EPIZOOTIC ASSOCIATION OF *VORTICELLA* WITH THE DEVELOPING EGGS OF *PUNTIUS CONCHONIUS*

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PERITRICHOUS ciliates enter into an ectoparasitic association with fish eggs. Such an association has been frequently reported from the skin and gills of both freshwater and marine fishes[1–4] but there are only a few such reports[5] involving fish eggs or early life history stages. The present communication reports an infestation by *Vorticella* (protozoa; ciliata; peritrichida) on the fertilized eggs of the freshwater fish, *Puntius conchonius*. The influence of this association on the hatching ability of the eggs of this fish is described.

During November 1984, a large number of eggs of *P. conchonius* were collected from a previously marked breeding site at Gho-manasan, 14 km northwest of Jammu, (32.67 lat.N; 74.79 long.E). The eggs lay scattered in algal mats in the form of beaded strings, with a glossy appearance. The eggs were transported in open containers (with frequent change of water) to the laboratory where the fertilized eggs and the developing embryoes were sorted out. Of the 450 fertilized eggs, 15 unusual bluish eggs were noted (table 1). These eggs carried, on their surface, a tuft of peritrichously ciliated protozoa (*Vorticella*). The number of vorticella infesting the eggs varied between 100 and 200 (table 1), with approximately half of the infested lot carrying 161 to 180 protozoa per egg. The infested eggs appeared healthy and showed normal development; however, the infestation appeared to affect their hatching duration (table 2).

The infested eggs were incubated at 22°C in a "SEW" fish-egg hatching incubator. An equal number of uninfested eggs were incubated separately under similar conditions of temperature, DO, FCO_2, and pH. All the infested and control eggs yielded perfect hatchlings. The morphology of the larvae from both the infested eggs and the controls was identical.

Table 1 *Infestation index of Vorticella spp. on fertilized eggs of P. conchonius*

No. of protozoa per egg (infestation index)	Less than 100	101–120	121–140	161–180	181–200
No. of eggs affected	0	2	3	7	3
Percentage of infested eggs with different levels of infestations	00.0	13.3	20	46.6	20

Table 2 *Hatching duration and egg mortality in infested and non-infested eggs of P. conchonius*

No. of eggs	Hatching duration (h)	Egg mortality (%)	Hatching success (%)
Infested eggs:			
5	80	0	100
5	78	0	100
5	70	0	100
Uninfested eggs:			
20	89	0	100
20	85	0	100
20	78	0	100

In both the cases, the larvae were elongated, slender, transparent, broad anteriorly, tapering posteriorly and initially devoid of mouth; eyes were well marked; head was transversely oblong and adherent to the yolk-sac; the pectoral fin bud lay attached to the dorsal side of the yolk-sac; 29–30 myotomes were formed at hatching, of which 19–20 were pre-anal, the rest post-anal. No abnormal hatchling was recovered from either of the experimental sets.

The hatching period of the infested eggs was shorter than that of the non-infested control eggs. On an average, the infested fertilized eggs hatched normal larvae 8 hours earlier than did the uninfested eggs (table 2).

Since only a very small percentage of the fertilized eggs was infested, it would appear that the association was purely incidental. Nevertheless, the finding that hatching in the case of infested fertilized eggs occurred significantly quicker than in non-infested fertilized eggs of the fish, *P. conchonius* suggests that growth of *Vorticella* on the eggs of this fish does not affect the survival of the eggs or of the developing embryos. It may accelerate the pace of embryonal development of the fish in question. Although the eggs provide only a substrate for the sessile protozoa, the latter in turn possibly alters the microhabitat of the eggs in some unknown manner, and accelerates the rate of development of the embryo(s) within the infested

egg(s) and brings the egg-embryo complex to the growth stage at which the embryo ordinarily hatches.

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STUDIES ON CHANGES IN CONTENT AND FRACTIONATION OF TOTAL SOLUBLE PROTEIN IN THE COTYLEDON AND EMBRYO OF GERMINATING SUNFLOWER (*HELIANTHUS ANNUUS* L.) SEEDS

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ALTHOUGH studies on the multifarious aspects of germination of main crop seeds are available reports on sunflower seeds are scanty. The present work was therefore undertaken to outline the metabolic changes of protein initiated during the early stages of germination.

The transport of total soluble proteins from cotyledons to the embryonic parts increases with germinating time[1-4]. The total protein extracts in cotyledon as well as embryo of different seeds during germination after precipitation with various concentrations of ammonium sulphate differ widely and the concentration of ammonium sulphate necessary to elute the fractions of the total soluble proteins depends on the stage of germination[5-7].

'Peredovik' (EC 68415) variety obtained from the seed farm of this University was used. The seeds, immediately after harvest, were dried in sun. As the seeds were found to be most viable in a month or two after harvest, this experiment was carried out during this period.

Healthy seeds were soaked in sterile glass-distilled water for 6 h and allowed to germinate on a wet Whatman No. 1 filter paper spread on a petri dish of 15 cm diameter. About 30–35 seeds were taken in each petri dish and allowed to germinate in a BOD incubator in dark at 30°C. The seeds were taken out from incubation at 24, 48, 72, 96 and 120 h intervals of germination and the decoated seeds were dissected into the cotyledon and the embryo parts. The dissected parts were then wiped out of any adhering water and dried in an oven at 45–50°C. The dried materials were then ground in a sieve grinder at 40 mesh, packed in sealed polythene packets and stored in a desiccator at 4–5°C. When required, the dry powdered materials were used for analysis.

The protein was estimated by the colorimetric method of Lowry *et al*[8] as modified by Hartree[9]. The total soluble proteins were fractionated into three sub-fractions by the salting out technique, using ammonium sulphate as the precipitant.

Analysis of the total soluble proteins and their ammonium sulphate fractions is reported in tables 1 and 2. As the seeds pass through, from the initial to the final stage of germination, the total soluble protein decreased in the cotyledon and increased in the embryo. The percentage of increase in embryo protein was 12 and that of decrease in cotyledon protein was about 45.

Ammonium sulphate fraction reveal the following:

(a) Irrespective of the time of germination, 50% (or more) of the total soluble proteins are present as proteins precipitated by 50% saturation of ammonium sulphate (fraction A). The rest is distributed as proteins precipitated by 100% ammonium sulphate saturation (fraction B) and as proteins remaining in the supernatant solution after treatment with ammonium sulphate (fraction C). This is true for both cotyledon and embryo (tables 1 and 2).

(b) The percentage of total soluble protein, present as fraction A protein, instead of remaining the same, increases with increase of hours of germination and reaches a high value of 80% at the late hours of germination. On the other hand, the percentage of fraction B and fraction C proteins decreases with increase of hours of germination. The percentage of fraction B protein falls from 20 to 9.5 in cotyledon and from 27 to 7 in embryo. On the other hand, the percentage of fraction C protein decreases from 20 to 8 in the cotyledon part; in embryo part, however, the percentage of fraction C