ABSTRACT

Title of Dissertation: IDENTIFICATION, LIFE HISTORY, AND ECOLOGY OF PERITRICH CILIATES AS EPIBIONTS ON CALANOID COPEPODS IN THE CHESAPEAKE BAY

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Epibiotic relationships are a widespread phenomenon in marine, estuarine and freshwater environments, and include diverse epibiont organisms such as bacteria, protists, rotifers, and barnacles. Despite its wide occurrence, epibiosis is still poorly known regarding its consequences, advantages, and disadvantages for host and epibiont. Most studies performed about epibiotic communities have focused on the epibionts' effects on host fitness, with few studies emphasizing on the epibiont itself.

The present work investigates species composition, spatial and temporal fluctuations, and aspects of the life cycle and attachment preferences of Peritrich epibionts on calanoid copepods in Chesapeake Bay, USA. Two species of Peritrich

ciliates (*Zoothamnium intermedium* Precht, 1935, and *Epistylis* sp.) were identified to live as epibionts on the two most abundant copepod species (*Acartia tonsa* and *Eurytemora affinis*) during spring and summer months in Chesapeake Bay. Infestation prevalence was not significantly correlated with environmental variables or phytoplankton abundance, but displayed a trend following host abundance.

Investigation of the life cycle of *Z. intermedium* suggested that it is an obligate epibiont, being unable to attach to non-living substrates in the laboratory or in the field. Formation of free-swimming stages (telotrochs) occurs as a result of binary fission, as observed for other peritrichs, and is also triggered by death or molt of the crustacean host. Attachment success of dispersal stages decreased as telotroch age increased, suggesting that colonization rates in nature may be strongly dependent on intense production of telotrochs by the epibiont ciliates.

Laboratory experiments demonstrated that *Z. intermedium* colonizes equally adult and copepodite stages of *A. tonsa* and *E. affinis*. The epibiont is also able to colonize barnacle nauplii and a harpacticoid copepod, when these were the only living host available, but fails to colonize non-crustacean hosts, such as the rotifer *Brachionus calyciflorus* or polychaete larvae. When the epibiont could choose between adults of *A. tonsa* and alternate hosts from the zooplankton community, it always colonized preferentially its primary host, with only a few telotrochs attaching to other crustaceans (barnacle nauplii and harpacticoid copepod), and to rotifer eggs, suggesting that specific cues may be involved in host selection by this epibiotic species.

IDENTIFICATION, LIFE HISTORY, AND ECOLOGY OF PERITRICH CILIATES AS EPIBIONTS ON CALANOID COPEPODS IN THE CHESAPEAKE BAY

by

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2003

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DEDICATION

To my husband Eduardo,

for his love, support, and advice during

the development of this project.

ACKNOWLEDGMENTS

I would like to thank the following people and institutions for their help during the development of this project, and also in other related areas of my life.

Dr. Wayne Coats, for the opportunity to develop this research at the Smithsonian Environmental Research Center, for the advice, support and friendship, and for the encouragement to continue pursuing my scientific interests with enthusiasm.

Dr. Eugene Small, for the advice, support, and friendship throughout this project, and for stimulating my interest in ciliate systematics. I also thank my other Committee members Dr. Marie Bundy, Dr. Darcy Lonsdale, Dr. Michael Roman, Dr. Diane Stoecker, and Dr. Stephen Wolniak for constructive suggestions, helpful advice and encouragement to pursue this research.

My husband Eduardo Eizirik, for his love and constant support during this project and over the years, and particularly for the great advice and help in proofreading this dissertation, as well in the formatting of some of the included figures and reference list.

My parents, Hermilo and Stela, and my sister Silvia, for their love and support throughout my life, which have been of fundamental importance for me to become who I am.

My parents-in-law Cláudio and Marisa, and my sister-in-law Mariana for their love and support during the development of this project, and in the last 10 years of my life.

All my other family members, for the support and encouragement over the years.

My friends and past and current colleagues at the Protistan Ecology Laboratory, for their help and advice during the development of this research. In particular, I thank Tamieka Armstrong, Yvan Bettarel, Sean Cooney, Michael Goodison, Sarah Jardeleza, John Miller, Myung Gil Park, Suzanna Ribblett, and Gabriela Smalley for helpful discussions related to this project.

Tim Maugel and Sarah Jardeleza for their help with the Scanning Microscopy.

Dr. John Clamp for his help with the identification of the epibiont and for interesting discussions about Peritrich taxonomy.

Deborah Morrin-Norlund, and Lois Reid, for their friendship and administrative help at several moments during the development of this research.

My friends Gila Bar-Gal, Marina Carreiro e Silva, Michele Diez, Anne Innis, Tonya Rawlings, Emma Teeling, and Karen Yee for their help and support during the development of this project.

I thank Yvan Bettarel, Eduardo Eizirik, Anne Innis, John Miller, and Tonya Rawlings for critically reading and providing helpful suggestions to different portions

of this dissertation. I am also grateful to Tonya Rawlings, Darrick Sparks, and Karen Yee for helping me with sample collection.

The first four years of this work were supported by a fellowship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. Additional support was provided by the Smithsonian Institution, and the Department of Biology, University of Maryland, College Park. Support to attend scientific meetings during the development of this project was provided by the University of Maryland, College Park, and the Smithsonian Environmental Research Center.

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CHAPTER 1

GENERAL INTRODUCTION

Overview of Epibiosis

Epibiotic relationships involving planktonic crustacea 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 and Finlay, 1989; Abelló and Macpherson, 1992; Iyer and Rao, 1995; Hanamura, 2000; Gilbert and Schröder, 2003; Song, Al-Rasheid, and Hu, 2003). In spite of its wide occurrence, epibiosis remains poorly understood with respect to its ecological implications for both the host and the epibiont. Most studies of crustacean epibiosis have been performed in freshwater systems, with very few focusing on marine and estuarine environments (Carman and Dobbs, 1997). In general, these studies have stressed aspects of the ecology and life history of the host, with less emphasis on the epibiont. Examination of species composition, abundance, and life history of epibionts in aquatic environments is recognized as an important tool to better understand seasonal occurrence of epibiosis, host-epibiont population dynamics, and epibiont substrate specificity (Fenchel, 1965; Wahl, 1989; Threlkeld, Chiavelli, and Willey, 1993; Carman and Dobbs, 1997; Hanamura, 2000). However, most prior reports of crustacean epibiosis have not considered the relationship from the perspective of the epibiont.

Implications of an epibiotic relationship

Historically, epibiosis has been viewed as a commensal relationship between two or more organisms; however, several studies have suggested that epibionts can

have a deleterious effect on the host. For example, Kankaala and Eloranta (1987) found that the cladoceran *Daphnia longispina* and its epibiont *Vorticella* sp., a peritrich ciliate, grazed on particles within the same size-range and suggested that the epibiont and host competed for food. Since *Vorticella* sp. had 50-80% higher feeding rates than *D. longispina*, the epibiont seemed to have a competitive advantage that might reduce host fitness. Similarly, Xu and Burns (1991) reported that, under food limiting conditions, the peritrich ciliate epibiont *Epistylis daphniae* reduced reproduction, growth, and survivorship of its host, the freshwater copepod *Boeckella triarticulata*. The observed effects may reflect an additional energy cost for the host, which was more pronounced in a food-depleted environment.

Weissman, Lonsdale, and Yen (1993) recorded slower sinking rates for the copepod *Acartia hudsonica* when infested by the solitary peritrich *Rhabdostyla* sp., suggesting that epibiont burden may increase drag forces, thereby hindering locomotion and increasing energy expended by the host. In addition to reducing host swimming speed, epibionts may increase host visibility and thus susceptibility to predation. For example, Willey, Cantrell, and Threlkeld (1990) observed that cladocerans and copepods carrying the euglenoid epibiont *Colacium calvum* were selectively preyed upon by visually-oriented fish (*Menidia beryelina*) and pump-filter feeders (*Dorosoma cepediarum* and *Tilapia aurea*). In a similar study, Chiavelli, Mills, and Threlkeld. (1993) reported that planktivory by young fish (*Perca flavescens* and *Dorosoma cepedianum*) was associated with a decline of *Daphnia* populations (including *D. pulex* and *D. galeata mendotae*) infested by the epibionts *Colacium calvum*, *Colacium vesiculosum* and *Synedra cyclopum*.

Detailed information about epibionts can also provide important insights into the biology and ecology of host organisms. For example, Abelló and McPherson (1992) used the age of epibiont hydrozoan colonies (*Stegopoma plicatile*) and individual barnacles (*Poecilasma kaempferi*) to show that adult males of the anomuran crab *Lithodes ferox* have a shorter intermolt period than females. In a similar study, Gili, Abelló, and Villanueva (1993) used growth rates of the epibiont barnacle *Poecilasma kaempferi*, and the hydroid *Stegopoma plicatile* to determine carapace age and intermolt duration of the crab *Bathynectes piperitus*.

When colonizing living substrates, epibionts have to adapt to morphological fluctuations of the host 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. Also, reproduction of the epibiont often occurs while it is attached to the host's carapace, resulting in a positive burden increase associated to host's intermolt time. Willey and Threlkeld (1995) demonstrated that the epibiotic burden of *Colacium calvum* and *Vorticella campanula* on *Daphnia galeata mendotae* and *Daphnia pulex*, respectively, increased linearly and could provide useful information about the intermolt time of the host. In addition, they observed that *V. campanula* produced free-swimming stages immediately before its host started the molting process, and suggested that this behavior allows the epibiont to re-colonize the same substrate right after the host's molt. The same pattern was not observed in *C. calvum*, which exhibited a lag time between host's molt, formation of free-swimming stages, and re-colonization. The behavior observed in *V. campanula* could be

attributed to possible chemical stimulus released by the host before the molt and detected by the epibiont as a signal to form telotrochs.

As mentioned previously, few studies have focused on the epibiont aspect of the epibiotic relationship, yet available information does provide glimpses of interactions that can influence epibiont populations. When more than one species of epibiont colonize a host, competition for space and/or resources can occur. This appears to be the case for caprellid amphipods (*Caprella andreae*) and barnacles (*Chelonibia testudinaria*) associated with loggerhead sea turtles (Caine 1986). On hosts with high densities of epibionts, amphipods appeared to have a negative impact on the barnacles, preying upon newly settled barnacle spats. Competition among conspecific epibionts may also occur, as suggested by Reynoldson's (1950) observation that the average cell size of peritrich epibionts (*Urceolaria mitra*) decreased when hosts (the flatworm *Polycelis tenuis*) were heavily infested. In other cases, epibiosis may provide the epibiont with a refuge from predation, as demonstrated for the rotifer *Brachionus rubens*. When living on *Daphnia carinata, B. rubens* is less susceptible to predation by the carnivorous rotifer *Asplanchna intermedia* (Iyer and Rao 1995). Other biotic and abiotic factors may also affect epibiont success. For example, fluctuations in rainfall and bacterial concentrations are reflected by parallel changes in populations of *Urceolaria mitra* associated with *Polycelis tenuis* (Reynoldson 1955).

Several authors have raised the issue of host specificity when considering epibiosis. In some instances, epibionts appear specific to only one or two host species and sometimes fail to attach to non-living substrates, as reported for *Lagenophrys*

lunatus attached to shrimps in the genus *Palemonetes* (Clamp, 1973), for *Vorticella microstoma* colonizing *Scapholeberis kingi* (Henebry and Ridgeway, 1979), and for *Epistlylis pygmaeum* colonizing *Brachionus calyciflorus* and *Brachionus angularis* (Gilbert and Schröder, 2003). In contrast, other epibionts (e.g. *Lagenophrys denisi*) are able to colonize living host species (e.g. the crayfish *Cambarellus patzcuarensis*), as well as non-living substrates (Mayén-Estrada and Aladro-Lubel, 2000). The assessment of host specificity may help in understanding the seasonal occurrence of epibiosis in aquatic environments, encouraging further studies about the epibiont's life cycle, such as occurrence of encystment when the specific host is not available.

Epibiosis at an ecosystem level

Epibiont populations may play relevant roles at the ecosystem level. For example, Baldock (1986) estimated that the biomass of the peritrichs *Opercularia* spp., *Epistylis breviramosa*, *E. racemosa* and *Vorticella campanula* associated with Trichoptera larvae (*Brachycentrus subnubilus*) exceeded that of free-living ciliates in similar environments. When abundant, epibionts may even help fuel higher trophic levels. Interestingly, the algal epibiont *Korshicoviella gracilipes* on *Daphnia pulicaria* forms free-swimming, dispersal, and unattached overwintering stages that can represent an important food source for the host when more typical planktonic prey is at low abundance levels (Barea-Arco, Perez-Martinez, and Morales-Barquero 2001).

Henebry and Ridgeway (1979) reported that the prevalence of peritrich and suctorian epibionts on crustacean zooplankton reflected the eutrophic condition of their study site, raising the possibility that protistan epibiosis might be used as a

bioindicator for water quality. Commensalism between ciliates and invertebrate hosts has been suggested before as an indicator of water pollution. For example, Antipa (1977) demonstrated that the obligatory commensal ciliates *Conchophtirus curtus* and *Heterocinetopsis uniondarum* could disappear from their bivalve hosts *Lampsilis ventricosa* and *Anodonta grandis* when exposed to sewage contamination. In spite of this, the occurrence of epibiosis has not so far been included in the assessment of water pollution or in the establishment of water quality indices.

Epibiosis in the Chesapeake Bay

The only account of ciliate epibionts associated with zooplankton of Chesapeake Bay is that of Herman and Mihursky (1964), who reported *Zoothamnium* sp., a stalked, colonial peritrich, as a common epibiont on the most abundant calanoid copepod, *Acartia tonsa*, in the Patuxent River (a subestuary of the Chesapeake Bay). Infestation rates on *A. tonsa* reached 100% from early March to mid-April, and apparently only this copepod species, among the zooplankton community, was found carrying epibionts. In addition, partial examination of samples collected in the previous year in the same months (March and April) revealed that *Zoothamnium* was present on *A. tonsa*, but infestation rates were not quantified, and no other details were provided. Even for the focal year of the study, the analysis was limited to only one station in the Patuxent River on three sampling dates.

Although it was observed in that study that *Zoothamnium* only infested *A. tonsa* even when this was not the most abundant species in the zooplankton community, no further investigation about host specificity or ability to colonize other

substrates was performed. Also, the authors mentioned that most of the epibionts were attached to the appendages, but no quantitative approach was taken to verify density of colonies on different body parts or preferential colonization of a determined site. In addition, no attempt was made to identify the epibiont to species level, or to survey the distribution of epibiosis throughout the Chesapeake Bay and across life history stages of the copepod. Higher sinking rates for dead infested vs. non-infested fixed copepods were recorded by Hermann and Mihursky (1964), suggesting that the peritrichs might put hosts at a disadvantage in the natural environment, however, no further experiments to identify other possible deleterious effect of epibionts on copepods were carried out. The lack of detailed information about identity, biology and ecology of the epibiont, and potential deleterious effects on the host, limits the conclusions that can be drawn from this initial study about occurrence of epibiosis in the Chesapeake Bay, as well as any comparison with the occurrence of epibiosis in other aquatic systems.

Techniques applied in peritrich identification

An important consideration when working with peritrich epibionts is careful identification of species. Since identification of peritrich species can be difficult and time consuming, epibionts are often characterized only to the genus level, as is the case for the peritrich associated with copepods in Chesapeake Bay (Herman and Mihursky, 1964). In some instances, even generic identifications are questionable, due to insufficient or inappropriate examination of specimens.

Identification of peritrich species is typically based on light microscopic examination of living specimens (e.g. Kahl, 1935; Precht, 1935; Fauré-Fremiet, 1930). However, some recent studies of free living and epibiont peritrichs have demonstrated that techniques such as cytological staining and scanning electron microscopy are also valuable approaches (e.g. Bauer-Nebelsick, Bardele, and Ott, 1996; Green and Shiel, 2000). Cytological techniques like Protargol silver staining reveal cortical characters such as kinetosomes, ciliary and cortical microtubules, nuclei and mitochondria (Zagon and Small, 1970; Montagnes and Lynn, 1987). In peritrichs, unlike most other ciliates, body ciliature is highly reduced and cannot be used to differentiate genera and/or species. On the other hand, oral structure has proven to be conserved within species, thus being useful for identification. For example, Zagon and Small (1970) showed with Protargol staining that the oral structure of *Carchesium polypinum* was consistent within the population of a polluted stream in the United States and was the same as a population of the same species found in Europe, suggesting that this morphological character may be suitable for identification of peritrichs in general, as has been suggested by Lom (1964). Gross (1986) was able to distinguish four different species in the genus *Zoothamnium* based on the structure of the oral apparatus stained with Protargol combined with morphological characteristics of the living and stained colony, demonstrating the importance of the oral structure for peritrich identification.

Topography of the cell, shape of the peristome and features of the scopula are characteristics that have also been used for peritrich species descriptions and can be better observed by utilizing scanning electron microscopy (SEM). Even though the

majority of peritrich species has been described using morphological characters from living cells, a growing number of recent studies have used SEM as an additional tool to observe such characteristics in the sessile and motile stages of peritrichs. These observations may be useful to when comparing different stages in the life cycle and identifying peritrichs to species level. For instance, Valbonesi (1989) used SEM to study morphological characteristics of the peritrich *Zoothamnium intermedium*, an epibiont on *Acartia clausi*, and could distinguish two different free-swimming morphotypes, which he tentatively identified as microgamont and telotroch forms. Even though it is not clear from this study whether the two observed forms are different morphotypes or only stages of telotroch formation, the use of SEM was shown to provide relevant information for detailed morphological studies of peritrichs.

Despite the use of light microscopy, staining techniques and/or scanning microscopy, the majority of studies of epibiotic relationships involving crustacea have failed to identify the precise species of ciliate epibionts, especially peritrichs (e.g. Foster, Sarphie, and Hawkings, 1978; Nagasawa, 1986). Identification of epibionts to species level allow detailed studies from the epibiont's perspective, including species composition, temporal and spatial distributions of epibionts and dynamics of their natural populations. Moreover, it would facilitate comparison of epibiont species among different aquatic environments, as well as the investigation of the occurrence of the same species as free-living versus epibiont forms in different systems.

The genus *Zoothamnium*

The peritrich genus *Zoothamnium* was first described by Bory de St. Vincent in 1826. There are approximately 50 described species belonging to this genus (Corliss, 1979), which is characterized by a colonial form and arboroid appearance. In general, the colony possesses a continuous myoneme that runs from the basal stalk to the branches, enabling the organism to contract as a single unit. Due to this diagnostic characteristic, and to the presence of a continuous membrane connecting all the cells in the colony, the genus *Zoothamnium* is thus recognized to be a coenobium and not a simple colony like other colonial genera in the order Sessilina, and is now placed in a separate Family (Zoothamnidae) within the SubClass Peritrichia (Lynn and Small, 2000). Even though the cellular organization of the species in the genus *Zoothamnium* would be more accurately described as a coenobium, to conform to the prevalent usage in the current and old literature, the terms "coenobium" and "colony" will be used as synonyms throughout this dissertation when referring to this genus.

Two kinds of coenobial organization can be identified in species within the genus *Zoothamnium*: (i) coenobia where all zooids are capable of metamorphosing into free-swimming stages (telotrochs), and (ii) colonies where zooids have different sizes (macro- and microzooids) and one type of zooid (macrozooids, or, at times, specialized microzooids) is capable of forming dispersal stages (telotrochs). This second type is called heteromorphic. The coenobial development of a heteromorphic species in the genus *Zoothamnium* was first comprehensively described by Fauré-Fremiet, (1930) who investigated *Z. alternans*. He observed that the colony is composed by axial macrozooids and several lateral microzooids. The axial

macrozooids would be responsible for the formation of free-swimming stages, that he called “ciliospores”, and the microzooids would be involved in the nutrition of the colony and in the formation of sexual stages (microgamonts). A few years later, Summers (1938) also investigated the development of several colonies of *Z. alternans* and suggested that there are at least four types of zooids in a single colony: (i) a single apical macrozooid, which is characterized by the bigger size and position within the colony; (ii) several microzooids that are involved in the nutrition of the colony; (iii) terminal microzooids at the tip of each branch that could give rise to microgamonts; and (iv) axial microzooids that can foster telotroch stages, which can transform into a new colony.

In species in the genus *Zoothamnium* where all zooids have the potential to form telotrochs, no size difference is observed in zooids within the colony, and the coenobial development is similar to other colonial genera such as *Carchesium* and some species in the genus *Epistylis*. The only difference is the division of the shared branched myoneme, as well as zooid extension into the stalk, which occurs in the genus *Zoothamnium* but is not observed in the contractile colonial genus *Carchesium* where the myoneme is not continuous throughout the branches. Nagasawa (1987) described the coenobial development of an unidentified species in the genus *Zoothamnium* attached to a calanoid copepod. Despite the lack of species level identification, she was able to observe that all the zooids in the coenobium were approximately of the same size and potentially capable of forming telotrochs. The development of a new coenobium starts with the settlement of a telotroch that attaches to the substrate, starts to secrete a stalk, transforms into an adult zooid, and undergoes

binary fission (Succhard, 1979). After the division is completed the new daughter cells will remain in the same stalk and will elongate until reaching the normal vegetative size and shape of the organism. Only after that the common stalk and myoneme begin to divide forming a new colony. Examples of species that possess this kind of colony development include *Zoothamnium intermedium* Precht, 1935; and *Zoothamnium arbuscula*. For this latter species see especially Succhard, 1979.

Scope of the Present Research

Most of the prior work on ciliate epibionts of planktonic crustacea has been limited to short-term observations of natural populations (with few exceptions, e.g. Green, 1974; Chiavelli, Mills, and Threlkeld, 1993) and has largely emphasized possible effects of epibiosis on host organisms. Few studies have considered important aspects of epibiont biology, such as species composition, abundance, life history, morphology, and attachment mechanisms. In an attempt to address these issues, the present study aims to: (i) identify and characterize species of peritrich epibionts living on Chesapeake Bay copepods using live observations, Protargol staining, and SEM techniques; (ii) determine the prevalence and distribution of peritrich epibionts on planktonic crustacea along the main axis of the Chesapeake Bay; (iii) examine relationships among epibiont prevalence, host abundance and environmental variables; (iv) characterize the biology and life history of *Z. intermedium*; and (v) assess substrate specificity of *Z. intermedium*.

In Chapter 2, I characterize morphologically (using live observations, Protargol staining and Scanning Electron Microscopy) *Zoothamnium intermedium*, a peritrich

ciliate attached to populations of *Acartia tonsa* and *Eurytemora affinis* from the Chesapeake Bay. I also provide a morphological characterization (using Protargol staining) of *Epistylis* sp., another peritrich attached to *A. tonsa*. I present a comprehensive redescription of *Z. intermedium* and comparisons with other described species in the genus *Zoothamnium*.

In Chapter 3, I investigate the spatial and temporal distribution of *Z. intermedium* and *Epistylis* sp. as epibionts on calanoid copepods in the Chesapeake Bay, and perform correlation analyses between infestation prevalence and host abundance relative to environmental variables. I also provide data on the distribution of epibiont colonies on different body parts and life history stages of the hosts.

In Chapter 4, I explore different aspects of the life cycle of *Z. intermedium* including (i) formation, survivorship and attachment success of telotroch stages, (ii) growth and development of the coenobium, and (iii) the ability of *Z. intermedium* to attach to non-living substrates in the field. I also provide comparisons of biological aspects observed in the epibiont with other free-living peritrichs.

In Chapter 5, I assess the ability of *Z. intermedium* to colonize alternate hosts from the zooplankton community, as well as test for preferential attachment to different life history stages of its primary hosts (*A. tonsa* and *E. affinis*), and for preferential attachment to *A. tonsa* when exposed to alternate hosts. In addition, I explore predatory relationships between hosts and free-swimming stages of *Z. intermedium*, and compare my results with those found for other epibiont species.

In Chapter 6, I review the main results described in chapters 2, 3, 4, and 5, and discuss the potential relevance and impacts of these findings for the study of epibiosis in aquatic environments, particularly those addressing estuarine ecosystems.

CHAPTER 2

MORPHOLOGICAL CHARACTERIZATION OF *ZOOTHAMNIUM INTERMEDIUM* PRECHT, 1935 AND *EPISTYLIS* SP. (CILIOPHORA, PERITRICHIA) ATTACHED TO CALANOID COPEPODS IN THE CHESAPEAKE BAY, USA

ABSTRACT

I have more completely characterized morphologically *Zoothamnium intermedium* Precht, 1935, a peritrich epibiont on the copepods *Acartia tonsa* and *Eurytemora affinis* in the Chesapeake Bay, and *Epistylis* sp colonizing populations of *A. tonsa* in the same ecosystem. The colonial sessile *Z. intermedium* possesses a bell shaped zooid that ranges in size *in vivo* from 31.2 to 54.7 μm x 16.7 to 31.3 μm. The cell body has a single contractile vacuole that empties its contents into the infundibulum. A "C shaped" macronucleus lies in the upper half of the body. Colonies of *Z. intermedium* can have up to 30 zooids per main stem stalk, but most of them have 2-4 zooids. The oral ciliature, revealed by the Protargol method, consists of an outer haplokinety and an inner polykinety that performs about 1 ½ turns around the peristomial disk before entering the infundibulum. Three infundibular polykineties, each possesses three rows of kinetosomes, were identified. Scanning electron microscopy shows an annular pattern of ridges and, between ridges, scattered irregularly distributed pores on the zooid cell membrane. *Epistylis* sp. has a "C shaped" macronucleus that lies close to the peristomial opening. The oral ciliature is similar to *Z. intermedium*, but the haplo- and polykineties perform 1 ¼ turns before entering the infundibulum. Three oral polykinetids were identified each consisting of three rows of kinetosomes. Morphological comparisons between specimens of *Z. intermedium* attached to *A. tonsa* and *E. affinis*, are presented along with comparisons with other described species in this genus.

INTRODUCTION

Sessile peritrich ciliates are commonly found attached to a variety of non-living and living substrates in fresh, brackish and marine habitats. Living hosts (i.e. basibionts) often constitute a suitable attachment site for peritrichs and other sessile organisms since the movement of the substrate organism can supply the epibionts with nutrients and facilitate removal of waste material (Felgenhauer and Schram, 1978; Wahl, 1989). Crustacea are the most common basibionts for various species of peritrichs, and several studies have been focused on this relationship involving planktonic or benthic hosts (e.g. Fenchel, 1965; Clamp, 1973; 1988; 1994; Green, 1974; Foster, Sarphie, and Hawkings, 1978; Nagasawa, 1986; Green and Shiel, 2000)

Despite the well-documented occurrence of peritrichs as epibionts on planktonic crustacea in a variety of aquatic environments (Foster, Sarphie, and Hawkings, 1978; Nagasawa, 1986; Willey and Trelkeld, 1993; Weissmann, Lonsdale and Yen, 1993; Hanamura, 2000), only a few studies have identified epibionts to species level (Henebry and Ridgeway, 1979; Valbonesi and Guglielmo, 1988; Xu and Burns, 1991; Xu, 1992; 1993). Species identification of peritrich ciliates can be difficult and time consuming because of superficial original descriptions or incomplete redescriptions, as pointed out by Leitner and Foissner (1997). Also, most of the available species descriptions of non-loricate peritrichs are based on live observations only, although studies have shown that characters like oral infraciliature are diagnostic across genus/species boundaries, and are useful for identification (Zagon and Small, 1970; Zagon, 1971; Gross, 1986).

When working with peritrich epibionts, precise identification of species would be valuable to studies emphasizing species composition, host specificity and seasonal occurrence of infestation because it allows comparisons of temporal and spatial distribution of epibiotic species in different aquatic environments.

The peritrich genus *Zoothamnium* Bory de St. Vincent, 1826 contains approximately 50 species (Corliss, 1979), which are mostly described, based on the examination of living forms, that emphasize aspects of the colony such as branching pattern, size of basal and lateral stalks, and length and width of the zooid (Kahl, 1935; Precht, 1935). Despite the wide use of staining techniques and electron microscopy in taxonomy of various species of ciliates (e.g. Foissner, Berger, and Schaumburg, 1999; Foissner, Agatha, and Berger, 2002), only a few studies have reported morphological aspects of species in the genus *Zoothamnium* using cytological stains and scanning or transmission electron microscopy (Laval, 1968; Couch, 1978; Succhard, 1979; Gross, 1986; Valbonesi, 1989, Bauer-Nebelsick, Bardele, and Ott, 1996; Hu and Song, 2001; Song, Al-Rasheid and Hu, 2002), and even in some of these reports detailed information about morphology or species identification is lacking.

Zoothamnium intermedium Precht, 1935 was first described as an epibiont on amphipods and isopods in the Kiel Bay, Germany (Precht, 1935); the original description was based on live specimens only, emphasizing gross morphological characters such as size of the zooid and branching pattern of the colony. A more detailed description of this species was given by Valbonesi and Guglielmo (1988) and Valbonesi (1989) who provided morphological observations using light and

scanning electron microscopy of the trophont (sessile) and telotroch (free-swimming) stages collected from the Lagoon of Venice in Italy. However, all morphological data from light microscopy were based only on formalin-fixed organisms with no comparison to live specimens, and no use of any staining technique.

The genus *Epistylis* Ehrenberg, 1830 contains approximately 100 described species (Corliss, 1979), most of which are believed to live as epibionts on plants, crustaceans, insects, and rotifers in freshwater or marine environments (Sládecek, 1986). Similar to other peritrichs, most species in the genus *Epistylis* are described based on live observations of morphological characters, with only a few studies including the use of staining techniques (e.g Lom, 1964; Fernández-Galiano and Carrascosa, 1989).

In this study, I characterize morphologically two species of peritrich epibionts (*Zoothamnium intermedium*, and *Epistylis* sp.) attached to calanoid copepods (*Acartia tonsa* and *Eurytemora affinis*) in Chesapeake Bay, USA, and provide a redescription of *Z. intermedium* using live observations from cultured specimens, Protargol staining from specimens from cultures and the field, and scanning electron microscopy from cultures. I also compare the morphology of colonies of *Z. intermedium* attached to *A. tonsa* and *E. affinis*, in order to assess whether or not the same species of epibiont colonizes both host species.

MATERIALS AND METHODS

Collection of Field Specimens and Staining: *A. tonsa* and *E. affinis* were collected at nine stations along the main axis of the Chesapeake Bay, USA. Stations were sampled monthly during spring and summer of 1994-1996 and 1999-2000, using a 35 μm plankton net (30 cm in diameter) to obtain an integrated sample from the upper 10-15m in the water column at day time. A 250-ml subsample of each net tow was preserved with modified Bouin's fixative (Coats and Heinbokel, 1982) at a final concentration of 5%. Copepods were examined using a dissecting microscope and infested individuals were removed from the samples and stained using the Protargol technique (Montagnes and Lynn, 1993). Due to the large size of copepods, filters were omitted from the preparation and infested individuals were placed in small baskets (approximately 10 copepods/basket) made out of screw cap Polypropylene tubes with a removed bottom. The center of the cap was also removed and replaced by a screen with a 253μm-mesh net. The entire staining procedure was run using the baskets, except the final steps (Xylene and Xylene + Permount®) where copepods were removed from the baskets with a glass Pasteur pipette and placed inside screw cap glass jars. To mount permanent slides, copepods were placed on a glass slide in a drop of mounting medium (Permount®), and a cover slip was pressed gently on top of the specimen.

Cultures of *Acartia tonsa*, *Eurytemora affinis* and *Z. intermedium*: *A. tonsa* and *E. affinis* were collected from the Patuxent River (a tributary of the Chesapeake Bay) using a plankton net with a mesh size of 35 μm. Live copepods were brought to the

laboratory and examined for the presence of ciliate epibionts. Copepod cultures were initiated by placing 30 non-infested males and 30 non-infested females in 4-liter beakers (3 cultures for each species) containing 2 liters of 15 psu (practical salinity units) Patuxent River water that had been screened through a 20μm-mesh Nitex screen. As food for the copepods, *Thalassiosira weissflogii* and *Isochrysis galbana* grown in f/2 medium (Nerad and Daggett, 1992) were added to the beakers at final concentrations of $1X10^3$ cells/ml and $3X10^3$ cells/ml, respectively. Algal prey and copepod cultures were maintained at 20^oC, with cool white fluorescent lamps providing a 14 h light, 10 h dark cycle. Copepod cultures were transferred once a week to a fresh medium with algal prey, by concentration in a 20μm-mesh net.

Cultures of ciliate epibionts (*Zoothamnium intermedium*) were initiated by placing five infested copepods, *A. tonsa* or *E. affinis* into two established cultures of corresponding host species. *Z. intermedium*-copepod cultures were kept in the same growth conditions described above and transferred to a fresh medium once a week.

Morphology of living and stained epibionts: Live peritrichs (a total of 32 coenobia of *Z. intermedium* attached to each host) obtained from laboratory cultures were observed and photographed using a Axioscope microscope equipped with an Axiocam digital camera (Zeiss Inc.). Images were captured electronically and measurements of the peritrichs were performed using Axiovision software (Zeiss Inc. version 2.0.5). Telotrochs (free swimming stages) were also obtained from the cultures (telotroch formation was induced by killing infested copepods, see Chapter 4) for morphological measurements. To facilitate videotaping and measurements, a relaxing solution that

chelates calcium (Clamp and Coats, 2000) was added to the water until telotroch motility was slowed down but cell shape was preserved. A total of 30 telotrochs obtained from colonies attached to each host species were videotaped using a ZVS 3C75DE camera attached to an Axioscope microscope (Zeiss Inc.).

Stained specimens in the trophont stage (a total of 70 *Z. intermedium*, 40 attached to *A. tonsa* and 30 attached to *E. affinis*; and 30 *Epistylis* sp. (attached to *A. tonsa* from field samples) were observed using an Axioscope microscope (Zeiss Inc.) and measured with a filar micrometer. Drawings of living specimens were based on micrographs, and those of stained specimens were made using a drawing tube mounted to a microscope.

Scanning Electron Microscopy: Epibionts and hosts were obtained from cultures, washed five times in < 0.45 μm 15 psu water, and fixed in 1% Osmium Tetraoxide (OsO_4) for one hour. Copepods and epibionts were washed in double-distilled water to remove the fixative, dehydrated in a graded ethanol series, critical-point dried in liquid carbon dioxide using a DCP-1 Critical Point Dryer (Denton Company), and coated with a gold-palladium alloy, using a DV-503 Vacuum Evaporator. Specimens were visualized using an AMRAY 1820D Scanning Microscope (AMRAY Corp.), and the number of ridges on the pellicle, was assessed in 30 zooids from colonies attached to each copepod host.

Data Analyses: As data were normally distributed and passed the test of homogeneity of variance, Two-Way Analysis of Variance was performed to assess differences in

morphological measurements between *Zoothamnium intermedium* attached to *A. tonsa* and *E. affinis*. Measured characters were analyzed as factor A, and host species factor B. Due to a positive interaction (AXB), pairwise multiple comparisons were performed using the Tukey test. The analysis was performed using Sigma Stat Version 2.0 (SPSS, Inc.) with data reported in the text as mean $\pm$ standard deviation of the mean (SD), or ranges when appropriate.

RESULTS

Morphological analyses revealed that one species of coenobial peritrich belonging to the genus *Zoothamnium* Bory de St. Vincent, 1826 colonized populations of *Acartia tonsa* and *Eurytemora affinis* in the Chesapeake Bay, while a colonial peritrich genus, *Epistylis* Ehremberg, 1838, colonized only *A. tonsa*. The species belonging to the genus *Zoothamnium* was identified as *Zoothamnium intermedium* Precht, 1935 based on a comparison of the morphology of the coenobium, morphology and size of the zooids, and epibiotic way of life. The characters that could be compared with the original description of *Z. intermedium* are based on live specimens only, and below I provide a redescription of this species based on live observations, Protargol staining and scanning electron microscopy (Figures 2.1 to 2.3 and Tables 2.1, 2.2, 2.3 and 2.4). *Epistylis*, on the other hand, could not be identified to species level since it was not possible to make live observations. Protargol staining observations had been previously published for only 5 species in the genus *Epistylis* (Lom, 1964; Fernández-Galiano and Carrascosa, 1989) but none of them matched the

morphology observed for the specimens of this genus attached to *A. tonsa* from the Chesapeake Bay. Therefore, below I present morphological characters observed for *Epistylis* sp. from Protargol staining (Figure 2.4 and Table 5), without assigning a species name.

Morphological comparisons between *Z. intermedium* attached to *A. tonsa* and *E. affinis*: Two Way ANOVA comparisons of morphological characters measured from silver stained *Z. intermedium* attached to *A. tonsa* and those on *E. affinis* did not reveal any significant difference in the measured characters (Table 2.1). The number of pellicle ridges above and below the telotroch band revealed by scanning microscopy also did not show any statistically significant difference (One Way ANOVA) between *Z. intermedium* attached to the two host species (Table 2.2). On the other hand, comparisons between live specimens attached to *A. tonsa* and *E. affinis* showed significant difference ($p<0.05$) in four characters (Table 2.3): (i) length of the zooid from peristomial lip, (ii) length of basal stalk, (iii) length of the first order branch, and (iv) size of the gap present between the attachment site and the beginning of the secreted of the myoneme. Since there was no difference in fine structure detectable by Protargol staining or scanning electron microscopy, and since Protargol staining of the specimens from cultures attached to *A. tonsa* and *E. affinis* did not show any statistical significant difference between cultured and specimens from the field (Table 2.4), size of zooid and length of colony branches, *in vivo*, do not provide enough support to consider the Chesapeake Bay *Zoothamnium* sp. a different species in the genus *Zoothamnium*. Therefore the redescription of *Zoothamnium intermedium* presented

below will include range of measurements taken from colonies attached to both host species.

Family Zoothamniidae Sommer, 1951
Genus *Zoothamnium* Bory de St. Vincent, 1826

***Zoothamnium intermedium* Precht, 1935**

Morphology of Live specimens. The coenobium is dichotomously branched, with alternate branches terminating at different levels. The basal stalk is the longest in the colony and had a smooth surface without ridges or protuberances (Figs. 2.1 A, B, and E, 2.2 A, 2.3 A). The length of the basal stalk and first order branches was highly variable and a statistically significant difference in stalk length was observed between specimens attached to *A. tonsa* and *E. affinis* (Table 2.3). The diameter of the basal stalk and first order branches is similar, and no significant difference from specimens attached to both host species was observed (Table 2.3). A continuous thin myoneme extended from the basal stalk to the branches and to each zooid, allowing the coenobium to contract as an entire unit in a zigzag fashion, typical of the genus *Zoothamnium* is identified (Figs. 2.1 B, and E, 2.2 A). The attachment disk and the most basal part of the main stalk were the only two structures in the colony that lacked myonemes (Fig. 2.1 B). The space between the attachment site and the tip of the myoneme was highly variable (Table 2.3), and the mean size of this gap is significantly different between specimens attached to *A. tonsa* (24.0 μm $\pm$ 22.0 μm) and *E. affinis* (13.0 μm $\pm$ 5.7 μm). Coenobia could have up to 30 zooids, but most of them had 2 or 4 zooids (mean: 2.7 $\pm$ 1.0, Table 2.3) that were similar in size and capable of forming telotrochs. Zooids are inverted bell-shaped (Figs. 2.1 A, and C,

2.2 A, 2.3 B) and ranged in length from 31.2 to 54.7 μm (total length including all specimens attached to *A. tonsa* and *E. affinis*, see Table 2.3) and in width from 16.7 to 31.1 μm (width of the body at midpoint). The peristomial disk had a diameter similar to the body width (Table 2.3), and it possessed a moderately thick peristomial lip (Figs. 2.1 C, 2.2 A) that hung over the body when the cell was fully extended. The epistomial disk is slightly elevated (Fig. 2.1 C) and is round on its surface, with a width that was ~ ¾ the width of the peristomial disk. A single contractile vacuole is located below the peristomial lip and discharges its contents into the infundibulum (Figs. 2.1 D, 2.2 A). A transverse pattern of ridges on the pellicle was visible in the living specimen (Figs. 2.1 C; 2.2 A), as well as a "C-shaped" macronucleus that lies in the upper half of the cell close to the peristomial lip (Fig. 2.2 A).

Free-swimming stages (telotrochs) were round in shape and possess a single row of cilia located near the aboral end (Fig. 2.3 E). Telotrochs range in size, *in vivo*, from 26.7 to 31.6 μm in length and from 25.8 to 35.7 μm in width (Table 2.5). Protargol stained telotrochs were observed in field samples from colonies attached to *A. tonsa* and *E. affinis*, but no measurements were performed since the presence of the dispersal stage was not observed in a high frequency. No statistically significant difference was observed in size of telotrochs originating from colonies attached to *A. tonsa* and *E. affinis* (Two-Way ANOVA; $p > 0.05$).

Morphology of fixed and stained specimens. Infraciliary and nuclear characteristics of *Z. intermedium* are well recognizable in Protargol stained specimens. The micronucleus possesses a round shape (Figs. 2.1 F, 2.2 A, and B) and always lies close

to the macronucleus (Figs. 2.2 A and B). The macronucleus has the same configuration ("C-shaped") observed in live specimens, and is always located in the upper half of the zooid, close to the peristomial lip (Figs. 2.1 E and F, 2.2 C). A total of 21 somatic myonemes that extended from the scopula to the epistomial disk are present in the cell body (Figs 2.1 H, 2.2 B). The oral infraciliature is typical for the genus *Zoothamnium* and sessiline peritrichs, in general, with an outer haplokinety and an inner polykinety (PK1) that performes *ca* 1 ½ turns around the peristomial disk before entering the infundibulum through the oral opening. Inside the infundibulum, the haplo- and polykineties run together for a short distance with the former separating at a 90^{o} angle from the latter and running parallel to the lower end of the cell (Fig. 2.2 C). In addition to PK1, the infundibular polykineties PK2 and PK3 were identified (Figs. 2.1 G, 2.2 C and D): PK1 consists of three rows of kinetosomes that are equal in length and continue as the peristomial polykinety at the oral end. PK2 also consisted of three rows of kinetosomes, that were shorter in length in comparison to PK1. The central row of kinetosomes in PK2 is shorter than the two adjacent rows at the oral end, with the third row slightly diverging obliquely form the other two. The PK2 rows do not merge with the oral peristomial kinety and a gap between PK1 and PK2 could be observed at the oral end. All PK2 rows terminate at the curvature of PK1 leaving a space that ranged in size between 1.2 and 3.0 μm (Table 2.2). PK3 consists of three short rows of kinetosomes of equal size that terminate at the aboral end of PK2 extending about ¼ of the length PK2 towards the oral end of the cell (Figs. 2.2 C and D).

Scanning electron microscopy revealed that pellicular pores are irregularly distributed over the cell surface (Fig. 2.3 C). A mean of 40 parallel pellicular ridges (± 3.5; range 36 - 50) are present between the peristomial lip and trochal band and 20 (± 1.7; range 17 - 25) are observed between the telotroch band and scopula (Figs 2.3 B and C). The trochal band consists of three closely spaced ridges that encircle the zooid near the aboral end. Scanning electron microscopy also revealed several bacteria colonizing the attachment disk and the stalk of *Z. intermedium* as can be seen in Figure 2.3 D.

Family Epistylididae Kahl, 1933

Genus Epistylis Ehrenberg, 1838

***Epistylis* sp.**

Morphology of stained specimens. Colony with alternate branches terminating at different levels are supported by a long, thin, non-contractile basal stalk (28.7 μm ± 18.4 μm X 4.3 μm ± 0.9 μm; Table 2.5). Colonies could have up to 15, inverted bell-shaped zooids that are similar in size (30.7 μm ± 5.1 μm X 18.5 μm ± 3.2 μm; Table 2.6). Nuclear characteristics and buccal infraciliature are easily recognizable in Protargol-stained specimens. The macronucleus is "C-shaped" and is located in the upper half of the cell, close to the peristomial opening (Figs. 2.4 A and C). The micronucleus is round shaped and lies closed to the macronucleus (Fig. 2.4 C). The general features of the oral infraciliature is typical of sessiline peritrichs with an outer haplokinety and an inner polykinety that perform about 1 ¼ turns before entering the

infundibulum. Inside the oral opening the haplokinety run parallel to the polykinety for a short distance and completes one turn before ending at the wall of the infundibulum (Fig. 2.4 D). Three infundibular polikineties are present inside the infundibulum (PK1, PK2, and PK3). PK1 is formed by three rows of kinetosomes that are equal in length and merge with the peristomial kinety at the oral end (Figs. 2.4 D and E). PK2 consists of three rows of kinetosomes equal in length to each other, but shorter than PK1. These rows do not merge at the oral end, leaving a gap between PK1 and PK2 (Figs. 2.4 D and E). PK2 terminates at the curvature of PK1. PK3 is formed by three short rows of kinetosomes equal in size that terminate at the aboral end of PK2. PK3 extends about 1/3 of PK2 towards the oral end of the cell (Figs. 2.4 D and E). Only one row of kinetosomes is present in the trochal band (Figs 2.4 A, B, and C). The pellicle presented a reticulated pattern of striations (Fig. 2.4 B) and approximately 28 horizontal ridges ($\pm$ 2.4; Table 2.6) are observed between the oral end of the cell and trochal band, and 18 ($\pm$ 2.0; Table 2.6) are present between the trochal band and scopula.

DISCUSSION

The genus *Zoothamnium* Bory de St. Vincent, 1826 is characterized by the presence of a continuous myoneme that extends from the basal stalk to the zooids, enabling the entire coenobium to contract as a single unit (Corliss, 1979; Curds, Gates, and Roberts, 1983). After being compared with original descriptions of several species in the genus *Zoothamnium*, the species investigated in the present study was identified as *Zoothamnium intermedium*. Although the original description of *Z.*

intermedium (Precht, 1935) is very brief and based only on the measurements of few characters, almost all of them fall in the same range of size observed in the investigated species. The only exception is the size of the gap present between the attachment site and the beginning of the secreted myoneme, which in the present species is highly variable, but in the original description provided by Precht (1935) is cited as being always constant (~12 μm). Since there is no reference to sample number in the original description and this character proved to be quite variable among colonies (encompassing the value originally described), it is a conservative position to assume that the species investigated in the present study is the same as that originally described as *Z. intermedium*. Other species in the genus *Zoothamnium* also present a similar coenobium shape and size, but other morphological features were not compatible with the species of *Zoothamnium* observed in the present study (see Table 2.7). For example, *Zoothamnium parasiticum* Stein, 1859 has the same overall colony shape and branching pattern observed in *Z. intermedium*, but the heavy peristomial lip and the size of the zooid (~ 70 μm; Kahl, 1935) are considerably different from the species investigated here. *Zoothamnium carcini* Kent, 1881 also presents a similar colony shape, but the zooids are approximately 100 μm in size when fixed (Kahl, 1935). *Zoothamnium rigidum* Precht, 1935 and *Zoothamnium hiketes* Prech, 1935 are also similar to *Z. intermedium*, differing only on the size of the zooids, with both former species being larger than the latter (Prech, 1935).

Gross (1986) characterized morphologically, by live observations and Protargol staining, four species in the genus *Zoothamnium* collected on glass slides from Chesapeake Bay. One of these species was identified as *Zoothamnium alternans*

Claparède and Lachmann, 1858, and differs from *Z. intermedium* basically by the presence of micro- and macrozooids, overall shape of the colony, and arrangement of the oral polykinetids. The other three morphospecies of *Zoothamnium* were not named, but differ from *Z. intermedium* in the size and overall shape of the colonies, size of the zooids, and arrangement of oral polykinetids.

A more recent description of *Zoothamnium intermedium* was provided by Valbonesi and Guglielmo (1988) and Valbonesi, (1989) who found the species attached to copepods and shrimp zoea in a Lagoon in Italy. Although the species description was based on formalin-fixed organisms, the measurements obtained for those specimens fall approximately in the same range recorded for stained specimens in the present study. Also, overall shape of the colony and of individual zooids, shape and position of macro- and micronucleus, and the epibiotic way of life are the same observed for *Z. intermedium* attached to copepods in Chesapeake Bay. Scanning electron microscopy observations also revealed an annular pattern of ridges and a scattered pattern of pores distributed on cell the membrane (Valbonesi, 1989) as was observed in the present study for *Z. intermedium*. Investigations of the motile stage of *Z. intermedium* by Valbonesi (1989) revealed two different morphotypes defined on the basis of the length of the cilia in the trochal band as telotroch (with short cilia in the trochal band) and microgamont (with long cilia in the trochal band). Interestingly, in the present study I was able to identify the telotroch stage of *Z. intermedium* as round cells with cilia in the trochal band measuring on average 4 μm in length (range 2.5-6.4 μm, see Table 2.5), approximately the same size reported by Valbonesi (1989) for what he classified as microgamont. The free-swimming cells observed and

measured *in vivo* in the present study were ready to attach as soon as they contacted a suitable host (personal observation), indicating that they were the dispersal stage and not a microconjugant (microgamont). No other motile form was observed in the present study. Therefore, I suggest that the telotroch stage of *Z. intermedium* has a round shape and a single row of long cilia in the trochal band. The motile stage presenting short cilia as described by Valbonesi (1989) might be the microconjugant (which was not observed in the present study) or cells detached at an early stage of telotroch formation (due to specimen preparation), with not fully developed cilia in the trochal band.

The genus *Epistylis* Ehrenberg, 1830 is a colonial peritrich characterized by a non-contractile stalk. In the colony, only the zooids are able to contract due to the presence of a somatic myoneme (Corliss, 1979). Since I was not able to observe *Epistylis* from the Chesapeake Bay "*in vivo*", no comparisons with original species descriptions based on live specimens could be made. Therefore, I compared the arrangement of oral polykinetids in the investigated species to those of five other species of *Epistylis* characterized with Protargol preparations, but none of which corresponded to pattern observed here. For example, the rows of kinetosomes of PK1 and PK2 in *Epistylis digitalis* Ehremberg, 1838 do not fuse together at the oral end , and PK1 and PK3 terminate at the same level in the infundibulum, with PK2 ending at the curvature of PK1 (Lom, 1964), This arrangement is different from the one observed for *Epistylis* in the present study, in which PK2 and PK3 terminate at the same level and at the curvature of PK1. The oral architecture of *Epistylis lwofii* investigated with Protargol preparations revealed that the three oral polykinetids

terminate at the same level inside the infundibulum (Lom, 1964) differing from *Epistylis* observed in the present study. Oral architecture of two other species of *Epistylis* epibionts (*Epistylis lacustris* Imhoff, 1884 and an unidentified species) was also investigated by Lom (1964) based on Protargol stains. In both *Epistylis lacustris* and *Epistylis* sp., PK3 was not observed, a character that was easily recognizable in *Epistylis* attached to *A. tonsa* in this study. Also, *E. lacustris* presents a belt shaped structure highly impregnable by Protargol, which is absent in the *Epistylis* from Chesapeake Bay. *Epistylis plicatilis*, Ehrenberg, 1830 also differs from the *Epistylis* investigated in the present study by the presence of only two rows of kinetosomes in P3 (Fernández-Galiano and Carrascosa, 1989), instead of three.

In summary, this study provided a redescription of a species in the genus *Zoothamnim* epibiont on copepods in Chesapeake Bay, which was recognized as *Zoothamnium intermedium*. Since the original description is based on very few characters and no sample number is available in that publication, direct and detailed comparisons of *Z. intermedium* from the Kiel Bay (location type) with the *Zoothamnium* examined here would be required to confirm definitively whether or not they are the same species. Also, molecular analyses comparing *Z. intermedium* attached to the two copepod hosts may be performed in the future to investigate if they indeed comprise a single genetic unit, as suggested by the morphological results presented here. *Epistylis* sp. attached to *A. tonsa* in Chesapeake Bay may be an undescribed species; detailed comparisons to other members of the genus (including *in vivo* observations, as well as measurements of morphological characters using live

specimens, Protargol staining and scanning electron microscopy) are necessary to either assign it a new name or to classify it as one of the currently known species.

Table 2.1. Measurements of field, Protargol stained colonies of *Zoothamnium intermedium* attached to *Acartia tonsa* (At) and *Eurytemora affinis* (Ea). A total number of 40 colonies attached to *A. tonsa* and 30 colonies attached to *E. affinis* were measured from the months with highest incidence (July 1994, March, April, May and August 1996). No statistically significant difference was found between the species for the measured characters.

Character	Species	Mean (μm)	SD (μm)	Mode	CV (%)	Range
Total length of the body from	At	32.3	± 4.5	25.0	13.9	22.8 – 41.2
epistomial disk to aboral end	Ea	30.6	± 4.6	28.9	15.0	23.9 – 43.5
Width of the body below the	At	22.6	± 2.5	21.2	11.1	17.8 – 28.5
peristomial lip	Ea	21.5	± 2.5	21.4	11.6	16.9 – 28.3
Width of the body at midpoint	At	22.5	± 3.2	22.0	14.2	16.6 – 30.3
between oral and aboral ends	Ea	20.1	± 3.8	22.5	18.9	12.9 – 30.8
Distance between trochal band	At	8.1	± 2.3	7.4	28.3	4.5 – 14.6
and scopula	Ea	7.8	± 1.5	7.2	19.2	5.5 – 12.4
Length of basal stalk	At	38.6	± 20.3	29.5	52.6	7.4 – 83.9
	Ea	34.4	± 25.4	26.2	73.8	7.4 – 86.5
Width of basal stalk	At	7.1	± 2.4	5.3	33.8	4.8 – 17.3
	Ea	6.8	± 1.8	6.0	26.5	3.6 – 9.9
Distance of myoneme from	At	20.4	± 13.8	20.0	67.6	1.2 – 50.0
attachment point	Ea	20.8	± 18.6	10.2	89.4	1.9 – 73.2
Length of micronucleus	At	2.5	± 0.4	2.5	16.0	1.5 – 3.4
	Ea	2.3	± 0.4	2.0	17.3	1.6 – 3.4
Width of micronucleus	At	2.4	± 0.4	2.3	16.7	1.7 – 3.0
	Ea	2.3	± 0.5	2.2	21.7	2.7 – 6.9
Width of macronucleus at	At	4.2	± 1.0	3.0	23.8	2.3 – 6.5
midpoint	Ea	4.8	± 1.1	4.3	22.9	2.7 – 6.9
Distance between ends of	At	2.0	± 0.4	1.7	20	1.2 – 3.0
OPK1 and OPK2	Ea	2.0	± 0.3	1.8	15.0	1.4 – 2.5
Number of zooids in the	At	5.5	± 2.8	2.0	50.9	2.0 – 13.0
colony	Ea	6.2	± 2.8	4.0	45.2	2.0 – 14.0

Table 2.2. Number of annular ridges on the membrane of *Zoothamnium intermedium* attached to *Acartia tonsa* (At) and *Eurytemora affinis* (Ea). A total of 30 zooids attached to each host species were observed.

Character	Species	Mean	SD	Mode	CV (%)	Range
Number of Ridges from the peristome to the trochal band	At	39.4	2.1	37	5.4	37 – 45
	Ea	40.1	3.5	38	8.7	36 – 50
Number of Ridges from the trochal band to the scopula	At	20.0	1.7	19	9.0	17 – 25
	Ea	20.0	1.5	20	7.0	18 – 25

Table 2.3. Measurements of cultured live colonies of *Zoothamnium* sp. attached to *Acartia tonsa* (At) and *Eurytemora affinis* (Ea). A total number of 32 colonies were measured for each character in each copepod species. Asterisks indicate characters where there is a statistically significant difference between the two host species ($p<0.05$)

Character	Species	Mean (μm)	SD (μm)	Mode	CV (%)	Range
Total length of the body from	At	41.0	± 5.5	43.0	13.4	31.2 - 53.0
epistomial disk to aboral end	Ea	44.9	± 4.8	43.6	10.7	35.8 – 54.7
Length of body from peristomial	At	34.0	± 5.9	38.0	17.3	20.7 - 44.3
lip to aboral end *	Ea	38.7	± 5.2	38.2	13.4	26.8 – 49.9
Width of the body below the	At	23.0	± 2.2	23.8	9.6	18.2 – 28.8
peristomial lip	Ea	23.1	± 2.4	21.9	10.4	18.2 – 28.1
Width of the body at midpoint	At	21.2	± 2.8	22.8	13.2	16.7 – 27.4
between oral and aboral ends	Ea	23.2	± 3.1	21.4	13.4	16.7 – 31.3
Width of peristomial lip	At	25.8	± 2.7	25.2	10.5	18.3 – 31.5
	Ea	26.5	± 2.4	29.1	9.0	21.5 – 30.8
Thickness of peristomial lip	At	4.1	± 1.2	3.6	29.7	2.5 – 7.7
	Ea	3.6	± 0.6	4.1	16.7	2.5 – 4.4
Width of epistomial disk	At	18.0	± 2.2	16.8	12.2	11.9 – 23.6
	Ea	18.6	± 2.1	19.0	11.3	13.5 – 22.8
Width of scopula	At	7.3	± 2.0	5.1	27.4	4.3 – 11.8
	Ea	8.1	± 1.6	5.8	19.7	4.8 – 8.2
Length of basal stalk *	At	43.3	± 21.8	12.6	50.3	13.0 – 105.4
	Ea	28.9	± 12.2	20.0	42.4	10.3 – 60.5
Width of basal stalk	At	10.2	± 1.5	10.0	14.7	7.3 – 14.6
	Ea	10.1	± 1.8	10.2	17.8	4.7 – 13.4
Length of lateral stalk *	At	38.0	± 27.2	22.0	71.6	5.8 – 115.1
	Ea	25.2	± 14.9	10.7	59.1	6.7 – 62.9
Width of lateral stalk	At	9.3	± 2.1	11.6	22.6	0.9 – 11.6
	Ea	9.3	± 1.8	10.6	19.3	6.3 – 13.9
Distance of myoneme from	At	24.7	± 22.0	16.8	89.1	3.7 – 115.1
attachment point *	Ea	13.7	± 5.7	15.0	41.6	4.1 – 23.6
Thickness of myoneme	At	2.0	± 0.4	1.8	20.0	1.3 – 3.0
	Ea	2.3	± 0.5	2.5	21.7	1.6 – 3.7
Total number of zooids in the	At	2.7	± 0.9	2.0	33.0	2.0 – 6.0
colony	Ea	2.7	± 1.0	2.0	37.0	2.0 – 6.0
Total number of branches in the	At	2.1	± 1.0	2.0	47.6	1.0 – 5.0
colony	Ea	2.5	± 1.4	1.0	56.0	1.0 – 6.0

Table 2.4. Measurements of cultured, Protargol stained colonies of *Zoothamnium* sp. attached to *Acartia tonsa* (At) and *Eurytemora affinis* (Ea). A total number of 30 colonies were measured for each character in each copepod species. Asterisks indicate characters where there is a statistically significant difference between the two host species ($p<0.05$).

Character	Species	Mean (μm)	SD (μm)	Mode	CV (%)	Range
Total length of the body from	At	31.1	± 4.7	25.0	15.2	22.0 – 39.5
epistomial disk to aboral end	Ea	30.3	± 5.1	29.0	17.0	25.7 – 35.4
Width of the body below the	At	22.0	± 3.4	21.2	15.5	14.7 – 28.5
peristomial lip	Ea	21.5	± 3.0	21.4	13.5	17.0 – 28.3
Width of the body at midpoint	At	21.7	± 4.3	22.0	20.1	12.3 – 30.3
between oral and aboral ends	Ea	20.3	± 4.1	22.5	20.4	16.0 – 30.8
Distance between trochal band	At	8.0	± 2.0	7.4	25.1	4.9 – 14.6
and scopula	Ea	7.8	± 2.0	7.2	26.2	4.0 – 12.4
Length of basal stalk	At	39.6	± 25.2	46.6	63	8.5 – 137.8
	Ea	35.0	± 22.6	26.2	64.6	10.0 – 86.5
Width of basal stalk	At	6.8	± 2.2	7.0	31.8	4.8 – 17.3
	Ea	6.8	± 1.5	6.0	22.4	5.0 – 9.9
Distance of myoneme from	At	23.1	± 15.6	11.0	67.6	2.2 – 63.3
attachment point	Ea	19.9	± 13.2	4	66.9	4.0 – 48.4
Length of micronucleus	At	2.5	± 0.4	2.5	15.9	1.9 – 3.4
	Ea	2.3	± 0.5	2.0	19.9	1.5 – 3.4
Width of micronucleus	At	2.5	± 0.3	2.3	12.6	2.0 – 3.1
	Ea	2.4	± 0.5	2.0	22.4	2.0 – 3.5
Width of macronucleus at	At	4.1	± 0.9	3.0	21.1	2.9 – 6.0
midpoint	Ea	4.7	± 1.0	4.0	21.4	3.5 – 6.9
Distance between ends of	At	2.1	± 0.4	1.8	19.4	1.2 – 3.0
OPK1 and OPK2	Ea	2.1	± 0.3	2.0	16.4	1.5 – 2.6

Table 2.5. Measurements of live telotrochs from cultured *Zoothamnium intermedium* attached to *Acartia tonsa* (At) and *Eurytemora affinis* (Ea). A total of 30 telotrochs originating form colonies attached to each host species was measure for each character.

Character	Species	Mean (µm)	SD (µm)	Mode	CV (%)	Range
Length of the body	At	27.7	2.2	26.8	8.1	23.7 – 31.5
	Ea	28.2	2.3	26.3	8.2	24.2 – 31.6
Width of the body	At	28.6	1.9	26.3	6.7	26.3 – 32.1
	Ea	28.9	2.2	29	7.7	25.8 – 33.7
Length of the cilia*	At	4.0	± 0.8	4.6	20.6	2.5 – 6.2
	Ea	4.0	± 0.9	4.0	21.7	2.5 – 6.4

*Length of cilia in the trochal band was measured from Lugol's fixed cells

Table 2.6. Measurements of field, Protargol stained colonies of *Epistylis* sp. attached to *Acartia tonsa*. A total number of 30 colonies were measured from the months with highest infestation prevalence (August and September 1994, and September 1996).

Character	Mean (μm)	SD (μm)	Mode	CV (%)	Range
Total length of the body from epistomial disk to aboral end	30.7	± 5.1	29.2	16.7	20.0 – 45.7
Width of the body below the peristomial lip	17.0	± 2.5	16.4	14.7	13.4 – 22.5
Width of the body at midpoint between oral and aboral ends	18.5	± 3.2	20.4	17.4	12.2 – 24.3
Distance between trochal band and scopula	11.2	± 2.3	12.5	20.2	6.7 – 14.9
Length of basal stalk	28.7	± 18.4	20.0	64.0	10.0 – 44.9
Width of basal stalk	4.3	± 0.9	4.0	21.4	2.8 – 6.4
Length of micronucleus	2.1	± 0.3	2.0	13.0	1.5 – 2.7
Width of micronucleus	2.2	± 0.3	2.2	12.7	1.8 – 2.6
Width of macronucleus at midpoint	3.3	± 0.6	3.2	18.9	2.4 – 4.5
Number of zooids in the colony	7.9	± 3.2	10.0	41.2	2 - 15

Table 2.7. Morphological comparison between *Zoothamnium intermedium* and other similar species in the genus *Zoothamnium*.

Character	**Z. intermedium**[a]	**Z. parasiticum**[b]	**Z. carcini**[c]	**Z. rigidum**[d]	**Z. hyketes**[e]	**Z. alternans**[f]	**Z. *sp1***[g]	**Z. *sp2***[h]
Size of the zooid (μm)	45	70	100	95	55	53	58	70
Branching pattern	alternate	alternate	alternate	alternate	alternate	alternate	alternate	dichotomous
Presence of macrozooids	No	No	No	No	No	Yes	No	No
Shape of macronucleus	"C" shaped	Horse-shoe shaped	Not indicated	"C" shaped	"C" shaped	"C" shaped	Horse-shoe shaped	Horse-shoe shaped
Position of contractile vacuole	Below the peristomial lip	In the upper half of the cell	Not indicated	In the upper half of the cell	In the upper half of the cell	Not indicated	Not indicated	Not indicated
Distance of myoneme from attachment point	Highly variable (2.2 – 63.3 μm)	Not indicated	Not indicated	Not indicated	Constant (15 μm)	Not indicated	Not indicated	Not indicated

a: this study
b and c: Kahl, 1935
d and e: Precht, 1935
f, g and h: Gross 1986

FIGURE LEGENDS:

Figure **2.1 A -2.1 H**: Photomicrographs of *Zoothamnium intermedium* attached to *A. tonsa* and *E. affinis* from life (**2.1 A-2.1 D**; Differential Interference Contrast) and after Protargol staining (**2.1 E-2.1 H**; Bright Field). **2.1 A.** Colonies at low magnification attached to the abdomen of the host. **2.1 B.** Colony with two zooids attached to the head of the host. Arrow shows the continuous myoneme in the basal stalk. **2.1 C.** Detailed zooid: arrow shows the peristomial lip, arrowhead shows the infundibulum, and double arrowheads show the elevated epistomial disk. **2.1 D.** Detailed zooid showing the contractile vacuole close to the peritomial lip. **2.1 E.** Colony after Protargol staining. Arrow shows the myoneme in the basal stalk and branches; arrowhead shows basal bodies in the telotroch band. **2.1 F.** Detailed zooid after Protargol staining showing the macronucleus (arrow) and the micronucleus (arrowhead). **2.1 G.** Zooid at high magnification showing the structure of the oral polykinetids (P) 1, 2, and 3. **2.1 H.** Zooids showing stained sphincter myoneme delimiting the peristomial opening (arrow). Arrowhead shows somatic myonemes close to the peristome. Bars = 50 μm (**2.1 A**); 20 μm (**2.1 B, 2.1 C, 2.1 D, 2.1 E**); 10 μm (**2.1 F, 2.1 G, 2.1 H**)

Figure **2.2 A-2.2 D:** Morphology of *Zoothamnium intermedium* epibiont on *Acartia tonsa* and *Eurytemora affinis* from life (Fig. **2.2 A**) and after Protargol staining (Fig. **2.2 B-2.2 C**). **2.2 A.** General aspect of the colony. **2.2 B.** Detail of the zooid showing somatic myonemes (SMY), macronucleus (MAC) and micronucleus (MIC). **2.2 C.**

Zooid at high magnification showing arrangement of the oral polykinetids (PK1, PK2, PK3). **2.2 D.** Detail of the oral polykinetids at high magnification. CV, contractile vacuole; FV, food vacuole; g, germinal row; INF, infundibulum; MAC, macronucleus; MIC, micronucleus; MY, myoneme; ODK, oral dikinetid; OPK, oral polykinetid; SCP, scopula; SMY, somatic myoneme; STK, stalk; STR, striae; TB, telotroch band. Bars = 20 μm (**2.2 A**); 10 μm (**2.2 B, 2.2 C**); 1 μm (**2.2 D**)

Figure **2.3 A-2.3 E**: Scanning Electron Micrographs of *Zoothamnium intermedium* attached to *Acartia tonsa* and *Eurytemora affinis*. **2.3 A.** Colonies at low magnification (730X), arrow shows the telotroch band. **2.3 B.** Detail of the zooid showing the parallel striae pattern on the pellicle (arrow) and attached bacteria (arrowhead). **2.3 C.** Zooid at very high magnification (13.900X) showing the scattered pattern of pores on the pellicle (arrowheads) and the parallel striae. **2.3 D.** Stalk showing the attachment disk on the surface of the host (arrow) and bacteria. **2.3 E.** Telotroch of *Z. intermedium* showing cilia in the telotroch band (arrow). Bars = 100 μm (**2.3 A**); 10 μm (**2.3 B, 2.3 C, 2.3 D**); 1 μm (**2.3 E**)

Figure **2.4 A-2.4 E**: Photomicrographs after Protargol (**2.4 A, 2.4 B**) and morphology (**2.1 C-2.1 E**) of *Epistylis* sp. attached to *Acartia tonsa*. **2.4 A.** Colony at low magnification attached to the abdomen of the host. **2.4 B.** Detail of the zooid showing a reticulate pattern of striae on the pellicle (arrowhead) and telotroch band (arrow). **2.4 C.** Zooid showing somatic myonemes (SMY), macronucleus (MAC) and micronucleus (MIC). **2.4 D.** Zooids at high magnification showing the arrangement of

the oral structure. **2.4 E.** Detail of the oral polykinetids (PK1, PK2, PK3) at high magnification. g, germinal row; MAC, macronucleus; MIC, micronucleus; ODK, oral dikinetid; OPK, oral polykinetid; SMY, somatic myoneme; STK, stalk; STR, striae; TB, telotroch band. Bars = 20 μm (**2.4 A**); 10 μm (**2.4 B, 2.4 C, 2.4 D**); 1 μm (**2.4 E**).

Figure 2.1.

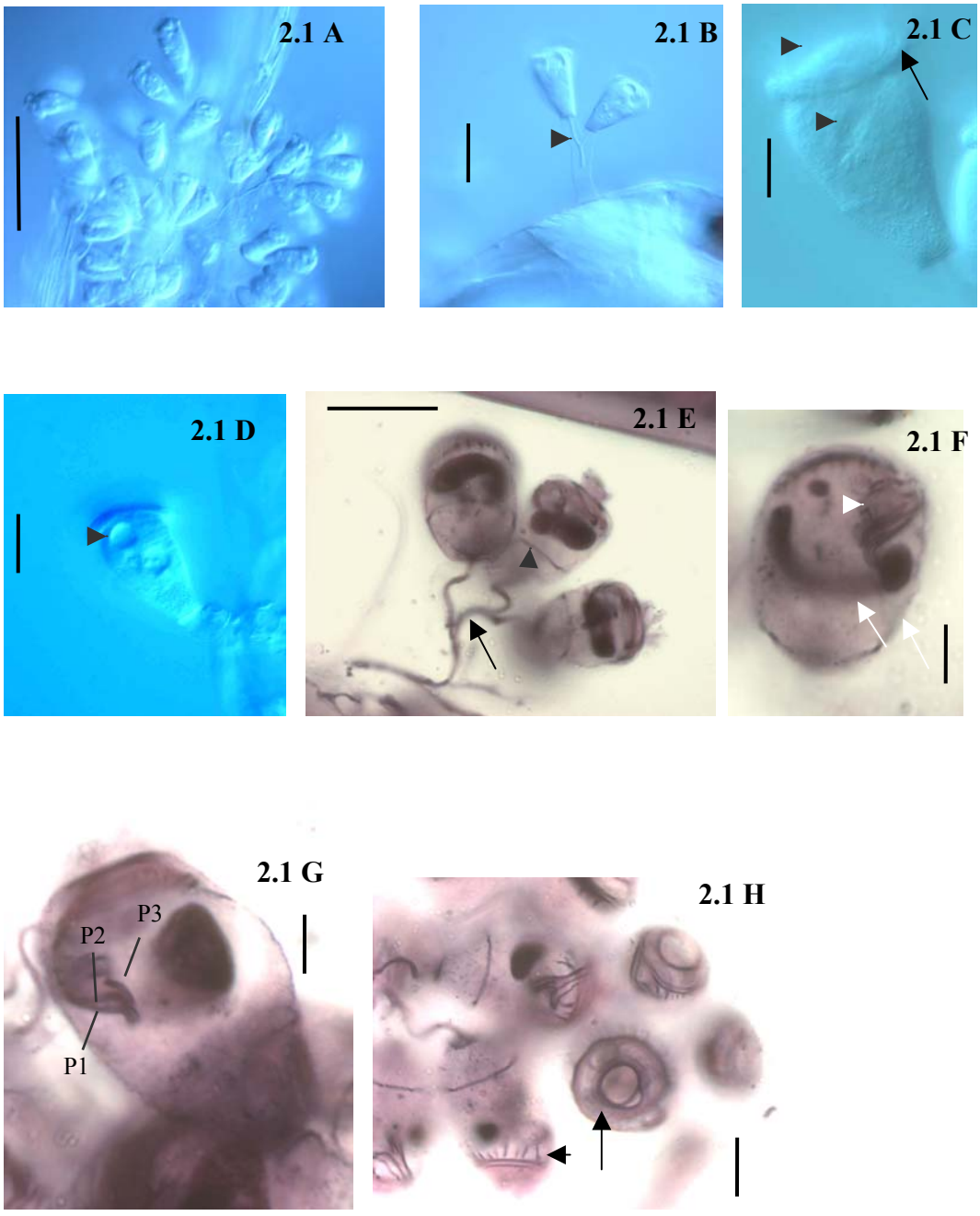
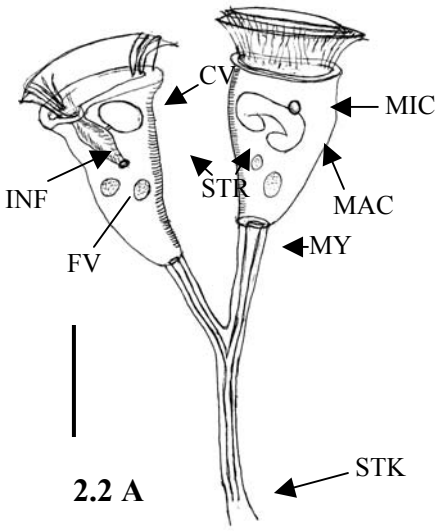


Figure 2.2



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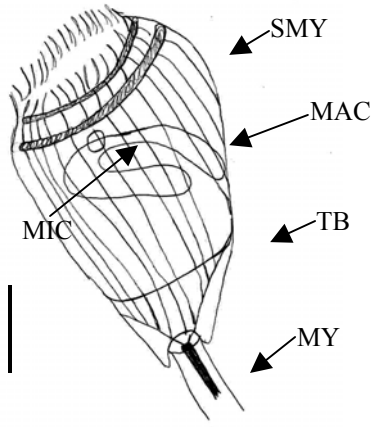

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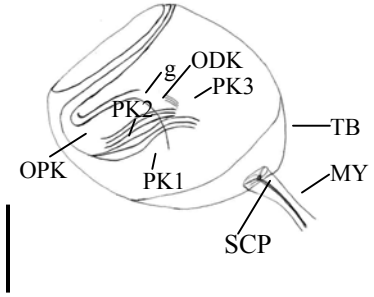

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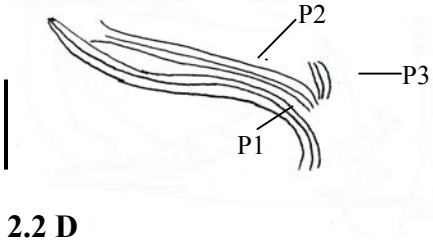
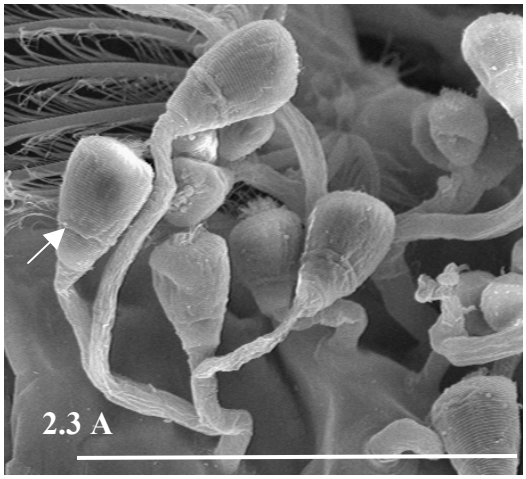
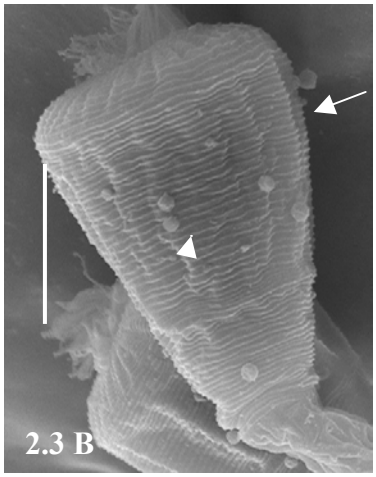
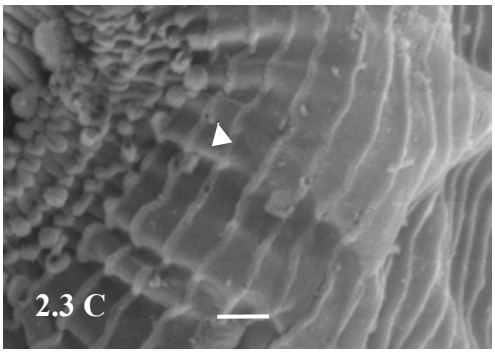


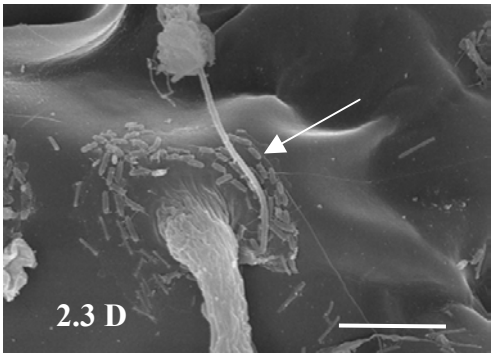
2.2 D

Figure 2.3









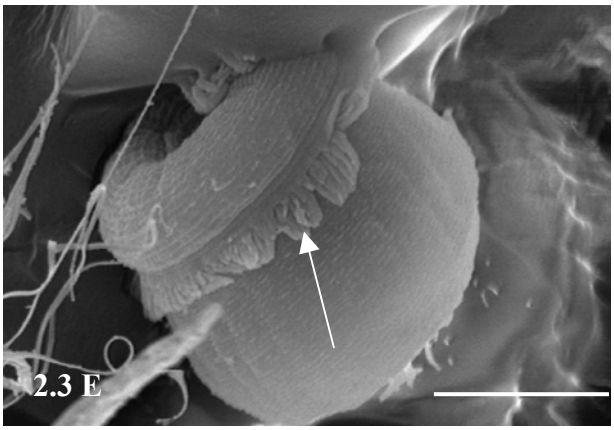
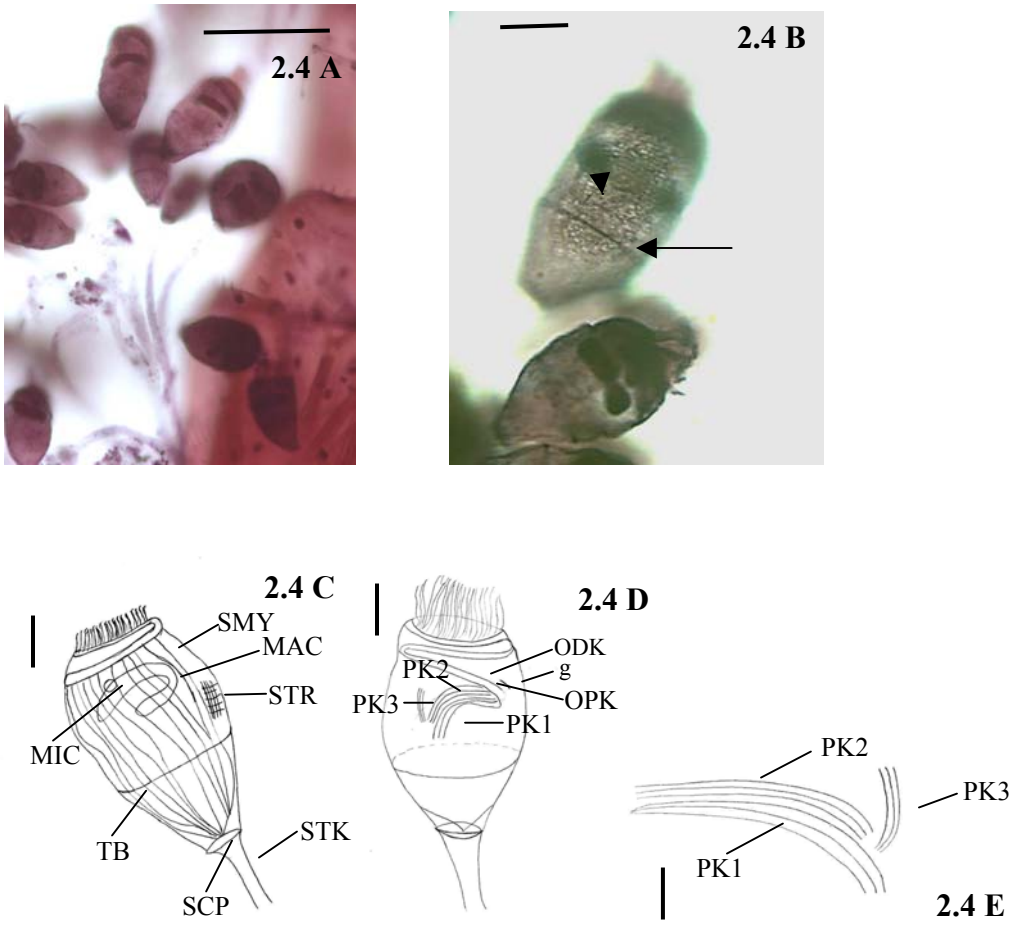


Figure 2.4


2.4 A
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CHAPTER 3

SPATIAL AND TEMPORAL PATTERNS IN THE OCCURRENCE OF PERITRICH CILIATES AS EPIBIONTS ON CALANOID COPEPODS IN THE CHESAPEAKE BAY, USA

ABSTRACT

I investigated temporal and spatial patterns of distribution in two peritrich ciliates (*Zoothamnium intermedium* and *Epistylis* sp.) living as epibionts on calanoid copepods (*Acartia tonsa* and *Eurytemora affinis*) in Chesapeake Bay. Net tow samples collected along the main axis of the Bay were analyzed to estimate occurrence of epibionts on copepods and to explore relationships among infestation prevalence, host abundance and environmental variables. *Z. intermedium* and *Epistylis* sp. colonized populations of *A. tonsa* during spring and summer months, while only *Z. intermedium* colonized *E. affinis* during spring. Occurrence of epibionts on copepods showed a high interannual variation, marked seasonality, and geographic heterogeneity. Extensive statistical analyses rejected simple scenarios of interactions between epibiosis, environmental variables, and host density, suggesting a more complex dynamics for the system. Overall, epibiont infestation density (colonies/host area) and load (zooids/host area) were higher on copepodites than on adults for both host species suggesting a preferential attachment to juveniles or a higher predation pressure on adult stages. Infestation density and load of both epibiont species were higher on cephalothorax and abdomen of *A. tonsa* and *E. affinis* in comparison to antenna and swimming legs, suggesting that ciliates can more easily colonize less active parts.

INTRODUCTION

Epibiotic relationships involving planktonic crustacea as basibionts (i.e. hosts) are widespread in marine environments and encompass a variety of organisms as epibionts, including bacteria, algae, and ciliates (Gibson 1978; Hanamura, 2000; Nagasawa, Simidu, and Nemoto 1985; Turner, Postek, and Collard 1979). Historically, epibiosis has been viewed as a commensal relationship, however several studies have suggested that epibionts can have deleterious effects on the host. These effects include decreased reproduction, growth, and survivorship (Xu and Burns 1991), increased susceptibility to predation (Chiavelli, Mills, and Threlkeld 1993; Weissman, Lonsdale, and Yen 1993; Willey, Cantrel, and Threlkeld 1990), and starvation due to competition with the epibionts (Kankaala and Eloranta 1987). In other cases, the epibiont itself may serve as food for the host. For example, *Daphnia* spp. readily prey on free-swimming stages of their epibionts (Al-Daheri and Willey 1996; Barea-Arco, Pérez-Martinez, and Morales-Barquero 2001). Thus, from an ecosystem perspective, epibiosis may interfere with fitness of the host organism, or fuel higher trophic levels. In addition, epibionts often occur on planktonic crustacea under eutrophic conditions, suggesting that the prevalence of epibionts on zooplankton might be used as an index of water quality (Henebry and Ridgeway 1979).

Few studies have addressed temporal and spatial patterns in the occurrence of epibionts that infest planktonic crustacea. Most of these are from freshwater environments and have considered algal or ciliate epibionts on relatively small temporal (annual or subannual) and spatial (from few meters to a couple kilometers) scales (Chiavelli, Mills, and Threlkeld 1993; Green 1974; Strinadel and

Ebert 1997; Xu 1992). While numerous accounts exist for ciliates as epibionts of marine zooplankton, little information is available on the prevalence of epibionts in zooplankton populations (Weissman, Lonsdale, and Yen 1993), and data on seasonality and geographic distribution of epibionts is almost lacking.

The only record of ciliate epibionts associated with zooplankton of Chesapeake Bay, the largest estuary in the United States, is that of Herman and Mihursky (1964). They reported *Zoothamnium* sp., a stalked, colonial peritrich, as a common epibiont on the most abundant calanoid copepod, *Acartia tonsa*, in the Patuxent River, a sub-estuary of the Chesapeake Bay. Samples collected on three dates between early March and mid-April revealed 100% infestation of *A. tonsa*, but other zooplankton species encountered during the study were not infested. Despite several studies have focused on the ecology of Chesapeake Bay zooplankton, no data are available on the seasonal, interannual, or spatial distribution of ciliate epibionts associated with *Acartia tonsa* or other zooplankton species.

Here I describe seasonal occurrence of peritrich ciliates as epibionts on Chesapeake Bay populations of *Acartia tonsa* and *Eurytemora affinis* by analyzing net tow samples taken over a five-year period. I also report epibiont density and load on copepod life-history stages and body parts by estimating number ofcolonies and zooids distributed over the host and on different body parts. Finally, I consider relationships among epibiont prevalence, host abundance, and environmental variables.

MATERIALS AND METHODS

Field Sampling. The occurrence of peritrich epibionts on *Acartia tonsa* and *Eurytemora affinis* was determined from samples collected at nine stations (one sample per station) along the main axis of the Chesapeake Bay (Fig. 3.1; filled circles; Table 3.1). Stations were sampled monthly in spring and summer of 1994-1996 and 1999-2000, using a 35-µm-mesh plankton net (30-cm diameter) to obtain a vertically integrated sample over the upper 10-15 m of the water column (a total of 21 samples/station during the study period). A 250-ml subsample of each net tow was fixed with modified Bouin's fluid (Coats and Heinbokel 1982) at a final concentration of 5% and examined using a dissecting microscope (Bausch and Lomb; 30X magnification) to determine the presence of ciliate epibionts on host organisms. Vertical CTD-O_2 (conductivity, temperature, depth, and dissolved oxygen) profiles were taken at each station using shipboard instrumentation (R/V Cape Henlopen; CTD Type Seabird 911 Plus).

Epibiont, Host, and Phytoplankton Population Assessments. Infestation prevalence (i.e., percent hosts with epibionts) was obtained for adults and copepodites of each host species by scoring all individuals in the sample as either infested or non-infested. Epibionts present on each host were tentatively identified to genus based on key morphological features characterized by Lynn and Small (2000). Infestation density (i.e., number of peritrich colonies/host area) and infestation load (i.e., number of peritrich zooids/host area) were then determined for samples where epibiont prevalence was $\geq$ 5% for *E. affinis* and $\geq$ 10% for *A. tonsa*. For these data, 30 adults and 30 copepodites were selected from each sample and examined using a Zeiss RA

microscope (100X) to enumerate colonies/host and zooids/colony. The position of colonies on host organisms (i.e., antennae, cephalothorax, abdomen, and swimming legs) was recorded, and the dimensions (length and width) of host body parts were measured using a calibrated ocular micrometer. The area of host body parts was calculated from mean length and width measurements assuming a cylindrical shape (i.e., $A= (2\pi W)*L$), as recommended by Mauchline (1998). To confirm epibiont identification, 2-3 representative specimens were taken from each sample and silver-stained using the Protargol technique (Montagnes and Lynn, 1993). Stained specimens were identified to genus or species using criteria described in Chapter 2.

Since net tows used to assess epibiont prevalence were not suitable for determining copepod densities, estimates of host abundances were obtained from the Chesapeake Bay Monitoring Program (CBMP) Database (http://www.chesapeakebay.net). Nine CBMP stations (Fig. 3.1; filled triangles; Table 3.1), one close to each location sampled for presence of epibionts, were selected for analysis involving basibiont abundance. Data on phytoplankton abundance (cells/ml in surface water) were also obtained for the nine CBMP stations.

Data Analyses. Multivariate Correlation analyses (Spearman-Rho correlation) were performed to evaluate inter- and intra-annual trends in epibiont prevalence relative to host abundance, phytoplankton abundance, and environmental variables (i.e., temperature, salinity, and dissolved oxygen). Phytoplankton abundance used in these analyses was based on cells smaller than 10 μm in diameter, as the ciliate epibionts encountered in this study typically ingested bacteria to nanoflagellate-sized prey (Corliss, 1979). Kruskal-Wallis ANOVA on ranks was performed to discern

differences in epibiont density (colonies/mm^2) and load (zooids/mm^2) among host body parts, with Dunn's test used for pairwise comparisons of means. Correlation analyses were performed using JMPIN Version 4.04 (SAS Institute), Analyses of Variance were run using SigmaStat Version 2.0 (SPSS Inc.) and data are reported in the text as mean $\pm$ standard error of the mean (SE).

RESULTS

The coenobial and colonial peritrichs, *Zoothamnium intermedium* Precht, 1935 and *Epistylis* sp., respectively, colonized Chesapeake Bay populations of *Acartia tonsa* (adults and copepodites) during spring and summer months of 1994-2000. Adults and copepodites of *Eurytemora affinis* were also infested by *Z. intermedium,* but not by *Epistylis* sp. Other copepod species (e.g. *Cyclops* spp., *Oithona* spp., *Diaptomus* spp.), rotifers (e.g. *Brachionus* spp.,*Synchaeta* spp., *Trichocerca* spp.), cladocerans (e.g. *Evadne* spp., *Podon polyphemoides*), and barnacle nauplii (Balanidae) were also present in the samples, but did not harbor ciliate epibionts.

Infection prevalence for *Z. intermedium* on *A. tonsa* reached appreciable levels (> 5%) at mid to high salinity (10 to 25 psu), regardless of temperature (Fig. 3.2A). Infestations of *Epistylis* sp. on *A. tonsa* also exceeded 5% at higher salinities (16-26 psu), but only when water temperature was above 23^{o}C (Fig. 3.2B). Interestingly, *Z. intermedium* and *Epistylis* sp. did not co-occurr in any sample. By contrast, *Z. intermedium* on *E. affinis* showed high prevalence (>5%) over a broad range of temperatures (8-23^{o}C) and salinities (7 to 24 psu; Fig. 3.2C).

Occurrence of peritrich epibionts on Chesapeake Bay copepods exhibited high interannual variation, with yearly mean prevalence ranging from a low of 0.1% ± 0.3 (median = 0.2; n = 27) to a high of 5.2% ± 1.4 (median = 1.7; n = 63) for *Z. intermedium* on *A. tonsa*, 0.1 ± 0.1 (median = 0.1; n = 45) to 0.8 ± 0.3 (median = 0.9; n = 45) for *Epistylis* sp. on *A. tonsa*, and 0.2 ± 0.1 (median = 0.7; n = 45) to 1.5 ± 0.4 (median = 0.2; n=63) for *Z. intermedium* on *E. affinis* (Fig. 3.3A). Host taxa also showed considerable variation in annual mean abundance across the study period, but there was no clear relationship between epibiont prevalence and host abundance (cf. Fig. 3.3A and B). In addition, epibionts exhibited marked seasonality with peak prevalence of *Z. intermedium* occurring in early spring and late summer on *A. tonsa*, but only in spring on *E. affinis* (Fig. 3.3 C). Infestations of *Epistylis* sp. on *A. tonsa* were only present in late summer and early fall. Seasonal fluctuations in epibionts were not clearly related to host abundance, with high prevalence of *Z. intermedium* on *A. tonsa* occurring when host density was either high (13.2 ± 2.1, n=45; August) or low (3.2 ± 1.4, n=9; March). The spring maximum in prevalence of *Z. intermedium* on *E. affinis*, however, did coincide with the maximum monthly abundance for that host (cf. Fig. 3.3C and D).

Epibionts also showed geographic heterogeneity along the main axis of the Bay, with mean prevalence for *Z. intermedium* on *A. tonsa* being highest in the lower Bay (4.3 ± 1.9 to 5.8 ± 3.1 for stations 707-744; Fig. 3.3E) and decreasing abruptly at station 804 northward (range: 0.3 ± 0.1 to 1.8 ± 0.7). *Epistylis* sp. infestations of *A. tonsa* were less common than *Z. intermedium* on *A. tonsa*, but followed the same pattern of decreasing prevalence from south to north (range: 0.1 ± 0.1 to 1.4 ± 0.8;

Fig. 3.3E). By contrast, *Z. intermedium* was most prevalent on *E. affinis* in the upper Bay (0.9 $\pm$ 0.5 and 1.1 $\pm$ 0.7 for stations 908 and 858, respectively; Fig. 3.3E), with consistently lower values (range: 0.2 $\pm$ 0.2 to 0.4 $\pm$ 0.) south of station 845. Host density appeared to have little influence on the spatial distribution of *A. tonsa* epibionts, as mean abundance for this copepod was relatively stable along the axis of the Bay (range: 4.5 $\pm$ 1.4 to 10.9 $\pm$ 2.5; Fig. 3.3F). *E. affinis*, however, exhibited peak concentrations in the upper Bay (6.2 $\pm$ 1.5 L^{-1} at station 908) that coincided with maximum epibiont prevalence (1.2 + 0.7; Fig. 3.3F).

Contour plots for mean occurrence of peritrich epibionts (Fig. 3.4) showed infestation foci for *Z. intermedium* on *A. tonsa* in the lower Bay during late summer and on *E. affinis* in the upper Bay during spring. Those foci coincided with temporal-spatial regions of elevated host abundance, suggesting a density dependent epibiont-host interaction (cf. Fig. 3.4 A and B for *A. tonsa* and C and D for *E. affinis*). High prevalence of *Z. intermedium* on *A. tonsa* in early spring (late March and April), however, did not coincide with high host densities, nor did areas of high host abundance in late spring (May and early June) support increased levels of epibiosis (Fig. 3.4A). A density dependent interaction was also indicated for Z. *intermedium*, but not for *Epistylis* sp., when data for epibiont prevalence on *A. tonsa* were grouped into categories of low (<5 L^{-1}), medium (>5-15 L^{-1}), and high (> 15 L^{-1}) host abundance (Fig. 3.5). Correlation analysis, however, failed to show a significant relationship between infestation prevalence and copepod abundance when data for stations was pooled across years for each epibiont-host pair ($p > 0.05$; Table 3.1).

Also, no significant correlation was observed between environmental variables, phytoplankton abundance, and occurrence of epibiosis ($p > 0.05$).

Infestation density and load: Estimates of epibiont density (colonies/host) and load (zooids/colony) were obtained from three stations in 1994 (724 and 707 in May; 724 in September), eight stations in 1996 (908, 858, 845 in March; 858 in April; 744, 724, 707 in August; 724 in September), and one station in 2000 (724 in September).

Ciliates colonized all major body parts of *A. tonsa* and *E. affinis*, and a statistically significant difference was observed in infestation density of *Z. intermedium* between adults and copepodites of the two host species (ANOVA Kruskal-Wallis, $p<0.05$; Fig. 3.6 A) with higher numbers of colonies per body area occurring on copepodite stages. The same pattern was observed for *Epistylis* sp. where colony density was significantly higher on copepodites than on adults of *A. tonsa* (ANOVA Kruskal-Wallis; $p<0.05$; Fig 3.6 A). However when mean number of zooids per epibiont colony was considered no difference was observed for *Z. intermedium* colonizing adults and copepodites of *A. tonsa* (ANOVA Kruskal-Wallis; $p>0.05$; Fig 3.6 B). On the other hand, the number of zooids/mm^2 was higher on adults than on juveniles of *E.affinis* (ANOVA Kruskal-Wallis; $p<0.05$; Fig 3.6 B) displaying the same pattern observed for infestation density in this host species. Mean number of zooids per *Epistylis* sp. colony was not significantly different for adults and copepodites of *A.tonsa* in a similar pattern observed for *Z. intermedium* colonizing the same host species. Infestation load of *Z. intermedium* was significantly higher on copepodites than on adults of *A. tonsa* (ANOVA Kruskal-Wallis; $p<0.05$; Fig 3.6 C),

but no significant difference was observed in infestation load of *Z. intermedium* on adults and copepodites of *E. affinis* (ANOVA Kruskal-Wallis; $p>0.05$; Fig 3.6 C). Likewise, load of *Epistylis* sp. was not significantly different between adults and copepodites of *A. tonsa* (ANOVA Kruskal-Wallis; $p>0.05$; Fig 3.6 C).

When epibiont colonization was analyzed across host body parts, higher infestation density and load were observed on the cephalothorax and abdomen in comparison to more mobile parts (antenna and swimming legs). For example, infestation density and load of *Z. intermedium* were significantly higher on the cephalothorax and abdomen of *A. tonsa* in comparison to antenna and swimming legs (ANOVA Kruskal-Wallis, $p<0.05$; Fig. 3.7 A,C). Also, a significantly higher density of *Z. intermedium* colonies was observed on the abdomen of *E. affinis* when compared to the other body parts (ANOVA Kruskal-Wallis, $p<0.05$; Fig. 3.8 A), with an infestation load significantly higher on both cepahlothorax and abdomen of when compared to antenna and swimming legs (ANOVA Kruskal-Wallis, $p<0.05$; Fig. 3.7 C). A higher density of *Epistylis* sp. was recorded on the abdomen of *A. tonsa* (ANOVA Kruskal-Wallis, $p<0.05$; Fig. 3.8 A) in comparison to the other body parts, while infestation load was significantly higher on cephalothorax and abdomen when compared to antenna and swimming legs (ANOVA Kruskal-Wallis, $p<0.05$; Fig. 3.8 C). When the mean number of zooids per epibiont colony was considered, a significant difference was observed on the cephalothorax and abdomen of the two copepod species in comparison to antenna and swimming legs (ANOVA Kruskal-Wallis, $p<0.05$; Fig. 3.7 B) for both epibiont species. Analyses of epibiont infestation

density, load, and mean number of zooids per colony by year, station, and host life stage revealed the same pattern observed for the combined data.

DISCUSSION

Zoothamnium intermedium and *Epistylis* sp. were the only species of peritrich ciliates colonizing populations of *Acartia tonsa* and *Eurytemora affinis* in the Chesapeake Bay during the study period. *Z. intermedium* was originally described as an epibiont on amphipods and isopods from the Kiel Bay (Precht, 1935), and later was found attached to copepods and barnacle nauplii in the Lagoon of Venice, Italy (Valbonesi and Guglielmo, 1989). The genus *Epistylis* is also a common peritrich found as an epibiont on crustaceans in freshwater (Willey and Threlkeld, 1993) and estuarine environments (Turner, Postek, and Collard, 1979), with several species having been originally described from crustacean hosts (Kahl, 1935; Precht 1935).

Epibionts in the present study colonized only adults and copepodites of *A. tonsa* (both *Z. intermedium* and *Epistylis* sp.) and *E. affinis* (*Z. intermedium* only), not being observed attached to other zooplankton species present in the samples. The lack of colonization of other substrates may be due to host specificity, high preference for these two host species, or to a host-epibiont density dependent relationship, since *A. tonsa* and *E. affinis* are the dominant copepod species in the Bay (Brownlee and Jacobs, 1987). Interestingly, Valbonesi and Guglielmo (1988) reported *Z. intermedium* colonizing several species of planktonic crustacea (e.g. *Acartia latisetosa Calanipeda aquadulcis,* and the zoea stage of the shrimp *Hippolyte longirostris*) from Lagoon of Venice, Italy, but high infestation prevalence was observed only on the

most abundant copepod species (*Acartia clausi*), also supporting a host-epibiont density dependent relationship. In addition, other studies demonstrated that these species, which were considered to be specific of one host, could colonize different substrates in different systems. For example, Henebry and Ridgeway (1979) in an assessment of the epibiont community attached to planktonic crustacea from a lake in Illinois observed that *Vorticella microstoma* infested only the cladoceran *Scapholeberis kingii*, failing to colonize other hosts and non-living substrates. By contrast, the same species of *Vorticella* has been reported heavily colonizing the cyanobacteria *Microcystis aeruginosa* in a lake (Pratt and Rosen, 1983) and also being able to attach to non-living surfaces in a river in Spain (Garcia et al., 1989).

High epibiont prevalence has generally been observed when host species are more abundant (Mohlemberg and Kaas, 1990; Xu, 1992; Chiavelli, Mills, and Threlkeld, 1993), however, some studies failed to show a clear relationship between epibiont prevalence and substrate density (Lopez et al., 1998; Hanamura, 2000). In Chesapeake Bay, prevalence of *Z. intermedium* and *Epistylis* sp. was not directly correlated to host abundance, although an increase in prevalence with increasing substrate abundance was observed for *Z. intermedium* attached to *A. tonsa* when host densities were categorized (Fig. 3.5). One possible explanation for the absence of strong correlation is that *Z. intermedium* and *Epistylis* sp. are obligatory epibionts on *A. tonsa* and *E. affinis*, and factors like fluctuations in phytoplankton and bacteria populations, influx of freshwater in the system and pollution in addition to host abundance could be governing the colonization of these two copepod species by epibionts. In this case, infestation prevalence will be higher when ideal conditions are

met, but epibionts will always be present in very low (sometimes undetectable) numbers. The lack or very low infestation prevalence of *Z. intermedium* and *Epistylis* sp. observed during the study period could support this hypothesis.

In epibiotic communities, strong positive host density dependence suggests high specificity of the epibiont species to the host (Threlkeld, Chiavelli, and Willey, 1993). On the other hand, correlation between host abundance and epibiont prevalence for some substrates but not for others can indicate that some organisms may function as habitat source and others may act as sink habitats (Threlkeld, Chiavelli, and Willey, 1993). Pulliam (1988) defined sink habitats as patches where mortality rates exceed reproductive rates and source habitats as places where reproduction exceeds mortality. In his model, he predicts that an organism migrates from an overpopulated source habitat to an empty sink habitat because it is more advantageous to colonize a poorer habitat than not being able to find a substrate at all. Also, if the number of migratory organisms from the source habitat is large and the reproductive deficit in the sink habitat is small, the majority of the population will occur in the sink habitat.

Z. intermedium and *Epistylis* sp. colonized only populations of *A. tonsa* and *E. affinis* not being observed on any other zooplankton. In this case, if these two copepod species act as habitat sink, the source habitat could be an organism from the benthic community, which was not assessed in the present study. The interannual variation observed in this study could also support the idea of copepods as sink habitats, since migration from source habitat presents high fluctuation and is only seen when population density in the source habitat is very high (Pulliam, 1988).

The absence of *Epistylis* sp. from most samples, or its restriction to specific times of the year, suggest the possibility of a competition with *Z. intermedium*, since these two epibiont species never co-occurred. Ebert, Hottinger, and Pajunen (2001) observed a competition between *Epistylis helenae* and *Vorticella octava* attached to *Daphnia* spp., mainly because *V. octava* disturbed the surrounding environment and required more space especially due to its ability to contract. Like *V. octava*, *Z. intermedium* has a contractile stalk, being able to contract the entire colony at once, while *Epistylis* sp. is a non-contractile peritrich. This morphological difference might have led to an interespecific competition for space between *Z. intermedium* and *Epistylis* sp. favoring the former. The hypothesis that Z. *intermedium* is a better competitor is based on the observation that *Epistylis* sp. abundance was restricted to late summer and early fall and that this species was absent when *Z. intermedium* was abundant at these times of the year. On the other hand, the temporal and spatial segregation observed for the two epibiont species might not be only associated with competition for space. Biotic and abiotic factors could also be influencing the relationship, favoring one species in detriment of the other.

Z. intermedium appears to be euryhaline, and eurythermal since the species colonized Chesapeake Bay copepods at salinities that ranged from 3 psu to 22 psu over a wide range of temperature (4^{o}C to 26^{o}C). A similar result was reported for an unidentified species of *Zoothamnium* colonizing crayfishes in an estuary in India, where it attached to hosts at salinities ranging from 0 to 34 psu (Jayasree, Jankiran, Madahavi, 2001). Although this species of *Zoothamnium* was found in a broad range of salinity, high infestation prevalence was observed only at salinities lower than 16

psu. Epibiont species may suffer environmental stress depending on the capability of their hosts to live in or migrate to freshwater and marine environments (Wahl, 1989). Thus, epibionts that survive in a broad range of salinity and temperature, for example, will be able to maintain a wide geographical distribution as observed for *Z. intermedium* in the Chesapeake Bay. By contrast, *Epistylis* sp. exhibited a more restricted range of salinity (11 to 27 psu) and temperature (22^{o}C to 25^{o}C), colonizing only *A. tonsa* during the studied period. This restricted distribution of *Epistylis* sp., however, could also be due to competition with *Z. intermedium* and not only to tolerance to salinity or temperature.

Z. intermedium and *Epistylis* sp. attached to the entire body of their copepod hosts, but overall infestation density and load of *Z. intermedium* and *Epistylis* sp. was significantly higher on copepodites than on adults for both *A. tonsa* and *E. affinis*. This result differs from what was found for other systems where high infestation was observed on the adult stages of planktonic crustacea (Xu, 1992; Willey and Threlkeld, 1993). Higher density of colonies and zooids on copepodite stages is a counterintuitive result because colonies are shed with the cast skin every time copepodites molt, forcing epibionts to find another substrate. Accordingly, adult copepods should constitute a more stable substrate for epibionts because they do not molt and can accumulate a higher density of colonies.

One possible explanation for the difference in infestation density observed between adults and copepodites in the Chesapeake Bay, is that heavily infested adults could have a higher predation risk than juvenile stages, since epibionts would increase the apparent size of adults and make them more visible to fish that prey

visually. Willey, Cantrel and Threlkeld (1990) demonstrated that prevalence of *Colacium vesiculosum* on *Daphnia* spp. was significantly lower in experimental tanks containing fish than in control tanks, suggesting that epibionts made the host more susceptible to predation by increasing size and contrast. Moreover, epibionts may affect swimming behavior and escape reaction by increasing drag forces, and making hosts also more susceptible to fishes that are pump filter feeders (Willey, Cantrel, and Threlkeld, 1990; Chiavelly, Mills and Threlkeld, 1993).

An alternate hypothesis is that juveniles are more susceptible to infestation due to differences in swimming behavior or speed in comparison to adults, or perhaps free-swimming stages of epibionts are less vulnerable to copepodite predation when compared to adults. Experiments are necessary to discern if epibionts colonize preferentially copepodite stages of *A. tonsa* and *E. affinis* or if the difference observed from field samples just reflects the predation pressure that infested adults suffer in nature.

When presence of epibionts was analyzed across host body parts, infestation density and load were higher on the cephalothorax and abdomen of both host species when compared to antennae or swimming legs. Epibionts have been reported to attach to sites of the host where chances of gathering food are high. For example, Evans, Sicko-Goad, and Omair (1979) found that the suctorian *Tokophrya quadripartita* attached especially to the head of *Limnocalanus macrurus*, and attributed this preference to the carnivorous habits of this ciliate. Also, *Colacium vesiculosum* colonized the post abdomen of *Daphnia* spp. suggesting that the epibiont was able to take up dissolved nutrients eliminated by its host. The preferential attachment

observed for *Z. intermedium* and *Epistylis* sp., however, is not apparently related to availability of food, but might be related to the mobility of body parts, since copepods use antennae and legs for swimming and food gathering. The presence of epibiont colonies on antennae and swimming legs demonstrates that once epibionts attach to these body parts they can grow, but the significantly lower colony density suggests that these mobile parts are harder to colonize than cephalothorax and abdomen.

In the present study, I documented the spatial and temporal patterns of occurrence of peritrich epibionts on copepods in Chesapeake Bay and showed that peaks of infestation prevalence occur during early spring and late summer. Statistical analyses rejected any simple scenario of interaction between infestation prevalence, host abundance and environmental variables, suggesting that complex interactions govern this epibiotic system. Interestingly, copepodite stages had higher density of epibiont colonies when compared to adults implying a preferential colonization on the juvenile stages or a high predation pressure on the adult stages in nature. In addition, epibiont colonies were denser on the cephalothorax and abdomen of copepod hosts when compared to antenna and swimming legs, demonstrating that more mobile parts are harder to colonize. Future research will explore preferential epibiont attachment to copepodite stages of *A. tonsa* and *E. affinis*.

Table 3.1. Latitude and longitude of stations where host abundance (Chesapeake Bay Program) and infestation prevalence were assessed.

Abundance	Infestation Prevalence
39’26N 76’22W	39’07N 76’20W
39’20N 76’25W	38’57N 76’23W
38’59N 76’20W	38’44N 76’26W
38’33N 76’18W	38’34N 76’25W
38’08N 76’24W	38’18N 76’17W
37’35N 76’15W	38’03N 76’13W
37’14N 76’12W	37’43N 76’10W
37’13N 76’08W	37’23N 76’05W
36’59N 76’09W	37’07N 76’06W

Table 3.2. Total number of *A. tonsa* infested by *Zoothamnium intermedium* and *Epistylis* sp., and *Eurytemora affinis* colonized by *Z. intermedium* found in each station during the study period.

Station	Samples Analyzed	Total Number of *A. tonsa* infested by *Z. intermedium*	Total Number of *A. tonsa* infested by *Epistylis* sp.	Total Number of *E. affinis* infested by *Z. intermedium*
908	24	158	14	246
858	24	80	4	309
845	24	117	5	118
834	24	120	0	33
818	24	91	5	12
804	24	192	12	29
744	24	1350	42	20
724	24	623	252	14
707	24	893	81	20

Table 3.3. Correlation coefficient and p values obtained from Spearman Rank Correlation analyses for host abundance, environmental variables and infestation prevalence of *Zoothamnium intermedium* attached to *Acartia tonsa* and *Eurytemora affinis* and *Epistylis* sp. attached to *A. tonsa* in 1994-1996 and 1999-2000 in the Chesapeake Bay. C = correlation coefficient (r^2); p = p value; n=number of samples

VARIABLES	INFESTATION PREVALENCE		
	Z. intermedium attached to *A. tonsa*	*Z. intermedium* attached to *E. affinis*	*Epistylis* sp. attached to *A. tonsa*
Abundance of *A. tonsa*	C = 0.04 p = 0.5 n= 216	C = -0.1 p = 0.1 n = 216	C = 0.09 p = 0.1 n = 216
Abundance of *E. affinis*	C = 0.1 p = 0.1 n= 216	C = 0.5 p = 0.1 n = 216	C = -0.02 p = 0.2 n = 216
Salinity	C = -0.08 p = 0.2 n= 216	C = -0.3 p = 0.1 n = 216	C = 0.1 p = 0.06 n = 216
Temperature	C = -0.3 p = 0.1 n= 216	C = -0.3 p = 0.1 n = 216	C = 0.1 p = 0.1 n = 216
Dissolved Oxygen	C = 0.2 p = 0.1 n= 216	C = 0.02 p = 0.6 n = 216	C = 0.2 p = 0.1 n = 216
Chlorophyll *a*	C = 0.2 p = 0.1 n= 216	C = -0.07 p = 0.2 n = 216	C = 0.2 p = 0.1 n = 216
Abundance of Non-diatom < 10 µm	C = -0.1 p = 0.06 n= 216	C = -0.03 p = 0.6 n = 216	C = -0.03 p = 0.6 n = 216
Abundance of Non-diatom > 10 µm	C = 0.009 p = 0.8 n= 216	C = 0.2 p = 0.1 n = 216	C = -0.06 p = 0.3 n = 216

FIGURE LEGENDS

Figure **3.1**. Map of Chesapeake Bay showing stations where infestation prevalence was estimated from (●) and routine stations of the Chesapeake Bay Monitoring Program that were used to determine host abundance (▲).

Figure **3.2**. Infestation Prevalence (%) relative to salinity (psu) and temperature (^{o}C) profiles: (**A**) *Z. intermedium* attached to *A. tonsa*; (**B**) *Epistylis* sp. attached to *A. tonsa*; (**C**) *Z. intermedium* attached to *E. affinis*. (**.**) for infestation prevalence <5%, (◓) for infestation prevalence >5<10%,(⬤) for infestation prevalence >10%

Figure **3.3**. Infestation Prevalence (%) of *Zoothamnium intermedium* and *Epistylis* sp.(**A, C, E**), and abundance (copepod/L) of *Acartia tonsa* and *Eurytemora affinis* (**B, D, F**) in a temporal and spatial distribution.

Figure **3.4**. Mean infestation prevalence (%) of *Zoothamnium intermedium* attached to *A. tonsa* (**A**) and *E. affinis* (**C**), and mean abundance of *A. tonsa* (**B**) and *E. affinis* (**D**) (copepod/L) between 1994-1996 and 1999-2000 along the main axis of the Chesapeake Bay.

Figure **3.5**. Mean infestation (%) of *Z. intermedium* and *Epistylis* sp. at different categories of *A. tonsa* abundance (copepod/L).

Figure **3.6**. Infestation density (colonies/mm^2), mean number of zooids per colony, and infestation load (zooids/mm^2) of *Z. intermedium* and *Epistylis* sp. attached to adults and copepodites of *A. tonsa* and *E. affinis*. Asterisks indicate a statistically significant difference ($p < 0.05$).

Figure **3.7**. Infestation density (colonies/mm^2), mean number of zooids per colony, and infestation load (zooids/mm^2) of *Z. intermedium* and *Epistylis* sp. attached to cephalothorax, abdomen, antenna, and swimming legs of *A. tonsa* and *E. affinis*. Asterisks indicate a statistically significant difference ($p < 0.05$).

Figure 3.1

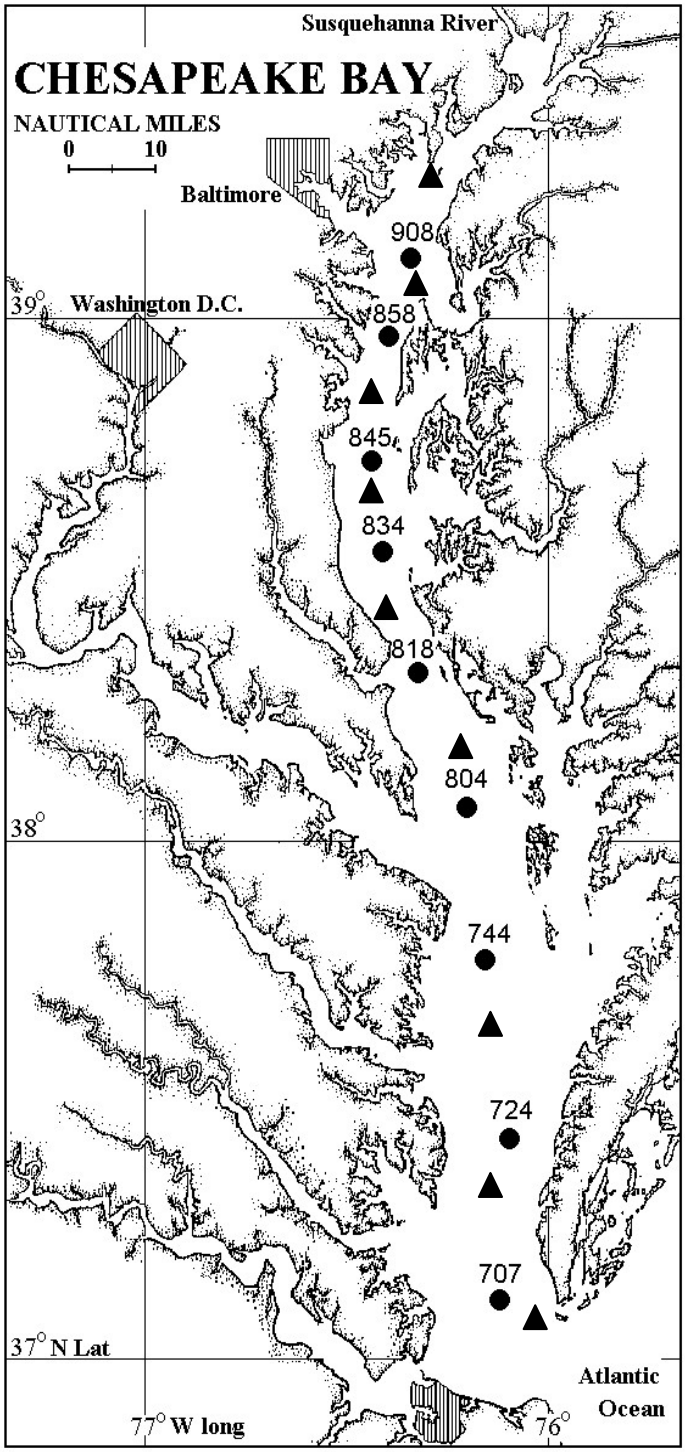

Susquehanna River
CHESAPEAKE BAY
NAUTICAL MILES
0 10
Baltimore
908
39°
Washington D.C.
858
845
834
818
804
38°
744
724
707
37° N Lat
Atlantic
Ocean
77° W long
76°

Figure 3.2

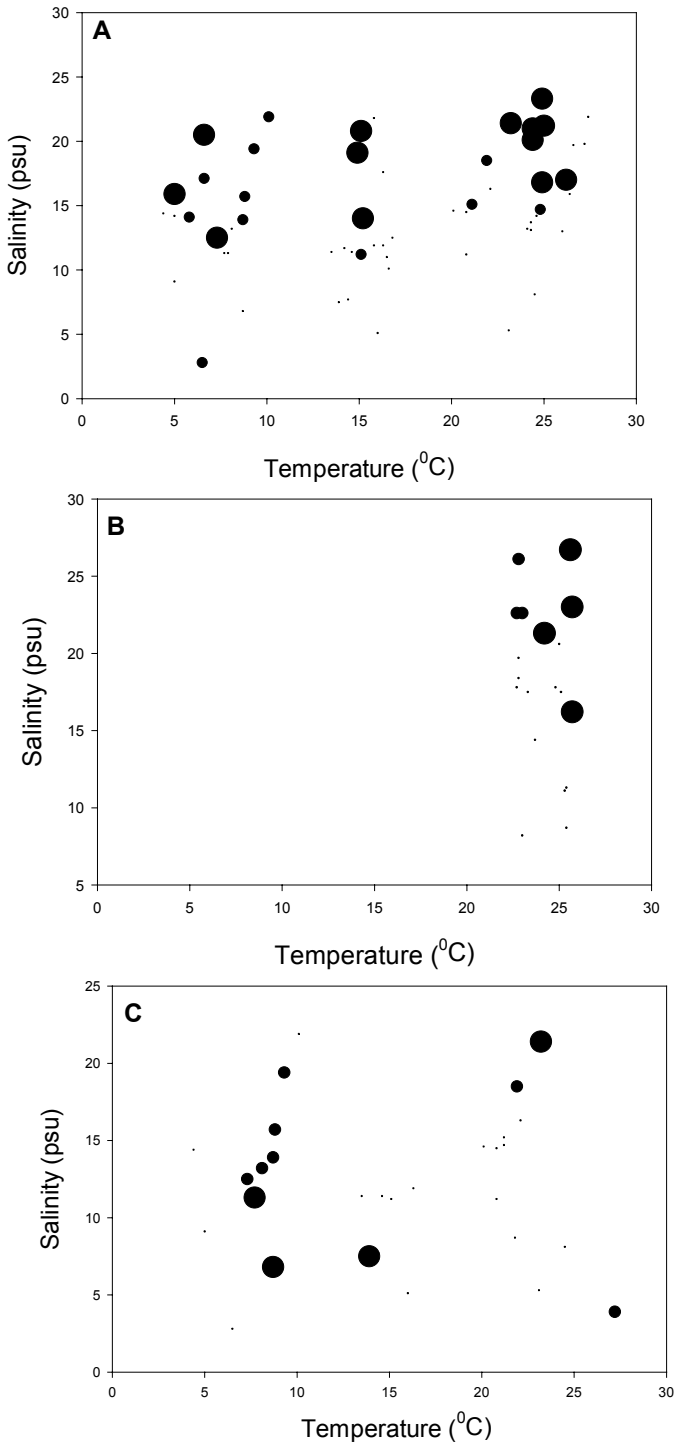

A
Salinity (psu)
Temperature (^{0}C)
B
Salinity (psu)
Temperature (^{0}C)
C
Salinity (psu)
Temperature (^{0}C)

Figure 3.3

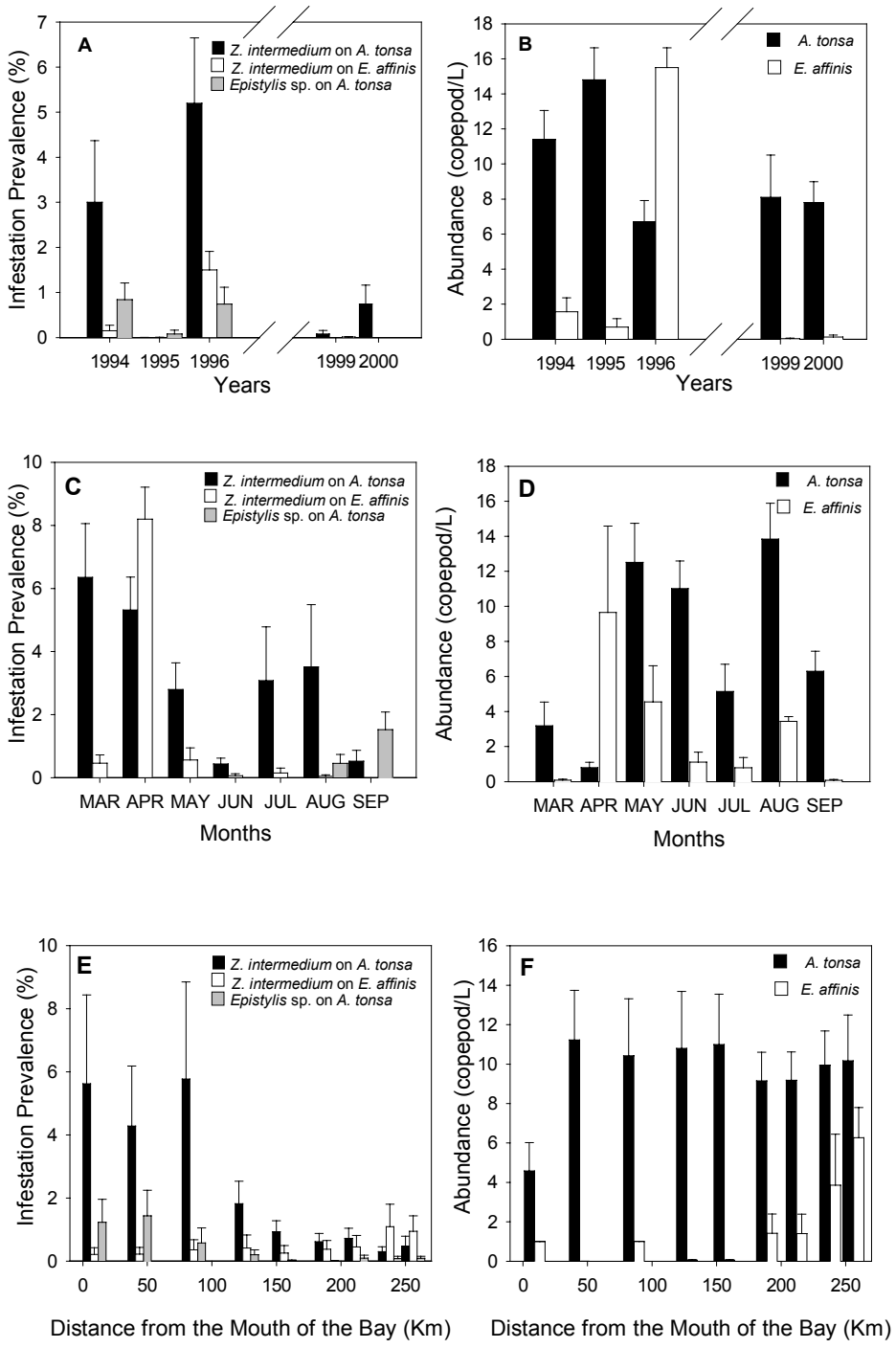

A
Z. intermedium on A. tonsa
Z. intermedium on E. affinis
Epistylis sp. on A. tonsa
Infestation Prevalence (%)
1994 1995 1996 1999 2000
Years
B
A. tonsa
E. affinis
Abundance (copepod/L)
1994 1995 1996 1999 2000
Years
C
Z. intermedium on A. tonsa
Z. intermedium on E. affinis
Epistylis sp. on A. tonsa
Infestation Prevalence (%)
MAR APR MAY JUN JUL AUG SEP
Months
D
A. tonsa
E. affinis
Abundance (copepod/L)
MAR APR MAY JUN JUL AUG SEP
Months
E
Z. intermedium on A. tonsa
Z. intermedium on E. affinis
Epistylis sp. on A. tonsa
Infestation Prevalence (%)
Distance from the Mouth of the Bay (Km)
F
A. tonsa
E. affinis
Abundance (copepod/L)
Distance from the Mouth of the Bay (Km)

Figure 3.4

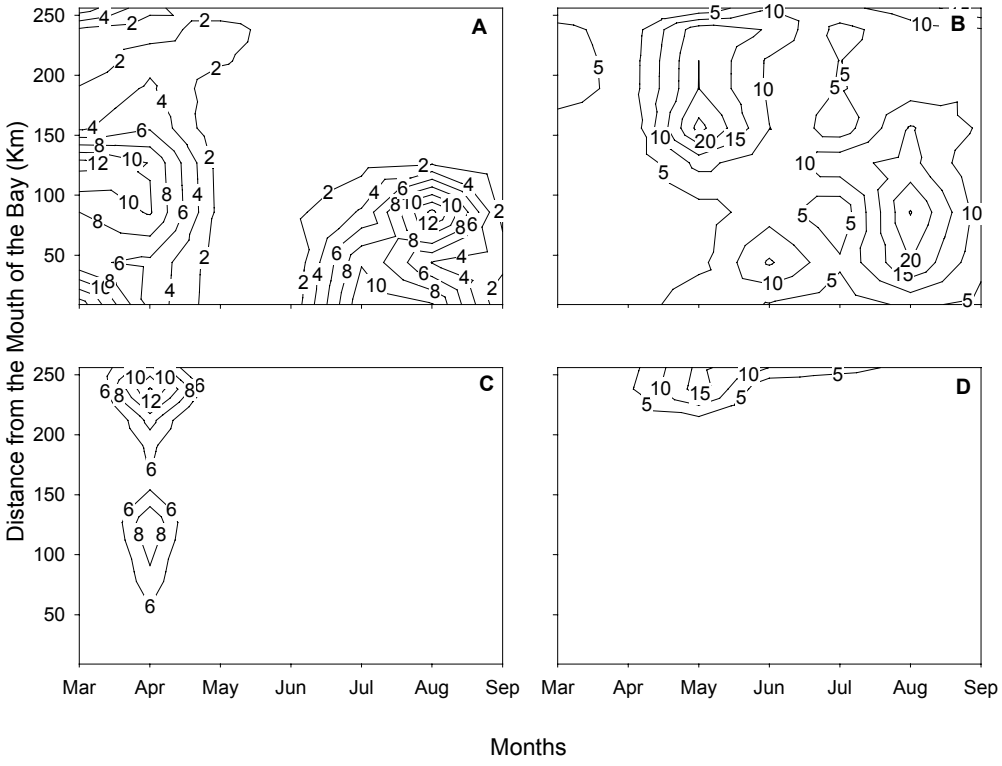

A
B
C
D
Distance from the Mouth of the Bay (Km)
250
200
150
100
50
Mar
Apr
May
Jun
Jul
Aug
Sep
Months

Figure 3.5

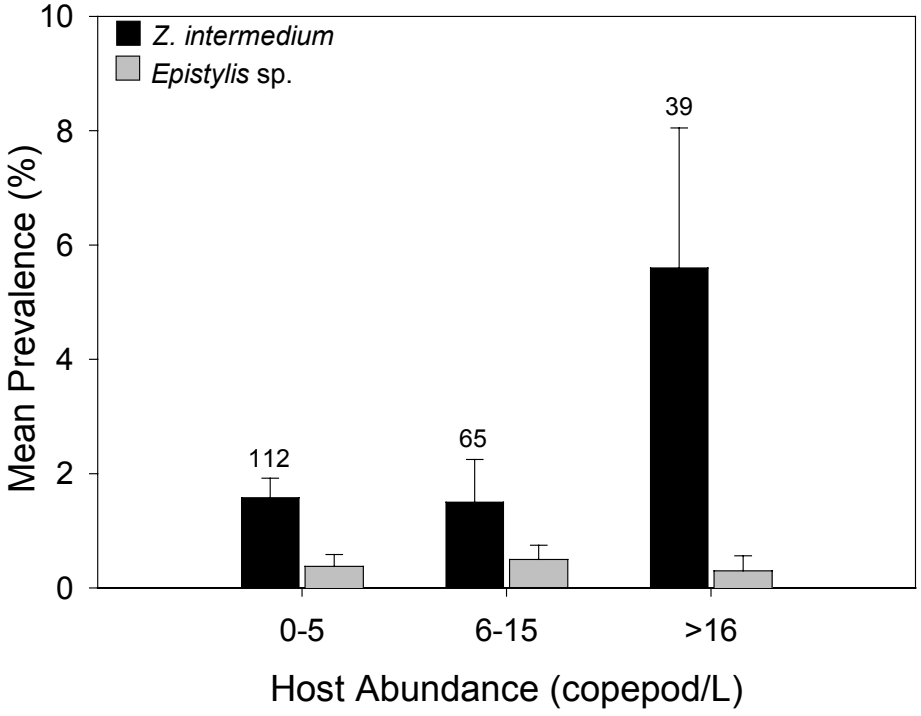

10
8
6
4
2
0
Mean Prevalence (%)
Z. intermedium
Epistylis sp.
112
65
39
0-5
6-15
>16
Host Abundance (copepod/L)

Figure 3.6

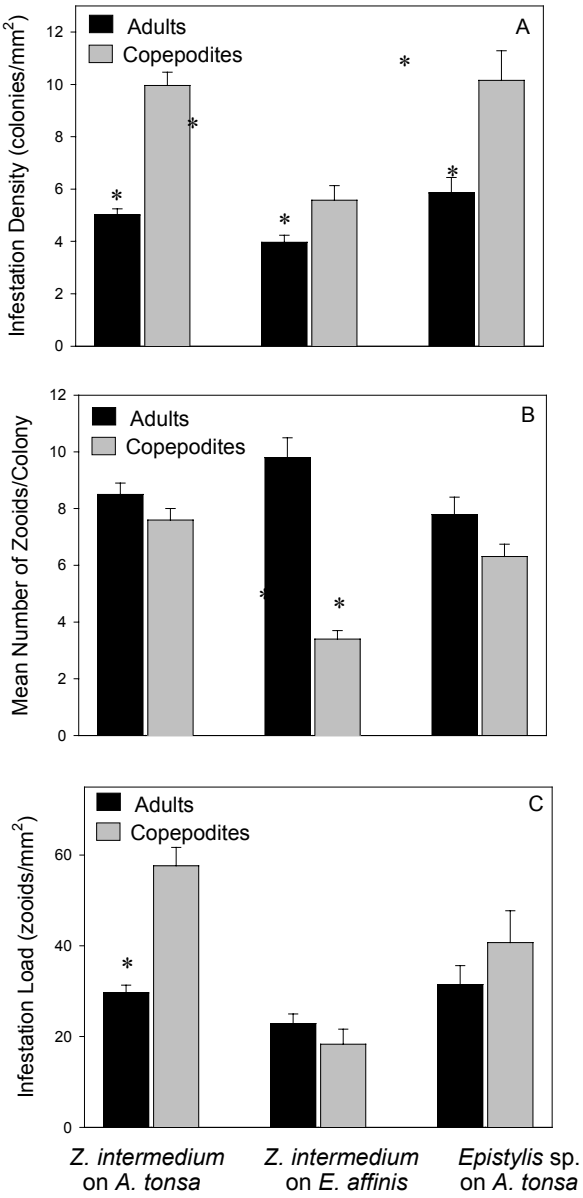
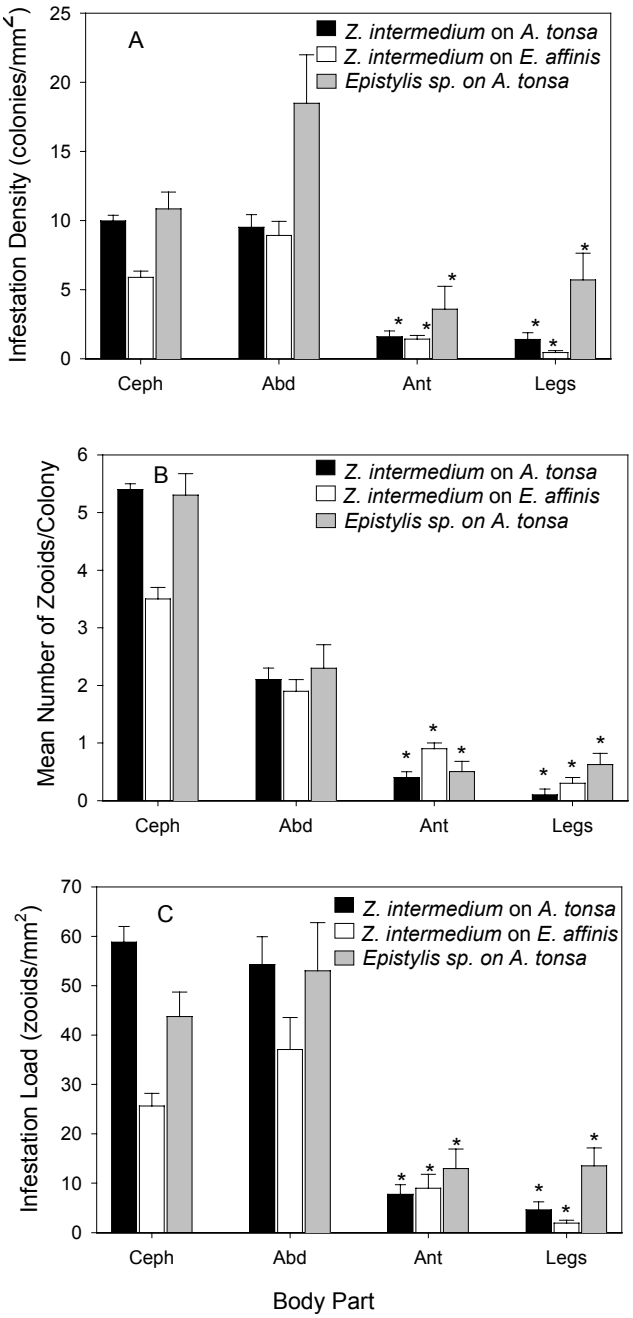

Adults
Copepodites
A
Infestation Density (colonies/mm2)
0
2
4
6
8
10
12
*
*
*
*
*
*
B
Mean Number of Zooids/Colony
0
2
4
6
8
10
12
*
*
C
Infestation Load (zooids/mm2)
0
20
40
60
*
Z. intermedium on A. tonsa
Z. intermedium on E. affinis
Epistylis sp. on A. tonsa

Figure 3.7


A
Z. intermedium on A. tonsa
Z. intermedium on E. affinis
Epistylis sp. on A. tonsa
Infestation Density (colonies/mm2)
25
20
15
10
5
0
Ceph
Abd
Ant
Legs
B
Mean Number of Zooids/Colony
6
5
4
3
2
1
0
C
Infestation Load (zooids/mm2)
70
60
50
40
30
20
10
0
Body Part

CHAPTER 4

TELOTROCH FORMATION, SURVIVORSHIP, ATTACHMENT SUCCESS, AND GROWTH OF THE EPIBIOTIC PERITRICH *Zoothamnium intermedium* PRECHT, 1935

ABSTRACT

In the present study I investigated aspects of the life cycle of the peritrich *Zoothamnium intermedium*, an epibiont on calanoid copepods in the Chesapeake Bay. Controlled laboratory experiments were designed to characterize induction of formation, survivorship, and attachment of free-swimming stages (telotrochs), as well as growth rates of the trophont stage attached to its primary host (the copepod *Acartia tonsa*). Results showed that formation of telotroch stages started two hours after the death of the host, and within seven hours more than 90% of the zooids had formed dispersal stages and left the substrate. Induction of telotroch formation occurred only following the death of the copepod to which the epibiont was attached, suggesting that a combination of mechanical and chemical cues is involved in triggering formation of dispersal stages in *Z. intermedium*.

Telotrochs of *Z. intermedium* were able to survive for 14 hours in the absence of their primary copepod hosts (*A. tonsa* and *Eurytemora affinis*) independent of the copepod species from which the telotrochs had dispersed. In addition, no attachment to non-living substrates was observed in either the laboratory or the field, strongly indicating that this species of peritrich is an obligate epibiont. Attachment success of dispersal stages decreased as their age increased; the highest attachment frequency was attained by three-hour old telotrochs, suggesting that colonization rates in nature may strongly depend on the production of free-swimming stages by the ciliate.

Maximum growth rates of *Z. intermedium* were 0.8 day^{-1} with a bacterial or algal primarily based diet. Colonization rates (rate of change of colonies/host) were

lower than growth rates (0.4 day^{-1}) and reached a plateau at high concentrations of food. The rate of change in zooids per colony (proliferation rate) did not reach a plateau in the algal primarily based diet study, offering a support to the theory that epibiont abundance and frequency increases with increased environmental eutrophication.

The experiments and results presented here may serve as a tool for the design of studies assessing epibiont host specificity, as well as a model for the investigation of the life cycle of free-living and epibiont peritrichs in other systems.

INTRODUCTION

The occurrence of peritrich ciliates colonizing planktonic crustacea is well documented (Hermann and Mihursky, 1964; Green, 1974; Henebry and Ridgeway, 1979; López et al., 1998), with the majority of the reports focusing specially on the effects of these epibionts on host survivorship and reproduction (Kankaala and Eloranta, 1987; Xu and Burns, 1991; Xu, 1992; Weissman, Lonsdale and Yen, 1993). However, studies emphasizing aspects of the peritrich life cycle such as formation and survivorship of free-swimming dispersal stages, ability to colonize multiple hosts or non-living substrates, or growth rates of these ciliates are still lacking.

The life cycle of sessile peritrichs is composed, generally, by a stalked feeding stage, the trophont, which is attached to a substrate, and a free-swimming dispersal stage, called telotroch. Telotroch formation in coenobial, colonial or solitary peritrichs can be a result of asexual reproduction or a direct transformation of the zooids, which frequently occurs in response to unfavorable environmental conditions such as low concentration of oxygen or direct disturbance of the organism. In the case of peritrichs living as epibionts, telotroch formation could also be triggered by the death or molt of the host substrate.

Induction of telotroch formation in laboratory cultures of peritrichs has been performed by briefly exposing cells to air (Nakajima, Hashimoto, and Asai, 1986), by changing the culture medium (Rose and Finley, 1976), by disturbing mechanically the colonies (Succhard, 1979), or by treating the trophont stage with a combination of chemicals (de Baufer et al., 1999). However, such experiments were all performed

with peritrichs attached to non-living substrates and no data on formation of dispersal stages are available for those ciliates colonizing living organisms.

Studies emphasizing epibiont host specificity have pointed out that some peritrichs are able to attach to only one or two host species, failing to colonize non-living substrates (Clamp, 1973; Henebry and Ridgeway, 1979; Gilbert and Schröder, 2003). By contrast, some authors demonstrated that some epibionts are opportunistic, being able to attach to living or non-living substrates (Mayén-Estrada and Aladro-Lubel, 2000). However, only a few studies have investigated the ability of a peritrich epibiont to attach to non-living substrates in the laboratory and/or in the field (Henebry and Ridgeway, 1979; Mayén-Estrada and Aladro-Lubel, 2000).

Assessment of community composition and growth rates of free-living peritrichs have been the subject of only a few studies in freshwater environments, such as streams and activated sludge plants (Curds and Vandyke, 1966; Taylor, 1983; Kusuoka and Watanabe, 1987; Harmsworth, Sleigh, and Baker, 1992; Harmsworth and Sleigh, 1993). One of the most comprehensive studies of growth *in situ* of peritrichs in the field was performed by Kusuoka and Watanabe (1987) in an urban stream in Japan. They sampled colonies of *Carchesium polypinum* and individuals of *Vorticella microstoma* attached to transparent plastic films and found growth rates of 1.37 day^{-1} and 1.01 day^{-1} for the two peritrichs respectively. Regarding peritrichs as epibionts, only one study so far performed by Gilbert and Schröder (2003) has dealt with growth rates of peritrichs attached to living substrates in the laboratory. The authors observed an exponential growth of colonies of *Epistylis pygmaeum*, from a

single zooid, attached to the rotifer *Brachionus angularis* , but no actual growth or colonization rates were calculated for the trophont stage of this species.

In the present study, I have investigated induction of telotroch formation, survivorship, attachment success, growth rate, and the ability of *Zoothamnium intermedium*, a peritrich epibiont attached to *Acartia tonsa* and *Eurytemora affinis* from the Chesapeake Bay, USA, to colonize non-living substrates in the field. To determine if telotroch formation in *Z. intermedium* is associated with chemical and/or mechanical cues released by the host in which the peritrich is attached, or to some cues present in the environment, or to impairment or reduced swimming of the host, a series of experiments was performed, where infected copepods were killed, injured, or tethered or exposed live to dead non-infested hosts. To investigate the survivorship of telotrochs and the ability of them to attach to non-living substrates in absence of their primary hosts, laboratory experiments where the abundance and attachment of telotrochs were monitored over time were carried out. The ability of *Z. intermedium* to attach to non-living substrates in the field was also investigated by sampling the *aufwuchs* community of a tributary of the Chesapeake Bay over a period of seven weeks. To determine if attachment success decreases as age increases, telotrochs of different ages were exposed to their primary host *A. tonsa* and attachment was monitored over time. Finally, to follow the sequence of telotroch formation, survivorship, and attachment success, growth rates of *Z. intermedium* colonizing *A. tonsa* were carried out with two diets, bacteria only and algae as the main sources of food.

MATERIALS AND METHODS

Cultures of *Acartia tonsa*, *Eurytemora affinis* and their epibionts: *A. tonsa* and *E. affinis* were collected from the Patuxent River (a tributary of the Chesapeake Bay) brought live to the laboratory and examined for the presence of ciliate epibionts. Copepods were transferred to 4-liter beakers (3 cultures for each species) containing 2 liters of < 0.45 μm filtered seawater with salinity adjusted for 15 psu, and maintained as described in Chapter 2. Cultures of *Zoothamnium intermedium* were also initiated and maintained following the methods described in Chapter 2.

1.0. Telotroch formation following death of the host: The formation of free-swimming stages (telotrochs) in the peritrich epibiont *Zoothamnium intermedium* following host substrate death was investigated by performing a series of three identical experiments. Infested *A. tonsa* and *E. affinis*, 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 the epibionts remained alive. Dead hosts and attached epibionts were placed in plastic Petri dishes (in each of the three runs containing 5 replicates with one copepod each) filled with 10 ml of < 0.45μm 15psu water, and the number of zooids/host was assessed using a dissecting microscope. Dishes were placed in an incubator at 20^{o}C and the number of zooids attached to the host was assessed hourly. The experiment was terminated when more than 90% of the zooids had formed telotrochs and left the host.

1.1. Telotroch formation induced by dead copepods: To determine if telotroch formation of the epibiont *Zoothamnium intermedium* can be induced by the exposure of an infested copepod to epibiont-free dead copepods, a series of experiments was conducted. Non-infested *A. tonsa* obtained from laboratory cultures were killed as described above. Dead copepods were placed in plastic containers filled with < 0.45µm 15psu water with a final concentration of 1, 2 and 10 dead, clean hosts per ml. The experiment with 10 dead hosts/ml was carried out in a multi-well culture chamber, with a final volume of water of 1 ml per replicate. The experiments conducted with 1 and 2 dead hosts/ml were carried out in Petri dishes with a final volume of water of 10 ml per replicate. Epibiont colonies attached to *A. tonsa* obtained from laboratory cultures were enumerated, and infested hosts were placed in chambers containing dead copepods at a final concentration of one infested host per experimental unit. Experiments for each dead host concentration were run three times with five replicates each. Experimental containers were placed in an incubator at 20^{o}C and the total number of attached zooids was assessed hourly for 7 hours (see Results for rationale on selecting time).

1.2 Telotroch formation triggered by inability of host to swim: To assess if telotroch formation in *Z. intermedium* can be induced by slowing down the movement of the host, infested *A. tonsa* from laboratory cultures were tethered with a cat hair (approximately 1 cm in length). One infested *A. tonsa* was placed in a drop of water on a glass slide, the number of zooids per epibiont colony was assessed, and the excess of water was removed. Both tips of the cat hair were dipped into an ethyl

cyanoacrilate-based glue (commercially known as "crazy glue"), and one of them was attached to the copepod (to a region where no epibiont colonies were present). The tethered copepod was immediately transferred to a Petri dish filled with 10 ml of < 0.45μm 15psu water with a final concentration of one infested host per experimental unit, and the second tip of the hair was glued to the container's wall above the water line (while the copepod remained submerged). As a control for telotroch formation, a tethered infested *A. tonsa*, with a known number of attached zooids, was killed by pressing gently a Pasteur pipette in the middle of the host's cephalothorax (in a place where no epibiont colonies were present). Control and treatment units had five replicates each and the experiment was run three times. Experimental containers were placed in an incubator at 20^{o}C and number of attached zooids in each replicate was assessed hourly for 7 hours.

1.3. Telotroch formation induced by host injury: To determine if telotroch formation in *Z. intermedium* is induced by impaired swimming of the host and/or exudates present in host wounds, one swimming leg and one antenna were removed with a forceps from infested *A. tonsa* obtained from laboratory cultures. After limb removal, the number of zooids per epibiont colony was assessed for each host, and infested copepods were placed in Petri dishes filled with 10 ml of < 0.45μm 15psu water, with a final concentration of one host per experimental container. The experiment was run three times with five replicates each. Dishes were placed in incubator at 20^{o}C and the number of attached zooids was assessed hourly for 7 hours.

1.4. Telotroch formation induced by inability of the host to swim and by the presence of non-infested dead hosts: To assess if telotroch formation can be induced by a combination of reduced swimming by the host and a chemical cue released by dead copepods in the environment, infested *A. tonsa* obtained from laboratory cultures were tethered with a cat hair, as described above, the number of zooids per copepod was assessed, and hosts were placed in a multi-well chamber filled with 1 ml of < 0.45µm 15psu water at a final concentration of one tethered host per well. Non-infested *A. tonsa* also obtained from laboratory cultures were killed with a cover slip as described above and placed in the wells containing a tethered infested host at a final concentration of 10 dead hosts per experimental unit. Chambers were kept in the same conditions described for the other experiments and number of zooids per host was assessed hourly for 7 hours.

2.0. Survivorship of *Z. intermedium* in the absence of live hosts: To determine how long the free-swimming stages of *Zoothamnium intermedium* are able to survive in the absence of the two host species, and also if they were able to attach to the bottom and walls of the experimental chamber, 30 infested *A. tonsa* and *E. affinis* (with a load of about 100 zooids each) obtained from laboratory cultures were killed as described above. Dead hosts were placed in glass Petri dishes (3 replicates with 10 copepods each) filled with 30 ml of < 0.45µm 15psu water. Dishes were placed in an incubator at 20^{o}C, and after 3 hours (see Results for rationale on selecting time), dead copepods with their remaining attached zooids were removed from the chambers using a glass Pasteur pipette. Abundance of telotrochs in the water was assessed over time by

taking 2-ml samples at two-hour intervals, preserving them with one drop of acid Lugol's, and counting the number of cells using a Sedgewick-Rafter chamber (one entire chamber was counted for each replicate). The experiment was terminated when the average concentration of cells/ml in the three replicates was ≤ 2. Attachment of *Z. intermedium* to the bottom and walls of the experimental dish was verified by scanning the dish (all replicates) in a dissecting microscope.

3.0 Attachment of *Z. intermedium* to coverslips in the field: To extend the previous experiment regarding the ability of *Z. intermedium* to attach to non-living substrates, and to determine if this species is a common member of the benthic community, cover-slip traps were sampled from the field and analyzed for the presence of *Z. intermedium* during a period of seven weeks. Small rectangular boxes (6 cm long X 3.5 cm wide) made with Plexiglas®, with open sides, and six grooves in the internal sides, each capable of holding 2 cover slips (22 mm X 22 mm), were used as traps. Inside each box, 12 cover slips were placed back to back along the grooves and a rubber band was crossed over the open sides to assure that the cover slips would stay in place. Two sets of traps were suspended in the Rhode River (a tributary of the Chesapeake Bay) from the dockside at the Smithsonian Environmental Research Center ($38^{o}46$'N and $76^{o}52$'W), Edgewater, Maryland, on April 15th, 21st, 28th, May 12th, 23rd, and June 3rd 2003. On the first three sampling dates, traps were left in the field for a period of seven days to allow enough time for colonization, which is dependent of water temperature. For all other sampling dates, traps were left in the

field for a period of four days. At the time of recovery, traps were directly placed in a beaker filled with water from the site and taken to the laboratory.

Two cover slips from each set of traps were preserved with modified Bouin's fixative at a final concentration of 5% (Coats and Heinbokel, 1982), and four cover slips from each set were placed in small Petri dishes filled with water from the collection site for observation of live organisms with an inverted microscope (Invertscope, 25X magnification; Zeiss Corp.). Five fields of view in each cover slip were inspected for the presence of colonies of *Zoothamnium*. The total number of colonies and zooids in each field of view was recorded and the abundance of *Zoothamnium* per area was calculated. The Protargol staining technique (Zagon and Small, 1970) was applied to the preserved material to confirm the identification of the *Zoothamnium* species that were observed alive and attached to the cover slips. Filters were omitted from the preparation and cover slips were run throughout the procedure and mounted on glass slides. Protargol slides were observed using light microscopy, and morphological characteristics such as organization of the oral apparatus were observed for each *Zoothamnium* colony and compared to the described morphology of *Z. intermedium* attached to copepods (see Chapter 2).

To assess the occurrence of *Z. intermedium* on copepods, plankton samples were also collected from each site at the time of cover-slip recovery. Horizontal net tows were taken using a plankton net with 202-μm mesh size and 50-cm in diameter. A 125 ml sub-sample from the net tow was fixed with modified Bouin's fluid (Coats and Heinbokel, 1982) at a final concentration of 5 %, and examined using a dissecting microscope (Bausch and Lomb; 30X magnification) to determine the presence of *Z.*

intermedium on adults and copepodites of *Acartia tonsa* and *Eurytemora affinis*. Identification of *Z. intermedium* was confirmed by staining infested copepods randomly selected from the samples (~5 copepods/sample). The Protargol staining technique (Zagon and Small, 1970) was run following the procedure described in Chapter 2 and diagnostic characteristics of *Z. intermedium* (the same as those described in Chapter 2) were observed using light microscopy. Infestation prevalence (percentage of copepods carrying epibionts) was calculated for adults and copepodites of both host species.

4.0 Attachment success of *Z. intermedium* to *A. tonsa*: To determine if telotroch attachment success decreases as its age increases, infested *A. tonsa* (60 copepods with a load of about 50 zooids each) were obtained from laboratory cultures and were killed as described in experiment 1.0. Dead hosts were placed in a glass Petri dish with 30 ml of 15 psu filtered water (< 0.45µm). Petri dishes were placed in an incubator at 20^{o}C, and after three hours, dead copepods with their remaining zooids were removed from the container with a glass Pasteur pipette. A 2 ml sample was taken from the Petri dish, fixed with acid Lugol's and counted using a Sedgewick-Rafter chamber to assess the initial concentration of telotrochs/ml. A final concentration of 1 telotroch/ml was added to each of 15 scintillation vials filled with 24 ml of 15 psu filtered water (< 0.45µm). Non-infested, adult *A. tonsa* obtained from laboratory cultures were added to the experimental vials (one copepod/vial). Scintillation vials were placed randomly in a plankton wheel (rotating at two rpm) and three replicates were fixed with acid Lugol's every two hours for 10 hours. To assess attachment rate, preserved copepods

were removed from the sample with a Pasteur pipette and observed with light microscope and the number of zooids attached to the host was recorded. The rest of the sample was settled for at least 24 hours and after that it was reduced to a volume of 10 ml, transferred to a settling chamber and settled for another 24 hours. The number of telotrochs that did not attach to the available host was counted using an inverted microscope (Invertscope; 100X magnification; Zeiss Corp.). The assessment of telotrochs that did not attach in addition to the attached zooids would allow an estimate of predation rates of copepods on free-swimming stages of their epibionts. The same procedure was repeated in separate experiments where telotrochs entered the experiments after 5, 7, and 9 hours after the death of the original hosts.

5.0. Growth rates of *Z. intermedium* feeding on bacteria: To assess growth rates of *Z. intermedium* feeding on bacteria alone, the development of clonal ceonobia was observed over a period of 96 hours. Adult females of *A. tonsa* were exposed to telotrochs of *Z. intermedium*, and hosts carrying a single zooid were transferred to glass Petri dishes filled with 150 ml of <0.45μm 15 psu water, enriched with varying amounts of Cerophyl® to reach different bacterial concentrations. As food for the copepods, the diatom *Thalassiosira weisflogii* was added at a final concentration of $1X10^3$ cells/ml. The experiment had a total of five treatments (bacterial concentration of 10^4, 10^5, 10^6, 10^7, and 10^8) with three replicates per treatment and one infested host per replicate. Experimental chambers were kept in an incubator at 20°C with photoperiod of 14:10 light:dark hours. Copepods were observed every 24 hours for a period of four days and the total number of attached zooids; number of coenobia per

host and mean number of zooids per coenobia were recorded. Growth rates (rate of change in number of epibiont zooids) were calculated using the following formula: $\ln(N_t) = \ln(N_0) + rt$, where, N_t is the number of zooids at time t; N_0 is the number of zooids at time 0; t is the period of time in days; and r is the growth rate. Colonization rates (rate of change in number of epibiont coenobia) were calculated using the same formulae as suggested by Kusuoka and Watanabe (1987): $\ln(N_t') = \ln(N_0') + r' t$, where, N_t' is the number of epibiont coenobia at time t; N_0' is the number of epibiont coenobia at time 0, t is the period of time in days; and r' is the colonization rate. Proliferation rates (rate of change in the mean number of epibiont zooids/coenobium) were calculated using the same formulae: $\ln(N_t'') = \ln(N_0'') + r'' t$; where N_t'' is the mean number of epibiont zooids/coenobium at time t; N_0'' is the mean number of epibiont zooids/coenobium at time 0; t is the time in days; and r" is the proliferation rate.

A water sample (5 ml) from each replicate was taken at the beginning (T_0) and at the end of the experiment (T_4), and preserved with formaldehyde for enumeration of bacteria using the DAPI staining technique (Porter and Feig, 1980). Counts were performed using an Axioscope equipped with optics epifluorescence (Zeiss Corp.). For each replicate, duplicate counts were carried out up to a total of 200 cells/count. Enumeration of background bacteria was performed by replicate counts of blank filters (for each concentration) and the result was included in the calculation of bacterial concentration.

5.1. Growth rates of *Z. intermedium* feeding on *Isochrysis galbana* based diet: To determine growth rates of *Z. intermedium* feeding primarily on the haptophyte algae

Isochrysis galbana, the development of coenobia originated from a single zooid was observed over a period of 96 hours. The experimental set up used in this experiment was the same used to assess growth rates of the epibiont feeding on bacteria. The experiment had a total of six concentrations of *I. galbana* (10^1, 10^2, 10^3, 10^4, 10^5, and 10^6 cells/ml) with three replicates per treatment and one infested *A. tonsa* per replicate. The number of zooids and coenobia per host and mean number of zooids per coenobia were recorded every 24 hours for 96 hours. A water sample (5 ml) from each replicate was taken at every observation point and fixed with acid Lugol's for enumeration of *I. galbana*. Concentration of algal food was assessed by direct counts using a Palmer-Maloney counting chamber. Triplicate counts were performed for each replicate to a total of 100 cells/count.

Data analyses: As the data passed normality and homogeneity of variance tests, regression analyses to determine the best-fit model and slopes of the curves were run in the telotroch formation, survivorship and attachment success experiments. Time was used as independent variable and percentage of zooids in the colonies, telotrochs in the water, or zooids attached were the dependent variables. Analyses of Variance were performed on the slope of the curves to discern differences in telotroch formation and survivorship between the two host species and in attachment success among different telotroch ages. Statistical Analyses were performed using SPSS for Windows Version 11.0 (SPSS Inc.) and Sigma Stat Version 2.0 (SPSS, Inc.). Data is plotted as means $\pm$ SE (standard error of the mean), and for all statistical tests the level of alpha used was 0.05.

RESULTS

Telotroch Formation and Survivorship (Experiments 1.0 – 2.0): Zooids of *Zoothamnium intermedium* attached to *A. tonsa* started to form dispersal stages two hours after the death of the host (Figure 4.1). In three hours, *ca* 50% of the zooids in the coenobia had formed telotrochs and left the host substrate; within seven hours, more than 90% of the cells had formed free-swimming stages. The same pattern was also observed for zooids of *Z. intermedium* attached to *E. affinis*, as observed in Figure 4.1. Regression analyses showed that telotroch formation in *Z. intermedium* attached to both host species fits a model of exponential decay, followed by a brief lag phase (Figure 4.1). No statistically significant difference was observed in the slopes of the curve of telotroch formation between the two host species (ANOVA, $p > 0.05$).

Formation of dispersal stages in *Z. intermedium* attached to live *A. tonsa* was not induced by the presence of dead, non-infested *A. tonsa* in the environment, independent of the concentration of dead copepods (Fig. 4.3). Tethering of the live infested host also failed to trigger telotroch formation in *Z. intermedium* (Figure 4.2). On the other hand, formation of dispersal stages in the dead, tethered hosts (controls) followed a similar pattern observed for non-tethered, dead *A. tonsa* and *E. affinis* (see Figure 4.1 and 4.2). Telotroch formation in *Z. intermedium* was also not induced by impairment of host swimming or tethering of a living host in presence of dead non-infested copepods. Figure 4.3 shows a summary of the results obtained for the experiments inducing formation of free-swimming stages in *Z. intermedium*.

Telotrochs of *Z. intermedium* were able to survive a short period of time in the absence of their primary hosts *A. tonsa* and *E. affinis*. After seven hours following the death of the host (i.e. 7-hour old telotrochs), 80 % of the telotroch population was still present in the water (Figure 4.4). This number decreased to 50 % in 9-hour-old telotrochs and reached 20 % in 11-hour-old telotrochs. After 14 hours following the death of the host (14 hour-old telotrochs), the concentration of telotrochs in the water was $\leq$ 2 cells/ml (Figure 4.4). No attachment was observed to the walls or bottom of the experimental container in the experiments with telotrochs originated from coenobia attached to both *A. tonsa* or *E. affinis*. Similarly to telotroch formation, regression analyses indicated that survivorship curves of *Z. intermedium* telotrochs fit in an exponential decay model (R^2=0.93) followed by a lag phase. No statistically significant difference was observed for the slopes of the curves of telotroch survivorship for *A. tonsa* and *E. affinis* (ANOVA, $p > 0.05$).

Attachment of *Z. intermedium* to non-living substrates in the field (Experiment 3.0): Live observations and Protargol staining revealed that four species in the genus *Zoothamnium* (two of them tentatively identified as *Z. alternans* and *Z. arbuscula*) were present in the benthic community of the Rhode River during a period of seven weeks between April and June 2003, but none of them was identified as the same epibiont species (*Zoothamnium intermedium*) that colonizes populations of *A. tonsa* and *E. affinis* in the Chesapeake Bay and its tributaries. Assessment of the number of coenobia and zooids of *Zoothamnium* per substrate area showed that this genus was the dominant Peritrich from April 15th to April 21st, but was outnumbered by

Vorticella spp. on May 12th, showing an increase in subsequent samplings as shown in Figure 4.5 A. During the same period, *Z. intermedium* was found attached to adult and juvenile stages of *A. tonsa* and *E. affinis* at an infestation rate that varied between 4.5 and 39% for adults and juveniles of *A. tonsa* and 6 and 25% for adults and copepodites of *E. affinis* (Figure 4.B). No infestation was detected for adults of *A. tonsa* on some sampling dates in April, May and June (see Figure 4.B). Infestation prevalence was very low for adults and copepodites of *E. affinis* in samples from, May 12th as shown in Figure 4.5 B.

Attachment success of *Z. intermedium* to *A. tonsa* (Experiment 4.0): Attachment rate of *Z. intermedium* on *A. tonsa* reached a maximum of 32% for 3-hour old telotrochs, with this number decreasing by more than 50% for 5-hour old telotrochs, which displayed a maximum rate of 11% (Figure 4.6A). Older telotrochs had an even lower success, for instance, only 9% of the 7-hour old telotrochs, and 6% of the 9 hour-old teltrochs were able to attach to an available host (Figure 4.6A). Regression analyses demonstrated that attachment success in *Z. intermedium* fits an exponential growth model ($R^2 = 0.97$) with an exponential rise to a plateau (Figure 4.6A). Analysis of Variance (ANOVA) for the slopes of the regression showed that attachment rate was significantly higher ($p < 0.05$) for 3-hour old telotrochs when compared with older cells. A similar result was found for 5-hour old telotrochs, for which attachment rate was significantly higher than in 7- and 9-hour old telotrochs (ANOVA, $p < 0.05$). No statistically significant difference was observed in attachment success between 7- and 9-hour old telotrochs (ANOVA, $p > 0.05$).

Analyses of the number of telotrochs that remained in the water (Figure 4.6B) revealed a pattern similar to that observed for telotroch survivorship, indicating that predation of *A. tonsa* on free swimming stages of its epibiont *Z. intermedium* is negligible. Regression analyses showed that the curves obtained for non-attached telotrochs in the water fit three different models according to the starting age of the free-swimming cells. For instance, the curve for 3-hour old telotrochs fits a linear model (R^2= 0.99), while curves for 5- and 7-hour old telotrochs fit a model of exponential decay (R^2 = 0.93 and 0.98, respectively). The regression for 9-hour old telotrochs best fits the cubic model (R^2 = 0.99). Analysis of Variance performed to compare the slopes of the curves demonstrated that all ages are significantly different from each other (ANOVA, $p < 0.05$).

Growth of *Z. intermedium* on bacterial and algal diets (Experiment 5.0 and 5.1): The growth rates of *Z. intermedium* (number of zooids/host/unit of time), feeding on bacteria alone and algae are presented in Figure 4.7 A and B. In both diets a similar pattern was observed where growth rates increased with increasing food concentration until a threshold was reached. Additional increases in food concentration either resulted in no change in growth (Figure 4.7 A) or in a decrease in growth rate (Figure 4.7 B). In the experiment with bacteria as the main food, the maximum growth rate (μ) estimated from the mean growth rates of three replicates was 0.85 day^{-1} (SE = 0.007) and was reached at a concentration of 10^6 cells/ml. In the experiment with algae as the main food, the maximum growth rate, estimated as in the bacteria experiment, was 0.83 day^{-1} (SE = 0.03) and was reached at a concentration of 10^5

cells/ml. The maximum doubling rate of *Z. intermedium* was 1.20 and 1.23 per day in the experiments with algae and bacteria respectively.

Figure 4.7 C and D show the colonization rates (increasing of colonies/host) of *Z. intermedium* on *A. tonsa* feeding on bacteria and algae respectively. With both diets, colonization rates increased with an increasing food concentration until a maximum was reached. Further increases in food concentration, in both cases, resulted in a decrease in colonization rates after reaching this maximum (Figure 4.7 C and D). In the experiment with bacteria, the maximum colonization rate was 0.50 day^{-1} (SE = 0.04) and was observed at a concentration of 10^7 cells/ml, one order of magnitude higher than the observed growth rates. Colonization rates in an algal-based diet were similar to those observed for bacteria alone. The maximum rate was 0.51 day^{-1} (SE = 0.01) and was observed at the same concentration for which maximum growth rates were achieved (10^5 cells/ml).

The mean number of zooids per coenobium (proliferation rate) of *Z. intermedium* was also similar for bacterial and algal diets (Figure 4.7 E and F). With a diet based on bacteria, the maximum proliferation rate was 0.44 day^{-1} (SE = 0.04) and was reached at a concentration of 10^6 cells/ml (Figure 4.7 E). Differently from the bacterial diet and from growth and colonization rates, proliferation rate on an algal diet did not reach a plateau, and the highest rate of 0.42 (SE = 0.038) was recorded at 10^6 cells/ml (Figure 4.7 F). Algae concentration remained fairly constant throughout the study, as can be seen in figures 4.8 A and B.

DISCUSSION

The present study investigated induction of telotroch formation, survivorship, attachment success, ability to colonize non-living substrates, and growth rates of the peritrich epibiont *Zoothamnium intermedium* attached to calanoid copepods in the Chesapeake Bay, USA. This is the first comprehensive study focusing on aspects of the life cycle of an obligate peritrich epibiont, some of these aspects have been investigated for free-living species of peritrichs.

Formation of free swimming stages in the epibiont peritrich *Zoothamnium intermedium* was induced by killing the infested host, with no telotroch formation observed when the host was tethered, injured, or when clean dead hosts were present in the environment. Telotrochs were also formed in this species as a result of binary fission, as was indicated by the appearance of new colonies in the growth rate experiment (Figure 4.7). Transformation of trophonts into dispersal stages in peritrich epibionts has been demonstrated to follow the death of or molt of the substrate (Clamp, 1973), and as in free-living peritrichs, to form as a product of binary fission during asexual reproduction (Nagasawa, 1986). In addition to a result of binary fission, telotroch formation in peritrichs attached to Crustacea can occur before or after the host sheds its molt, as was reported by some authors who observed this aspect of the epibiont life cycle. For example, Fenchel (1965) observed that all species of the ciliates chonotrich and peritrich ciliates (including species of *Epistylis* and *Zoothamnium*) attached to the amphipod *Gammarus* spp. formed free-swimming cells prior to the host's molt, colonizing the same substrate immediately after the molt. Green (1974) observed that the population density of *Epistylis helenae* attached to the

cladoceran *Daphnia pulex* decreased before the end of the host's instar. He also demonstrated that most of the stalks in the colony were empty before the molting process started, suggesting that some cue could be detected by the epibiont, which in turn would form dispersal stages and be available for recolonization of the same host individual when its cast skin was shed. Willey and Threlkeld (1995), also observed that *Vorticella campanula* reached a positive burden immediately after its cladoceran host, *Daphnia pulex* casted its carapace. By contrast, Clamp (1973) reported that *Lagenophrys lunatus* attached to species of shrimp in the genus *Palemonetes* started the process of forming telotrochs after the host has cast its molt. The time was not quantified but several hours were needed to complete telotroch formation and evacuation of the old carapace. In the present work, observations of infested molts demonstrated that *Zoothamnium intermedium* started forming telotrochs after the old carapace had been shed by the copepod hosts, and not beforehand as was demonstrated for other peritrichs including species in the genus *Zoothamnium* (Fenchel, 1965). Although the time taken to form telotrochs from the shed carapace was not quantified in the present study, observation of old carapaces and quantification of time of telotroch formation following the death of the host suggest that, as described for *Lagenophrys lunata* (Clamp, 1973), several hours would be required for *Z. intermedium* to form dispersal stages and leave the host's old exoskeleton.

Despite these observations, the nature of the mechanisms involved in triggering telotroch formation following the death or the molt of the host is still not well understood. The results obtained for *Z. intermedium* in this study suggest that a

combination of chemical and mechanical stimuli coming from the individual host the epibiont is attached to, is responsible for the triggering of telotroch formation in this species of peritrich (Figure 4.3). Interestingly, production of dispersal stages in free-living peritrichs is sometimes associated with changes in environmental conditions such as low oxygen concentration, or high carbon dioxide tension (Bick, 1972). It was demonstrated for *Vorticella* spp. that when the oxygen concentration falls below 2 mg/l these organisms form free-swimming stages and leave their stalks behind to find a more suitable substrate. Formation of telotroch stages of *Z. intermedium*, in the present study, seemed to be linked to cues coming from the host and not to changes or even signals released in the environment. At same time, since change in pH, bacterial concentration, and dissolved oxygen concentration were not measured in the performed experiments, these environmental parameters might also be involved in the whole process of telotroch formation in this epibiont species.

Although the results obtained from the induction of telotroch formation experiments were not conclusive with respect to the exact stimulus received by the epibiont, if any, this study demonstrated an efficient and reproducible method of massive production of telotrochs that can be used to investigate morphological developmental, and ecological aspects of *Z. intermedium*. The usefulness of a similar approach was pointed out by Vachianno et al. (1992) who developed a method of telotroch formation for *Vorticella convallaria* attached to non-living substrates. Interestingly, in their experiment with *V. convallaria* a very high concentration of telotrochs (about 1000 cells/ml) was formed two hours after the cells were rinsed in a particular medium, while zooids of *Z. intermedium* started forming telotroch two hours

after the death of the host, reaching a maximum within seven hours following copepod's death (Figure 4.1 A and 4.1 B). This suggests that free-living peritrichs show a faster response to changes in their surrounding environment, while some epibionts may need to receive a mechanical, chemical or even electrical signal from their substrate organism to start forming dispersal stages.

Trophont stages of peritrich ciliates undergo morphological changes, such as disintegration of the oral cilia, production of a ciliary wreath at the aboral pole of the cell, and modification of the cell shape, to transform into telotrochs (Succhard, 1979). The dispersal stage does not feed, and after being released from the stalk the cells have to find a suitable substrate for attachment in a period that, generally, goes from hours to a few days (as inferred from Fenchel, 1965; Clamp, 1973). In peritrichs that can colonize a variety of substrates, it is not a difficult task to find a suitable site for attachment in such restricted time, but for those that display host or substrate specificity this limited period can be critical. Vacchiano et al. (1992) observed that approximately 70% of a population of *Vorticella convallaria* telotrochs introduced to an experimental container with appropriate medium attached and transformed into stalked zooids within two hours. By contrast, some telotrochs from peritrich epibionts, when isolated from their original host, and not presented with a new one, are not able to transform into trophont stages and die after a few days, as observed by Fenchel (1965) when studying epibiont fauna in Gammarid amphipods. In the present study, telotrochs of *Z. intermedium* lived for 14 hours in the absence of their primary hosts, independent of the copepod species from which telotrochs originated. The lack of telotroch attachment to the walls or bottom of the experimental container suggests

that *Z. intermedium* is an obligate epibiont that needs a living substrate to start metamorphosing into the trophont stage. In the Chesapeake Bay, this species of epibiont was found colonizing only *Acartia tonsa* and *Eurytemora affinis* even when other planktonic species were also abundant (see Chapter 3), but whether *Z. intermedium* specifically targets these two copepod species, or living hosts in general, could only be determined by experimentally exposing telotrochs to other potential hosts from the zooplankton community.

Selection of suitable substrates for settlement has been the subject of several studies including invertebrate larvae, especially barnacle species. In general, invertebrate larvae show a pattern of extensive initial exploration of the substrate and finally they settle where they encounter more individuals of their own species (Knight-Jones and Crisp, 1953). This searching and settlement behavior would help avoid unfavorable environments where the adult will have a lower chance of survival, since early post-settlement mortality sometimes can be extremely high (Gosselin and Qian, 1996). This similar pattern of settling next to conspecifics displayed by different species of larvae was suggested to be convergent (Knight-Jones and Crisp, 1953), and might be the same utilized by telotrochs of peritrich ciliates (Langlois, 1975). Peritrich epibionts can choose their host based on availability of space and access to resources (facultative), or based on chemical attraction to a specific host or group of hosts, not being able to attach to non-living substrates (obligate). *Zoothamnium intermedium* found as epibiont on Chesapeake Bay copepods is an example of obligate epibiont, since it failed to attach to glass surfaces in the laboratory and to glass cover-slips in the field, as was demonstrated by live and Protargol staining observations of

peritrichs attached to cover-slips sampled from nature. At the same time, *Z. intermedium* was colonizing copepods, demonstrating that free-swimming stages were available for potential colonization of non-living substrates if possible. Although *Z. intermedium* was not found attached to cover slips, four other species in the genus *Zoothamnium* (including *Z. arbuscula* and *Z. alternans*) were found to be very abundant from April to the beginning of May, when they started to be outnumbered by *Vorticella* spp. Gross (1986) in a study characterizing the morphology by Protargol staining of *Zoothamnium* species from the Patuxent River, a subestuary of the Chesapeake Bay, also found four species of this genus colonizing slides, but none of them presented morphological characteristics similar to those observed for *Z. intermedium* attached to copepods.

Although Green (1974) had suggested that the specificity among epibionts is much more related to the site of attachment on the host than to a host species, more recent studies have shown that this is not always the case for peritrichs and other sessile ciliates. For example, Henebry and Ridgeway (1979), investigating the epibionts attached to zooplankton in an eutrophic lake, observed that *Vorticella microstoma* was able to attach to one species of cladoceran and was never found on cover slips, suggesting that it constitutes an obligate epibiont. Nolting and Rustige (1998), sampling the ciliate fauna attached to amphipods in two rivers in Germany, found that 12 out of 22 species of epibionts were able to attach to cover slips. Among the 10 species that were obligate epibionts, they found two species of peritrichs in the genus *Lagenophrys* (*L. nassa* and *L. ampulla*), again demonstrating substrate specificity of members of this group.

Studies focusing on host-parasite assemblages have demonstrated that the age of the infective stage of the parasite and sometimes the age of the host are important factors determining parasite infestation success (Theron, Rognon, and Pages, 1998; Cho et al., 1999). Although epibionts are not true parasites, the existence of an optimum infestation period would be compatible with the fact that in several planktonic assemblages epibiont prevalence has a strong correlation with host abundance (Threlkeld, Chiavelli, and Willey, 1993). Experiments of attachment success performed in the present study showed that the ability of telotrochs of *Zoothamnium intermedium* to find and attach to a host decreases as telotroch age increases. The maximum success was attained by three-hour-old telotrochs, which displayed an attachment frequency significantly higher than older telotrochs (Figure 4.7 A). These results indicate that high infestation rates observed in the field at certain times of the year (see Chapter 3) would not be dependent only on the abundance of the host species, but also on the production of dispersal stages by the epibiont, since the amount of time to form telotrochs, and for them to find, and to attach to a host is very limited. Attachment success of free-living peritrichs was investigated in *Vorticella convallaria* and it was demonstrated that approximately 70% of the telotrochs settled at the bottom of experimental dishes within two hours of exposure, with almost 100% of settlement occurring within 12 hours (Vacchiano et al., 1992). Telotrochs of *Z. intermedium*, however, showed a maximum attachment success within six hours of exposure to a preferred host, and this rate remained constant up to 10 hours of exposure. Interestingly, the attachment rate observed for *Z. intermedium* was much lower than that observed for *V. convalaria* and could be attributed to predation

pressure on the telotrochs. However, examination of the contents of the experimental containers demonstrated that predation was negligible, so that the curves obtained for telotrochs that did not attach (i.e. remaining in the water) were similar to the ones obtained for telotroch survivorship (Figures 4.4 and 4.7 B). Predation on free-swimming stages of epibiont was reported for the algae *Korshikoviella gracilipes* attached to *Daphnia pulicaria*, and was regarded as a major source of phytoplankton for the host, since in cold months the abundance of phytoplankton is low (Barea-Arco, Pérez-Martínez, and Morales-Barquero, 2001). Even though telotrochs of *Z. intermedium* are in the range of particle size grazed by copepods, some adaptation to the epibiotic life perhaps makes them less vulnerable to predation by the host.

Studies focusing on growth rates of peritrichs in the laboratory or in the field are scarce (Curds and Vandyke, 1966; Sudo and Aiba, 1971; Kusuoka and Watanabe,1987) however, these ciliates are important consumers of bacteria in aquatic environments, especially eutrophic habitats, due to their high metabolic rates (Fenchel, 1987). *Z. intermedium*, in the present study, had a maximum population growth rate of approximately 0.8 day^{-1} (Figure 4.7 A and B) when exposed to diets based primarily on bacteria or *Isochrysis galbana*, demonstrating that this peritrich is able to grow at approximately the same rate when the main available food is bacteria or small algae. No other study assessing growth rates of a species in the genus *Zoothamnium* is yet available, but, when compared to another peritrich, the growth rates of *Z. intermedium* are lower than those found for *Carchesium polypinum* in the field (1.4 day^{-1}; Kusuoka and Watanabe, 1987), or in the laboratory (1.8 day^{-1}; Sudo and Aiba, 1971). On the other hand, the growth rates found for *Z. intermedium* were similar to

those reported for other species of ciliates. For example, Jack and Gilbert (1993) found maximum growth rates of 0.87 day^{-1} for the oligotrich ciliate *Strobilidium gyrans*, and 0.86 day^{-1} for *Euplotes eurystomus* feeding on a cryptomonad algae at a temperature of 20°C.

The only other record of growth rates estimated in the laboratory for a peritrich epibiont is that of Gilbert and Scröder (2003), who observed colony development of *Epistylis pygmaeum* attached to *Brachionus angularis*. They reported an exponential increase in zooids and colonies originated from a single attached zooid, but the actual growth rates were not calculated for the trophont stage.

The maximum rate at which *Z. intermedium* on *A. tonsa* formed new colonies (colonization rates) was approximately 0.4 day^{-1} at a concentration of 10^7 bacteria cells/ml or 10^6 algal cells/ml (Figure 4.7 C and D), and was much lower than the actual growth rate (zooids/host) for this species. Interestingly, Kusuoka and Watanabe (1987) found colonization rates of *C. polypinum* higher than the actual growth rate estimated for the same species, due to migration of telotrochs from other sites, since their experiment was performed *in situ*. By contrast, in the present study no other population of *Z. intermedium* was present and new colonies were formed from telotrochs originated by binary fission from the original zooid, or in later stages by direct transformation of the trophont stage.

Proliferation rates (i.e. number of zooids/colony) of *Z. intermedium* reached a plateau when these peritrichs had bacteria as the main food (Figure 4.7 E). On the other hand, in the algal-based diet the number of zooids per colony continued to increase with higher food concentration (Figure 4.7 F). This observation is in

agreement with the fact that abundance of certain species of epibionts varies with the degree of eutrophication of aquatic environments (Henebry and Ridgeway, 1979; Mannesmann and Rustige, 1994). Mannesmann and Rustige (1994) found a strong correlation between increase in epibiont abundance on the amphipod *Gammarus pulex* and the increase in organic load. Although they only tested polluted environments, the correlation between load and eutrophication observed by them in the field and suggested by laboratory experiment in the present study, could provide some insights about the relationship between epibiont load and organic pollution.

In summary, this study described several aspects of the life cycle of the peritrich epibiont *Zoothamnium intermedium*, and the results presented here may serve as tools for the design of detailed laboratory experiments focusing on ecological aspects of this relationship such as host specificity. Moreover, the experiments described here could be used as models for studying aspects of the life-cycle of free-living and epibiont peritrichs in other systems.

FIGURE LEGENDS:

Figure **4.1**:Telotroch formation of *Zoothamnium intermedium* following the death of the host. Symbols represent means of three experiment runs with five replicates each. Error bars represent the Standard Error of the Mean.

Figure **4.2**:Telotroch formation of *Z. intermedium* attached to tethered *Acartia tonsa* (open circles) compared to telotroch formation on dead *A. tonsa* (filled circles). Symbols represent means of three experiment runs with five replicates each. Error bars represent the Standard Error of the Mean.

Figure **4.3**:Summary of the results obtained for all experiments inducing telotroch formation on the peritrich epibiont *Zoothamnium intemrmedium* attached to *Acartia tonsa*. Symbols represent means of three experiment runs with five replicates each. Error bars represent the Standard Error of the Mean.

Figure **4.4**:Survivorship of telotrochs of *Zoothamnium intermedium* in absence of living host. Circles represent a telotroch population originated form *Acartia tonsa* and squares represent a telotroch population originated from *Eurytemora affinis*. Symbols represent means of three replicates and error bars represent the Standard Error of the Mean.

Figure **4.5**:Attachment of peritrich ciliates to cover slips and copepods in the field. (**A**) Number of peritrich zooids/ mm^2 of cover-slip recovered in six sampling dates (between April 15th and June 3rd 2003) from the Rhode River, a tributary of the Chesapeake Bay. Bars are mean numbers of zooids calculated from eight replicates and error bars are the Standard Error of the Mean. (**B**) Infestation Prevalence (%) of *Z. intermedium* on adults and copepodites of *A. tonsa* and *E. affinis* collected from the Rhode River in six sampling dates between April 15th and June 3rd 2003.

Figure **4.6**: Attachment success of telotrochs (at different ages) of *Zoothamnium intermedium* to *Acartia tonsa*. (**A**) Percentage of attached zooids over time. Symbols represent means of three replicates and error bars are the Standard Error of the Mean. (**B**) Percentage of telotrochs of *Z. intermedium* that remained in the water after termination of the experiment. Symbols represent means of three replicates and error bars are the Standard Error of the Mean.

Figure **4.7**: Growth, colonization and proliferation rates of *Zoothamnium intermedium* attached to *Acartia tonsa*. Panels **A, C, E** show results obtained with a diet based primarily on bacteria, and panels **B, D, F** represent a diet based primarily on *Isochrysis galbana*. Symbols are the means of three replicates and error bars are the Standard Error of the Mean.

Figure **4.8**: Concentration of *Isochrysis galbana* observed throughout the growth rate experiment. Panel **A** shows fluctuation of *I. galbana* at concentrations of 10^1, 10^2, and 10^3 cells/ml, and panel **B** shows concentrations of 10^4, 10^5, and 10^6.

Figure 4.1

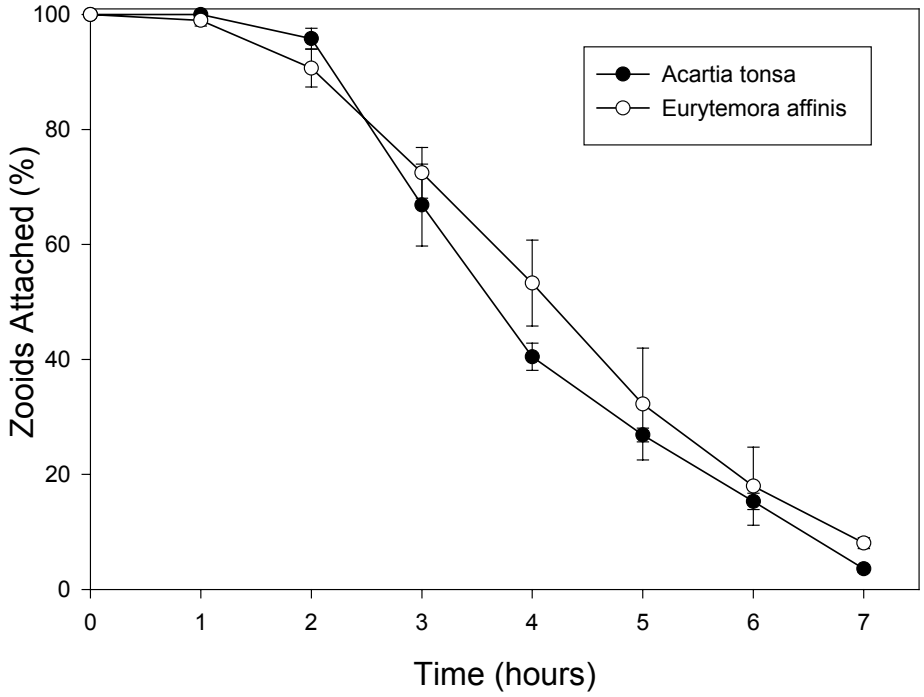

Acartia tonsa
Eurytemora affinis
Zooids Attached (%)
Time (hours)
0
20
40
60
80
100
0
1
2
3
4
5
6
7

Figure 4.2

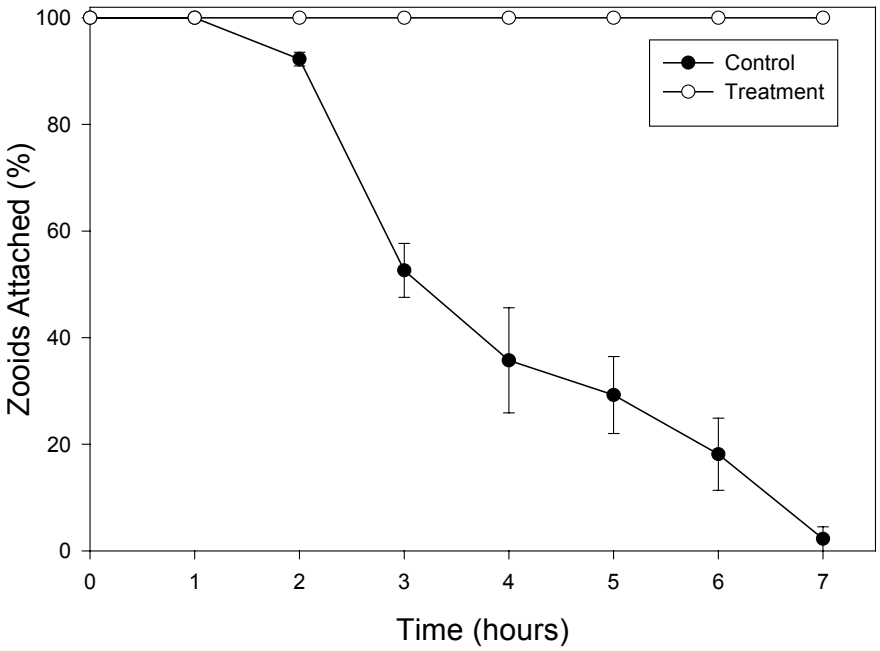

Zooids Attached (%)
100
80
60
40
20
0
0
1
2
3
4
5
6
7
Time (hours)
Control
Treatment

Figure 4.3

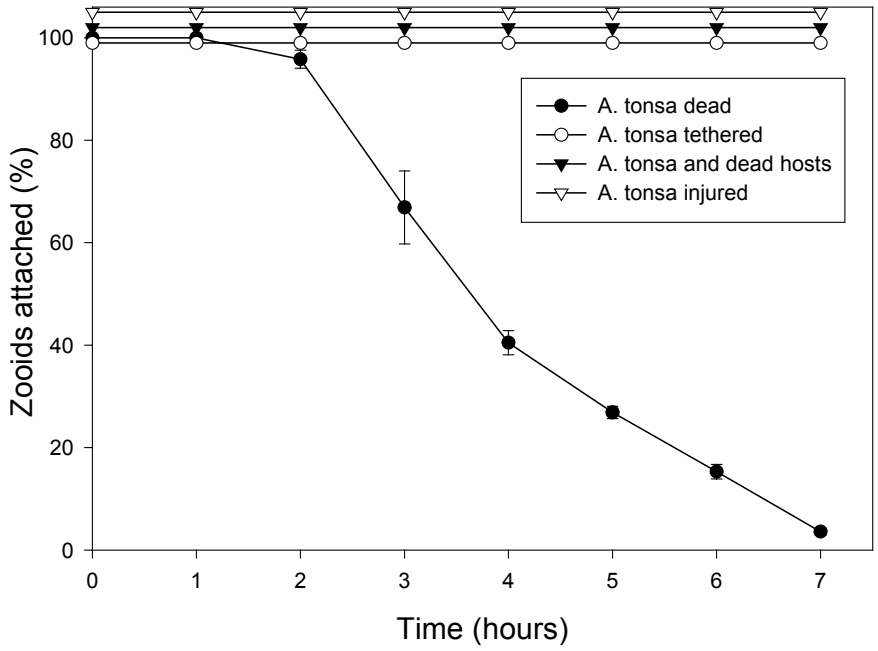

Zooids attached (%)
Time (hours)
A. tonsa dead
A. tonsa tethered
A. tonsa and dead hosts
A. tonsa injured
0
20
40
60
80
100
0
1
2
3
4
5
6
7

Figure 4.4

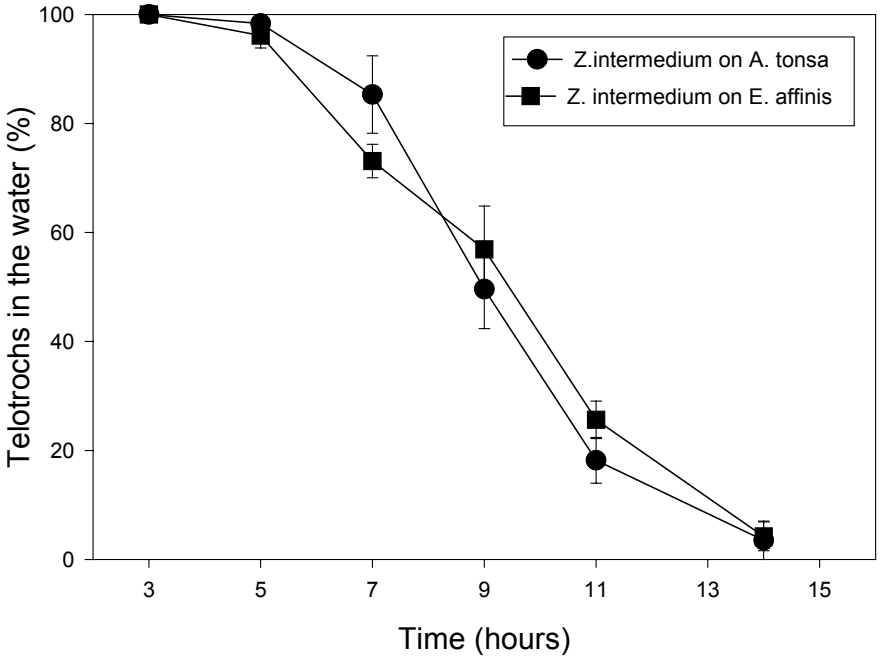

Z.intermedium on A. tonsa
Z. intermedium on E. affinis
Telotrochs in the water (%)
Time (hours)
0
20
40
60
80
100
3
5
7
9
11
13
15

Figure 4.5

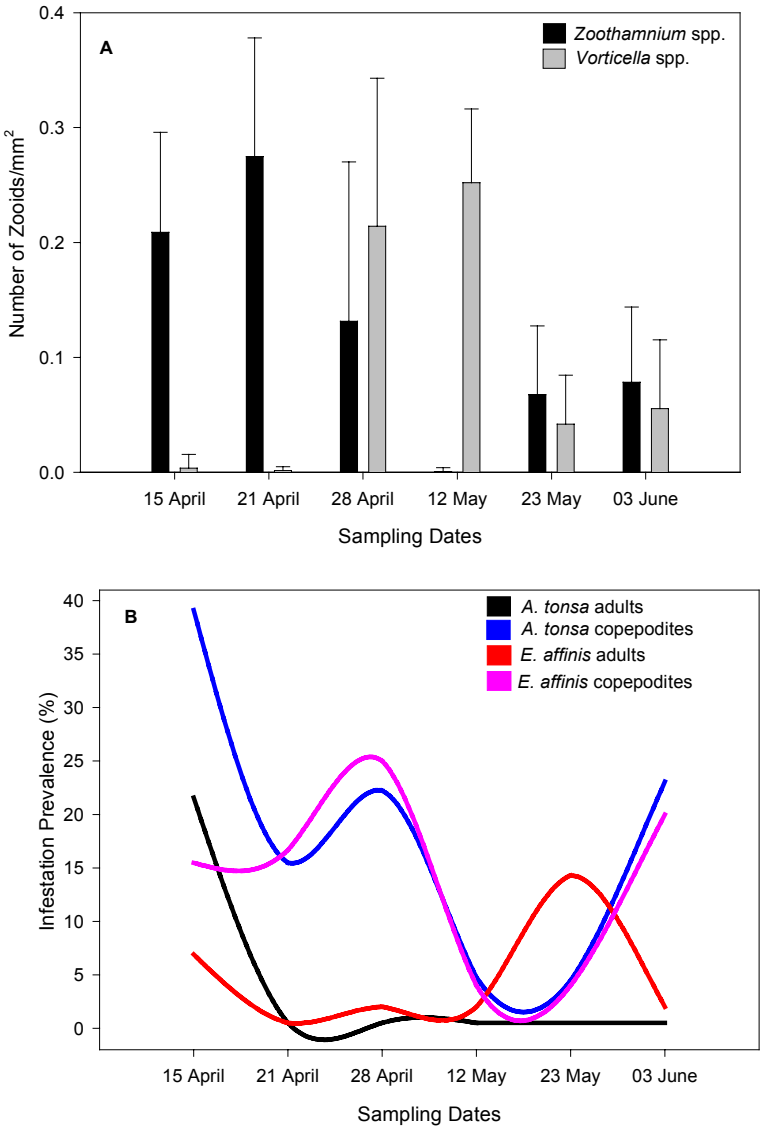

A
Zoothamnium spp.
Vorticella spp.
Number of Zooids/mm2
0.4
0.3
0.2
0.1
0.0
15 April
21 April
28 April
12 May
23 May
03 June
Sampling Dates
B
A. tonsa adults
A. tonsa copepodites
E. affinis adults
E. affinis copepodites
Infestation Prevalence (%)
40
35
30
25
20
15
10
5
0
15 April
21 April
28 April
12 May
23 May
03 June
Sampling Dates

Figure 4.6

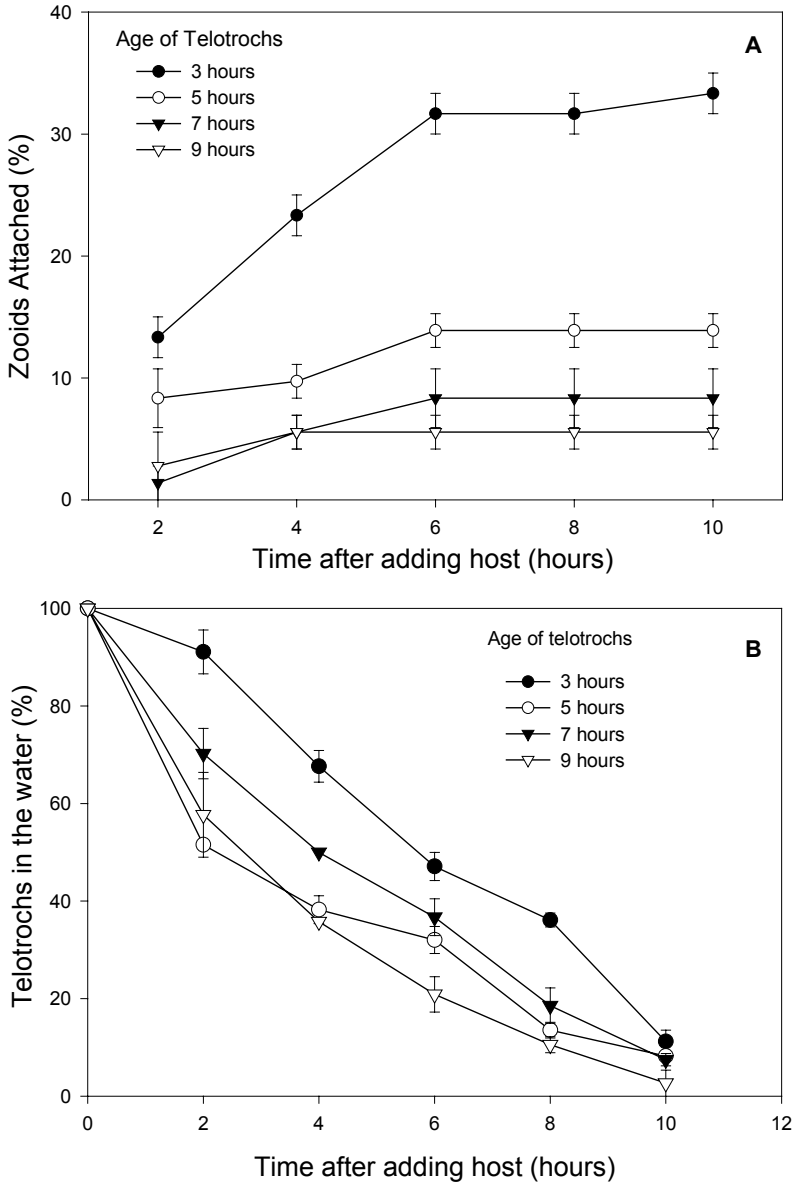

Age of Telotrochs
3 hours
5 hours
7 hours
9 hours
A
Zooids Attached (%)
Time after adding host (hours)
Age of telotrochs
3 hours
5 hours
7 hours
9 hours
B
Telotrochs in the water (%)
Time after adding host (hours)

Figure 4.7

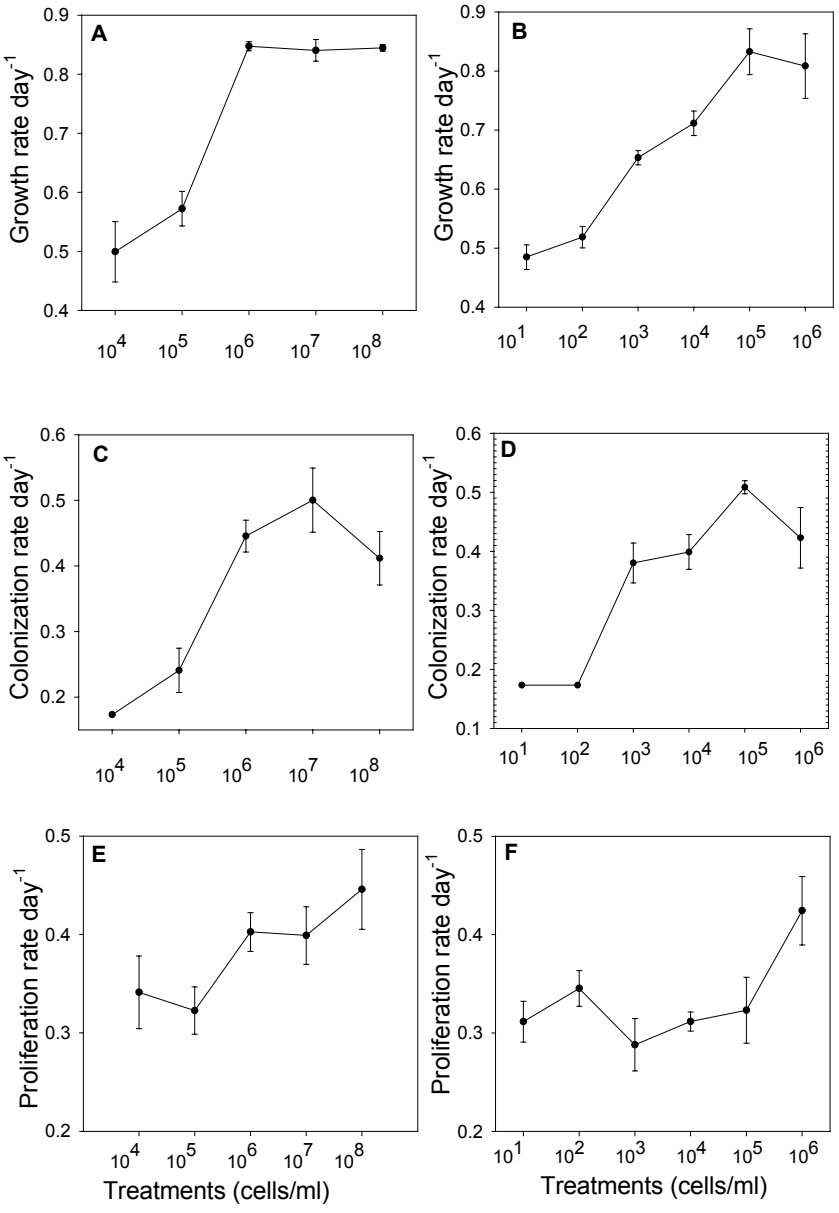
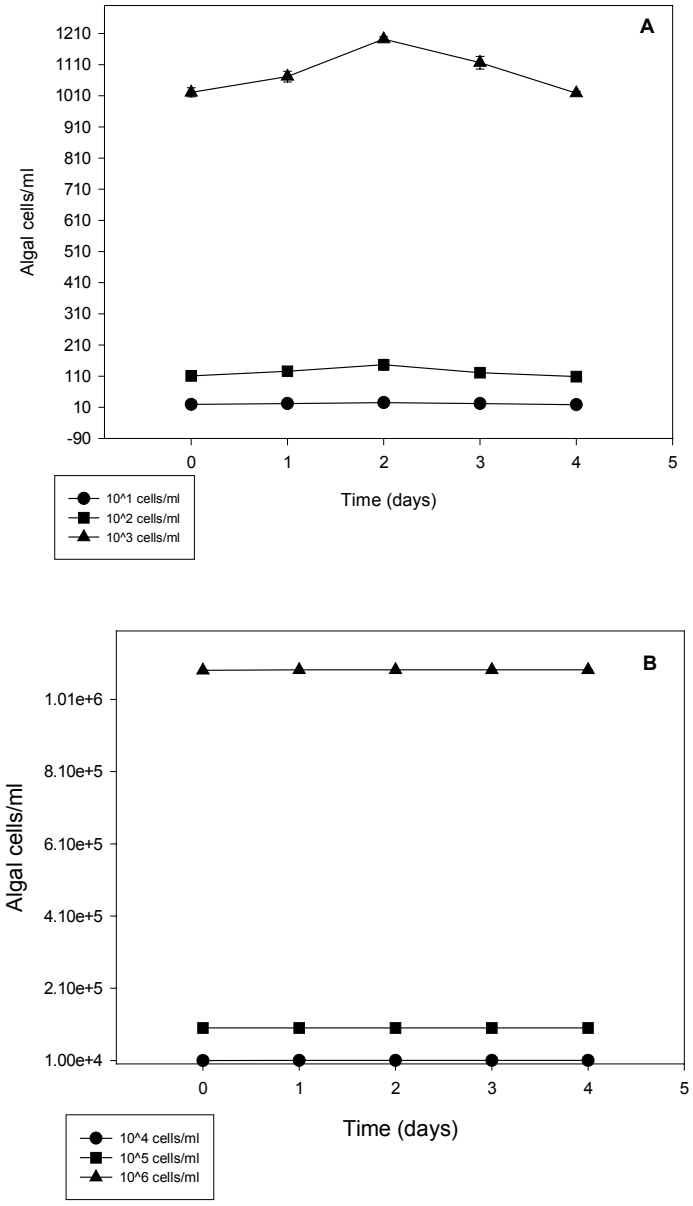

A
B
C
D
E
F
Growth rate day-1
Colonization rate day-1
Proliferation rate day-1
Treatments (cells/ml)
Treatments (cells/ml)

Figure 4.8


A
1210
1110
1010
910
810
710
610
510
410
310
210
110
10
-90
Algal cells/ml
0
1
2
3
4
5
Time (days)
10^1 cells/ml
10^2 cells/ml
10^3 cells/ml
B
1.01e+6
8.10e+5
6.10e+5
4.10e+5
2.10e+5
1.00e+4
Algal cells/ml
0
1
2
3
4
5
Time (days)
10^4 cells/ml
10^5 cells/ml
10^6 cells/ml

CHAPTER 5

ATTACHMENT PATTERNS OF THE EPIBIOTIC PERITRICH *Zoothamnium intermedium* PRECHT, 1935

ABSTRACT

In the present study I investigated the occurrence of preferential attachment of the epibiont peritrich *Zoothamnium intermedium* on calanoid copepods in the Chesapeake Bay. Controlled laboratory experiments tested preferential colonization of the epibiont on adult versus juvenile stages of its primary hosts *Acartia tonsa* and *Eurytemora affinis*, as well as the epibiont's ability to colonize other hosts from the zooplankton community (the rotifer *Brachionus plicatilis*, barnacle nauplii, polychaete larvae, and a harpacticoid copepod) in presence or absence of *A. tonsa*. Results demonstrated that there is no preferential colonization by *Z. intermedium* of juvenile or adult stages of *A. tonsa* or *E. affinis*. Also, no selective attachment was observed when adults or copepodites were compared between the two host species, but growth rates of the epibiont were significantly higher on adults of the rather pelagic *A. tonsa* when compared to the epibenthic *E. affinis*. *Z. intermedium* was able to colonize barnacle nauplii and the harpacticoid copepod in the absence of *A. tonsa* or *E. affinis*, but no attachment was observed to *B. plicatilis* or polychaete larvae. Although the epibiont was able to attach to alternate hosts, the frequency of attachment was significantly lower for these species (15% for barnacle nauplii and 7% for the harpacticoid copepod) when compared to *A. tonsa* and *E. affinis*. *Z. intermedium* colonized preferentially *A. tonsa* when simultaneously exposed to this species and an alternate host. Although no attachment of *Z. intermedium* was observed in the experiment with *B. plicatilis*, in the presence of *A. tonsa* the epibiont was able to attach to the eggs of the rotifer. These results suggested that chemical, behavioral, and

possibly mechanical cues are involved in the recognition of the host by the peritrich epibiont *Z. intermedium*.

INTRODUCTION

Stalked peritrich ciliates are known for their ability to colonize the surface of zooplankton in marine, estuarine and freshwater habitats (e.g. Nagasawa, 1987; Weissmann, Lonsdale, and Yen, 1993; Gilbert and Schröder, 2003). The implications of this epibiotic relationship regarding effects on the host, and specific colonization by the ciliate in the field have received considerable attention. For example, Hermann and Mihursky (1964) reported that 100% of the population of *Acartia tonsa* was infested by the colonial peritrich *Zoothamnium* in the Patuxent River, USA, while only a few individuals of *A. clausii* were observed carrying epibionts. On the other hand, Green (1974), studying the epibiotic community of freshwater cladocera, observed that epibionts had preference for specific sites in different hosts, but in general they were able to colonize a variety of host species. A similar observation was made by López et al. (1998), who found *Epistylis* sp. attached to several zooplankton crustacea in a tropical lake. Although these reports suggest that host specificity in epibiotic assemblages varies across different systems, studies involving controlled experiments testing the ability or preference of peritrich epibionts to colonize different planktonic are very limited.

The only study focusing on host specificity of a peritrich ciliate using laboratory experiments is that of Gilbert and Schröder (2003). They investigated preferential attachment of *Epistylis pigmaeum* to rotifers and cladocerans, and found that the epibiont can colonize a variety of hosts, but would not attach to non-living substrates. In that study, the initial concentration of dispersal stages of the epibiont

employed in each host combination was not determined, which makes it difficult to compare across experiments since colonization could be affected by encounter rates between epibiont and host.

In the present study I investigated the ability of *Zoothamnium intermedium* (a colonial peritrich epibiont colonizing populations of *Acartia tonsa* and *Eurytemora affinis* in the Chesapeake Bay, USA) to attach to other potential hosts from the zooplankton community. I also assessed preferential attachment of the epibiont to juvenile and adult stages of the two primary host species, and tested for preferential attachment to *A. tonsa* in comparison to other hosts. The hypotheses to be tested by this study were: (i) *Z. intermedium* will attach preferentially to copepodites of *A. tonsa* relative to adult stages (see Chapter 3); (ii) when exposed to potential hosts, it will be able to attach to crustacea only; and (iii) it will colonize preferentially *A. tonsa* when having a choice between this primary host and other species from the zooplankton community.

MATERIALS AND METHODS

Cultures of *Acartia tonsa*, *Eurytemora affinis*, and *Zoothamnium intermedium*: Cultures of *A. tonsa* and *E. affinis* were initiated with 60 adults each (30 males and 30 females) collected from the Patuxent River (a tributary of the Chesapeake Bay). Copepods were placed in 4-liter beakers filled with < 0.45 μm filtered seawater with salinity adjusted to 15 psu, and maintained as described in Chapter 2. Cultures of *Z. intermedium* and its hosts were also initiated and kept as described in Chapter 2.

Culture of Alternate Hosts: A culture of the rotifer *Brachionus plicatilis* was initiated with 10 adult females collected from the Patuxent River. Rotifers were placed in 600 ml beakers filled with 15 psu filtered seawater (< 0.45 μm) and maintained in the same conditions as the infested and non-infested cultures of copepods. As food for the rotifers, *Isochrysis galbana* grown in medium F/2 (Nerad and Daggett, 1992) was added to the culture at a final concentration of $1X10^5$ cells/ml. The organisms were kept in the laboratory for at least one week before they were used in the experiments. The species of harpacticoid copepod that was used in the experiments was also collected from the Patuxent River. Cultures were initiated with 10 males and 10 females that were placed in 1-liter beakers filled with filtered 15 psu seawater (< 0.45 μm). Harpacticoid copepods were maintained in the laboratory in the same conditions and with the same food concentration used for the other copepod hosts (see Chapter 2). Barnacle nauplii and polychaete larvae were collected from the Patuxent River and maintained in the laboratory in 250 ml beakers in the same conditions described for the other alternate hosts. Since these organisms are larval stages, animals were kept in laboratory cultures for two days before they were used in the experiments. Barnacle nauplii were kept in a culture enriched with a concentration of $3X10^5$ cells/ml of *Thalassiosira weissflogii* grown in F/2 medium (Nerad and Daggett, 1992); the culture of polychaete larvae was also enriched with *T. weissflogii*, but at a final concentration of $1X10^3$ cells/ml.

Preferential attachment of *Z. intermedium* to adults *versus* copepodites of *A. tonsa* and *E. affinis*: To test if *Z. intermedium* colonizes preferentially adults or copepodites

of its primary hosts (*A. tonsa* and *E. affinis*), infested *A. tonsa* (60 copepods with a load of about 60 zooids each) were obtained from the laboratory cultures and killed as described in Chapter 4. Dead copepods with attached zooids were placed in a glass beaker filled with 25 ml of < 0.45 μm 15 psu water. Beakers were placed in an incubator at 20^{o}C, and after three hours, copepods with their remaining attached zooids were removed from the beaker with a glass Pasteur pipette. A 2-ml sample was taken from the beaker, fixed with acid Lugol's and enumerated in a Sedgwick-Rafter counting chamber to assess the concentration of telotrochs/ml. A final concentration of 2 telotrochs per ml was added to 12 scintillation vials (12 treatment replicates) filled with 24 ml of 15 psu filtered water (< 0.45 μm). Non-infested adults and copepodites (Stages IV and V) of *A tonsa* obtained from laboratory cultures were added to the experimental vials (1 adult and 1 copepodite/vial), which were then closed and placed in a plankton wheel rotating at 2 rpm. After six hours, vials were removed from the wheel and contents were fixed with 8 drops of acid Lugol's. To assess infestation load (number of zooids/mm^2), preserved hosts were removed from the vials with a Pasteur pipette, observed with light microscope, and the number of zooids attached to each host was recorded. Dimensions of the host's body parts (length and width of cephalothorax, abdomen, antenna, and swimming legs) were measured using a calibrated ocular micrometer, and the area was calculated assuming a cilindrical shape as described in Chapter 3. The rest of the sample was settled for at least 24 hours and after that the sample was reduced to 10 ml and settled for an additional 24 hours. The same experimental set up was repeated for adults versus copepodites of *E. affinis*.

Preferential attachment to *A. tonsa versus E. affinis*: To investigate if *Z. intermedium* attaches preferentially to *A. tonsa* or *E. affinis*, infested *A. tonsa* (60 adults with a load of ~ 50 zooids each) obtained from the cultures were killed and epibiont telotrochs produced as described above, and added to the experimental units at a final concentration of 2/ml. Non-infested adults of *A. tonsa* and *E. affinis* obtained from the cultures were added to the scintillation vials, that were then placed on a plankton wheel (rotating at two rpm) in an incubator at 20^{o}C. The experiment was terminated in six hours and the contents of the vials were fixed with acid Lugol's (10 drops/ vial). Infestation load was assessed as described above and samples from T_0 were reduced to 10 ml and settled for 24 hours, and the number of inoculated telotrochs was counted with an inverted microscope. The same experimental set up was repeated to compare copepodite stages of *A. tonsa* and *E. affinis*.

Growth rates of *Z. intermedium* on *A. tonsa* and *E. affinis*: To assess if growth rates of *Z. intermedium* vary according to the host it is attached to, adult females of *A. tonsa* and *E. affinis* were exposed to telotrochs of the epibiont (produced as described above), after which females carrying a single zooid were separated for the experiment. The experimental design was the same used to assess growth rates of *Z. intermedium* (see Chapter 4) and will be described here briefly. Females (three from each host species) carrying a single zooid of *Z. intermedium* were transferred to six Petri dishes filled with 150 ml of < 0.45 µm 15 psu water enriched with *Isochrysis galbana* at a final concentration of 10^5 cells/ml (see Results of Chapter 4). Experimental containers

were placed in an incubator at 20°C with a photoperiod of 14:10 hours light:dark. Growth, colonization and proliferation rates (see Chapter 4 for details) were assessed every day for 4 days and a 5 ml water sample was taken and preserved with Lugol's for algal enumeration. Concentration of algal food was assessed by direct counts using a Palmer-Maloney counting chamber. Triplicate counts were performed for each replicate to a total of 100 cells/count.

Attachment to alternate hosts: To test if *Z. intermedium* is able to colonize additional members of the zooplankton community in absence of its primary copepod hosts, a series of experiments was carried out, in which telotrochs were exposed separately to four different species: the rotifer *Brachionus plicatilis*, barnacle nauplii, polychaete larvae, and a harpacticoid copepod. In each experimental set, infested *A. tonsa* (60 copepods with a load of approximately 50 zooids/host) from laboratory cultures were killed, telotrochs were obtained, and the number of telotrochs/ml was assessed as described in the experiment above. A final concentration of 1 telotroch/ml was added to six scintillation vials (3 T_0 and 3 treatment replicates) filled with 24 ml of 15 psu filtered water (< 0.45 μm). Alternate hosts obtained from laboratory cultures were added to the vials at a final concentration of 1 host/ml, with exception of the experiment with *B. plicatilis*, which had a final concentration of 3 hosts/ml. Scintillation vials were placed on a plankton wheel (rotating at two rpm) in an incubator at 20°C, and the experiment was terminated after six hours. As a control for telotroch competence (ability to attach), experiments with *A. tonsa* or *E. affinis* were run at the same time with identical conditions to those used for attachment to alternate

hosts. The contents of the vials were fixed with 10 drops of acid Lugol's and infestation load was assessed as described above. Samples were settled and telotrochs that did not attach to the hosts were counted using an inverted microscope. The following calculation was made to assess number of telotrochs lost during the experiment: IC –FC –TA, where: IC is the initial concentration of telotrochs (obtained from enumeration of T_0); FC is the final concentration of telotrochs in the water (obtained by enumeration of telotrochs in the settled sample); and AT is the number of telotrochs that attached to a host (obtained by direct enumeration of attached zooids).

Preferential attachment of *Z. intermedium* when exposed to *A. tonsa* and alternate hosts: To investigate if *Z. intermedium* colonizes preferentially *A. tonsa* when exposed to alternate hosts from the zooplankton community, a series of experiments was performed in which telotrochs were exposed to adults of *A. tonsa* and to the same four host species tested in isolation in the experiment described above (*B. plicatilis*, barnacle nauplii, polychaete larvae, and a Harpacticoid copepod). In each experimental set, *Z. intermedium* telotrochs were produced from infested *A. tonsa* (60 copepods with a load of ~ 60 zooids each), and their concentration (cells/ml) assessed, as described above. A final concentration of 2 telotrochs/ml was added to 15 scintillation vials (3 T_0 and 12 replicates) filled with 24 ml of 15 psu filtered water (< 0.45 μm). Non-infested *A. tonsa* and alternate hosts were added to the scintillation vials at a final concentration of 1 *A. tonsa* and 1 alternate host/ml, with exception of the experiment with *B. plicatilis* in which 10 individuals were added to each experimental unit due to possible predation of *A. tonsa* on this rotifer species. Vials

were placed on a plankton wheel (rotating at 2 rpm) in an incubator at 20^{o}C, and the experiment was terminated after six hours. Contents of the vials were fixed with acid Lugol's (10 drops/vial) and infestation load was assessed as described in the experiments with adults and copepodites of *A. tonsa* and *E. affinis*. Dimensions of the host body parts (length and width of the body and legs for barnacle nauplii; length and width of cephalothorax, abdomen, and swimming legs for harpacticoid copepod; length and width of the body and area of the eggs for *B. plicatilis*) were measured for each individual with a calibrated ocular micrometer, and the area available for attachment was calculated assuming a triangular, cylindrical, and rectangular /spherical shape for barnacle nauplii, harpacticoid copepod, and *B. plicatilis* body/eggs respectively. Samples were settled and telotrochs that did not attach to the hosts were counted using an inverted microscope. Number of telotrochs lost during the experiment was calculated in the same way described above. In the experiment with *B. plicatilis*, the final number of individuals was also assessed from the settled samples to evaluate predation of *A. tonsa* on this rotifer species.

Data analyses: As data passed tests for normality and homogeneity of variance, Student's t tests were performed to assess any preferential attachment of *Z. intermedium* to copepodite and adult stages of *A. tonsa* and *E. affinis*, and preferential colonization of *A. tonsa* in comparison to alternate hosts. Analyses of Variance (ANOVA) were carried out to test differences in colonization rates of telotrochs of *Z. intermedium* when exposed to *A. tonsa*, *E. affinis* and alternate hosts from the zooplankton community, as well as to assess differences in telotroch loss during the

experiment. When a significant difference among the treatments was observed, pairwise comparisons were perfomed using Tukey test. Data in the text is reported as mean $\pm$ SE (standard error of the mean). The analyses were performed using Sigma Stat Version 2.03 (SPSS Inc).

RESULTS

Preferential colonization and growth rates of *Z. intermedium* attached to *A. tonsa* and *E. affinis*: As had been observed from field samples (see Chapter 3) *Z. intermedium* was able to colonize the whole body of *A. tonsa* and *E. affinis*, including antennae and swimming legs. Cephalothorax and abdomen were the most colonized sites, with almost all copepods having zooids attached to these two body parts. No statistically significant difference was observed in the mean number of zooids/mm^2 of *Z. intermedium* attached to adult versus copepodite stages of *A. tonsa* or *E. affinis* (t Test; $p > 0.05$), as shown in Figure 5.1 A and B. Telotrochs of *Z. intermedium* also colonized equally adults and juvenile stages when the two host species were compared, as can be observed in Figures 5.1 C and D. However, when comparing growth rates of *Z. intermedium* attached to adults of both host species, a statistically significant difference was observed between *A. tonsa* and *E. affinis*. Growth rates of 0.8 day^{-1} were observed for *Z. intermedium* attached to *A. tonsa*, while the epibiont attached to *E. affinis* grew at a rate of 0.6 day^{-1} (t Test $p < 0.05$; Figure 5.2 A). Colonization rates were also significantly higher for *Z. intermedium* attached to *A. tonsa* (0.4 day^{-1}; Figure 5.2 B) than to *E. affinis* (0.3 day^{-1}; t Test $p < 0.05$).

Interestingly, proliferation rates did not show a statistically significant difference between hosts (Figure 5.2 C; t Test; $p > 0.05$).

Attachment to alternate hosts: Telotrochs of *Z. intermedium* were able to attach to barnacle nauplii and to a harpacticoid copepod in absence of their primary hosts (Figure 5.3 A). By contrast, no attachment was observed to non-crustacean hosts (polychaete larvae and the rotifer *Brachionus plicatilis*) when they were the only substrate available (Figure 5.3 A). *Z. intermedium* colonized only the dorsal surface of the barnacle nauplii, with no zooids found attached to the legs or ventral portion. Differently from *A.tonsa* and *E. affinis*, zooids of *Z. intermedium* attached only to the cephalothorax of the harpacticoid copepod, with no attachment observed on the abdomen, antennae or swimming legs. Although *Z. intermedium* was able to colonize barnacle nauplii and a Harpacticoid copepod, the percentage of zooids attached to these hosts (15.4% and 8% respectively) was significantly lower that the percentage observed for *A. tonsa* (39%) or *E. affinis* (36%; ANOVA; $p < 0.05$). No significant difference in zooid attachment was observed between *A. tonsa* and *E. affinis* (ANOVA; $p > 0.05$). The percentage of telotrochs lost during the experiment was significantly higher in the experiments with alternate hosts (ANOVA, $p < 0.05$; Figure 5.3 B) in comparison to the experiments with *A. tonsa* and *E. affinis*. The values obtained in the experiment with barnacle nauplii (64 % $\pm$ 1.28; Figure 5.3 B) and the harpacticoid copepod (57 % $\pm$ 2.6; Figure 5.3 B) were higher than those obtained for telotroch survivorship (see Chapter 4, Figure 4.4), suggesting that these two host species may be preying on free-living stages of *Z.intermedium*. No statistically

significant difference was observed in the percentage of telotrochs lost in the experiments with *A. tonsa* and *E. affinis* (Figure 5.3 B), and the values were similar to those observed in the experiment with *A. tonsa* presented in Chapter 4 (Figure 4.6 B).

Preferential attachment of *Z. intermedium* when exposed to *A. tonsa* versus other potential hosts: *Zoothamnium intermedium* attached preferentially to adults of *A. tonsa* when given the choice between this primary and alternate hosts. Similar to the observed in the experiments with alternate hosts alone, *Z. intermedium* was able to colonize barnacle nauplii and the harpacticoid copepod, but in both cases the number of zooids per mm^2 was significantly lower than that observed for *A. tonsa* (t Test; $p < 0.05$; Figure 5.4 A and B). No attachment was observed to polychaete larvae in the presence of *A. tonsa* (Figure 5.4 C). Interestingly, telotrochs of *Z. intermedium* were able to colonize *Brachionus plicatilis* (0.5 zooids/mm^2) in the presence of *A. tonsa* (Figure 5.4 D). Zooids of *Z. intermedium* were observed exclusively on the eggs of *B. plicatilis*, with no colonization recorded on the body. Even though attachment to this rotifer species was observed in this experiment, the number of zooids per mm^2 was significantly higher on *A. tonsa* (t Test; $p < 0.05$). Enumeration of telotrochs lost during the experiment revealed no statistically significant difference among the treatments (ANOVA, $p > 0.05$; Figure 5.5) and similar patterns to those observed for telotroch survivorship (see Chapter 4; Figure 4.4). This suggests that predation pressure on telotrochs by alternate hosts may be negligible in presence of *A. tonsa*. No predation of *A. tonsa* on *B. plicatilis* was observed during the course of the

experiment, since all 10 individuals from each replicate could be recovered at the end of the incubation.

DISCUSSION

The results of the present study demonstrated that *Z. intermedium* colonizes equally adults and copepodites stages of its primary hosts, *A. tonsa* and *E. affinis*, but that growth rates and colonization rates were significantly higher when the epibiont attached to *A. tonsa*. This ciliate also was shown to be able to attach to other crustaceans commonly found in the zooplankton community of the Chesapeake Bay. However, when offered an option to attach to *A. tonsa* or alternate hosts, *Z. intermedium* always colonized preferentially the former.

Laboratory studies testing preferential attachment of epibionts to different life history stages of the host are lacking; however, several observations from the field suggested that adults are always more heavily colonized than juveniles (Green, 1974; Chiavelli, Mills and Threlkeld, 1993; Gilbert and Schröder, 2003). This fact is attributed not to preferential epibiont colonization, but to a larger size of the adults and, in the case of crustacean hosts, to a high molt frequency, which decreases or is completely absent in the adult stages. For example, Xu (1993) observed that *Epistylis daphniae* was able to colonize mature and immature stages of *Moina macrocopa* in a polluted stream, but old specimens were frequently saturated with epibionts. This difference was compared and attributed to the length of the intermolt duration in this cladoceran, which is much longer in old than in young adults or juveniles. Similarly, Green and Shiel (2000) reported higher infestations of *Trichodina* sp. on adults of two

species of calanoid copepods in comparison to young stages. In fact, early copepodite stages (CI to CII) were never found carrying epibionts in that study, which was attributed to their smaller size and high molting rates. The most intuitive explanation for higher epibiont load on adults in comparison to juveniles of crustacea is the duration of the intermolt stage, but other factors such as swimming behavior, characteristics of the exoskeleton or secretion of chemicals could be different between young and adult stages, and may influence the behavior of certain species of epibionts. Moreover, those studies just compared epibiont load between adult and young stages instead of density of epibionts per unit of area, which could yield a completely different result. Interestingly, observation of field samples revealed higher density of *Z. intermedium* on copepodite than on adult stages of *A. tonsa* and *E. affinis* from Chesapeake Bay (Chapter 3), implying a preferential attachment to the juvenile stages. However, laboratory experiments presented here demonstrated that *Z. intermedium* colonizes equally adults and copepodites of both host species, suggesting that adults might be suffering a higher predation pressure in the field than young stages (see Chapter 3). Field studies investigating susceptibility to predation of zooplankton infested by epibionts, suggested that fish selectively prey upon heavily infested organisms (Willey, Cantrel and Threlkeld, 1990) due to their larger size or to slower escape responses in comparison to non-infested or lightly colonized ones. Another possible explanation for the difference observed in Chesapeake Bay copepods is a higher attachment rate to copepodites in the field, or even higher growth and colonization rates of *Z. intermedium* when attached to young stages of its primary host.

Data from field studies have indicated preferential colonization of certain hosts by some species of epibiont, or complete avoidance of others. For instance, López et al. (1998) reported that the ciliate *Epistylis* sp. was able to colonize a variety of zooplankton hosts, but completely avoided calanoid copepods. Likewise, Chiavelli, Mills, and Threlkeld (1993) found an exclusive selection by the epibionts *Colacium calvum* and *Synedra cyclopum* towards species in the genus *Daphnia* in comparison to other zooplankton present in Oneida Lake. Laboratory experiments performed by Gilbert and Schröder (2003) also suggested that *Epistylis pigmaeum*, primarily attached to the rotifer *Brachionus angularis*, was able to colonize a variety of hosts, but demonstrated a strong preference for rotifers, some species of cladocerans and a copepod, with no complete avoidance detected for any tested species. Although field samples of *Z. intermedium* colonizing copepod populations in the Chesapeake Bay revealed higher infestation prevalence of the epibiont on *A. tonsa* in comparison to *E. affinis* (see Chapter 3), thus implying a preferential attachment to this copepod species, laboratory experiments demonstrated that *Z. intermedium* colonizes equally both host species with no apparent preference for either of them.

Interestingly, when growth and colonization rates of *Z. intermedium* attached to *A. tonsa* and *E. affinis* were assessed, a significant difference was recorded for the two host species, with higher rates observed for the epibionts attached to *A. tonsa*. Growth rates in ciliates are influenced by temperature, salinity, and by the concentration and availability of food in the environment (Fenchel, 1987). Peritrich epibionts have a better chance of encountering adequate food concentrations due to constant movement of the host, than peritrichs attached to non-living substrates that

are dependent on the food availability passively surrounding them. On the other hand, the habits of the host may directly influence the growth of the epibionts by different access to more or less suitable food. For example, the copepod *Eurytemora affinis* can be considered an epibenthic organism (Jones et al., 1990), spending most of its time in bottom waters, while *Acartia tonsa*, usually swims freely in the water column. In Chesapeake Bay, *E. affinis* is the most abundant copepod in the estuarine turbidity maximum zone (ETM), and its retention in that zone is possible due to its feeding, behavioral, and reproductive characteristics (Roman, Holliday, and Sanford, 2001). On the other hand, *A. tonsa*, the most abundant zooplankton in Chesapeake Bay (Brownlee and Jacobs, 1987), is not retained in this region especially due to its reproductive strategies (Roman, Holliday, and Sanford, 2001). This difference in behavior between *E. affinis* (inhabiting bottom waters) and *A. tonsa* (spending more time in the water column), was also observed in laboratory cultures, and could have contributed for the difference in growth rates of the epibiont observed in the experiments comparing these two host species.

Z. intermedium was able to attach to other crustacean hosts in the absence of *A. tonsa* or *E. affinis*. The attachment success, however, was much lower than that observed for its primary hosts (Figure 5.3). By contrast, no attachment to non-crustaceans host was observed in the absence of the *A. tonsa*. *Z. intermedium* also preferred *A. tonsa* when offered the choice between this host and other members of the zooplankton community. Interestingly, the epibiont was able to attach to the eggs of *B. plicatilis* in presence of *A. tonsa*, but the reason why this was observed is not known. One possible explanation is the presence of chitin in the shell of the eggs of

some rotifers (including *Brachionus* species) and not in the integument, which consists primarily of keratin (Bender and Kleinow, 1988). The presence of chitin may be one of the required cues for settlement and, since it is present only in the eggs, the epibiont would be attracted to this attachment site. A similar result was reported by Gilbert and Schröder (2003) for *Epistylis pigmnaeum* attached to rotifers. They observed that the epibiont had a strong preference for colonizing the eggs, attaching to the body only when the eggs were overcrowded. However, this does not explain why *Z. intermedium* in this study did not attach to the eggs of the females when *A. tonsa* was not present. Another possibility is that *A. tonsa* releases some chemical in the environment that cues settlement and telotrochs could have mistaken the host due to the spread of the cue in the experimental container, or the ciliates need both chitin and other chemical cue from the copepod host to be able to attach to other hosts.

The nature of the preference that some epibionts display for the outer covering of certain species of crustaceans is unknown, but a theory proposed by Shomay (cited by Clamp, 1973) suggests that some epibionts are restricted to certain crustacean groups due to the affinity of the carapace for water. A peritrich colonizer would be attracted by a hydrophilic carapace while it would be repelled by a hydrophobic one. This theory offers an explanation only about the absence of peritrich epibionts from certain groups of crustacea, but it does not address the fact that some peritrichs are specific to one species, genus or family. Moreover, the mechanisms involved in the selection of hosts are probably much more complex than a simple attraction or repellence caused by the amount of water present in the exoskeleton. Chemistry of the

substrate as well as chemical cues released by the host may be playing an important role in the selection of an attachment site by peritrichs and other groups of epibionts.

Z. intermedium in the present study demonstrated no attachment preference when exposed to its two primary copepod hosts from the field. In contrast, the peritrich always colonized preferentially *A. tonsa* when exposed to this copepod species versus other potential hosts. While *Z. intermedium* attached preferentially to *A. tonsa*, it demonstrated to be able to colonize other species from the zooplankton community as well, showing that although some chemical, morphological or mechanical characteristics are more attractive in *A. tonsa*, they are not completely specific to this copepod species. Further studies addressing the release of chemical cues by the host and biochemical aspects of *A. tonsa* and *E. affinis* exoskeleton are needed to shed more light on the mechanisms mediating host selection in *Z. intermedium*.

FIGURE LEGENDS

Figure **5.1**: Number of zooids of *Zoothamnium intermedium* per unit of area of primary hosts *Acartia tonsa* and *Eurytemora affinis*: (**A**): Preferential attachment to adult versus copepodite stages of *A. tonsa*; (**B**): Preferential colonization of adult versus juvenile stages of *E. affinis*; (**C**): Preferential attachment to adults of *A. tonsa* versus *E. affinis*; (**D**): Preferential attachment to copepodites of *A. tonsa* versus *E. affinis*. Bars represent means of 12 replicates and error bars are the Standard Error of the Mean.

Figure **5.2**: Growth of *Zoothamnium intermedium* attached to *Acartia tonsa* and *Eurytemora affinis*. (**A**): Growth rates (zooids/host); (**B**): Colonization rates (colonies/host); (**C**): Proliferation rates (mean number of zooids/colony). Bars represent means of 3 replicates and error bars are the Standard Error of the Mean. Asterisks indicate a statistically significant difference ($p < 0.05$).

Figure **5.3**: Ability of *Zoothamnium intermedium* telotrochs to colonize *Acartia tonsa*, *Eurytemora affinis* and alternate hosts from the zooplankton community. (**A**): Percentage of zooids attached to *A. tonsa*, *E. affinis*, Barnacle nauplii, Harpacticoid copepods, Polychaete larvae, and *Brachionus plicatilis*; (**B**): Percentage of zooids lost during the experiment. Bars represent means of 12 replicates and error bars are the Standard Error of the Mean. Asterisks indicate a statistically significant difference ($p < 0.05$).

Figure **5.4**: Number of zooids of *Zoothamnium intermedium* per unit area of the host. (**A**): Preferential attachment of *Z. intermedium* to *A. tonsa* versus Barnacle nauplii; (**B**): Preferential colonization of *Z. intermedium* on *A. tonsa* versus a Harpacticoid copepod; (**C**): Preferential attachment of *Z. intermedium* to *A. tonsa* versus Polychaete larvae; (**D**): Preferential attachment of *Z. intermedium* to *A. tonsa* versus *Brachionus plicatilis*. Bars represent means of 12 replicates and error bars are the Standard Error of the Mean. Asterisks indicate a statistically significant difference ($p < 0.05$).

Figure **5.5**: Percentage of zooids of *Zoothamnium intermedium* lost, and attached to a host during the experiment of preferential attachment to *Acartia tonsa* and alternate hosts from the zooplankton community.

Figure 5.1

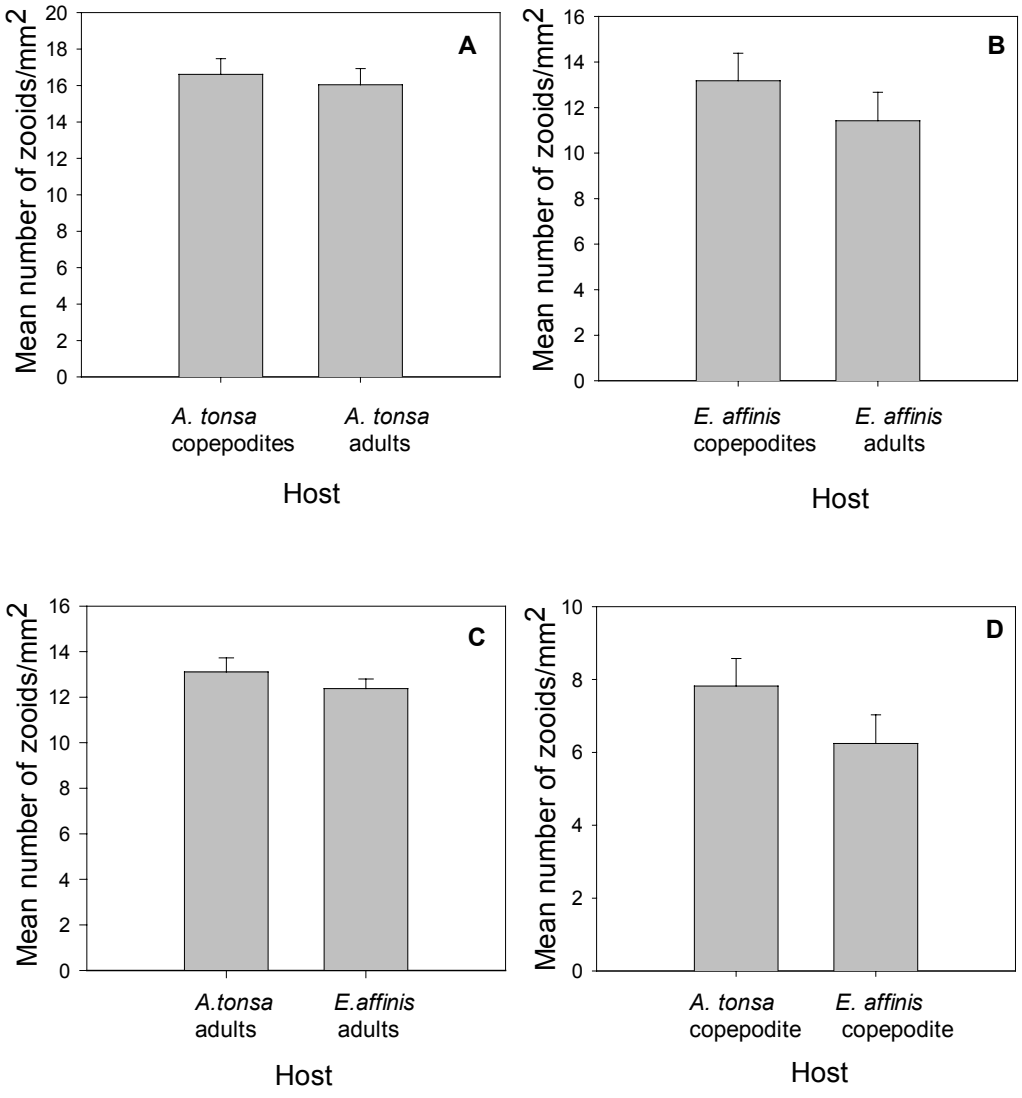

A
Mean number of zooids/mm2
20
18
16
14
12
10
8
6
4
2
0
A. tonsa copepodites
A. tonsa adults
Host
B
Mean number of zooids/mm2
16
14
12
10
8
6
4
2
0
E. affinis copepodites
E. affinis adults
Host
C
Mean number of zooids/mm2
16
14
12
10
8
6
4
2
0
A.tonsa adults
E.affinis adults
Host
D
Mean number of zooids/mm2
10
8
6
4
2
0
A. tonsa copepodite
E. affinis copepodite
Host

Figure 5.2

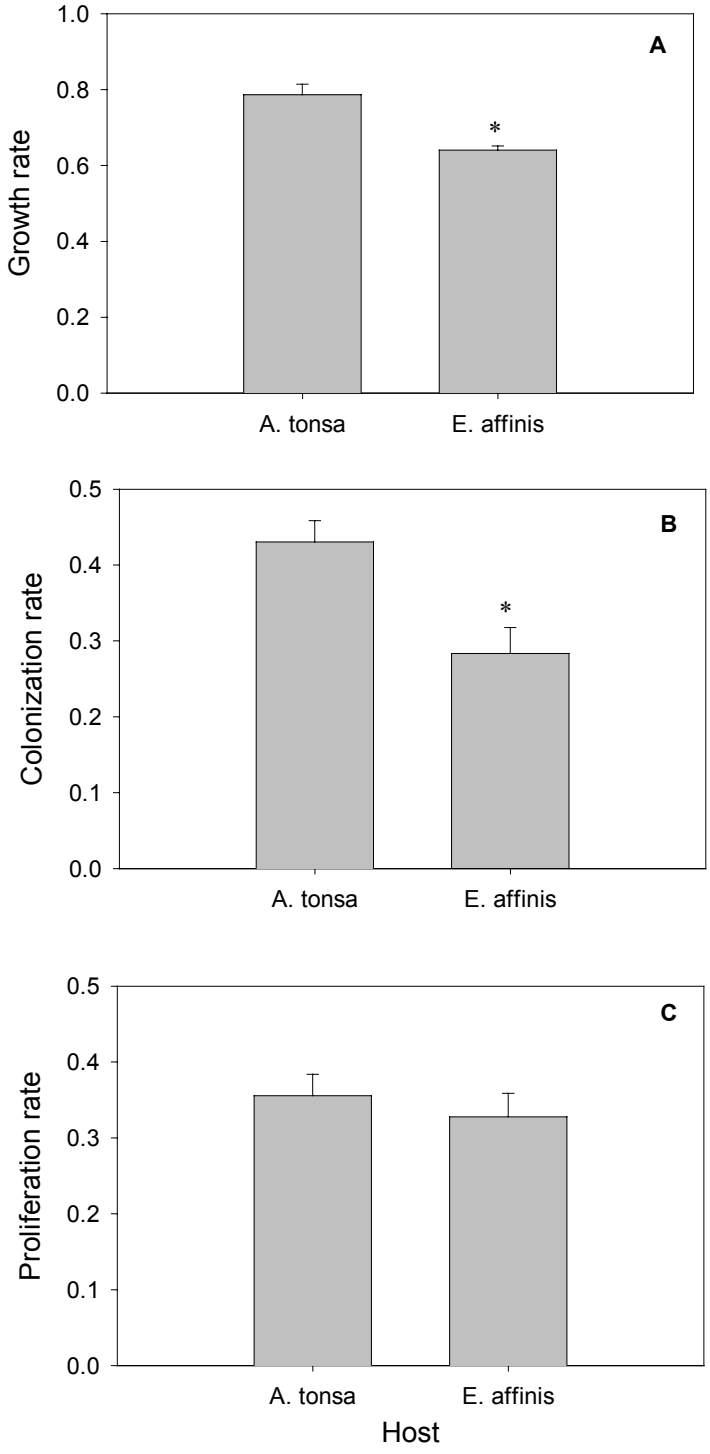

A
Growth rate
1.0
0.8
0.6
0.4
0.2
0.0
*
A. tonsa
E. affinis
B
Colonization rate
0.5
0.4
0.3
0.2
0.1
0.0
*
A. tonsa
E. affinis
C
Proliferation rate
0.5
0.4
0.3
0.2
0.1
0.0
A. tonsa
E. affinis
Host

Figure 5.3

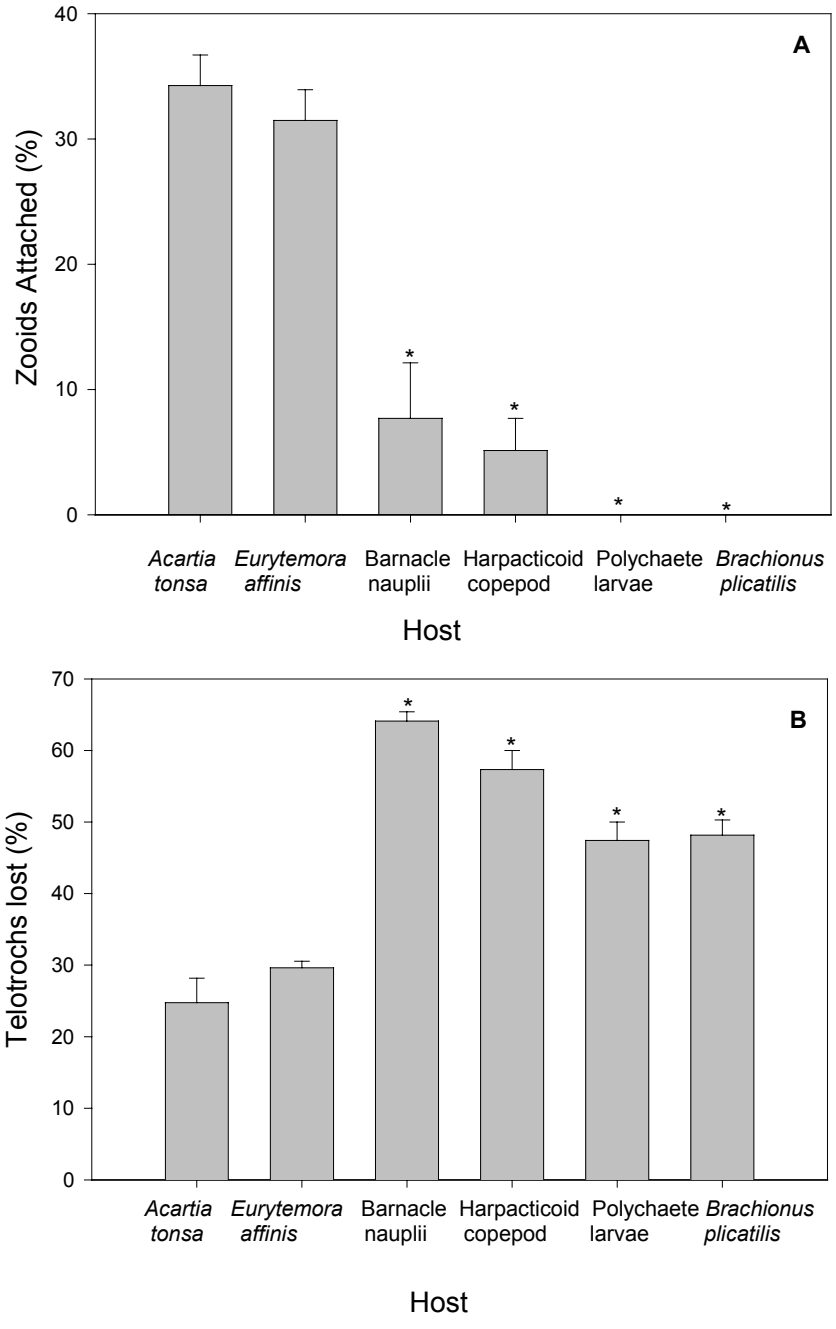

A
Zooids Attached (%)
40
30
20
10
0
*
*
*
*
Acartia tonsa
Eurytemora affinis
Barnacle nauplii
Harpacticoid copepod
Polychaete larvae
Brachionus plicatilis
Host
B
Telotrochs lost (%)
70
60
50
40
30
20
10
0
*
*
*
*
Acartia tonsa
Eurytemora affinis
Barnacle nauplii
Harpacticoid copepod
Polychaete larvae
Brachionus plicatilis
Host

Figure 5.4

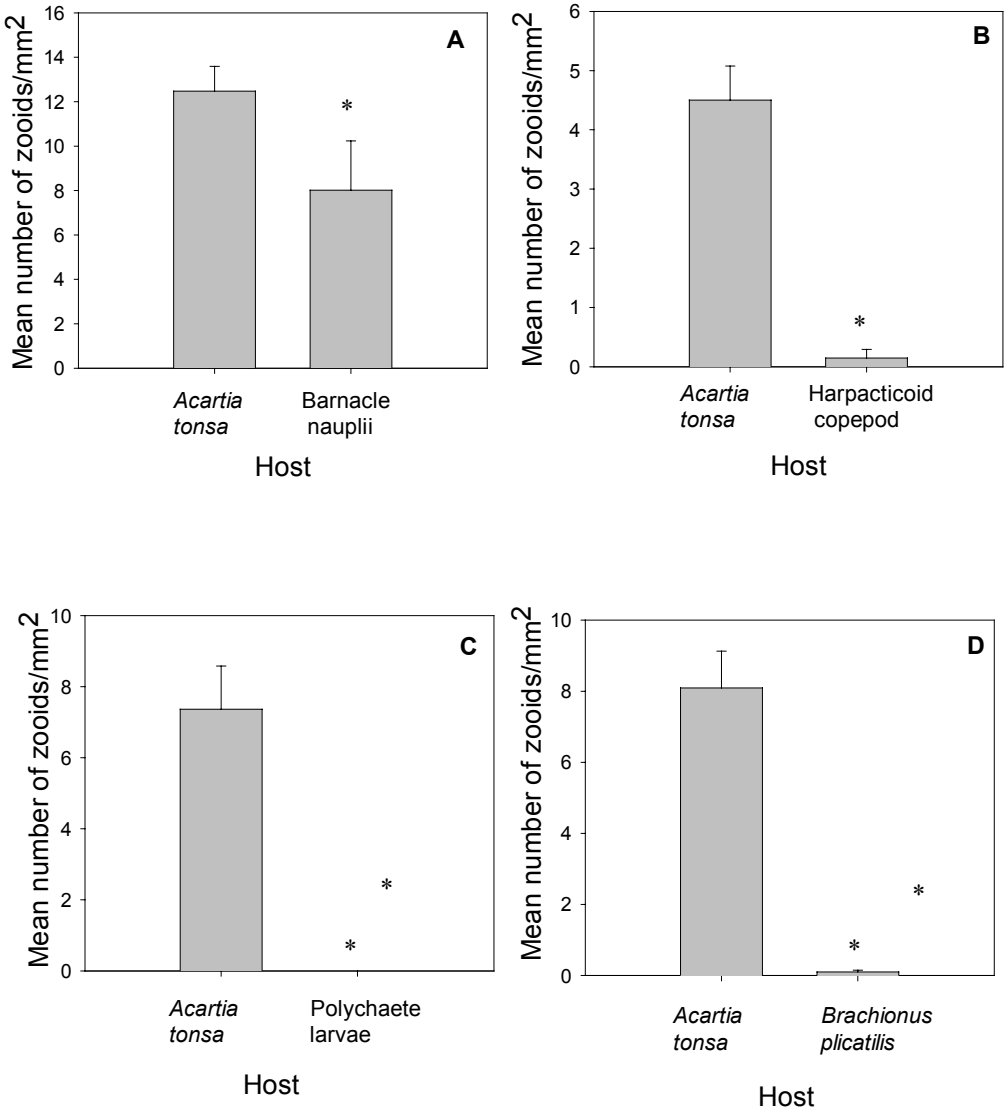
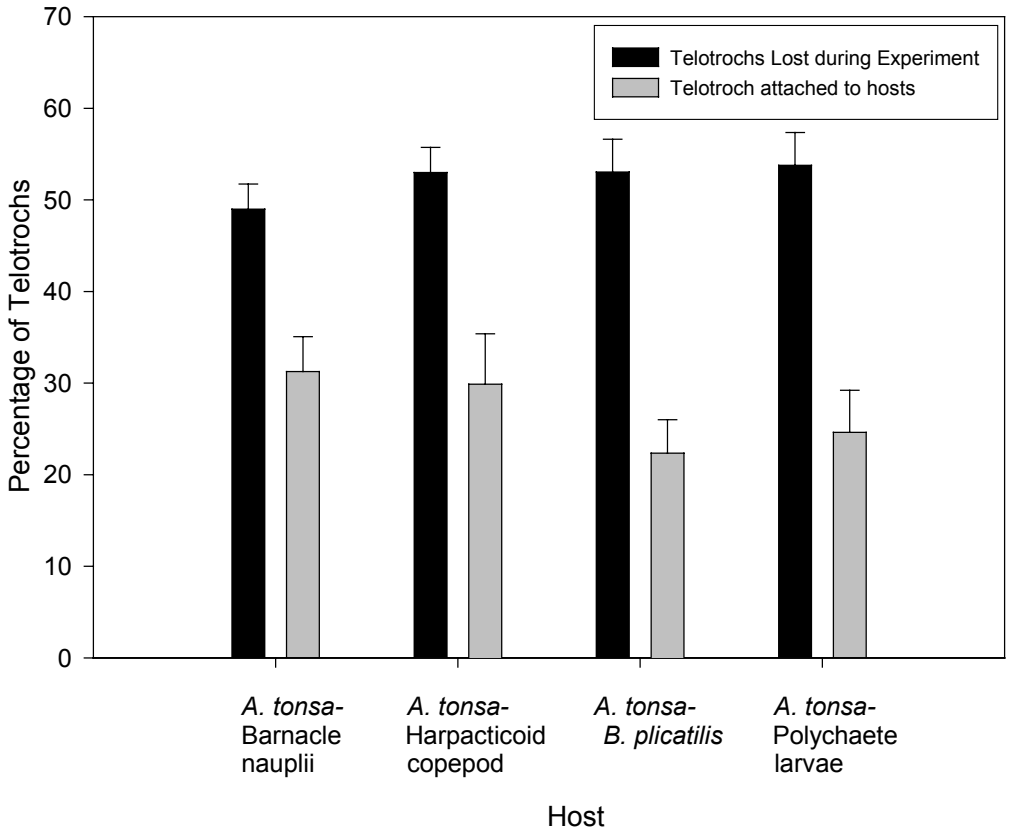

A
Mean number of zooids/mm2
16
14
12
10
8
6
4
2
0
*
Acartia tonsa
Barnacle nauplii
Host
B
Mean number of zooids/mm2
6
5
4
3
2
1
0
*
Acartia tonsa
Harpacticoid copepod
Host
C
Mean number of zooids/mm2
10
8
6
4
2
0
*
*
Acartia tonsa
Polychaete larvae
Host
D
Mean number of zooids/mm2
10
8
6
4
2
0
*
*
Acartia tonsa
Brachionus plicatilis
Host

Figure 5.5


70
60
50
40
30
20
10
0
Percentage of Telotrochs
Telotrochs Lost during Experiment
Telotroch attached to hosts
A. tonsa- Barnacle nauplii
A. tonsa- Harpacticoid copepod
A. tonsa- B. plicatilis
A. tonsa- Polychaete larvae
Host

CHAPTER 6

GENERAL DISCUSSION

Epibiotic relationships in aquatic environments have been known for more than a century. Ryder (1883), in a report published in Science magazine focusing on protist parasites in oysters, mentioned the existence of colonies of an organism identified as *Zoothamnium* attached to the back and legs of the oyster-crab *Pinnotheres osterum*. The author suggested that the relationship would be advantageous for the host, since it could feed on zooids that detached from the colony. Since then, reports and investigations of epibiotic relationships emphasizing seasonal occurrence of epibionts, issues about host specificity and possible deleterious effects that epibionts may have on the host have tried to shed light on the nature and consequences of this ecological association. Although the occurrence of epibiotic relationships has been documented frequently, especially in freshwater environments, some aspects such as life history stages of the epibiont, mechanisms of attachment, and assessment of host specificity are still not well explored (Carman and Dobbs, 1997).

The initial goals of the present study were to characterize morphologically the ciliate epibiont community on calanoid copepod populations from the Chesapeake Bay, USA, and to assess patterns of spatial and temporal occurrence of epibiosis in the same environment. The results presented in Chapters 2 and 3 revealed that two species of peritrich ciliates, identified as *Zoothamnium intermedium* and *Epistylis* sp., are common epibionts on *Acartia tonsa* and *Eurytemora affinis* populations during the spring and summer months of the year. The most abundant species of peritrich attached to copepods in the Bay was identified as *Zoothamnium intermedium* Precht, 1935. As pointed out by Carman and Dobbs (1997), the identification of epibiont species and comprehensive studies about species composition in epibiotic

communities are still lacking especially in marine environments. This is also the case in estuarine systems such as the Chesapeake Bay. As discussed in Chapter 2, species identification of peritrichs can be difficult and time consuming due to incomplete original descriptions and redescriptions. The application of cytological techniques such as Protargol staining, Scanning Electron Microscopy in peritrich identification (e.g. Zagon and Small, 1970; Gross, 1986; Leitner and Foissner, 1997; Song, 1997; Hu and Song 2001) has provided useful knowledge on relevant diagnostic characteristics that may help with species comparison from different habitats in the world. At the same time, it is important to provide diagnostic features and measurements of reliable morphological characters observed in live specimens, so that researchers who are not trained to apply staining techniques or electron microscopy would be able to correctly identify peritrichs to species level (Leitner and Foissner, 1997). In addition, characters from live specimens are required for comparisons with original descriptions.

Occurrence of epibionts on organisms from zooplankton communities has been widely reported in the literature (e.g. Sherman and Schaner, 1965; Green, 1974; Willey, Cantrel, and Threlkeld, 1990; Chiavelli, Mills, and Threlkeld, 1993; Weissman, Lonsdale, and Yen, 1993; Gilbert and Schröder, 2003), but only few studies investigated seasonal occurrence of epibiosis over a period of one year (e.g. Green, 1974; Xu, 1992; Chiavelli, Mills, and Threlkeld, 1993; Willey and Threlkeld, 1993; Barea-Arco, Pérez-Martinez, and Morales-Barquero, 2001). Most of these studies observed a strong correlation between epibiont prevalence and host abundance, while others reported a neutral or negative relationship with substrate availability.

Threlkeld, Chiavelli and Willey (1993) suggested that the existence of a positive or negative correlation between epibiont prevalence and host abundance could be explained by the existence of source and sink habitats (Pulliam, 1988). A negative correlation with a given host species would suggest that substrate is a sink habitat for that particular epibiont. In other words, the epibiont is able to colonize that given substrate, but reproduction and migration is very reduced or does not occur at all, while in a source, or preferred habitat, the epibiont is capable of completing its life cycle and to migrate and colonize other hosts. The authors pointed out that some exceptions to this pattern could be due to selective predation on infested hosts or to poor sampling.

Infestation prevalence of *Zoothamnium intermedium* from Chesapeake Bay did not show any significant correlation with abundance of its copepod hosts, *Acartia tonsa* and *Eurytemora affinis* (see Chapter 3), but indicated a trend of infestation peaks following high densities of the host. From the point of view suggested by Threlkeld, Chiavelli, and Willey (1993), the copepod hosts might be hypothesized to be a sink habitat for *Z. intermedium* with perhaps benthic living or non-living substrates serving as source habitat. However, laboratory experiments and observations in this study revealed that *Z. intermedium* could reproduce and disperse when attached to *A. tonsa* (see Chapter 4, growth rates experiments) and *E. affinis* (observation from laboratory cultures), demonstrating that copepods are not sink habitats in the studied system. Moreover, *Z. intermedium* was not able to attach to non-living substrates in the laboratory nor in the field (see Chapter 4), suggesting that this epibiont has an obligate relationship with living hosts.

The dynamics of infestation prevalence could be dependent on other abiotic and biotic factors in addition to host abundance. One of the factors that could help explain patterns of infestation observed in the field is epibiont production. Temperature, salinity, dissolved oxygen, availability of food, and strategies of dispersal may be determinant factors for the increase or decrease in epibiont prevalence. For example, dispersal stages of sessile peritrichs are unable to feed, therefore surviving from only a few hours to a few days (Fenchel, 1965; Clamp, 1973) if they do not find a suitable substrate for attachment. The survival time for telotrochs of *Z. intermedium* was shown to be approximately 14 hours at 20^0 C(see Fig. 4.4), implying the the epibont has to find a new substrate in a very short period for the epibiont to be able to find another substrate. Moreover, it was demonstrated that the attachment success of telotrochs of *Z. intermedium* decreases as telotroch age increases (see Chapter 4, Fig. 4.6 A), suggesting that it is extremely important to find a host immediately after the formation of the dispersal stage. Despite the short-lived telotroch and a highest attachment frequency recorded in the laboratory of approximately 37%, infestation prevalence of *Z. intermedium* in the field was found to be as high as 70% on *A. tonsa* (see Chapter 3). This high prevalence observed in the field might occur as a combination of high host abundance and high epibiont production fueled by different biotic and abiotic factors, whose complexity could not be fully characterized with the extent of sampling and available variables analyzed in this work (see Chapter 3). Therefore, I suggest that *Z. intermedium* is an obligate epibiont of living substrates and its abundance is dependent on the availability of the host substrate, as well as on the

production of dispersal stages, which may be directly related to salinity, temperature and availability of food.

Host specificity is another subject commonly discussed in studies of epibiotic relationships in zooplankton communities, especially because epibionts often colonize one or more groups or species of hosts in a given environment. Wahl and Mark (1999) suggested that most epibionts exhibited a facultative relationship with their living host based on a survey of the literature and on field experiments with artificial microhabitats carried out in the Baltic Sea. The majority of the epibiont species surveyed were invertebrates, but unidentified species of *Vorticella* and *Zoothamnium* were also found in the field study, and were reported to colonize a variety of living (especially macrophytes) and non-living substrates. Green (1974) also reported that the majority of epibionts he found in freshwater cladocerans were not specific to one host, with exception of *Colacium vesiculosum* that, in the studied environment, was found colonizing only *Daphnia magna*. A similar situation was reported by Henebry and Ridgeway (1979), who found *Vorticella microstoma* only attached to *Scapholeberis kingii* in a polluted lake in Illinois, and by Hermann and Mihursky (1964) who found *Zoothamnium* sp. colonizing only *Acartia tonsa* in the Patuxent River, even when it was not the most abundant species. Field samples analyzed in the present study revealed that *Z. intermedium* was only attached to *A. tonsa* and *E. affinis* (see Chapter 3) even when other zooplankton species were abundant. On the other hand, laboratory experiments demonstrated that the ciliate epibiont is able to attach to additional potential hosts from the zooplankton community (e.g. barnacle nauplii, and Harpacticoid copepod; see Chapter 5) in presence or absence of its primary host *A.*

tonsa. Moreover, observation of live samples collected from the field showed that, when infestation prevalence on *A. tonsa* and *E. affinis* was *ca.* 100%, *Z. intermedium* was able to attach to barnacle nauplii and rotifer eggs in the field. A similar observation was pointed out by Green (1974), who suggested that heavy infestation of a preferred host species could lead to transient association with other living organisms present in the environment. From the results obtained in the analyses of field samples (Chapter 3), laboratory experiments (Chapters 4 and 5) and field study (Chapter 4), *Z. intermedium* seems to be an obligate epibiont of living substrates, perhaps with preference for crustacean hosts. The fact that the epibiont was able to attach to eggs of *B. plicatilis* in the presence of *A. tonsa* can be due to the release of chemical cues by the copepod, the presence of chitin in the shell of the eggs, and/or overcrowding of the copepod with temporary attachment to the rotifer (see discussion in Chapter 5). The lack of attachment of *Z. intermedium* to other zooplankton species observed in samples from the field (Chapter 3) could be due to non-saturation (i.e. no overcrowding) of the primary hosts in the surveyed period, or to insufficient sampling.

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, Sicko-Goad, and Omair (1979) observed that the suctorian *Tokophrya quadripartita* attached preferentially to the head of its host, the copepod *Limnocalanus macrurus*, an ideal site to come to contact with prey. Clamp (1973) also observed that the peritrich *Lagenophrys lunatus* exhibited strong selection for some areas of the body (especially the uropods) of its shrimp host that received more flux of water. *Colacium calvum* also attached preferentially to the anal region of its

host, the cladoceran *Daphnia* spp,. possibly to take advantage of the material excreted by the substrate (Chiavelli, Mills, and Threlked, 1993). Preferential attachment of epibionts to males or females was pointed out by Sherman and Schaner (1965) who observed only slight to moderate infestation of *Paracineta* sp. on males of the copepod *Meritridia lucens*. Females, in contrast, were always heavily infested by the suctorian epibiont. Xu and Burns (1991) also reported that the peritrich epibiont *Epistylis daphniae* attached preferentially to females of the copepod *Boeckella triarticulata*, with very low infestation rates in males. Weissman, Lonsdale and Yen (1993) did not find any preference of the peritrich *Rhabdostyla* sp. for age, stage or site in the body of the copepod *Acartia hudsonica*. In the present work, *Z. intermedium* was also able to attach to the cephalothorax, abdomen, antennae and swimming legs of its copepod hosts, but density of colonies and zooids were higher on the abdomen of both host species (see Chapter 3), and there was no apparent preference for males or females. Interestingly, a high density of epibiont colonies was found on copepodite stages of the hosts, suggesting a preferential attachment to juvenile stages (Chapter 3). This observation is unique (especially for copepods that have a terminal molt), and surprising since studies that compared infestation prevalence throughout life stages reported higher epibiont infestation on adults of crustacean hosts than on juvenile forms (Sherman and Schaner, 1965; Mohlemberg and Kaas, 1990; Xu, 1993; Chiavelli, Mills, and Threlkeld, 1993). However, subsequent laboratory experiments demonstrated that *Z. intermedium* does not colonize preferentially copepodite or adult stages of either host species (see Chapter 4), suggesting that the pattern observed in field samples reflects other interactions that may be occurring in nature. One possible

explanation is the selective predation on heavily infested adults, since a large number of colonies could make the individuals more visible for fish that prey by sight, and also hamper locomotion, thus affecting their swimming escape reaction. Surveys and manipulations in the field demonstrated that the prevalence of euglenoid epibionts on cladocerans and copepods was significantly reduced in the presence of pump-filter feeders or fish that prey by sight, in comparison to sites without zooplankton predators (Willey, Cantrel and Threlkeld, 1990; Willey, Willey, and Threlkeld, 1993). In the case of small euglenoids, the attraction to predators possibly comes from the bright green color of the chloroplast inside the epibiont cell. *Z. intermedium* would not have on copepods the color effect that epibionts like *Colacium* spp. may have on their host, but the high number of colonies may lead the host to appear larger for predators, and potentially easy to capture.

Frequent molts, death, and sometimes desiccation of the substrate organism are some of the unstable conditions that epibionts have to face (Wahl, 1989). To be successful, the epibiont must be able to form dispersal stages, leave the old substrate and find another suitable host to start another cycle. For example, some peritrich epibionts are able to start forming free-swimming stages prior to the completion of the molt cycle of their crustacean hosts (Fenchel, 1965; Green, 1994; Willey and Threlkeld, 1995), while others remain attached to the molt and only after it is shed do they produce dispersal stages (Clamp, 1973). In the present study, results of experiments stimulating telotroch formation in *Z. intermedium*, suggested that a combination of chemical, mechanical and perhaps electrical cues coming from the host to which the epibiont is attached is probably needed to trigger telotroch formation

in this peritrich (Chapter 4). Observations of copepod exuvia from laboratory cultures revealed that *Z. intermedium*, differently from other peritrichs attached to crustaceans, starts forming telotroch stages after the host has cast the old skin. The mechanisms 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 (Clamp, 1973)

The inferences and conclusions obtained in this work may provide some insights about the complexity of epibiotic relationships. The experiments presented here may serve as models that can be employed in other systems to assess aspects of the life-cycle and host specificity of epibionts or free-living peritrichs. Suggestions for future studies of the *Z. intermedium*-copepod association in Chesapeake Bay include: (i) the assessment of potential deleterious effects that the epibiont might have on the fitness of the host, including effects on reproduction and survivorship; (ii) the analysis of potential biochemical recognition and affinity of the epibiont by elements present on the surface of the host; and (iii) investigation of possible demographic impacts that this epibiotic relationship might have on copepod populations and its consequence for the ecosystem as a whole.

GLOSSARY

Some of the technical terms used in this dissertation are defined below:

Basibiont – substrate organism which is host to the epibiont.

Coenobium – a type of colonization in which all the colonial cell-like units are interconnected a continuous, common cell membrane.

Colonization rate – increase in the number of colonies/host per unit of time.

Dikinetid – organellar complex in ciliates composed of two approximately adjacent kinetosomes, associated cilia, and infraciliary organelles. See kinetid.

Epibiont – organism that grows attached to a living surface.

Germinal row – line of non-ciliated kinetosomes associated with the terminal portion of the infraciliary base of the haplokinety. It plays a productive role in stomatogenesis in Peritrichs.

Growth rate – change in the number of zooids/host per unit of time. It is a function of the colonization and proliferation rates.

Haplokinety – a double row of kinetosomes associated with the oral ciliature and exhibiting a zigzag pattern.

Infestation density – number of epibiont colonies/unit of area of the host.

Infestation load – number of epibiont zooids/unit of area of the host.

Infestation prevalence – percentage of host population carrying epibionts in a given time.

Infundibulum – section of the oral cavity in peritrich ciliates. It is usually funnel-shaped, and contains the oral infraciliature.

Kinetid – elementary organizational unit of the ciliate cortex (region underneath the membrane that contains the ciliary organelles). Consists of one or more kinetosomes and associated structures.

Kinetosome – basal body. Cylindrical organelle consisting of 9 peripheral microtubular triplets and 2 inner microtubules. Basal termination of cilia (and flagella).

Kinety – longitudinally oriented row of a single, paired, or multiple kinetosomes, their cilia, and other associated cortical organelles.

Oral dikinetid (ODK) – Dikinetid located to the right side of the cytostome in the oral region of ciliates. See Dikinetid.

Oral polykinetid (OPK) – Polykinetid located to the left side of the cytostome in the oral region of ciliates. See Polykinetid.

Peristome – circular region of the zooid apical surface which opens to the infundibulum in peritrich ciliates.

Polykinetid - organellar complex in ciliates composed of more than two kinetosomes, associated cilia, and infraciliary organelles.

Proliferation rate – change in the mean number of epibiont zooids/colony attached to a host per unit of time.

Scopula – compound organelle located at the aboral pole of sessiline peritrich ciliate zooids that may function as a holdfast organelle, or as a source of the secreted stalk.

Telotroch – free-swimming, dispersal stage found in sessiline peritrichs.

Telotroch band – synonym of trochal band. Ring of cilia and kinetosomes near the posterior (aboral) end of a telotroch.

Trophont – mature, vegetative, sessile feeding adult stage in the peritrich life cycle.

Zooid – individual cell or cell like unit in a peritrich colony or coenobium.

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