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FIRST DESCRIPTION OF EPIZOIC CILIATES (SESSILIDA STEIN, 1933) ON BATHYPOREIA LINDSTRÖM, 1855 (CRUSTACEA: AMPHIPODA) AND INFESTATION PATTERNS IN BRACKISH AND MARINE WATERS

BY

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ABSTRACT

During a reexamination of macrozoobenthic samples from a long-term monitoring program in the Dutch Oosterschelde and Westerschelde, the abundant presence of peritrich ciliates on *Bathyporeia* Lindström, 1855, specimens was discovered. Of the more than 3500 *Bathyporeia* specimens investigated, 44 % contained ciliates. Although *Bathyporeia sarsi* Watkin, 1938 was significantly more often infested than *Bathyporeia pilosa* Lindström, 1855, these differences in infestation rates were largely due to differences between water bodies with higher infestation rates in the polyhaline than in the mesohaline reach. Observation of additionally collected living specimens and freshly preserved material showed that at least two, and likely three, species of ciliates are present of which two might be undescribed so far. One of the observed species matches *Zoothamnium nanum* Kahl, 1933. A second species belongs to the genus *Epistylis* Ehrenberg, 1830, but does not seem to match a so far known species. This also accounts for a possible third species belonging to the genus *Zoothamnium* Bory de St. Vincent, 1826, deviating from *Z. nanum* amongst others in the habitus of the stalk. The front part of *Bathyporeia* spp. and the antennae in particular, significantly more often harbored ciliates than the remainder of the body, where additional ciliates were only found on the ventral site. This shows that the peritrich ciliates benefit from the water currents induced by the basibiont providing food items, but might also indicate that *Bathyporeia* spp. benefits from the presence of the epibionts as they are most prevalent on the easiest to clean

body parts. Analyses of densities and distributions of epibiont - and basibiont species gave first indications of the ecological niche of the peritrich ciliate communities.

INTRODUCTION

Epibiosis is the association between two organisms: the epibiont and the basibiont. Epibionts include those organisms that during the sessile phase of their life cycle are attached to the surface of a living substratum; i.e. the basibiont which lodges the epibiont (Fernandez-Leborans & Von Rintelen, 2010). A diverse group of organisms that can often be found as epibionts is the group of sessile peritrich ciliates (Sessilida Stein, 1933). These unicellular organisms can temporarily or permanently settle on basibionts where they attach to the surface with stalks. Whereas a lot of species belonging to the order of the Sessilida (recently Utz et al. (2010) has proposed a split up of the order of Sessilida into the orders of Vorticellida and Opercularida on genetic evidence) are not substrate specific and can be found on either living and non-living substrates, several species seem to have a preference for living substrates. Moreover a substantial number of these epibiont Sessilida appear to have a clear preference for certain basibiont species or might even be species specific (Fernandez-Leborans & Tato-Porto, 2000). In the last case there may be a functional relationship between the epibiont and basibiont species that at least for the epibiont will have positive effects often going beyond the presence of an attachment site (Utz, 2003).

Crustaceans are frequently found to be basibiont species for a range of organisms (Wahl, 2009) and particularly for peritrich ciliates (Fernandez-Leborans, 2009). It is therefore surprising that so far no protozoan epibionts are recorded for the genus *Bathyporeia* Lindström, 1855, as several *Bathyporeia* species are common and abundantly present at least in Europe and North-western America (Horton et al., 2013). The sole indication in scientific literature that peritrich ciliates indeed use *Bathyporeia* as a substrate is the recording of dõUdekem dõAcoz (2004) that ramified colonies of peritrich ciliates are often observed on the appendages of *Bathyporeia* species, sometimes in large numbers. A closer look to the frequently found *Bathyporeia* specimens in the samples of one of our (Monitor Taskforce of the Royal Netherlands Institute of Sea Research, NIOZ) benthic community sampling programs in the South-western Netherlands learned that epizoic ciliates were abundantly present. It was wondered whether temporal and spatial patterns in infestation rates of *Bathyporeia* specimens could be observed and whether ciliate densities and distributions on the basibiont specimens might clarify the ecology of the epibionts and the epibiotic interaction in particular. To achieve this, samples from several years, from different water bodies with different abiotic conditions, containing different potential basibiont *Bathyporeia* species in different densities, were analyzed. Additional *Bathyporeia* specimens were collected for unpreserved and alive observation of the epibionts, to allow taxonomic identification.

MATERIAL & METHODS

Benthic community samples

As part of the MWTL program (Monitoring of the Water State of the Netherlands) commissioned by the Directorate General óRijkswaterstaatô of the Dutch Ministry of Infrastructure and Environment, the research assistants of the Monitor Taskforce of the NIOZ-Yerseke (NIOO-CEME before 2012) took 975 benthic community samples in the Oosterschelde (Eastern Scheldt) and Westerschelde (Western Scheldt) during the period 2010-2012. The Oosterschelde is a semi-enclosed marine tidal bay and the Westerschelde is the Dutch part of the Scheldt estuary covering the mesohaline and polyhaline zones, both situated in the South-western part of the Netherlands. Each year 130 samples were taken in the Oosterschelde and 195 in the Westerschelde according to a random stratified sampling scheme based on a habitat classification system (Ecotope mapping according to ZES.1; Bouma et al., 2005) between the middle of August and the end of October. Sample intensity was higher in ecotopes with more variable and diverse communities resulting in relatively more samples taken from intertidal ï than from subtidal habitats (for sampling details see: Escaravage et al., 2013). Each sample consists of the residual macrofauna specimens of three cores with a diameter of 10 cm of the top 30 cm sediment taken within an area of 1 m^2 and sieved over a 1 mm mesh. Intertidal samples were taken during low water using a handcorer while subtidal cores were taken from a Reineck boxcore deployed from a vessel. Subtidal samples from particularly sediments rich in shell fragments did not always reach a depth of 30 cm but covered at least the top 20 cm of the sediment. Sampling residues were fixed with 4 % buffered and neutralized formalin and stained with Rose Bengal after which the macrofauna specimens were sorted and identified in the laboratory as much as possible to the species level (or to the closest reliable taxonomic level). Identified organisms were stored in ethanol (70 %). Additionally sediment samples of the top 8 cm of the sediment were taken for grain size analyzes using a Malvern Mastersizer (i.e. laser diffraction methodology). For salinity indication only a distribution in mesohaline (average salinity between 4 and 18) and polyhaline sites (average salinity between 18 and 30) was made for the Westerschelde, with the border between the two zones near the line Hansweert-Perkpolder. The Oosterschelde is marine (salinity around 30) entirely. Samples containing *Bathyporeia* were reexamined for this study to qualify and quantify the possible presence of epizoic ciliates.

Bathyporeia reexamination

All *Bathyporeia* specimens collected during the years 2010-2012 were retrieved from the storage after which they were identified, using a binocular microscope, to the species level according to Lincoln (1979) and dôUdekem dôAcoz (2004). Juvenile specimens who could not be identified to the species level (and were therefore only identified to the genus level) were treated as a separate group. Retrieved incomplete specimens were not taken into consideration. Besides taxonomic identification also the gender was noted. On each *Bathyporeia* specimen the number of individual ciliates (i.e. counting each zooid of colonies as an individual) was estimated. Therefore 4 density classes were distinguished: no ciliates present (category 0), 1 up to 25 ciliates present (category 1), 26 ï 75 ciliates present (category 2), more than 75 ciliates present (category 3). The estimations were done after initial training on a set of random samples (to encounter different ciliate densities on *Bathyporeia* specimens) where besides estimations the exact numbers of ciliates were counted. Estimation of ciliate density classes instead of counting was done to speed up the classification process and was performed using 400x magnification (and 1000x magnification for parts that were not clear at first sight due to stain and/or impurities in samples). Besides that ciliate numbers were estimated, also the positioning of ciliates on parts of the bodies of the *Bathyporeia* specimens was recorded. Therefore 4 regions (as indicated in fig. 1 for a female *Bathyporeia elegans* Watkin, 1938) were distinguished, namely the antennae region (position 1), the mouth region (position 2), the gnathopods, pereopods and pleopods region (position 3) and the uropods region (position 4), for which the presence or absence of ciliates was recorded. On the remainder of the body (i.e. the dorsal side consisting of head, pereon and pleon as indicated in fig. 1 in white) ciliates have not been observed during this study.

Identification of ciliates

Initially we did not succeed in identifying the epizoic ciliates even to the genus level, as the preservation with formalin results in the contraction of the zooids which does not allow having a proper look at for instance the oral infraciliature. Therefore additional specimens of *Bathyporeia* containing ciliates were collected, but this time brought alive to the laboratory, where behavior (i.e. the contractions) and more details on the habitus could be studied using a binocular microscope. Besides those ciliates were contracting, also several specimens had their ciliature crown permanently opened which allowed making pictures of the details. On October the 9th, 2013, specimens of *Bathyporeia sarsi* Watkin, 1938 were collected on an intertidal sand flat called óGalgeplaatô (51°33ô03ò N ï 003°58ô17ò E) in the Oosterschelde. On April the 25th, 2014, and June the 28th, 2016, additionally specimens of *Bathyporeia pilosa* Lindström, 1855 were collected on an intertidal sand flat called óDen Inkelô (51°25ô30ò N ï 004°04ô11ò E) in the Westerschelde. At both sites several *Bathyporeia* specimens were collected; most of them contained ciliates, which were generally several solitary individuals and occasionally colonies sharing a branched stalk. A few specimens of *Bathyporeia* contained a lot of ciliates which were then more often colony-forming with multiple branches. To identify the ciliates the identification keys of Kahl (1935), Patterson (1978) and Curds et al. (1983) were used. Additionally at several stages, experts in the field (i.e. Gregorio Fernandez-Leborans, Alan Warren and John C. Clamp, who we thank for their kind help) were consulted, by sharing photo material and eventually also preserved material.

Statistics

To identify patterns in relative numbers of ciliates related to the basibiont species and sample site characteristics, a Principal Component Analysis (an indirect gradient analysis for datasets with a short gradient length as indicated by a DCA) was performed using Canoco for Windows version 4.5. To test for potential differences in relative ciliate numbers or the presence or absence of ciliates between the different regions, species, genders, basins, salinities and years, (multiple) t-tests were applied using the statistical software NCSS version 8 or Microsoft Excel 2010. Spatial patterns were visualized using Geographic Information Systems (ArcMap 10.2).

RESULTS

Identification of ciliates

Initially it was uncertain if the ciliates observed on *Bathyporeia* would contract, as the studied specimens consisted of preserved material (e.g. fig. 2a, b). This was however very likely due to the pedunculated (from singular specimens to pairs and occasional tree-shaped colonies) oval shaped bodies with the presence of cilia surrounding a mouth and the frequently present spasmoneme in the stalks (fig. 2b). Such organisms typically belong to the order of the Sessilida (or Vorticellida according to the proposed new classification by Utz et al. (2010) on basis of genetic evidence), family Vorticellidae Ehrenberg, 1838 (Kahl, 1935; Patterson, 1978). Initially the preserved specimens were identified as most likely belonging to the genus *Zoothamnium*. As the macrobenthos samples including the peritrich ciliates were preserved in formaldehyde and stained with Rose Bengal, for many specimensô characteristics for identification were not clear. Collecting new material and studying specimens alive revealed the abundant presence of specimens without spasmonemes (not being an artefact of preservation) as well. Occasionally specimens were observed that were contracting, while other individuals in the same colony where not. We did however never observe the presence of discontinuous spasmonemes or the presence of only short spasmonemes. Branches of colonies not contracting with the rest might therefore be the result of local degradation of the spasmoneme. From the (unstained) samples collected in 2014 and 2016 three types of peritrich ciliates could be identified.

There are small colonies having continuous spasmonemes running through stalks of which the cortex has conspicuous transverse folding. The branching of the colonies is irregular (asymmetric) and primary and secondary stalks are relatively short (e.g. fig 2a,b). This matches the description as given by Kahl (1935) for peritrich ciliates commonly present on marine amphipods in the Kieler Bucht at that time, called *Zoothamnium nanum* Kahl, 1933. Precht (1936) confirms the presence of the species on *Gammarus locusta* Linnaeus, 1758, *Microdeutopus gryllotalpa* Costa, 1853, *Jaera marina* Fabricius, 1780 (accepted as *Jaera (Jaera) albifrons* Leach, 1814) and *Gammarus duebeni* Lilljeborg, 1852, in the Kieler Bucht and indicates that *Z. nanum* is probably restricted to Arthrostraca (generally indicating the orders Amphipoda and Isopoda) hosts. Fernandez-Leborans & Gabilondo (2005) record *Z. nanum* also for other species of the genus *Gammarus* Fabricius, 1775. Figure 2d shows *Zoothamnium nanum* as observed during this study in detail.

There are small colonies and individuals without a spasmoneme in the stalk that have a peristome of the epistyliform type. This type of ciliate seems to belong to the genus of *Epistylis* Ehrenberg, 1830, but in contrast to many *Epistylis* species lack longitudinal striations on their relatively long stalks (fig. 2e). The specimens did not exactly match any of the described species and might be a so far undescribed *Epistylis* species. A few species are known to occur on amphipods: e.g. *E. humilis* (as indicated as such by Kahl, 1935), *E. gammari* Precht, 1935 and *E. jaerae* Precht, 1935 (Precht, 1935). *E. cf. ovum* (as indicated as such by Bierhof & Roos, 2007 and Fernandez-Leborans, 2009) is observed on *Gammarus tigrinus* Sexton, 1939 in the Netherlands. *Gammarus tigrinus*, although not present at the localities where *Bathyporeia* were collected for the current study, is known to be present in at least the most Eastern part of the Western Scheldt (Faasse & Van Moorsel, 2003) and in the fresh to slightly brackish waters in the vicinity of the Oosterschelde and the Westerschelde (Wolff, 2005).

Additionally there are specimens that belong to the genus *Zoothamnium* as well, forming small colonies with relatively short secondary stalks like *Z. nanum*. However contrary to *Z. nanum*, the stalk cortex is relatively smooth and unfolded and morphological details of the zooids seem to be different (indicating that the specimens are not just a developmental variant of the first). As the habitus (fig. 2f) did not match any of the descriptions given in literature (i.e. referred in the current article) it might be an undescribed species as well.

Infestation patterns for *Bathyporeia* spp.

On a total of 975 samples over three years, 225 samples contained *Bathyporeia* specimens. In total 3745 *Bathyporeia* specimens were found of which 2950 could be identified as *B. pilosa*, 179 as *B. sarsi*, 7 as *B. elegans*, 5 as *Bathyporeia guilliamsoniana* Bate, 1857 and 6 as *Bathyporeia pelagica* Bate, 1856. Further 598 *Bathyporeia* specimens could not be identified to the species level, generally because they were juveniles. Fifteen percent of the *Bathyporeia* specimens could be identified as a (adult) male.

On 44 % of all collected *Bathyporeia* specimens, ciliates were found. For the individual species the infestation rates equal 43 % for *B. pilosa*, 54 % for *B. sarsi* and 44 % for the specimens that could not be identified to the species level. For the relative rare species in the macrobenthos samples from the Oosterschelde and Westerschelde, the infestation rates equal 0, 14 and 60 % for respectively *B. pelagica*, *B. elegans* and *B. guilliamsoniana*.

When ciliates were present those were more often found on the antennae then on the other identified parts of the body of *Bathyporeia* (fig. 3). At low infestation rates (up to 25 ciliates present) ciliates were significantly more often present on the antennae than in the other regions, except for the region of gnathopods, pereopods and pleopods (which however represents a much larger surface than the antennae). At intermediate infestation rates (26 - 75 ciliates), ciliates were significantly more often present on the antennae than in the other body regions. It was only at high infestation rates (> 75 ciliates) when ciliates were nearly always present on the antennae, that ciliates were also present in almost 80 % of the cases in the mouth ï and the gnathopods, pereopods and pleopods region. The uropods were significantly less popular with ciliates only present in about 30 % of the cases for the heavily infested specimens. The uropods region was the only region for which the likelihood of presence of ciliates did not significantly increase with the infestation rate, except for the dorsal part consisting of head, pereon and pleon on which ciliates were never observed.

Infestation rates related to species and environment

A PCA indicates that average ciliate numbers on *Bathyporeia* specimens are larger in the presence of either (adult) *B. sarsi* or *B. pilosa* than in the presence of *Bathyporeia* sp., which largely consists of juvenile specimens (fig. 4). The relation between ciliates and adult *Bathyporeia* specimens is basically the result of a higher chance on the presence of ciliates on one of the specimens when more *Bathyporeia* specimens are present ($y=0.153 \ln(x) + 0.429$, $R^2 = 0.242$, $p < 0.001$), than a relation between densities. No significant relation was found between *Bathyporeia* numbers and average infestation rates in a univariate regression analysis ($y = 0.086 x + 39.0$, R2 = 0.003, ns). Additionally the multivariate analyses indicate that the average ciliate numbers might be on average higher in polyhaline waters than in mesohaline waters, and/or in the Oosterschelde than in the Westerschelde. Ciliate numbers appear also to be higher in shallow waters; i.e. highest infestation rates are always observed in the intertidal zone whereas occasional low densities are observed on *Bathyporeia* from the subtidal range. Ciliate numbers appear also to be higher at lower longitudes which suggest higher average ciliate numbers closer to the North Sea compared to the more land inwards parts of the systems. However, also the niche of the *Bathyporeia* species might play a role here. *B. sarsi* was present in 5.4 and 4.9 % of the Oosterschelde and polyhaline Westerschelde samples respectively, but only in 1.2 % of the mesohaline Westerschelde samples. *B. pilosa* was typically present in higher densities, e.g. 22.5 ± 4.1, 18.1 ± 2.9 and 3.8 ± 1.7 specimens per samples where present in the polyhaline - and mesohaline Westerschelde and Oosterschelde respectively, compared to on average (± standard error) 2.7 ± 0.6 , 1.4 ± 0.4 and 2.4 ± 0.5 specimens of *B. sarsi*.

T-testing shows that relative average infestation rates are indeed significantly higher for *B. sarsi* than for *B. pilosa* (fig. 5). It is found that the infestation rate for females and juveniles (that can be either male or female) is slightly but significantly higher than for males. A difference in infestation rate between species can potentially be a salinity effect or a difference in infestation rates between systems (waterbodies). Figure 5 shows that when only the polyhaline reaches are compared, infestation rates are significantly higher in the Oosterschelde than in the Westerschelde. This difference appears to be even larger when only the specimens of *B. pilosa* (the most abundant species in both systems) are considered for the two entire systems. Figure 7 gives an overview of the distribution and abundances of *Bathyporeia* and the relative infestation rates. The overview clearly indicates that *Bathyporeia* are restricted to certain areas due to habitat suitability. It is also clear that in general all areas and even almost all samples containing *Bathyporeia* are infested with ciliates to a certain level. Overall infestation rates are not positively related to the *Bathyporeia* densities as indicated before. For instance in several of the Oosterschelde samples, high infestation rates were observed whereas *Bathyporeia* densities were not that high. In the eastern part of the Westerschelde there are several samples with high *Bathyporeia* densities, however with relative low infestation rates and *vice versa*.

Although ciliates were observed from the mesohaline to the polyhaline reaches, as is one of their basibiont species *B. pilosa*, there are differences in infestation rates present between these two salinity reaches. This might be due to differences in fitness of ciliates at different salinities or due to differences in susceptibility of the basibiont species. It was observed that infestation rates of *B. pilosa* were significantly higher in the polyhaline zone than in the mesohaline zone in the Westerschelde. We have however to take into account that infestation rates may differ strongly between years (fig. 6). For separate years, significant differences between the polyhaline and mesohaline zone are only present in 2010 and 2011. The overall difference for the entire period of three years was especially due to a huge difference in 2010 (fig. 6). The lack of differences in infestation rates between the two salinity zones in 2012 are due to a significant increase of the infestation rate in that year in the mesohaline zone.

DISCUSSION

Three species of epizoic ciliates

Although not described in detail before (only mentioned in general terms by dôUdekem dôAcoz (2004) to be common on the appendages of *Bathyporeia* species), the current study shows that peritrich ciliates are common on large parts of the *Bathyporeia* populations present in meso- and polyhaline waters. Although in this study predominantly specimens of *B. pilosa* and *B. sarsi* are investigated, there is no reason to assume this is different for other estuarine *Bathyporeia* species (as indicated by the few specimens of three other *Bathyporeia* species investigated in this study). As *Bathyporeia* populations from very different habitats are studied in two diversified and heterogeneous systems for several years it is likely that in line with dôUdekem dôAcoz (2004) the observations on infestation patterns are representative for estuarine *Bathyporeia* populations in a larger geographic region (e.g. Western Europe). Colleagues from the NIOZ in Texel could confirm the common presence of ciliates on *Bathyporeia* (predominantly *B. sarsi*) from the Wadden Sea as well.

As indicated, the observed ciliates belong to two genera: *Zoothamnium* and *Epistylis*. As infestation and distribution patterns of the ciliates are analyzed on bases of preserved and stained material it was impossible to identify species of ciliates present. The characteristics that could be distinguished, e.g. besides solitary individuals the presence of small colonies with irregular branches with relatively short primary and secondary stalks and the shape of the zooids, suggests that at least *Zoothamnium nanum* was frequently among them. Additional observations of material alive collected from two sites in the Oosterschelde and the Westerschelde in different years indicates that at least three different species of peritrich ciliates might be present on *Bathyporeia*. As these observations, although from different systems and different years, might spatially and temporally be too restricted, it is uncertain to what extent the species identifications reflect the epizoic ciliate populations on *Bathyporeia* as inventoried for the years 2010-2012. The results however show the abundant presence of epizoic ciliates on *Bathyporeia* in Dutch estuarine and marine waters, with the presence of more species and *Zoothamnium nanum* as an important candidate to be the most common species. Besides, two species were observed: *Zoothamnium* sp. and *Epistylis* sp., that do not exactly match descriptions of known species and might be so far undescribed species, possibly restricted or particularly related to *Bathyporeia* of estuarine and marine waters.

Mutual epibiont ï basibiont interactions

There are several theories about impacts and benefits of epibiosis for both epibionts and basibionts (e.g. Wahl, 2009). However, at least for relationships involving peritrich ciliates, several of the potential effects and to what account they are of importance, is unclear. At least from the ciliate point of view it is clear that settlement on *Bathyporeia* specimens, besides the sec provision of substrate to attach to, provides free transport in the horizontal plane and into and out of the top layer of the sediment to and from the water layer (e.g. Nicolaisen & Kanneworff, 1969; De-la-Ossa-Carretero et al., 2012). Ciliates being active filter feeders can profit from favorable hydrodynamic conditions that they can reach via the basibiont (Bickel et al., 2012). Also the movement of the *Bathyporeia* specimens creates water current from which the ciliates can profit. Additionally, *Bathyporeia* species (like other amphipods) actively create water current with their pleopods along the ventral part of the body to provide oxygen rich water to the gills (Wijnhoven et al., 2003). There are no indications that the observed epizoic ciliates are parasites of *Bathyporeia*, active feeding on its host, as no ciliate tissue like fibers or vessels seem to penetrate into the *Bathyporeia* tissue. The distribution of the ciliates solely on the ventral site and on the different podae and antennae shows that on the one hand ciliates profit from the water currents, but on the other hand attach to the more protected and or flexible parts at the ventral site of the body where they are not easily rubbed off when the basibionts move through the sediment matrix. The fact that ciliates are never observed on the dorsal parts, i.e. head, pereon and pleon, indicates that they are not only easily rubbed off there, but that the *Bathyporeia* body surface might be such that it is difficult or impossible for ciliates to attach there. The highest densities of ciliates are centered around the mouth and the front part of the *Bathyporeia* specimens. This may indicate that the ciliates profit from the feeding behavior and the frequent loss of small food particles by the basibiont into the water current passing the ciliates and that they are in need of a certain water current (e.g. Clamp, 1973).

The benefits for the basibiont are less clear, or it should be some kind of camouflage or benefits from reduced buoyancy (e.g. Wahl, 2009). But there might be benefits as it is likely that at least during the initial ciliate attachment phase, *Bathyporeia* specimens have the opportunity to get rid of ciliates at least from several extremities as they want, as specimens frequently clean themselves with their gnathopods. This is at least efficient for al kind of particles that stick in between and to the hairs (Coleman, 1989). No indications were found that easier to clean body parts harbored less ciliates. On the contrary, antennae might be most easy to clean (Coleman, 1989), but in general were infested the most. It is also unclear what happens during molting: Does this release *Bathyporeia* from its epibionts, or can ciliates change substratum (i.e. from the old to the new exoskeleton) as observed for other species (Utz & Coats, 2005; Bickel et al., 2012)? Epibiont species might even chemically detect the onset of molt and evacuate and/or form swarmers in time as indicated by amongst others Fenchel (1965) and Clamp (1973). Other possible benefits of the presence of epibionts for basibionts like *Bathyporeia*, are mentioned by Wahl (2009): Like the production of nutrients or vitamins by the ciliates, available for the basibiont. Definitely more research is needed to get more insight in the epibiont ï basibiont interactions for *Intranstylum* ï *Bathyporeia* couples, and ciliate ï Amphipoda interactions in terms of benefits in general.

The ecological niche of epizoic ciliates on Bathyporeia

Significantly more epizoic ciliates were found on average on *B. sarsi* than on *B. pilosa*, but there was also a higher infestation rate for *B. pilosa* from the Oosterschelde than from the Westerschelde. Infestation rates of *B. pilosa* from the Westerschelde suggest a slight preference of the observed ciliates (if predominantly one species) for polyhaline instead of mesohaline conditions and possibly a preference for stable salinity conditions (e.g. like in the Oosterschelde) than fluctuating salinity conditions like in the Westerschelde (Cozzoli et al., 2013). It is however uncertain if infestation rates indicate a certain preference of for instance *Zoothamnium nanum* (as the most likely candidate representing a large share of the observed ciliates on *Bathyporeia*) or differences in susceptibility of *Bathyporeia* under different conditions. In the latter case it is also of importance if the presence of epibionts is beneficial for *Bathyporeia* and whether the crustaceans to some extent can regulate infestation rates actively.

Other aspects that might be of importance in determining average infestation rates are nutrient availability (Chiavelli et al., 1993) and the amount and type of suspended matter in the water column that might interfere with feeding, although there are strong indications that for most ciliates this is of little importance (Boenigk & Novarino, 2004). Related to food availability, the exact timing of sampling and year to year differences in timing of food availability peaks, as these will be highly seasonal, are of importance. As timing of sampling during each of the three years and in the different parts of the two sampled systems did at most differ a few days (always less than one week) year to year differences in environmental conditions and foodweb developments may be of importance. From long-term total phytoplankton measurements in the Western Scheldt, we know that the onset of phytoplankton peak densities could be observed in the second week of May in 2010, 2011 and 2012 in the middle of the system (Waterbase, 2016). High concentrations however lasted till about the 3rd week of July in 2010, till the end of July in 2012 and till halfway August followed by a second small peak in September in 2011. This might indicate higher and/or longer high food availability in 2011, although phytoplankton species composition, but also other microorganism concentrations and spatial differences will be of importance. Infestation rates as observed by us were not specifically deviating between 2011 and the other two years. To get a better understanding of the infestation rate ï food availability relation, a monitoring and/or experiments focused on these aspects is necessary. Additional certain sediment characteristics, although the sediment should be suitable for *Bathyporeia* in the first place, might implicate a certain friction that may appear due to scouring, to be unfavorable for the epibionts (Wahl, 1989). Also contaminant levels might influence the susceptibility of *Bathyporeia* specimens, but simultaneously might impact the fitness and/or survival of the epibionts too (e.g. Mohr, 1952; Utz, 2003).

At this moment it is still unclear whether the epizoic ciliates observed and *Zoothamnium nanum* in particular can also survive a certain period without being attached to *Bathyporeia*, as observed for other ciliate species (Bickel et al., 2012), or even has a solitary life stage either in the water column or attached to sediment. If a phase of stalk-less or solitary ciliates without a host exists for the observed epizoic ciliate species, these most likely are present in or on sediments where *Bathyporeia* populations thrive. In that case, hydrodynamic conditions and natural sediment disturbance might be of importance in determining the local success of *the Bathyporeia*-related ciliate species. It has to be noticed however that various environmental conditions like differences in hydrodynamics and sediment conditions are available for the ciliate populations (also in the water bodies studied), but that the suitability of an area is also limited by the tolerance range of *Bathyporeia*. It seems that at least the optimum for *B. pilosa* and *B. sarsi* and therefore potentially also for the related ciliate species lies in an environment with intermediate dynamics where the sediment consists of fine - to muddy fine sand. This all remains to a certain extent speculative at this stage, as many factors coincide, e.g. turbidity, nutrient loads and contaminant loads are likely higher in the Westerschelde than in the Oosterschelde. However also the specifics, and not solely the quantity, of nutrients and pollutants might be of importance. Highest infestation rates being observed in the intertidal zone might indicate that *the Bathyporeia*-related epizoic ciliates are to a certain extent susceptible to low oxygen contents that might occasionally occur in the typical subtidal *Bathyporeia* habitats. Also *Bathyporeia* is likely susceptible to low oxygen content, but specimens can cope with, react and adjust to small scale gradients (Sandberg, 1994), whereas epizoic ciliates, if not actively disengaging, are completely dependent of the behavior of their host.

The current study clearly shows the common presence of epizoic ciliates on *Bathyporeia* in estuarine and marine environments. It seems that several species are present on *Bathyporeia* of which *Zoothamnium nanum* might be the most common species in the Dutch estuarine and marine waters. Besides, two other possibly undescribed species: *Zoothamnium* sp. and *Epistylis* sp. were observed. The current study gives first indications of the ecology of the *Bathyporeia*-related epizoic ciliate species and their interactions with their basibiont species. Future work could include identification of the epibionts using molecular techniques (e.g. sequencing of the small subunit of the rRNA as suggested by John C. Clamp; personal communication). A next step would be to clarify spatial and temporal epizoic species assemblages on *Bathyporeia* based on inventories of alive (unpreserved) material. To make an additional step in understanding the life cycle and population developments of the *Bathyporeia*-related epizoic ciliate species and relations with environmental characteristics, additional field observations and likely experimental research is essential.

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REFERENCES

BICKEL, S.L., K.W. TANG & H.-P. GROSSART, 2012. Ciliate epibionts associated with crustacean zooplankton in German lakes: distribution, motility, and bacterivory. Front. Microbiol., **3(243)**: 1-11.

BIERHOF, M.J. & P.J. ROOS, 1977. Sedentary ciliates from two Dutch freshwater *Gammarus* species. Bijdr. Dierk., **46**: 151-170.

BOENIGK, J. & G. NOVARINO, 2004. Effect of suspended clay on the feeding and growth of bacterivorous flagellates and ciliates. Aquat. Microb. Ecol., **34**: 181-192.

BOUMA, H., D.J. DE JONG, F. TWISK & K. WOLFSTEIN, 2005. Zoute wateren EcotopenStelsel (ZES.1); voor het in kaart brengen van het potentiële voorkomen van levensgemeenschappen in zoute en brakke rijkswateren: 1-156. Report RIKZ 2005.024 (LnO, Middelburg) (in Dutch).

CHIAVELLI, D., E.L. MILLS & S.T. THRELKELD, 1993. Host preference, seasonality, and community interactions of zooplankton epibionts. Limnol. Oceanogr., **38**: 574-583.

CLAMP, J.C., 1973. Observations on the host-symbiont relationship of *Lagenophrys lunatus* Imamura. J. Protozool. **20**: 558-561.

COLEMAN, Ch.O., 1989. Burrowing, grooming, and feeding behavior of *Paraceradocus,* an Antarctic Amphipod genus (Crustacea). Polar Biol., **10**: 43-48.

COZZOLI, F., T.J. BOUMA, T. YSEBAERT & P.M.J. HERMAN, 2013. Application of non-linear quantile regression to macrozoobenthic species distribution modelling: comparing two contrasting basins. Mar. Ecol. Prog. Ser., **475**: 119-133.

CURDS, C.R., M.A. GATES & D.McL. ROBERTS, 1983. British and other freshwater ciliated Protozoa; Part II; Ciliophora: Oligohymenophora and Polyhymenophora; Keys and notes for the identification of the free-living genera. In: D.M. Kermack & R.S.K. Barnes (eds.), Synopses of the British Fauna, No. 23. (Cambridge University Press, Cambridge).

DE-LA-OSSA-CARRETERO, J.A., Y. DEL-PILAR-RUSP, F. GIMÉNEZ-CASALDUERO, J.L. SÁNCHEZ-LIZASO & J.-C. DAUVIN, 2012. Sensitivity of amphipods to sewage pollution. Est. Coast. Shelf Sci., **96**: 129-138.

DõUDEKEM DôACOZ, C., 2004. The genus *Bathyporeia* Lindström, 1855, in western Europe (Crustacea: Amphipoda: Pontoporeiidae). Zool. Verh. Leiden, **348**: 3-162.

ESCARAVAGE, V., H. HUMMEL, D. BLOK, A. DEKKER, A. ENGELBERTS, O. VAN HOESEL, L. KLEINE SCHAARS, R. MARKUSSE, T. MELIEFSTE, W. SISTERMANS & S. WIJNHOVEN, 2013. Macrozoöbenthosonderzoek MWTL in de Delta 2012; Waterlichamen: Oosterschelde en Westerschelde (najaar). Rapportage in het kader van Monitoring Waterstaatkundige Toestand des Lands (MWTL): 1-20 + Annexes. Monitor Taskforce Publication Series 2013ï 23; RWS report BM 13.14 (NIOZ, Yerseke) (in Dutch).

FAASSE, M., G. VAN MOORSEL, 2003. The North-American amphipods, *Melita nitida* Smith, 1873 and *Incisocalliope aestuarius* (Watling and Maurer, 1973) (Crustacea: Amphipoda: Gammaridea), introduced to the Western Scheldt estuary (The Netherlands). Aquat. Ecol., **37**: 13-22.

FENCHEL, T., 1965. On the ciliate fauna associated with the marine species of the amphipod genus *Gammarus* J. G. fabricius, Ophelia, **2**: 281-303.

FERNANDEZ-LEBORANS, G., 2009. A review of recently described epibiosis of ciliate Protozoa on Crustacea. Crustaceana, **82**: 167-189.

FERNANDEZ-LEBORANS, G. & R. GABILONDO, 2005. Hydrozoan and protozoan epibionts on two decapod species, *Liocarcinus depurator* (Linnaeus, 1758) and *Pilumnus hirtellus* (Linnaeus, 1761), from Scotland. Zool. Anzeiger **244**: 59-72.

FERNANDEZ-LEBORANS, G. & M.L. TATO-PORTO, 2000. A review of the species of protozoan epibionts on crustacean. I. Peritrich ciliates. Crustaceana, **73**: 643-683.

FERNANDEZ-LEBORANS, G. & K. VON RINTELEN, 2010. Biodiversity and distribution of epibiontic communities on *Caridina ensifera* (Crustacea, Decapoda, Atyidae) from Lake Poso: comparison with another ancient lake system of Sulawesi (Indonesia). Acta Zool., **91**: 163-175.

HORTON, T., J. LOWRY, M. COSTELLO & D. BELLAN-SANTINI, 2013. *Bathyporeia* Lindström, 1855. In: T. Horton, J. Lowry & C. de Broyer (eds.), World Amphipoda database. (Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=101742 on 31-12-2013).

KAHL, A., 1935. Urtiere oder Protozoa; I: Wimpertiere oder Ciliata (Infusoria); Eine Bearbeitung der freilebenden und ectocommensalen Infusorien der Erde, unter Ausschluß der marinen Tintinnidae. Teil 30 in Dahl, Dahl & Bischoff (eds.) Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise. Verlag von Gustav Fischer, Jena, p. 1- 886 (In German).

LINCOLN, R.J., 1979. British marine Amphipoda: Gammaridea: 1-658. (British Museum (Natural History), London).

MIRONOVA, E.I., I.V. TELESH & S.O. SKARLATO, 2014. Ciliates in plankton of the Baltic Sea. Protistology, **8**: 81-124.

MOHR, J.L., 1952. Protozoa as indicators of pollution. Sci. Monthly, **74**: 7-9.

NICOLAISEN, W. & E. KANNEWORFF, 1969. On the burrowing and feeding habits of the amphipods *Bathyporeia pilosa* Lindström and *Bathyporeia sarsi* Watkin. Ophelia, **6**: 231-250.

PATTERSON, D.J., 1978. Kahlôs keys to the ciliates. A translation by D.J. Patterson of the keys to the level of subgenus, originally published in Kahl, A., Wimpertiere oder Ciliata in Dahlôs Die Tierwelt Deutschlands Parts 18 (1930), 21 (1931), 25 (1932 and 30 (1935). University of Bristol, UK, p. 1-89.

PRECHT, H., 1936. Epizoen der Kieler Bucht. Nova Acta Leopoldina, **3**: 405-475 (In German).

SANDBERG, E., 1994. Does short-term oxygen depletion affect predator-prey relationships in zoobenthos? Experiments with the isopod *Saduria entomon*. Mar. Ecol. Prog. Ser., **103**: 73-80.

UTZ, L.R.P., 2003. Identification, life history, and ecology of peritrich ciliates as epibionts on calanoid copepods in the Chesapeake Bay. PhD dissertation, University of Maryland, USA, p. 1-187.

UTZ, L.R.P. & D.W. COATS, 2005. Spatial and temporal patterns in the occurrence of peritriche ciliates as epibionts on calanoid copepods in the Chesapeake Bay, USA. J. Eukaryot. Microbiol., **52**: 236-244.

UTZ, L.R.P., T.L.L. SIMAÕ, L.S.L. SAFI & E. EIZIRIK, 2010. Expanded phylogenetic representation of genera *Opercularia* and *Epistylis* sheds light on the evolution and higher-level taxonomy of peritrich ciliates (Ciliophora: Peritrichia). J. Eukaryot. Microbiol., **57**: 415ï420.

WAHL, M., 1989. Marine epibiosis. I. Fouling and antifouling: some basic aspects. Mar. Ecol. Prog. Ser., **58**: 175-189.

WAHL, M., 2009. Epibiosis: ecology, effects and defenses. In: M. Wahl (ed.), Marine hard bottom communities: patterns, dynamics, diversity, and change: 61-72. (Springer, Heidelberg).

WATERBASE, 2016. Rijkswaterstaat Waterbase ï Historic water quantity and water quality data made available by Rijkswaterstaat, Dutch Ministry of Infrastructure and the Environment. Available from: http://live.waterbase.nl/waterbase_wns.cfm?taal=nl (in Dutch) Accessed on June 6th, 2016.

WIJNHOVEN, S., M.C. VAN RIEL, G. VAN DER VELDE, 2003. Exotic and indigenous freshwater gammarid species: Physiological tolerance to water temperature in relation to ionic content of the water. Aquat. Ecol., **37**: 151-158.

WOLFF, W.J., 2005. Non-indigenous marine and estuarine species in The Netherlands. Zool. Med. Leiden, **9**: 1-116.

Figure captions:

Fig. 1. Schematic drawing of a female *Bathyporeia elegans* after dôUdekem dôAcoz (2004) with indication of the distinguished regions on the body where the presence or absence of ciliates was recorded. No ciliates have been observed in the region indicated in white.

Fig. 2. Photographs of peritrich ciliates on *Bathyporeia* sp.; a) Typical colonies of peritrich ciliates (most likely *Zoothamnium nanum*) attached to a peduncle of an antenna from *Bathyporeia pilosa* (specimen stained with Rose Bengal and preserved in formaldehyde) (1000x magnification); b) Typical colonies of peritrich ciliates (most likely *Zoothamnium nanum*) in detail (on a specimen stained with Rose Bengal and preserved in formaldehyde) (4000x magnification); c) Individual and small colonies of alive *Zoothamnium* sp. on peduncles from antenna 2 of an alive specimen of *Bathyporeia sarsi* (1000x magnification); d) A singular alive specimen of *Zoothamnium nanum* on *Bathyporeia sarsi* (4000x magnification) with its cilia out; e) Singular alive peritrich ciliates on *Bathyporeia pilosa* (4000x magnification) showing a specimen without a spasmoneme (*Epistylis* sp.); f) Singular and couples of peritrich ciliates on *Bathyporeia pilosa* (4000x magnification) where the lower specimen belonging to the genus *Zoothamnium* lacks transverse folds (*Zoothamnium* sp.).

Fig. 3. Presence (0 = óalways absentô; 1 = óalways presentô) of ciliates in the in fig. 1 indicated regions of the body of *Bathyporeia* specimens (Region 1 = antennae region; region 2 = mouth region; region 3 = gnathopods, pereopods and pleopods region; region 4 = uropods region) for 3 ciliate density classes (Category 1 equals 1-25, category 2 equals 26-75 and category 3 equals >75 ciliates per *Bathyporeia* specimen). Significant differences (p<0.05) are indicated with different characters.

Fig. 4. Principal Component Analysis (PCA) of *Bathyporeia* species densities and average ciliate numbers per specimen related to óenvironmentalô characteristics (i.e. substrate type (fine sand; muddy fine sand; other soft sediment substrate), salinity range (mesohaline; polyhaline), water body (Oosterschelde; Westerschelde) and location (depth; longitude as relative distance to the North Sea), sample year, total *Bathyporeia* density)).

Fig. 5. Relative number of ciliates per *Bathyporeia* specimen (all species included unless otherwise indicated), calculated as the average density class (deviating from 0 = no ciliates to 3 = more than 75 ciliates) ± standard error per sample, comparing: a) the species *B. pilosa* and *B. sarsi*; b) the gender groups males and the total of females and juveniles; c) the Oosterschelde and the polyhaline part of the Westerschelde; d) the entire waterbodies Oosterschelde and Westerschelde. All graphs show differences that are significant (p<0.05).

Fig. 6. Relative number of ciliates per *Bathyporeia pilosa* specimen, calculated as the average density class (deviating from 0 = no ciliates to 3 = more than 75 ciliates) ± standard error per sample, comparing the different years for the mesohaline and the polyhaline zone of the Westerschelde. Significant differences (p<0.05) are indicated with different characters.

Fig. 7. Positioning of MWTL samples with *Bathyporeia* present taken during 2010-2012 in the Oosterschelde and Westerschelde in the South-western part of the Netherlands with an

indication of their total numbers and the relative ciliate infestation rates. The color gradient in each of the two systems indicates the relative water depth with the deepest parts in darkest. Specimens for alive observation were collected at óDen Inkelô and óGalgeplaatô. The small map shows all MWTL samples taken during the same years.

Fig. 1.

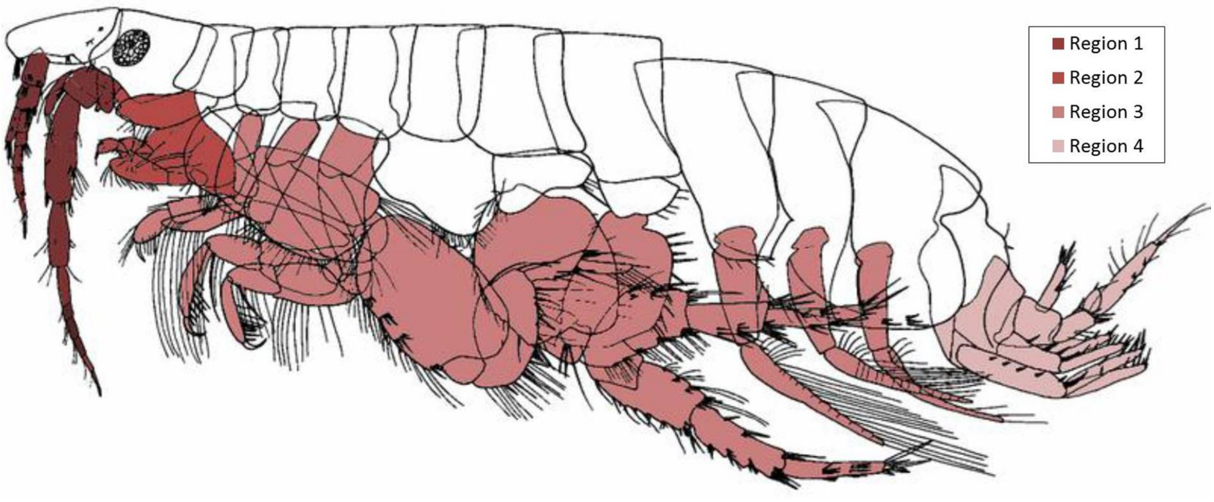


Fig. 2.

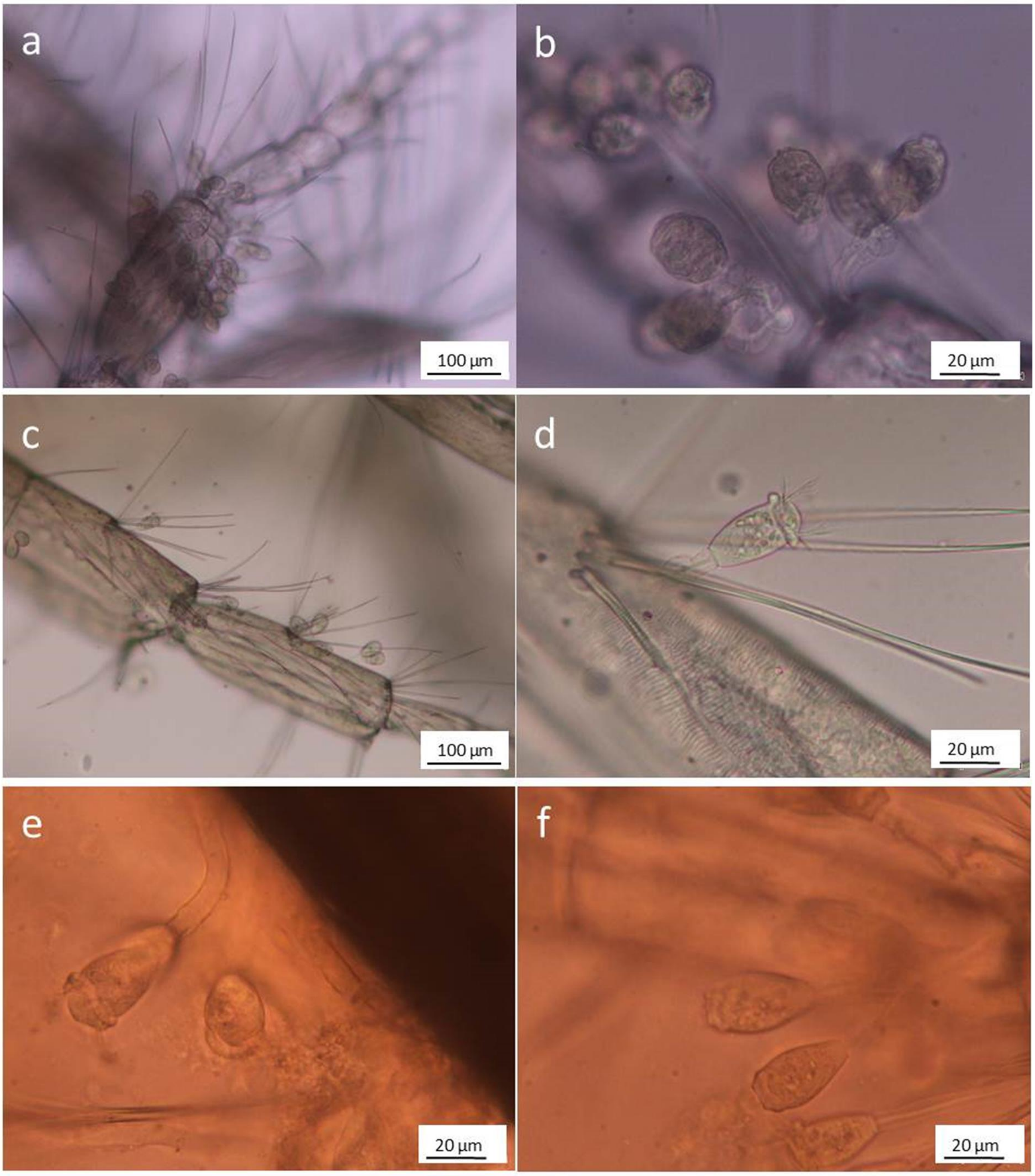


Fig. 3.

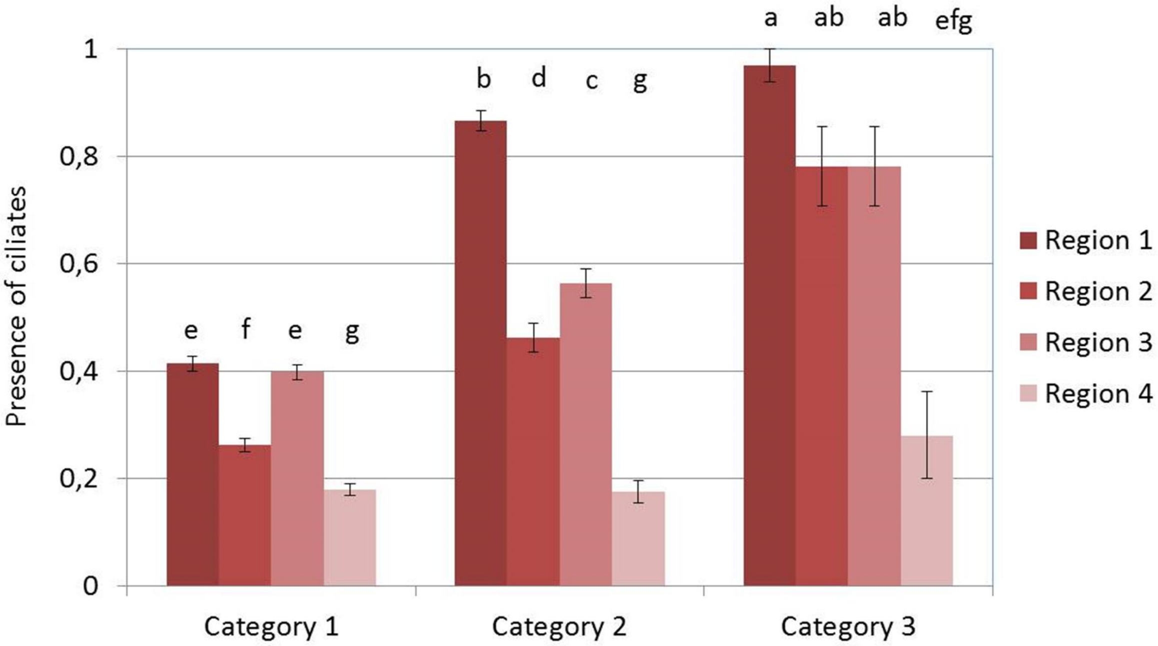
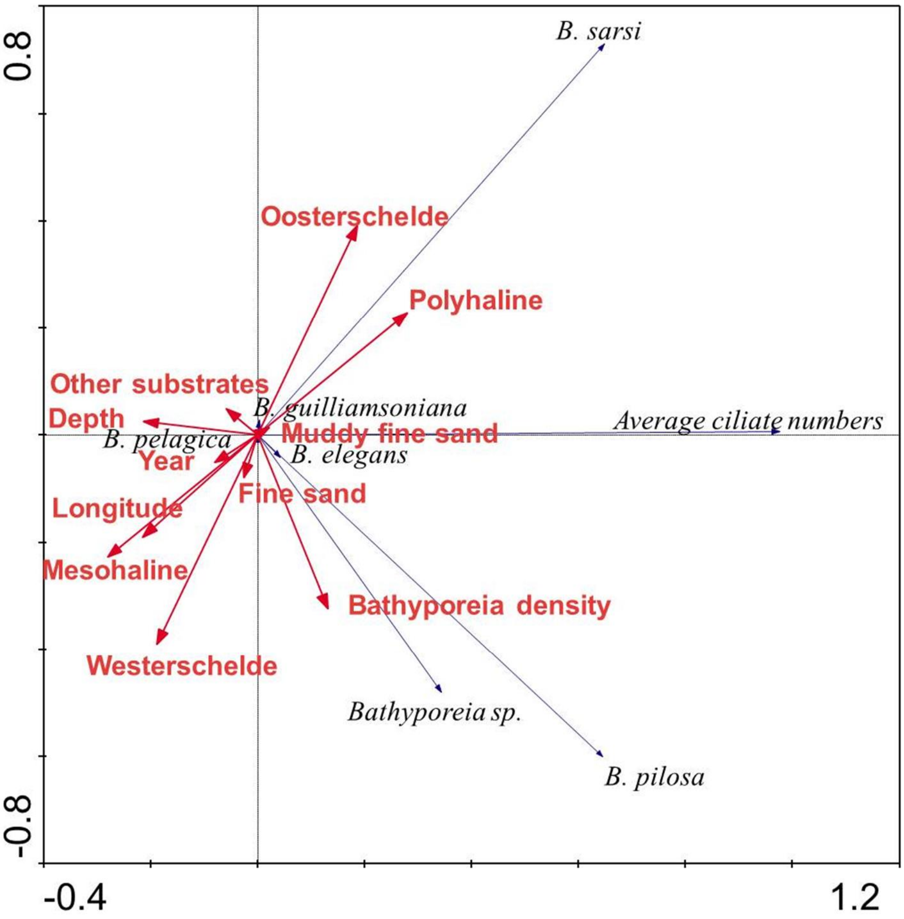

a
ab
ab
efg
b
d
c
g
e
f
e
g
1
0,8
0,6
0,4
0,2
0
Presence of ciliates
Category 1
Category 2
Category 3
Region 1
Region 2
Region 3
Region 4

Fig. 4.



B. sarsi
Oosterschelde
Polyhaline
Other substrates
B. guilliamsoniana
Depth
Average ciliate numbers
B. pelagica
Muddy fine sand
Year
B. elegans
Fine sand
Longitude
Mesohaline
Bathyporeia density
Westerschelde
Bathyporeia sp.
B. pilosa
0.8
-0.8
-0.4
1.2

Fig. 5.

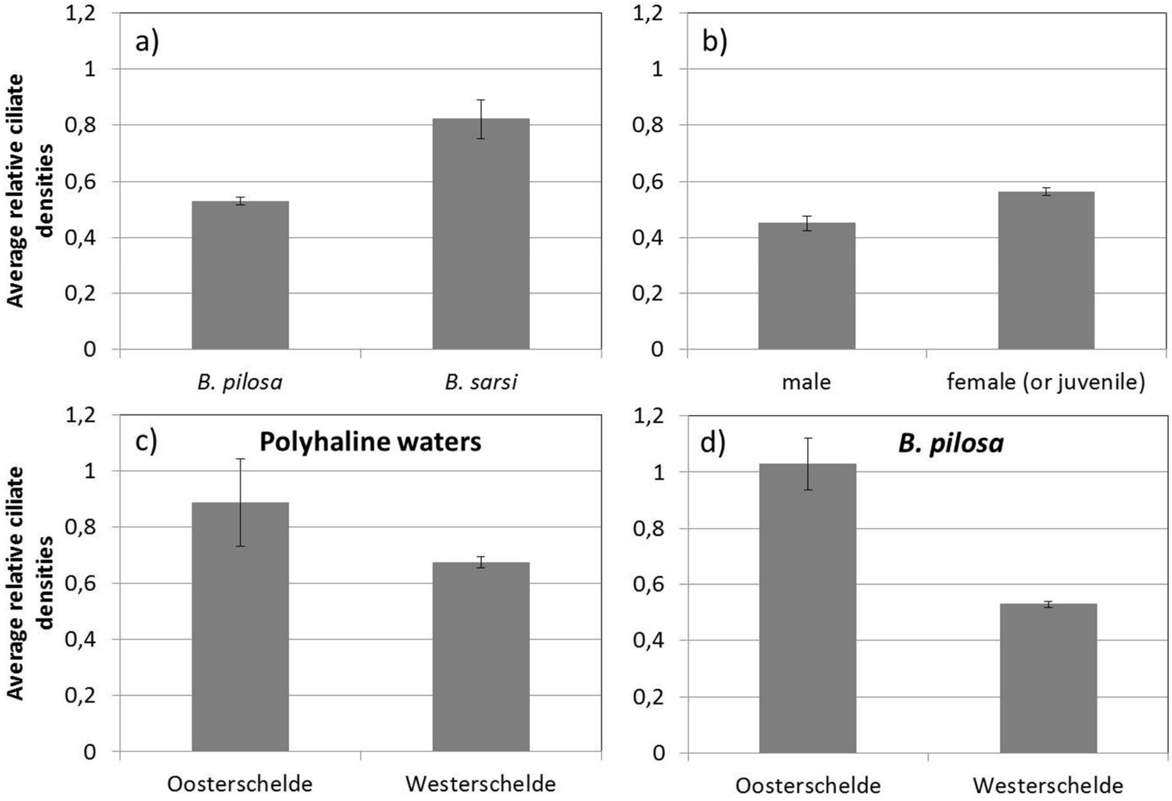

Average relative ciliate densities
1,2
1
0,8
0,6
0,4
0,2
0
a)
b)
B. pilosa
B. sarsi
male
female (or juvenile)
c)
Polyhaline waters
d)
B. pilosa
Oosterschelde
Westerschelde
Oosterschelde
Westerschelde

Fig. 6.

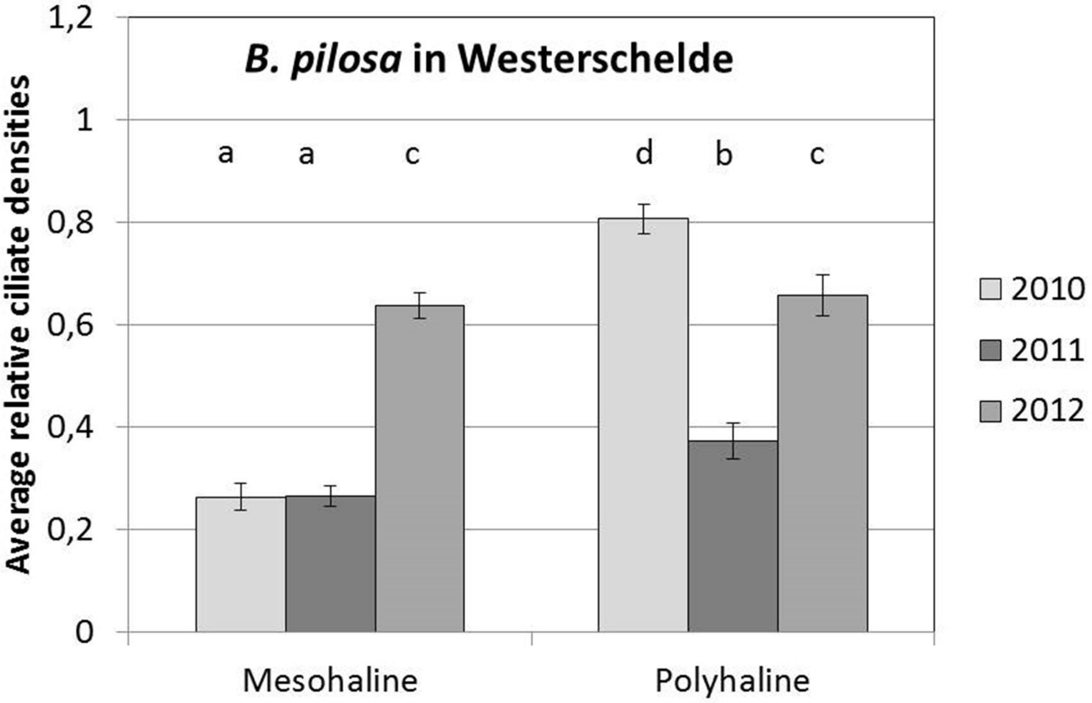
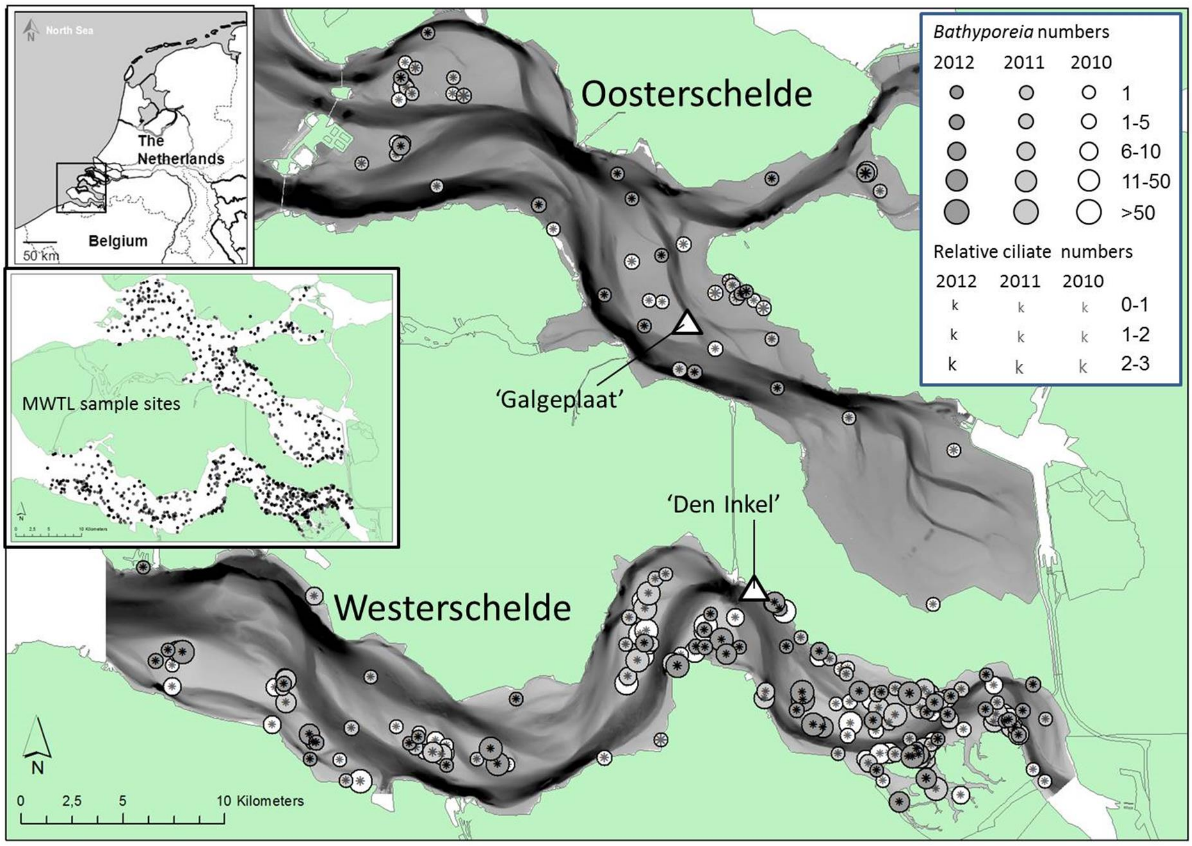

B. pilosa in Westerschelde
Average relative ciliate densities
1,2
1
0,8
0,6
0,4
0,2
0
a
a
c
d
b
c
Mesohaline
Polyhaline
2010
2011
2012

Fig. 7.


North Sea
The Netherlands
Belgium
50 km
MWTL sample sites
Oosterschelde
'Galgeplaat'
'Den Inkel'
Westerschelde
N
0 2,5 5 10 Kilometers
Bathyporeia numbers
2012 2011 2010
1
1-5
6-10
11-50
>50
Relative ciliate numbers
2012 2011 2010
0-1
1-2
2-3