

THE ROLE OF MOTION IN THE FORMATION OF FREE-LIVING STAGES AND ATTACHMENT OF THE PERITRICH EPIBIONT *Zoothamnium intermedium* (CILIOPHORA, PERITRICHIA)

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ABSTRACT

Precht, 1935 is a common peritrich epibiont infesting calanoid copepods in the Chesapeake Bay, USA. Formation of free-living stages (telotrochs) in sessile peritrichs can be triggered by environmental factors or, in the case of epibionts, death or molt of the host. By contrast, colonization mechanisms in peritrichs are not well understood, but free space and chemical cues from the substrate are probably involved. In the present paper, we investigated, through controlled experiments, the effects of motion on the formation of free-living stages and colonization of substrates by the peritrich epibiont *Zoothamnium intermedium*. Results revealed that zooids of *Z. intermedium* started to form telotrochs after two hours following the death of the host, and within seven hours more than 90% had formed dispersal stages, and left the substrate independent of the constant movement of the medium. Telotrochs of *Z. intermedium* were able to colonize live *Calanoides* (Poppe, 1880), but failed to attach to egg sacs when removed from the host. These results suggest that mechanical, chemical, and maybe electrical cues are involved in the formation of dispersal stages and colonization of substrates by this species of epibiont.

Key words: ciliates; epibiosis; copepods; estuary; zooplankton.

RESUMO

O papel do movimento na formação de estágios livre-natantes e colonização de substratos no peritríquio epibionte *Zoothamnium intermedium* (Ciliophora; Peritrichia)

A espécie de peritríquio *Zoothamnium intermedium* Precht, 1935 é frequentemente encontrada como epibionte em copépodos calanóides na Chesapeake Bay, EUA. A formação de estágios livre-natantes (telotróquios) em peritríquios sésseis pode ser induzida por fatores ambientais, ou no caso de epibiontes, por morte ou muda do hospedeiro. Mecanismos de colonização de substratos em peritríquios não são totalmente compreendidos, mas acredita-se que disponibilidade de espaço e sinais químicos do substrato estejam envolvidos no ritual de reconhecimento. No presente trabalho, foi investigado através de experimentos em laboratório, os efeitos do movimento na formação de telotróquios e na colonização de substratos na espécie de peritríquio epibionte *Z. intermedium*. Resultados revelaram que a formação de telotróquios ocorreu duas horas após a morte do hospedeiro e em sete horas mais de 90% dos zoóides formaram estágios de dispersão e abandonaram o substrato, independente do constante movimento do meio em que se encontravam. Telotróquios de *Z. intermedium* colonizaram o copépodo *Calanoides* (Poppe, 1880), mas não foram capazes de colonizar os sacos ovíferos deste mesmo crustáceo quando separado do corpo. Estes resultados sugerem que talvez sinais químicos, mecânicos e talvez elétricos estejam envolvidos na formação de telotróquios e na colonização de substratos por parte desta espécie de epibionte.

Palavras-chave: ciliados; epibiose; copépodos; estuário; zooplâncton.

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INTRODUCTION

Epibiotic relationships involving planktonic crustaceans are a widespread phenomenon in marine, estuarine and freshwater environments, and encompass a variety of epibiont organisms including protists, bacteria, hydrozoa, barnacles, and rotifers (FENCHEL, 1965; GREEN, 1974; FENCHEL; FINLAY, 1989; ABELLÓ; MACPHERSON, 1992; IYER; RAO, 1995; HANAMURA, 2000; GILBERT; SCHRÖDER, 2003; SONG et al., 2003). In spite of its wide occurrence, epibiosis remains poorly understood with respect to its ecological implications for both the host and the epibiont (WEISSMANN et al. 1993; AL-DHAHERI; WILLEY, 1996; CARMAN; DOBBS, 1997).

When colonizing living substrates, epibionts have to adapt to morphological fluctuations of their hosts such as growth and molting, and be able to form motile stages and seek new substrates when these changes occur (WAHL, 1989). In the case of crustacean hosts, epibionts must be able to leave their host and re-colonize the same or another substrate, after each molt.

Peritrich ciliates are common epibionts on planktonic crustaceans, and this association has been well documented in the literature (e.g. HERMANN; MIHURSKY, 1964; GREEN, 1974; HENEGBRY; RIDGEWAY, 1979; LÓPEZ et al., 1998). The life cycle of sessile peritrichs includes an attached stage known as trophont, and a free-swimming, dispersal stage called telotroch. Formation of telotroch in sessile peritrichs could be triggered by environmental factors such as low oxygen concentration, direct disturbance of the organism, or, in the case of epibionts, death or molt of the host.

Mechanisms of recognition of a living substrate or specific hosts by epibionts are still unknown, but it is believed that chemical, mechanical, or even electrical cues may be involved in the recognition of the substrate for colonization, as well as in the process of formation of free-living stages when the host molts or dies (WAHL, 1989). For example, some species of peritrich epibiont appear to be specific of one host and sometimes fail to attach to non-living substrates (CLAMP, 1973; HENEGBRY; RIDGEWAY, 1979; GILBERT; SCHRÖDER, 2003), while others are able to colonize non-living substrates, as well as living host species (MAYÉN-ESTRADA; ALADRO-LUBEL, 2000).

The ciliate peritrich Precht, 1935 is a common epibiont infesting the

calanoid copepods Dana, 1848 and (Poppe, 1880) in the Chesapeake Bay, USA. This species is able to colonize the whole body of the copepod, including swimming legs and eggs of (an egg-carrier species). Preliminary studies have demonstrated that telotroch formation in this epibiont species can be triggered by death of the copepod host (UTZ, 2003) suggesting that the lack of motility could be involved in the signal to leave the colonized substrate.

is also able to colonize different species of crustaceans, but fails to attach to non-crustacean hosts (UTZ, 2003), suggesting that not only movement, but also some characteristics in the pellicle of the substrate organism are involved in the settlement of this epibiont species.

We hypothesized that will form free-swimming stages independent of the motion of the surrounding medium, and that telotrochs will be able only to attach to the free-swimming and not to its egg sac.

MATERIALS AND METHODS

Cultures of *Acartia tonsa*, *Eurytemora affinis* and *Z. intermedium*: and were collected from the Patuxent River (a tributary of the Chesapeake Bay) using a plankton net with mesh size of 202 μm , brought live to the laboratory and examined for the presence of ciliate epibionts. Clean (without epibionts) and infested copepods (carrying) were separated from the samples, and cultures were initiated by placing 30 non-infested and 30 infested copepods from each species in 4 liter beakers filled with two liters of < 0.45 μm filtered seawater with salinity adjusted for 15 psu. was identified by analyzing characters of the cell and the colony in vivo and stained by the Protargol technique (MONTAGNES; LYNN, 1987). Full description of characters is found in Utz (2003). Copepods were fed with a mixture of (Grunow, 1880) and (Stein, 1878) grown in f/2 medium (NERAD; DAGGETT, 1992) at a final concentration of 1×10^3 cells/ml and 3×10^3 cells/ml, respectively. Algal and copepod cultures were maintained in incubator at 20°C, with cool white fluorescent lamps providing a 14 h light, 10 h dark cycle. Copepod cultures were transferred to a fresh medium with algal prey twice a week by concentration in a 20 μm -mesh net. Figure 1 shows attached to its hosts.

Telotroch formation inhibited by movement: To determine if telotroch formation is inhibited by the motion of the medium surrounding the epibiont and dead host, laboratory experiment was conducted. Infested *Paramecium* obtained from laboratory cultures were placed on a glass slide and a cover slip was gently pressed on top of the copepod. This procedure killed the host, but not the epibionts. Dead hosts and epibionts were placed in plastic Petri dishes (in each of three runs containing one copepod per dish and five replicates) filled with 10 ml of $< 0.45 \mu\text{m}$ filtered seawater with salinity adjusted for 15 psu. Petri dishes were placed on a shaker table (Lab-Line # 141740, Melrose Park, Illinois) rotating at four rpm. Agitator and experimental units were placed in an incubator at 20°C, and the number of attached zooids was assessed hourly during seven hours (time necessary for 90% of the zooids to form telotrochs and leave the dead host according to Utz (2003)).

Attachment of *Zoothamnium intermedium* to *E. affinis* eggs: To investigate if movement is involved in substrate choice by the peritrich epibiont *Zoothamnium intermedium*, a controlled experiment was developed. Infested *Paramecium* obtained from laboratory cultures were killed as described above to obtain free-swimming stages of *Paramecium*. Non-infested, ovigerous females of *Paramecium* obtained from cultures were placed on a glass slide and the egg sac was removed with a needle. Females and egg sacs were placed in distinct plastic Petri dishes (two runs with five replicates for each treatment: females and egg sacs) filled with 10 ml of $< 0.45 \mu\text{m}$ filtered seawater with salinity adjusted for 15 psu. Telotrochs of *Zoothamnium intermedium*, at a final concentration of two cells/ml, were inoculated in the dishes with *Paramecium* and with egg sacs. Experimental units were placed in an incubator at 20°C and the number of attached zooids was assessed every two hours for eight hours.

Data analyses: Tests of normality and homogeneity of variances were performed, and regression analyses were run for both experiments to determine the best-fit model of the curves. Time was used as independent variable, percentage of attached zooids was used as dependent variable. Statistical Analyses were performed using SPSS for Windows Version 11.0 (SPSS Inc.) and Sigma Stat Version 2.0 (SPSS, Inc.), and data were plotted as means \pm SE (standard error of the mean).

RESULTS

Zooids of *Zoothamnium intermedium* started to form telotrochs two hours after the death of the host and within seven hours 90% of the zooids had formed free-swimming stages as can be observed in Figure 2. No statistically significant difference was observed among different runs of the same experiment. Regression analyses showed that formation of telotroch in a moving medium fits a model of exponential decay with a brief lag phase. The pattern observed in the experiment suggests that the movement of the water does not inhibit the formation of dispersal stages in this species of epibiont.

Telotrochs of *Zoothamnium intermedium* were able to attach to females of *Paramecium* in a period of eight hours, but no attachment to the egg sacs was observed in the same period of time, as can be seen in Figure 3. Regression analyses demonstrated that attachment of free-swimming stages of *Zoothamnium intermedium* to its host follows an exponential growth model with an exponential rise to a plateau (Figure 3). The results observed in this experiment suggest that not only characteristics of the pellicle are involved in colonization of a substrate by the peritrich epibiont *Zoothamnium intermedium*, but a more complex interaction between pellicle and epibiont may be occurring in this association.

DISCUSSION

The present study investigates the role of motion in the formation and attachment of free-swimming stages of the peritrich epibiont *Zoothamnium intermedium*. According to Utz (2003), telotroch formation in this epibiont species occurs following the natural death or is induced by killing the host. When induced by the death of the host, the majority of the zooids form telotrochs within seven hours following the death of the substrate (UTZ, 2003). In the present study, the same pattern was observed (Figure 3) independent of the motion of the medium where the infested copepods were placed, suggesting the existence of a single or several cues coming from the dead host, in addition to movement and assuming that live motion was reproduced.

Telotroch formation in sessile, free-living or epibiont peritrichs occurs as a result of binary fission or from the need to leave the old and to find a new substrate for attachment (NAGASAWA, 1986). In peritrich epibionts on crustaceans, telotroch formation

can be a product of asexual reproduction or can occur immediately before or after the host has shed its molt. Fenchel (1965) reported that all species of peritrichs colonizing amphipods of the genus *Amphipoda* started forming telotroch prior to the host's molt, and were able to colonize the same host substrate just after its molt. On the other hand, Clamp (1973) observed that a species of *Amphipoda* attached to different species of shrimp in the genus *Stomatopoda* started forming telotrochs only after the host had cast its skin. Although these reports show different behaviors displayed by different species, the mechanism by which epibionts recognize that the host is about to molt or has molted are still unknown, but perhaps biochemical changes in the host's body fluids or hormonal fluctuation play an important role in this process (CLAMP, 1973).

The formation of free-swimming stages by the peritrich epibiont *Amphipoda* when in a medium moving constantly, investigated in the present work, suggests that maybe a combination of a mechanical, chemical, and even electrical stimuli probably coming from the host are involved in the formation of telotrochs in this epibiont species. Observations from old laboratory cultures demonstrated that epibionts having their stalks infested by a fungi formed telotrochs at a lower rate when compared to non-infested ones even when the host was killed by the same process described here. This observation could suggest that cues needed to start the process of telotroch formation may travel through the stalk until reaching the zooids in the colony. Perhaps the presence of fungi inhibits or causes a delay in the perception of the cue resulting in a much longer process in comparison to those epibionts that do not have fungal infestation.

Substrate selection for attachment has been the subject of several studies in benthic invertebrates, especially barnacles (BERTNESS et al., 1996). In general, invertebrate larvae exploit the substrate and settle close to individuals of their own species (KNIGHT-JONES; CRISP, 1953). This behavior would help to avoid places where adults would have little or lower chance of survival, since in some species post-settlement mortality can be very high (GOSSELIN; QUIAN, 1996). This pattern of settling close to congeners was also suggested to be utilized by telotrochs of free-living peritrichs (LANGLOIS, 1975).

Peritrich epibionts can choose a substrate to settle based on the availability of space (when facultative

epibionts) or they can be attracted by specific cues present in the substrate (when obligate epibionts). In addition, epibionts can also display preferential attachment to specific sites on the host's body, and in most cases, these preferred sites are related to advantageous uptake of food. For example, Evans and co-workers (1979) observed that the suctorian *Amphipoda* (Claparede and Lachmann, 1859) attached preferentially to the head of its host, the copepod *Amphipoda* Sars, 1863, an ideal site to come to contact with prey.

Amphipoda is capable of settling on the whole body of its host, without displaying preference for any specific site (UTZ, 2003). In the present study, it was demonstrated that *Amphipoda* was able to colonize *Amphipoda*, one of its primary hosts, but was not able to colonize its eggs when removed from the host. This result suggests that a combination of substrate chemistry, but also movement may be of importance for the settlement of this epibiont species.

The experiments and results presented in this study are the beginning and may serve as an experimental model to investigate thoroughly the mechanisms of recognition of suitable substrates by sessile peritrichs, epibionts or not.

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REFERENCES

- ABELLÓ, P.; MCPHERSON, E. Epibiosis and rhizocephalan infestation patterns in relation to the reproductive biology of *Amphipoda* (Filhol, 1885) (Anomura:Lithodidae). **Journal of Crustacean Biology**, Seminole, v. 12, n. 4, p. 561-570, 1992.
- AL-DHAHERI, R.S.; WILLEY, R.L. Colonization and reproduction of the epibiotic flagellate (Euglenophyceae) on *Amphipoda*. **Journal of Phycology**, Philadelphia, v. 32, p. 770-774, 1996.
- BERTNESS, M. D.; GAINES, S. D.; WAHLE, L. A. Wind-driven settlement patterns in the acorn barnacle *Amphipoda*. **Marine Ecology Progress Series**, Oldendorf, v. 137, p. 103-110, 1996.
- CARMAN, K. R.; DOBBS, F. C. Epibiotic microorganisms on copepods and other marine crustaceans. **Microscopy Research and Technique**, Hoboken, v. 37, p. 116-135, 1997.
- CLAMP, J. C. Observations on the host-symbiont relationships of *Amphipoda* Imamura. **Journal of Protozoology**, Lawrence, v. 20, n. 5, p. 558-561, 1973.

- EVANS, M.S.; SICKO-GOAD, L.; OMAIR, M. Seasonal occurrence of *Paramecium* (Suctorina) as epibionts on adult *Calanoida* (Copepoda: Calanoida) in Southeastern Lake Michigan. **Transactions of the American Microscopical Society**, Lawrence, v. 98, p. 102-109, 1979.
- FENCHEL, T. On the ciliate fauna associated with the marine species of the amphipod *Paramecium* J.G. Fabricius. **Ophelia**, Strenstrup, v. 2, n. 2, p. 281-303, 1965.
- FENCHEL, T. FINLAY, B. J. *Paramecium*: a mouthless ciliate with a symbiotic kitchen garden. **Ophelia**, Strenstrup, v. 30, n. 2, p. 73-95, 1989.
- GILBERT, J. J.; SCHRÖDER, T. The ciliate epibiont *Paramecium*: selection for zooplankton hosts, reproduction and effect on two rotifers. **Freshwater Biology**, London, v. 48, p. 878-893, 2003.
- GOSSELIN, L. A.; QIAN, P. Y. Early post-settlement mortality of an intertidal barnacle: a critical period of survival. **Marine Ecology Progress Series**, Oldendorf, v. 135, p. 69-75, 1996.
- GREEN, J. Parasites and epibionts of cladocera. **Transactions of the Zoological Society of London**, London, v. 32, p. 417-515, 1974.
- HANAMURA, Y. Seasonality and infestation pattern of epibiosis in the beach mysid *Paramecium*. **Hydrobiologia**, Dordrecht, v. 427, p. 121-127, 2000.
- HENEGBRY, M. S.; RIDGEWAY, B. T. Epizoic ciliated protozoa of planktonic copepods and cladocerans and their possible use as indicators of organic pollution. **Transactions of the American Microscopical Society**, Lawrence, v. 98, n. 4, p. 495-508, 1979.
- HERMAN, S. S.; MIHURSKY, J. A. Infestation of the copepod *Paramecium* with the stalked ciliate *Paramecium*. **Science**, Washington DC, v. 146, p. 543-544, 1964.
- IYER, N.; RAO, T. R. Epizoic mode of life in *Paramecium* Ehrenberg as a deterrent against predation by *Paramecium* Hudson. **Hydrobiologia**, Dordrecht, v. 313/314, p. 377-380, 1995.
- KNIGHT-JONES, E. W.; CRISP D. J. Gregariousness in barnacles in relation to the fouling of ships and to anti-fouling research. **Nature**, London, v.171, p. 1109-10, 1953.
- LANGLOIS, G. A. Effect of algal exudates on substratum selection by motile telotrochs of the marine peritrich ciliate *Paramecium*. **Journal of Protozoology**, Lawrence, v. 22, p. 115-123, 1975.
- LÓPEZ, C. et al. Epizoans on a tropical freshwater crustacean assemblage. **Marine Freshwater Research**, Collingwood, v. 49, p. 271-276, 1998.
- MAYÉN-ESTRADA, R.; ALADRO-LUBEL, M. A. First record of *Paramecium* (Ciliophora: Peritrichia) on the exoskeleton of crayfish *Paramecium*. **Journal of Eukaryotic Microbiology**, Lawrence, v. 47, p. 57-61, 2000.
- MONTAGNES, D. J. S.; LYNN, D. H. A quantitative Protargol Stain (QPS) for ciliates and other protists. In: Kemp, P. F.; Sherr, B. F.; Sherr, E. B.; Cole, J. J. **Handbook of Methods in Aquatic Microbial Ecology**, Boca Raton, Lewis Publishers, 1993. p. 229-240.
- NAGASAWA, S. The peritrich ciliate *Paramecium* attached to the copepod *Paramecium* in Tokyo Bay waters. **Bulletin of Marine Science**, Miami, v. 38, n. 3, p. 533-558, 1986.
- NERAD, T.; DAGGETT, P. Cultivation of selected photosynthetic cryptomonads. In: Lee, J. J.; Soldo, A. (Eds.). **Protocols in Protozoology**, Kansas, Allen Press, 1992, v. 1, A-6.1 -A6.5 p.
- SONG, W.; AL-RASHEID, K. A. S.; HU, X. Notes on the poorly-known marine peritrichous ciliate *Paramecium* Kahl, 1933 (Protozoa: Ciliophora) an ectocommensal organism from cultured scallops in Qingdao, China. **Acta Protozoologica**, Warszawa, v. 41, p. 163-168, 2002.
- UTZ, L. R. P. **Identification, life history, and ecology of peritrich ciliates as epibionts on calanoid copepods in the Chesapeake Bay**. College Park: University of Maryland, 2003. 175p. Doctoral Dissertation in Marine, Estuarine, and Environmental Sciences, University of Maryland at College Park. 2003.
- WAHL, M. Marine epibiosis: I. Fouling and antifouling some basic aspects. **Marine Ecology Progress Series**, Oldendorf, v. 58, p. 175-189, 1989.
- WEISSMAN, P.; LONSDALE, D. J.; YEN, J. (1993). The effect of peritrich ciliates on the production of *Paramecium* in Long Island Sound. **Limnology and Oceanography**, Lawrence, v.38, p. 613-622, 1993.

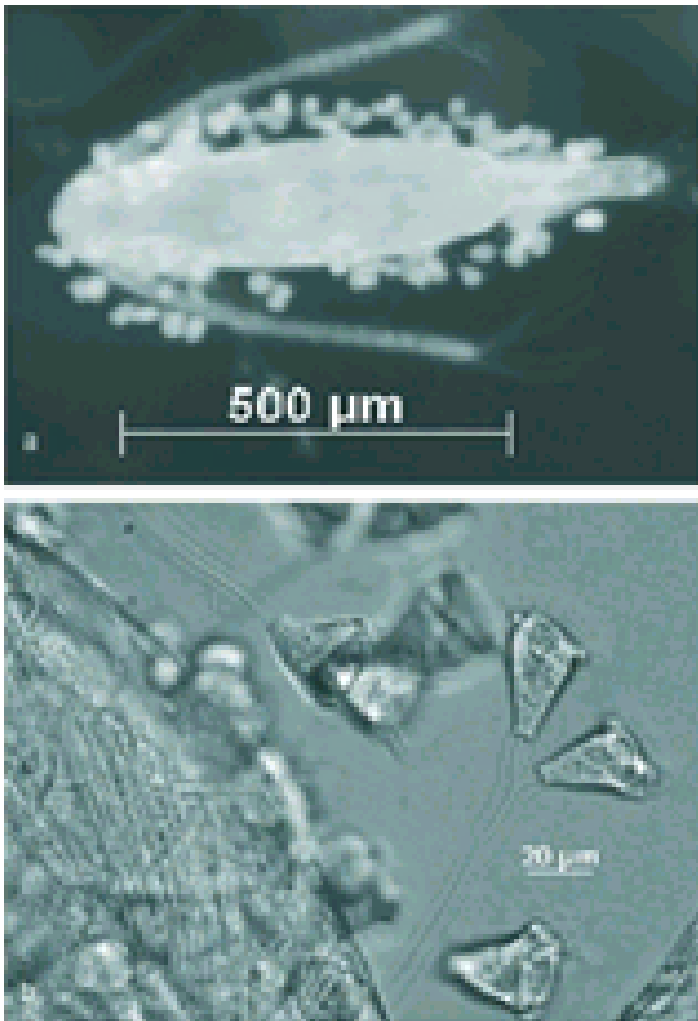


Fig. 1. Photomicrographs (dark and bright field) showing *Eurytemora affinis* attached to its hosts: (a) *Eurytemora affinis* attached to *Paracalanus crassirostris*; (b) Detail of a colony of *Eurytemora affinis* attached to *Paracalanus crassirostris*.

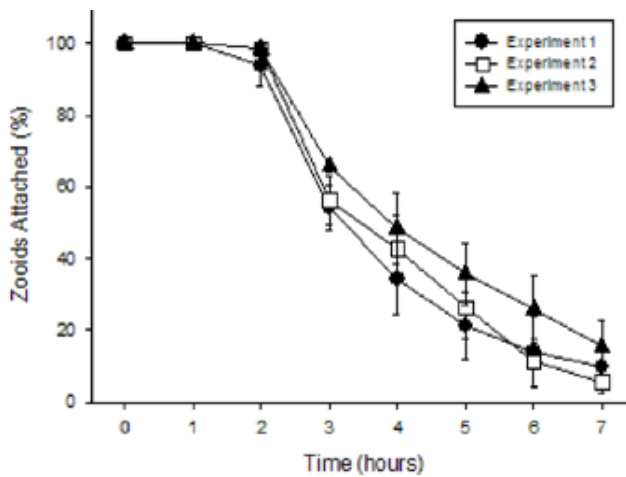


Fig. 2. Telotroch formation of *Eurytemora affinis* attached to *Paracalanus crassirostris* under constant agitation. Each line represents one experiment with five replicates. Error bars represent the Standard Error of the Mean.

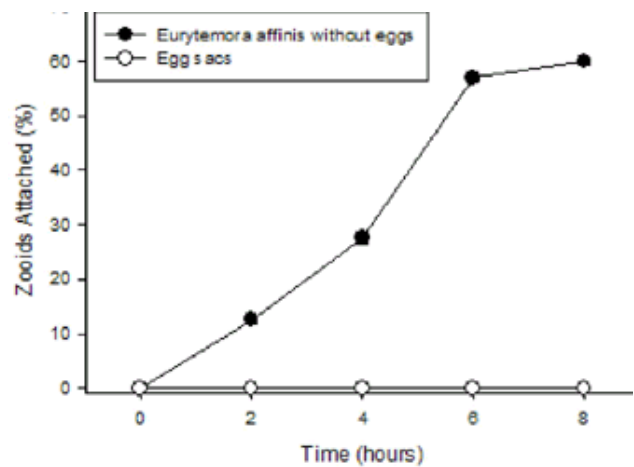


Fig. 3. Attachment of telotrochs of *Eurytemora affinis* to females of *Paracalanus crassirostris* (filled circles) and to eggs sacs removed from females of *Paracalanus crassirostris* (open circles). Symbols represent means of two experiment runs with five replicates each. Error bars represent the Standard Error of the Mean.