Studying phytoplankton parasites in a Baltic Sea spring bloom using imaging flow cytometry

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Abstract

Phytoplankton, important primary producers in aquatic environments, are known to be infected by several kinds of parasites. Chytridiomycota, or chytrids, are among the most common fungal parasites found to infect phytoplankton. They have been shown to have significant impacts on phytoplankton communities and species composition. However, even though phytoplankton parasites have been recognized for decades, many questions are still unresolved about their role and impact, especially in marine environments and in the Baltic Sea. With the help of imaging flow cytometry, such as Imaging FlowCytobot (IFCB), it is possible to continuously sample and analyse phytoplankton blooms. The IFCB takes water samples every ~20 minutes that are run through the machine, while taking images of all phytoplankton in the samples. The images are automatically classified using machine learning techniques, sorting them into categories based on morphology. Chytrid infections are possible to detect in these images, thus, IFCB can also be utilised to detect fungal parasites on phytoplankton.

In this study, phytoplankton parasites are studied using IFCB. The purpose of this thesis is to study the presence and impact of chytrid parasites on phytoplankton in the Baltic Sea, to increase the knowledge of phytoplankton parasites in the area. The material used in this study, provided by the Finnish Environmental Institute, was sampled in the Archipelago Sea with IFCB during spring bloom in 2021. The images were automatically classified in taxonomic groups, provided in daily or hourly folders. Four of these groups

were chosen for this study to be analysed further: Centrales, *Chaetoceros* spp. (provided in folders with chains and single cells separately), *Pauliella taeniata* and *Skeletonema marinoi*. The bloom period for each group was analysed for putative infections, with a set of predetermined selection criteria.

Putative infections were found on all groups, but the infection rates were lower than expected, especially on the dominating diatom *S. marinoi*. The most infected group was *P. taeniata*. Considering both infection rates and quality criteria, only Centrales and *P. taeniata* indicate actual chytrid infections, whereas infections on *S. marinoi* and *Chaetoceros* spp. are more uncertain. Further, there was no indication that the infection rates influence the growth rate of phytoplankton host blooms negatively. While temperature could explain infection abundances for most groups (except single-celled *Chaetoceros* spp.), nutrient changes did not show similar patterns. It seems, therefore, that infection abundances are more likely connected to changes in host bloom abundances than environmental changes. Future studies should include evaluation of the putative infections observed from images taken with IFCB, for example, by comparing chytrid infections detected on IFCB images with infections detected with microscopy. However, the potential of this methodology is huge, as automated phytoplankton and anomaly identification would enable more extensive research on phytoplankton parasites.

Key words

phytoplankton, phytoplankton parasitism, Chytridiomycota, chytrid parasites, infection, diatoms, Centrales, *Chaetoceros* spp., *Pauliella taeniata*, *Skeletonema marinoi*, imaging flow cytometry, Imaging FlowCytobot, IFCB

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1 Introduction

Phytoplankton, i.e., microscopic pelagic photosynthetic organisms, are important organisms at the base of the aquatic food web (Falkowski et al., 1998). Due to this, it is important to study phytoplankton communities and dynamics, to understand how they affect the marine environment. A fact that is often overlooked, however, is that phytoplankton are susceptible to parasitic infections, which can have large impacts on the phytoplankton community. The interaction between phytoplankton parasites and their hosts is still an understudied field of research within aquatic research (Frenken et al., 2017), especially in marine environments (Scholz et al., 2016).

This thesis is about fungal parasites on phytoplankton in a brackish environment: the Baltic Sea. This chapter starts with an introduction to phytoplankton, moving on to their parasites and the method imaging flow cytometry as a methodology to analyse phytoplankton and detect parasite infections on phytoplankton. At the end of this chapter, the purpose of this thesis and research questions are presented.

1.1 Spring phytoplankton in the Baltic Sea

Phytoplankton are among the most important primary producers in the world. Together with other aquatic autotrophic organisms, they are responsible for almost half of the global net primary production (Falkowski & Raven, 2007). They are also an important part of the aquatic food web and biogeochemical cycles such as carbon cycling (Falkowski et al., 1998). Phytoplankton in temperate ecosystems generally bloom in spring, summer, and sometimes in autumn. These blooms are often followed by an increase in the abundance of zooplankton, due to the increase of available food sources (Sommer et al., 2012). The spring blooms are triggered when light increases and nutrients become available in the euphotic zone at the end of winter (Sommer et al., 1986). The spring blooms can also be affected by overwintering zooplankton, as these might increase the grazing pressure on early spring blooms (Sommer et al., 2012).

In the Baltic Sea, the spring blooms consist mainly of diatoms, dinoflagellates and the mixotrophic ciliate *Mesodinium rubrum* (Hjerne et al., 2019). Among the most common groups and species of diatoms during spring blooms are *Melosira arctica, Thalassiosira levanderi, Chaetoceros* spp.*, Diatoma tenuis, Pauliella taeniata* and *Skeletonema marinoi*. Among the dinoflagellates, *Biecheleria baltica*, *Gymnodinium corollarium*, *Apocalathium malmogiense* and *Peridinella catenata* are a few of the most abundant spring bloom species (Spilling et al., 2018). The diatoms are generally the first phytoplankton to appear in spring blooms, while dinoflagellates appear later (Hjerne et al., 2019).

An earlier onset of spring blooms has been observed in the Baltic Sea, likely triggered by more sunshine, less wind and warmer temperatures (Almén & Tamelander, 2020; Hjerne et al., 2019). The phytoplankton spring blooms appear generally one to two weeks earlier in the Baltic Proper today than a few decades ago (Hjerne et al., 2019). A shift has occurred over the last few decades in the composition of the blooms, as the biomass of dinoflagellate has increased, while the biomass of diatoms has decreased (Hjerne et al., 2019; Klais et al., 2011; Spilling et al., 2018). The proportion of dinoflagellates relative to diatoms vary spatially in different areas in the Baltic Sea, as diatoms dominate in some areas while dinoflagellates dominate in others (Klais et al., 2011). Diatoms and dinoflagellates have slightly different patterns after bloom termination, as diatoms generally sink to the sediments to be buried and degraded, whereas dinoflagellates more often are integrated in the microbial food-web or produce cysts. Thus, a shift in species composition can also affect biogeochemical pathways and nutrient cycling (Klais et al., 2011; Spilling et al., 2018).

1.2 The role of phytoplankton parasites for bloom succession

An emerging question in phytoplankton community ecology is the role of parasitic infections for phytoplankton blooms. Phytoplankton are susceptible to several types of parasites, including viruses, fungi, protists and bacteria (Frenken et al., 2017; Park et al., 2004). Among these, viruses have previously been studied the most, but in recent decades

fungal parasites have received increasing attention (Frenken et al., 2017; Ibelings et al., 2004). Many fungal parasites of phytoplankton belong to the phylum Chytridiomycota, shortly referred to as chytrids (Frenken et al., 2017). They have been found to infect multiple species throughout the year (Gsell et al., 2022; Van den Wyngaert et al., 2022). Gsell et al. (2022) found that the average infection prevalence in a lake system was 2.78%, with the highest infection prevalence reaching 47.35%. Infection prevalence on diatom populations in freshwater has in some cases been found to exceed even 90% (Ibelings et al., 2011). Thus, as the host cell usually dies after being infected, chytrid parasites of phytoplankton can cause substantial mass mortalities among the phytoplankton community (Gsell et al., 2022; Ibelings et al., 2011).

Chytrid parasites have free-living zoospore stages that actively swim and search for a suitable host. Upon encounter with and recognition of a host cell, the zoospore attaches to the host. This attachment leads to encystment of the zoospore on the host cell surface and a sporangium begins to develop (Figure 1). The parasite receives nutrients from its host through a rhizoidal feeding structure that penetrates the host cell and takes up nutrients via osmotrophy (Ibelings et al., 2004; Van den Wyngaert et al., 2017). Host-specificity varies, as the infection of chytrids is often considered highly host-specific, although there are also examples of parasitic chytrids that can infect several host species (Ibelings et al., 2004; Kagami et al., 2021; Rasconi et al., 2012; Van den Wyngaert et al., 2022). The sporangium develops and matures within a few days, after which it releases asexually produced zoospores (Ibelings et al., 2011; Van den Wyngaert et al., 2017). They can also form resting spores, especially when healthy, uninfected host cells are sparse (Van den Wyngaert et al., 2017).

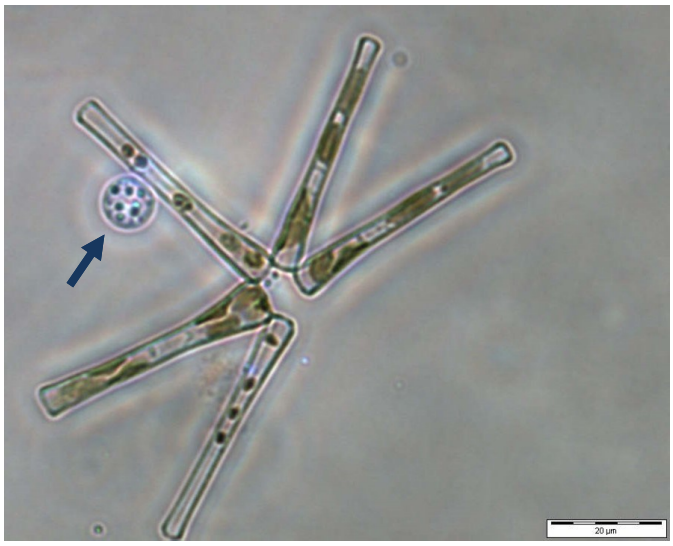


Figure 1. Infected freshwater diatom *Asterionella formosa*. The arrow indicates a chytrid sporangium. Image credit: Silke van den Wyngaert.

When the parasite population increases faster than the host population, an epidemic might arise. Some studies suggest that epidemics arise more easily when the growth conditions for the host species are poor (Ibelings et al., 2004). On the other hand, too small host populations limit the infection of parasites (Ibelings et al., 2011; Sommer et al., 2012). Both light availability and temperature have been reported as important factors affecting parasite epidemics (Gsell, de Senerpont Domis, Naus-Wiezer, et al., 2013; Ibelings et al., 2004). In many cases, however, it seems that the infection rate is mostly dependent on host cell density or the phytoplankton community composition rather than environmental factors per se (Ibelings et al., 2004; Rasconi et al., 2012). Some freshwater studies suggest that the most dominant phytoplankton species are the most susceptible within a community (Rasconi et al., 2012), but there are also studies that dispute this theory, showing that there is no difference in infection prevalence between more or less common species (Gsell et al., 2022).

Chytrids have a low infection rate in low water temperatures, which means that their host species have a window of opportunity to initiate the spring bloom while the water temperatures are still low, before infection rates start to increase. This time lag between the start of spring bloom followed by epidemic onset has been observed in several studies (Alacid et al., 2017; Gsell, de Senerpont Domis, Naus-Wiezer, et al., 2013; Ibelings et al., 2011). Ibelings et al. (2011) found that warmer winters resulted in a smaller window of

opportunity for the phytoplankton, and consequently the bloom magnitude was much smaller. This in turn resulted in absence of epidemic for the chytrids as the host densities were too low. Warmer temperatures due to climate change might therefore result in a change in the parasite-host interactions (Ibelings et al., 2011; Rohrlack et al., 2015). There is often a minimum threshold value of host density required for epidemics to occur, due to the limited searching ability of the chytrid zoospores. The reported thresholds vary between studies, depending on species and environmental conditions (Ibelings et al., 2004).

Chytrid parasites affect their hosts in several ways. They can cause mass mortalities in phytoplankton populations (Ibelings et al., 2011; Rasconi et al., 2012) and affect phytoplankton dynamics and succession (Sommer et al., 2012). Selective chytrid parasitism can affect the phytoplankton species competition if one phytoplankton species, that otherwise might dominate the bloom, is heavily infected, allowing other non-infected species to increase (Donk & Ringelberg, 1983). Additionally, some chytrid parasites have been found to prefer larger host cells to small ones (Ibelings et al., 2004) which might lead to a changed species composition of the phytoplankton community, when large dominating phytoplankton decrease due to parasitic infection, allowing smaller phytoplankton to increase (Sommer et al., 2012). Furthermore, high infection prevalence has been observed to promote genetic diversity in phytoplankton host populations, due to negative frequency-dependent selection (Gsell, de Senerpont Domis, Verhoeven, et al., 2013).

Parasitic chytrids can also have an impact on the aquatic food web. Some larger phytoplankton are unavailable for grazing by zooplankton, but infection of these species can result in carbon and nutrients being directly transferred to the parasites and further up to zooplankton through the consumption of the parasite zoospores (Kagami et al., 2014; Klawonn, Van den Wyngaert, et al., 2021). During epidemics, the biomass of parasites can be quite high, and thus the parasitic zoospores can be an important food source for zooplankton (Kagami et al., 2014). Chytrid infection might also mechanically fragment large inedible phytoplankton, making them available for grazing by zooplankton (Ibelings et al., 2004; Kagami et al., 2014). Together, these parasite-mediated pathways are referred

to as the fungal shunt (Klawonn, Van den Wyngaert, et al., 2021) and mycoloop (Kagami et al., 2014).

Although parasitism on phytoplankton has been known and studied for several decades (Canter & Lund, 1948; Donk & Ringelberg, 1983; Ibelings et al., 2004), there are still many questions that remain to be resolved regarding the role of parasites, and the interaction between parasites and their phytoplankton hosts (Frenken et al., 2017; Ibelings et al., 2004; Park et al., 2004). The role of parasites is often overlooked when studying phytoplankton blooms and interactions, especially in marine studies (Gleason et al., 2011; Scholz et al., 2016). Most studies on phytoplankton parasitism have been conducted in freshwater systems, although parasites on phytoplankton also occur in marine environments (Comeau et al., 2016; Hassett & Gradinger, 2016; Park et al., 2004). In the Baltic Sea, only two chytrid parasite species infecting dinoflagellate species have been described (Karpov et al., 2021; Reñé et al., 2022). To my knowledge, there are no previous studies on infection rates or the impact of chytrid parasites on phytoplankton populations in the Baltic Sea.

1.3 Imaging flow cytometry

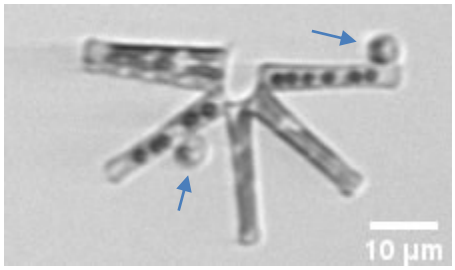
Thanks to modern techniques, it has become possible to study phytoplankton and other aquatic microorganisms more extensively. Instead of manual sampling during spring blooms, it is possible to continuously sample and analyse the emerging blooms and their succession with imaging flow cytometry (Kraft et al., 2021; Sosik & Olson, 2007). The most commonly used imaging flow cytometer for continuous deployments is the Imaging FlowCytobot, IFCB (Olson & Sosik, 2007). The IFCB takes samples continuously (new sample every ~20 minutes) and takes images of the phytoplankton when triggered by chlorophyll *a* fluorescence. This results in a large volume of images produced, which are then analysed using machine learning algorithms (Kraft et al., 2022; Olson & Sosik, 2007; Peacock et al., 2014). These images are also stored for additional future analysis.

Different kinds of automated classification of phytoplankton make it possible to analyse much larger samples and observe successional patterns. Sosik and Olson (2007) defined

22 categories of different taxonomic groups, from images taken with IFCB in a marine area of Massachusetts, USA. They then used a support vector machine (SVM) as learning algorithm to sort the different phytoplankton in respective categories. Comparison with manual classification showed that the SVM classifier was relatively accurate in identifying the different phytoplankton, with an overall classification accuracy of 88%. Kraft et al. (2022), on the other hand, used a convolutional neural network (CNN) model to classify images taken by IFCB in the Baltic Sea, using a test image data set of 63 000 images divided into 50 different phytoplankton taxonomic categories. The CNN classifier performance was very high for the test samples, with an overall F1-score of 0.95. When they applied the CNN classifier on natural samples, the performance dropped slightly but was still high (F1-score 0.82).

With the IFCB results, Sosik and Olson (2007) could plot time series abundances, observing temporal bloom patterns and changes for certain diatom species. Similarly, Kraft et al. (2022) used the IFCB data and CNN classifier during summer of 2021, continuously assessing the cyanobacterial abundances in the Baltic Sea, to publish up-to-date information online for public accessibility on the situation of harmful algal blooms. It is possible to calculate temporal changes in phytoplankton biomass, for example, during the bloom season or on even larger temporal scales comparing seasons to each other (Kraft et al., 2021; Olson & Sosik, 2007). IFCB is a powerful tool to detect bloom dynamics and drivers of bloom formation (Kraft et al., 2021).

In the images provided by the IFCB, it is also possible to distinguish parasitic infection. Peacock et al. (2014) used imaging flow cytometry to detect a parasitic nanoflagellate infecting the marine diatom *Guinardia delicatulaa*. Chytrid parasites can also be detected with the IFCB (Figure 2). The high temporal resolution of the IFCB allows novel opportunities to capture and analyse rapid parasite-phytoplankton interactions compared to classical phytoplankton bloom monitoring studies.

A 

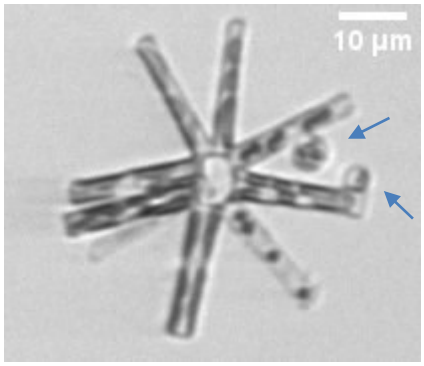
B 

Figure 2. IFCB images of the freshwater diatom *Asterionella formosa* infected by parasitic chytrids (**A-B**). Arrows indicate the chytrids. Image credit: Silke van den Wyngaert.

1.4 Purpose and problem statements

The purpose of this thesis is to study the presence and impact of chytrid parasites on phytoplankton in the Baltic Sea during the spring bloom. It is important to increase the knowledge about the role of parasites for phytoplankton blooms as it has relevance for food web interactions in the Baltic Sea ecosystem. To my knowledge, there are no published studies on this topic from the Baltic Sea region. At this stage, it is highly relevant to gain an understanding of what species are infected, and what the ecological implications might be.

The problem statements are:

1. Which phytoplankton species are infected by parasites and what are the infection rates?
2. Does parasite infection have an effect on the phytoplankton host blooms?
3. When does epidemic outbreak occur relative to the phytoplankton host bloom?
4. Are epidemic outbreaks connected to changes in environmental conditions?

From these questions, the following hypotheses are formulated:

H1: Parasites infect the most common phytoplankton species in the Baltic Sea.

H2: Parasites cause a decrease in the host population size.

H3: The parasitic epidemic peaks together with the peak of the phytoplankton host bloom, although slightly temporally lagged.

H4: Epidemic peaks correlate with changes in environmental conditions.

2 Materials and methods

2.1 Study area and data collection

The data used in this study has been collected at Utö Atmospheric and Marine Research Station in the Archipelago Sea (59°46.84′ N, 21°22.13′ E) by the Finnish Environment Institute. The station is run by the Finnish Meteorological Institute together with the Finnish Environment Institute. The island is situated on the outer edge of the Archipelago Sea towards the Baltic Proper (Figure 3). Meteorological and hydrographic observations have been recorded at Utö for more than 100 years (Laakso et al., 2018).

The data was collected during spring season in 2021, using an Imaging FlowCytobot (IFCB; McLane Research Laboratories, Inc., United States). Samples were taken and analysed continuously (5 ml every ~20 minutes), resulting in large amounts of data. Each sample flows through a 150 µm mesh at the instrument inlet to prevent it from clogging (Kraft et al., 2021). Chlorophyll *a* fluorescence was used as a trigger for the IFCB to take images of the cells flowing through the machine, resulting in images only on phytoplankton, ranging from ~5 µm to ~300 µm filament length (Kraft et al., 2021). The image resolution is roughly 3.5 pixels per µm (Kraft et al., 2022).

Temperature measures were collected in the same flow-through system that the IFCB is connected to and are available in high frequency. Samples of inorganic dissolved nutrient concentrations (NO_2, NO_3 and PO_4) were collected manually, therefore having lower frequency throughout the spring bloom. Missing values between nutrient data points were estimated with linear interpolation (Junninen et al., 2004), to allow comparison and statistical analyses with the infection abundance data.

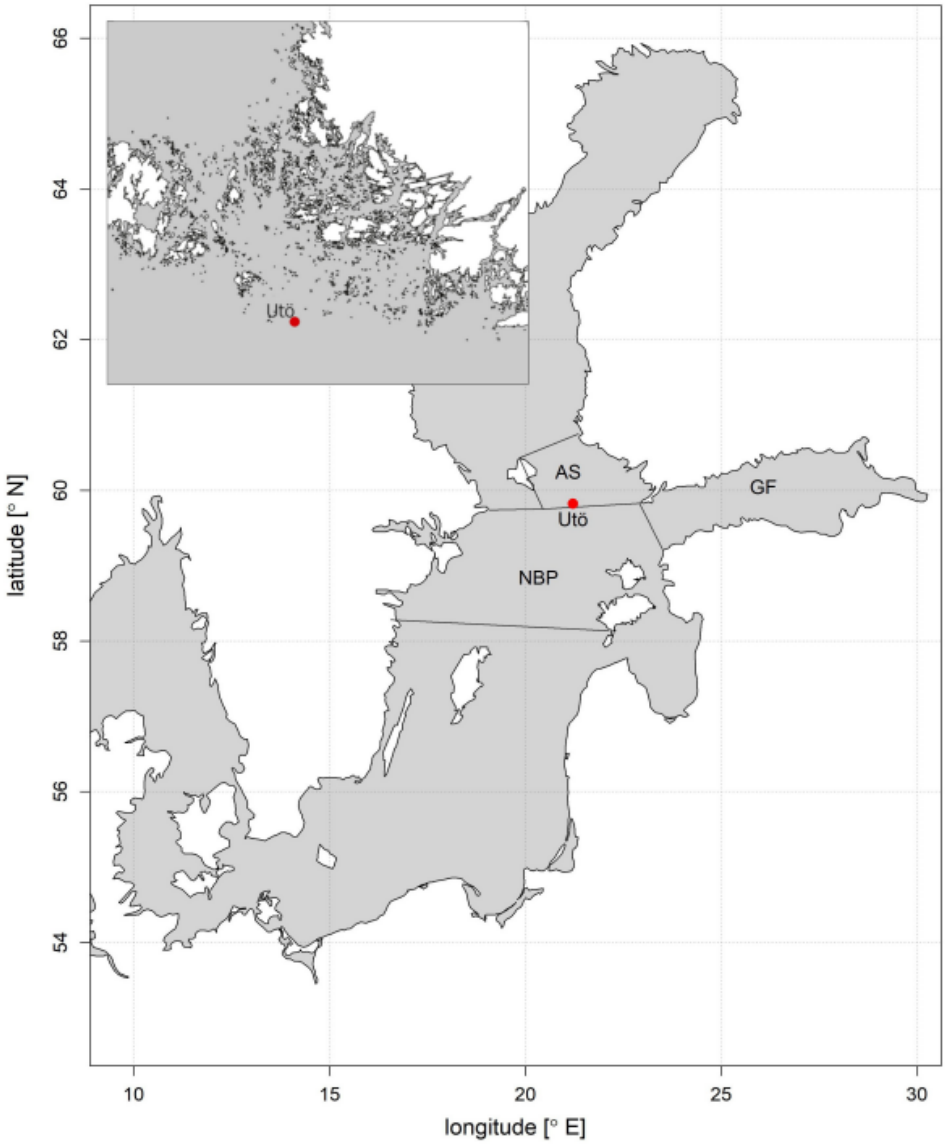


Figure 3. Map of the location of Utö in the Baltic Sea and the nearby sea areas. AS = Archipelago Sea, NBP = Northern Baltic Proper, GF = Gulf of Finland. The sampling area in more detail is seen in the upper left corner. From Kraft et al. (2021).

The technique used for phytoplankton identification was Convolutional Neural Network (CNN). This is a machine learning technique that can classify images automatically using example images. In this case, a labelled image data set of about 63 000 pictures of phytoplankton in 50 different categories was used, representing the most common phytoplankton taxa in the Gulf of Finland and the Northern Baltic Proper (Kraft et al., 2022). Some taxonomic groups contain single species, whereas some groups are difficult

to distinguish on species level via images only and are, therefore, classified on higher taxonomic levels. The images provided by the IFCB and classified with CNN, are saved in daily or hourly taxa-specific folders.

2.2 Data analysis

2.2.1 Data description

For the data analyses, four different categories were chosen and analysed for parasite infection: Centrales, *Chaetoceros* spp., *Skeletonema marinoi* and *Pauliella taeniata* (Figure 4). All of these are different kinds of diatoms. *Chaetoceros* spp. was further analysed as two distinct groups, one group with chains of cells and one group with single-celled images. The study period was limited to cover the bloom period for each category, ranging overall between 25 March to 17 May (Figure 5).

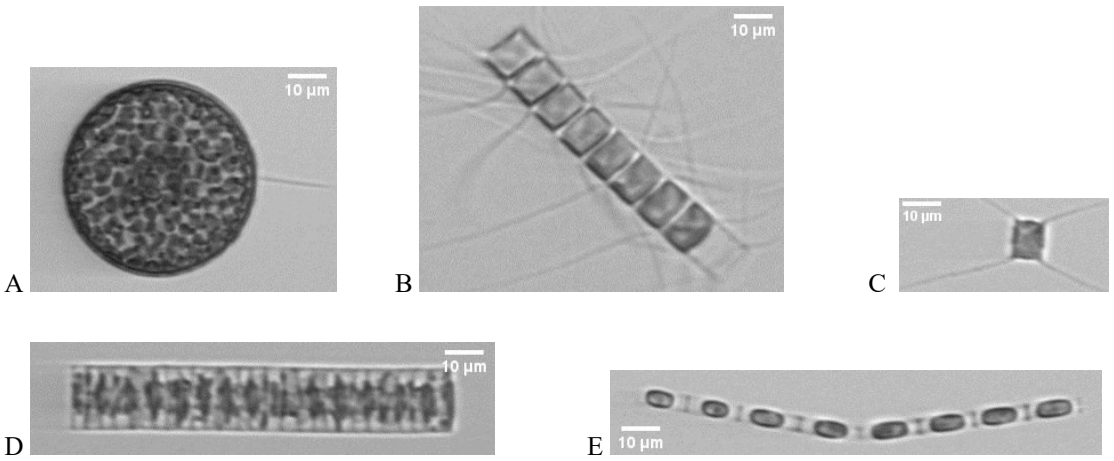


Figure 4. IFCB example images of the four chosen taxa. **A)** Centrales, **B)** chain of *Chaetoceros* spp., **C)** single-celled *Chaetoceros* spp., **D)** *Pauliella taeniata*, **E)** *Skeletonema marinoi*.

The images were provided in hourly folders. There was a large difference in total number of images among the categories, as some groups were more abundant than others (Figure 5). Since *P. taeniata* was the first species to be analysed, and the total number of images was relatively low, all images in the bloom were analysed. For the other categories, only a certain number of images were selected for analysis. Up to 100 images were analysed

every third hour during the spring bloom for each group. This meant that a maximum of 800 images per day for each species were analysed. As the blooms of the chosen species and taxa differed in duration, so did the total number of analysed days. When the number of images within an hour did not reach a total of 100 images, fewer images were analysed for that specific hour. When the total number of images within a certain hour exceeded 100 images, only the first 100 images in that folder were analysed.

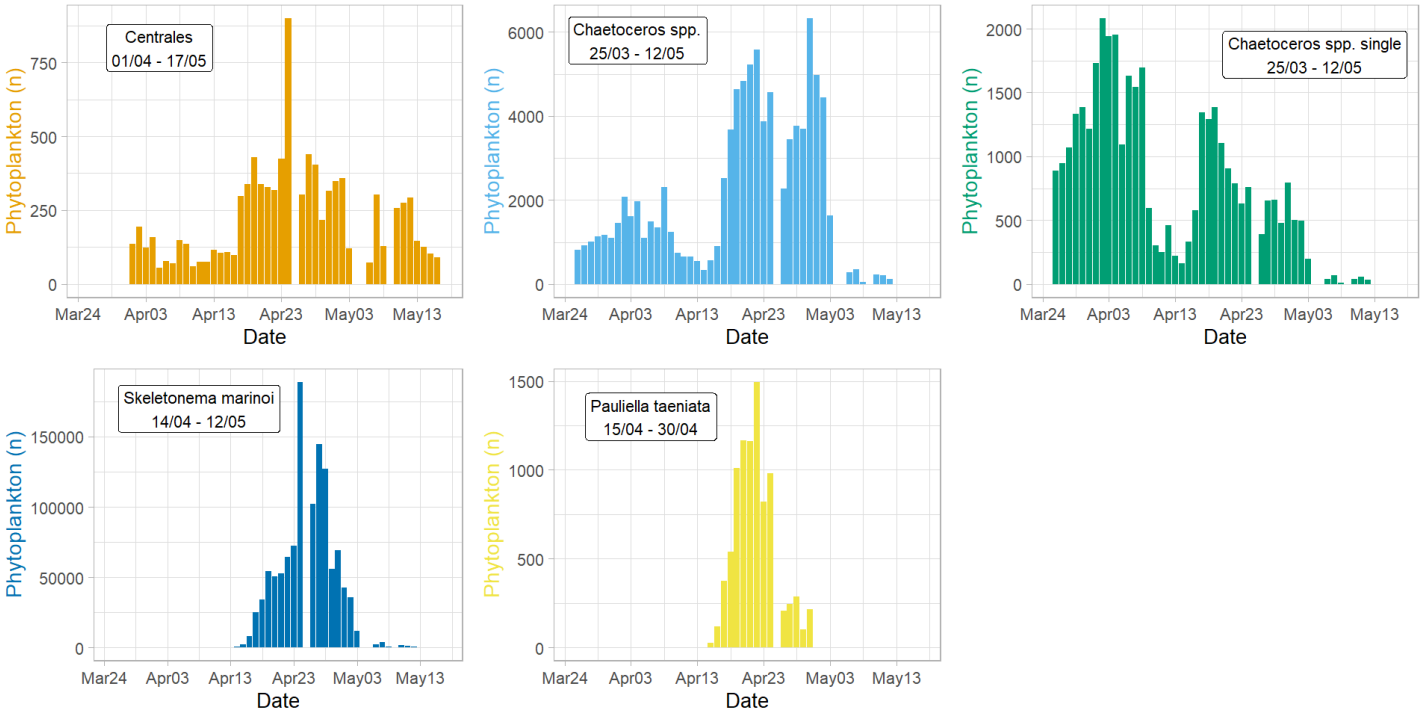


Figure 5. Bloom periods with phytoplankton abundances for all analysed taxa. The unit n stands for number of images. Note that the x-axis shows the same date interval in all graphs but the scale on the y-axis differs.

2.2.2 Definition of the criteria for putative infections

Images of phytoplankton with putative infections were copied and saved in separate folders. Chytrid sporangia have a dominantly spherical and quite uniform shape, and it is difficult to distinguish species level without genetic analysis (Van den Wyngaert et al., 2017); thus, the results show putative chytrid infections in general, not on species level. Four mandatory criteria were chosen as a basis for image selection, according to current

knowledge on chytrid morphology. These include criteria on the shape, location, colour, and size of the sporangium (Table 1).

Prior to the image analysis, a cross-validation was done with three different hourly folders of *Pauliella taeniata* IFCB images, together with my supervisory team, to evaluate if the mandatory criteria were appropriate to identify the putative parasite infections. One hourly folder was chosen at the beginning of the bloom, one during the bloom peak and one at the end of the bloom period. The total number of images was 89. From these, eight images in total were assigned as putative infections (9.0%). Five of these images (5.6%) were assigned as putative infections by all of us.

Table 1. Criteria for putative infections.

Chytrid sporangium criteria	**Description**
Shape	Round / Oval
Location	Touching the host cell
Colour	Grey or with visible granular contents (not completely black)
Size	2-30 µm

2.2.3 Manual selection

After going through the bloom periods for each species, the selected images of putative infections were analysed manually, and a data set was created with information for each image on infection stage and image quality (Table 2). For Centrales, additional information on size was added, i.e., whether the infected host cell was large or small. This was determined by comparing the image size to the other images sizes of Centrales within the same hourly folder. The reason for doing this was to see whether most of the putative infections were found on large Centrales, which could indicate infections on the large centric diatom *Thalassiosira baltica*. Three additional categories were initially considered in the analysis but were not included in the results of this thesis: the location of infection on host, number of infections per image and whether the infected host cell was dead/degraded or not.

Table 2. Description of qualitative variables for each putative infection.

Qualitative variable	Values	Description
Infection stage	1 = Zoospore	Small size, light grey
	2 = Developing sporangium	Small size, dark grey or visible contents
	3 = Mature sporangium	Large size and visible contents
	4 = Empty sporangium	Large size and no contents and/or translucent
Image quality	1 = Good	Image quality is good, and the infection is relatively certain
	0 = Poor	Image is blurred and/or infection is very uncertain
Centrales size	NA	Host cell is comparatively small or average size
	"big"	Host cell is comparatively large

2.2.4 Statistical analyses

The data was analysed initially by calculating infection rate and absolute infection abundance among the phytoplankton. Daily infection rate (R) was calculated as the number of putative infections (i) divided by the total number of analysed images (A) per day (Eq. 1). To express the infection rate in percentage, R was then multiplied by 100.

$$R = i / A \quad (1)$$

The daily absolute infection abundance (I) was calculated by multiplying the daily infection rate (R) with the host abundance (H), i.e., the total number of images per day (Eq. 2). This is considered as a proxy for parasite abundance on the host population, although slightly underestimated as each image with putative infection is calculated as one infection, but the images would sometimes include several putative chytrid sporangia.

$$I = R * H \quad (2)$$

The data were plotted to show infection rate over time and temporal dynamics of infections. As the putative infections were not normally distributed, only non-parametric tests were used. The non-parametric Spearman's rank correlation coefficient was used to test whether there were correlations between the infection abundance and total host

abundance, infection rate and total host abundance, or infection abundance and environmental data. The non-parametric Theil-Sen regression model was used to test whether total host abundance could explain the infection abundance, or whether the infection rate could explain host abundance decline. This non-parametric test is a median-based linear model, and it is robust against outliers (Fernandes & G. Leblanc, 2005; Gsell et al., 2022). The Theil-Sen estimator was also used to test whether temperature changes or changes in nutrient concentrations could explain the infection abundance. Correlation tests were performed prior to the regression models, to test whether the regression model was applicable on the data. Significance level $p < 0.05$ was used both for correlations and linear regressions.

All statistical analyses were performed in R version 4.1.1 (R Core Team, 2021). Prediction models were calculated using the predict function in the car package (Fox & Weisberg, 2019) to plot confidence intervals. For graphics, package ggplot2 (Wickham, 2016) was used and the mblm package (Komsta, 2019) was used for the Theil-Sen regression.

3 Results

In this chapter, the research questions will be revisited, and the results of the analyses will be presented. Prior to that, the qualitative data results about the putative infections will be presented.

3.1 Qualitative data results

3.1.1 Quality criteria

For each image, the quality was noted as either of good or poor quality. Good quality images have more certain putative infections than poor quality images, which are blurred or have bad resolution, or in other ways represent very uncertain infection. The results presented here show the overall quality ratio for the whole analysed period, since the

distribution of images of good and poor quality was relatively evenly distributed throughout the bloom periods (Figure 6).

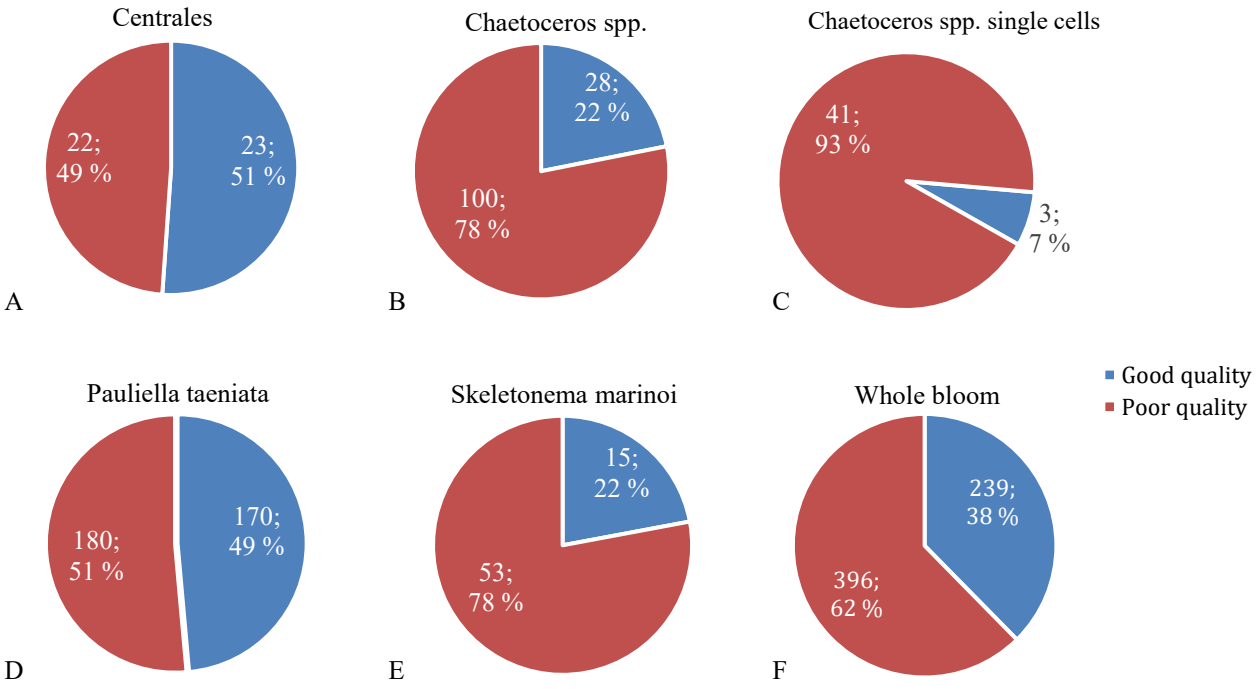


Figure 6. Quality of the images with putative infections. Images were labelled as poor quality when image resolution was blurred or infection very uncertain. The first value indicates number of images per category, the second value indicates the corresponding percentage. **A)** Centrales, **B)** *Chaetoceros* spp., **C)** *Chaetoceros* spp. single cells, **D)** *Pauliella taeniata*, **E)** *Skeletonema marinoi*, **F)** total bloom.

There were both poor and good quality images among all taxa analysed. Looking at all groups together, 38% of the images were of good quality (Figure 6f). However, the quality differed considerably between categories. For *P. taeniata* and Centrales, about half of the putative infections were of good quality. For the rest of the groups, the good quality ratio was much lower, especially for the group with single-celled *Chaetoceros* spp., with only 7% good-quality images.

3.1.2 Infection stages

For each image with putative infection, the infection stage was noted (Figure 7). Zoospores, developing sporangia, and mature sporangia were found among the putative

infections for all taxa. Empty sporangia were found only among the infections on *P. taeniata*, Centrales. and single-celled *Chaetoceros* spp.

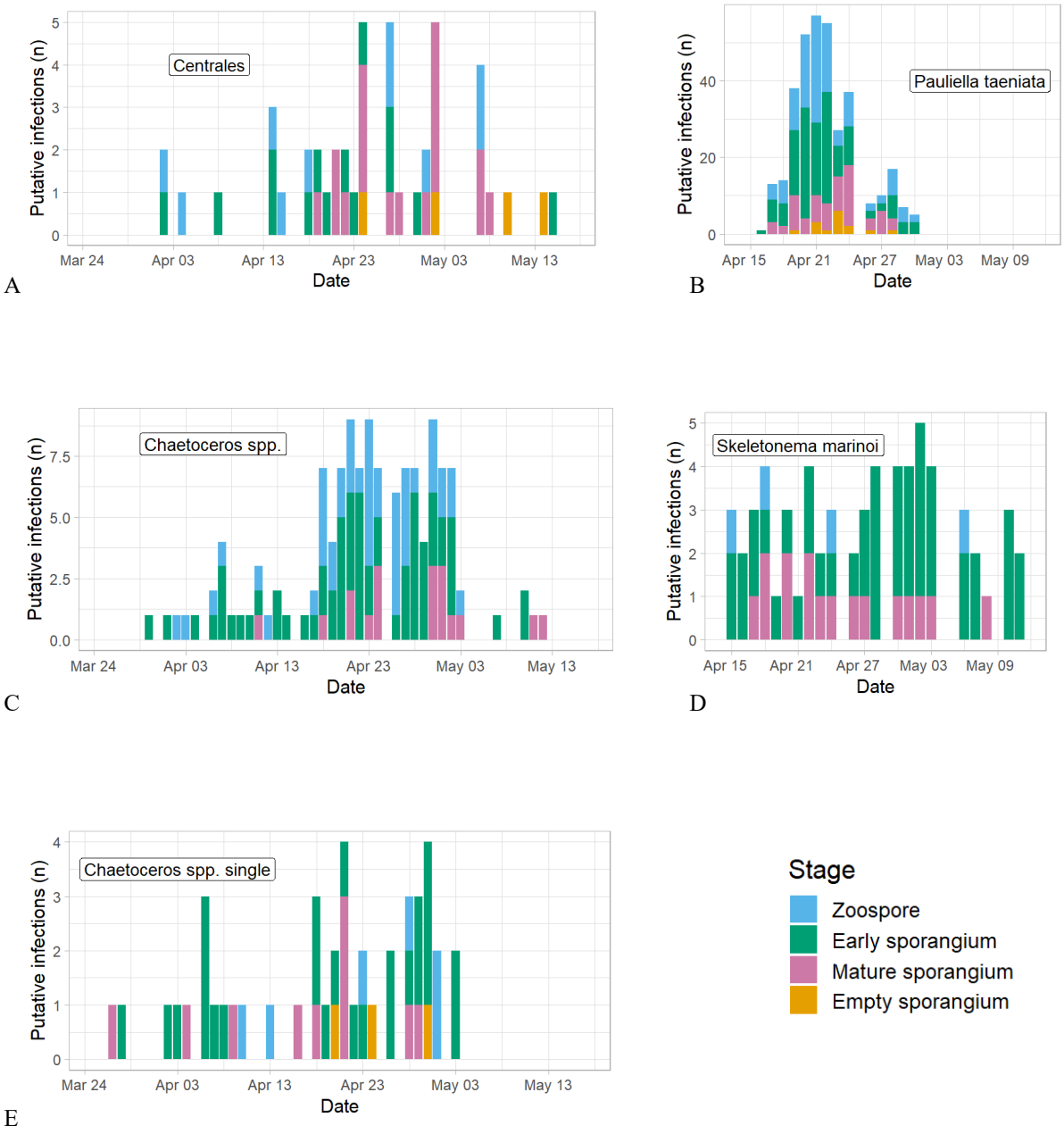


Figure 7. Infection stages of the putative infections. Each infection is labelled as either zoospore, early sporangium, mature sporangium, or empty sporangium. The unit n stands for number of images. **A)** Centrales, **B)** *Pauliella taeniata*, **C)** *Chaetoceros* spp., **D)** *Skeletonema marinoi*, **E)** *Chaetoceros* spp. single cells. Note that the x-axis is the same for *Chaetoceros* spp., single-celled *Chaetoceros* spp. and Centrales, but differs from *Pauliella taeniata* and *Skeletonema marinoi*. The y-axis differs for all groups.

3.1.3 Centrales size distribution

For all putative infections on Centrales, an additional qualitative variable was added on the size of the host cell. By comparing the image sizes as a measure on cell size, it was possible to determine whether the putative infection was found on the larger host cells or on smaller ones (Figure 8).

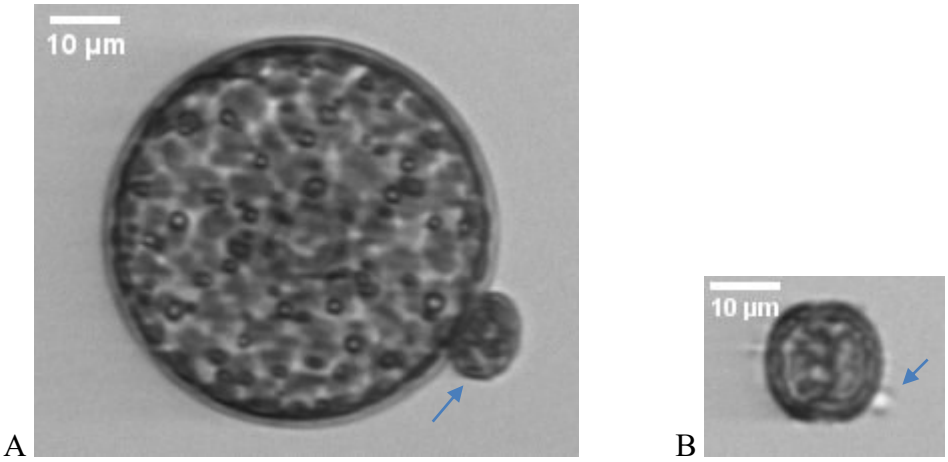


Figure 8. IFCB example images of putative infection on large **(A)** and small host **(B)**, belonging to Centrales. Arrows indicate the putative infections.

There was a larger proportion of bigger sized Centrales infected (62%) than smaller sized Centrales (38%) (total number of infected cells n = 45). Thus, putative infections on larger sized Centrales hosts were more abundant than on smaller sized Centrales (Figure 9).

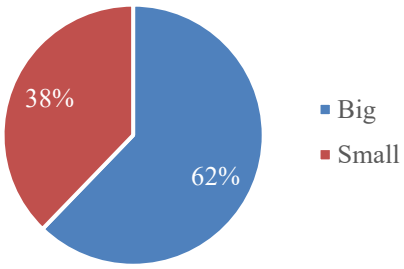


Figure 9. Size distribution of infected hosts belonging to Centrales.

3.2 Which phytoplankton species are infected by parasites and what are the infection rates?

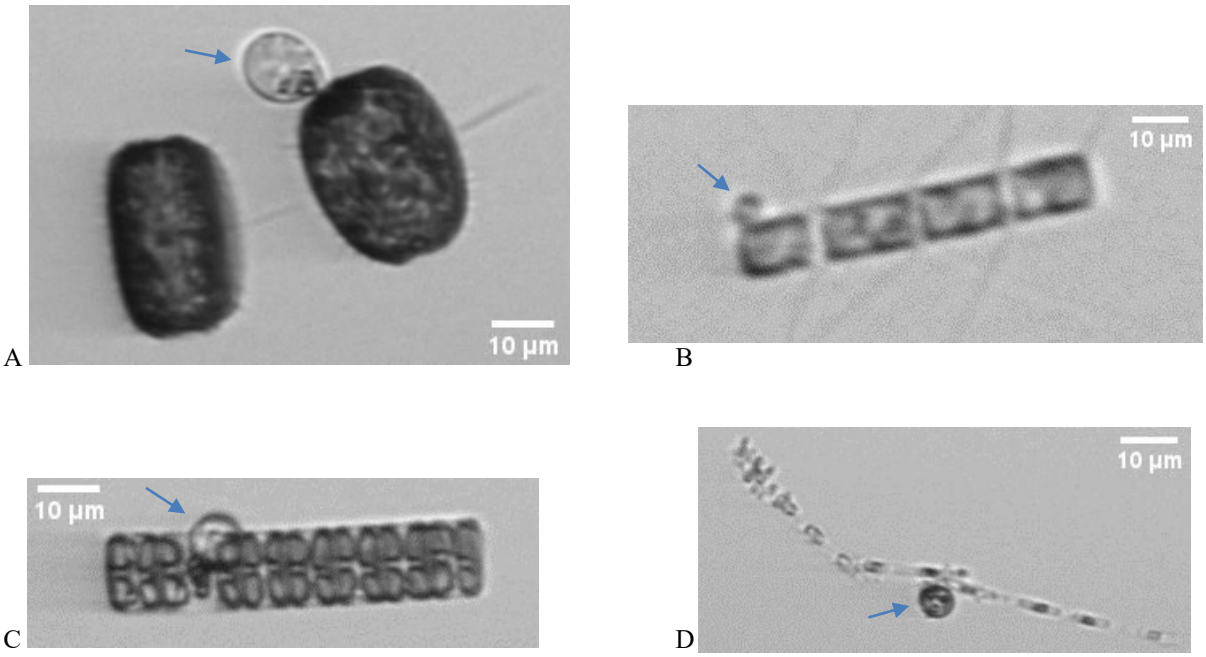


Figure 10. IFCB example images of putative infections on **A)** Centrales, **B)** *Chaetoceros* spp., **C)** *Pauliella taeniata* and **D)** *Skeletonema marinoi*. Putative infections are indicated by the arrows.

Overall, putative parasitic infections were found in all taxa analysed (Figure 10). The dominant species among the analysed taxa was *S. marinoi* (Figure 11a), but it did not have the highest infection rate. The highest daily infection rates were found on the much less abundant Centrales and *P. taeniata* (Figure 11b).

The infection rates were relatively low in all groups (Table 3). The two lowest mean infection rates were found among single-celled *Chaetoceros* spp. (0.46% ± 0.47 SE) and *S. marinoi* (0.54% ± 0.40 SE). *Pauliella taeniata* had the highest mean infection rate (3.80% ± 1.05 SE).

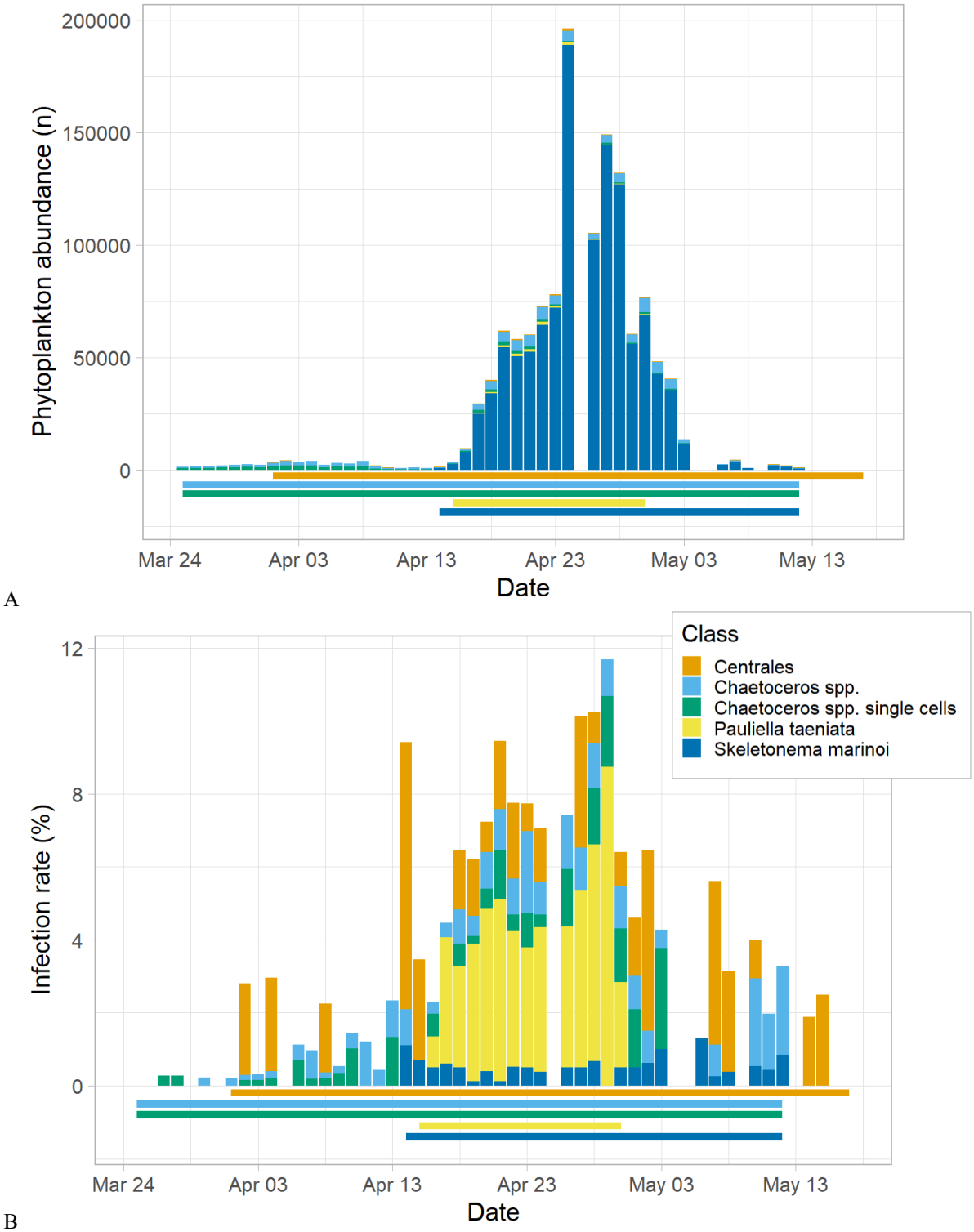


Figure 11. Phytoplankton host abundances (**A**) and infection rates per taxa (**B**). Horizontal bars below indicate the total number of days included in the analysis for each group, i.e. the length of the bloom period. The unit n stands for number of images.

Table 3. Summary statistics of the putative infections. Put. inf. = number of images with putative infections found; Analysed = total number of images analysed; Abs.inf. = absolute number of infections; Tot.bloom = total number of phytoplankton images; Mean rate (%) = mean infection rate for the whole period expressed in percentages; SD = standard deviation of the mean rate, SE = standard error of the mean rate; Max. rate (%) = maximum daily infection rate.

Species	**Put. inf**	**Analysed**	**Abs. Inf.**	**Tot. bloom**	**Mean rate (%)**	**SD**	**SE**	**Max. rate (%)**
Centrales	45	3196	137	9498	1.21	1.63	1.48	7.32
Chaetoceros spp.	128	19 140	761	92 457	0.68	0.65	0.79	2.44
Chaetoceros spp. single cells	44	12 029	137	36 860	0.46	0.66	0.97	2.78
Pauliella taeniata	350	8751	350	8751	3.80	2.05	1.05	8.74
Skeletonema marinoi	68	14 083	5161	1 153 431	0.54	0.29	0.40	1.29
TOTAL	635	57 199	6546	1 300 997	1.34	1.05	0.94	

3.3 Does parasite infection have an effect on the phytoplankton host blooms?

Statistically significant correlations were found between infection abundance and host abundance for Centrales ($p < 0.001$), *Chaetoceros* spp. ($p < 0.001$), *S. marinoi* ($p < 0.001$), *P. taeniata* ($p < 0.001$) and for all groups ($p < 0.001$). The strength of the correlation was strongest for *P. taeniata* (Table 4). The single-celled images of *Chaetoceros* spp. showed no significant correlation between infection and *Chaetoceros* spp. abundance ($p = 0.0668$) and was, therefore, not included in the regression models.

Table 4. Correlations between infection abundance and host abundance. Statistically significant values ($p < 0.05$) indicated in bold.

Group name	**S**	**p-value**	**ρ**
Centrales	4316.3	**< 0.001**	0.67
Chaetoceros spp. chains	3851.3	**< 0.001**	0.75
Chaetoceros spp. single	10994	0.0668	0.28
Pauliella taeniata	28	**< 0.001**	0.95
Skeletonema marinoi	442	**< 0.001**	0.83
Whole bloom	3001	**< 0.001**	0.86

The median-based regression model was used to test whether host abundance significantly predicted infection abundance for Centrales, *Chaetoceros* spp., *P. taeniata*, *S. marinoi* and whole bloom. The overall regression was statistically significant for all the different groups ($p < 0.001$), and it was found that host abundance significantly predicted infection abundance (Figure 12).

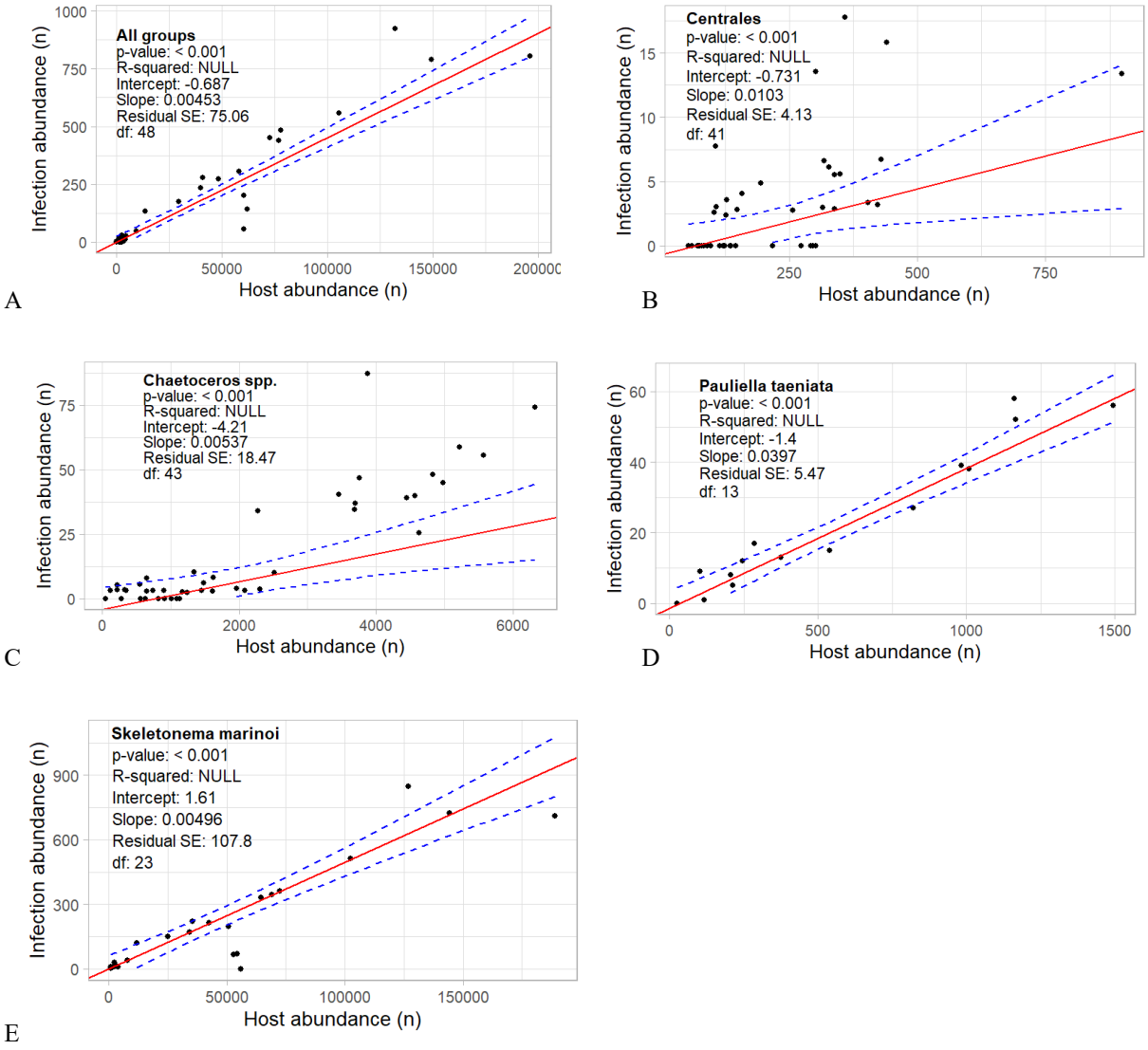


Figure 12. Theil-Sen regressions of infection abundance and host abundance. The unit n stands for number of images. **A)** Whole bloom, **B)** Centrales, **C)** *Chaetoceros* spp., **D)** *Pauliella taeniata*, **E)** *Skeletonema marinoi*.

Statistically significant correlations were found between host abundance and infection rate for Centrales ($p < 0.005$). There were no statistically significant correlations between host abundance and infection rate for *Chaetoceros* spp., *Chaetoceros* spp. single cells, *S. marinoi*, *P. taeniata* or whole bloom (Table 5).

Table 5. Correlations between host abundance and infection rate. Statistically significant values ($p < 0.05$) indicated in bold.

Group name	**S**	**p-value**	**ρ**
Centrales	7263.4	**0.00237**	0.45
Chaetoceros spp. chains	11064	0.0716	0.27
Chaetoceros spp. single	14259	0.692	0.061
Pauliella taeniata	428	0.397	0.24
Skeletonema marinoi	3488.8	0.0944	-0.34
Whole bloom	18653	0.471	0.10

Since there was no correlation between infection rate and host abundance for the other groups, Centrales was the only group included in the regression models. There was a statistically significant positive relationship between host abundance and infection rate ($p < 0.001$). However, since the relationship was positive, there was no indication that infections on Centrales caused a decline in the host population (Figure 13).

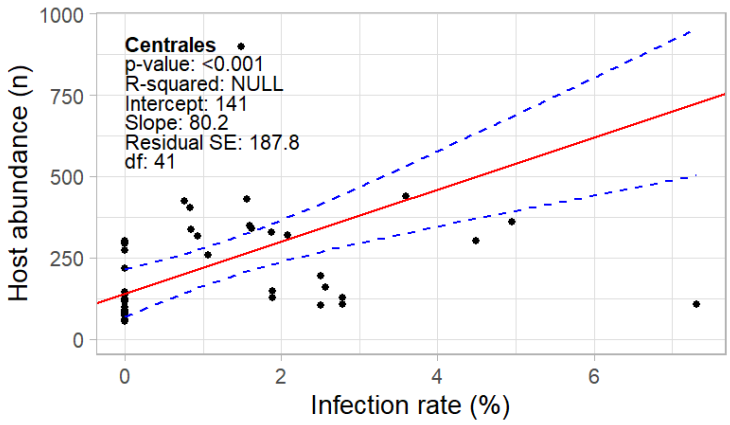


Figure 13. Theil-Sen regression of infection rate and host abundance on Centrales. The unit n stands for number of images.

3.4 When does epidemic outbreak occur relative to the phytoplankton host bloom?

By comparing the peak of the infection rate to the peak of the host bloom, it is possible to determine whether the infection epidemic peaks simultaneously as the host bloom peaks, or with a temporal lag.

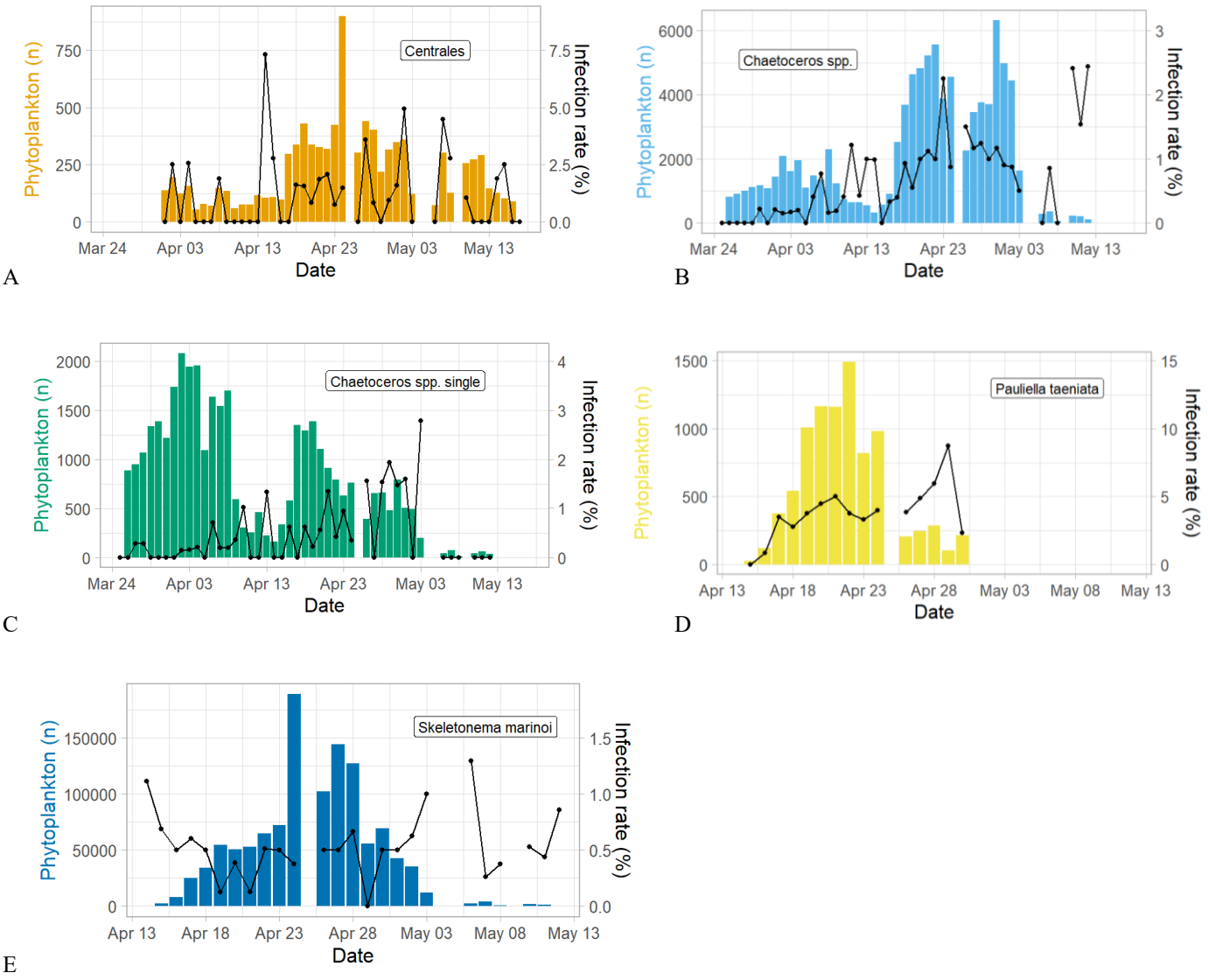


Figure 14. Infection rates for each group. Bars indicate bloom abundances (left y-axis). The unit n stands for number of images. The black lines and points indicate infection rate (right y-axis). Note that both the x- and y-axis differ between groups. **A)** Centrales, **B)** *Chaetoceros* spp., **C)** single-celled *Chaetoceros* spp., **D)** *Pauliella taeniata*, **E)** *Skeletonema marinoi*.

Looking at the different groups, there seemed to be two different patterns. The infection rate of *S. marinoi* and *P. taeniata* both peaked slightly after the peak of their host's bloom, while the infection rates of Centrales and *Chaetoceros* spp. seemed to follow the host bloom formation without a time lag (Figure 14). The infection rate on single-celled *Chaetoceros* spp., however, did not peak together with the host bloom peak, but rather temporally lagged. The two species *S. marinoi* and *P. taeniata* had one main host bloom peak respectively, whereas both Centrales and *Chaetoceros* spp. peaked more than once during the bloom period.

3.5 Are epidemic outbreaks connected to changes in environmental conditions?

The environmental data used in the analyses were temperature and nutrient conditions. During the spring period analysed, the temperature increased steadily, while the nutrient conditions decreased (Figure 15). As the nutrients are correlating with each other (variables NO_2, NO_2 + NO_3 and PO_4), only one of them (NO_2 + NO_3, Figure 15c) was used in the analysis, and the other variables were assumed to show similar correlations with infection abundance.

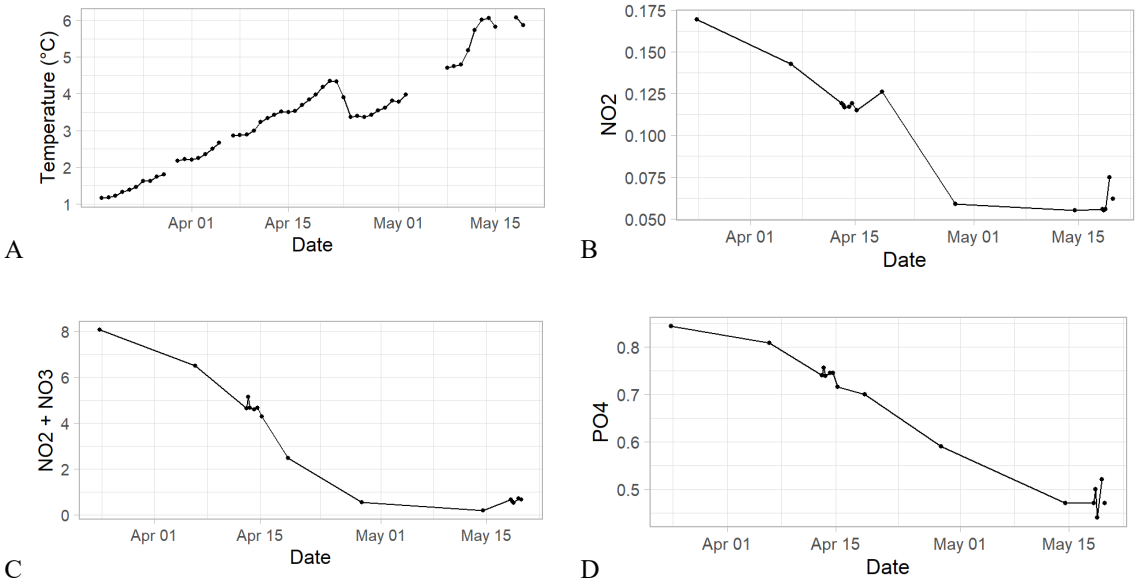


Figure 15. Environmental data. **A)** temperature shown in degrees Celsius, **B)** NO_2 = nitrite, **C)** NO_2 + NO_3 = nitrite + nitrate, **D)** PO_4 = phosphate.

Further, only the environmental data that showed an indication of linear relationship with infection abundance, were used in these analyses. This was tested by checking for correlations between 1) infections and temperature changes and 2) infections and nutrient conditions for each group.

Chaetoceros spp. ($p < 0.001$), *P. taeniata* ($p < 0.01$), *S. marinoi* ($p < 0.05$) and total bloom ($p < 0.05$) showed statistically significant correlations between infection abundance and temperature (Table 6). There were no significant correlations found for single-celled *Chaetoceros* spp. or Centrales, hence, they were not included in the regression analyses.

Table 6. Correlations between infection abundance and temperature. Statistically significant values ($p < 0.05$) indicated in bold.

Group name	**S**	**p-value**	**ρ**
Centrales	6721.4	0.228	0.20
Chaetoceros spp. chains	4301.9	**< 0.001**	0.60
Chaetoceros spp. single	8537.1	0.218	0.20
Pauliella taeniata	192	**0.00953**	0.66
Skeletonema marinoi	2580	**0.0339**	-0.46
All groups	8328.3	**0.0142**	0.37

The median-based linear model was used to test whether temperature could explain infection abundance. Overall regression between infection abundance and temperature was statistically significant for all groups included in the analysis (Figure 16). The relationship was positive for *Chaetoceros* spp., *P. taeniata* and whole bloom, but negative for *S. marinoi*.

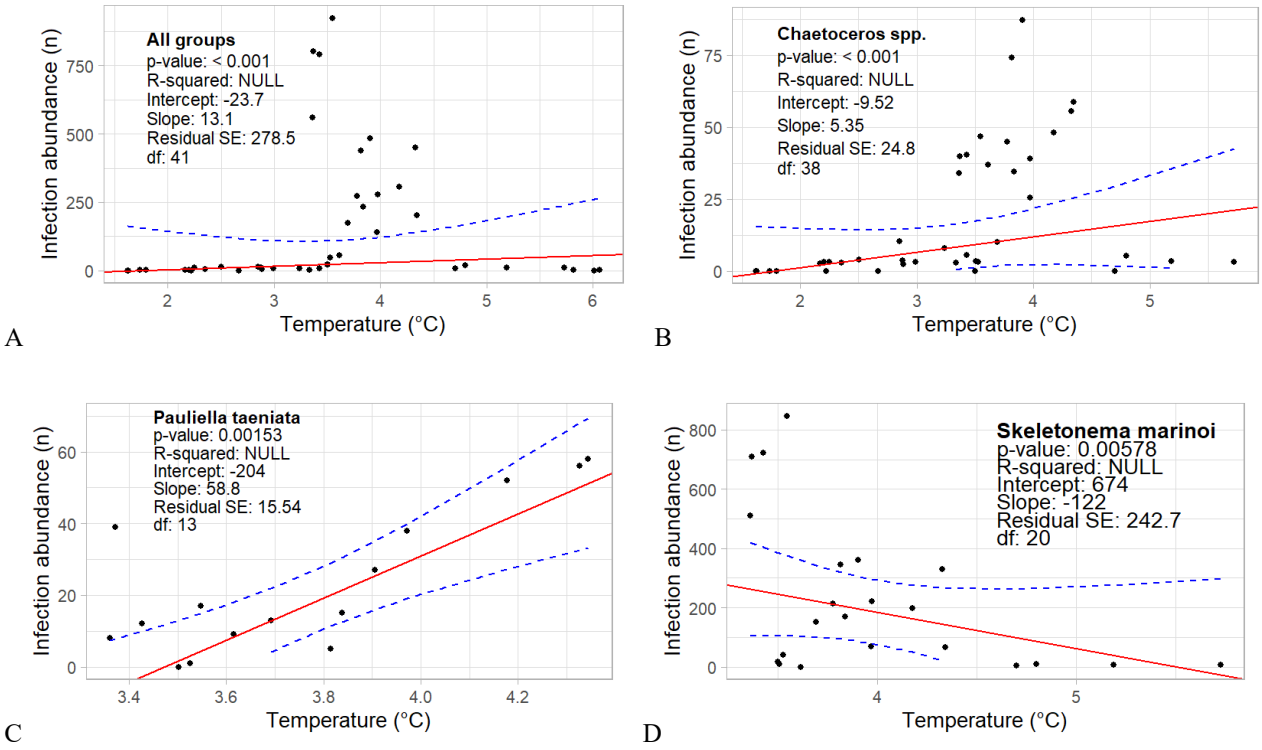


Figure 16. Theil-Sen regressions of temperature and infection abundance on **A)** total bloom, **B)** *Chaetoceros* spp., **C)** *Pauliella taeniata*, **D)** *Skeletonema marinoi*. The unit n stands for number of images.

The correlation tests between infections and nutrients showed that there were statistically significant negative correlations between *Chaetoceros* spp. ($p < 0.001$) and whole bloom ($p < 0.05$) infections and nutrient changes (Table 7). For the rest of the groups, no statistically significant correlations were found.

Table 7. Correlations between infections and nutrient data ($NO_2 + NO_3$). Statistically significant values ($p < 0.05$) indicated in bold.

Group name	**S**	**p-value**	**ρ**
Centrales	14780	0.459	-0.12
Chaetoceros spp. chain	22991	**< 0.001**	-0.52
Chaetoceros spp. single	16831	0.477	-0.11
Pauliella taeniata	572	0.944	-0.02
Skeletonema marinoi	1934	0.216	0.26
All groups	27832	**0.0169**	-0.34

The median-based linear model was used to test whether nutrients can explain infection abundance. Overall, the regression between infection abundance and nutrient changes was statistically significant for both *Chaetoceros* spp. ($p < .001$) and whole bloom ($p < .001$; Figure 17).

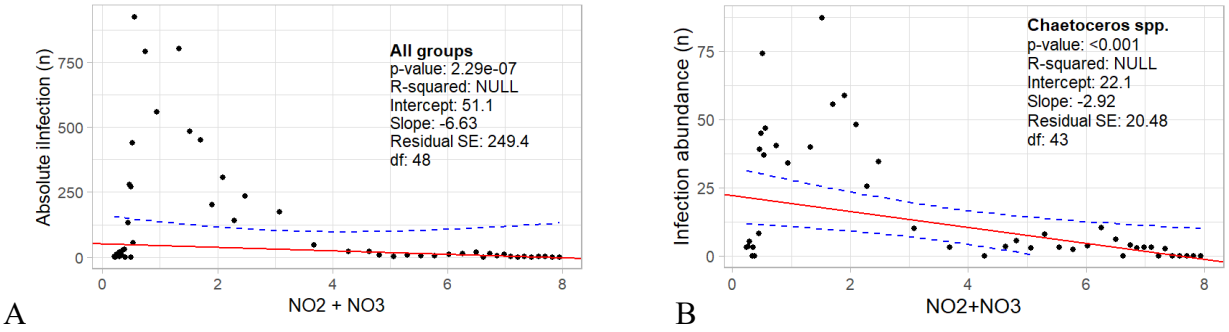


Figure 17. Theil-Sen regressions of nutrients and infection abundance for **A)** whole bloom, **B)** *Chaetoceros* spp. The unit n stands for number of images.

4 Discussion

In the following sections, first the qualitative criteria will be discussed, and each hypothesis will be revisited. Finally, the methodology is reviewed, and at the end of this chapter, a few suggestions for future studies are made.

4.1 Results discussion

4.1.1 Assessing quality criteria and infection stages

The results from the quality criteria illustrate the high uncertainty of the putative infections among all groups, as the percentages with images of low certainty ("poor quality") were dominant for almost all groups. It was only for *P. taeniata* and Centrales that about half of the images could be classified as "good quality", that is, more likely to contain real infections. Similarly, the distribution of the putative infections according to

infection stage also gives an indication of which taxa with putative infections are more likely to contain true infections than others. Looking at a whole bloom period, one would expect to find all infection stages present, but for several taxa no empty sporangia were found at all. The absence is worth noting when thinking about the validity of the putative infections.

Combining these results together, can give a better indication of which taxa are most likely to show the most valid results. For example, even though the putative infections of *P. taeniata* were only 48.6% "good quality", it had the highest certainty compared to the other groups, and the infection stage distribution also included empty sporangia. Adding this to the fact that *P. taeniata* also had the highest mean infection rates, suggests that this species might be a good example of a species in the Baltic Sea infected by chytrids, or at least a good candidate to start analysing further. Centrales shows the same indication, as it had about the same amount of good quality images as *P. taeniata,* and there were empty sporangia among its infection stage distribution. On the other hand, since *S. marinoi* and *Chaetoceros* spp. both included only about 22% "good quality" images, and neither had any empty sporangia in their infection stage distributions, it implies that they are much less likely to be infected by chytrids than for example *P. taeniata*.

4.1.2 Infected Centrales size

As most infections were found on larger host cells of Centrales, this could indicate infection on *Thalassiosira baltica*, which is a large diatom belonging to the order Centrales. *Thalassiosira* sp. has previously been shown to be susceptible to chytrid parasites (Gutiérrez et al., 2016). However, the method used here to obtain the ratio large vs small, was qualitative and should not be interpreted as definite. Rather, it can give a hint to where it would be reasonable to look further for fungal infections. Besides, only the ratio of infected host sizes was calculated, not considering the total amount of larger and smaller host sizes within the whole sample. If the large host cells, that had a higher infection ratio than smaller host cells, are also more common in general, the difference might simply describe that large cells are more abundant. However, if the larger host cells are less common among the general bloom, this might suggest that they are more susceptible to infection.

4.1.3 Infected species and the infection rates

Overall, putative infections were found among all studied taxa, although the infection rates were very low compared to infection rates found in lake systems (Gsell et al., 2022; Ibelings et al., 2011; Van den Wyngaert et al., 2022). This needs further investigation, to find out the reasons for the seemingly low infection rates on the studied phytoplankton in the Baltic Sea. This study only included data from one spring bloom, and conducting the same approach on another year might give very different results, as infection dynamics on phytoplankton blooms can vary considerably from year to year (Gsell et al., 2022). For example, if one species was heavily infected one year, it might be less abundant the next year. Further studies could also include and quantify grazers, such as micro- and mesozooplankton, to study whether a potential top-down control by grazers on the parasitic zoospores (Kagami et al., 2014), cause lower infection rates.

Interestingly, the dominant diatom species *S. marinoi* was not the most infected species among the groups analysed but had rather a very low infection rate. Thus, the hypothesis that the most dominant species is the most infected (H1) is rejected. This is contrary to studies in lake systems, where it is often the most dominant species that is most susceptible to infection (Rasconi et al., 2012). However, it is important to keep in mind, that there is no certain consensus on this, as other studies suggest that there is no relationship between phytoplankton dominance and infection prevalence (Gsell et al., 2022). Yet, when the host population is small, the probability of finding suitable hosts diminishes for the chytrid parasites (Ibelings et al., 2011). In any case, this is an important finding, and it suggests that more research is needed to determine the reasons behind the low infection rates of *S. marinoi*.

One possible explanation could be that perhaps *S. marinoi* has some chemical or physical resistance against infections. There are, however, previous studies in fully marine systems that have observed chytrid infections on *Skeletonema* sp. (Garvetto et al., 2019). An alternative explanation could be that the brackish water in the Baltic Sea is not optimal for the chytrid species of *S. marinoi* to survive, which would allow the diatom to be so successful during the spring blooms. In other words, the brackish environment of the Baltic Sea might provide a so-called parasitic refuge for this diatom. The dominance of

the less susceptible *S. marinoi* in turn could potentially explain the low infection rates on more susceptible species, such as *P. taeniata*. When the susceptible host population is too low, for example, due to species competition, parasite transmission is hampered and an epidemic is unlikely (Rasconi et al., 2012). On the other hand, even low infection rates might still further reduce the host species' ability for competitiveness, causing a positive feedback loop for the dominance of other species, in this case *S. marinoi*.

Another thing to note about the putative infections on *S. marinoi* is that the species has sexual stages that are quite similar in shape and size to chytrid infections (Godhe et al., 2014), which might impact the validity of the sorted putative infections. This could mean that the true infection rates on *S. marinoi* in the Baltic Sea are even lower than found in this study. Genetic diversity among populations of *S. marinoi* (Sjöqvist et al., 2014) might make it more difficult for the chytrid parasite to adapt to the host and become epidemic (Agha et al., 2018).

4.1.4 Effect of infections on host bloom

Although statistically significant correlations were found between infection prevalence and host abundance for most of the groups analysed, there were no indications that the infections have a negative impact on the host population growth. Thus, the second hypothesis that the chytrid parasites would have a negative impact on the host bloom (H2) is rejected. Analysing a spring bloom from another year might give other results, however, as the infection prevalence vary from year to year (Gsell et al., 2022).

Furthermore, as the infection rates were quite low in general, it is disputable whether one can talk about an epidemic whatsoever in this case. However, as discussed above, even low infection rates could impact their host species' competitiveness in a phytoplankton community, impacting the species composition of the bloom. Thus, further studies are needed to measure the impact of infections on the host blooms.

4.1.5 Host and infection peaks

For *P. taeniata*, which had the highest infection rates compared to the other groups, the peak in infection rate occurred slightly after the host bloom peak, which suggests a

temporally lagged density dependence. This is similar to host and infection dynamics found in Gsell et al. (2013), where prevalence of infection peaked after the host bloom peak, when host abundances were declining. The peak in infection rate for *S. marinoi* shows the same indication. For both *Chaetoceros* spp. and Centrales, however, the peak in infection rate occurred around the same time as the host bloom peak. The hypothesis that the infection rate peak occurs slightly after the host bloom peak (H3) is confirmed for *P. taeniata* and *S. marinoi*, and partly confirmed for *Chaetoceros* spp. and Centrales.

Here it is important to note that *P. taeniata* and *S. marinoi* are single species, whereas *Chaetoceros* spp. and Centrales include several different species, which might affect the results. For example, as *Chaetoceros* spp. and Centrales have several peaks, it could indicate the presence of different species peaking at different times.

4.1.6 Infections and environmental changes

The correlations between infection abundance and environmental changes varied. The infections of Centrales and single-celled *Chaetoceros* spp. did not show any correlations with neither temperature changes nor nutrient conditions. For the other groups, *P. taeniata*, *S. marinoi*, *Chaetoceros* spp. and looking at the whole bloom, it was found that temperature could significantly explain infection abundance. For *P. taeniata*, *Chaetoceros* spp. and whole bloom, this relationship was positive, which is consistent with previous findings that chytrids increase with warmer temperatures (Gsell, de Senerpont Domis, Naus-Wiezer, et al., 2013; Ibelings et al., 2011). For *S. marinoi*, however, the relationship was slightly negative.

Looking at nutrients, only *Chaetoceros* spp. infections and total bloom infections were found to correlate with nutrient changes. The infection abundance did seem to increase with decreasing nutrients but looking at the regression plots, the curve does not seem to be linear but rather negatively exponential, suggesting the linear regression as model fitting was not optimal (Figure 17). Further studies could explore other statistical analysis options to find a more suitable model.

Overall, however, the correlations between infection abundance and nutrient changes does not seem equally important as an explanatory variable as temperature change or host

abundances. A more reasonable explanation might be that a growing phytoplankton community in spring would cause decreasing nutrient concentrations in the water, rather than infection abundance itself being connected to nutrient changes (Rasconi et al., 2012). This would mean that the negative correlations found between *Chaetoceros* spp. infection abundance and nutrients, and whole bloom infection abundance and nutrients, are indirect.

Even though some statistically significant correlations were found between taxa-specific infection abundance and environmental changes, the abundances remained very low throughout the bloom period. Thus, the hypothesis that the epidemic peaks are connected to environmental changes (H4) is rejected. However, as the linear models were not appropriate to use for testing the impact of the environmental variables on all groups, some combinations were in this case excluded. To improve the analysis and include all variables, one could perform analyses for non-linear relationships such as generalized mixed models.

4.2 Methods discussion

Since this study was one of the first of its kind in the Baltic Sea, there is no previous research from the region to compare the results with. This study also serves as a testing of the methodology of detecting chytrid infections on phytoplankton with imaging flow cytometry. Without any validation of the putative infections, there is no way to be certain that the images sorted in this study were in fact images of actual infections. To develop this method further, some way of validating the results would be important. This could be done for example by comparing putative infections detected with IFCB images, to manually detected infections by microscopy or with staining methods (Klawonn, Dunker, et al., 2021) from the same sample.

One could argue that the number of images analysed per day was too low and a larger effort might allow higher probability of infection detection. However, there was no reference study done in this area and field, and thus, the number of images was instead decided according to what would be feasible to go through manually within the scope of a master's thesis. Further, if there would be an infection rate of for example 5-10%, at

least 5 in 100 images would be expected to be infected, for some samples less and for some more. Thus, 100 images every third hour, with a maximum of 800 images per day, was determined to be adequate for the purpose of this thesis. Additionally, in lake studies on phytoplankton parasites, the Utermöhl technique is often utilized, where sometimes even less cells are analysed (Donk & Ringelberg, 1983; Gsell et al., 2022; Rasconi et al., 2012).

Another question is whether sorting the first 100 images within an hour might be biased against size, as larger cell aggregates might sink faster. Due to the many hours per day, it was decided that it should not affect the results in this case. Moreover, there might be some difference in sinking rate of infected versus non-infected cells, for example if infected host cells sink slower or faster than other cells, or the bigger cells sink the fastest. The difference was nevertheless assumed to be negligible.

For this thesis, only image abundance was used as a variable, and not biomass data. The image sizes varied a lot, as the chain-building characteristic of some phytoplankton results in largely differing chain sizes. Including biomass data might give more accurate results and it would be possible to analyse whether there is a size difference for parasite susceptibility. Biovolume information can be computed from the images taken by the IFCB and then converted to biomass (Kraft et al., 2022). This is something to consider in further studies.

Although there is a high uncertainty of the validity of the images as infected diatoms, the results of this thesis are still an important and necessary step for developing an automated methodology for finding anomalies on phytoplankton blooms, which would further contribute to the knowledge on phytoplankton bloom formation and dynamics between host and parasites. By developing such automated detection method, it would be possible to study much larger samples in a much shorter time than analysing everything manually, which would enable to collect larger datasets and to perform more comprehensive analyses.

4.3 Future outlook

This thesis was an important step in increasing the knowledge on phytoplankton parasites in the Baltic Sea. However, many unanswered questions remain that still need answers, and further research is needed. In this thesis, only one spring season was included, focusing only on a few diatom species in the Baltic Sea. In the future, a similar but improved approach might be applied to other species and taxa, for example dinoflagellates or cyanobacterial species. It would also be important to include several years and blooms, studying larger temporal scales, to detect changes over time. Also looking at blooms throughout the year would be important, not only during spring bloom.

Overall, an increased number of studies on phytoplankton parasitism in the Baltic Sea would be important, to identify with more certainty which species are infected by chytrid parasites, and what their impact is on the phytoplankton community in the Baltic Sea.

5 Conclusions

Putative infections were found on Centrales, *Chaetoceros* spp., *P. taeniata* and *S. marinoi*. Overall, the infection rates were lower than expected, especially on the dominating diatom *S. marinoi*. Taking the quality criteria into consideration, only Centrales and *P. taeniata* indicate actual chytrid infections, whereas infections on *S. marinoi* and *Chaetoceros* spp. are more uncertain.

Future studies would need to evaluate the validity of the putative infections observed from images taken with imaging flow cytometry, but the potential of this methodology is huge. With automated phytoplankton and anomaly identification, it is possible to analyse larger samples more efficiently, allowing more extensive research. In the future, we could possibly identify chytrid infection automatically using IFCB.

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Summary in Swedish – Svensk sammanfattning

En studie av växtplanktonparasiter under en vårblomning i Östersjön med hjälp av bildflödescytometri

Inledning

Växtplankton är mikroskopiska fotosyntetiserande organismer i akvatiska förhållanden. Tillsammans med andra akvatiska autotrofa organismer utgör de hälften av den globala nettoprimärproduktionen (Falkowski & Raven, 2007) och de utgör basen för akvatiska näringsvävar (Falkowski m.fl., 1998). Växtplankton förekommer året runt och stora förekomster av växtplankton i ett område kallas för algblomning. Om våren inleds den så kallade vårblomningen av växtplankton när solljuset ökar och mängden näringsämnen i vattnen ökar efter vintern (Sommer m.fl., 2012). I Östersjön domineras vårblomningen främst av kiselalger och dinoflagellater (Hjerne m.fl., 2019).

Växtplankton kan infekteras av flera olika typer av parasiter, bland annat olika virus, bakterier och svampar, men det är fortfarande ett relativt förbisett område inom studier

kring växtplankton. De senaste decennierna har dock växtplanktonparasiter fått alltmer uppmärksamhet och man har börjat inse vilken inverkan de kan ha på växtplankton-samhällen (Frenken m.fl., 2017; Park m.fl., 2004). I denna studie är svampparasiter på växtplankton i fokus. De hör vanligen till pisksvamparna, Chytridiomycota (Frenken m.fl., 2017). Dessa svampparasiter orsakar epidemier hos växtplankton, där stora delar av en växtplanktonpopulation kan vara infekterade. I sötvattensystem har man mätt infektionsnivåer där så mycket som 90 % av en algpopulation är infekterad (Ibelings m.fl., 2011).

Pisksvamparna producerar så kallade zoosporer med flageller, som aktivt kan röra sig och leta efter värdorganismer, i detta fall olika arter av växtplankton. Vid kontakt med en lämplig värdorganism fäster zoosporen sig på ytan och infekterar sin värd. Pisksvampen tar näring av sin värdorganism och mognar på några dagar till ett sporangium, som bildar nya zoosporer (Van den Wyngaert m.fl., 2017). Eftersom värdcellen oftast dör av infektionen, kan det leda till epidemier med hög mortalitet bland infekterade växtplankton under algblomningar. Detta kan i sin tur leda till en förändrad dynamik bland växt-planktonsamhällen, där infekterade arter minskar vilket ger utrymme för andra arter att öka i antal (Sommer m.fl., 2012). Svampparasiter på växtplankton kan också påverka akvatiska födovävar eftersom stora epidemier av parasiterna gör att de själva blir föda åt djurplankton (Kagami m.fl., 2014).

Trots en ökad uppmärksamhet de senaste decennierna finns det fortfarande flera obesvarade frågor gällande svampparasiternas inverkan på växtplanktonsamhällen. Majoriteten av forskning har gjorts i sötvattensystem, vilket betyder att svampparasiter i marina miljöer är ännu mer okända (Gleason m.fl., 2011). Det finns dessutom endast ett fåtal tidigare studier kring svampparasiter på växtplankton i Östersjön (Reñé m.fl., 2022).

Flödescytometri är en automatiserad metod för att studera, analysera och sortera celler. Denna metod har idag utvecklats till att även kunna producera bilder av cellerna i ett prov, en så kallad bildflödescytometri. Denna metod används alltmer inom forskning av växt-planktonsamhällen. En av de vanligaste maskinerna som används för detta syfte är Imaging FlowCytobot (Olson & Sosik, 2007). Bilderna som produceras i denna analys lagras sedan i en databas och klassificeras i taxonomiska grupper med hjälp av algoritmer

och maskininlärningsteknik. Denna automatiserade provtagning och analys av växtplanktonsamhällen möjliggör bättre och mer noggranna analyser av algblomningar och interaktioner mellan växtplankton och deras miljö.

Syfte och forskningsfrågor

Syftet med undersökningen är att studera svampparasiter på växtplankton i Östersjön. Det vore viktigt att öka kunskapen om dessa i Östersjön i och med att det finns väldigt lite tidigare forskning kring svampparasiter på växtplankton i Östersjön.

Utgående från syftet har fyra forskningsfrågor formulerats:

F1: Vilka växtplankton är infekterade av pisksvampar i Östersjön och hur stor är infektionsgraden?

F2: Har parasitism någon effekt på vårblomningar av växtplankton i Östersjön?

F3: När når infektionsepidemin sin högsta nivå i jämförelse med växtplanktonblomningen?

F4: Kan infektionsepidemins början kopplas till förändringar i miljöförhållanden?

Följande fyra hypoteser formulerades baserat på forskningsfrågorna:

H1: Svampparasiterna infekterar de mest allmänt förekommande växtplanktonen i Östersjön.

H2: Parasiterna orsakar mindre algblomningar hos de infekterade arterna.

H3: Infektionsepidemin följer växtplanktonblomningen med en viss fördröjning.

H4: Infektionsepidemins början korrelerar med förändringar i miljöförhållanden.

Metod och material

Materialet till denna studie har samlats in av Finlands miljöcentral vid Meteorologiska institutets atmosfär- och havsforskningsstation på Utö (Figure 3). Datainsamlingen skedde under våren 2021 med hjälp av en Imaging FlowCytobot (härefter IFCB; McLane Research Laboratories, Inc., United States). Ett vattenprov på ca 5 ml tas av maskinen var 20:e minut, varpå det analyseras automatiskt utan behov av manuell övervakning. Maskinen triggas till att ta en bild vid förekomst av klorofyll *a*, vilket resulterar i en samling bilder av enskilda celler eller kedjor av växtplankton som finns i vattenprovet. Dessa bilder skickas till en databas som klassificerar dem i separata digitala mappar enligt

morfologiska skillnader. Resultatet är ett stort material av bilder på växtplankton, sorterade enligt taxonomisk nivå för hela vårblomningen 2021.

Utifrån detta material valde jag att fokusera på fyra grupper kiselalger: Centrales, *Chaetoceros* spp., *Skeletonema marinoi* och *Pauliella taeniata*. *Chaetoceros* spp. analyserades som två distinkta grupper: kedjor med *Chaetoceros* spp. och enskilda celler av *Chaetoceros* spp. Tidsperioderna som inkluderades i analysen begränsades till gruppernas specifika vårblomningar, som skedde under olika datum under våren 2021. Totalt analyserades 100 bilder var tredje timme per grupp under den valda tidsperioden, dvs. maximalt 800 bilder per dag för varje grupp. De bilder som tydde på en svamp-infektion på en växtplanktoncell kopierades och sparades i separata mappar för vidare analys.

Utgående från bilderna på de förmodade infektionerna kunde en daglig infektionsfrekvens (R) räknas ut för de olika grupperna genom att dividera antalet bilder med förmodade infektioner (i) med det totala antalet analyserade bilder med växtplankton (A) per dag, se ekvation 1.

$$R = i / A \quad (1)$$

Ett dagligt infektionsantal (I) kunde beräknas genom att multiplicera den dagliga infektionsfrekvensen (R) med det totala växtplanktonantalet (H), dvs. det totala antalet bilder per dag (ekvation 2).

$$I = R * H \quad (2)$$

Resultaten analyserades vidare genom att grafiskt visualisera infektionsfrekvens och växtplanktonförekomst, samt genom korrelations- och regressionsanalyser. För de statistiska analyserna användes icke-parametriska test: Spearmans rangkorrelation och Theil-Sens medianbaserade regression.

Undersökningens resultat

Resultaten visar att förmodade svampinfektioner hittas bland alla analyserade grupper, men infektionsfrekvensen varierar ganska mycket (Table 3). Framför allt var det inte den mest dominerande växtplanktonarten *S. marinoi* som visade störst infektionsfrekvens,

utan den mycket mindre allmänna *P. taeniata* (Figure 11). Därmed förkastas hypotes 1 att de mest dominerande arterna är mest infekterade.

Regressionsanalysen mellan växtplankton och infektion var statistiskt signifikant för Centrales, *P. taeniata*, *S. marinoi,* kedjor av *Chaetoceros* spp. samt hela växtplankton-samhället, men inte för encelliga *Chaetoceros* spp. Detta innebär att en ökning i växtplankton kan förklara en ökning i infektionsantal för alla grupper utom enskilda celler av *Chaetoceros* spp. (Figure 12). Däremot kunde det inte påvisas att svampinfektions-frekvensen hos växtplankton påverkar sina värdorganismers algblomningar negativt (Table 5), vilket innebär att hypotes 2 förkastas.

Alla analyserade grupper visar tecken på ett densitetsberoende förhållande mellan infektion och värdorganism, dvs. infektionsfrekvensen ökar med ökande växtplankton-populationer. Det var dock endast hos *P. taeniata* och *S. marinoi* som epidemin ökade med en viss fördröjning efter algblomningens ökning, medan de andra gruppernas infektioner mer eller mindre följde växtplanktonens ökning (Figure 14). Hypotes 3 att infektionsepidemin följer algblomningen med en viss fördröjning bekräftas därför delvis.

Regressionsanalysen mellan temperatur och infektion visade att en ökande temperatur kan förklara ökande infektion hos *P. taeniata*, kedjor av *Chatoceros* spp. samt för alla grupper totalt, men inte hos *S. marinoi,* Centrales eller encelliga *Chaetoceros* spp. (Figure 16). Samband mellan förändringar i näringskoncentration och infektionsökning hittades endast hos *Chaetoceros* spp. och för alla grupper gemensamt, men effekten var väldigt låg. Eftersom infektionsförekomsten var så låg hos alla grupper konstateras det dock att det inte förekom en infektionsepidemi och därför förkastas hypotes 4 att början av epidemin påverkas av förändringar i miljöfaktorer.

Diskussion

Förmodade infektioner hittades bland alla grupper som analyserades i denna studie, men infektionsfrekvenserna var mycket lägre än förväntat och vad som är vanligt i bland annat sötvattensystem (Gsell m.fl., 2022). Vidare studier kunde inkludera andra arter och typer av växtplankton, för att öka bredden på studieområdet. Bland annat kunde man studera huruvida svampparasiter hittas på dinoflagellater eller cyanobakterier. Det vore också

viktigt att undersöka varför *S. marinoi* hade så få infektioner trots att det är en väldigt vanlig kiselalg i Östersjön. Möjligen har arten något slags försvar mot parasitiska angrepp.

Eftersom infektionerna var så få kan man inte tala om epidemier i de här fallen. Viktigt att notera är ändå att denna studie endast fokuserade på en enda vårblomning. Andra tider på året eller andra års vårblomningar kan visa helt andra resultat gällande infektion än vad som hittades våren 2021. Förändringar i temperatur kan påverka infektionernas ökning eller minskning och är viktigt att ta i beaktande i och med klimatförändringen (Ibelings m.fl., 2011).

Denna studie var en av de första i sitt slag, och de förmodade infektionerna som hittades kan inte med säkerhet antas vara äkta svampinfektioner. Vidare utveckling av denna metodologi skulle kräva en validering av sorteringsskedet, till exempel genom att jämföra sorterade förmodade infektioner ur ett vattenprov taget med IFCB, med observerade infektioner i mikroskop ur samma vattenprov. Resultaten är ändå viktiga för utvecklingen av bildflödescytometri som metodologi, som trots allt är en relativt ny metod inom växtplanktonforskning. Bildflödescytometri har en enorm potential i och med att man med hjälp av automatiserad provtagning och analys av vattenprover kan effektivera data-insamlingen och analysskedet, vilket ökar möjligheterna till att analysera växtplankton-samhällen mer ingående.

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