Intertidal microphytobenthos of Te Ihutai/Avon-Heathcote Estuary (areas adjacent to the oxidation ponds)

prepared for

Avon-Heathcote Estuary Ihutai Trust

by

Yuriy Malakhov

Christchurch, November 2019

MARINE ECOLOGY
RESEARCH GROUP
UNIVERSITY OF CANTERBURY, NZ

UC
UNIVERSITY OF
CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

Executive Summary

For more than 100 years, the upper part of Te Ihutai/Avon-Heathcote estuary has been subjected to wastewater discharge. This has significantly deteriorated the environmental health of the area and made it hugely unappealing for leisure and recreation. The intense proliferation of microphytobenthos (MPB) was one of the signs of it. In 2010, the wastewater was diverted from the estuary into the newly-built oceanic outfall. However, the 2010-2011 earthquakes struck soon after the outfall construction was completed. These earthquakes altered the estuary's hydrological regime and caused its temporarily enrichment with raw sewage. After all these perturbations, Ihutai came back on the track of recovery; however, there are still some concerns about the continuing eutrophication of the estuary. Thus, the main aim of this study was to assess the present state of MPB communities in the upper reaches of Ihutai, and to report on a set of benthic environmental health indicators – sediment total nitrogen (TN) and phosphorus (TP) concentrations, and redox potential discontinuity (RPD) in sediment. The report also provides basic information on MPB and their ecology and sums up previous studies of the estuary MPB.

The main findings of the study are:

- Present-day MPB communities of the upper Ihutai reverted back to normal and look typical for this kind of habitat (brackish mudflats). Diatoms comprise 90-100% of the communities and the rest is made up of filamentous and unicellular cyanobacteria.
- MPB populations appear diverse and do not indicate any localised point-source nutrient enrichment, i.e. wastewater seepage. The average MPB biomass is low but seems to be enough to sustain dense populations of grazing snails.
- The measured environmental parameters show signs of moderate eutrophication of the studied area. Natural features of this part of the estuary (muddy sediment, reduced flushing) increase the risks of worsening the situation.

Regular estuarine health monitoring is recommended to continue tracking the recovery of the estuary. A more detailed study of nutrient budgets of the estuary may be needed to identify the current sources of eutrophication.

Background

1. *The ecological role of microphytobenthos.*

Microphytobenthos (MPB), also known as benthic microalgae, are an important autotrophic component of coastal soft-sediment ecosystems (MacIntyre et al. 1996). In estuaries, MPB dominate bare, unvegetated sediments of intertidal flats, extend into the subtidal channels, form thick mats in fringe salt marshes, and intersperse seagrass beds (Paterson et al. 2003). Sometimes inconspicuous, these biofilms contain a rich and varying mixture of different photosynthetic microorganisms, among which diatoms are usually the most common and abundant group (Admiraal 1984). However, other microorganisms, such as cyanobacteria, euglenoids and dinoflagellates, can be equally important in the formation of MPB communities under some conditions (Paterson and Hagerthey 2001, Scholz and Liebezeit 2012, Semcheski et al. 2016).

MPB can account for more than 50% of total primary production in shallow estuaries (Underwood and Kromkamp 1999). They are subjected to direct grazing by benthic micro-, meio- and macrofauna (Montagna et al. 1995, Miller et al. 1996). MPB biofilms also facilitate the microbial loop, providing detrital organic matter for the secondary bacterial production (van Oevelen et al. 2006). In some situations, wave and tidal action can re-suspend a significant part of MPB into the water column, making it available for the filter-feeders and zooplankton consumption (de Jonge and van Beusekom). Microalgal biofilms are highly nutritious and it was shown that they significantly add even to the diet of some shorebird species (Kuwae et al. 2012).

MPB contribute to a variety of other crucial ecosystem services, such as nutrient cycling (Sundbäck et al. 2000, Cook et al. 2004), carbon sequestration (Oakes and Eyre 2014), and sediment stabilisation (Passarelli et al. 2014, Hubas et al. 2018). MPB also strengthen ecosystem resilience (Thrush et al. 2012). For example, MPB facilitate ecosystem recovery after nuisance seaweed blooms and associated macrofauna die-offs by ensuring a food source for newly colonising grazers (Larson and Sundbäck 2008).

2. *Previous studies of Ihutai/Avon-Heathcote MPB.*

Te Ihutai or Avon-Heathcote estuary is located near Christchurch city (ca. 400,000 people). It is fed by two rivers: Ōtākaro/Avon and Ōpāwaho/Heathcote. The estuary is a relatively large (ca. 8 km^2), predominantly intertidal, shallow (average depth 1.4 m) and permanently open tidal lagoon (Hollever and Bolton-Ritchie 2016, Hume et al. 2016). The tidal cycle is semidiurnal, and the water temperature annually fluctuates between 10 °C (winter) to 20 °C (summer). High tide water salinity typically ranges between 3 psu (near river mouths) and almost 34 psu (closer to the ocean).

The estuary was long subjected to sewage discharge, until it was diverted to an ocean outfall in 2010. Today, most of the pollution originates from diffuse sources in the heavily modified urban catchment of Ihutai and enters the estuary via riverine inflow, groundwater seepage, and stormwater runoff. Ihutai bathymetry and morphology was heavily impacted by the powerful earthquakes of 2010-2011, which resulted in the increase of the intertidal flats area in the estuary (Measures et al. 2011). The earthquakes induced sediment liquefaction and brought huge amounts of sand on the surface of estuarine bed. Surprisingly, the new sediments facilitated the recovery of the AH from many years of wastewater pollution and buried the old eutrophied sediments in many parts of the estuary (Zeldis et al. 2011, Skilton 2013).

In its present state, Ihutai is a moderately eutrophic estuary with a variety of intertidal sediment habitats. The sediment composition of intertidal flats ranges from firm sand and mud/sand to soft and very soft mud. There is a reasonably large and healthy seagrass bed in the southern lower part of the estuary, and many smaller seaweed and shellfish beds that are scattered throughout the middle part of Ihutai (Hollever and Bolton-Ritchie 2016).

The Ihutai ecology has been extensively studied in the past 100 years. However, the prevalent majority of such studies was focused on macroinvertebrates, fish and birds. Microalgae received much less attention from the researches and were just briefly considered in the number of post-graduate student theses and local environmental reports (Thompson 1929, Bruce 1953, Williams 1960, Webb 1965, Bennington 1971). These early studies recorded presence of common diatom and non-diatom genera in the estuary and provided some information on their distribution.

Linzey (1944) was one of the first to mention the formation of a yellow-green slime on the mud surface in the estuary as a result of great *Euglena* Ehrenberg abundances in the sediment. Steffensen (1974) later identified these microalgae as *E. obtusa* F. Schmitz and *E. salina* Liebetanz and carried out a more detailed study of their distribution in the estuary and lower banks of the Heathcote river.

The first detailed study of Ihutai benthic diatoms was done by McClatchie et al. (1982). After studying a single site in the estuary, they found 53 diatom species in its sediments, of which 25 taxa were recorded as new for New Zealand's algae flora. In a series of manipulative experiments, they also found that grazing by the deposit-feeding snail *Amphibola crenata* Gmelin, 1791 increases MPB species richness, while significantly decreasing cell density. These observations were interpreted as a possible qualitative community adaptation to grazing-induced stress.

The most systematic and recent attempt of investigating Ihutai MPB biodiversity was undertaken by Cane (1997). She identified 66 MPB taxa from five microalgae phyla, including 19 genera not previously recorded from the estuary. However, two-thirds of these identifications did not extend below the genus level. The sampling was done along the 200 m transect, which ran across the three tidal zones (upper, mid, and lower shore) and was laid out between the outlets of Bromley oxidation ponds. Cane's thesis thoroughly documented the spatial and seasonal changes in MPB distribution along the transect, and to date, this is the most comprehensive study of Ihutai MPB before the wastewater diversion and the earthquakes of 2010-2011.

Materials and Methods

The twelve study sites were adjacent to the Bromley oxidation ponds and stretched between Windsurfer's Reserve car park and the area opposite to the South Brighton jetty (Pleasant Point yacht club). All sites lied in the mid tide zone (within 35-180 m away from the shore) and their selection was based on visual assessment of sediment texture. The sampling was done on 30 September 2019. Each site was sampled for benthic chlorophyll *a* (Chl *a*), MPB community structure, sediment nutrients concentration, and RPD.

Benthic Chl *a* (MPB biomass proxy) cores were taken to a depth of 2 cm with custom-made plastic corers of 3 cm inner diameter. Cores were individually wrapped in aluminium foil, labelled, and transported on ice to a freezer within 2 h, then stored at -20° C until analysis. These were then freeze-dried and extracted with 90% aqueous acetone solution, following the procedures outlined in Lorenzen (1967) and Colijn and Dijkema (1981). To ensure good mixing of the solvent with the sediment, the extraction tubes were shaken for 1-2 minutes on a vortex mixer. The resulting Chl *a* concentrations were expressed in $\mu g/cm^2$.

For the MPB community analysis, a second set of equivalent cores was taken immediately next to the Chl *a* ones. These were put into plastic containers, transported to the laboratory on ice and fixed with 4% formaldehyde solution in artificial Ca^{2+}/Mg^{2+}-free seawater (de Jonge 1979). Then, MPB were pre-concentrated using the gradient centrifugation in colloidal silica Ludox HS-40 (Blanchard et al. 1988, Méléder et al. 2007, Xu et al. 2010). Ludox is slightly denser (1.3 g/ml) than MPB cells and because of that it effectively separates them from the much heavier sediment grains during the centrifugation. After the centrifugation, MPB suspensions were examined under Olympus BH-2 compound microscope, equipped with a differential interference contrast (DIC) system. The individual microalgae cells and colonies were photographed using a ZEISS Primo Star microscope, equipped with an imaging system. Relative abundances of MPB taxa were estimated using Sedgewick-Rafter counting chamber as described in Karlson et al. (2010).

Sediment nutrients cores were taken to a depth of 5 cm with custom-made plastic corers of 6 cm inner diameter. These cores were put into clean, nutrient-free containers and delivered to R.J. Hill Laboratories Ltd. for chemical analyses. TN was analysed by catalytic combustion at 900 °C using an Elementar CNHO analyser. TP was analysed by inductively coupled plasma mass spectrometry after nitric and hydrochloric acid digestion of the air-dried sediment.

RPD measurements were done on site, following the guidelines of National Estuary Monitoring Protocol (Robertson et al. 2002). At each site, a sediment core (6 cm inner diameter) was taken to a depth of 10 cm, extruded onto a white tray, and carefully split lengthwise. The change in colour and texture was then studied with particular attention to the occurrence of any black (anoxic) zones. The average distance (in mm) from the core surface to these zones was then recorded as the depth of RPD layer.

Results

The sediment texture of the sampled sites varied from muddy sand to sandy mud and soft mud (Fig. 1). The sandier sites were located at the southern end of the sampling area, in the vicinity of Sandy Point. These locations had quite high densities of mud snails and were littered with shell hash. Further north, the sediment patches started changing between sandy mud and soft mud. Mud snail density somewhat decreased at these sites but was still high. No readily apparent sediment biofilms were detected within the entire study area. Opportunistic seaweeds had fragmentary occurrence and did not cover the significant portions of the sediment.

The Chl *a* concentrations ranged between 1.29 and 32.5 $\mu g/cm^2$. The median concentration was 3.29 $\mu g/cm^2$ and only two sites near Sandy Point exceeded the concentrations of 3.9 $\mu g/cm^2$ (Fig. 2). RPD was recorded between 0 and 23 mm depth. The median RPD depth was 13 mm. Only two very muddy sites had RPD of 0 mm (Fig. 3).

Most of the study sites ($n = 7$) had less than 500 mg/kg of TN in their sediment. These concentrations were below the chemical analyser minimum detection limit, and thus the exact concentrations of TN were hard to estimate. All samples with higher TN concentrations (500 - 1200 mg/kg) were collected from the muddier, northern part of the sampling area (Fig. 4). Likewise, the sediment TP concentrations were higher in the muddy sites (Fig. 5). Overall, they ranged between 260-810 mg/kg.

Different diatom genera dominated the MPB communities at all sites (Fig. 6). They made up 100% of the community composition in half of the samples. In the rest of the samples, these microalgae were accompanied by small numbers (1-10%) of filamentous and coccoid cyanobacteria. Overall, the MPB communities of all sites appeared diverse and no monospecific microphyte populations were detected.

Diatoms were represented with a variety of life forms, among which small motile pennate naviculoids were the most common (Fig. 7). One example of such algae is genus *Diploneis* Ehrenberg ex Cleve, whose small cells were observed quite often in Ihutai samples (Fig. 8). Tychoplanktic diatoms, which can be re-suspended into the water column, were another common finding (Fig. 9). However, their abundances were markedly lower compared to the naviculoids and they did not occur at every studied site either. Large (ca. 150-200 μm length) sigmoid diatoms, such as genera *Pleurosigma* W. Smith and *Gyrosigma* Hassal, were also quite frequent in the upper reaches of the estuary (Figs. 10-11). Cyanobacteria occurred at the sites where at least some amount of sand was present in the sediment composition. Filamentous forms were more common and coccoid cyanobacteria were found only at two sites (Fig. 12).

Discussion

Diatoms are one of the major groups of microalgae. They are unicellular and occur either solitary or in small colonies. All diatom cells are covered with intricate silicon frustules (shells). Each species has a unique frustule morphology; however, its fine details are often blurred with cell contents and extracellular slime. Precise diatom species identification is done by the examination of specially prepared diatom slides or with the scanning electron microscopy. This is a tedious and time-consuming process, which was beyond the scope of this study. Sediment-inhabiting diatoms have a great variety of life modes. Most of them are capable of gliding movement on sediment surface (e.g. naviculoids and sigmoids on Figs. 7-8 & 10-11) and express regular vertical migrations in the upper sediment layers (Consalvey et al. 2004). Some diatoms lack the ability to move and grow attached to the sand grains. The third group – tychoplankters – can occur both in the sediments and in the water column (Fig. 9). These diatoms may or may not move, they are lightweight and easily re-suspended into the overlying water by wind and/or tidal currents (Admiraal 1984).

Cyanobacteria, sometimes called blue-green algae, are one of the most ancient lineages of photosynthetic organisms. Their cells are prokaryotic and have a much simpler level of organisation than other microalgae. Some cyanobacterial species have immobile spherical (coccoid) cells, which grow either solitary or joined together in a colony (Fig. 12C). Other species grow as long filamentous colonies (Fig. 12A-B). The latter creep over the sediment, sometimes forming thick biofilms on its surface. Precise cyanobacterial species identification requires a combination of culturing and molecular approaches, which was beyond the scope of this study (Johansen and Casamatta 2005). Cyanobacteria are quite common in the marine littoral throughout the world, but their diversity and ecology are significantly understudied (Hoffmann 1999). Generally, cyanobacteria tend to occur more frequently in the sandy sediments of estuarine intertidal (Semcheski et al. 2016). This seems to be the case for this study as well. Cyanobacteria were often found at the muddy sand or sandy mud sites but were almost absent in the soft mud samples (cf. Figs. 1 & 6).

It appears that the MPB community of the upper Ihutai part has undergone dramatic changes since the wastewater diversion in 2010. For example, Cane (1997) documents a year-round presence of thick brown, green, and blue-green biofilms in the area. These biofilms covered significant portions of her transect and had high abundances of euglenoids and filamentous cyanobacteria. She also reports frequent blooms of microscopic green algae on the sediment surface and in the mid-zone tide pools. No such phenomena can be seen now and no euglenoid or green algae were found during the 2019 sampling. On the other hand, the observations of diatom and cyanobacterial taxa generally match the 1997 records. However, it seems that filamentous cyanobacteria have become significantly reduced in their numbers and do not constitute a notable part of the current MPB assemblages.

Overall, the 2019 MPB community of the upper Ihutai part appears fairly typical for the brackish mudflats, which experience low hydrodynamic stress (Paterson and Hagerthey 2001). It is reasonably diverse and demonstrates an ample variety of photosynthetic microorganisms. The diatom composition of the sandier sites generally resembled that of the other parts of the estuary. The muddier sites were marked by the higher frequency of *Pleurosigma* and *Gyrosigma* species. The largest difference between the studied area and the rest of the estuary is a complete absence of euglenoid algae in the mid-tide zone (Malakhov, unpublished). The nature of the substrate, lower salinity, and higher grazing pressure are the main drivers of these differences.

On average, the MPB biomass of the sampled sites (Chl *a*) was quite low (Fig. 2). Nevertheless, it was able to sustain abundant mud snail populations. The two muddy sand sites had somewhat unusually high Chl *a* concentrations of 32.5 and 9.2 $\mu g/cm^2$. Such values were previously measured around the estuary; however, they are normally associated with dense bright-green biofilms on the sediment surface (Malakhov, unpublished). No readily apparent biofilms were present in case of these two outliers, and it is possible that such great Chl *a* values result from buried seaweed fragments or shell hash pieces covered in epiphytes.

RPD depth indicates a transitioning from oxidising aerobic to reducing anaerobic condition in the sediment. Increased muddiness is correlated with shallower RPD depths and makes sediment more prone to the anoxia (Jørgensen and Revsbech 1985). The shrinking of oxygenated zone seriously affects sediment habitability and leads to the accumulation of toxic hydrogen sulphide and ammonia (Sutula et al. 2014). Reduced oxygenation is also linked to the accumulation of nutrients in the sediment (TN and TP), which can fuel opportunistic seaweed blooms and boost eutrophication (Coelho et al. 2004, Garcia-Robledo et al. 2016).

The current guidelines for monitoring New Zealand estuaries consider RPD of <10 mm as an indicator of poor environmental health, within 10-30 mm – of fair conditions; TN concentrations between 500-2000 mg/kg are considered good and < 500 mg/kg – very good; TP concentration between 200-500 mg/kg – good, and between 500-1000 mg/kg – fair (Robertson et al. 2002). All upper Ihutai sites fell within these limits and thus demonstrated signs of moderate eutrophication. Seepage of nutrient-rich groundwaters from the adjacent oxidation ponds can contribute to this and influence the upper Ihutai nutrient regime. Groundwater seepage appears either as distinct liquified "wetter" sediment or as large pools of standing water. It is markedly different in salinity, nutrient composition and other physical and chemical properties. When present, it alters MPB patches and increases the surrounding MPB community biomass (Waska and Kim 2010, Welti et al. 2015). No obvious seepage phenomena were observed in the sampled mid-tide zone and no abnormalities in

MPB community composition (e.g. dense monospecific microalgae populations) were detected. Seepage effects are highly localised, and it is possible that they may be more pronounced in the upper intertidal (~10-15 m away from the shore) rather than at the mid tide level. More detailed sampling (salinity, pH, detailed nutrient analysis) would be needed to detect and confirm the presence of oxidation ponds seepage.

Conclusion / Recommendations

This study offers a glimpse into the diversity and ecology of MPB of the upper Ihutai/Avon-Heathcote estuary. Historically, these areas were heavily impacted by wastewater discharge and river pollution. This led to the formation of atypical MPB proliferations, which smothered the sediment and decreased the appeal of the area. The contemporary MPB communities correspond more with their habitat. Clearly, such dramatic transformation is an effect of the 2010 wastewater diversion. Nevertheless, the upper part of the estuary is still moderately eutrophic, and its natural features (i.e. increased muddiness, reduced tidal flushing) have the potential of worsening the eutrophication. Thus, the environmental health of this area must be monitored regularly, and appropriate measures must be taken to mitigate these risks (e.g. reducing the amount of inflowing nutrients in river water). A more detailed study of the nutrient balances of this area may be needed to identify and assess the current sources of eutrophication.

Acknowledgements

I would like to thank Prof Islay Marsden and the Ihutai Trust for offering me a student scholarship to conduct this research. I am also grateful to Spencer Virgin for his help in the field.

Figures

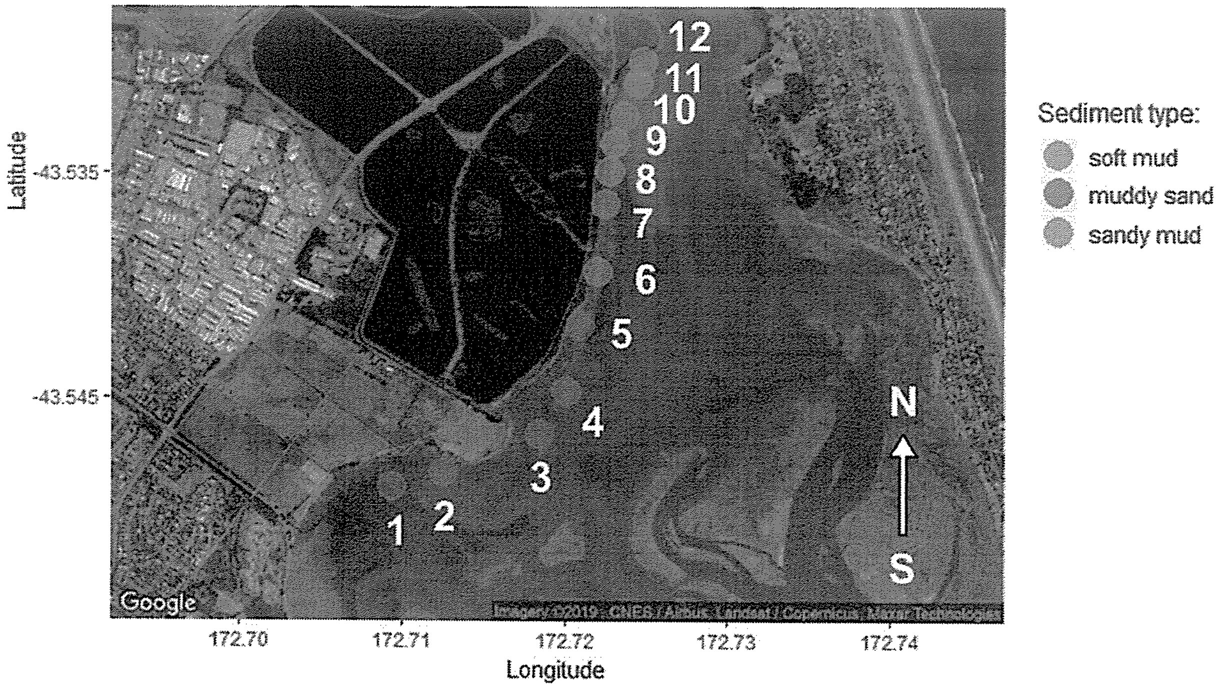

Figure 1. Sediment types of the upper part of the estuary. Numbers indicate site locations.

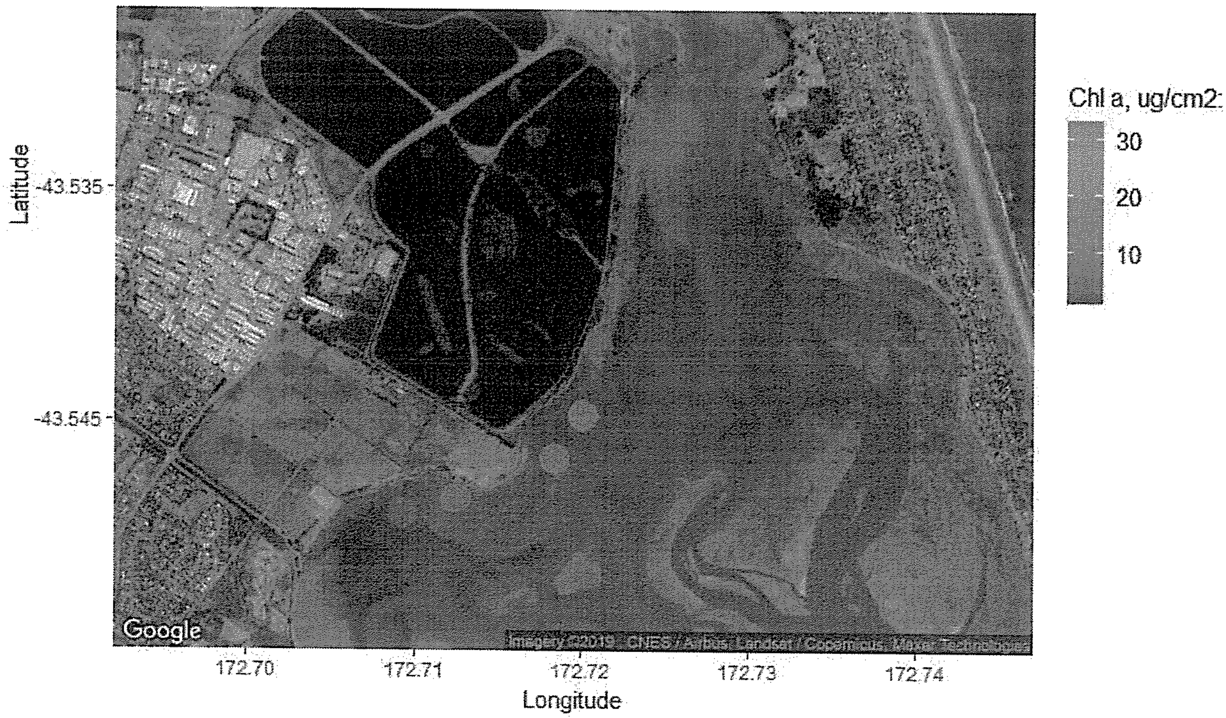

Figure 2. Chl *a* concentrations in the sediments of the upper part of the estuary.

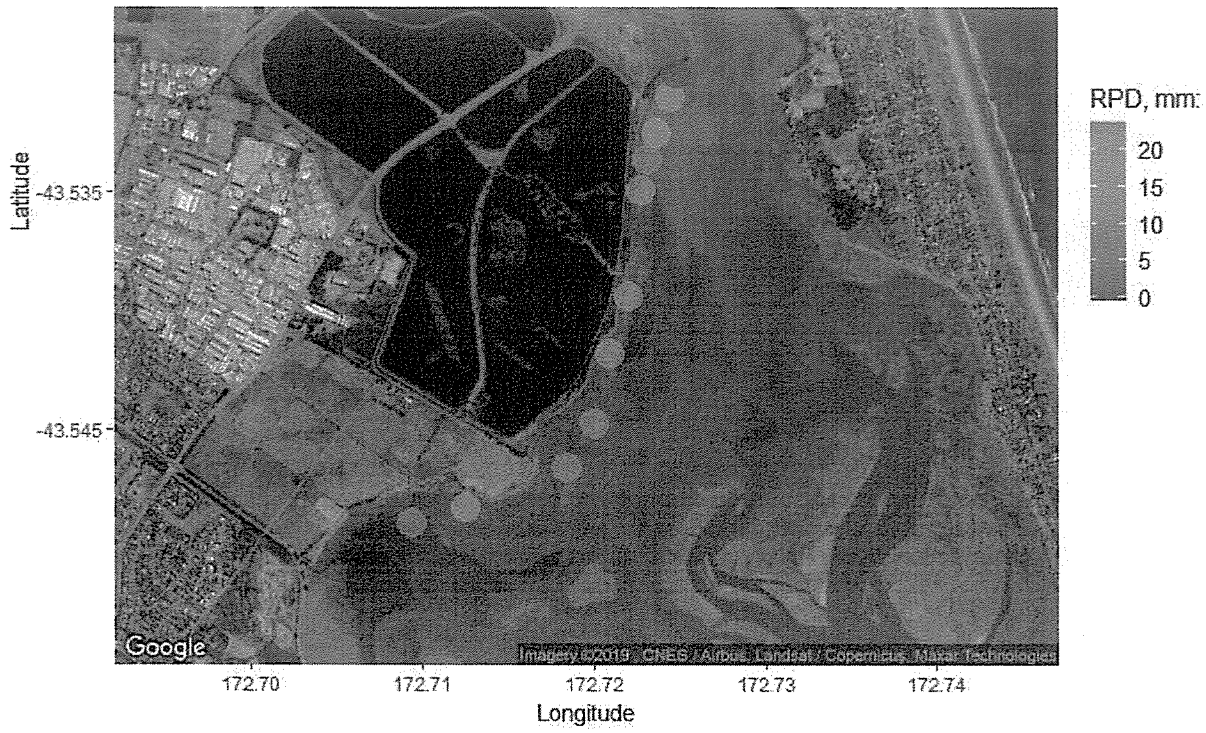

Figure 3. RPD depth in the sediments of the upper part of the estuary.

Figure 4. TN concentrations in the sediments of the upper part of the estuary.

Figure 5. TP concentrations in the sediments of the upper part of the estuary.

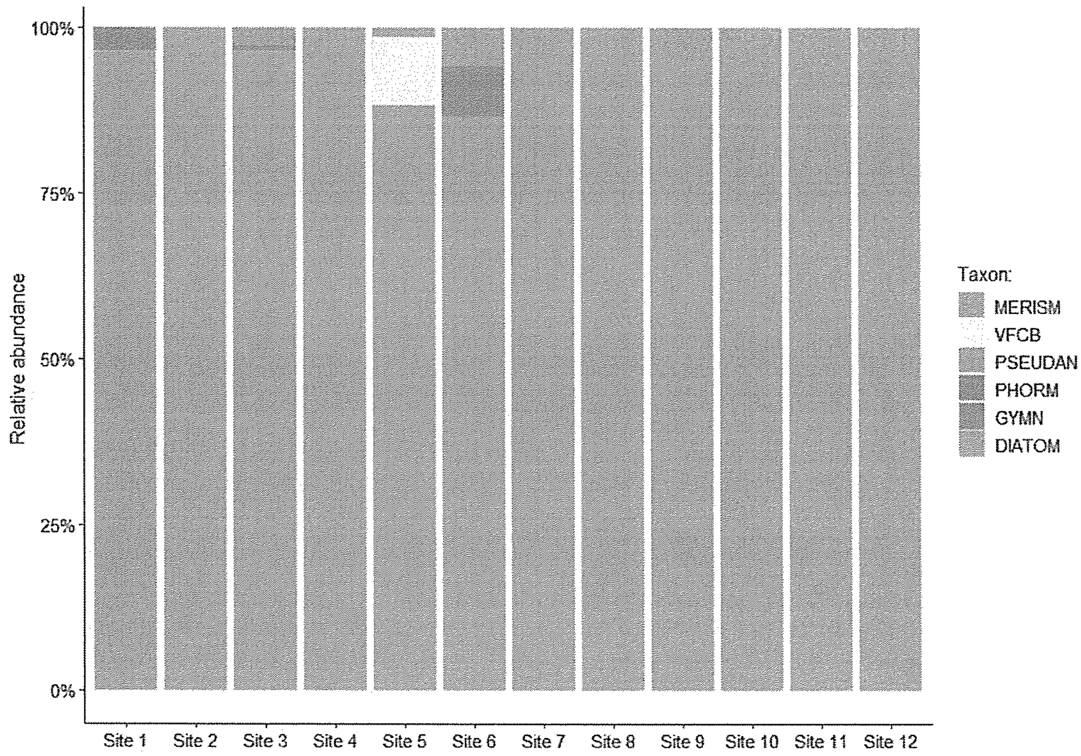

Figure 6. MPB communities of the upper part of the estuary. MERISM – gen. *Merismopedia* Meyen (cyanobacteria), VFCB – very fine cyanobacteria, PSEUDAN – family *Pseudanabaenaceae* K. Anagnostidis & J. Komárek, PHORM – gen. *Phormidium* Kützing ex Gomont (cyanobacteria), GYMN – genus *Gymnodinium* F. Stein (dinoflagellates), DIATOM – diatoms.

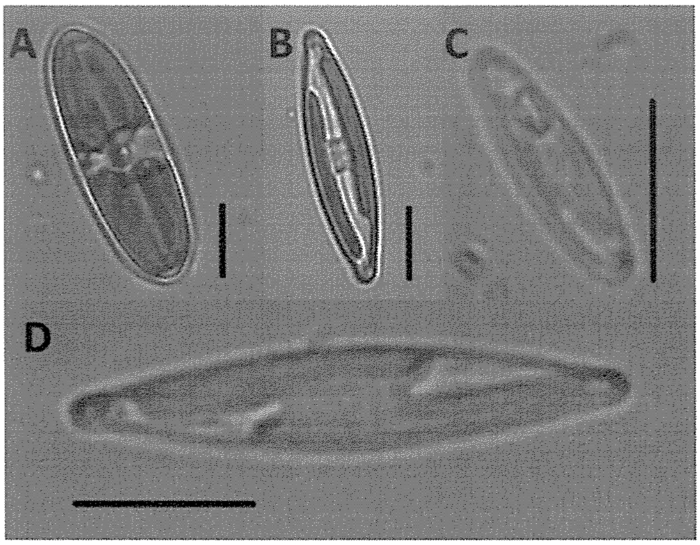

Figure 7. Naviculoid diatoms. A & B – live cells under 400x magnification; C & D – fixed cells, 1000x magnification. Scale bars – 10 μm.

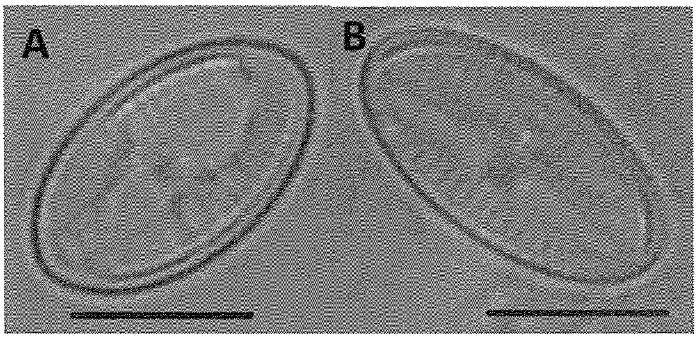

Figure 8. *Diploneis sp.* A – valve view of a fixed cell with two parietal chloroplasts; B – empty silicon frustule (valve view). 1000x magnification, scale bars – 10 μm.

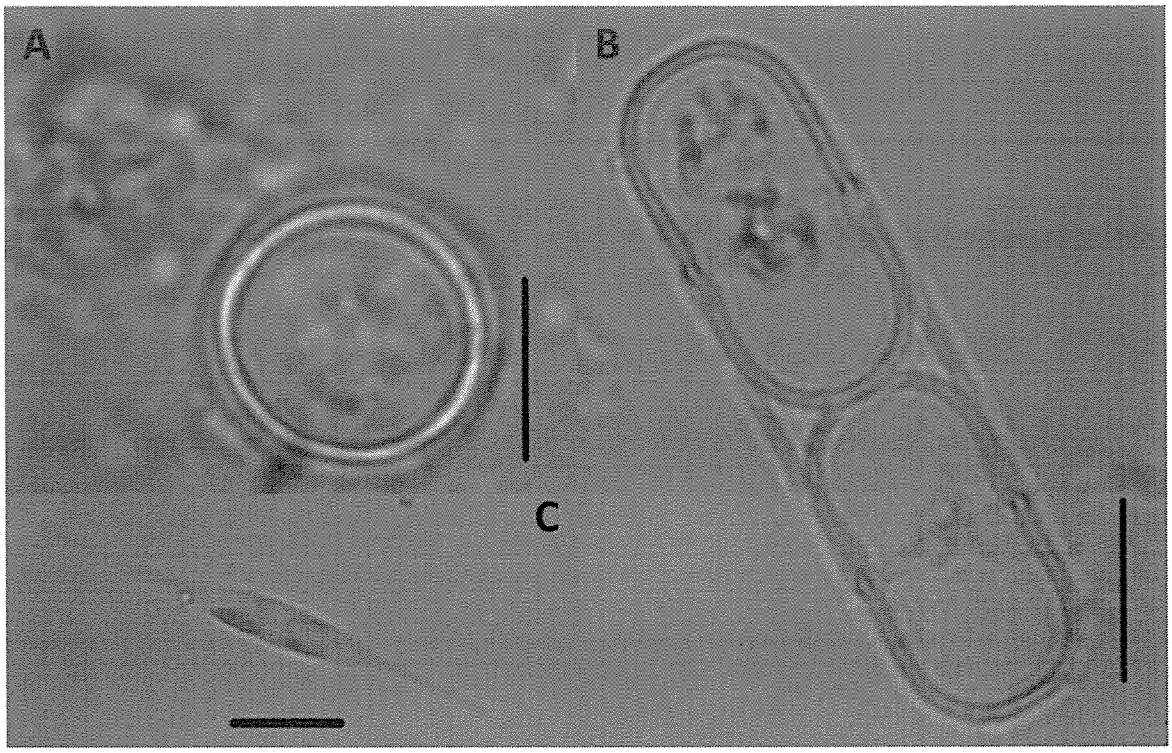

Figure 9. Tychoplanktic diatoms. A – valve view of a centric diatom (fixed, 1000x magnification); B – girdle view of a centric diatom colony (fixed, 1000x magnification); C – *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin (live cell, 400x magnification). Scale bars – 10 µm.

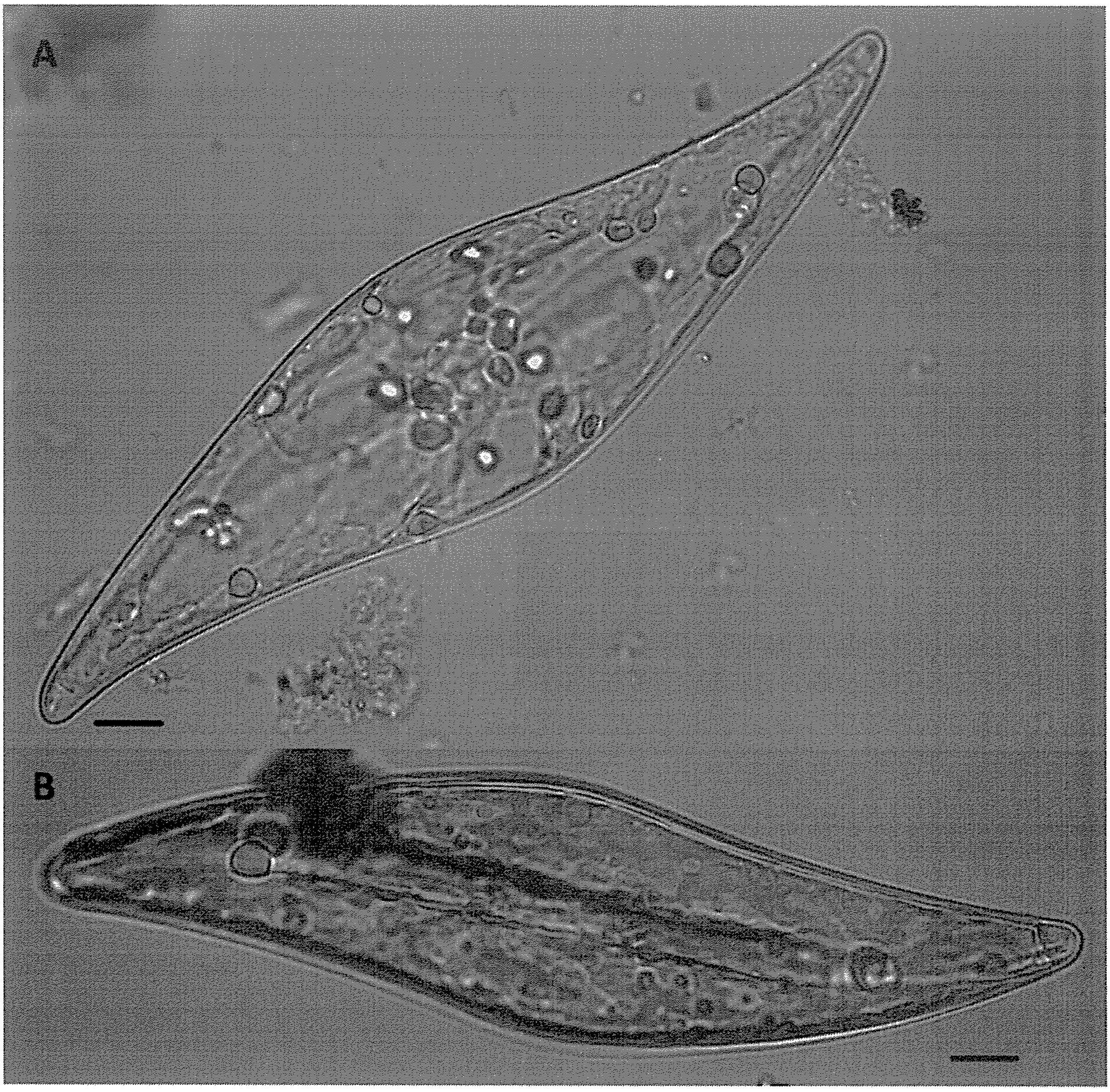

Figure 10. Genus *Pleurosigma* W. Smith. A – valve view of a fixed cell (400x magnification); B – live cell (400x magnification). Scale bars – 10 μm.

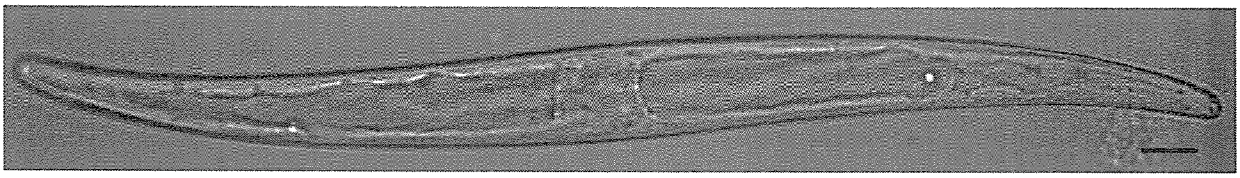

Figure 11. *Gyrosigma sp.* Valve view of a fixed cell (400x magnification, scale bar – 10 μm).

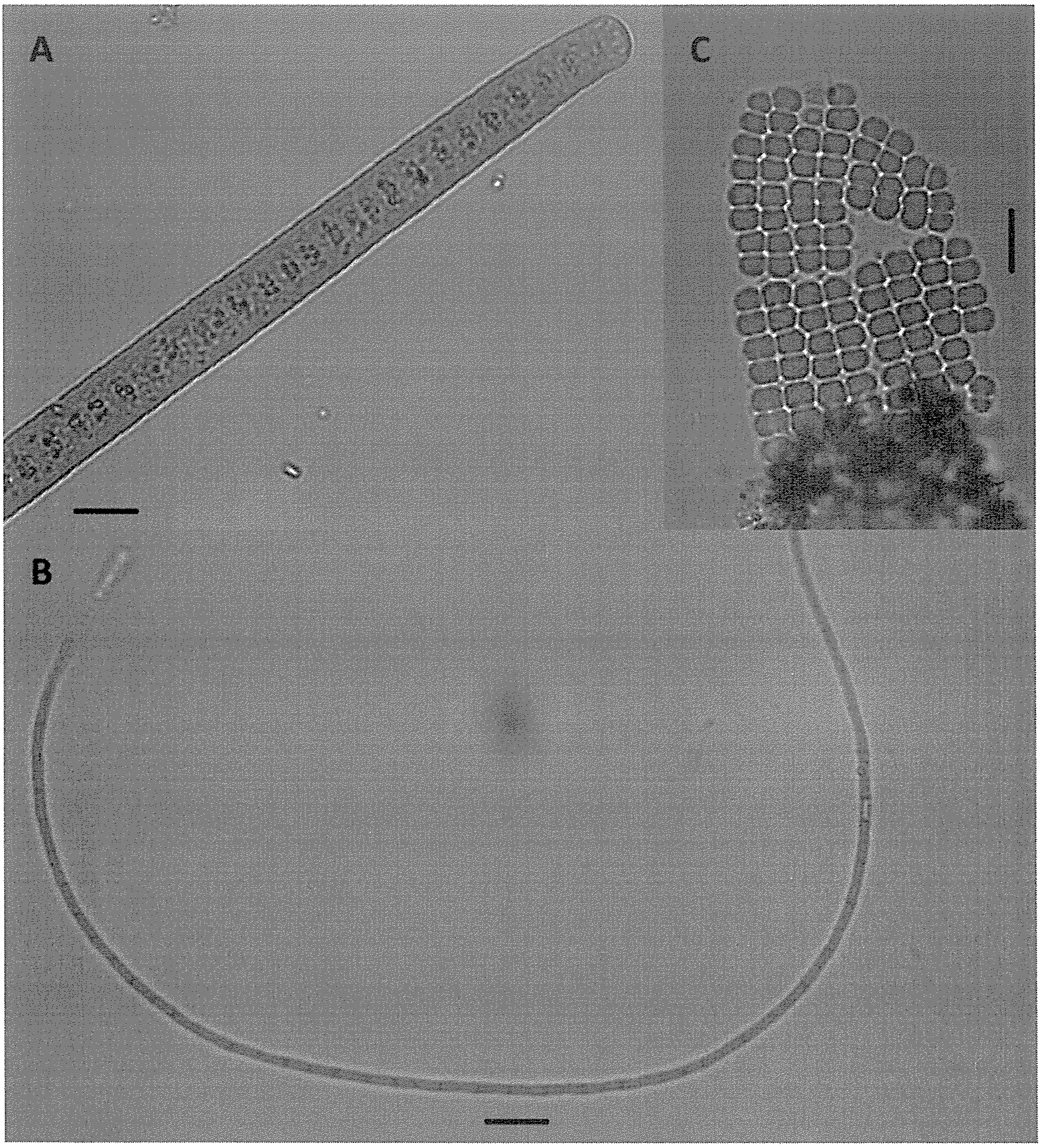

Figure 12. Cyanobacteria of the Ihutai sediments. A – apical fragment of the *Phormidium sp.* filament; B – very thin filamentous cyanobacterium; C – flat tabular colony of *Merismopedia sp.* Scale bars – 10 µm.

References

Admiraal, W. 1984. The ecology of estuarine sediment-inhabiting diatoms. *Progress in phycological research* **3**: 269-322.

Bennington, S. L. 1971. A diversity study to investigate the extent of recovery after pollution in an estuarine environment. University of Canterbury, Christchurch, New Zealand.

Blanchard, G., M. J. Chretiennot-Dinet, A. Dinet, and J.-M. Robert. 1988. Méthode simplifiée pour l'extraction du microphytobenthos des sédiments marins par le gel de silice Ludox. *Comptes rendus de l'Académie des sciences. Série 3, Sciences de la vie* **307**: 569-576.

Bruce, A. 1953. Report on a biological and chemical investigation of the waters in the Estuary of the Avon and Heathcote Rivers. Christchurch Drainage Board, Christchurch, New Zealand.

Cane, R. P. 1997. A taxonomic survey and ecological study of the benthic microalgae in the sediments of the Avon-Heathcote Estuary, Christchurch, New Zealand. University of Canterbury, Christchurch, New Zealand.

Coelho, J., M. Flindt, H. S. Jensen, A. Lillebø, and M. Pardal. 2004. Phosphorus speciation and availability in intertidal sediments of a temperate estuary: relation to eutrophication and annual P-fluxes. *Estuarine, Coastal and Shelf Science* **61**: 583-590.

Colijn, F., and K. S. Dijkema. 1981. Species composition of benthic diatoms and distribution of chlorophyll a on an intertidal flat in the Dutch Wadden Sea. *Marine Ecology Progress Series* **4**: 9-21.

Consalvey, M., D. M. Paterson, and G. J. Underwood. 2004. The ups and downs of life in a benthic biofilm: migration of benthic diatoms. *Diatom Research* **19**: 181-202.

Cook, P. L., A. T. Revill, E. C. Butler, and B. D. Eyre. 2004. Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling. *Marine Ecology Progress Series* **280**: 39-54.

de Jonge, V. 1979. Quantitative separation of benthic diatoms from sediments using density gradient centrifugation in the colloidal silica Ludox-TM. *Marine Biology* **51**: 267-278.

de Jonge, V., and J. van Beusekom. 1995. Wind-and tide-induced resuspension of sediment and microphytobenthos from tidal flats in the Ems estuary. *Limnology and Oceanography* **40**: 776-778.

Garcia-Robledo, E., J. Bohorquez, A. Corzo, J. L. Jimenez-Arias, and S. Papaspyrou. 2016. Dynamics of inorganic nutrients in intertidal sediments: porewater, exchangeable, and intracellular pools. *Frontiers in Microbiology* 7.

Hoffmann, L. 1999. Marine cyanobacteria in tropical regions: diversity and ecology. *European Journal of Phycology* **34**: 371-379.

Hollever, J., and L. Bolton-Ritchie. 2016. Broad scale mapping of the Estuary of the Heathcote and Avon Rivers/Ihutai. Environment Canterbury.

Hubas, C., C. Passarelli, and D. M. Paterson. 2018. Microphytobenthic Biofilms: Composition and Interactions. Pages 63-90 *in* P. G. Beninger (ed.). *Mudflat Ecology*. Springer International Publishing, Cham.

Hume, T., P. Gerbeaux, D. Hart, H. Kettles, and D. Neale. 2016. A classification of New Zealand's coastal hydrosystems. National Institute of Water & Atmospheric Research Ltd.

Johansen, J. R., and D. A. Casamatta. 2005. Recognizing cyanobacterial diversity through adoption of a new species paradigm. *Algological studies* **117**: 71-93.

Jørgensen, B. B., and N. P. Revsbech. 1985. Diffusive boundary layers and the oxygen uptake of sediments and detritus. *Limnology and Oceanography* **30**: 111-122.

Karlson, B., C. Cusak, and E. Bresnan. 2010. Microscopic and molecular methods for quantitative phytoplankton analysis. UNESCO.

Kuwae, T., E. Miyoshi, S. Hosokawa, K. Ichimi, J. Hosoya, T. Amano, T. Moriya, M. Kondoh, R. C. Ydenberg, and R. W. Elner. 2012. Variable and complex food web structures revealed by exploring missing trophic links between birds and biofilm. *Ecology Letters* **15**: 347-356.

Larson, F., and K. Sundbäck. 2008. Role of microphytobenthos in recovery of functions in a shallow-water sediment system after hypoxic events. *Marine Ecology Progress Series* **357**: 1-16.

Linzey, J. T. 1944. A short study of the hydrography of the Estuary of the Avon and Heathcote Rivers. *Transactions of the Royal Society of New Zealand* **73**: 365-376.

Lorenzen, C. J. 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnology and Oceanography* **12**: 343-346.

MacIntyre, H. L., R. J. Geider, and D. C. Miller. 1996. Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries* **19**: 186-201.

McClatchie, S., S. Juniper, and G. Knox. 1982. Structure of a mudflat diatom community in the Avon-Heathcote Estuary, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **16**: 299-309.

Measures, R., M. Hicks, U. Shankar, J. Bind, J. Arnold, and J. Zeldis. 2011. Mapping earthquake induced topographical change and liquefaction in the Avon-Heathcote Estuary. National Institute of Water & Atmospheric Research, Christchurch, New Zealand.

Méléder, V., Y. Rincé, L. Barillé, P. Gaudin, and P. Rosa. 2007. Spatiotemporal changes in microphytobenthos assemblages in a macrotidal flat (Bourgneuf Bay, France). *Journal of Phycology* **43**: 1177-1190.

Miller, D. C., R. J. Geider, and H. L. MacIntyre. 1996. Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. II. Role in sediment stability and shallow-water food webs. *Estuaries* **19**: 202-212.

Montagna, P. A., G. F. Blanchard, and A. Dinet. 1995. Effect of production and biomass of intertidal microphytobenthos on meiofaunal grazing rates. *Journal of Experimental Marine Biology and Ecology* **185**: 149-165.

Oakes, J. M., and B. D. Eyre. 2014. Transformation and fate of microphytobenthos carbon in subtropical, intertidal sediments: potential for long-term carbon retention revealed by 13C-labeling. *Biogeosciences* **11**: 1927-1940.

Passarelli, C., F. Olivier, D. M. Paterson, T. Meziane, and C. Hubas. 2014. Organisms as cooperative ecosystem engineers in intertidal flats. *Journal of Sea Research* **92**: 92-101.

Paterson, D. M., and S. E. Hagerthey. 2001. Microphytobenthos in Constrasting Coastal Ecosystems: Biology and Dynamics. Pages 105-125 *in* K. Reise (ed.). *Ecological Comparisons of Sedimentary Shores.* Springer, Berlin-Heidelberg.

Paterson, D. M., R. Perkins, M. Consalvey, and G. J. Underwood. 2003. Ecosystem function, cell micro-cycling and the structure of transient biofilms. Pages 47-63 *in* W.E. Krumbein, D.M. Paterson, G.A. Zavarzin (eds.) *Fossil and Recent Biofilms: A Natural History of Life on Earth.* Springer, the Netherlands.

Robertson, B. M., P. Gillespie, and R. Asher. 2002. Estuarine environmental assessment and monitoring: a national protocol. Cawthron Institute.

Scholz, B., and G. Liebezeit. 2012. Microphytobenthic dynamics in a Wadden Sea intertidal flat – Part II: Seasonal and spatial variability of non-diatom community components in relation to abiotic parameters. *European Journal of Phycology* **47**: 120-137.

Semcheski, M. R., T. A. Egerton, and H. G. Marshall. 2016. Composition and diversity of intertidal microphytobenthos and phytoplankton in Chesapeake Bay. *Wetlands* **36**: 483-496.

Skilton, J. E. 2013. Invertebrate responses to large-scale change: impacts of eutrophication and cataclysmic earthquake events in a southern New Zealand estuary. University of Canterbury, Christchurch, New Zealand.

Steffensen, D. A. 1974. Distribution of *Euglena obtusa* Schmitz and *E. salina* Liebetanz on the Avon-Heathcote estuary, Christchurch. *Mauri Ora* **2**: 85-94.

Sundbäck, K., A. Miles, and E. Göransson. 2000. Nitrogen fluxes, denitrification and the role of microphytobenthos in microtidal shallow-water sediments: an annual study. *Marine Ecology Progress Series* **200**: 59-76.

Sutula, M., L. Green, G. Cicchetti, N. Detenbeck, and P. Fong. 2014. Thresholds of adverse effects of macroalgal abundance and sediment organic matter on benthic habitat quality in estuarine intertidal flats. *Estuaries and Coasts* **37**: 1532-1548.

Thompson, E. F. 1929. An introduction to the natural history of the Avon-Heathcote estuary and Brighton Beach, Canterbury, New Zealand: A study in littoral ecology. University of Canterbury, Christchurch, New Zealand.

Thrush, S., J. Hewitt, and A. Lohrer. 2012. Interaction networks in coastal soft-sediments highlight the potential for change in ecological resilience. *Ecological Applications* **22**: 1213-1223.

Underwood, G. J. C., and J. Kromkamp. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Advances in Ecological Research* **29**: 93-153.

van Oevelen, D., K. Soetaert, J. J. Middelburg, P. M. Herman, L. Moodley, I. Hamels, T. Moens, and C. H. Heip. 2006. Carbon flows through a benthic food web: integrating biomass, isotope and tracer data. *Journal of Marine Research* **64**: 453-482.

Waska, H., and G. Kim. 2010. Differences in microphytobenthos and macrofaunal abundances associated with groundwater discharge in the intertidal zone. *Marine Ecology Progress Series* **407**: 159-172.

Webb, B. F. 1965. Report on a biological investigation of the waters in the Estuary of the Avon and Heathcote Rivers. Christchurch Drainage Board, Christchurch, New Zealand.

Welti, N., D. Gale, M. Hayes, A. Kumar, M. Gasparon, B. Gibbes, and D. Lockington. 2015. Intertidal diatom communities reflect patchiness in groundwater discharge. *Estuarine, Coastal and Shelf Science* **163**: 116-124.

Williams, M. 1960. Report on a biological investigation of the waters in the Estuary of the Avon and Heathcote Rivers. Christchurch Drainage Board, Christchurch, New Zealand.

Xu, K., Y. Du, Y. Lei, and R. Dai. 2010. A practical method of Ludox density gradient centrifugation combined with protargol staining for extracting and estimating ciliates in marine sediments. *European Journal of Protistology* **46**: 263-270.

Zeldis, J., J. Skilton, P. South, and D. Schiel. 2011. Effects of the Canterbury earthquakes on Avon-Heathcote Estuary/Ihutai ecology. Environment Canterbury, Christchurch, New Zealand.

Appendix

A copy of the nutrient analysis report from R. J. Hill Laboratories Ltd. Samples IHU1 – IHU10 were collected from Sites 3 – 12 of the study, IHU11 – from Site 2, and IHU12 – from Site 1.

Hill Laboratories
TRIED, TESTED AND TRUSTED

R J Hill Laboratories Limited
28 Duke Street Frankton 3204
Private Bag 3205
Hamilton 3240 New Zealand

T 0508 HILL LAB (44 555 22)
T +64 7 858 2000
E mail@hill-labs.co.nz
W www.hill-laboratories.com

Certificate of Analysis

Page 1 of 2

Client:	School of Biological Sciences	**Lab No:**	2254516 SPv1
Contact:	Dr I Marsden	**Date Received:**	07-Oct-2019
	C/- School of Biological Sciences	**Date Reported:**	11-Oct-2019
	Private Bag 4800	**Quote No:**	101717
	Christchurch 8140	**Order No:**	663065
		Client Reference:	
		Submitted By:	Dr I Marsden

Sample Type: Sediment

Sample Name:		IHU 1 30-Sep-2019	IHU 2 30-Sep-2019	IHU 3 30-Sep-2019	IHU 4 30-Sep-2019	IHU 5 30-Sep-2019
Lab Number:		2254516.1	2254516.2	2254516.3	2254516.4	2254516.5
Total Recoverable Phosphorus	mg/kg dry wt	260	330	370	430	720
Total Nitrogen*	g/100g dry wt	< 0.05	< 0.05	< 0.05	< 0.05	0.08

Sample Name:		IHU 6 30-Sep-2019	IHU 7 30-Sep-2019	IHU 8 30-Sep-2019	IHU 9 30-Sep-2019	IHU 10 30-Sep-2019
Lab Number:		2254516.6	2254516.7	2254516.8	2254516.9	2254516.10
Total Recoverable Phosphorus	mg/kg dry wt	560	780	490	530	810
Total Nitrogen*	g/100g dry wt	0.06	0.09	< 0.05	0.05	0.12

Sample Name:		IHU 11 30-Sep-2019	IHU 12 30-Sep-2019			
Lab Number:		2254516.11	2254516.12			
Total Recoverable Phosphorus	mg/kg dry wt	300	370	-	-	-
Total Nitrogen*	g/100g dry wt	< 0.05	< 0.05	-	-	-

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.
Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Sediment

Test	Method Description	Default Detection Limit	Sample No
Environmental Solids Sample Drying*	Air dried at 35°C Used for sample preparation. May contain a residual moisture content of 2-5%.	-	1-12
Environmental Solids Sample Preparation	Air dried at 35°C and sieved, <2mm fraction. Used for sample preparation. May contain a residual moisture content of 2-5%.	-	1-12
Total Recoverable digestion	Nitric / hydrochloric acid digestion. US EPA 200.2.	-	1-12
Total Recoverable Phosphorus	Dried sample, sieved as specified (if required). Nitric/Hydrochloric acid digestion, ICP-MS, screen level. US EPA 200.2.	40 mg/kg dry wt	1-12
Total Nitrogen*	Catalytic Combustion (900°C, O2), separation, Thermal Conductivity Detector [Elementar Analyser].	0.05 g/100g dry wt	1-12

IANZ ACCREDITED LABORATORY

This Laboratory is accredited by International Accreditation New Zealand (IANZ), which represents New Zealand in the International Laboratory Accreditation Cooperation (ILAC). Through the ILAC Mutual Recognition Arrangement (ILAC-MRA) this accreditation is internationally recognised.
The tests reported herein have been performed in accordance with the terms of accreditation, with the exception of tests marked *, which are not accredited.