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GUT HISTOLOGY, NUTRITIONAL PHYSIOLOGY AND HOST SPECIFICITY OF THE FRESHWATER TEMNOCEPHALID *Paracaridinicola platei* (TURBELLARIA, TEMNOCEPHALIDA) (FERNANDO, 1952) BAER, 1953

by

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INTRODUCTION

Paracaridinicola platei is an ecto-commensal temnocephalid, found within the branchial chamber or crawling about in a leech-like manner on the anterior region of its host-the shrimp. Using its posterior suckers the animal adheres to its host and explores the food resources of different locations as the shrimp darts about. Temnocephalids within the branchial chamber of the host obviously make use of the continuous gill ventilating current which brings in minute organisms for feeding.

Plate (1914) has identified two species of temnocephalids namely *Paracaridinicola platei* and *Monodiscus parvus* in the Kandy Lake. Studies hitherto on these organisms however, have been meagre.

Fernando (1945, 1952) has described the basic morphology and aspects of glycogen storage in *P. platei*. Later, Breckenridge and Nathanael (1988) and Nathanael 1990 (a) and (b) have conducted studies on the vitelline glands, biology and population dynamics of this interesting ecto-commensal. The present study is focused on the histology of the alimentary canal, some aspects of digestive physiology and host specificity in *P. platei*.

Study site and habitat

Specimens of *P. platei* for the present study were collected from the Kandy Lake which is an ornamental man-made lake situated between 80° 39' E and 7° 18" N at an elevation of 510m above mean sea level. This lake is characterized by the paucity of aquatic macrophytes. The circumference of the lake is about 3.25 km, with a maximum depth of 14m, and situated in a valley flanked by steep hills. The drainage area therefore cannot be reckoned to be large. Although a small quantity of untreated sewage flows into the lake, sewage from the city as such, has no access. It can therefore be inferred that the inflow of organic matter and nutrients into the lake is small. (De Silva and De Silva, 1984).

The climate is mainly equatorial and is influenced by the South-west (May to September) and the North-east (September to December) monsoons, which are governed by wind and wind direction. The normal pattern of rainfall is blurred by the incidence of freak inter-monsoonal convectional rains.

Temnocephlids were collected from a sampling site 16 metres long with a width of one metre. This site was mainly sandy with plenty of roots and decaying matter. Two branches of a kumbuk tree *Terminalia arjuna* overhang this sampling site, shading it from direct sunlight. The embankment around this site was not steep, and the water at times appeared to be turbid, but there were no signs of human interference in the form of feeding of fish or the dumping of refuse. No significant fluctuation in temperature or pH in the water was observed-the water temperature varying between 26° C - 30° C, and the pH between 7.1-8.5. The pH was measured using a portable electronic pH meter by collecting water samples at a depth between 50-100 cm. The shrimp *Caridina fernandoi* which forms the habitat of the ecto-commensal is found along the edges of the Kandy Lake, where the intensity and flow of water is rather low. They are found swimming or resting among the roots, overhanging grass or among the washed out debris on the shore. Generally, the temnocephalids are found within the branchial chamber and also externally on the anterior region of the shrimp. Although not markedly gregarious, they may be found in groups of two or three near the eyes, on the rostrum or antennae.

MATERIALS AND METHODS

A scoop net which could be swept along the water's edge was used for collecting shrimps for the study. Shrimps gathered this way were quickly transported to the laboratory in glass jars. Temnocephalids present on the anterior region of the shrimp were picked out with a pair of fine jeweller's forceps. The carapace was then removed and temnocephalids within the branchial chamber were also picked out for examination.

Routine histological and histochemical procedures were employed for a study on the process of digestion and food reserves. For routine histological observations, the animals were fixed in Bouins, Heidenhains Susa and Formol calcium (Pearse, 1968), processed in the usual manner, embedded in paraplast and sectioned serially at a thickness of 5 microns. The sections were then stained with Haematoxylin/Eosin, Halmi's and Azan (vide Humason, 1967).

Specific histochemical tests involved those for protein using Bromphenol blue (Pearse, 1968) and for glycogen identified with the PAS/diastase procedure and with Best's carmine (Spicer, et al., 1967) while the distribution of lipids was studied by staining sections of formol fixed specimens with Sudan Black B (Humason, 1967).

For host specificity studies in the laboratory, ten specimens each of three species of shrimps- *Caridina fernandoi Caridina simoni* (from the Kandy Lake) and *Caridina pristis* (from the Maha oya stream, Peradeniya), were reared separately in well aerated glass jars, at room temperature. All specimens were approximately of the same size and were free of ecto-commensals. An equal number of ecto-commensals were then introduced into each of these jars, and the progress of infection was monitored, at pre-determined intervals of three, seven, eleven and fifteen days. Parallel observations were made under precisely the same experimental conditions in a collateral study, using mixed populations of two host groups *(C. fernandoi/C. simoni and C. fernandoi/C. pristis)* which were subjected to ecto-commensal infection.

RESULTS

The food and feeding mechanism of *P. platei* have been dealt with in an earlier publication. (See Nathanael (1990a).

It was found that regurgitation of the gut contents occurred when fresh specimens are flattened on a glass slide by the application of slight pressure on the cover slip. There was no difficulty in identifying rotifers, crustacean larvae, ciliate protozoans and unicellular plant material in the gut contents. The plant material may have been ingested directly or else come from the guts of ingested food organisms. However, quantification and identification of individual food items was not feasible since the gut contents were in a partially digested state.

Gut Histology

The alimentary canal of *P. platei* consists of a mouth, pharynx and blind sac-like intestine. The intestinal wall of the single layered gastrodermis is composed predominantly of large columnar phagocytic cells. There are also spherical to oval glandular cells which stain very intensely with Bromphenol blue. (Fig. 1) A few of these cells are horizontal and lie below the gastrodermis. They open *via* long necks in between the columnar cells into the gastrodermal lumen.

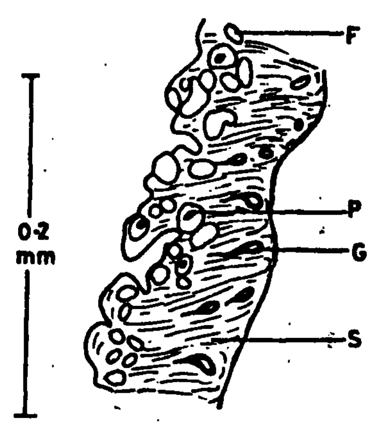

Fig. 1. Part of transverse section of gut of *P. platei* stained with Bromphenol blue.

Digestive Physiology

The columnar phagocytic cells appear to commence phagocytosis immediately, as soon as food enters the intestine. The fragmented food particles become enclosed in food vacuoles, where they undergo digestion and absorption. The cytoplasm of each columnar cell contains between four to seven food vacuoles of different sizes. The diameter of these vacuoles range from 0.0066-0.0017µm. As food is engulfed, the columnar cells become loaded with food vacuoles which gradually pass down toward the basal region. In animals where food has been in the process of digestion for sometime, the entire gastrodermis takes on the appearance of a highly vacuolated syncytium. As digestion progresses and the number of food vacuoles increase, the height of the gastrodermal syncytium increases as well, and the intestinal lumen appears constricted. The contents within the food vacuoles gradually condense, become homogeneous and form darkly staining compact masses.

On the basis of histological and histochemical tests it was noted that the substance within the vacuole was strongly proteinaceous and eosinophilous, since it stains a deep blue with Bromphenol blue, and a deep pink with Eosin. It also stains an intense red with Azan and orange with Halmi's. The presence of acid and alkaline phosphatases within the vacuoles is taken to be an indication that the food is completely hydrolysed into soluble constituents by enzymatic processes (For details see Nathanael (1990 a).

FOOD RESERVES

Staining with Best's carmine showed that glycogen deposits were present in the musculature of the pharynx, within the parenchyma, and also in varying degrees within the food vacuoles. Staining for lipids indicated that the gastrodermis contained lipid droplets, as well. The amount of lipid present was variable and probably depended on the type of food ingested and the degree of feeding and digestion.

HOST SPECIFICITY

Laboratory observations on the survivorship of *P. platei* on the three species of shrimps studied showed that there was a gradation-with best survival on *C.fernandoi* and least on *C. simoni* (Fig. 2). In the case of the mixed populations, it was evident that at first the ecto-commensals randomly clambered onto the nearest shrimp. With time however, (one week) it was apparent that the ecto-commensals showed a marked preference for *C.fernandoi*. At the end of the two week period the mixed population of *C. fernandoi* revealed the total absence of ecto-commensals on *C. simoni* and a 70% survivorship on *C. fernandoi*. Examination of the mixed population containing *C. fernandoi* and *C. pristis* revealed that only 10% of the *C. pristis* were infected in contrast to *C. fernandoi* where all were infected.

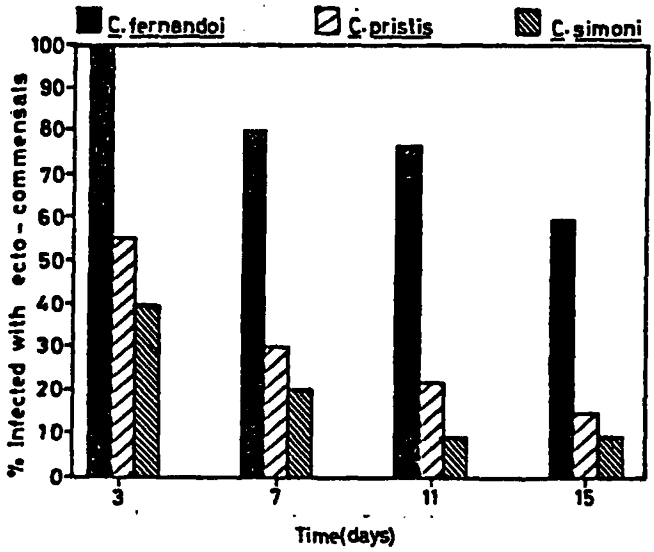

Fig. 2. Survivorship of *Paracaridinicola platei* on the three species of atyid shrimps.

DISCUSSION

The fresh-water habitat of the Kandy Lake does not appear to have influenced the basic pattern of nutrition of *P. platei*, which is very similar to that of free living rhabdocoels. The only point of difference, is the greatly increased emphasis on the formation and storage of glycogen, since Jennings (1968) has found that free living forms generally store only small amounts of food reserves in the form of lipids.

P. platei lives in a lake where food is plentiful, and has made use of the host as a means of efficiently exploiting the food resources of the environment with minimum wastage of energy. The host shrimp is used as a secure feeding platform, and the animal is effortlessly transported to different locations (especially at night) when the shrimps are active., In addition, the gill ventilating current provides a continuous source of food to the developing young.

Judging from the nature of the diet, mode of digestion and presence of food reserves it is pertinent to infer from this study that the animal is capable of living a completely independent life. Since it does not use the host's food or digestive enzymes, the host confers only a temporary benefit to the animal. With the death of the host the ecto-commensal just drops off, and attaches itself to leaves or other debris, crawling about in a leech-like manner until another suitable host is found. Although examination of the branchial chamber revealed no damage to host tissues due to feeding of the ecto-commensals, it is conceivable that two or three ecto-commensals attaching themselves to the antennae of the host could impede its activities.

The structure of the alimentary canal, the diet and feeding mechanism of *P. platei* are very similar to free living rhabdocoels and show no marked deviation from the typical turbellarian pattern. The sequential stages of digestion too appear to be typically turbellarian (Jennings, 1974) involving both acid and alkaline phosphatases. The ecto-commensal mode of life therefore does not appear to cause any significant divergence from the pattern of nutrition observed among free living forms.

Unlike free-living predatory turbellarians where lipids form the main food reserve *P. platei* has food stored in the form of glycogen, as well as lipids. In symbionts like *Paravortex scrobiculariae* (Jennings, 1981), the metabolism depends on anaerobic glycolysis, and so they store large amounts of glycogen. The fact that *P. platei* does not live in an anaerobic habitat probably explains why this form of metabolism does not take place. It is therefore possible that the food reserves stored are used up in other ways. While living on the host shrimp these ecto-commensals have to overcome problems of dispersal and establishment of their progeny on new hosts. This is accomplished mainly by the production of a large number of eggs. Since food appears to be readily available to this animal the food reserves are probably set aside for eventual inclusion in eggs, or for use as an energy-releasing substrate during egg production. It is also possible that the presence of these food reserves enable the animal to be active during critical periods, when there are fluctuations in host activity.

In the context of host specificity the observations of Nappi and Crawford (1984) on the occurrence and distribution of the turbellarian *Syndesmis* in sea urchins are interesting. They report the absence of *Syndesmis* in the sea urchin *Tripneustes ventricosus*, a species found in large numbers in each of the study sites occupied by infected urchins of the species *Lytechinus variegatus*. The authors suggest that there my be certain physiological differences which account for this type of host specificity. This may be true for *P. platei* as well, where *C. fernandoi* appears to be the favoured host under both natural and artificial conditions.

Our results are not at variance with those of Jennings (1968) who studied feeding, digestion and food storage of two temnocephalids, *T. brenesi* (from Costa Rica) and *T. novae* zealandiae (from New Zealand). The only difference is that unlike *P. platei* neither *T. brenesi* or *T. novae zealandiae* have any significant reserves of fat or glycogen in the parenchyma or gastrodermis, although both substances were found in the reproductive organs. In all these cases it has been found that the ecto-commensal mode of life has not caused any significant change in the general pattern of nutrition characteristic of free-living forms living in similar habitats.

The concept of commensalism provides a useful background against which more turbellarian associations could be considered. Turbellarians are involved in a variety of associations with other animals ranging from ecto and ento commensalism to true parasitism. It has been discovered that the umagillid rhabdocoel *Syndesmis fransciscana* living in the gut and coelom of the sea urchin *Lytechinus variegatus* in Jamaica, supplements its diet of protozoa by ingestion of host coelomocytes (Jennings and Mettrick, 1968). This may be an indication that the parasitic habit arose from commensalism-needing only a shift in dietary preference. Parasitic turbellarians like *Fecampia, Kronborgia* and *Oikiocolax* in contrast, show complete dependence on the host for nutrition. The typical rhabdocoel alimentary canal is reduced or absent, and the uptake of nutrients is by absorption through the body surface. In the case of *Kronborgia* and *Oikiocolax* (Jennings, 1971) the host suffers parasitic castration, resulting in eventual death in the former case.

It is hoped that the present study would promote future research on similar animal relationships in fresh water organisms, where food, shelter or both provided by the host, are made use of to different degrees.

SUMMARY

This study deals with some aspects of the nutrition and host specificity of *Paracaridinicola platei* (Fernando, 1952) Baer, 1953 an ecto-commensal temnocephalid found in the Kandy Lake of Sri Lanka, a tropical man-made lake with an area of about 18 ha.

Observations have been made on the histology of the alimentary canal and nutritional physiology and ecology.

Glycogen and lipids constitute the main food reserves, of *P. platei.* Laboratory studies on host specificity revealed that this ecto-commensal has a definite preference for the atyid shrimp *Caridina fernandoi*. A brief comparison is made with free living, endo-commensal and parasitic turbellarians found in similar habitats. This study is also important in confirmation of observations on glycogen storage in temnocephlids which cannot be found in free-living turbellaria. It is felt that future research along these lines on similar relationships in freshwater habitats could yield illuminating results.

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