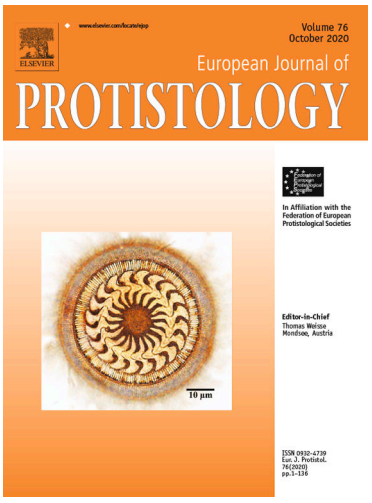

Investigating the epibiotic peritrich *Zoothamnium intermedium* : seasonality and distribution of its relationships with copepods in chesapeake bay (USA)

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Investigating the epibiotic peritrich *Zoothamnium intermedium* Precht, 1935: seasonality and distribution of its relationships with copepods in Chesapeake Bay (USA)

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Abstract

Zoothamnium intermedium is an obligate epibiont ciliate and has been found in a diverse array of hosts and environments. Different studies have reported conflicting distribution patterns and host preferences, even though studies in Chesapeake Bay have suggested that the ciliate has a strong host specificity for two calanoid copepod species. We examined the life cycle, host preferences, and ecological conditions conducive to *Z. intermedium* presence on copepods in Chesapeake Bay, the largest estuary in North America. The York River tributary was sampled biweekly from fall 2014 through summer 2015 for plankton, peritrichs and bacteria in the water column. Bacterial abundance in the water column peaked in fall and late spring, coinciding with increased abundance and species richness of non-epibiont peritrichs. Among the plankton, only the calanoid copepods *Acartia tonsa* and *Centropages hamatus* were colonized by *Z. intermedium*. The peritrich epibiont displayed higher colonization rates on *C. hamatus* even when *A. tonsa* was far more abundant. Multivariate correlation analysis of infestation prevalence on *A. tonsa* showed a strong correlation with dissolved oxygen, salinity and water temperature. Such correlations,

along with differences in host species biology, might be driving the seasonality of this epibiotic relationship.

Keywords: epibiosis, host interaction, Peritrichia, microbial ecology,

Introduction

Epibiosis, broadly defined as one species living and growing on the body surface of another, is widely observed in aquatic systems between unicellular epibionts from bacteria to algae, flagellates and ciliates, and multicellular hosts including crustaceans (Carman and Dobbs 1997). This life strategy often requires investment in special attachment mechanisms (Bickel et al. 2012) and some epibiont species exhibit host specificity (Gilbert and Shröder 2003), suggesting that the life cycle and ecological functions of these epibionts are strongly dependent on the particular hosts. Epibionts can derive considerable benefits from their attachment to hosts, such as increased mobility and filtration efficiency (Magagnini and Verni 1988, Regali-Seneghim and Godinho 2004, Pane et al. 2014). On the other hand, the effects of epibiosis on hosts are less clear. Although some investigators reported that colonization presents no measurable consequences for the hosts (Scott and Thune 1986, Hudson and Lester 1992), others have observed detrimental effects on the host's fecundity, feeding, locomotion, reproduction, growth and survivorship, and increased predation risk and sensitivity to contaminants (Kankaala and Eloranta 1987, Weissmann et al. 1993, Threlkeld and Willey 1993, Hanamura et al. 2010, Bickel et al. 2012).

Zoothamnium intermedium is a common peritrich epibiont, first described over 80 years ago (Precht 1935). Its zooids are bell-shaped and it attaches to a host's cuticle, forming clonal colonies. It was found on ascidians, shrimp and amphipods in Kiel Bay, Germany, where it was first discovered (Precht 1935), and it was subsequently reported to colonize a variety of crustacean hosts from other parts of the world (Valbonesi and Guglielmo 1988, Fernandez-Leborans and von Rintelen 2010, Nekuie Fard et al. 2015). In North American waters, the ciliate uses the planktonic copepods *Acartia tonsa* and *Eurytemora affinis* as its primary hosts, colonizing other hosts such as barnacle nauplii and harpacticoid copepods only in the absence of suitable primary hosts (Utz

and Coats 2008). While capable of forming short-lived telotroch stages for dispersion, *Z. intermedium* otherwise is thought to reside entirely on the carapaces of these crustaceans, in a symbiosis that defies facile characterization. Most likely, the ciliate is primarily a commensal, benefitting from the food environment made accessible by planktonic crustaceans swimming through the water column.

Planktonic copepods play a vital role in the marine pelagic food web, both as prey for fish larvae and other zooplanktivorous consumers, as well as grazers of phytoplankton (Turner 2004). *Acartia tonsa* is the dominant copepod species in Chesapeake Bay and a major component of fish diets (Sedlacek and Marcus 2005, Chen and Hare 2008), but eutrophication and overfishing have adversely affected this species by creating a favorable environment for its predators such as ctenophores, and causing non-predatory mortality due to hypoxia (Keister et al. 2000, Purcell and Decker 2005, Kimmel et al. 2006, Condon and Steinberg 2008, Kimmel et al. 2012, Elliot et al. 2013). Anthropogenic impacts also affect water temperatures (Lomas et al. 2005), and elevated temperatures are known to prompt other ciliate taxa to infest copepods (Walkusz and Rolbiecki 2007).

Utz and Coats (2005) found that *Z. intermedium* reached over 6% colonization prevalence on *A. tonsa* in March along the main axis of Chesapeake Bay in Maryland, while Peng (2013) found up to 78% colonization prevalence in the York River in lower Chesapeake Bay at the same time of the year. Although both studies found *Z. intermedium* colonizing *A. tonsa* in Chesapeake Bay, those authors investigated different habitats, with Utz and Coats (2005) sampling in deeper waters, along the main axis of the bay, and Peng (2013) sampling in a shallow coastal zone of the York River. Such differences in habitat could explain the lower number of *A. tonsa* copepods available as host in the samples of Utz and Coats (2005).

Zoothamnium intermedium colonization can have consequences for both the hosts and the broader food webs in which these hosts reside, yet the potentially important role of colonization by this epibiont has received little attention from parasitologists and ecologists over the years. Previous research on *Z. intermedium* has reported conflicting data on its seasonality and host preferences (Precht 1935, Valbonesi and Guglielmo 1988, Utz and Coats 2005, Fernandez-Leborans and Von Rintelen 2010, Nekuie Fard et al. 2015). By examining the epibiont-host dynamics in relation to the plankton community compositions, we sought to provide a better spatial-temporal resolution on the driving factors of this relationship. To achieve this, we surveyed target host species, as well as other members of the plankton, including bacteria, peritrichs and alternative potential plankton hosts. We also applied molecular methods to confirm the identification of the peritrich epibiont. The findings altogether improve our understanding of the ecological significance of peritrich epibiosis in Chesapeake Bay.

Materials and Methods

Collection and Preservation of Samples

The York River, a tributary of Chesapeake Bay, USA, is a partially mixed microtidal sub-estuary (Lin and Kuo 2001). Biweekly samples were collected from September 2014 to August 2015 from a fishing pier at Gloucester Point (37.247N, 76.499W), in the mesohaline part of the estuary where salinity is typically 15 or more. Zooplankton were collected by five-minute tows of a plankton net (0.5-m diameter; 200-μm mesh). Subsamples were fixed with 8% formaldehyde (Dias et al. 2009) for enumeration and selected colonized copepod specimens with 95% ethanol for molecular analysis. Major planktonic taxa were quantified and microscopically identified to order level (Steinberg and Condon 2009). The zooplankton fixed in formaldehyde were examined

under a dissecting microscope for the presence of *Z. intermedium*. The specimens fixed with 95% ethanol were also screened for the epibiont. Colonized hosts were further identified by DNA analysis (see below). The epibiont was microscopically identified as *Z. intermedium* by its colonial coenobium, alternate branching pattern, contractile stalk, continuous spasmoneme, bell-shaped zooid, absence of a macrozooid, and zooid length (Utz et al. 2008). The specimens were also used to enumerate epibiont colonies per host and zooids per colony (*sensu* Utz and Coats 2005). Epibiont species identification as well was further confirmed by DNA analysis (see below).

Other sessile peritrichs, *i.e*. those that are not obligate epibionts on other organisms, were sampled by submerging glass slides for two-week intervals during the same time period by suspending them from a pier just down-estuary from the plankton sampling site; afterward the slides were screened for peritrichs and colonies present within a cover slip area (Safi et al. 2014).

Water quality data were obtained from a monitoring station at the sampling site as a part of the Virginia Estuarine and Coastal Observing System (VECOS). Water temperature, salinity, dissolved oxygen, pH, and total chlorophyll were recorded every 15 minutes during the study period, both at the water surface and bottom.

Water samples for bacterial cell counts were collected with 5-mL Falcon® tubes and fixed with freshly prepared 1% paraformaldehyde at 4°C for 30-60 minutes. Samples were then frozen in liquid nitrogen and kept at -80°C. Samples were later thawed, stained with a 1% solution of SYBR-I (ThermoFisher, Waltham, MA) in the dark for 10 minutes, and then run through a BD Influx cell sorter (Benton Dickson, San Jose, California, USA), following Gasol and Del Giorgio (2000). Flow cytometry of samples was run for 3-5 minutes each, and data were acquired in log mode until around 10,000 events. The volume delivered to the cell sorter was calculated as the difference between the remaining volume and the initial volume.

DNA Analyses

Since we did not observe any clear morphological differences between the colonies from the two hosts, we further investigated the potential molecular diversity among them. A PCR-sequencing approach was used to confirm the identification of *Zoothamnium* epibiont specimens found on different copepods. We designed primers targeting the SSU rDNA gene region, VIMS-ZISSU-82F (5'-CGAAACTGCGAATGGCTCAT-3') and VIMS-ZISSU-1616R (5'-TTTGCAGGGACGTAATCAGCAC-3'), which were expected to produce an amplicon of ~1500 bp. DNA from peritrich-colonized zooplankton subsamples fixed in ethanol was extracted with a DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol.

Each PCR tube contained 3-10 ng of template DNA (3-10 ng), 1x PE buffer, bovine serum albumin 10 mg/mL, 10mM of each primer, and 0.6 U of AmpliTaq DNA polymerase (ThermoFisher, Waltham, MA) for a total volume of 25 μL. PCR was then run with the following cycling parameters: 1 cycle at 94°C for 4 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 1 min, and 72 °C for 2 min, with a final extension of 72 °C for 10 min. PCR products were checked by agarose electrophoresis and sent to Macrogen, Inc. (Rockville, Maryland, USA) for purification and direct Sanger sequencing.

For confirmation of peritrich-colonized zooplankter identification, a ~700-bp region of the mitochondrial cytochrome oxidase I gene (mtCO1) was amplified using primers LCO1490 and HCO2198 following parameters described in Folmer et al. (1994). Products were again analyzed by agarose electrophoresis and sent to Macrogen Inc. for purification and sequencing.

SSU rDNA sequences from peritrich epibionts were aligned with other *Zoothamnium* sequences available on GenBank using MAFFT version 7.407 (Katoh et al. 2002, Katoh and

Standley 2013) in Mesquite version 3.6 (build 917) (Maddison 2008), with refinement by eye. Sequence similarity search for copepod mtCO1 sequences was done by BLAST (Altschul et al. 1990).

Statistical Analyses

Multivariate correlation analysis (Spearman-Rho correlation) was performed using R v.3.6.1 (Team 2019) to evaluate relationships between epibiont prevalence (*i.e.* percentage of hosts colonized) and environmental variables. Two-way ANOVA tests were also performed in R to evaluate the differences in infestation density (colonies per host) and load (zooids per colony) between copepod host species and water quality parameters. All life stages (i.e. copepodite or adult) of the hosts were accounted for.

Results

Distribution of Zoothamnium intermedium

Zoothamnium intermedium was found on the calanoid copepods *Acartia tonsa* and *Centropages hamatus*, mostly colonizing the cephalothorax and abdomen of these hosts. It showed a seasonally varied pattern of host preference in the York River (**Figs. 1, 2, 3,** and **4**). It was found to colonize both copepod species in spring, but only *A. tonsa* during summer and only *C. hamatus* during fall and winter (**Fig. 5**). Interestingly, the epibiont was observed to colonize only *C. hamatus* even when *A. tonsa* was 300 times more abundant than *C. hamatus*. Mean prevalence of colonization varied from 0 to 20% in *A. tonsa*, but it exceeded 35% in *C. hamatus*. The number of colonies per individual host specimen varied from 0 to 19 on *A. tonsa*, with 1 to 73 zooids per colony, and 0 to 11 colonies with 1 to 52 zooids per colony on *C. hamatus*, but those numbers varied throughout the year, and no colonization was found in October and November (**Fig. 6**).

The plankton tows also retrieved a variety of unidentified calanoid copepods, as well as cyclopoid and harpacticoid species. Other zooplankton taxa included Rotifera, Polychaeta, Cladocera, Decapoda and Cirripaedia larvae, Amphipoda, Isopoda, and Mysidacea. The peritrich epibiont, however, was not found on any of these taxa, even during periods of high abundance of other potential hosts, such as different copepod species (September to November and June to August) and cirripedia larvae (December to February).

Salinity at the collection site ranged from 10.5 to 16.0, and temperature from 6.2 to 27.3°C (**Fig. 7**). Bacterial concentrations in the water column ranged from 9.9×10^5 to 5×10^6 cells mL^{-1}, with abundance peaks in fall and late spring. The concentrations of non-epibiont peritrichs were synchronous with bacterial concentrations in the water column, with spring and summer peaks (**Fig. 6**). Concentration and species richness of sessile peritrichs decreased markedly during the colder months, possibly due to the decrease in bacterial concentration as well as temperature. From December 2014 to March 2015, no peritrich ciliates were found on the submerged glass slides.

Rank correlation analyses showed significant negative correlations of infestation prevalence on *A. tonsa* with dissolved oxygen ($p = 0.03$) and salinity ($p = 0.004$), and positive correlation with water temperature ($p = 0.044$), and a nearly significant negative correlation with total suspended solids ($p = 0.052$) (**Table 2**). In contrast, infestation prevalence on *C. hamatus* was not correlated with any of the environmental variables. The ANOVA tests retrieved no significant differences for infestation density and zooid load between *A. tonsa* and *C. hamatus* for the studied period.

Genetic Identification

A total of four SSU rDNA epibiont sequences and three copepod mtCO1 sequences were analyzed. The *Zoothamnium* epibiont sequences of ~1435 bp were deposited in GenBank (MH374528, MH374523, MH374506, and MH376893). When compared to the original *Z.*

intermedium SSU rDNA sequence (KF790904), they scored ≥ 99.85% similarity in BLAST search.

Copepod mtCO1 sequences of ~660 bp were deposited in GenBank as *Acartia tonsa* (MH493899) and *Centropages hamatus* (MH549185). Both species showed a high percentage of identification of sequences on BLAST, ≥ 99.84 and 99.31, respectively, and have been previously reported for the sampling area (Van Engel and Tan 1965).

Discussion

The peritrich epibiont *Z. intermedium*, confirmed by SSU rDNA sequencing, colonized only the calanoid copepods *A. tonsa* (**Fig. 1**) and *C. hamatus* (**Fig. 2**), which were confirmed by mtCO1 sequence analysis. While no traditional protargol staining was performed to evaluate the species infraciliature of the ciliate, previous studies found no differences in the oral apparatus morphology among the specimens found on different hosts (Utz and Coats 2005). *In vivo* morphometry was also consistent among the specimens found on both copepod hosts. Furthermore, this was the only epibiont from genus *Zoothamnium* found colonizing the copepods collected throughout the sampling period. None of the other potential host taxa was colonized by *Z. intermedium* in our study, which is also consistent with previous observations (Utz and Coats 2005). The high similarity among the epibiont SSU rDNA sequences (≥ 99.85%) is evidence that this is indeed the same peritrich species colonizing both copepods.

Colonization rates were appreciable in *A. tonsa* (up to 11% during spring) and substantially higher in *C. hamatus* (~30% in spring and up to 70% in fall) even when *A. tonsa* abundances were 1-2 orders of magnitude higher than *C. hamatus* in the samples (**Fig. 4**). This may be attributed to differences in how the two species occupy the water column. While *A. tonsa* is considered a pelagic species (Nagasawa et al. 1987, Gaudy et al. 2000), *C. hamatus* has been described as more of an

epibenthic copepod (Beyst et al. 2000, Vallet and Dauvin 2004). During this study, the total suspended solids and turbidity were generally higher in the bottom layer than in the surface layer (**Fig. 7**), which may reflect a higher food supply for *Z. intermedium* attaching to an epibenthic copepod host. A similar behavior was reported by Jones et al. (2018), who reported density and prevalence of an *Epistylis* epibiont on an estuarine copepod varied with turbidity and organic matter content. Nevertheless, we failed to find significant correlations between epibiont prevalence and total suspended solids or turbidity (**Table 2**). However, we did find a significant negative correlation between the infestation prevalence on *A. tonsa* and dissolved oxygen ($p = 0.03$) and salinity ($p = 0.004$), as well as a significant positive correlation with water temperature ($p = 0.044$). Such correlations were not reported in previous studies of *Z. intermedium*, but research on other peritrich epibionts of copepods show some similar patterns. The presence of certain peritrich species has been generally regarded as an indication of saprobiotic conditions in freshwater systems (Curds and Cockburn 1970, Sládecek 1981, Salvado et al. 1995), as have the presence of certain peritrich epibionts (Cabral et al. 2018). However, little has been published on the relationship of peritrichs with dissolved oxygen in estuarine and marine environments, except for Hudson and Lester (1992), who found a significant positive correlation between peritrich epibionts on prawns and biological oxygen demand. Fortunately, more data are available on the relationships of salinity and temperature with epibiosis. Jones et al. (2019), in laboratory studies, found that high salinity was responsible for the mortality of an *Epistylis* sp. and a combined effect of high salinity and turbidity significantly affected its survivorship. Goh et al. (2019) investigated another *Zoothamnium* sp. epibiont of copepods and found that infestation was more prevalent after the establishment of a power plant, which increased the sea surface temperature of their study site by 0.58°C.

None of the other analyzed plankton taxa (Rotifera, Polychaeta, Cladocera, Decapoda and Cirripaedia larvae, Amphipoda, Isopoda, and Mysidacea) were colonized by the peritrich epibiont. The investigation of other plankton taxa aimed to validate previous reports of host preference for the species with multiple crustacean hosts, as well as to confirm laboratory experiments in which *Z. intermedium* preferred the calanoid copepods *A. tonsa* and *E. affinis* as hosts (Utz and Coats 2008). Other host biological factors, such as the vertical distribution of copepods and epibionts in the water column, as well as velocity shear due to swimming movements, were suggested to be important in determining epibiont prevalence. However, these are also different among *A. tonsa* and *C. hamatus* (Mauchline 1998). Host density, known to modulate infestation rates for other peritrich epibionts in a freshwater lake (Xie et al. 2001), also does not seem to be a factor. As mentioned earlier, the epibiont was found colonizing only *C. hamatus* even when *A. tonsa* was much more abundant. Conversely, *Z. intermedium* was found colonizing both copepod species when that host ratio was closer to 1. A similar pattern was reported with *Zoothamnium* sp. and copepods *Centropages abdominalis* and *Acartia clausi* by Nagasawa (1986) in a saline lake.

The epibiont species also showed different seasonal trends from the non-epibiotic sessile peritrichs found in the same period, the latter of which seemed to be synchronized to bacterial abundances in the water column (**Fig. 8**). This was expected, since peritrichs are primarily bacterivorous (Henebry and Ridgeway 1979, Stabell 1996). It was interesting, however, to find *Z. intermedium* colonizing *C. hamatus* in periods of low availability of food, *i.e.*, bacteria, and low water temperatures (**Fig. 4**). Epibionts can benefit from a reduced boundary layer around them relative to free-living forms, while improving their feeding rates when compared to non-epibionts, as they save energy from not generating their own feeding currents (Reynoldson 1955, Bickel et al. 2012). Additionally, the epibiotic life strategy can be advantageous for *Z. intermedium* in the

aforementioned scenario for using the host motility to better explore food patches (Kankaala and Eloranta 1987).

The same does not apply to the host, however. There are numerous reports of damage from *Zoothamnium* species on copepod hosts (Herman and Mihurski 1964, Feigebaum 1975, Couch 1983, Scott and Thune 1986, Nagasawa 1986, Hudson and Lester 1992, Souissi et al. 2013), especially in food-limited environments (Xu and Burns 1991). Colonial peritrichs have been shown to be detrimental to another *Acartia* species, *A. hudsonica*, potentially affecting its population fitness by decreasing sinking and egg production rates, as well as survival of nauplii (Weissman et al. 1993). Although our sampling method was not designed specifically for epibenthic species, the number of individuals retrieved for *C. hamatus* and *A. tonsa* was drastically different. Van Engel and Eng-Chow Tan (1965) investigated copepod abundance and composition in Chesapeake Bay, and reported *C. hamatus* as "one of the less numerous species", with April being its period of greatest abundance, which is consistent with our data.

It is not possible to determine if the original *Z. intermedium* described by Precht (1935) is the same species we found in our samples, but if that is indeed the case, it indicates a cosmopolitan distribution and variable host specificity. However, without definitive genetic evidence as we presented here, the peritrich could be easily misidentified or confused with other *Zoothamnium* species (Sun et al. 2012, Shen et al. 2016). As shown in our results, *Z. intermedium* exhibited strong host specificity and seasonality in Chesapeake Bay. By understanding the driving factors in this relationship, we can make better predictions on specific threats to copepod host species accordingly.

Here we documented the distribution of *Z. intermedium* on calanoid copepods from the York River in Chesapeake Bay. The epibiont was found only on *C. hamatus* from fall to spring, but

shifted to a mixed host preference with *A*. *tonsa* from spring through summer. Statistical analysis revealed that the epibiont colonization on *A*. *tonsa* had a strong negative correlation with dissolved oxygen and salinity, as well a strong positive correlation with water temperature. Next steps of the research should explore other water bodies, so other environments and potential hosts can be investigated.

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Table 1 Number of copepods retrieved from biweekly collections and percent colonized. *At*/*Ch* is the ratio of *Acartia tonsa* to *Centropages hamatus* found in the samples. A value of 1 was added for *C. hamatus* on 9/12 to avoid division by 0.

Table 2 Correlation coefficients (C) and p-values (*p*) for Spearman's rho rank correlations between infestation prevalence and water quality parameters in the York River (VA) between September 2014 and July 2015. *p*-values < 0.05 in bold. *n* = number of pooled samples). DO = dissolved oxygen, TSS = total suspended solids, Wtemp = Water temperature. 'b' corresponds to bottom and 's' to surface.

Fig. 1. Copepod host *Acartia tonsa* (female adult) displaying heavy colonization by *Zoothamnium intermedium*.

Fig. 2. Copepod host *Centropages hamatus* (male adult) displaying heavy colonization by *Zoothamnium intermedium*.

Fig. 3. Detail of a *Zoothamnium intermedium* colony on a copepod host.

Fig. 4. *Zoothamnium intermedium* colony separated from host.

Fig. 5. Colonization of *Zoothamnium intermedium* on copepod hosts relative to host abundance. Left axis shows mean colonization (number of colonized individuals/total number of individuals, in percentage) of hosts *Acartia tonsa* and *Centropages hamatus*. Right axis is the ratio of host *A. tonsa* to *C. hamatus* abundance ratio, represented by the dashed line. Data obtained from the York River from September 2014 to August 2015.

Fig. 6. Monthly means of infestation density (a) and infestation load (b) on copepod hosts in the York River from September 2014 to August 2015. Line represents standard errors.

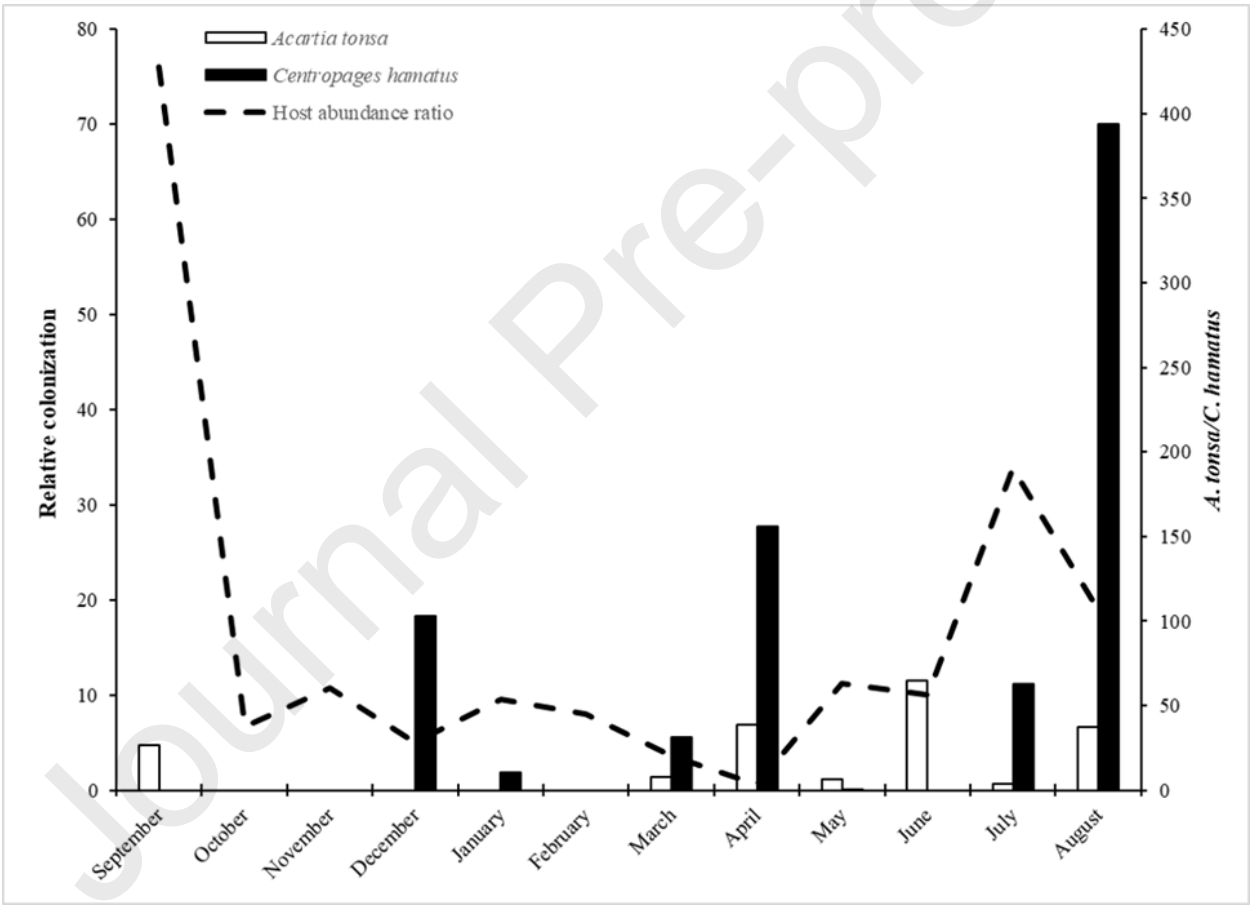
Fig. 7. Water quality parameters from the sampling site in the York River from September 2014 to August 2015. Solid lines represent bottom measurements, dashed lines represent surface measurements.

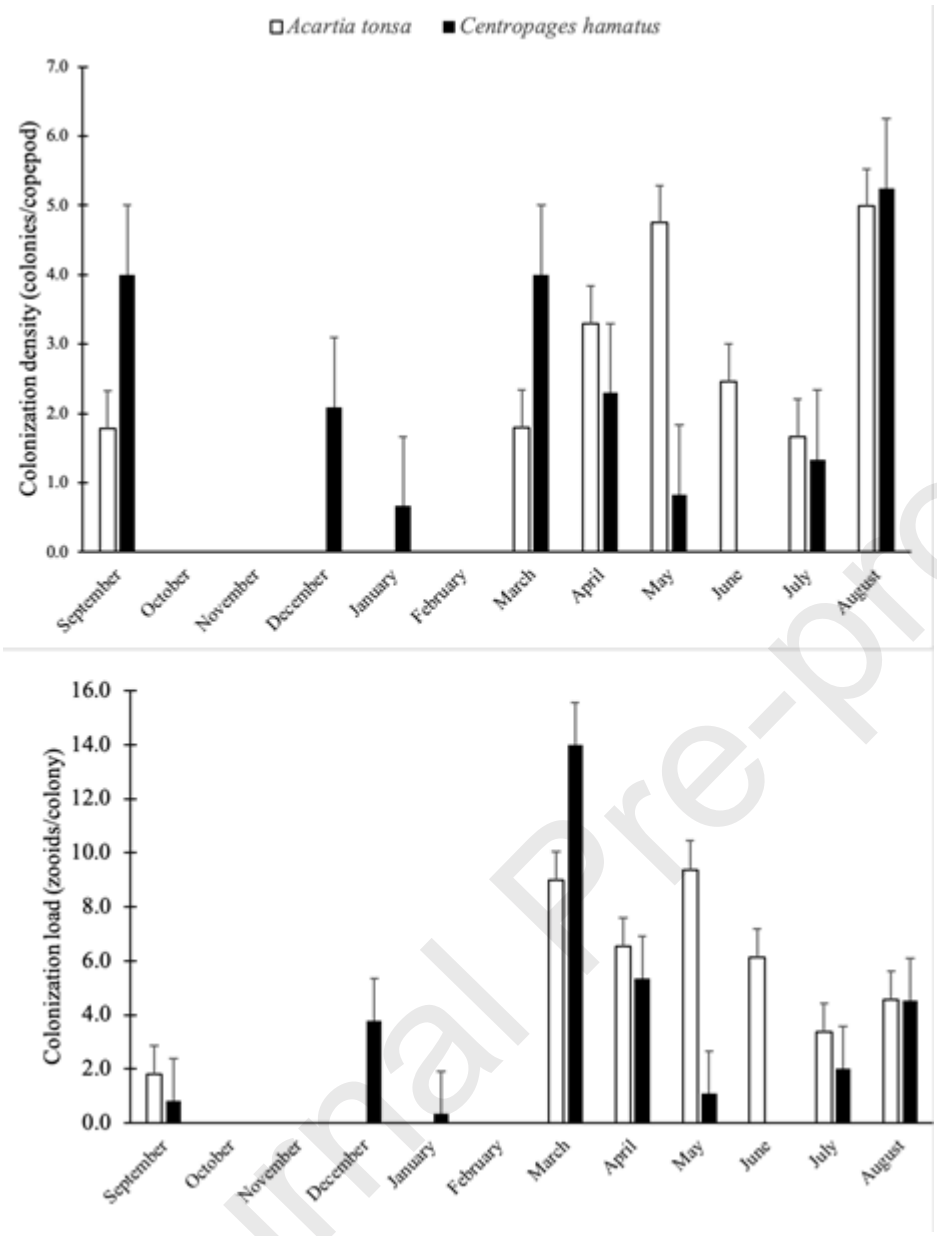
Fig. 8. Mean colonization density and species richness found on submerged glass slides deployed every two weeks in the York River from September 2014 to August 2015. Solid line represents peritrich abundance (colonies cm^{-2}), dashed line represents peritrich species diversity (species cm^{-2}). Grey area represents number of bacterial cells $x10^3$ mL^{-1}.

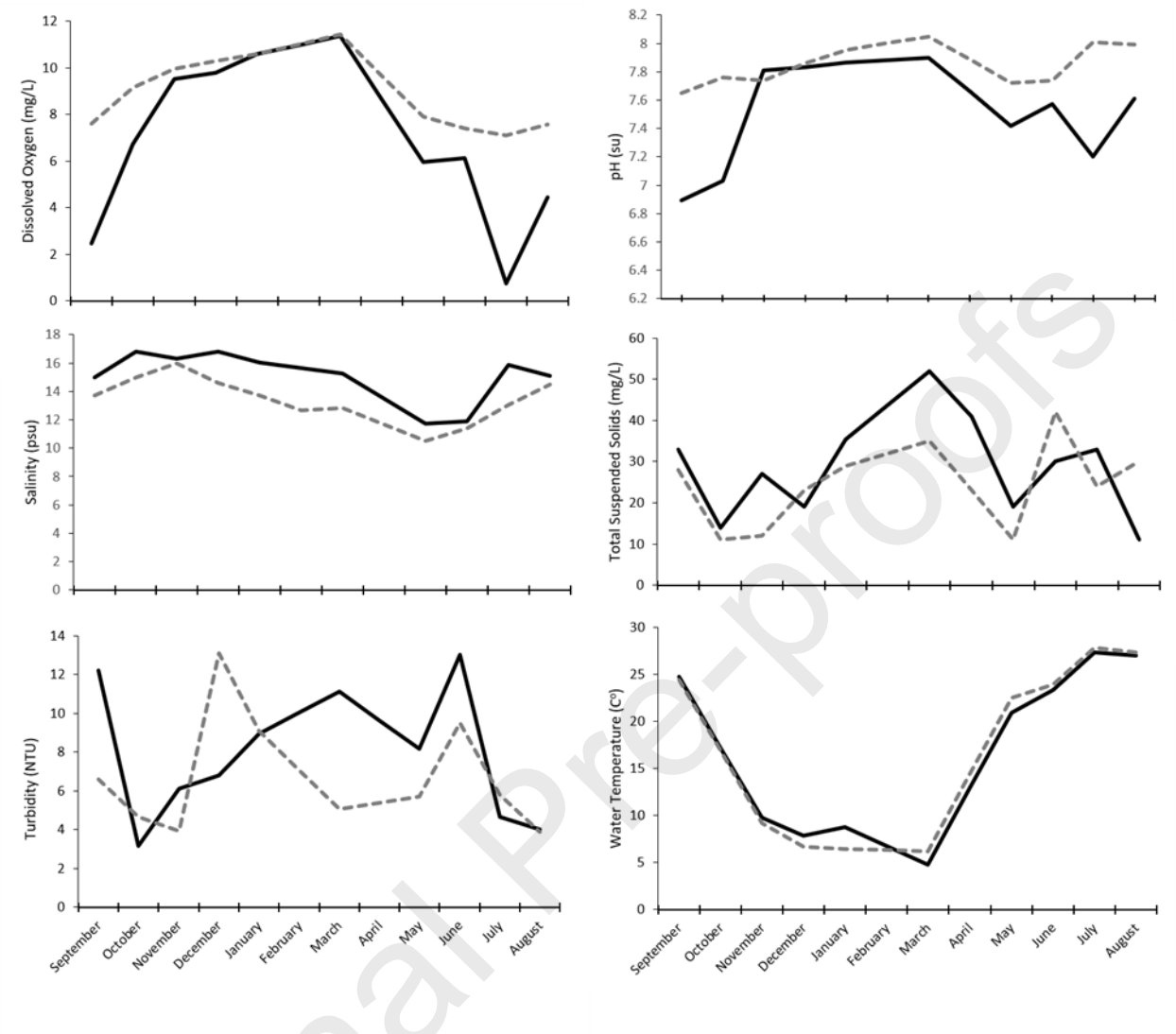
Table 1. Number of copepods retrieved from biweekly collections and percent colonized. *At*/*Ch* is the ratio of *Acartia tonsa* to *Centropages hamatus* individuals found in the samples. Numbers for *A. tonsa* and *C. hamatus* are shown as number of colonized copepods/ total number of copepods of that species, followed by (% copepods colonized). A value of 1 was added for *C.*

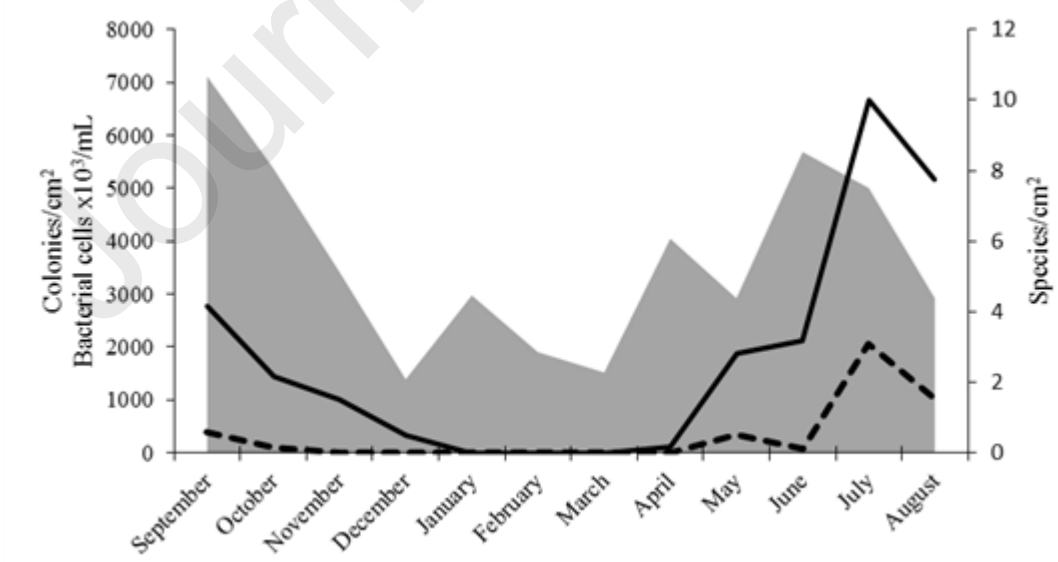
hamatus on 9/12 to avoid division by 0. “Other copepods” refers to all other species present in the sample that were not *A. tonsa* or *C. hamatus*.

Table 2. Correlation coefficients (C) and p-values (*p*) for Spearman’s rho rank correlations between infestation prevalence and water quality parameters in the York River (VA) between September 2014 and July 2015. *p*-values<0.05 in bold. *n* = number of pooled samples). DO = dissolved oxygen, TSS = total suspended solids, Wtemp = Water temperature. ‘b’ corresponds to bottom and ‘s’ to surface.









Collection date	Other copepods	*A. tonsa*	*C. hamatus*	*At/Ch*
12-09-2014	97	42/449 (9.35%)	0/0 (0.0%)	449
26-09-2014	14	0/407 (0.0%)	0/1 (0.0%)	407
10-10-2014	113	0/326 (0.0%)	0/6 (0.0%)	54.3
24-10-2014	167	0/360 (0.0%)	0/17 (0.0%)	21.2
07-11-2014	220	0/543 (0.0%)	0/39 (0.0%)	13.9
21-11-2014	313	0/1284 (0.0%)	0/12 (0.0%)	107
05-12-2014	42	0/1153 (0.0%)	12/45 (26.67%)	25.6
19-12-2014	15	0/613 (0.0%)	2/20 (10.00%)	30.7
02-01-2015	29	0/165 (0.0%)	0/4 (0.0%)	41.3
14-01-2015	11	0/89 (0.0%)	0/1 (0.0%)	89
29-01-2015	194	0/565 (0.0%)	1/18 (5.56%)	31.4
14-02-2015	171	0/608 (0.0%)	0/9 (0.0%)	67.6
27-02-2015	151	0/559 (0.0%)	0/16 (0.0%)	34.9
13-03-2015	331	1/275(0.36%)	0/12 (0.0%)	22.9
26-03-2015	93	4/158 (2.53%)	1/9 (11.11%)	17.6

09-04-2015	98	4/29 (13.79%)	0/18 (0.0%)	1.6
23-04-2015	84	0/48 (0.0%)	5/9 (0.0%)	5.3
09-05-2015	344	19/850 (2.24%)	3/36 (0.0%)	23.6
22-05-2015	20	7/412 (1.70%)	0/4 (0.0%)	103
06-06-2015	26	67/347 (19.65%)	0/5 (0.0%)	68.2
18-06-2015	35	9/265 (3.40%)	0/6 (0.0%)	44.2
02-07-2015	53	2/136 (1.47%)	0/0 (0.0%)	128
17-07-2015	208	0/128 (0.0%)	0/1 (0.0%)	128
31-07-2015	109	4/945 (0.42%)	1/3 (33.33%)	315
14-08-2015	101	5/55 (9.09%)	1/1 (100%)	55
28-08-2015	180	85/783 (10.86%)	2/5 (40.00%)	156.6

Variable	Depth	Infestation Prevalence	
		A. tonsa	*C. hamatus*
DO	b		C=-0.131, *p*=0.532, n=25
	s	C=-0.435, ***p*=0.03**, n=25	
pH	b		C=0.055, *p*=0.794, n=25
	s	C=0.001, *p*=0.995, n=25	
Salinity	b		C=-0.184, *p*=0.379, n=25
	s	C=-0.551, ***p*=0.004**, n=25	
TSS	b		C=-0.108, *p*=0.608, n=25
	s	C=0.393, *p*=0.052, n=25	
Turbidity	b		C=-0.102, *p*=0.627, n=25
	s	C=-0.147, *p*=0.483, n=25	
Wtemp	b		C=0.082, *p*=0.698, n=25

	s	C=0.406, ***p*= 0.044**, n=25	

Author Contribution Statement

Lúcia S.L. Safi (LSLS) was responsible for the conceptualization, sampling, analyses and writing of this project. Kam W. Tang (KWT) was responsible for the primary investigation and writing. Ryan B. Carnegie (RBC) was involved in data analysis and writing, as well as funding acquisition.