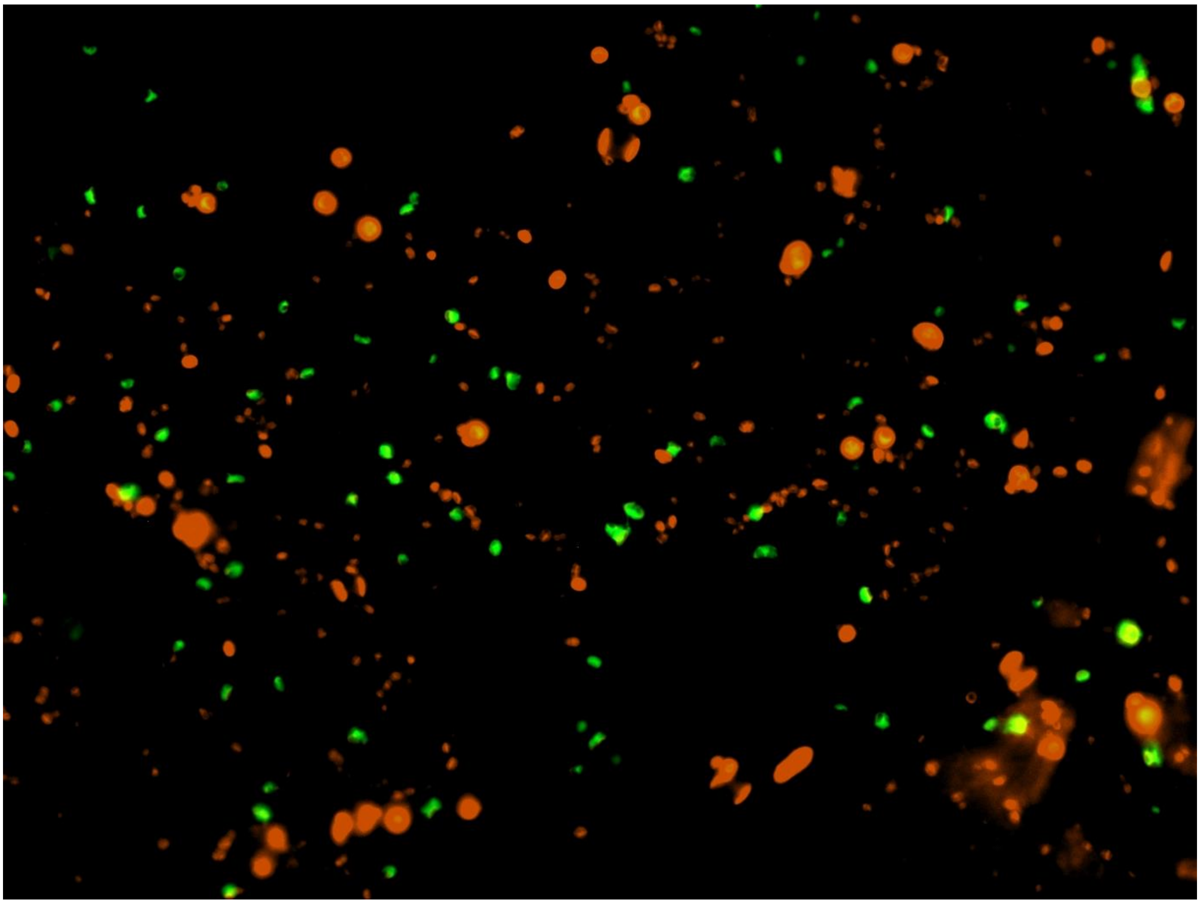
Influence of environmental factors on the distribution and dynamics of pico- and nanoplanktonic protists



Dr. Katarzyna Piwosz

Habilitation dissertation

Appendix 5: Summary of Professional Accomplishments

1. Name and Surname

Katarzyna Piwosz

2. Diplomas, degrees conferred in specific areas of science, including the name of the institution which conferred the degree, year of degree conferment, the title of the Ph.D. dissertation

18 June 2004 – **Master of Science in Oceanography**, Marine Biology, Faculty of Biology, Geography and Oceanology, University of Gdańsk;

31 March 2006 – **Master of Science in Marine Microbiology**, International Max Planck Research School of Marine Microbiology (Marmic);

9 December 2009 – **Ph.D. in Earth Science**, Oceanology, Institute of Oceanology Polish Academy of Science in Sopot. Ph.D. dissertation title: *Diversity of bacterivorous nanoflagellates in the Gulf of Gdańsk during the summer season and their interactions with pelagic bacterial communities*. Supervisors: prof. Jakob Pernthaler (University of Zurich), prof. dr. hab. Jan Marcin Węsławski (Institute of Oceanology PAS).

3. Information on employment in research institutes or faculties/departments

1 January 2009 – 31 March 2009: **the University of Zurich**, Limnological Station, Institute of Plant Biology, Switzerland, **assistant** (40% employment);

8 June 2009 – 31 December 2009: **Institute of Oceanology PAS**, Department of Marine Ecology, **assistant** (full-time employment) in the project: *Climate effects on planktonic food quality and trophic transfer in Arctic Marginal Ice Zones* (Norwegian Research Council project no. 178766/S30);

Since 1 February 2010: **National Marine Fisheries Research Institute (NMFRI),** Department of Fisheries Oceanography and Marine Ecology,

- **senior specialist**, initially half-time employment, since 1 January 2011 full-time employment;
- **adjunct** since 6 June 2011;
- **deputy of the department head** from 1 September 2014 till 30 April 2015 (I resigned after accepting a position abroad), tenure position since 1 February 2015, unpaid leave on my request from 1 June 2015 until 31 December 2020.

I was employed in the NMFRI statutory projects: *Ecology of benthos in the Vistula Lagoon* (project no. O-156) and *Co-occurrence of freshwater and marine populations of bacteria and nanoflagellates in the Vistula Lagoon* (project no. O-160); **two international projects of which I was the leader:** *CBOL: Seasonal differences in diversity of bacteria, and pico- and nanoplanktonic protists in three zones of the Vistula River estuary* (the Ministry of Science and Higher Education (MSHE) project no. 795/N-CBOL/2010/0) and *DEMONA: Diversity and ecology of mixotrophic nanoflagellates in the Gulf of Gdańsk* (Polish-Swiss Research Programme, project no. PSPB-036/2010), and of the NMFRI statutory project led by me: *Changes at the base of trophic webs in the south Baltic* *due to the anthropogenic pressure: analysis of the NMFRI database on primary production, phytoplankton, and bacterioplankton* (project no. P9-2/15).

1 June 2015 – 31 December 2020: **Institute of Microbiology, Czech Academy of Sciences**, Centre Algatech, prof. Michal Koblížek's Laboratory of Anoxygenic Phototrophs, Czechia, **adjunct**, full-time employment in projects: *How fast is the microbial loop?* (Czech Science Foundation (GAČR), project no. 13-11281S), *PhotoGemm+: Phototrophic Gemmatimonadetes* (GAČR, project no. 19-28778X) and *ALGATECH plus* (Ministry of Education, Youth, and Sport, project no. LO1416). **Leader of two projects**: *Factors regulating the phototrophic activity of the freshwater community of Betaproteobacteria* (GAČR, project no. 15-12197S) and *AAPs rule! Aerobic anoxygenic phototrophs: their role in carbon utilization under the light in freshwater ecosystems* (GAČR, project no. 18-14095Y).

4. Description of the scientific achievement set out in art. 219 para 1 point 2 of the Act

a) Title of the scientific achievements

Influence of environmental factors on the distribution and dynamics of pico- and nanoplanktonic protists.

My scientific achievement consists of six thematically related articles published in prestigious scientific journals indexed by the Institute for Scientific Information in Philadelphia, USA, of which I was the first and corresponding author.

b) List of publications contributing to scientific achievement.

1. **Piwosz K,** Wiktor JM, Niemi A, Tatarek A, Michel C. 2013. *Mesoscale distribution and functional diversity of picoeukaryotes in the first-year sea ice of the Canadian Arctic.* **The ISME Journal** 7 (8): 1461-1471

My contribution to this work included presenting the hypothesis and conceptualization of the study, preparing the grant application to the MSHE (project no. 695/N-ARCTICNET/2010/0), conducting the CARD-FISH procedure and microscopic analysis that are presented in figures 3b and 4, and in table 2, conducting the statistical analyses that are presented in figures 2 (Mann-Whitney test) and 5 (non-parametric multidimensional scaling: nMDS), and in table 3 (Spearman correlations), interpretation of the results, preparation of figures 2-5, preparation of tables 1-3, writing the manuscript, and preparation of the supplementary materials available on-line (tables S1-S2). I estimate my contribution to the work to be 75%.

2. **Piwosz K**, Spich K, Całkiewicz J, Weydmann A, Kubiszyn A, Wiktor J. 2015. *Distribution of small phytoflagellates along an Arctic fjord transect.* **Environmental Microbiology** 17 (7): 2393-2406

My contribution to this work included presenting the hypothesis and conceptualization of the study, conducting the CARD-FISH procedure, interpretation of the results, preparation of figures 5-6, preparation of tables 1 and 2, writing the manuscript, and preparation of the supplementary materials available on-line (tables S1-S3). I estimate my contribution to the work to be 64%.

3. **Piwosz K,** Kownacka J, Ameryk A, Zalewski M, Pernthaler J. 2016. *Phenology of cryptomonads and the CRY1 lineage in a coastal brackish lagoon (Vistula Lagoon, Baltic Sea).* **Journal of Phycology** 52 (4): 626-637

My contribution to this work included the hypothesis and conceptualization of the study, obtaining funding from the Polish Ministry of Science and Higher Education (project no. 795/N-CBOL/2010/0) and the Polish-Swiss Research Programme (project no. PSPB-036/2010), leading these projects sampling, performing phylogenetic analysis shown in figure 2, designing a new oligonucleotide probe for the CRY1 lineage, conducting the CARD-FISH procedure and microscopic analysis that are presented in figures 3E-6, and in table 2, performing the statistical analyses that are presented in figure 7 (distance-based redundancy analysis: db-RDA), interpretation of the results, preparation of figures 2-7, preparation of tables 1-2, writing the manuscript, and preparation of the supplementary materials available on-line (figures S1-S2). I estimate my contribution to the work to be 90%.

4. **Piwosz K,** Całkiewicz J, Gołębiewski M, Creer S. 2018. *Diversity and community composition of pico- and nanoplanktonic protists in the Vistula River estuary (Gulf of Gdansk, Baltic Sea).* **Estuarine Coastal and Shelf Science** 207: 242-249

My contribution to this work included presenting the hypothesis and conceptualization of the study, obtaining funding from the Polish Ministry of Science and Higher Education (project no. 795/N-CBOL/2010/0) and the Polish-Swiss Research Programme (project no. PSPB-036/2010), leading these projects, sampling, contributing to molecular work, analysis of data presented in figures 3-4, and in table 1, performing statistical analyses (Principal Component Analysis (PCA, figure 2), db-RDA (figure 5), the test of homogeneity of dispersions (PERMDISP), Permutational multivariate analysis of variance (PERMANOVA), Spearman correlations and Mantel tests (tables 2-3)), interpretation of the results, preparation of figures 2-5, preparation of tables 1-3, writing the manuscript, and preparation of supplementary materials available on-line. I estimate my contribution to the work to be 63%.

5. **Piwosz K.** 2019. *Weekly dynamics of abundance and size structure of specific nanophytoplankton lineages in coastal waters (Baltic Sea).* **Limnology and Oceanography** 64 (5): 2172-2186

I am the only author of this work. I collected samples, conducted the molecular and phylogenetic analysis, designed new oligonucleotide probes for the genus *Haptolina* (Haptophyta) and species *Apedinella radians* and *Pseudopedinella elastica* (Pedinellids), conducted the CARD-FISH procedure and microscopic analysis that are presented in figures 2-3, conducted the size-distribution analysis based on measurements of over 11 000 cells, which are presented in figures 4-5, conducted statistical analysis (db-RDA), results of which are shown in figure 6, interpreted the results, prepared all figures and table, wrote the manuscript, and prepared the supplementary material available online (figures S1-S4 and tables S1-S2). I obtained the funding from the Polish Ministry of Science and Higher Education (project no. 795/N-CBOL/2010/0) and the Polish-Swiss Research Programme (project no. PSPB-036/2010). Moreover, the open access fee was covered by project ALGATECH plus (LO1416) funded by the Ministry of Education, Youth, and Sport of the Czech Republic, in which I was an investigator.

6. **Piwosz K**, Shabarova T, Pernthaler J, Posch T, Šimek K, Porcal P, Salcher MM. 2020. *Bacterial and eukaryotic small-subunit amplicon data do not provide a quantitative picture of microbial communities, but they are reliable in the context of ecological interpretations.* **mSphere** 5: e00052-20

My contribution to this work included presenting the hypothesis and conceptualization of the study, obtaining funding from the Czech Science Foundation (project no. 18-14095Y), leading the project, collecting samples for the eukaryotic data set, conducting the CARD-FISH procedure and microscopic analysis that are presented in figures 1A-B and supplementary figures S1-S5, performing the statistical analysis (linear regression, Spearman correlation, Distance-based Linear Models: DistLM) that are presented in figures 1C-D, 2C-D, tables 1-3, interpretation of the results, preparation of all figures, preparation of tables 1-3, writing the manuscript, preparation of the supplementary materials available on-line (figures S1-S8, tables S1-S2). I estimate my contribution to the work to be 60%.

Bibliometric summary:

Article number, abbreviated journal name, year of publication	**Citations**		**IF**	**IF5**	**MSHE points**
	WoK	**Scopus**			
1. ISME J, 2013	35	36	9.267	9.296	140
2. Environ Microbiol, 2015	14	10	5.932	6.288	140
3. J Phycol, 2016	8	8	2.608	2.809	100
4. Estuar Coast Shelf Sci, 2018	4	4	2.611	2.975	100
5. Limnol Oceanogr, 2019	2	2	3.778	4.278	140
6. mSphere, 2020	4	6	4.282	4.466	100
Sum	**67**	**66**	**28.478**	**30.112**	**720**

The number of citations based on the Web of Knowledge (WoK) and Scopus databases accessed on 3rd of November, 2020. Impact factor (IF) and five-year IF (IF5) are given for the year of publication, and 2019 for the publication from 2020. Points of the Ministry of Science and Higher Education (MSHE points) are based on the appendix to the announcement of the MSHE issued on 31 July 2019.

c) Summary and discussion of the aim and results of the above-listed publications and their application potential.

Planktonic protists are the key component of aquatic environments. They contribute approximately 40% to the biomass of all living organisms in the oceans (Bar-On et al., 2018), and about 30% to the global primary production (Field et al., 1998; Armbrust et al., 2004). Unicellular algae form the base of pelagic food webs. They fix inorganic carbon that either directly flows to the higher trophic levels when algae are grazed by zooplankton, or it is released to the surrounding water in form of dissolved organic matter (DOM), where it drives bacterial production (Azam et al., 1983; Pernthaler, 2005;

Azam and Malfatti, 2007). It is estimated that up to 40% of primary production enters microbial food webs and is utilized by prokaryotic microorganisms (bacteria and archaea) (Fouilland and Mostajir, 2010). Prokaryotes are consumed by small (< 5 µm) mixotrophic and heterotrophic flagellates (Porter et al., 1985; Zubkov and Tarran, 2008; Šimek et al., 2014), which are themselves a food source for larger protists (Piwosz and Pernthaler, 2011; Grujčić et al., 2018; Šimek et al., 2020). These larger protists are grazed by zooplankton, allowing some portion of the primary production that was initially released as DOM to enter the higher trophic level (Azam and Malfatti, 2007). The proportion of small species in phytoplankton communities increases as a result of the ongoing climate change (Daufresne et al., 2009; Morán et al., 2010), resulting in a decrease in the total phytoplankton biomass over the last century (Boyce et al., 2010). This phenomenon has consequences for both macro- and microbial food webs and, therefore, for the functioning of ecosystems.

The cell size of planktonic protists ranges over three orders of magnitude. The smallest known eukaryote, *Ostreoccous taurii* (Mamiellophyceae), is only 0.8 µm, while planktonic Radiolaria can reach up to 1 mm (Dennett et al., 2002; Finkel et al., 2010; Biard et al., 2016). Three planktonic size fractions are recognized: microplankton (> 20 µm), nanoplankton (2-20 µm), and picoplankton (< 2 µm). Biodiversity and ecology of the largest fraction that includes diatoms, dinoflagellates, ciliates, and radiolarians, to name a few, is relatively well known. Species belonging to these groups can be identified based on conspicuous morphological features using light microscopy. In contrast, pico- and nanoplanktonic protists are much less studied. Their morphological diversity is low, and sample fixation often deforms the fragile cells (Jeuck et al., 2017). Identification to the species level requires observations of both live specimens and ultrastructures with electron microscopy (Novarino, 2012), which makes the efficient processing of numerous samples required for ecological studies infeasible. The enormous phylogenetic diversity of pelagic protists was realized only after the application of molecular methods, such as cloning and sequencing of 18S rRNA genes isolated directly from the environment (Lopez-Garcia et al., 2001; Moon-van der Staay et al., 2001). The rapid development of high-throughput sequencing (HTS) methods has enabled to obtain the sequences of millions of 18S rRNA genes from a single sample, allowing for the detection of very rare lineages (de Vargas et al., 2015). These discoveries have completely altered our knowledge of protistan diversity and changed the view on the evolution of eukaryotic organisms (Burki et al., 2020).

However, high-throughput sequencing methods do not provide accurate information on the contribution of specific lineages to microbial communities (Smith et al., 2017). Such quantitative data can be obtained with catalyzed reporter deposition – fluorescence *in situ* hybridization (CARD-FISH, Not et al., 2002, Pernthaler et al., 2004). This method applies specific oligonucleotide probes that bind to rRNA molecules in ribosomes, allowing for visualization and identification of microbial lineages at a single cell level (Amann and Fuchs, 2008). CARD-FISH enables not only to estimate the abundance of selected pico- and nanoplanktonic protists (Lim et al., 1999) but also their cell size, biomass, and trophic role based on the food vacuole content (Piwosz and Pernthaler, 2010, 2011; Grujčić et al., 2018; Šimek et al., 2020). While an enormous amount of information on the phylogenetic diversity of protists has accumulated during the last 20 years, the knowledge on the distribution and phenology of specific clades, their dynamics, and their dependence on environmental conditions is still negligible. Based on the ecological phenomena described for microplankton, it is frequently speculated that the smaller fractions also show cyclic seasonal patterns driven by changes in temperature, salinity, and nutrient concentrations. However, studies verifying these assumptions are scarce. Therefore, these were the questions I focused on in my habilitation dissertation.

The scientific achievement described in my habilitation dissertation consists of six thematically related articles published in prestigious scientific journals indexed by the Institute for Scientific Information in Philadelphia (US), as listed in point 4b. **Their overarching goal was to elucidate the effects of environmental factors on the distribution and dynamics of pico- and nanoplanktonic protists**. I formulated three hypotheses:

1. Environmental factors affect the distribution and dynamics of specific lineages and community composition of pico- and nanoplanktonic protists;
2. Spatial distribution and community composition of pico- and nanoplanktonic protists along environmental gradients is driven both by the response to the changing environmental factors (species sorting) and the inflow of new specimens with water masses (mass effects);
3. Seasonal changes in the composition of pico- and nanoplanktonic communities result from temporal niche separation of specific lineages and are caused by their response to the fluctuating environmental conditions.

These hypotheses were verified based on the field studies in aquatic environments of different salinity: first-year sea-ice brines, polar seas, the brackish Baltic Sea and Vistula Lagoon, and the freshwater Vistula and Elbląg Rivers. Moreover, these results provided new knowledge on the morphology of novel lineages of pico- and nanoplanktonic protists and their potential role in microbial food webs. My research builds on the state of the art molecular techniques (high throughput sequencing of 18S rRNA gene amplicons, CARD-FISH), and had an interdisciplinary character, combining ecology, phylogenetics, and multidimensional statistical analysis. They were funded from seven research projects (including four international) financed through national and international competitions, in which I was an investigator or the leader:

1. *'KONGHAU: The Kongsfjord-Hausgarten transect case study: Impact of climate on Arctic marine community structures and food webs.* (2010) **An international project** 658/W-HAUSGARTEN/2010/0 funded by the MSHE. Role: **investigator**;
2. *ARCTICNET: Mesoscale distribution of sympagic and pelagic protists in the sea ice cover of the Canadian Archipelago*. (2010-211). **An international project** 695/N-ARCTICNET/2010/0 funded by the MSHE. Role: **investigator**;
3. *Co-occurrence of freshwater and marine populations of bacteria and nanoflagellates in the Vistula Lagoon*. (2011) A statutory project of the NMFRI no. O-156. Role: **Main investigator**;

4. *CBOL: Seasonal Differences in Diversity of Bacteria, and Pico and Nanoplanktonic Protists in Three Zones of the Vistula River Estuary*. (2010-2014) **An international project** 795/N-CBOL/2010/0 funded by the MSHE. Role: **Project leader**;
5. *DEMONA: Diversity and Ecology of Mixotrophic Nanoflagellates in the Gulf of Gdańsk.* (2012-2015) **An international project** PSPB-036/2010, supported by Switzerland through the Swiss Contribution to the enlarged European Union (Polish-Swiss Research Programme). Role: **Project leader**;
6. *Changes at the base of trophic webs in the south Baltic due to the anthropogenic pressure: analysis of the NMFRI database on primary production, phytoplankton, and bacterioplankton*. (2015) A statutory project of the NMFRI no. P9-2/15. Role: **Project leader**;
7. *AAPs rule! Aerobic anoxygenic phototrophs: their role in carbon utilization under the light in freshwater ecosystems*. (2018-2020) **A junior project for young scientists** no. 18-14095Y funded by the Czech Science Foundation (GAČR). Role: **Project leader**.

The first article contributing to my habilitation dissertation was published in **The ISME Journal**, a prestigious journal of the International Society for Microbial Ecology (**Piwosz** et al. (2013) *Mesoscale distribution and functional diversity of picoeukaryotes in the first-year sea ice of the Canadian Arctic*. ISME J 7: 1461-1471). This study focused on the influence of environmental factors on the distribution of picoplanktonic protists in the first-year sea-ice in the Canadian Arctic. This type of sea-ice is projected to be the only form within the next two decades due to the ongoing global warming (IPCC, 2014), which will significantly alter the functioning of the Arctic ecosystems (Kędra et al., 2015). Our study was the first to apply the CARD-FISH method to investigate specific lineages of picoplanktonic protists in this unique habitat. These microorganisms probably freeze in during the sea ice formation in autumn, as it was shown in the microplanktonic fraction (Różańska et al., 2008). Their total abundance turned out to be an order of magnitude higher than previously estimated (Bachy et al., 2011). Chlorophytes (Chlorophyta) and cryptophytes (Cryptomonadales) were the dominant groups, each accounting for about 30% of the total abundance. This contradicted the earlier reports on the dominance of haptophytes (Haptophyta) (Alou-Font et al., 2013). Moreover, Pavlovales, reported from the sea-ice for the first time, turned out the most abundant haptophytes. All analysed groups of algae (those mentioned above and bolidophytes (family Bolidophyceae) contained bacteria in food vacuoles, indicating they were mixotrophic. Heterotrophic bacterivorous flagellates were less abundant than mixotrophs. Flagellates from the MAST-2 and MAST-6 lineages (Stramenopiles) were reported for the first time in the sea ice. Interestingly, the MAST-2 flagellates were three orders of magnitude more numerous than in the pelagic zone of the open ocean, suggesting that the first-year sea-ice may be their preferred habitat. Another novel observation was a high abundance of parasitic Syndiniales (Dinoflagellata), which indicates a significant impact of parasitism on the communities of sympagic algae. Altogether, these results demonstrate the importance of the picoplanktonic protists and their diverse trophic roles in the first-year sea ice. Statistical analyses showed that both abiotic (thickness of the snow cover) and biotic (chlorophyll-*a* concentration, the abundance of other groups) factors correlated with the abundance of some lineages, positively verifying the first hypothesis of this habilitation dissertation, and suggesting the existence of complex ecological interactions between picoplanktonic protists in the first-year sea-ice.

I pursue my research on the effect of environmental factors on the distribution of pico- and nanoplanktonic protists in the Arctic in the marine waters of the western Spitsbergen. It was also the first study using the CARD-FISH method in this area. Its results were presented in the second article of my habilitation thesis and were published in **Environmental Microbiology**, a prestigious journal of the Society for Applied Microbiology (**Piwosz** et al. (2015) *Distribution of small phytoflagellates along an Arctic fjord transect*. EMI 17: 2393-2406). The study aimed to understand how the abundances of specific algal lineages change along the environmental gradients from the open ocean to the inner basins of Kongsfjorden. I focused on groups with cell sizes below 10 µm because they account for over 95% of the abundance and biomass of pico- and nanoplankton in these waters (Not et al., 2005; Iversen and Seuthe, 2011). The abundances of the studied lineages were the highest in the open sea. Similarly, their percentage contribution to the total abundance of small algae decreased from 65% at the most outer station to 40% inside the fjord. Chlorophytes were the most abundant group at all stations, followed by Haptophyta dominated by *Pheocystsis* spp. (Prymnesiophyceae, Phaeocystales). This genus has two main morphological forms: colonies, which often form blooms in spring, and free-living flagellates. The ecology of the latter form is less understood due to the difficulties in reliable identification using light microscopy (Rousseau et al., 2007). We showed that *Pheocystsis* spp. was the dominant haptophyte also in summer. Interestingly, in a concurrent study on microphytoplankton communities using light microscopy this genus was not recorded (Kubiszyn et al., 2014). Moreover, the presence of the Pavlovales family was observed for the first in the waters of the western Spitsbergen. The innovative use of the fuzzy clustering methods in phytoplankton research combined with the redundancy analysis indicated that abundances of the studied algal lineages were affected mainly by changes in the environmental conditions, while the transport with open ocean waters was less important. These findings support the first and second hypotheses of my habilitation dissertation. As the inflow of oceanic species with the Atlantic waters is an important factor affecting the microphytoplankton communities in Kongsfjorden (Hop et al., 2002; Wiktor and Wojciechowska, 2005), these results suggest that pico- and nanoplankton communities may differently response to changes in environmental conditions. This is important because the influx of warm Atlantic waters to the areas of West Spitsbergen intensifies as a result of climate warming (Walczowski et al., 2012).

The above-described research provided evidence that the distribution of pico- and nanoplanktonic protists is affected by environmental factors. However, as I showed in my doctoral dissertation (Piwosz, 2009; Piwosz and Pernthaler, 2010, 2011), the abundance of individual protistan lineages can vary over two orders of magnitude within a week. Since field campaigns in polar waters are challenging except for the summertime, conducting a seasonal study there would require resources that were unavailable for me. Therefore, in my subsequent research, I focused on the coastal waters of the Baltic Sea intending to enhance the knowledge of how environmental conditions affect the temporal dynamics of pico- and nanoplanktonic protists. In the first study from this area and the third included in my habilitation dissertation, I investigated the influence of environmental factors on the dynamics and distribution of cryptophytes in the Vistula Lagoon. These results were published in the **Journal of Phycology**, the flagship journal of the Phycological Society of America (**Piwosz** et al. (2016) *Phenology of cryptomonads and the CRY1 lineage in a coastal brackish lagoon (Vistula Lagoon, Baltic Sea)*. J Phycol 52: 626-637). I focused on cryptophytes (order Cryptomonadales), whose importance in the Vistula Lagoon had been documented (Dmitrieva and Semenova, 2012). Once again, this was the first study using the CARD-FISH method in these waters. Cryptophytes were the most abundant in the freshwaters of the Elbląg River in spring during a phytoplankton bloom. However, their highest contribution (approx. 40%) to the total abundance of nanophytoplankton was observed in summer at a salinity of approx. 2. Interestingly, cryptophytes were the dominant nanoplankton group according to the Utermöhl method using the light microscopy because this classical approach underestimated the abundance of algae smaller than 10 μm over 10-fold compared to CARD-FISH. In addition, the CARD-FISH method allowed to estimate abundances of specific lineages. Cryptomonoadaceae and Pyrenomonadaceae peaked in spring at the freshwater station, while species *Proteomonas sulcata* was the most abundant in August at the freshwater station and in autumn at stations with salinity between 2 and 4. Such differences indicate the niche differentiation between these lineages, which was also supported by the non-parametric redundancy analysis (dbRDA). The key environmental factors explaining the distribution and dynamics of the studied cryptophyte lineages were salinity and concentration of dissolved inorganic nitrogen (nitrate, nitrite, and ammonia). These results provided further evidence supporting the first and third hypotheses of my habilitation dissertation. However, the most important, although the unexpected, outcome was the discovery that the most abundant cryptophyte lineage in the Vistula Lagoon had been the so-called CRY1 clade, known only from environmental sequences. The phylogenetic analysis of the nuclear 18S rRNA gene for the first time confidently placed the CRY1 clade inside Cryptomonadales, as a sister branch to the known species of the Cryptophyceae family (with 100% bootstrap support). This enabled to design a new, specific oligonucleotide probe, which was subsequently used to estimate the abundance of this clade in the Vistula Lagoon, showing it accounted for up to 80% of all cryptophytes. Morphological analyses of almost 1000 cells hybridized with the new probe revealed that their average size was below 4 μm. Surprisingly, the members of the CRY1 clade turned out to be heterotrophic, in contrast to the species of family Cryptophytceae. This finding was of great significance for understanding the evolution of eukaryotes, as it provided the evidence against a hypothesis on the common origin of Cryptista, Haptista, and the so-called the SAR supergroup (Stramenopiles, Alveolata, Rhizaria) that was discussed at that time (Burki et al., 2012; Keeling, 2013; Petersen et al., 2014), and which has been finally rejected (Burki et al., 2020). Moreover, the discovery that up to 75% of all cryptophytes with a cell size below 5 μm (of which the CRY1 clade accounted for up to 100%) were heterotrophic and possibly bacterivorous, was equally unexpected. This was later confirmed in independent studies

(Grujčić et al., 2018; Šimek and in., 2020), changing the view on the role of cryptophytes in aquatic ecosystems.

The above-described study indicated salinity as an important factor affecting the abundance of cryptophytes in the Vistula Lagoon. However, due to political constraints, the samples could be collected only in the Polish part of the Lagoon, in waters with salinity below 4. Thus, to enhance the understanding of the influence of environmental factors on pico- and nanoplankton communities along the whole salinity gradient typical for the Baltic Sea (from 0 to 7), an annual study was conducted at three sites of contrasting salinity: in freshwaters of the Vistula river, at its mouth (salinity approx. 3.5) and in the open waters of the Gulf of Gdańsk (salinity > 7). These results were published in an internationally recognized journal **Estuarine Coastal and Shelf Science** (**Piwosz** et al. (2018a) *Diversity and community composition of pico- and nanoplanktonic protists in the Vistula River estuary (Gulf of Gdansk, Baltic Sea)* ECSS 207: 242-249). The phylogenetic diversity of pico- and nanoplanktonic protists was investigated using high-throughput sequencing (pyrosequencing) of the V4 fragment of the 18S rRNA amplified directly from the metatranscriptome. Pyrosequencing has now been replaced by sequencing on the Illumina platforms, but at that time it allowed to obtain longer sequence reads and thus better taxonomic resolution. The use of the metatranscriptome instead of the metagenome enabled us to focus on the active groups and to exclude dead organisms passively introduced to the Gulf of Gdańsk with waters of the Vistula River. It was the first such research in this area. 1237 protistan phylotypes at the 97% sequence similarity (OTUs: operational taxonomic units) were detected. So deep sequencing revealed for the first time the presence of Amoebozoa and Apusomonadida in the Vistula Lagoon, Pelagophyceae in the Gulf of Gdańsk, and Radiolaria (class Acantharea) in the Baltic Sea. The phylogenetic diversity of pico- and nanoplanktonic protists was similar in all seasons at all the stations, but the values of the evenness indexes (Pielou's and Shannon) were the highest in the Gulf of Gdańsk. The impact of the Vistula River on the composition of the pico- and nanoplankton community was restricted to its mouth (salinity about 3.5). Permutational analysis of variance (PERMANOVA) indicated that pico- and nanoplankton community composition did not differ significantly between these sites. Diatoms, Synurophyceae, and cryptophytes were the dominant groups. In contrast, communities in the Gulf of Gdańsk were significantly distinct from those occurring at lower salinity, and they were dominated by dinoflagellates, Mamiellophyceae, Picozoa, and *Telonemia*. The differences in the composition of communities of pico- and nanoplanktonic protists could be explained by the effect of temperature, salinity, nutrient concentrations, and the distance between stations during all seasons, as indicated by distance-based redundancy analysis. This suggests that their composition in the Gulf of Gdańsk was affected both by the environmental factors (species sorting) and the inflow of individuals from the Vistula River (mass effects), positively verifying the second hypothesis of my habilitation dissertation. The composition of the pico- and nanoplanktonic community in the Gulf of Gdańsk was more influenced by the environmental factors, while that at the mouth of the Vistula River by mass effects. These results enhanced our understanding of the impact of freshwater inflow, which is predicted to increase as a result of climate change (Kniebusch et al., 2019), on communities of pico- and nanoplanktonic protists in the brackish waters of the Baltic Sea.

The above study showed that the pico- and nanoplankton community composition was the most strongly affected by environmental conditions in the brackish waters of the Gulf of Gdańsk. Therefore, I continued my research in this area. I combined the high-throughput sequencing (HTS) and CARD-FISH to investigate the effect of environmental factors on the dynamics of pico- and nanoplanktonic protists both at the level of community and specific lineages. These results were presented in the fifth work contributing to my habilitation thesis, published in **Limnology and Oceanography**, the prestigious journal of the Association for the Sciences of Limnology and Oceanography (ASLO): **Piwosz** (2019) *Weekly dynamics of abundance and size structure of specific nanophytoplankton lineages in coastal waters (Baltic Sea)* L&O 64: 2172-2186). Weekly sampling allowed to detect 945 pico- and nanoplanktonic OTUs, which doubles the estimates for the Gulf of Gdańsk obtained in the study described above (Piwosz et al., 2018a). It is also 25% higher than the number of protistan species known from the entire southern Baltic Sea (below 700 according to Hallfors, 2004). The HTS data served also as a base to select 15 protistan lineages for detailed studies using CARD-FISH. I designed three new oligonucleotide probes. This combined approach allowed me to confirm the results from the third and fourth work of my habilitation dissertation on the presence of Pelagophyceae in the Gulf of Gdańsk and on the importance of cryptophytes from the CRY1 clade in spring. The abundance and biomass of all studied groups of protists substantially varied within one week, forming numerous short-term peaks during the season. Application of the newly designed probes for the genus *Haptolina* (and the probes for the genera *Prymnesium* and *Chrysochromulina* developed by Simon et al.,1997), and the species *Apedinella radians* and *Pseudopedinella elastica* (Pedinellales) showed that the re-occurring maxima of the abundance and biomass of haptophytes and pedinellids had been caused by consecutive peaks of the different genera or species. Such changes can be explained by the existence of the temporal niche separation between closely related lineages, as suggested in the third hypothesis of my habilitation dissertation. The cell size structure of pico- and nanoplanktonic protists (over 11 000 measured cells) also showed conspicuous temporal changes. However, variability in the cell-size decreased with the taxonomic level, and almost ceased for specific lineages, genera, or species. This suggests that the observed changes in the size structure of pico- and nanoplankton resulted from the changes in the composition of communities, and not from the physiological response of the studied lineages. The multivariate statistical analysis of data (dbRDA) indicated that the dynamics of the abundance and biomass were best explained by the concentrations of inorganic nutrients (nitrogen, phosphorous, and silica), the changes in the size structure by temperature, and the variability of the cell surface to volume ratios by the concentrations of phosphate. However, these general relationships varied between specific lineages. Altogether, these results provided further evidence for the first and third hypotheses of my habilitation dissertation.

Combining the HTS of the 18S rRNA gene amplicons and CARD-FISH techniques in the above-described study provided novel knowledge on the relationships between environmental conditions and community composition and abundance of specific lineages of pico- and nanoplanktonic protists. In addition, it created a unique opportunity to directly compare the results of these two molecular methods. HTS enables to obtain hundreds of thousands or even millions of reads from a single sample. It is currently the method of choice in the ecology of microorganisms, including pico- and nanoplanktonic protists. However, analyses using mock bacterial communities with a defined species composition showed that the contribution of sequence reads to the libraries does not correspond to the relative abundance of organisms in the community (Smith et al., 2017). In contrast, CARD-FISH allows to accurately estimate the abundance of studied lineages (Piwosz and in., 2018b). Since the support for the second hypothesis presented in my habilitation thesis comes mainly from the study that used only the HTS method (Piwosz et al., 2018a), I decided to thoroughly compare ecological models calculated based on HTS and CARD-FISH data, to verify the correctness of the conclusions from the fourth publication of my scientific achievement. The results of these analyses were published in **mSphere**, a recognized journal of the American Society for Microbiology (**Piwosz** et al. (2020) *Bacterial and eukaryotic small-subunit amplicon data do not provide a quantitative picture of microbial communities, but they are reliable in the context of ecological interpretations*. mSphere 5: e00052-20). The cooperation with scientists from the Institute of Hydrobiology of the Czech Academy of Sciences and the University of Zurich enabled to collect the largest data set obtained with both methods published so far, containing almost 900 records of bacterial and protistan relative abundances. This was the first such detailed study comparing these two approaches using natural communities of microorganisms from environmental samples. Similar comparisons for protists had been published before, but they were based on morphological analysis of species, for which the sequences of the18S rRNA gene are still unavailable in the database (Pitsch et al., 2019). The advantage of our large dataset was that the CARD-FISH method is also based on the sequence of the 18S rRNA genes, allowing for direct comparison with relative abundances obtained from the HTS data. In general, we confirmed the previous observations from the mock community studies that the percentages obtained with the HTS methods do not correspond to the actual relative abundances in the original samples. Therefore, they should not be used to infer the abundance and dynamics of individual microbial lineages. On the other hand, the conclusions drawn from multivariate statistical analyses at the level of entire microbial communities, including pico- and nanoplanktonic protists, were consistent for both HTS and CARD-FISH. Although this work did not directly test research hypotheses, it provided evidence not only for the correctness of the conclusions presented in the fourth work of my habilitation dissertation (Piwosz et al., 2018a) but also of other studies on the communities of pico- and nanoplankton protozoa, which currently usually are based solely on the HTS techniques. Its significance is evidenced by the fact that since it was published on-line in March 2020, the publication was downloaded almost 2,400 times and cited 6 times.

Pico- and nanoplanktonic protists dominate the abundance and biomass of eukaryotic plankton (Massana, 2011). Their enormous phylogenetic diversity (de Vargas et al., 2015) contributes to the various roles they play in ecosystems and food webs: from primary producers, through mixotrophs and heterotrophs with different food preferences, to symbionts and parasites (Worden et al., 2015). The quantitative importance of pico- and nanoplankton increases with anthropogenic climate warming (Daufresne et al., 2009; Morán et al., 2010), altering microbial food webs and consequently the carbon flow through aquatic ecosystems (Boyce et al., 2010). Therefore, understanding the influence of environmental factors on the distribution and dynamics of community composition of pico- and nanoplanktonic protists and of specific lineages is crucial for comprehending the ongoing and future changes in the biosphere. **The results of my interdisciplinary research, conducted mostly in international collaborations that contribute to my habilitation dissertation, were among the first, if not the first, on the ecology of specific lineages of pico- and nanoplanktonic protists in the studied ecosystems.** They revealed the presence, provided the abundance and biomass estimates, and indicated the trophic role of novel taxa in their environments. Moreover, they led to the unexpected discovery of heterotrophic cryptophytes from the CRY1 clade, which has been important not only for understanding the ecology of pico- and nanoplanktonic protists but also for the evolution of eukaryotes. The data analysis using multidimensional statistical methods allowed to show that the spatial distribution and dynamics of pico- and nanoplanktonic protists result not only from species sorting by environmental factors, such as temperature, salinity, or nutrient concentrations but also from mass effects and inflow of species with water masses, providing evidence to support the hypotheses tested in my habilitation dissertation. The results of my work were published in prestigious scientific journals listed in point 4b, and were presented at ten international conferences, such as the FEMS Congress of European Microbiologists, Symposium on Aquatic Microbial Ecology (SAME), International Congress of Protistology (ICOP), International Symposium on Microbial Ecology (ISME) and European Phycological Congress or EMBO | EMBL Symposium.

The presented basic research was not aimed at direct practical applications. Nevertheless, they may contribute to applied science. For instance, further study on the role of specific protists in microbial food webs could potentially be used in the restoration of water reservoirs or in wastewater treatment plants to eliminate pathogenic bacteria. Moreover, my results undoubtedly provide the base for subsequent field and experimental studies on the changes in the communities of pico- and nanoplanktonic protists caused by natural and anthropogenic pressure, the ecophysiological reaction of specific lineages to rising temperature, eutrophication, and pollution, or on their role in microbial food webs, which I hope to continue as an independent scientist, educating the next generation of scientists.

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5. Presentation of significant scientific activity carried out at more than one university, or scientific institution, especially at foreign institutions

My fascination with protists began during the third year of my master's studies at the Faculty of Biology, Geography, and Oceanography of **the University of Gdańsk** when I started scientific cooperation with Dr. Józef Wiktor from the **Institute of Oceanology of the Polish Academy of Sciences** (IO PAS). He was the research supervisor of my first master thesis entitled *'Summer phytoplankton in the waters of West Spitsbergen'*, officially supervised by Prof. Jan Marcin Węsławski. I participated in three scientific cruises as a student: one on the Baltic Sea and two to Spitsbergen. I presented the results of my master thesis at two scientific conferences. They also were published in scientific journals: Polish Polar Research (Wikor and **Wojciechowska**, 2005) and **Polar Biology** (**Piwosz** et al., 2009). The article in Polar Biology is the third most cited article published in 2009 and *it is in the top 1% of the articles with the highest number of citations published in this journal during the last years.*

When analysing the phytoplankton samples from Spitsbergen, I noticed the presence of numerous small flagellates, which I could not identify. I learned from the scientific literature about the importance of microorganisms in marine waters and the molecular methods used to study their ecology. To further enhance my knowledge on the subject, I enrolled in an online course on Microbial Ecology offered at the **University of Umeå**, and also in a General Microbiology course for the Biology students. After graduating from the University of Gdańsk, I successfully applied to the prestigious **International Max Planck Research School for Marine Microbiology (Marmic) at the Max Plack Institute in Bremen (Germany)**. At the same time, I started doctoral studies at the Institute of Oceanology of the Polish Academy of Sciences in Sopot. The subject of my second master thesis, which I did at the **Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI) in Bremerhaven** under the supervision of Dr. Katja Metfies and prof. Linda Medlin was the phylogenetic diversity of cryptophytes in the North Sea (title: *Cryptophyceae diversity by sequencing 18S rDNA clone libraries*). The thesis was reviewed by prof. Allan Cembella, who considered it excellent.

To continue my research on the diversity of nanoplanktonic flagellates in marine environments, I accomplished my studies in Bremen with a master's degree and started a collaboration with prof. Jakob Pernthaler from **the University of Zurich** in Switzerland, who agreed to supervise my doctoral dissertation entitled '*Diversity of bacterivorous nanoflagellates in the Gulf of Gdańsk during the summer season and their interactions with pelagic bacterial communities*.' I spent almost 2 years in Switzerland at the Limnological Station of the University of Zurich. My investigations and research stays in Switzerland were *financed by three scholarships awarded to me by the Swiss Hydrobiology and Limnology Foundation, a research fellowship from the University of Zurich, and a research project led by me and funded from the State Committee for Scientific Research*. Moreover, in 2009 I was employed at the University of Zurich as an assistant to accomplish my work. One of the key results from my Ph.D. research was the discovery of parasitic Syndiniales and heterotrophic Cercozoa in the Baltic Sea and the Gulf of Gdańsk. I was the first person in the world to observe the members of the MAST-6 lineage (Stramenopiles) visualized using a newly design specific probe and the CARD-FISH method. Based on the observations of their food vacuoles, I discovered that these heterotrophic nanoflagellates are not bacterivorous, as it was considered at that time, but omnivorous. The application of the CARD-FISH method revealed very high dynamics of the abundance of the studied nanoplanktonic flagellates. These results were presented at four scientific conferences (including ASLO Aquatic Sciences Meeting, and The International Council for the Exploration of the Sea Annual Science Conference: ICES ASC), and were published in prestigious international scientific journals: *Environmental Microbiology* (**Piwosz** and Pernthaler, 2010), *PlosOne* (**Piwosz** and Pernthaler, 2011), and *Limnology and Oceanography* (**Piwosz** et al., 2013).

The results obtained during my master's and doctoral studies were an inspiration to the research conducted for my habilitation dissertation, as described in detail in point 4. Upon submitting my doctoral dissertation to reviewers, I was employed as an assistant at the **Institute of Oceanology PAS** in Sopot in the project '*Climate effects on planktonic food quality and trophic transfer in Arctic Marginal Ice Zones*', funded by the Norwegian Research Council (project no. 178766 / S30). I was also involved in the preparation of two applications for international research projects, in which I was subsequently employed as an investigator. I co-authored two publications on the ecology of planktonic protists in the Arctic Ocean, both published in prestigious scientific journals: *Journal of Plankton*

Research (Kubiszyn et al., 2014) and *Environmental Microbiology* (**Piwosz** et al., 2015) that were the outcome of the project *KONGHAU: The Kongsfjord-Hausgarten transect case study: Impact of climate on Arctic marine community structures and food webs* (MSHE project No. 658/W-HAUSGARTEN/2010/0). At the same time, I established cooperation **with Dr. Christina Michelle and Dr. Andrea Niemi from the Fisheries and Oceans Canada** as we worked together in the project *ARCTICNET: Mesoscale distribution of sympagic and pelagic protozoa in the ice cover of the Canadian Archipelago* (MSHE project No. 695/N-ARCTICNET/2010/0). This collaboration resulted in the publication in the prestigious *The ISME Journal* (**Piwosz** et al., 2013). Both articles in which I am the first author are part of my habilitation dissertation (publications no. 1 and 2).

In 2010, shortly after defending my Ph.D., I was employed at the **Sea Fisheries Institute in Gdynia** (currently the **National Marine Fisheries Research Institute**: NMFRI), first as a senior specialist, and then as an adjunct at the Department of Fisheries Oceanography and Marine Ecology. Initially, I was involved in the work on the phenology of cryptophytes in the Vistula Lagoon, financed by the statutory project. These results were published in the *Journal of Phycology* (**Piwosz** et al., 2016; publication no. 3 of this habilitation dissertation). I established the CARD-FISH technique in my new working place. I also participated in research on planktonic and benthic bacteria in the Vistula Lagoon, the results of which are currently under review. At the same time, I started a collaboration with **prof. Simon Creer from the University of Bangor in Great Britain, and with dr. hab. Marcin Gołębiewski from the University of Nicolaus Copernicus in Toruń**. We worked in the joint international project *CBOL: Seasonal differences in the biodiversity of bacteria and nano- and picoplanktonic protists from three zones of the Vistula estuary* (MSHE project No. 795/N-CBOL/2010/0), led by me. The key finding of research on bacterial communities was that populations present along the investigated salinity gradient had consisted of sub-populations specific to certain conditions (temperature and salinity) that could be distinguished based on different variants of the 16S rRNA gene. Moreover, we showed that the adaptation of freshwater bacteria to the brackish water was rare but still more frequent than the adaptations of brackish bacteria to the riverine environment. These results were published in *Environmental Microbiology Reports* (Gołębiewski et al., 2017) and contributed to the habilitation dissertation of Dr. Marcin Gołębiewski. The research on the protistan communities contributes to my habilitation thesis (publication no. 4: **Piwosz** et al., 2018a; *Estuarine, Coastal and Shelf Science*). This international collaboration got me acquaint with the HTS methods as a tool to investigate the composition of natural microbial communities, which is one of the main techniques I use in my research until now.

Contemporarily with the research described above, I continued my collaboration with **prof. Jakob Pernthaler from the University of Zurich**. As part of the joint project *DEMONA: Diversity and ecology of mixotrophic flagellates in the waters of the Gulf of Gdańsk*, financed by the Polish-Swiss Research Program (project no. PSPB-036/2010), we studied pico- and nanoplanktonic protists in the Gulf of Gdańsk. This project funded my three research visits to the **University of Zurich** (five months in total). Its results were published in the prestigious international journals: *Limnology and Oceanography* (**Piwosz**, 2019; publication no. 5 of this habilitation) and *mSphere* (**Piwosz** et al., 2020; publication no. 6 of this habilitation). I also started cooperation with **prof. Klaus Jürgens from The Leibniz Institute for Baltic Sea Research Warnemünde** (IOW), who invited me for a three-month research internship in his group in 2014.

In 2015, I was employed as an adjunct at **the ALGATECH Centre of the Institut of Microbiology of the Czech Academy of Sciences in the Czech Republic**, in the laboratory of anoxygenic phototrophs led by prof. Michal Koblížek. My research expertise has been augmented with the area of the ecology and ecophysiology of aerobic anoxygenic phototrophic (AAP) bacteria. A characteristic feature of this functional group is its photoheterotrophic metabolism. This means that they can produce ATP from the energy of light absorbed on bacteriochlorophyll-containing reaction centres, but they do not fix carbon dioxide and thus require the organic matter to build the biomass. AAP bacteria are not a monophyletic group. Most of the described AAP species belong to the classes of Alphaproteobacteria and Gammaproteobacteria, but some species from phyla Chloroflexota and Gemmatimonadota are also known. During the first 2.5 years (2015-2017), I co-led the project '*Factors regulating the phototrophic activity of the freshwater community of Betaproteobacteria*' financed by the Grant Agency of the Czech Republic (GAČR, project no. 15-12197S), implemented in cooperation with **Dr. Vojtěch Kasalický from the Institute of Hydrobiology, Biology Centre of the Czech Academy of Sciences**. The main goal was to understand the environmental conditions influencing the expression of photosynthetic genes and the importance of photoheterotrophic metabolism in the genus *Limnohabitans* (Burkholderiales, Gammaproteobacteria), an abundant component of bacterioplankton in freshwater lakes. The cultured species and strains of this genus had shown a typical heterotrophic metabolism, and it was not until their genomes had been sequenced that their photoheterotrophic metabolic potential was realized. I was the first person to obtain and describe the photoheterotrophic phenotype in the species *Limnohabitans planktonicus*. Besides, I modified the standard FISH technique to preserve the autofluorescence of bacteriochlorophylls to identify active AAP bacteria in environmental samples. These achievements contributed to the study on photoheterotrophy in genus *Limnohabitans* and were published in *Applied and Environmental Microbiology* (Kasalický et al., 2018), a recognized journal of the American Society of Microbiology (ASM). I also participated in research on the effect of light intensity on photochemical processes and photoheterotrophic metabolism in species from other taxonomic groups: *Dinoroseobacter shibae* (Rhodobacterales, Alphaproteobacteria), *Sphingomonas* sp. (Sphingomonadales, Alphaproteobacteria), and *Gemmatimonas phototrophica* (Gemmatimonadales, Gemmatimonadales). The newly developed statistical models of the dependence of the assimilation rate of simple organic compounds on the irradiance developed for *D. shibae* were published in *Environmental Microbiology* (**Piwosz** et al., 2018). The results of research on the other two species are currently under review.

However, the focus topic of my work at that time was the ecology of photoheterotrophic bacteria in lakes. In 2016, I organized a regular sampling campaign on a nearby oligotrophic lake. I also established FISH, CARD-FISH, and HTS techniques in my new working place. Since 2018 these ecological studies have been conducted in frames of a project entitled '*AAPs rule! Aerobic anoxygenic phototrophs: their role in carbon* *utilization under the light in freshwater ecosystems*' funded by the Grant Agency of Czech Republic (GAČR, project no. 18-14095Y), of which I am the leader. Presently, my Ph.D. student, M.Sc. Cristian Villena-Alemany, analyses a four-year-long time series of biweekly collected samples to understand the dynamics of bacterial and AAP communities using amplicon sequencing of 16S rRNA and *pufM* genes. *PufM* is a marker gene for AAP bacteria encoding the M-chain of protein building photosystem reaction centres. The other aim of this project was to estimate the contribution of photoheterotrophic metabolism of AAP bacteria to the overall activity of pelagic microbial communities, what is important for understanding the carbon cycle in aquatic ecosystems. Thanks to the innovative approach and measurements of respiration and bacterial production in infrared light, which is used only by AAP bacteria, I showed that photoheterotrophy contributes on average 15% to the rates of microbial activity (Piwosz and Villena-Alemany, submitted). This means that the current assessments of microbial respiration in lakes are overestimated. I also led an experiment on the influence of light on the activity and composition of bacterial communities that was conducted in an international group of 17 scientists from around the world during the 10th GAP (Group of Aquatic Primary Productivity) workshop. We found that although the rate of primary production controlled the metabolic activity of bacteria, its rapid decrease did not cause changes in the bacterial communities. In contrast, their composition was driven by light intensity, which stimulated the growth of AAP bacteria. These results were published in *mSphere* (**Piwosz** et al., 2020b). This international cooperation will continue in 2021 as part of the project '*INNPP: Effect of decreasing inorganic N:P ratio on the plankton community*' awarded to me and financed by the European AQUACOSM program.

I also was involved in developing a method of normalizing amplicon reads using internal standards (ARNIS: amplicon reads normalization using internal standard). The main goal was to obtain a method that would allow for estimating growth rates of hundreds of bacterial phylotypes detected in sequencing libraries. Comparing the rated from ARNIS and CARD-FISH yield similar results, indicating a high potential of the new approach. These results were published in the prestigious *The ISME Journal* (**Piwosz** et al., 2018b). They also inspired me to conduct a more throughout study on the quantitative accuracy of the amplicon sequencing techniques, which is part of my habilitation dissertation (**Piwosz** et al. 2020, Publication No. 6). I subsequently led and participated in more experiments using the ARNIS approach, the results of which are still being analysed. I was also involved in other research conducted in the laboratory: on the expression of the *pufM* gene by natural AAP communities in lakes (Fecskeová et al., 2019), metagenomics studies on the ecology of Gemmatimonadetes (Mujakić et al., in review), or research on the phylogenetic diversity of pelagic bacteria in the Adriatic Sea (in cooperation with scientists from **the Institute of Oceanography and Fisheries in Split** (Šantić et al., in review). I am also advising in research on the regulation of photosynthetic genes and nitrogenase expression in diazotrophic cyanobacterium *Trichodesmium* in a project led by Dr. Meri Eichner (GAČR Project No. 20-02827Y Single-cell orchestration of nitrogen fixation and photosynthesis in *Trichodesmium*).

The results of the research described above were published mostly in recognized scientific journals and they presented at **32 scientific conferences (26 international)**, including prestigious conferences such as *ASLO Aquatic Sciences Meeting, FEMS Congress of European Microbiologists, SAME: Symposium on Aquatic Microbial Ecology, ICOP The International Congress of Protistology, and ISME International Symposium on Microbial Ecology*. I co-authored 46 presentations (36 as the first author), including 23 talks, 19 of which I presented myself. I was also invited to *deliver 3 lectures opening scientific sessions, and a plenary lecture closing the conference*. The full list of my conference presentations can be found in point 2 of Part II of the List of Scientific Achievements.

My scientific activity in the field of microbial ecology has been recognized internationally. In 2015, I was invited to join **an expert group developing and testing phytoplankton indicators of the environmental state of the Baltic Sea for the Helsinki Commission (HELCOM**; I resigned in mid-2015 due to acceptation of the position in the Czech Republic). In 2017, I became **the ISME Ambassador** (International Society of Microbial Ecology, publisher of the prestigious ISME Journal) for Poland. ISME ambassadors are invited for this function by the president of the society, and their mission is to support scientific events related to the ecology of microorganisms and to promote the Society. As part of my activity, I obtained financial support in the amount of EUR 500 each for five scientific conferences held in Poland. Besides, I helped the Polish Society of Microbiologists to obtain EUR 5,000 for the organization of their XXIX National Congress, planned for September 2021. **In 2020, my activity was recognized and I was endowed the ISME Ambassador of the Year award**.

I was invited **46** times by editors of **23** different international journals to review **37** scientific manuscripts. I reviewed for recognized journals in the field of microbial ecology and general biological sciences, such as *The ISME Journal, Environmental Microbiology, FEMS Microbial Ecology, Microbial Ecology,* and *Current Biology* (the full list can be found in point 8 of part II of the List of scientific achievements). Moreover, I was **a reviewer of** a **doctoral dissertation** by M.Sc. Mikel Aguirre entitled "Unraveling bacterioplankton ecology of the estuaries of Urdaibai and Bilbao in the Bay of Biscay by High-Throughput sequencing" defended at the University of the Basque Country (Universidad del País Vasco).

6. Presentation of teaching and organizational achievements as well as achievements in the popularization of science

Until now, I have been employed mainly in research institutes, thus I did not have an opportunity to teach students. Consequently, my achievements in this area are modest compared to those of scientists working at universities. Nevertheless, whenever possible, I gave **lectures about the ecology of microorganisms in sea waters for undergraduate and graduate students in Trójmiasto's universities and institutes**: the Institute of Oceanology of the Polish Academy of Sciences in Sopot, the Naval Academy, The Gdańsk University of Technology, the University of Gdańsk, and for **the participants of the summer school** at the ALGATECH Center. I also assisted Dr. Dai Long Vu in a statistical course in the R program held for employees of the Algatech Center. Besides, I supervised students of Oceanography at the University of Gdańsk, Mrs. Natalia Szymańska, and Natalia Czerniawska, and Mr. Adam Hanzlík, a student of Molecular Biology and Biochemistry of Organisms at the Charles University in Prague, during their internships at the NMFRI and the Algatech Center, respectively. Currently, I am the **Ph.D. advisor** for M.Sc. Izabela Mujakić (the supervisor is Prof. Michal Koblížek), a fourth-year student of the doctoral programme at the University of South Bohemia in České Budějovice (Ph.D. thesis title: Ecology of Gemmatimonadetes). I also **supervise** M.Sc. Cristian Villena-Alemany, a second-year student of this programme and a scholarship holder of the Erasmus + program (Ph.D. thesis title: Ecology of aerobic anoxygenic photoheterotrophic bacteria in freshwaters).

My organizational achievements include membership in the organizing and scientific committees of conferences and workshops. **I chaired a workshop** entitled '*Microbes in the Baltic: Small things, small sea, big questions*', held in November 2014 in Gdynia. This event was attended by over 50 microbiologists from all the countries situated over the Baltic Sea and from Switzerland. The invited plenary lectures were delivered by the internationally recognized scientists: prof. Lasse Riemann from the University of Copenhagen, prof. Jakob Pernthaler from the University of Zurich, prof. Veljo Kisand from the Institute of Technology of the University of Tartu, prof. Klaus Jürgens from the Institute for Baltic Sea Research in Warnemünde, and prof. Bärbel Müller-Karulis from the Baltic Sea Center of the University of Stockholm. I was also **a member of the local organizing committee** of the 10th GAP (Group of Aquatic Primary Productivity) workshop, and a member of the competition committees awarding Young Researcher Awards at three conferences: SEFS10 Symposium for European Freshwater Sciences (2017, Olomouc, Czech Republic), 37th International Conference of the Polish Phycological Society (2018, Kraków) and the Interdisciplinary Academic Conference on Environmental Protection (2019, Gdańsk, Poland). Moreover, I was **a member of the scientific committee** of the last of the mentioned conferences. Currently, I **am a member of the organizing committee** for the 11th Polish Hydromicrobiological Conference HYDROMIKRO2021, which is to be held next year in Sopot. Finally, I was **a founding member** of the Sopot Scientific Society and its **treasurer** in 2007-2010. Presently, together with the junior ISME ambassador, Dr. hab. Robert Czajkowski from the University of Gdańsk, we are working to establish the Polish Society for the Ecology of Microorganisms. I am a current or past member of other Polish and international scientific societies: International Society for Microbial Ecology, British Phycological Society, American Society for Microbiology, Polish Society of Microbiologists.

I was also involved in outreach activities promoting science. In 2008, I wrote an article about the algae growing on yachts for the '*Żagle'* magazine. I participated twice in the *Baltic Science Picnic*, where I presented algae living in the Baltic Sea. I am currently working on an album about protists living in the Baltic Sea, being prepared for the 100^{th} anniversary of the NMFRI.

7. Additional information about my professional career

My experience in molecular biology techniques allowed me to get involved in diagnosing patients infected with the SARS-CoV-2 virus during the COVID-19 pandemic in spring 2020. The Algatech Center of the Institute of Microbiology of the Czech Academy of Sciences, where I worked at that time, was analysing samples for a hospital in the Czech town of Jindřichův Hradec. I was responsible for the interpretation of RT-qPCR results and for reporting the diagnosis to the director of the institute. In total, almost 1,900 samples were tested in the period from the beginning of April to the end of May 2020.

8. Summary

My scientific achievements include **25** original scientific articles (**23** after obtaining the doctoral degree) published in journals indexed by the Philadelphia Institute of Scientific Information, most of which is the outcome of international cooperation**. I am the first author of 15 and the corresponding author of 9 of these articles**. The summary Impact Factor of these publications, based on the Journal Citation Reports (JCR), is **88.100** according to yearly values and **95.994** for the 5-year periods. The sum points according to the appendix to the announcement of the Ministry of Science and Higher Education issued on 31 July 2019 is **2 430**. The total citations count of these articles is **372** (317 without self-citations) according to the Web of Knowledge database, and **405** (359 without self-citations) according to the Scopus database (accessed on 3rd of November, 2020). **My Hirsh index is 10**. I have presented the results of my research at numerous conferences and symposia, authoring or co-authoring 46 talks and posters, including four invited lectures.

I was the leader of six research projects, including four international. The total quote of funding I received is about **4 000 000** PLN. I also participated as an investigator or the main investigator in another 12 projects. I have been awarded several awards and scholarships, including two scholarships from the European Molecular Biology Laboratory EMBL Advanced Training Center Corporate Partnership Program Fellowship, a Travel Award from the Federation of European Microbiological Societies (FEMS), and the International Society of Microbial Ecology award for the best Ambassador of the year 2019-2020. Moreover, I received five individual awards from the Director of the National Marine Fisheries Research Institute for the publication record.

Katarzyna Piwosz

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Applicant's signature