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Host specificity and biochemical changes in fishes owing to the infestation of the isopod, *Alitropus typus* M. Edwards (Crustacea : Flabellifera : Aegidae)

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Abstract. Data are presented to indicate the nature of host-specificity shown by the juveniles of the isopod, *Alitropus typus* M. Edwards and the biochemical changes in certain host fishes attending the infestation. Even though specific host preference is apparently lacking, the frequency of attack on different fishes varies. Thus among the 9 species of fishes tried, *Anabas testudineus* (Bloch) was found to be most severely attacked by these isopods whereas *Puntius sarana subnasutus* (Val.) was comparatively less affected. Proximate analyses on 5 different species of fish, viz., *Channa orientalis* (Cuvier), *Anabas testudineus* (Bloch), *Lebistes reticulatus* (Peters) ; *Tilapia mossambica* (Peters) and *Macropodus cupanus* (Cuv. and Val.) suggest depletion of biochemical constituents on account of infestation. An increase in the moisture content in the infested fish from those of the non-infested ones was noticed with the increase ranging from 1·19% in the female *T. mossambica* to 10·15% in the male *L. reticulatus*. A drastic fall in protein, glycogen and lipid was also observed in the infested fish tested. Among the various fishes tried, the minimum depletion of glycogen occurred in *C. orientalis* (1·44%) and the maximum in the female *M. cupanus* (7·16%). In the case of lipid, the male *L. reticulatus* showed only marginal reduction (0·96%) whereas *C. orientalis* showed 13·20% depletion. Regarding protein, the maximum reduction after infestation was observed in *A. testudineus* (20·91%). The possible reasons for the depletion of these biochemical constituents in the infested fish are discussed in detail.

Keywords. *Alitropus typus* infestation ; biochemical constituents in fish ; host-specificity.

1. Introduction

Scientific information on the host-specificity and variations in the biochemical composition of fishes parasitized by isopods is meagre. However, the morphological and behavioural changes occurring in the host fishes as a result of infestation of parasitic isopods, mainly of cymathoids, have been widely reported. These changes are partial atrophy of the gills of fishes (Borgea 1933; Bowman 1960), deformation of the mouth of the host, changes in the behaviour of the fish, its feeding and growth (Turner and Roe 1967; Kroger and Guthrie 1972), retardation of growth and deterioration in the general condition of the host (Akhmerov 1939, 1941; Krykhtin 1951; Petrushevski and Shulman 1961).

In recent years some attempts have been made to explain the biochemical changes in fishes owing to the attack of copepod parasites. Noteworthy among these are the publications of Kabata (1958), Abrosov *et al* (1963), Mann (1965), Natarajan and Nair (1976), and Susheela *et al* (1977). The paucity of published data on the effect of infestation of the isopod, *Alitropus typus* on different fishes prompted this study. This flabelliferan group which comes under the family Aegidae is a common inhabitant of the littoral regions of the fresh and brackish water tracts along the south-west coast of India. The juveniles of the species (body length ranging from 3·5 mm to 9 mm) lead a parasitic life inside the gills and opercular regions of various fishes feeding on the blood and mucus of the hosts. The present study thus aims at evaluating the nature of parasitism shown by the flabelliferans and also the biochemical changes in some of the fishes owing to the infestation of these animals. Observations were also made to test the host-specificity shown by these parasites when different fresh and brackish water fishes were offered as hosts.

2. Material and methods

With a view to studying the host-specificity, 9 species of fresh and brackish water fishes which are common in the littoral regions of fresh and brackish water habitats of *A. typus* were selected. They were *Anabas testudineus* (Bloch) and *Macropodus cupanus dayi* (Cuv. and Val.) belonging to the family Anabantidae; *Channa* (*Ophiocephalus*) *orientalis* (Cuvier) belonging to the family Channidae; *Rasbora daniconius* (Hamilton–Buchanan) and *Puntius sarana subnasutus* (Val) belonging to the family Cyprinidae; *Tilapia mossambica* (Peters), *Etroplus maculatus* (Bloch) and *Etroplus suratensis* (Bloch) belonging to the family Cichlidae and finally *Lebistes reticulatus* (Peters) belonging to the family Cyprinodontidae. Fifty juveniles of *A. typus* (body length ranging from 3·5 mm to 7·0 mm) were kept in a glass trough containing 4 litres of well water. One specimen from each of the above-mentioned species of fish was then introduced into the trough after measuring its body length. After 8 hours, each fish was taken out and the total number of attached parasites was counted. The test was repeated 5 times. The duration of the experiment was purposefully restricted to 8 hr, since by then, the guts of the parasites became filled with the ingested blood and mucus of the host and they showed a tendency to drop off.

The biochemical changes as a result of infestation were studied in 5 different species of fishes (selected on the basis of host-specificity experiments). These were *Channa orientalis* (young ones), *Anabas testudineus, Lebistes reticulatus, Tilapia mossambica* and *Macropodus cupanus.* Body length measurements and sex, wherever possible, were noted in all the fishes. Each fish was kept in a 1 litre cylindrical jar containing 750 cc of well water and 5 juveniles of *A. typus* were introduced into each jar and were allowed to infest the fishes for 8 hr. The number of parasites provided was restricted to 5 only since there was a possibility of the fish being killed by their attack if more numbers were introduced. Jars containing fishes without parasites were also kept as controls. For *L. reticulatus*, *T. mossambica* and *Macropodus cupanus*, 10 infested and non infested males and females each were taken, while for *C. orientalis* and *A. testudineus* where sexes could not be differentiated by external morphological characters, a total of 10 specimens each of the infested and non-infested fishes were chosen. After 8 hr of infestation, the parasites were removed from the host and 2 each of the infested fishes were kept in separate jars for 20 hr to study the rate of survival after the attack of the parasites. Young *C. orientalis* and *L. reticulatus* (male) did not survive this period.

Infested and non-infested fishes were killed and weighed accurately and then dried to constant weight for determining the moisture content. The dried fishes were then powdered and kept in the desiccator for the biochemical analyses. Small quantities of the powdered tissue were then weighed and assayed for glycogen employing the Anthrone reagent procedure of Seifter *et al* (1950). The total protein was estimated by Wong's Microkjeldhal method (1923) and the total lipids were determined by the chloroform–methanol method described by Heath and Barnes (1970).

3. Observation and results

Juveniles of *A. typus* attached to the gill chambers of fishes were found to feed on the blood and mucus of th hosts. The position of the juveniles inside the gill chamber of the host was also characteristic being usually attached to the upper part of the gill arcs facing the water flow or in the buccal cavity itself clinging to the floor of the mouth and again facing the water flow. In majority of cases, these isopods were typically found in one gill chamber only. Oxygen uptake in the parasitized gill chamber would naturally be reduced and possibly this accounted for the fact that only one gill chamber was parasitized. Infestation of both chambers would suffocate and kill the hosts. Occasionally a single fish attacked on both gills by a number of starving parasites, sometimes as many as 20, had been noticed and as a result the opercula of the fish were widely gaping and gills severely damaged leading to serious difficulties in respiration and even death.

The host-specificity tests showed that all the fishes exposed were attacked by these parasites (table 1) even though the frequency of attack varied in the different fishes studied (table 2). As may be seen, the host fishes chosen for the present study could be grouped into 5 categories depending on the total number of parasites found on them (this was done by comparing differences of mean percentages with the critical differences using 5% table value). Thus categories I and II consists of those fishes attacked by the largest number of parasites. *Anabas testudineus* with the maximum number of parasites attached into them falls under category I followed by *Macropodus cupanus*, *Tilapia mossambica* and *Channa orientalis* also subjected to heavy attack but not to the extent of *A. testudineus*. Category III includes *Lebistes reticulatus* and *Rasbora daniconius* which are also attacked by the parasites in fair numbers. Categories IV and V comprise those fishes with comparatively lesser number of parasites. Thus *Etroplus suratensis*

Table 1. *A. typus*: Incidence of parasites (percentage) on the body of different host fishes (Transformed values of the percentages to angles are shown in brackets).

Fishes provided	Replication %					
	1	2	3	4	5	Total
Lebistes reticulatus	5·56 (13·69)	13·33 (21·39)	6·52 (14·77)	4·55 (12·38)	11·90 (20·18)	82·41
Macropodus cupanus	16·67 (24·12)	2·22 (8·53)	10·87 (19·28)	13·64 (21·64)	16·67 (24·12)	97·69
Tilapia mossambica	8·33 (16·74)	15·56 (23·26)	8·70 (17·15)	6·82 (15·12)	21·43 (27·56)	99·93
Anabas testudineus	16·67 (24·12)	46·67 (43·11)	30·43 (33·46)	52·27 (46·32)	16·67 (24·12)	171·13
Puntius sarana subnasutus	2·78 (9·63)	2·22 (8·53)	6·52 (14·77)	4·55 (12·38)	0 —	45·31
Etroplus suratensis	0 —	0 —	8·70 (17·15)	2·27 (8·72)	19·05 (25·91)	51·78
Etroplus maculatus	19·44 (26·13)	0 —	4·35 (12·11)	0 —	2·38 (8·91)	47·15
Rasbora daniconius	22·22 (28·11)	4·44 (12·11)	16·22 (23·73)	2·27 (8·72)	2·38 (8·91)	81·58
Channa orientalis	8·33 (16·74)	15·56 (23·26)	8·70 (17·15)	13·64 (21·64)	9·52 (17·95)	96·74

Table 2. Analysis of variance.

	d.f.	SS	MSS	F. ratio
Total	44	4639·3197		
Replication	4	58·0049	14·5012	0·2205*
Treatments	8	2411·7751	301·4719	4·23**
Error	32	2169·5397	67·7981	

* Not Significant. ** Significant at 5% level.

falls under category IV and *Etroplus maculatus* and *Puntius sarana* under category V.

The data obtained on the variations in the biochemical constituents of fishes in different stages such as non-infested and infested with parasites are given in table 3. Parasite free *C. orientalis* and *A. testudineus* exhibited a moisture content of 84·85% and 68·79% and after infestation, the moisture content increased to 87·09% and 72·32% respectively. Water content of the males and females of *L. reticulatus*, *T. mossambica* and *M. cupanus* also increased in varying proportions after infestation (table 3).

Table 3. Changes in the biochemical constituents of host fishes due to the infestation of juvenile *A. typus*.

Duration of infestation : 8 hours   Total number of parasites provided to each fish : 5

Fish provided	Sex	Total no. taken	Mean body length (mm)	Water content (%)	Glycogen (%)	Lipid (%)	Protein (%)
*Channa orientalis** (Young ones)							
Non-infested	—	10	23·90	84·85	1·76	15·26	71·56
Infested	—	8	24·10	87·09	0·32	2·06	63·49
*Anabas testudineus**							
Non-infested	—	10	47·80	68·79	7·77	6·09	69·71
Infested	—	8	48·70	72·32	2·23	3·46	48·80
Lebistes reticulatus							
Non-infested	M	10	27·70	71·62	5·42	7·59	54·37
	F	10	31·00	72·36	6·89	16·56	63·59
Infested	M	8	28·57	81·77	0·17	6·63	50·41
	F	8	29·10	74·93	0·18	7·82	56·17
Tilapia mossambica							
Non-infested	M	10	49·00	72·79	5·18	9·85	64·81
	F	10	45·75	74·24	6·38	10·20	59·00
Infested	M	8	47·25	75·11	0·68	2·56	53·39
	F	8	46·25	75·43	0·93	6·44	51·80
Macropodus cupanus							
Non-infested	M	10	53·00	68·33	9·08	15·67	67·50
	F	10	45·00	64·53	10·39	18·29	68·32
Infested	M	8	56·70	70·89	3·83	12·44	47·88
	F	8	46·00	66·74	3·23	7·09	48·66

* Sex could not be differentiated by external character.

The glycogen content of the parasite free *C. orientalis* and *A. testudineus* exhibited 1·76% and 7·77% respectively which decreased to 0·32% and 2·23% after infestation. The same decreasing trend after infestation was exhibited by both the sexes of *L. reticulatus, T. mossambica* and *M. cupanus* also (table 3).

Regarding lipid reserves, the fishes exhibited considerable differences. Thus *C. orientalis* showed the maximum depletion after infestation, the percentage falling to 2·06% from 15·26%. This was followed by the male *T. mossambica* and the female *M. cupanus* and *L. reticulatus*. The minimum depletion occurred in the males of *M. cupanus* and *L. reticulatus* (table 3).

The protein content also showed reduction in the infested fishes. Here, the maximum depletion occurred in *A. testudineus* followed by both the sexes of *M. cupanus* and the minimum in the male *L. reticulatus* (table 3).

4. Discussion

In India, relatively very little is known about the harmful effects of parasitic isopods on fresh and brackish water isopods. The juveniles of *A. typus* has been recorded during the present study from the gill chambers of many freshwater fishes. Parasitic isopods were formerly considered as being absolutely host-specific so that each species of host was assumed to harbour a distinctive species of parasite (Baer 1952). However, further investigations made on this aspect revealed the fact that the host–parasite relationship was not as highly specialized as was formerly assumed and the relationship was found to be distinctly ecological and the infestation of these parasites occurred on appropriate hosts that appeared in the cycle fashion within a given biotope. Experimental observations made on the host-specificity shown by *A. typus* also revealed that it does not show any host preference as such and that it would attach readily to any fish that is found within reach. Both the speed of the fish and the levels at which it swims seem to influence the attack.

The results of the biochemical analyses reveal, that the fishes are seriously affected by the parasite. The tissue of the infested fish shows changes with respect to its biochemical composition. The moisture content increased in all the fishes owing to infestation. Although a marginal increase in the moisture content in the infested fish occurs due to handling and exercise, the major factor for the increase is due to the decrease of organic constituents such as protein, glycogen and lipid. The reasons for the wide variations in moisture content in different species and sexes are not clear even though it is generally accepted that in non-fatty fish an inverse relationship between protein and water content exists and Love (1970) first proposed the "protein-water line" for the muscles of non-fatty fishes. Similar observations were made in the present study also wherein a well-defined inverse relationship between protein and water content was discernible.

The depletion of glycogen in the infested fish was due to the feeding of blood by the parasites which utilize the blood sugar as a source of energy reserve. Glycogen may be utilized by the arthropod parasites for the synthesis of chitin and also for moulting (Faure–Fremict 1913; Renaud 1949). Thus the depletion of glycogen in the infested fish might be due to the result of absorption of blood sugars by the parasite for converting it into polysaccharides for the synthesis of chitin, which is essential for the formation of the exoskeleton. It should, however, be borne in mind that a certain reduction of glycogen in the host fish also occurred as a result of the stress which the fish underwent as a result of the clogging of the gill chambers by these parasites.

From the present study it would appear that the percentage reduction of protein in the infested fish was not as high when compared with glycogen and lipids. Here the maximum percentage reduction in protein occurred in *A. testudineus* and the minimum in the males of *L. reticulatus.* It is generally recognized that parasites living in oxygen-rich surroundings such as blood could theoretically derive most of their energy from the oxidation of lipids and proteins. Lipids accumulate throughout the development of arthropod parasites which can digest fats by the activation of the enzyme lipase (Levenhook 1951). Lipids require substantially more oxygen for the release of energy but this was provided by the host's arterial blood on which the parasite fed. But if the lipids and protein were both used for the production of energy, both the constituents should show parallel trends in quantitative increase or decrease (Barnes *et al* 1963). But in the present study lipids and protein behaved differently. Thus the percentage reduction of lipids in the host fishes was substantially higher than that of protein and this suggests that the energy requirement of the parasite is met to a great extent by lipids. But one could note an inverse relationship existing between the reduction of glycogen and protein in the infested fishes. In those fishes where a high reduction of glycogen occurred for meeting their energy requirements, the reduction of protein was kept to the minimum level and *vice versa.*

From the above-mentioned observations, it is clear that the worst sufferers of fish on account of the infestation of *A. typus* are the young ones of *C. orientalis* and the adults of *L. reticulatus* and many specimens belonging to these species succumbed to the attack of this parasite.

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* Original not seen.