

#### 4. Diversity-productivity relationships in estuarine microphytobenthos

Vyverman, W.<sup>1</sup>, Stal, L.J.<sup>2</sup>, Muyzer, G.<sup>3</sup>, Sabbe, K.<sup>1</sup>, Vanellander, B.<sup>1</sup>,  
Creach, V.<sup>2,4</sup>, Forster, R.M.<sup>2,4</sup>, Kromkamp, J.C.<sup>2</sup>

<sup>1</sup> Laboratory of Protistology & Aquatic Ecology, Ghent University, Belgium Krijgslaan 281 S8, 9000 Gent, Belgium, tel.: 32 9 264 85 88, email: Wim.Vyverman@ugent.be

<sup>2</sup> Centre for Estuarine and Marine Ecology, NIOO-KNAW, Yerseke, The Netherlands

<sup>3</sup> Department of Biotechnology, Delft University of Technology, Delft, The Netherlands

<sup>4</sup> Current address: CEFAS, Lowestoft Laboratory, Pakefield Road, Suffolk, UK, NR33 0HT

#### Abstract

Field studies at three intertidal mudflat stations within the Scheldt Estuary indicated an inverse relationship between microphytobenthos biomass and species diversity, while the relationship between diversity measures and primary productivity appeared to be site specific, with either a significant positive or a unimodal relationship between both parameters. Species counts and molecular fingerprinting using DGGE were largely congruent and revealed that taxonomic turnover among eukaryotic microbenthos is largely determined by salinity, water content, temperature, irradiance, and tidal elevation. Experimental studies revealed significant cryptic diversity among diatoms and demonstrated that both facilitative and antagonistic interactions play an important role in structuring microphytobenthos communities. Species interactions need to be taken into account when studying critical ecosystem processes such as productivity, stability, sediment stabilization and nutrient cycling in marine and estuarine sediments.

#### i. Problem

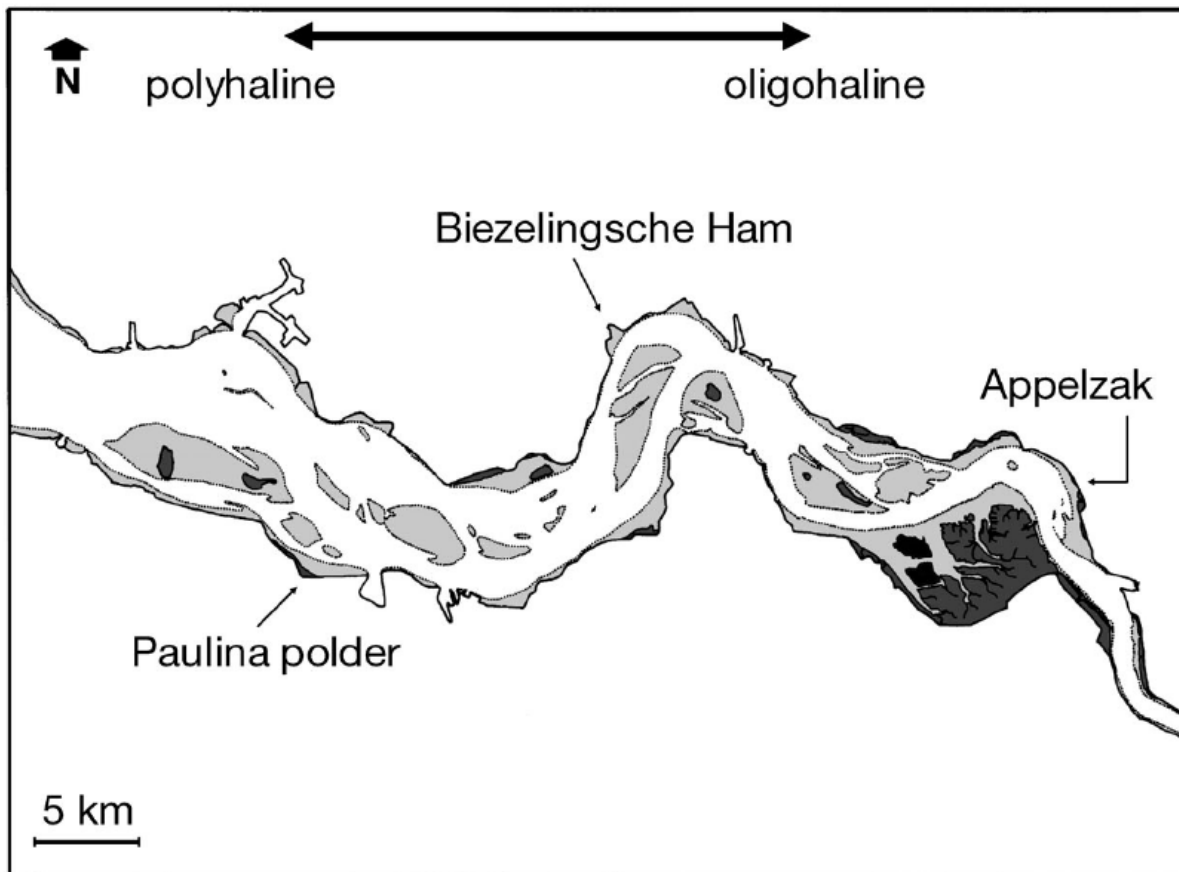
There has been a progressive loss of intertidal mudflats from the main estuaries of the North Sea due to industrial development, dredging activities and land reclamation. This trend may have important consequences for estuarine ecosystem functioning. Indeed, intertidal sediments are characterized by intense carbon and nutrient fluxes due to the primary production (PP) by microalgae, and respiration and remineralization by metazoan grazers and heterotrophic microorganisms. These mudflats often support the growth of dense biofilms of benthic diatoms, which may provide as much as 50 % of the total primary production of estuaries (Underwood & Kromkamp, 1999; Middelburg et al., 2000). While these benthic diatoms migrate into the sediment at high tide, they move to the surface when the sediment is emersed at low tide. Therefore, and in contrast to the turbid water column of estuaries where primary production is strongly limited by the reduced availability of light, high rates of photosynthesis are usually measured in intertidal sediments during daytime emersion. As a result, diatom growth may be unbalanced as nutrients can become limiting in the biofilm, leading to the exudation of copious amounts of extracellular polymeric substances (EPS) that form the biofilm matrix in which the

diatoms are embedded (Underwood & Paterson, 2003). In particular, acidic EPS have been suggested to serve as glue, binding the sediment particles (Decho, 2000). In this way, the diatom biofilm may render stability to the sediment, increasing its erosion threshold (de Brouwer et al., 2000). Furthermore, during inundation part of the EPS may be lost ('dissolved') into the water column or biofilms may slough off or be consumed by chemotrophic microorganisms and the diatoms themselves. Clearly, EPS and the diatoms represent an important source of carbon and energy in intertidal foodwebs.

Although these benthic communities of diatoms are very important, not much is known about their diversity and, in turn, how this diversity affects their function. From work on different mainly terrestrial ecosystems it has become increasingly clear that the biodiversity of an ecosystem and its functional features are intricately linked (Loreau, 2010). While much earlier research tried to understand how environmental constraints regulate diversity, the focus has shifted during the last decade to better understand how biodiversity affects ecosystem functioning. Understanding how altered diversity impacts the basic functions of ecosystems is a high priority on the international agenda, as loss in function will inevitably lead to serious consequences for the goods and services provided by these ecosystems.

## **ii. Aim**

The project 'Diversity-productivity relationships in microphytobenthos' (DIVPROD) was launched in 2002 with financial support of FWO and NWO in the framework of the Flemish-Dutch collaboration in coastal sea research. This project addressed the relationship between biodiversity of microphytobenthos and functional aspects of the ecosystem, i.e. primary productivity and stability of tidal sediments along a salinity gradient in the Westerschelde. As traditional diversity assessments of diatoms, like for any other group of microscopical organisms, are fraught with difficulties and usually capture only part of the true diversity, molecular approaches were pioneered in this project to better understand the nature of estuarine diatom diversity and to develop new methods for monitoring it. The project encompassed a combination of a broad-scale seasonal field study as well as mesocosm and laboratory experiments. The field study aimed to quantify the relationships between microphytobenthos species diversity, biomass and activity, and variation in climatic condition, salinity, sediment composition, water content, emersion time and nutrient levels. Experimental studies were designed to better understand diversity effects on biomass accumulation in artificial biofilm communities and to assess intra- and interspecific differences in niche differentiation. The project objectives were realized through a combination of a range of approaches and techniques, including HPLC pigment analysis, <sup>14</sup>C uptake and Pulse-Amplitude-Modulated (PAM) fluorescence to estimate biomass and productivity, while microscopical analysis and sequence analysis (18S rRNA, ITS, rbcL) and molecular techniques (PCR-DGGE and q-PCR) were used to assess diversity in field samples and experiments.



### iii. Results

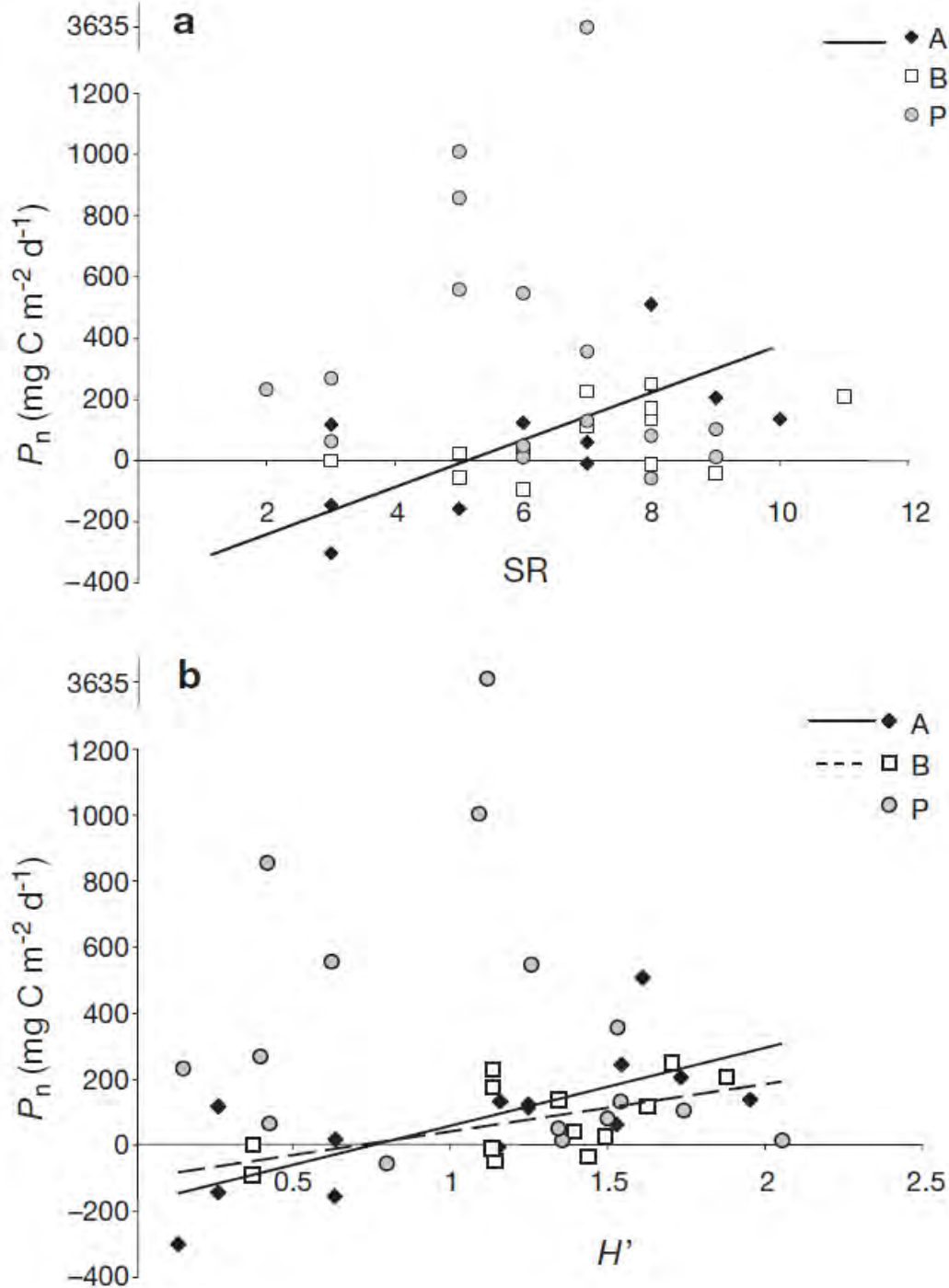
#### Field studies

In situ measurements of diversity and photosynthetic activity of diatom-dominated biofilms were investigated in 6 stations at three locations along the salinity gradient (Fig. E-4.1; Paulina Schor (PS) – salinity = 24, Biezelingse Ham (BH) – S=20 and Appelzak (AZ) – S=8.7).

**Fig. E-4.1:** Western Scheldt estuary showing locations of the sampling sites: Appelzak, Biezelingsche Ham, Paulina polder. After Forster et al. (2005)

At each location, a high- and low shore station were sampled nine times between March 2002 and September 2003. With the exception of station PS1 which had sandy sediment, all other stations were characterized by low median grain size (mostly between 50-100  $\mu\text{m}$ ). Diversity measures were calculated on the basis of relative cell counts down to morphospecies level. Biomass was estimated as chlorophyll a, and primary productivity (Pn) was modeled using a vertically resolved primary production model on the basis of measurements of photosynthetic activity, biomass and abiotic parameters (Forster et al., 2006). Species composition of benthic diatoms differed between sites along the salinity gradient of the estuary. As epipellic species were strongly correlated with photosynthetically active surface biofilm biomass and, hence, also with primary productivity, we focused on the diversity of this functional group. The results indicate that (1) biomass appears to be inversely related to the diversity of the biofilms (periods of low

biomass did not show low diversity [as reported for phytoplankton], possibly because these events were driven by grazing pressure and not by nutrient stress) and (2) relationships between diversity (species richness and Shannon index) and  $P_n$  appeared to be site specific, with either a significant positive or a unimodal relationship between both parameters (Fig. E-4.2).



**Fig. E-4.2:** Net primary production ( $P_n$ :  $\text{mg C m}^{-2} \text{d}^{-1}$ ) as a function of diversity indices: (a) species richness (SR) and (b) Shannon index ( $H'$ ), for Appelzak (A), Biezelingsche Ham (B) and Paulina polder (P). Linear regression: Panels a & b:  $R^2 = 0.5$ ,  $p < 0.01$ ,  $n = 13$  for Appelzak; Panel b:  $R^2 = 0.3$ ,  $p < 0.01$ ,  $n = 13$  for Biezelingsche Ham. After Forster et al. (2006).

The results confirmed that biomass and primary production were not interchangeable, as they did not show the same relationship with diversity. Changes in standing stock may be impossible to determine for each species, due to the open nature of the ecosystem. Moreover, the biomass represents the standing stock of organic matter, but may not reflect ecosystem metabolism if turnover rates are high. Furthermore, site-specific differences were also found, especially in the relationships of Pn and biodiversity. The causes of this variability in the relationship are multiple. The scale of the study seems to be one of the factors (Chase & Leibold, 2002), due to variability in abiotic parameters along the estuary, such as salinity, sedimentation dynamics and nutrient supply. All these parameters drive the composition of the diatom community, as well as that of associated chemotrophic microbial communities and consumers. Thus, the developmental history of the biofilm will influence the observed rates of ecosystem processes (Fukami & Morin, 2003).

In parallel, the composition and seasonal dynamics of biofilm-associated eukaryotic communities were analysed using microscopy and a genetic fingerprinting technique (PCR-DGGE) (Sahan et al., 2007). In the DGGE data, diatoms, ciliates, amoebae, copepods, nematodes, annelids and platyhelminthes were detected. Ordination analysis of the species counts and DGGE data were largely congruent and indicated that on the scale of the whole estuary (i.e. km scale) salinity, water content and seasonal factors, such as temperature and irradiance, were associated with patterns in the distribution of eukaryotic organisms, including epipelagic diatoms and micro- and meiofauna. This is in agreement with earlier reports on the distribution of micro-, meio- and macrobenthic communities in the Westerschelde (e.g. Soetaert et al., 1995, Hamels et al., 2004; Ysebaert et al., 2005). At smaller spatial scales, the position of sampling localities along the tidal exposure gradient appeared to be the main determinant of species turnover, in particular in the brackish reaches of the estuary.

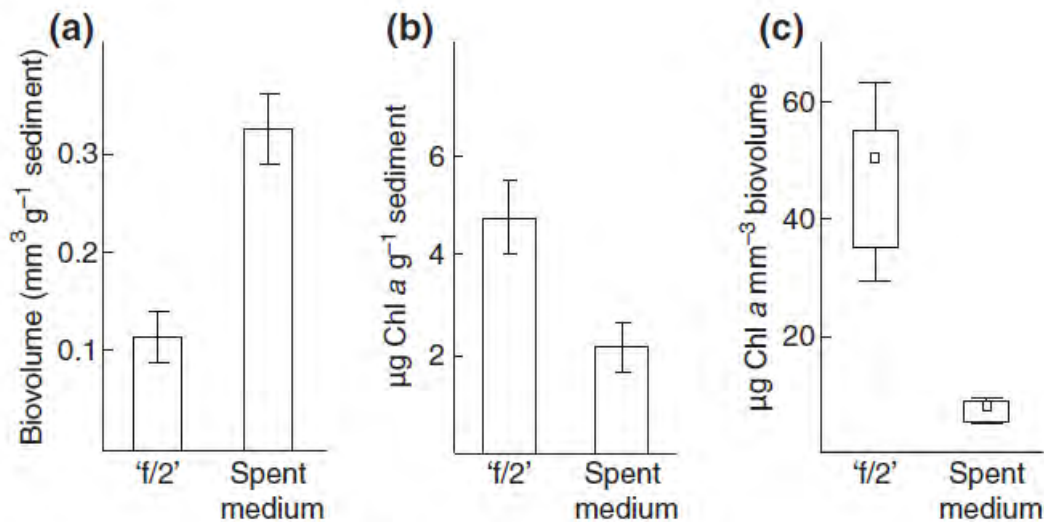
Sequencing of DGGE bands revealed the presence of different types of epipelagic diatoms although fewer taxa were identified than by microscopy. This may be due to the fact that PCR-DGGE does not detect genotypes if the abundance is <1 % (e.g. Muyzer et al., 1993) and suggests that microscopic analysis may have a higher resolution. However, it is also possible that microscopical analyses overestimate local diversity as also empty valves (dead cells) are identified. Although it was only possible to identify diatoms to the genus level using sequence similarity due to the poor representation of 18S rRNA gene sequences in the public databases, the diatom count data and the DGGE sequences agreed well. The affiliations obtained from DGGE sequences, *Entomoneis* (*Amphiprora*), *Pleurosigma* and *Dickieia*, are phylogenetically closely related to *Amphora*, *Gyrosigma* and *Staurophora* respectively. Molecular approaches thus provide a time- and cost-effective method to characterize microbial diversity, in particular for microorganisms that have fewer distinguishing morphological features (e.g. cyanobacteria, green algae). The increase of sequences of 18S rRNA genes in publicly accessible databases would further increase the potential of molecular analyses for micro-algae. The use of multiple primer sets could give a more representative picture of the communities. Although we used the 18S rRNA gene to estimate diversity, more variable regions (e.g., the ribosomal internally transcribed spacer, ITS), group-specific primers, or certain functional genes are needed to obtain a better resolution of the genetic diversity. In addition, expression profiling of functional genes will provide a useful tool to monitor microphytobenthos activity and its response to environmental stresses.

## Cryptic diversity

Evidence has accumulated during the last decade showing that many established diatom morpho-species actually consist of several, often sympatrically occurring pseudocryptic or truly cryptic species. This phenomenon raises important questions about niche partitioning between such closely related species. In this project we focused on the benthic diatom morpho-species *Navicula phyllepta* Kützing *sensu lato*, which is a widespread and common diatom in the Westerschelde estuary. We used the ribosomal ITS, 18S rRNA gene, and the RUBISCO LSU (*rbcL*) chloroplast gene sequence data together with cell wall morphology to show that populations of this species consist of several pseudocryptic species. Growth rate measurements in function of salinity showed that *N. phyllepta* strains assigned to the different species differed in their tolerance to low salinities (<5), which was reflected by their different (but widely overlapping) distribution in the estuary (Vanellander et al., 2009a). We developed a quantitative real-time PCR (qPCR) assay using TaqMan probes targeted to the internal transcribed spacer 1 (ITS1) to assess the spatial distribution and seasonal dynamics (Creach et al., 2006). Multiple regression analyses of the factors determining the abundance of the different species in field samples revealed that, in addition to salinity, sediment type and ammonium concentrations were probably equally important. Additionally, the seasonal pattern of the two forms of *N. phyllepta* showed an overlapping, but unique distribution along the estuary. Our results thus show that *N. phyllepta sensu lato* comprises different species with specialized ecophysiological characteristics, rather than generalists with a broad adaptability to different environmental conditions.

## Experimental studies

The potential effect of species diversity on ecosystem functioning was further addressed using microcosm studies (Vanellander et al., 2009b). Most experimental studies on microorganisms have used randomly assembled communities that do not resemble natural communities. It is therefore difficult to predict the consequences of realistic, non-random diversity loss. Therefore we used naturally co-occurring diatom species from intertidal mudflats to assemble communities with realistically decreasing diversity and analysed the effect of non-random species loss on biomass production. Our results demonstrate a positive biodiversity effect on production, with mixtures outperforming the most productive component species in more than half of the combinations. These strong positive diversity effects could largely be attributed to positive complementarity effects (including both niche complementarity and facilitation), despite the occurrence of negative selection effects which partly counteracted the positive complementarity effects at higher diversities. Facilitative interactions were, at least in part, responsible for the higher biomass production. For one of the species, *Cylindrotheca closterium sensu lato*, we showed its ability to significantly increase its biomass production in response to substances leaked into the culture medium by other diatom species. In these conditions, the species drastically reduced its pigment concentration, supporting the hypothesis that this species switched to mixotrophic growth in the presence of organic substrates excreted by other species (Fig. E-4.3). Additional studies on this species complex showed a widespread but varying capacity for mixotrophic growth as well as a distinct variation in temperature-dependent growth capacity among the several cryptic and pseudocryptic species (Vanellander et al., unpubl.).



**Fig. D-4.3:** Biovolume yield (a) and chlorophyll a yield (b) of a monoculture of *Cylindrotheca closterium* when grown in inorganic 'f/2' medium and in 'f/2' medium enriched with spent medium of a mixture of *Navicula* species. (c): boxplot of the chlorophyll a to biovolume ratio in both treatments. After Vanellander et al. (2009b).

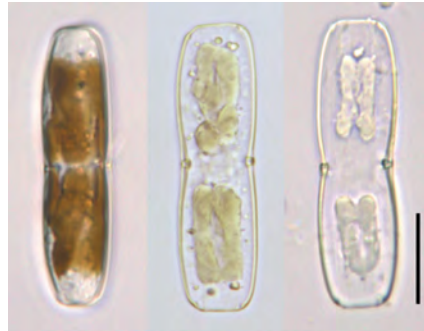
In conclusion, these experiments demonstrated that both species richness and identity can have strong effects on the biomass production of benthic diatom biofilms and that transgressive overyielding is common in these communities, whereby combinations of species are more productive than the yield of individual species. In addition to providing mechanistic evidence for facilitation, which is partly responsible for enhanced primary production, our results have implications for carbon cycling in sediments. Indeed, mixotrophic uptake of dissolved organic matter by diatoms may compete with carbon utilization by bacteria, further adding to the complexity of carbon cycling in intertidal sediments.

In addition to facilitative interactions, the organization of microphytobenthos communities appears to be strongly affected by antagonistic interactions. In laboratory experiments, we demonstrated allelopathic effects of an – as yet unidentified - *Nitzschia* species on several common species that occur sympatrically in the Westerschelde estuary. Furthermore, we demonstrated reciprocal, density dependent allelopathic interactions between this species and two other species, suggesting that antagonistic interactions are common among benthic diatoms. It is thus likely that these positive and negative interactions among diatoms contribute to the high spatial and temporal turnover in species composition in estuarine microphytobenthos within the constraints imposed by physical (e.g. Van der Wal et al., 2010) and other biological constraints (e.g., Hamels et al., 2004; De Troch et al., 2006) operating in these dynamic ecosystems.

#### iv. Conclusions

A general conclusion of this project is that there exists a scale-dependent, positive or unimodal relationship between microphytobenthos diversity and productivity. Micro- and mesocosm experiments further suggest that niche differentiation and facilitation are important mechanisms contributing to this relationship. Mixotrophy is probably one of the main mechanisms in explaining facilitative interactions in diatom biofilms. However, antagonistic interactions also appear to occur commonly in diatom-dominated biofilms and may contribute to spatial and

temporal turnover in these communities. The project further demonstrated that cryptic diversity is probably widespread in microphytobenthos, as was found in other functional and taxonomic groups of eukaryotic microorganisms. We showed that distributional patterns of cryptic species were largely overlapping at larger spatial scales, but that at the local scale and over time, niche segregation occurs between the studied gamodemes.



**Fig. D-4.4:** LM photographs of *Stauronella* sp. exposure to the toxic diatom *Nitzschia cf. pellucid*. Left: Healthy cell. Middle: *Stauronella* cell after 48h. exposure. Right: *Stauronella* cell after 72h exposure.

## v. Recommendations

The results obtained during the VlaNeZo project have provided deeper insights into the complexity of microphytobenthos communities and have highlighted some important mechanisms contributing to diversity-stability and diversity-productivity relationships in these biofilms. Since the conclusion of the VlaNeZo project, new technologies for rapid and cheap high throughput sequencing of metagenomes and metatranscriptomes have started to revolutionize our understanding of natural microbial communities. It is now becoming possible to link molecular processes occurring in populations of living cells to ecosystem processes. Future work should combine these new tools in field and laboratory-based studies to gain a deeper understanding of the role of environmental controls and various biological processes that determine the taxonomic and functional diversity of microbial communities. In particular, we need a better understanding of the drivers behind site-specific relationships between diversity and productivity. In turn, this will teach us more about the critical controls of important ecosystem processes such as productivity, stability, sediment stabilization and nutrient cycling in marine and estuarine sediments.

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