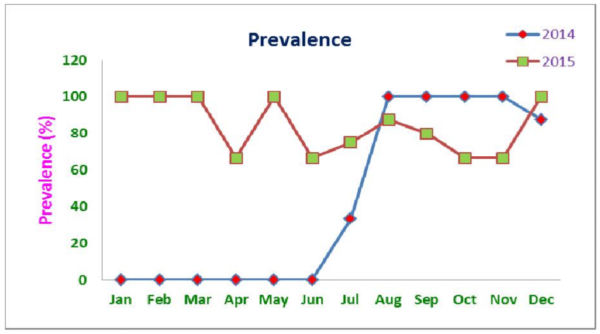


Fig.1a

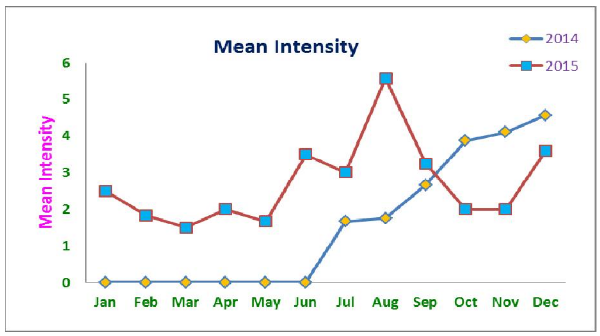


Fig.1b

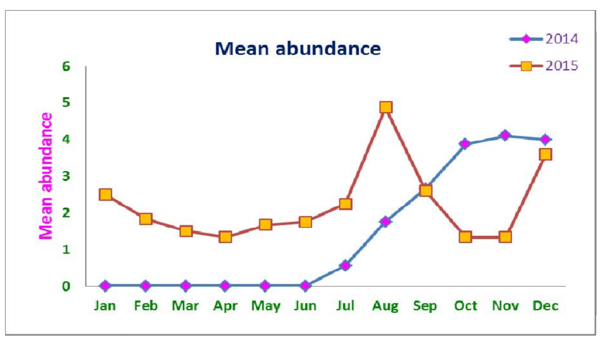


Fig.1c

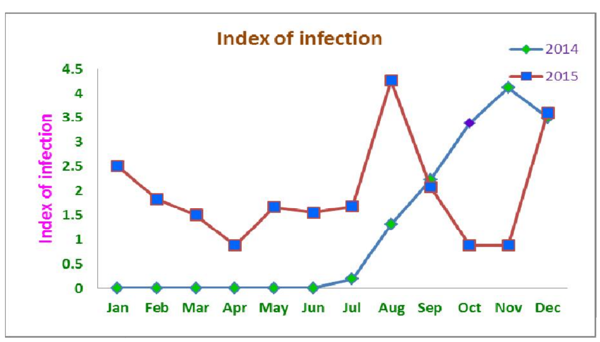


Fig.1d

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

Plate 4. Monthly population dynamics of cestode parasites in *Rhizoprionodon acutus*

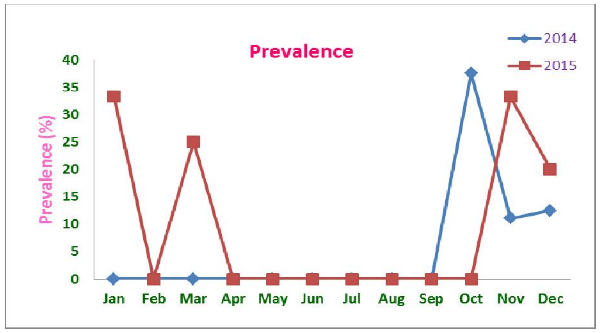


Fig.1a

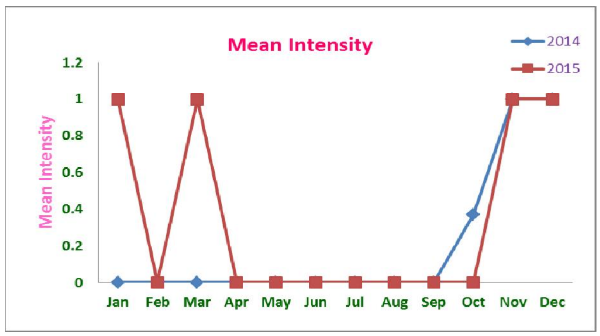


Fig.1b

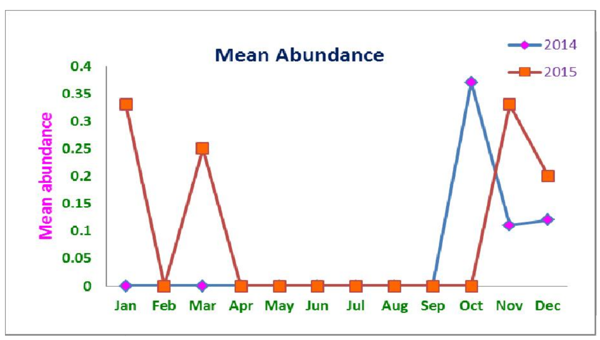


Fig.1c

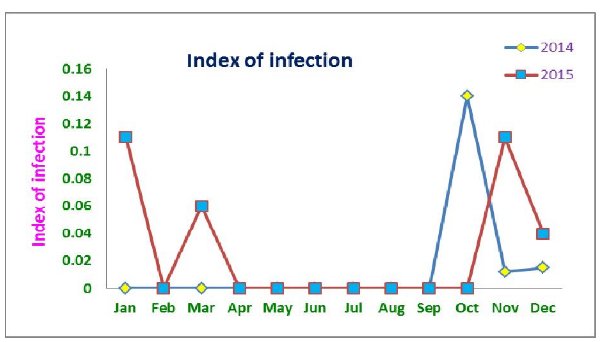


Fig.1d

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

Plate 5. Monthly population dynamics of nematode parasites in *Rhizoprionodon acutus*

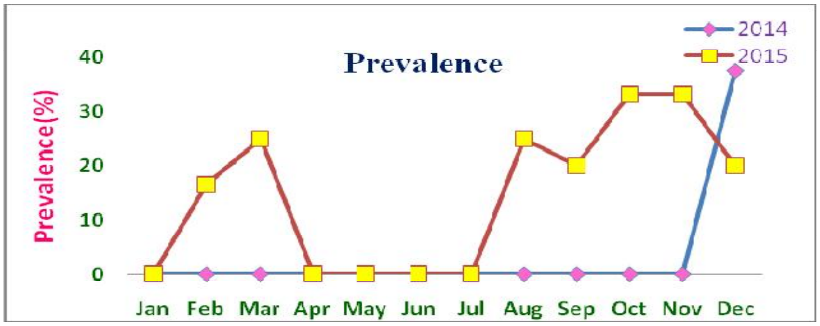


Fig.1a

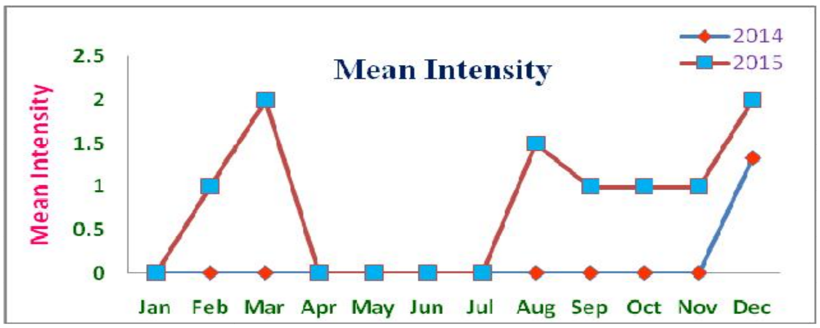
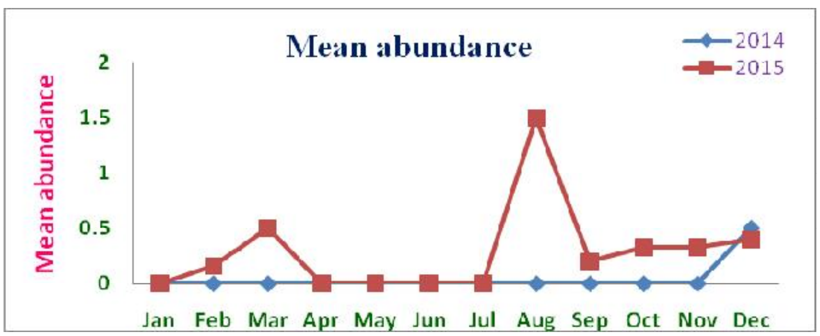


Fig.1b



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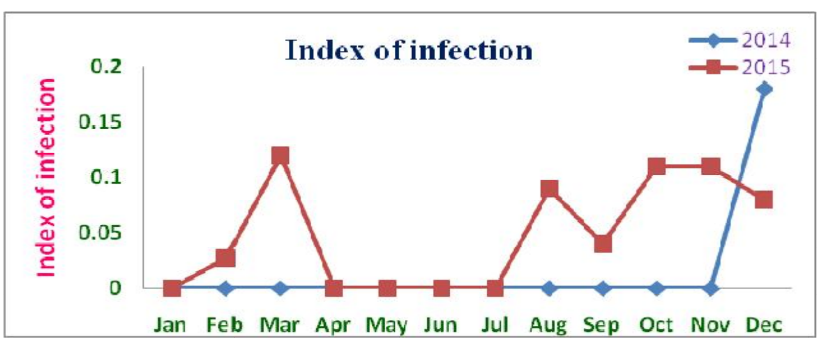


Fig.1d

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

Plate 6. Monthly population dynamics of copepod parasites in *Rhizoprionodon acutus*

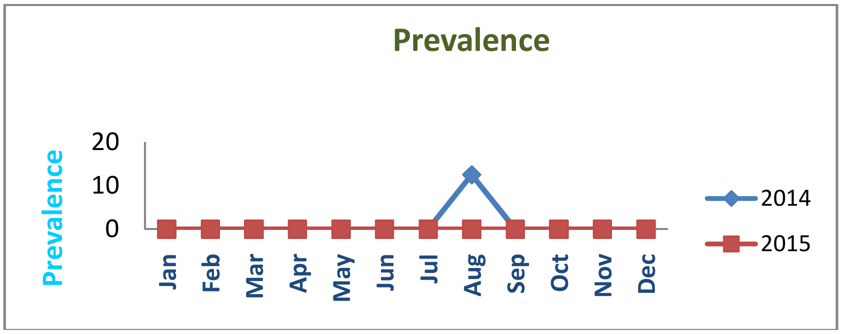


Fig.1a

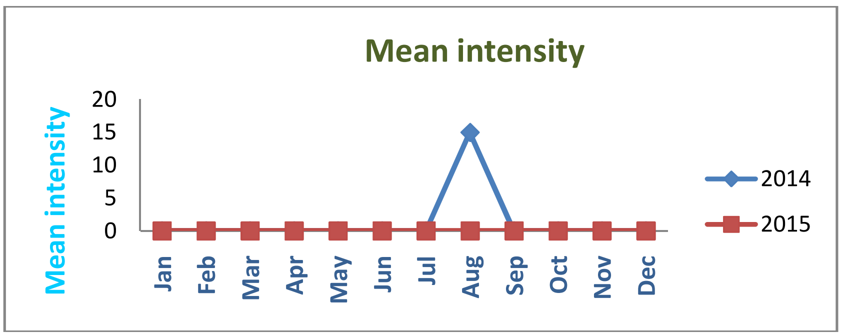
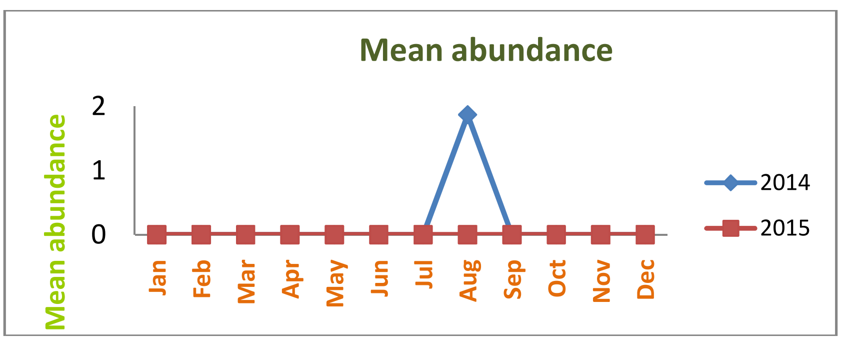


Fig.1b



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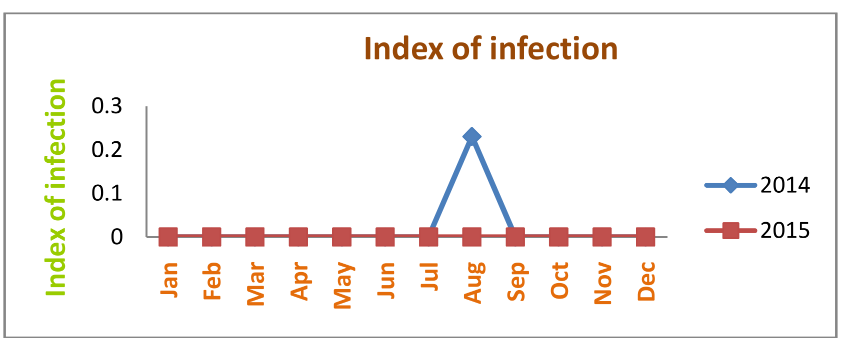

Fig.1d

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

Plate 7. Monthly population dynamics of isopod parasites in *Rhizoprionodon acutus*

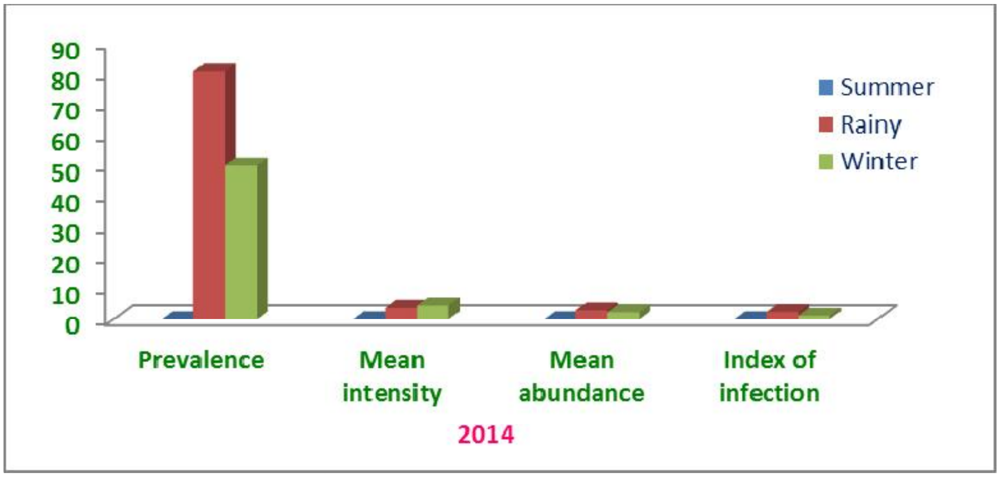

Fig. 1a

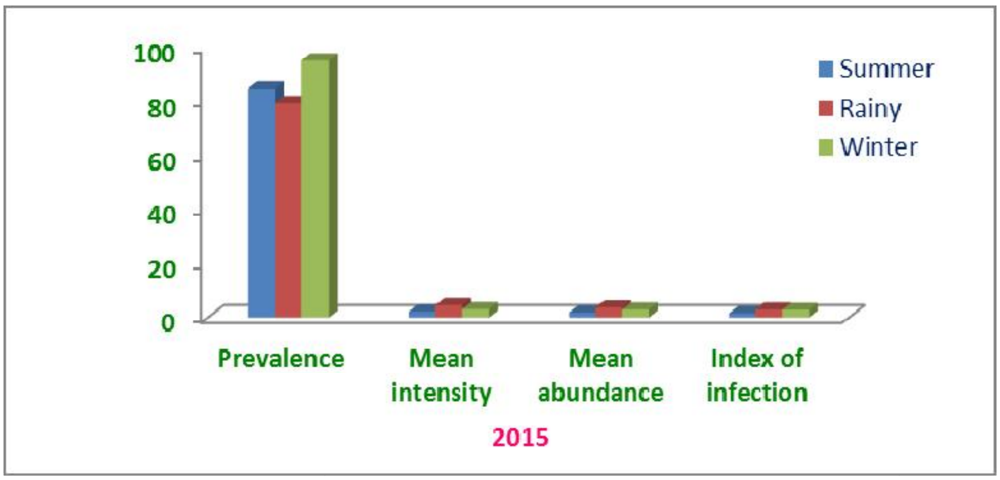

Fig. 1b

Plate 8. Fig. 1. Seasonal dynamics of overall parasitization in *R. acutus*

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

For the 2014 cycle, monogenean infection was noticed only in August. However, for the 2015 cycle, moderate to high prevalence values was noticed in January, August, September, November. Parasitization was nil from February to July for both the years. Mean intensity and mean abundance of monogenean parasites was highest in the month of August for both the cycles and negligible in rest of the months. Index of infection was nil for the 2014 cycle and only two months (January and August) of the 2015 cycle showed moderate values.

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

Prevalence of parasitisation with cestodes showed some variations in both the years. The 2014 cycle showed parasitisation only from July to December with high prevelance. However, 2015 cycle showed zig zag pattern with the highest prevalence (January-March and May) and rest of the months showed moderate values. The mean intensity of cestodes gradually increased from July to December for 2014 cycle whereas 2015 cycle showed highest MI value (5.57) in the month of August and rest of the months showed fluctuations in the values. Mean abundance and index of infection of cestodes were high in November for 2014 cycle and in August for 2015 cycle respectively.

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

The infection with nematodes is sporadic with an infection in only three months (October-December) of the 2014 cycle and four months (January, March, November and December) pf the 2015 cycle. Nematodes showed the highest prevalence, mean intensity, mean abundance and index of infection in October for 2014 cycle and in January and November of 2015 cycle respectively.

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

The infection with copepods is very sporadic with an infection in only one month (December) of the 2014 cycle and seven months (February, March and August to December). Copepods showed the highest prevalence, mean intensity, mean abundance and index of infection in December for 2014 cycle, however, 2015 cycle showed variations with highest prevalence and mean intensity being in March and December, highest mean abundance being in August and highest index of infection being in March respectively.

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

Infection with Isopods was very rare as it occurred only once in the month of August, 2014 entire two years study period. There was no infection of isopods in the year 2015.

3.2 Seasonal Dynamics of Parasites in *Rhizoprionodon acutus*

I. 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. However, 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, Figs. 1a & 1b). Recruitment of the parasites may take place after summer and reach their peak periods in the winter months.

4. 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 behaviour. Elasmobranchs were infected with a large number of parasites during the winter months when the temperature falls; 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. Rohde articulated the same view that infections are more in warm seas than in colder ones [29]. At moderate temperatures the zooplankton fauna may be rich when compared to high temperatures of summer months in tropical areas. The sea remains quiet and calm with very few disturbances during the winter months; hence the recruitment of infection may take place after summer and reach its peak in winter months. Thus, temperature and season play a crucial role in the recruitment of parasite fauna. The present study comes closer to the views of the Kennedy and Rohde where the prevalence of parasites in *R. acutus* was relatively high in the rainy and winter season than the summer season [27-29]. 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 Kennedy 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 [36]. 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]. However, mixed results on the impact of seasons on the incidence of parasitization for annual cycles 2014 and 2015 indicated insignificant influence of seasons on the parasitization which might be due to the drastic changes environmental conditions due to the anthropogenic activities. Thus, it can be concluded that the present work showed evident variations in the population dynamics statistics of the parasites within the host.

5. CONCLUSION

Marine fishes are very significant in commercial fisheries in various parts of the world including India. Population dynamic studies of parasites within the host fish will enable us to predict the behaviour of the parasites with respect to the changing environmental conditions. This study was conducted for two consecutive years to depict the nature of occurrence of parasites within *R. acutus* in terms of changing environmental condition such as temperature and seasons etc. 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 role of temperature and season in the recruitment of parasite fauna was very well documented. The prevalence of infection, mean intensity, mean abundance, index of infection was noticed to be high during the months of July to December due to low temperatures and low during the months of January-June because of high temperatures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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