Universität Bremen

CONSTRAINING THE ECOLOGICAL NICHE OF PLANKTONIC FORAMINIFERA IN THE ARCTIC

DISSERTATION

Zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften

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SUMMARY

The effects of global warming are especially pronounced in the Arctic: temperatures have increased at a rate twice as fast as in other regions of the world during the past century. This trend implies that the Arctic Ocean will likely become entirely ice-free during the summer before the end of this century. Paleoclimatic studies have shown that abrupt large-volume meltwater discharges into the Arctic Ocean and its surrounding seas, were capable of disturbing the global ocean circulation and triggering further climatic transformations. Hence, a better understanding of the past natural variability of the Arctic Ocean is needed for more accurate model predictions of future climate change. Planktonic foraminifera represent a powerful tool for palaeoceanographic reconstructions. Their fossil assemblages and the chemical composition of their calcite shells allow reconstructing the physical state of the ocean in the past. The correct interpretation of these paleo-reconstructions highly relies on a thorough understanding of species-specific ecology of living planktonic foraminifera in the water column as, for example, preferred depth habitat, calcification conditions, and biotic interactions. In the Arctic Ocean, due to the fragmented observations on this marine group, no consensus exists on the ecological preferences of the different species, hampering the correct interpretation of the paleosignal present in their shells. This thesis aims to extend the understanding of the ecology of Arctic planktonic foraminifera species by focusing on various levels of organismal biology and physiology.

To constrain the environmental and biological factors controlling the vertical distribution of the species *Neogloboquadrina pachyderma*, a compilation of 104 vertical density profiles from the Arctic Ocean and its marginal seas was investigated using a statistical approach (**Chapter 2**). Contrary to what has been previously assumed, no significant relationship between *N. pachyderma* depth habitat and depth of chlorophyll maximum was observed. The depth habitat of the species could instead be predicted with a model including sea-ice concentration, surface chlorophyll concentration, and days since ice-break-up as predictors explaining 33% of the observed variability.

The biotic interactions of *N. pachyderma* with the eukaryotic pelagic community were assessed using a single-cell metabarcoding approach (**Chapter 3**). The eukaryotic DNA present in 39 specimens and contextual seawater from the Baffin Bay was extracted, amplified, and sequenced. The analyses revealed that *N. pachyderma* is omnivorous as it lives and opportunistically feeds on diatom-fuelled aggregates. The data also showed a particularly high occurrence of reads belonging to Syndiniales in the foraminifera samples, suggesting that this widely distributed parasite could infect *N. pachyderma* and possibly influence its population dynamics.

To test the assumption that planktonic foraminifera can tolerate low salinity and record the chemical signature of past meltwater discharge events in their shells, specimens of *Neogloboquadrina incompta* were exposed to a gradient of salinities between 35 and 25 PSU (**Chapter 4**) as part of a culturing study. Survival was monitored over 26 days by measuring the extent of the rhizopodial network. The highest rhizopodial activity occurred at salinity levels between 35 and 31 PSU. The results indicated that the species can survive long-term exposure

to salinities as low as 28, but no rhizopodial activity and signs of cytoplasm degradation were observed in all specimens exposed to 25 PSU.

The responsiveness of Arctic planktonic foraminifera to current climate change was investigated by analysing a compilation of 51 species-resolved stratified population profiles collected in the Fram Strait between 1985 and 2015 (**Chapter 5**). The data revealed an ongoing Atlantification of the community not mirrored by changes in local environmental conditions. The abundance of Atlantic expatriates is instead rising because of processes favouring their growth in the Nordic Seas, the "source" area. On the contrary, the resident species *Turborotalita quinqueloba* showed declining density and habitat shoaling due to the ongoing extensive sea-ice export from the Arctic and associated cooling in the Fram Strait. These conditions favour the other resident species, the polar *N. pachyderma* being better adapted to the cold conditions of the area.

These results advance our understanding of the abiotic and biotic processes regulating the ecology of planktonic foraminifera in the Arctic Ocean and can be used to refine palaeoceanographic reconstructions in the polar regions and to improve predictions of future climate change.

ZUSAMMENFASSUNG

Die Auswirkungen der globalen Erwärmung sind in der Arktis besonders stark ausgeprägt: Die Temperaturen sind im vergangenen Jahrhundert doppelt so schnell gestiegen wie in anderen Regionen der Welt. Dieser Trend impliziert, dass der Arktische Ozean vor Ende diesen Jahrhunderts im Sommer wahrscheinlich völlig eisfrei sein wird. Paläoklimatische Studien haben gezeigt, dass abrupte großvolumige Schmelzwassereinleitungen in den Arktischen Ozean und die umliegenden Meere in der Lage waren, die globale Ozeanzirkulation zu stören und weitere klimatische Veränderungen auszulösen. Daher ist ein besseres Verständnis der natürlichen Variabilität des Arktischen Ozeans in der Vergangenheit erforderlich, um genauere Modellvorhersagen für künftige Klimaveränderungen treffen zu können. Untersuchungen an planktischen Foraminiferen stellen ein probates Mittel für paläoozeanographische Rekonstruktionen dar. Ihre fossilen Artenvergesellschaftungen und die chemische Zusammensetzung ihrer Kalzitschalen erlauben es, den physikalischen Zustand des Ozeans in der Vergangenheit zu rekonstruieren. Die korrekte Interpretation dieser paläozeanographischen Rekonstruktionen hängt in hohem Maße von einem gründlichen Verständnis der artspezifischen Ökologie lebender planktonischer Foraminiferen in der Wassersäule hinsichtlich z.B. bevorzugtem Tiefenhabitat, Kalzifizierungsbedingungen und biotischen Wechselwirkungen ab. Im Arktischen Ozean gibt es aufgrund der fragmentierten Beobachtungen an dieser Meeresgruppe keinen Konsens über die ökologischen Präferenzen der verschiedenen Arten, was die korrekte Interpretation des in ihren Schalen vorhandenen Paläosignals erschwert. Diese Arbeit zielt darauf ab, das Verständnis der Ökologie der planktonischen Foraminiferenarten der Arktis zu erweitern, indem sie sich auf verschiedene Ebenen der Biologie und Physiologie der Organismen konzentriert.

Um die ökologischen und biologischen Faktoren, die die vertikale Verteilung der Art *Neogloboquadrina pachyderma* kontrollieren, einzugrenzenwurde eine Zusammenstellung von 104 vertikalen Profilen zur Vorkommenshäufigkeit dieser Art aus dem Arktischen Ozean und seinen Randmeeren mit einem statistischen Ansatz untersucht (**Kapitel 2**). Entgegen bisheriger Vermutungen wurde keine signifikante Beziehung zwischen dem Tiefenhabitat von *N. pachyderma* und der Tiefe des Chlorophyllmaximums beobachtet. Das Tiefenhabitat der Art konnte stattdessen mit einem Modell vorhergesagt werden, das die Meereiskonzentration, die Chlorophyllkonzentration an der Oberfläche und die Tage seit Eisbruch als Prädiktoren enthält und die 33% der beobachteten Variabilität erklären.

Die biotischen Interaktionen von *N. pachyderm*a mit der eukaryotischen pelagischen Artengemeinschaft wurden mit einem Einzelzell-Metabarcoding-Ansatz bewertet (**Kapitel 3**). Die in 39 Proben und kontextbezogenem Meerwasser aus der Baffin Bay vorhandene eukaryotische DNA wurde extrahiert, amplifiziert und sequenziert. Die Analysen ergaben, dass *N. pachyderma* omnivor ist da sie umgeben von kieselalgenreichen Aggreaten zu leben scheint und sich opportunistisch von diesen ernährt. Die Daten zeigten auch ein besonders hohes Vorkommen von Syndiniales in den Foraminiferenproben, was darauf hindeutet, dass dieser weit verbreitete Parasit *N. pachyderma* infizieren und möglicherweise ihre Populationsdynamik beeinflussen könnte.

Um die Annahme zu überprüfen, dass planktische Foraminiferen niedrige Salinitäten tolerieren und in ihren Schalen die chemische Signatur vergangener Schmelzwasserausflussereignisse aufzeichnen können, wurden Proben von *Neogloboquadrina incompta* einem Gradienten von Salinitäten zwischen 35 und 25 PSU als Teil einer Laborstudie ausgesetzt (**Kapitel 4**). Ihr Überleben wurde über den Verlauf von 26 Tagen durch Messung der Ausdehnung des rhizopodialen Netzwerks überwacht. Die höchste rhizopodiale Aktivität trat bei Salzgehaltsniveaus zwischen 35 und 31 PSU auf. Die Ergebnisse deuteten darauf hin, dass die Spezies eine langfristige Exposition bei einem Salzgehalt von nur 28 überleben kann, jedoch wurden ausbleibende rhizopodiale Aktivität und Anzeichen von Zytoplasmaabbau bei allen Proben beobachtet, die einer Salinität von 25 PSU ausgesetzt waren.

Die Reaktionsfähigkeit der arktischen planktischen Foraminiferen auf den gegenwärtigen Klimawandel wurde durch die Analyse einer Zusammenstellung von 51 artenauflösenden, geschichteten Populationsprofilen untersucht, die zwischen 1985 und 2015 in der Framstraße gesammelt wurden (**Kapitel 5**). Die Daten zeigten eine laufende Atlantifizierung der Artengemeinschaft, die nicht durch Veränderungen der lokalen Umweltbedingungen widergespiegelt wird. Stattdessen steigt die Zahl der aus dem Atlantik migrierenden Individuen aufgrund von Prozessen, die ihr Wachstum in den Nordmeeren, dem "Quellgebiet", begünstigen. Im Kontrast dazu zeigt die ansässige Art *Turborotalita quinqueloba* eine abnehmende Häufigkeit und Habitatvertiefung aufgrund des anhaltenden umfangreichen Meereisexports aus der Arktis und der damit verbundenen Abkühlung in der Framstraße. Diese Bedingungen begünstigen die andere ansässige Art, die polare *N. pachyderma*, die besser an die kalten Bedingungen des Gebietes angepasst ist.

Diese Ergebnisse fördern unser Verständnis der abiotischen und biotischen Prozesse, die die Ökologie der planktischen Foraminiferen im Arktischen Ozean regulieren und können zur Verbesserung der paläoozeanographischen Rekonstruktionen in den Polarregionen und zur Verbesserung der Vorhersagen des zukünftigen Klimawandels genutzt werden.

OWN CONTRIBUTION TO MANUSCRIPTS

The first study (**Chapter 2**) has been designed by the candidate with contributions from all co-authors. The candidate compiled new vertical profiles of foraminifera abundance from plankton samples collected previously in the Baffin Bay and assembled a comprehensive dataset of published planktonic foraminifera counts from vertically resolved plankton tows in the Arctic and subarctic regions. The candidate extracted oceanographic data from satellite raster images and CTD profiles. He harmonised all data, designed and performed all statistical analyses, produced all figures and prepared the first draft of the manuscript. The analyses benefitted by contributions from all co-authors that provided insights regarding the interpretation of the data, and helped to structure the manuscript.

For the second study (**Chapter 3**), the candidate developed the research question and designed the sampling scheme, with advice from MK and RM. He collected all samples during the expedition MSM66 to the Baffin Bay, including the extraction of individual foraminifera from plankton tows and sampling of environmental DNA by water filtration, as well as preservation of the samples for subsequent analyses. With the assistance of RM, he developed an optimised protocol for DNA extraction from the samples and carried out all DNA extractions from single cells and from the environmental filters. He optimised PCR protocols for the extracts and prepared all samples for metabarcoding by next-generation sequencing, including primer design, primer tagging and PCR. With the help of AFG, he performed all bioinformatic analyses, including filtering and demultiplexing of raw reads, identification of amplicon sequence variants, their clustering and taxonomical assignment. The candidate extracted oceanographic data from satellite raster images and CTD profiles. He harmonised all data produced all the figures, and designed and implemented all statistical analyses of the metabarcoding data matrix. He interpreted the data and wrote the first draft of the manuscript that was commented by all co-authors.

For the third study (**Chapter 4**), the candidate designed the culturing protocol with advice from MK and planned the experiment in Tromsø in coordination with of KZ and TLR. The candidate organized the experimental setup including recovering and filtering culture water from the sampling site, preparing different dilutions for the experimental treatments and the algal mix for feeding. With the assistance of JM, he carried out the sampling on board of R/V Helmer Hensen, including isolation and taxonomic identification of the foraminifera. The candidate then carried out the experiment, recorded the observations, analysed the data, produced the figures, interpreted the results and wrote the first draft of the manuscript. All co-authors commented and contributed to improve the draft.

For the fourth study (**Chapter 5**), the candidate developed the research question and designed the study with the help of MK. He processed and analysed new plankton samples from the Fram Strait collected by KZ, KW and TLR. The candidate taxonomically identified the different species, produced new counts and combined with a newly developed compilation of published data from the study area. The candidate extracted oceanographic data from satellite raster images and CTD profiles, harmonised all data, designed and performed the statistical analyses, produced the figures, interpreted the data and produced the first draft of the manuscript with contribution from all co-authors.

Chapter 1

INTRODUCTION

1.1 A changing Arctic

Over the last century, the Earth's climate system faced dramatic transformations with the global average surface temperature rising by approximately 1°C (Pörtner et al. 2019). Anthropogenic emissions of greenhouse gases are the primary culprit behind the observed warming trend (Huber and Knutti 2012; Santer et al. 2013) and the effects are more pronounced in the Arctic where average air temperatures increased at 2.4 times the rate of the Northern Hemisphere average (Box et al. 2019), an effect known as Arctic amplification. As a result, Arctic sea ice cover is thinning and shrinking at increasing pace and magnitude, inducing profound changes in the polar ecosystem and to the arctic fauna (Fig.1-1) (WWF 2018; Box et al. 2019; Pörtner et al. 2019).

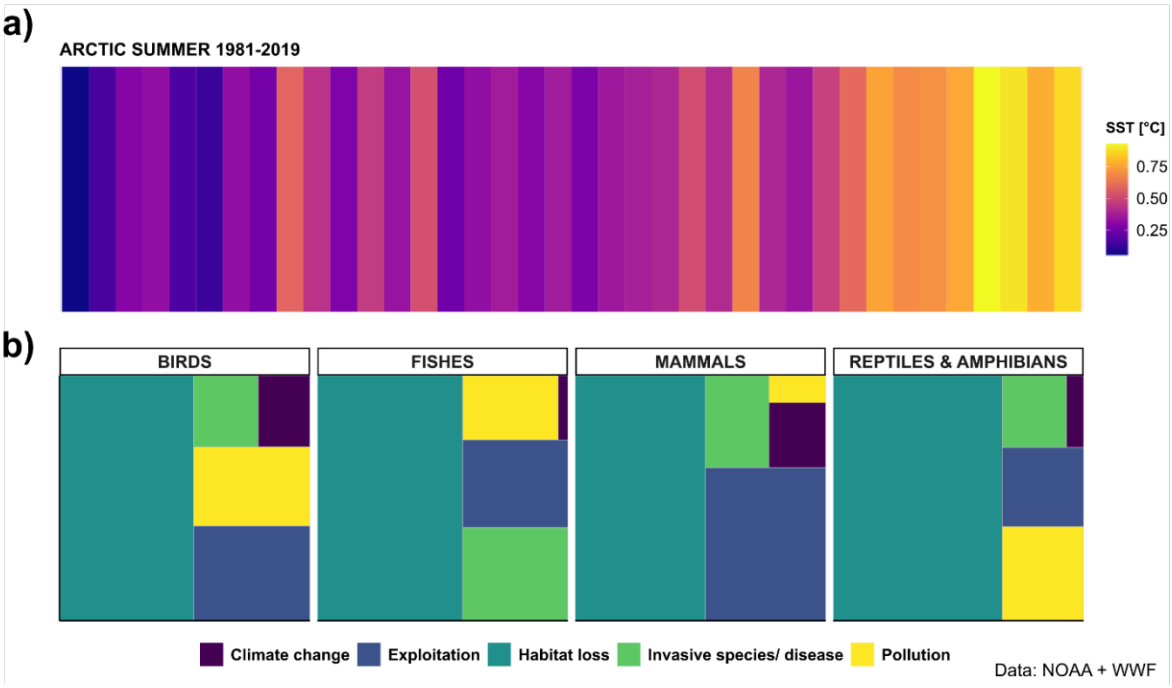

Figure 1-1 *a) Average Arctic Ocean summer sea surface temperatures for the period 1981-2019. Each stripe represents one year (data from NOAA Optimum Interpolation Sea Surface Temperature V2 [weekly resolution] (Reynolds et al. 2002). b) Tree maps showing the main threats to Arctic fauna. Data from the Living Planet Report (WWF 2018). Own visualisation.*

Sea ice biota and marine species are the most threatened as their habitat is collapsing (Karnovsky & Gavrilo, 2016; Laidre et al., 2015; Wassmann et al., 2011). The rapid ice loss of Arctic glaciers and the Greenland Ice Sheet is also significantly contributing to global sea-level rise, posing threats to coastal ecosystems but also human settlements and infrastructures (Pörtner et al. 2019). Model simulations predict that due to the freshening deriving from the sea

ice melt, the Arctic Ocean is expected to experience a decrease in surface mean carbonate saturation state and pH by more than 20% (Steinacher et al. 2009). The predicted acidification of Arctic waters will impact the survival of organisms that produce carbonate shells or skeletons like pteropods (Comeau et al. 2009; Koh et al. 2015), foraminifera (Manno et al. 2012), bivalves and cold-water corals (Fabry et al. 2009).

The environmental changes in the Arctic are already affecting marine biodiversity: poleward range shifts of non-native species have been observed in plankton (Kraft et al., 2013; Oziel et al., 2020; Schröter et al., 2019; Wassmann et al., 2015) and fishes (Andrews et al., 2018; Mecklenburg, Lynghammar, Johannesen et al., 2018). This trend is projected to intensify in the future and involve more marine species due to the progressive warming of polar waters (Beaugrand et al., 2019; Hastings et al., 2020; Ibarbalz et al., 2019).

The biophysical transformations faced by the Arctic ecosystem are unprecedented for the last century, and as polar amplification continues, the effects will extend beyond the Arctic boundaries (Box et al. 2019). Already during the 20th Century, pulses of freshwater from the Fram Strait and the Canadian Arctic caused the so-called "Great Salinity Anomalies" that influenced circulation patterns in the North Atlantic (Dickson et al. 1988; Belkin et al. 1998). On longer time-scales, deeper in the history of Earth's climate, paleoclimatic studies showed that abrupt large-volume discharges of freshwater into the Arctic Ocean and its surrounding seas were capable of disrupting the formation of the North Atlantic Deep Water, slowing down the global thermohaline circulation and consequently triggering wide-ranging climate change (Greene et al. 2008).

A better understanding of the past natural variability of the Arctic Ocean on time scales longer than the instrumental era is therefore needed for more accurate model predictions of the changes we can expect in a future shaped by both natural as well as anthropogenic climate (Polyak et al. 2010; Zhu et al. 2020).

The fossil remains of marine microorganisms from deep-sea sediments represent a useful resource for reconstructing the oceanographic history of the Arctic Ocean, in particular, the marine zooplankton group of planktonic foraminifera is commonly used as polar paleoceanographic proxy (Ericson 1959; Kellogg et al. 1978; Bond et al. 1993; Stein et al. 1994; Kohfeld and Fairbanks 1996).

1.2 Planktonic foraminifera: sentinels of change

Planktonic foraminifera are marine protists that produce a calcite shell (or test) around their cell (Fig. 1-2). The extant 47 morphospecies described occur globally in the world's oceans (Schiebel and Hemleben 2017) and display a well-defined biogeographic distribution which is mainly controlled by sea-surface temperature (Bé & Tolderlund, 1971; Morey et al., 2005). In the water column, the vertical distribution of these protists varies according to species-specific ecological niches primarily connected to physical vertical gradients that control light intensity, water temperature, oxygen availability and concentration of food (Fairbanks et al. 1980; Field 2004; Kucera 2007; Rebotim et al. 2017). Different preferred habitat depths also depend on

species life-history traits and reflect an array of different strategies (e.g., presence/absence of photosynthetic symbionts, food preference).

Planktonic foraminifera occur in low densities in the global ocean (Keeling and del Campo 2017), but they are unique among zooplankton because their calcite shells are well preserved in the marine sediment, providing an exceptional archive for investigating past climatic and ecological change across temporal scales (Lewandowska et al. 2020). The high sensitivity of planktonic foraminifera diversity to environmental forcing makes the comparison of fossil communities' composition an invaluable resource to elucidate climate change dynamics in time and space on time scales beyond the instrumental period.

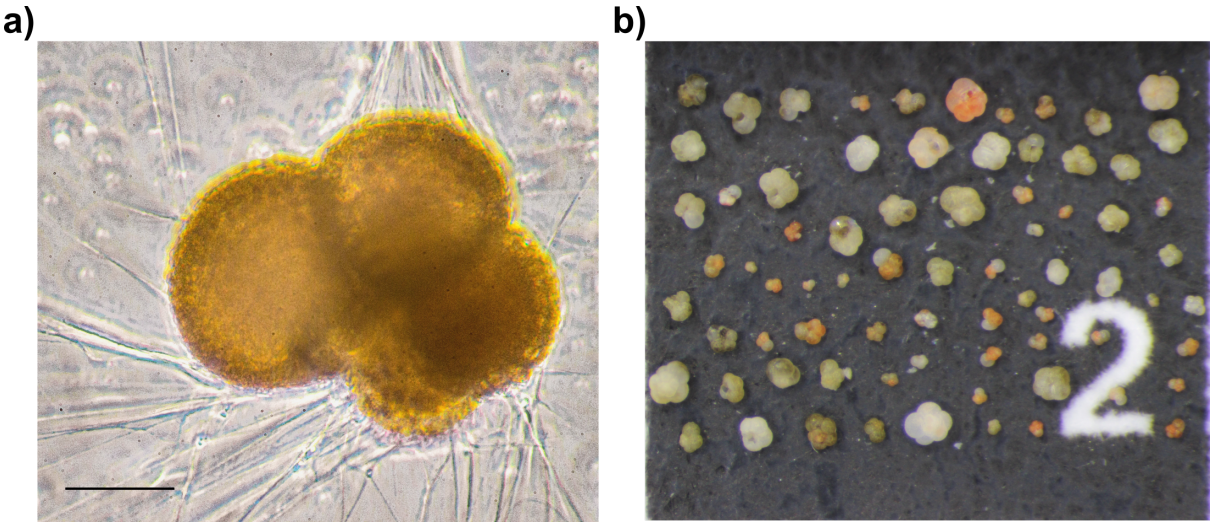

Figure 1-2 *a) Living planktonic foraminifera specimen. Scale bar = 50µm. Own picture. b) Planktonic foraminifera specimens sampled with a plankton tow in the Baffin Bay. The number 2 in the photo is 2 mm. Photo: Nina Wunder.*

Indeed, analyses of planktonic foraminifera census counts have shed light on past climatic transitions (Broecker et al. 1988, 1990). Moreover, the record of past planktonic foraminifera communities can serve as a baseline for previous marine ecosystem states that can be used to assess the impact of human influence on modern climate (Field et al., 2006; Jonkers et al., 2019; Spielhagen et al., 2011).

The chemical signature of the foraminifera shell is another extensively used paleoproxy as it provides information about the composition and history of the seawater and the environmental conditions at the time of calcification (Emiliani 1955; Nurnberg 1995; Ravelo and Hillaire-Marcel 2007; Pearson 2012). Such an approach allows reconstructions of paleotemperatures, paleosalinities, and paleochemistry of the oceans, which provide essential data for global circulation models that try to predict the response of the atmosphere-ocean system to changes such as atmospheric CO_2 increase (Erez, 2003).

However, the interpretation of the environmental signal preserved in planktonic foraminifera is not straightforward and requires a deep understanding of the species biology and ecology. Many different factors can affect the chemical signal of the shell as the presence of symbionts, or the shell-size or even the microenvironment where the calcite is secreted (Ezard et al. 2015; Fehrenbacher et al. 2018). Similarly, chemical and census data derived from sediment present challenges as the recovered assemblages represent in many cases composite pictures of species

inhabiting different seasonal and vertical habitats and thus, do not necessarily reflect the same environmental forcing (Jonkers & Kucera, 2019; Jonkers & Kucera, 2017; Lessa et al., 2019) (Fig. 1-3). Unfortunately, despite decades of study on planktonic foraminifera in the Arctic Ocean, many uncertainties remain, hampering our understanding of their ecological niches and therefore the correct interpretation of the paleosignal present in their shells.

1.3 Arctic planktonic foraminifera: the missing link

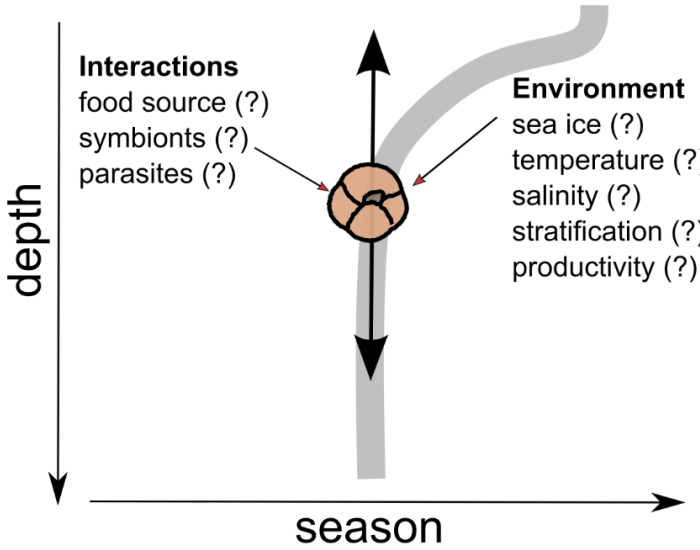

Figure 1-3 *Theoretical scheme of the influence of ecological and biological factors on the paloesignal recorded by planktonic foraminifera investigated in this thesis. The shaded grey line represents an idealized temperature profile. Own visualisation.*

The Arctic is one of the Earth's least understood biomes because of the logistic and economic challenges that limit the research activity in such a remote region (Colella et al. 2019). Indeed, for the first 80 years of the 20th century, most of the observations on pelagic zooplankton were based on samples collected from drifting ice platforms or ships frozen in the ice (Kosobokova & Hirche, 2009). Planktonic foraminifera were no exception, by using 200 µm mesh size nylon net from the drifting station Alpha, the pioneer micropaleontologist Allan Bé collected specimens of the most abundant polar species, *Neogloboquadrina pachyderma*, and described its different ontological stages in one of the earliest paper on the topic (Bé, 1960). With the advent of the big icebreakers in the 1980s, opportunities to investigate arctic planktonic foraminifera multiplied and with them, the possibility to carry out interdisciplinary research with better sampling design and allowing a better understanding of relationships between the structure of the pelagic communities and hydrographic processes (Carstens and Wefer 1992; Carstens et al. 1997; Volkmann 2000). Nevertheless, ice conditions still influenced the work at sea, impairing the building a spatially and temporally consistent record of repeated observations. This resulted in stark differences among studies on planktonic foraminifera ecological preferences. Even for the abundant *N. pachyderma,* no consensus existed on the main abiotic factors controlling its vertical distribution (Xiao et al. 2014), nor about its main biotic interactions (Volkmann 2000) (Fig. 1-3). This gap in ecological knowledge is also reflected in the recently published Protist Interaction Database (PIDA) that collects observations of protist-protist or protist-prokaryote ecological interactions (Bjorbækmo et al. 2020) and contains no entry for polar planktonic foraminifera species (Fig. 1-4). Ecological information on the favourite food source or the presence of symbionts in these polar protists could contribute to improving their use as paleoceanographic proxy (Bird et al. 2018). Moreover, the lack of established culturing protocols of these species under cold conditions hinders our understanding of their physiology

and ecological ranges. This is particularly important when it comes to reconstructing past meltwater influx from ice sheets, since reconstructions are based on the assumption that calcification occurs close to the surface, within the layer affected by the discharged meltwater. The paelosignal of salinity levels not conducive to foraminifera' survival or for their calcification, would not be recorded in the shell, resulting in a systematic underestimation of the true salinity anomaly.

Thus, clarifying the ecological preferences of planktonic foraminifera in the Arctic is a fundamental precondition for a comprehensive interpretation of paleoceanographic environments in Polar Regions.

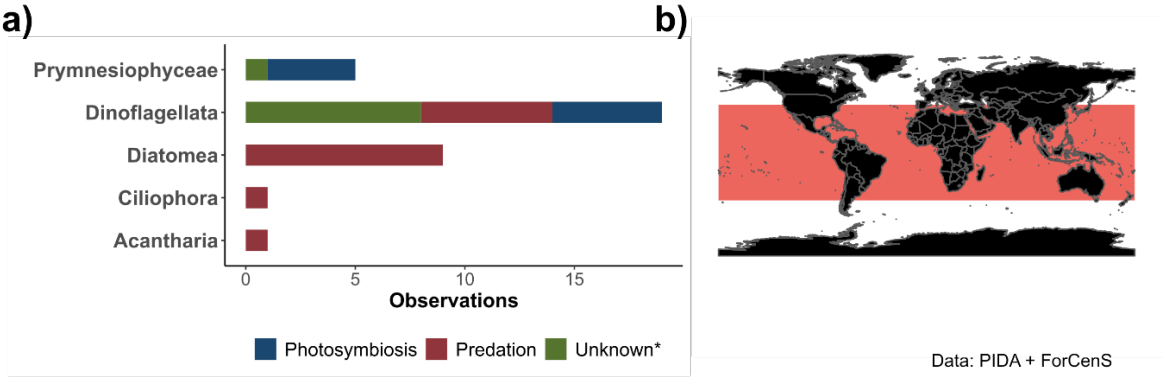

Figure 1-4 *a) Observed planktonic foraminifera interactions with other protists reported in PIDA. (*) Unknown symbiotic interaction b) Distribution range (red area) of planktonic foraminifera species included in PIDA derived from core-top sediment assemblages. Data from PIDA (Bjorbækmo et al. 2020) and ForCenS (Siccha and Kucera 2017). Own visualisation.*

1.4 Thesis Objectives and Outline

The aim of this thesis is to constrain the ecological niche of Arctic planktonic foraminifera species using a combination of different approaches including data synthesis, culturing, and metabarcoding analyses (Fig. 1-5). This work is a cumulative thesis consisting of four manuscripts. Two chapters have been accepted for publication: Chapter 2 has been published in the journal *Biogeosciences* while Chapter 4 has been accepted for publication in the journal *Polar Research*. Chapters 3 and 5 are in preparation for submission.

The manuscript in Chapter 2 is a study that aimed to constrain the factors controlling the depth habitat of the species *Neogloboquadrina pachyderma*, the dominant planktonic foraminifera species in the Arctic. Previous work showed that *N. pachyderma* in the northern polar regions has a highly variable depth habitat, complicating the interpretation of the paleoecological signal contained in its shells. The following working hypothesis was tested:

> h_1: Environmental factors are the main drivers of *N. pachyderma* vertical distribution in the Arctic

A compilation of new and existing population density profiles from 104 stratified plankton tow hauls collected in the Arctic and the North Atlantic oceans and associated oceanographic data were analysed to test h_1. The importance of environmental factors (mixed-layer depth, sea

surface temperature, sea surface salinity, chlorophyll-a concentration, and sea ice concentration) and ecological factors (synchronized reproduction and daily vertical migration) on *N. pachyderma* vertical distribution was assessed using a statistical approach.

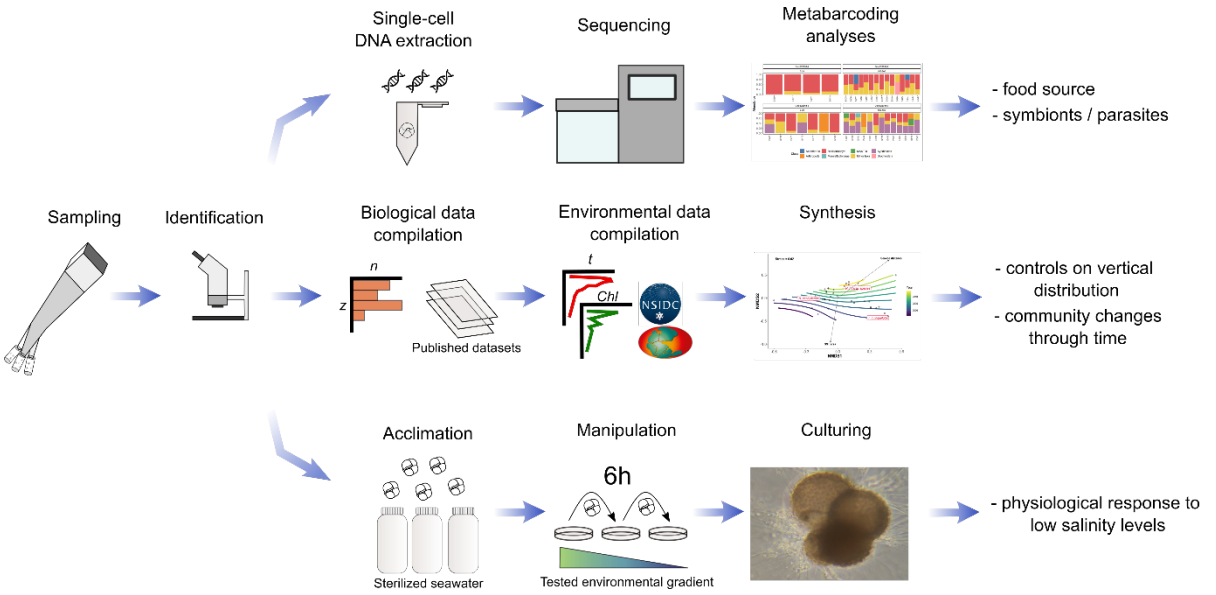

Figure 1-5 *Schematic illustration of the different approaches used in this thesis and the respective research questions addressed. Own visualisation.*

In Chapter 3, the biotic interactions of *N. pachyderma* with the pelagic eukaryotic community were investigated and the following working hypothesis was tested:

h$_2$*: N. pachyderma* is herbivorous and feeds on diatoms

The hypothesis was tested by assessing the eukaryotic community within the single foraminifera and in the contextual seawater using a metabarcoding approach on samples collected in the Baffin Bay during the MSM66 expedition in 2017.

In Chapter 4, the results of a culturing experiment on the species subpolar species *Neogloboquadrina incompta* are reported. Chemical signatures in the calcite of shells of this species have been previously used to trace and quantify past meltwater discharge events, but no experimental data exist on the range of salinities under which *N. incompta* can survive and thus, could potentially record. This study aimed to provide the first insight into the changes in the physiology and viability of *N. incompta* in response to different salinity conditions and to test the following working hypothesis:

h$_3$: *Neogloboquadrina incompta* can survive in low salinity conditions

An experimental approach was designed to test this hypothesis. Specimens of *N. incompta* were collected in the northern Norwegian Sea off Tromsø in October 2018. The foraminifera were exposed to a gradient of salinities between 35 and 25 PSU, and their response and survival were monitored over 26 days by measuring the extent of the rhizopodial network.

Chapter 5 aimed to constrain the changes in the community structure and distribution of planktonic foraminifera community in the Arctic. The study was based on a compilation of new and published data assembled to obtain a 30-years long time series of vertical abundance profiles of the planktonic foraminifera community from the Fram Strait. The resulting dataset was analysed to test the following working hypothesis:

> h_4: Changing Arctic climate is already affecting polar planktonic foraminifera diversity and distribution

Closing remarks and future perspectives are presented and discussed in Chapter 6.

Chapter 2

DEPTH HABITAT OF THE PLANKTONIC FORAMINIFERA *Neogloboquadrina pachyderma* IN THE NORTHERN HIGH LATITUDES EXPLAINED BY SEA-ICE AND CHLOROPHYLL CONCENTRATION

This work has been published in *Biogeosciences*, 12 Sep 2019.

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Data availability: Data on total concentrations of *N. pachyderma* from plankton tows during cruises NEWP-92, NEWP-93, ARK-IV/3, ARK-X/1, ARK-X/2, ARK-XI/2, M36/3, MSM09/2, ARK-XXVI/1 is available at https://doi.pangaea.de/10.1594/PANGAEA.905270. The table complete with data source and derived environmental data of the stations included in the study is available on Zenodo (DOI: 10.5281/zenodo.2653733).

Abstract

Neogloboquadrina pachyderma is the dominant planktonic foraminifera species in the polar regions. In the northern high latitude ocean, it makes up more than 90% of the total assemblages, making it the dominant pelagic calcifier and carrier of paleoceanographic proxies. To assess the reaction of this species to a future shaped by climate change and to be able to interpret the paleoecological signal contained in its shells, its depth habitat must be known. Previous work showed that *N. pachyderma* in the northern polar regions has a highly variable depth habitat, ranging from the surface mixed layer to several hundreds of meters below the surface, and the origin of this variability remained unclear. In order to investigate the factors controlling the depth habitat of *N. pachyderma*, we compiled new and existing population density profiles from 104 stratified plankton tow hauls collected in the Arctic and the North Atlantic oceans during 14 oceanographic expeditions. For each vertical profile, the Depth Habitat (DH) was calculated as the abundance-weighted mean depth of occurrence. We then tested to what degree environmental factors (mixed layer depth, sea surface temperature, sea surface salinity, chlorophyll *a* concentration and sea ice concentration) and ecological factors (synchronised reproduction and daily vertical migration) can predict the observed DH variability and compared the observed DH behaviour with simulations by a numerical model predicting planktonic foraminifera distribution. Our data show that the DH of *N. pachyderma* varies between 25 m and 280 m (average ~100 m). In contrast with the model simulations, which indicate that DH is associated with the depth of chlorophyll maximum, our analysis indicates that the presence of sea-ice together with the concentration of chlorophyll *a* at the surface have the strongest influence on the vertical habitat of this species. *N. pachyderma* occurs deeper when sea-ice and chlorophyll concentrations are low, suggesting a time transgressive response to the evolution of (near) surface conditions during the annual cycle. Since only surface parameters appear to affect the vertical habitat of *N. pachyderma,* light or light-dependant processes might influence the ecology of this species. Our results can be used to improve predictions of the response of the species to climate change and thus to refine paleoclimatic reconstructions.

2.1 Introduction

Neogoboquadrina pachyderma is the most abundant planktonic foraminifera in the Arctic Ocean and its marginal seas, where it also dominates the pelagic calcite production (Volkmann 2000; Schiebel et al. 2017). When the organism dies, its calcite shells sink to the seafloor and when preserved in the sediments, it serves as a source of information on the physical state of the ocean in the past (Kucera 2007; Eynaud 2011). To understand the origin of the paleoceanographic proxy signal and to predict the production of the species under varying physical conditions, including projected future change scenarios, it is important to constrain the factors that determine its vertical habitat. Previous work has shown that the seasonality of *N. pachyderma* production follows the timing of food availability, which is tightly linked with temperature (Jonkers & Kucera, 2015; Tolderlund & Bé, 1971). On the other hand, the vertical habitat of the species is variable and appears hard to predict (Xiao et al. 2014).

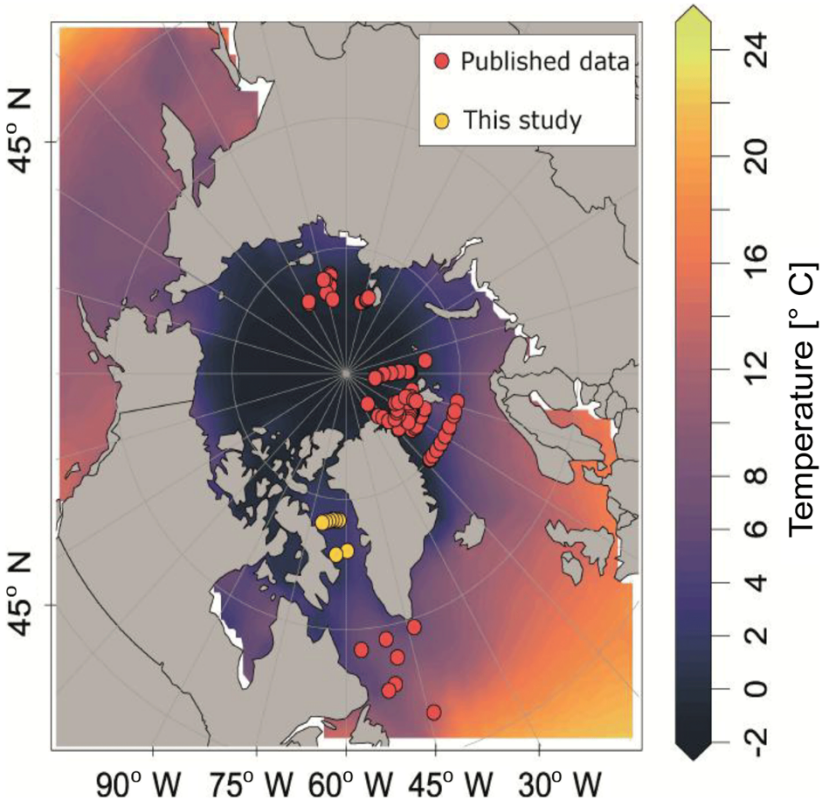

Figure 2-1 *Plankton net stations with vertically resolved N. pachyderma counts that were used in this study. Background colour indicates the mean summer sea surface temperature (SST) (data from World Ocean Atlas 2013, Locarnini et al., 2013).*

Previous studies proposed different abiotic factors as drivers of *N. pachyderma* vertical distribution including temperature (Carstens et al., 1997; Carstens and Wefer, 1992; Ding et al., 2014), density stratification (Simstich et al. 2003) and the depth of the subsurface chlorophyll maximum indicating food availability (Kohfeld and Fairbanks, 1996; Pados & Spielhagen, 2014; Volkmann, 2000). Next to environmental factors, the behaviour of the species itself, such as its ontogenetic vertical migration (Bijma, Erez, et al., 1990; Erez, 1991) and day/night migration (Field 2004), or morphologically hidden cryptic diversity (Weiner et al., 2012), could also influence the vertical habitat observed in a single profile. However, the Arctic and the North Atlantic are inhabited by a single *N. pachyderma* genotype (Type I) (Darling et al. 2007), indicating that the variable depth habitat of the species cannot be attributed to cryptic diversity. On the other hand, analysis of the size distribution of *N. pachyderma* shells in the Arctic by Volkmann (2000) suggested a synchronised reproduction around the full moon, with sexually mature individuals descending towards a deeper habitat to release gametes. Similarly, diel vertical migration (DVM) is known to confound observations of vertical distributions patterns of Arctic plankton (Berge et al. 2009). Although the only study on DVM in polar waters on *N. pachyderma* showed no evidence of this phenomenon (Manno and Pavlov 2014), it was based on observations during the midnight sun with relatively weak changes in light intensity and the existence of DVM in *N. pachyderma* during other times of the year cannot be firmly ruled out. Therefore, the influence of the two ecological patterns on the depth habitat of *N. pachyderma* has to be considered in the analysis of our compilation of vertical profiles.

The lack of consensus on potential drivers of habitat variability in *N. pachyderma* calls for a systematic approach synthesizing new and existing observations into the same conceptual framework. In addition, there is now an opportunity to compare observations with predictions

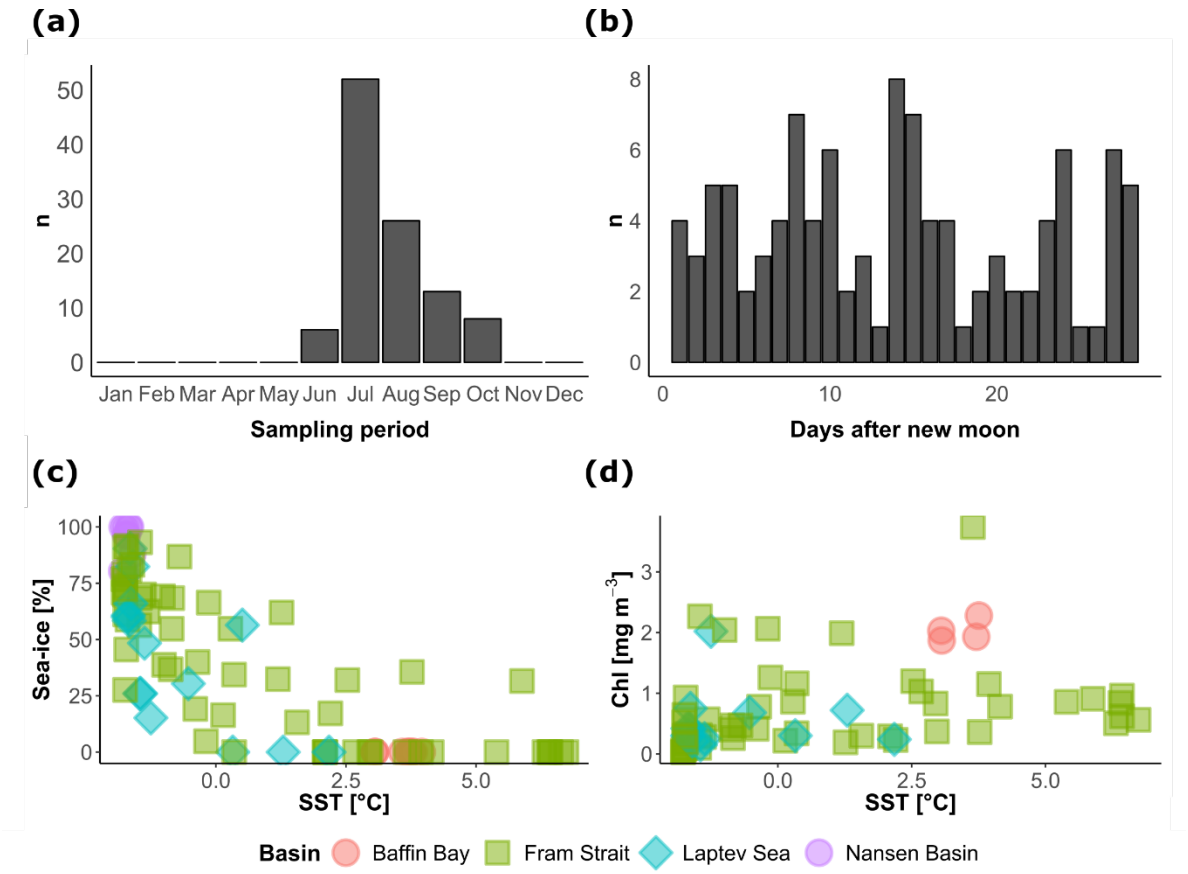

Figure 2-2 *Temporal and environmental coverage of the vertical profiles of N. pachyderma concentration included in the study. The distribution of (a) the months and (b) days of the synodic lunar cycle of sample collection, showing a summer bias but even coverage of the lunar cycle. The relationship between the environmental conditions during sample collection (c–d) indicates the extent of the sampled environmental space.*

of a numerical model in the same framework. This opportunity arises from the recently extended model PLAFOM2.0, which can predict the seasonal and vertical habitat of *Neogloboquadrina pachyderma* (Kretschmer et al. 2018). This model is driven by temperature, food concentration, and light availability (which matters only for species with symbionts). The species-specific food concentrations are simulated by the Community Earth System Model, version 1.2.2 (CESM1.2, Hurrell et al., 2013) at every time step and are subsequently used by PLAFOM2.0 to calculate the monthly carbon concentration of *N. pachyderma* and other four species of planktonic foraminifera.

Here, we assembled existing vertical population density profiles of this species from the Arctic and North Atlantic, combined these with new observations from the Baffin Bay and associated the observations with oceanographic data. Based an analysis of this dataset, we present a new concept that explains depth habitat variability in this important high-latitude marine calcifier. Next to three previously proposed environmental drivers of habitat variability (temperature, stratification, food availability), we also consider chlorophyll concentration at the surface as a measure of productivity, as well as salinity and sea-ice concentration. These parameters were included in order to test i) the possibility that the foraminifera are attracted to food at the surface, ii) the possibility of the foraminifera evading low salinity surface layers, and iii) the possibility that the foraminifera habitat responds to sea-ice related variability in light, atmospheric exchange and/or mixing.

2.2 Material and Methods

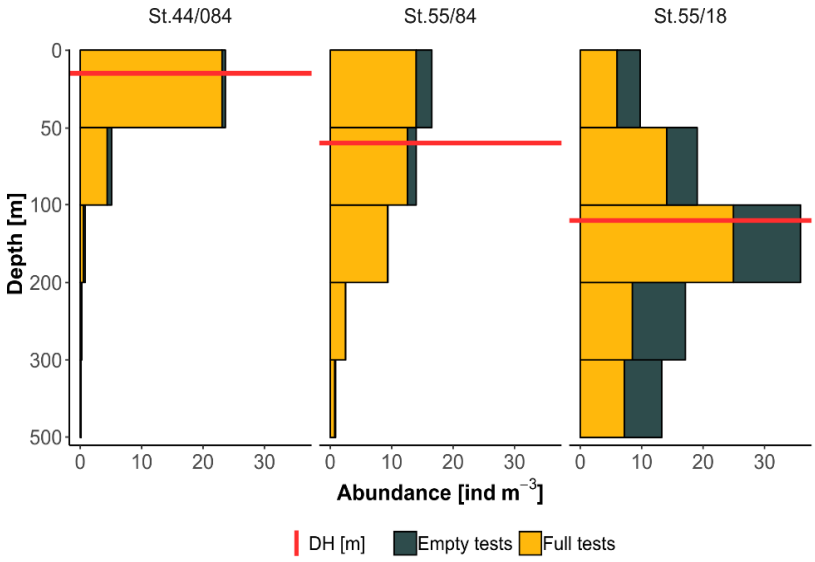

Figure 2-3 *Example of vertical profiles from three stations included in the study displaying shallow (left), intermediate (centre), and deep (right) depth habitat (DH).*

Our analysis is based on a synthesis of existing and new vertical abundance profiles of *N. pachyderma* from the high-northern latitudes. We exclude the Pacific Ocean because it is inhabited by a distinct genetic type of *N. pachyderma* with potentially different ecology (Darling et al. 2007). We compiled 97 population density profiles of *N. pachyderma* collected during 13 oceanographic expeditions between 1987 and 2011 (Fig.2-1). We excluded one profile from Jensen (1998), station 37/6, where the abundance maximum occurred anomalously deep (below 500 m) and which we thus suspect to reflect an error (i.e. due to sample mislabelling). We retained all other profiles, despite the differences in the sampling design (mesh size and vertical resolution of the sampled depth intervals) and in counted size fraction. The compilation is representative of the Eurasian Arctic Ocean and its marginal seas, as well as of the North Atlantic, but contains no data from the oceanographically distinct Baffin Bay. To fill this gap, we extended the compilation by generating new data from eight plankton tow profiles collected during the MSM09 cruise in 2008 (Fig.2-1). At all stations sampling was carried out down to 300 m using a multiple closing plankton net (HydroBios, Kiel) with a 50 × 50 cm opening and a 100 µm mesh (Kucera et al. 2014). The vertical distribution of planktonic foraminifera was resolved to nine levels by conducting two casts at each station (300–220 m, 220–180 m, 180–140 m, 140–100 m, 100–80 m, 80–60 m, 60–40 m, 40–20 m, 20–0 m). After collection, net residues from each depth were concentrated on board, settled and decanted, filled up with 37% formaldehyde to a concentration of 4% and buffered to pH 8.5 using pure solid hexamethylenetetramine ($C_6H_{12}N_4$) to prevent dissolution, and refrigerated. Specimens of planktonic foraminifera were picked from the wet samples under a binocular microscope and air-dried. All individuals in the fraction above 100 µm were counted and identified to species level following the classification of Hemleben et al. (1989) and Brummer and Kroon (1988). Full (cytoplasm-bearing) tests were counted separately and considered as living at the time of sampling. Counts were converted to concentration using the volume of filtered water determined from the product of towed intervals height and the net opening (0.25 m^2).

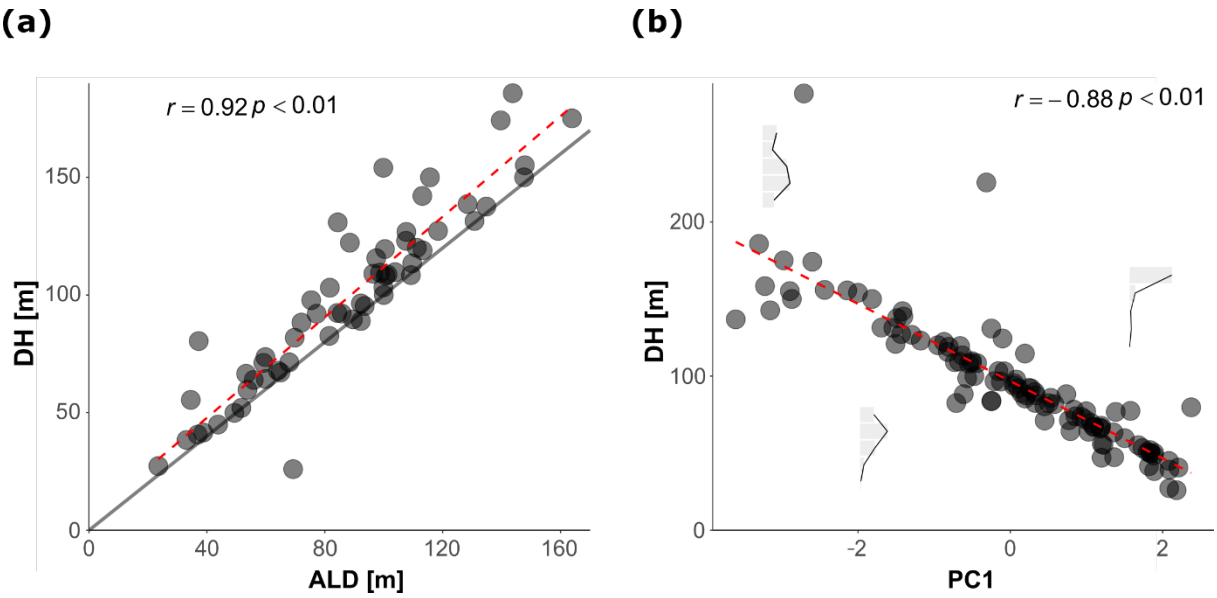

Figure 2-4 *(a) Relationship between the depth habitat (DH) and the average living depth (ALD). The dashed red line shows the linear fit while the solid line represents the 1:1 relationship between the two variables. (b) Relationship between the DH and the PC1 resulted from the PCA calculated on the normalized counts. The abundance profiles based on the standardized counts in the plot show examples of the shape of the vertical distribution of N. pachyderma for three values of PC1 loadings The dashed red line shows the linear fit.*

For the new profiles from the Baffin Bay, water temperature and salinity were measured with a conductivity–temperature–depth (CTD) device deployed before each plankton tow. A submersible fluorospectrometer (bbe Moldaenke GmbH) was used for the stations MSM09/457, MSM09/458, MSM09/460 and MSM09/462 to obtain vertical profiles of algae pigment concentrations from the surface to 300 meters depth (Kucera et al., 2014). For the remaining profiles from the literature, physical oceanographic data and chlorophyll *a* concentration profiles for each station were, if available, obtained from CTD profiles retrieved from the PANGAEA data repository using the R package "pangaear" (Simpson and Chamberlain, 2018, R Core Team, 2017). Sea surface parameters, sea surface temperature (SST), sea surface salinity (SSS) and surface chlorophyll concentration, were obtained from CTD profiles and Niskin bottles by averaging all the values from the first 5 meters. The depth of the chlorophyll maximum (DCM) was determined from vertical profiles of chlorophyll concentration obtained from either water column profiles or discrete measurements from Niskin bottles. The depth of the mixed layer (MLD), defined as the depth where in situ water density varied by more than 0.03 kg/m^3 as in De Boyer Montegut *et al.* (2004), was calculated from the CTD profile of each station using a custom function in R. No vertically resolved profiles of environmental variables were available for plankton net hauls collected during the expeditions NEWP93, ARK-IV/3, ARK-X/1, ARK-X/2, M36/3, and M39/4. These profiles could thus only be used for the analysis of ontogenetic and diel vertical migration. In addition to the in-situ data, daily sea ice concentrations for the location of all the 104 sites included were extracted from 25 × 25 km resolution passive microwave satellite raster imagery obtained from the National Snow and Ice Data Centre (Boulder, Colorado, USA) for 1979–2011 using a custom function in R (R Core Team 2017). We used the data to determine sea-ice concentration at the time of collection and also to retrieve the time after sea-ice break for all stations that were sea-ice free

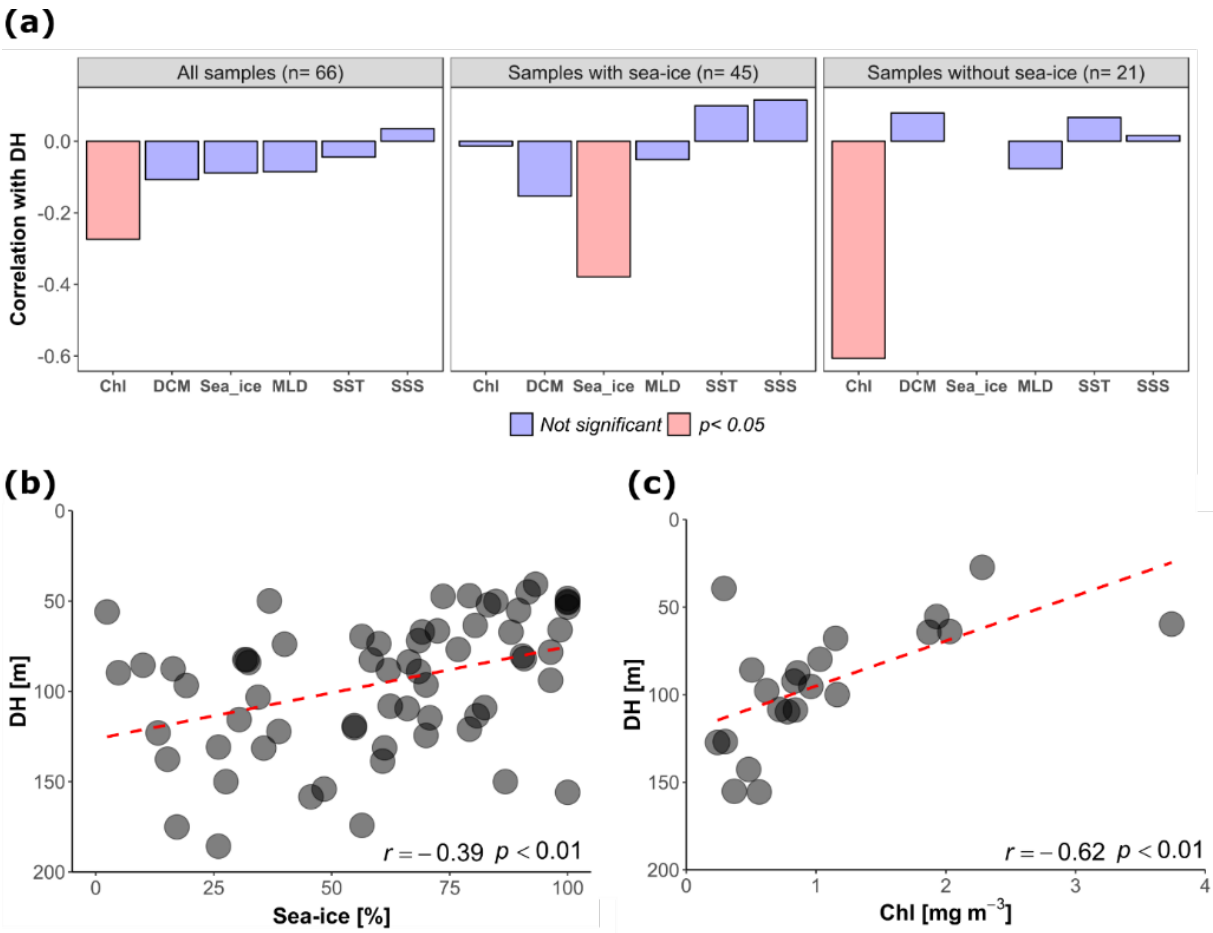

Figure 2-5 *Correlation between depth habitat (DH) and the environmental variables calculated at all the sites, in the subsets with sea ice and without sea ice (only sites where all the tested variables were available were considered). Chl: chlorophyll concentration at surface; Sea_ice: sea-ice coverage; DCM: depth of chlorophyll maximum; SST: sea surface temperature; MLD: depth of the mixed layer; SSS: sea surface salinity. (b) Relationship between DH and sea-ice concentration in the stations covered by sea ice (all the sites with available sea-ice data are shown, n=65). (c) Relationship between DH and chlorophyll concentration at the surface for the sea-ice-free stations (all the sites with available chlorophyll data are shown, n=22). The dashed red lines show the linear fit.*

at the moment of sampling. The date of the most recent sea-ice concentration maximum was used to retrieve the time by subtracting the days until the time of collection. Finally, the time of the collection was compared to the time of sunrise and sunset for each station determined using the R package "SunCalc" (Agafonkin and Thieurmel 2018) to distinguish day-time and night-time collections. The sampling date was used to determine the lunar day using the R package "lunar" (Lazaridis 2015).

The cross plots in Fig. 2-2 show how the final compilation of 104 profiles covers the environmental space and how the observations are spread across the seasons and the lunar cycle. The sampling is strongly biased towards the summer but the lunar cycle is completely covered. Most of the profiles were collected under midnight sun conditions, leaving only 28 profiles that could be used to test the diel vertical migration (Table 2-1). The profiles cover SST conditions between -2 and 7°C and contain profiles taken across the entire range of sea-ice concentrations. Since sea-ice concentration at the studied profiles was not linearly related to SST, the compilation should allow to assess the effect of the two variables independently (Fig.2-2c). Productivity, expressed as surface chlorophyll *a* concentration, is neither correlated with temperature. The most productive stations were located in the Baffin Bay and in the Fram Strait

Table 2-1 *Results of the t test performed on the samples collected in normal day–night conditions to assess the effects of DVM on DH.*

Time of the day	n	Mean DH (m)	Std. Deviation	t-value	p-value
Night	19	99.069	46.762	-1.82	0.08
Day	9	66.949	35.401		

with surface chlorophyll concentrations ranging between 2 and 4 mg m^{-3} (Fig. 2-2d). Surface salinity was mostly around 33 PSU, only in the Laptev Sea values dropped below 30 PSU.

To facilitate the analysis of depth habitat across density profiles with observations at different depth intervals, the density profiles were summarized into a single parameter, DH (depth habitat), which is the abundance-weighted mean depth calculated using the mid points of the collection intervals (Fig.2-3), as in Rebotim et al. (2017). The precision with which the DH can be determined is linked to the vertical resolution of the profiles. The combined analysis of casts with different vertical resolution therefore unavoidably introduces some random noise in the DH estimates, but this does not compromise the first order results of our study. Since counts of living and dead specimens were not available for all the stations, total counts were considered. However, where possible, we also derived the average living depth (ALD) to assess possible biases deriving from using total counts to constrain depth habitat. This comparison showed that ALD was highly correlated with DH and on average 11 meters shallower than DH, which thus represents a slight systematic overestimation of the actual living depth of *N. pachyderma* (Fig.2-4). Exceptions are stations MSM09/466, 55/84, and 36/069 where the observed ALD was deeper than DH due to the high number of dead specimens in the upper catch intervals. The appropriateness of a single parameter (DH) as an indicator of the distribution of *N. pachyderma* in the water column was further tested using a multivariate approach. We determined profile-standardized concentrations calculated for 5 depths (0-50, 50-100, 100-200, 200-300, 300-500) for all the stations and performed a principal component analysis (PCA) on the relative abundances in the sampling intervals using the R package "vegan" (Oksanen et al. 2018). The two first principle components explained 43% and 32% of the total variance in the relative abundance in the water column. The first axis exhibited negative loadings for the deeper intervals (100-200, 200-300, 300-500) and positive loadings for shallow intervals 0-50 and 50-100, indicating that it describes a depth-changing unimodal distribution (Fig 2-4b). Mapped on the PC1 loadings, DH showed a significant correlation (Pearson r = -0.88, p-value <0.01) indicating that all profiles had a single maximum and the depth distribution can be collapsed into a single variable (Fig 2-4b).

We start our analysis by considering the potential effect of DVM and the possibility of synchronised vertical ontogenetic migration associated with the lunar cycle. Despite its potential importance (Rebotim et al. 2017), we cannot analyse seasonal variation in depth habitat because only a single season was sampled. The influence of DVM on DH was assessed by dividing samples in two groups based on whether they were collected during the day or during the night. The two groups were tested for homoscedasticity (homogeneity in variances) using an F- test and then a t-test was performed to verify if there was a significant difference in

the DH of day and night populations. To investigate the effects of the lunar cycle on the depth habitat of *N. pachyderma,* we used a periodic regression following the approach described in Jonkers and Kucera (2015). In the next step, we analysed the relationship between DH and sea surface temperature, sea surface salinity, mixed layer depth, surface chlorophyll concentration, depth of chlorophyll maximum and sea-ice concentration. We use linear regression to assess if any of the variables individually predicts a significant part of the DH variability and the variables that showed significant correlation with DH were used to construct a multiple linear regression model allowing interactions. The use of linear regression assumes normality, which was tested, and linearity in the relationship, which is assumed, but prevents overfitting and therefore all estimates of goodness of fit in our models can be considered conservative.

Table 2-2 *Results of the periodic regression performed to assess the influence of the lunar cycle on DH.*

	Depth habitat (m)	
Predictors	*Estimates*	*p*
sin (Lunar day ^{R})	-8.41	0.171
cos (Lunar day ^{R})	-10.39	0.071
Observations	104	
R^2 / adjusted R^2	0.047 / 0.029	

2.3 Results

The DH values derived from the abundance profiles ranged from 26 m to 283 m with an average of 100 m (IQR= 54.95). The deepest observation comes from the Fram Strait, the shallowest from the Baffin Bay.

An independent-samples t-test revealed no evidence for an effect of diel vertical migration on the observed *N. pachyderma* vertical distribution (Table 2-1). Similarly, the periodic regression showed no significant effect of lunar phase on DH (p = 0.17, adjusted r^2 = 0.029) (Table 2-2). In the subsequent analyses we could thus focus on abiotic factors in explaining vertical habitat variability in *N. pachyderma*. Bivariate linear regressions against DH carried out on a subset of 66 profiles for which all of the tested environmental parameters were available yielded a significant relationship only for chlorophyll concentration at the surface (Fig. 2-5a). However, we noticed that profiles from stations where sea-ice was present appeared to show a relationship with sea-ice concentration and we thus carried out separate analyses for profiles with and without sea-ice. We found no significant correlation between DH and the variables SST, SSS, MLD and DCM neither in the complete data set nor in the subsets (Fig.2-5a). Chlorophyll

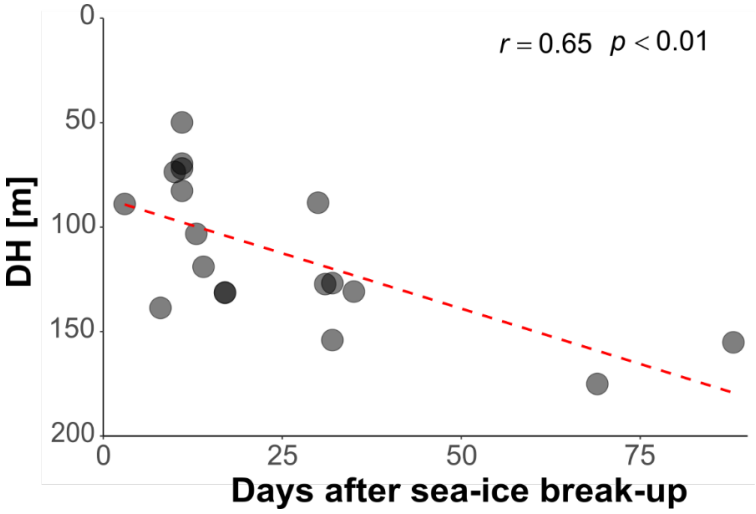

Figure 2-6 *Relationship between depth habitat (DH) and the time (days) after the sea-ice break-up. The dashed red line shows the linear fit.*

concentration at the surface appeared to be the only parameter showing significant negative correlation in both the complete dataset (r = -0.28, p < 0.05) and the sea-ice free subset (r = -0.60, p < 0.01). A negative correlation between DH and sea-ice concentration was observed in the subset including ice-covered stations (r = -0.38, p < 0.05). Following the initial variable selection, where only profiles for which all variables were available were considered, we then extended the analyses to all profiles where sea-ice concentration and/or chlorophyll concentration at the surface were available. These analyses confirm the significance of the relationships (Figs. 2-5b and c).

In the Arctic, the break-up of the sea-ice is normally followed by a pulse of productivity (Leu et al. 2015), making the two tested variables potentially causally connected in a time-transgressive manner. To test for the presence of such a relationship, we tested the relationship between DH and the number of days since sea-ice break-up. To decrease the collinearity between sea-ice and productivity, the analysis was restricted to 18 profiles from stations with chlorophyll concentrations <0.5 mg m^{-3}. This analysis shows that DH significantly increases with time after the sea-ice break-up (r = 0.65, p < 0.01) (Fig. 2-6). In the final step, we combined the three variables that individually showed significant effect on DH for at least one subset of the profiles and constructed a multiple regression model to predict the depth habitat of *N. pachyderma* based on sea-ice concentration and the interaction between chlorophyll concentration at surface and days after the sea-ice break. A linear formulation of the model is significant (p < 0.01) and the model explains 29 % of the depth habitat variability in *N. pachyderma* (adjusted r^2 = 0.29). Next, we tested a non-linear relationship, considering the log-normal nature of the DH. This model leads to a marginal improvement (adjusted r^2 = 0.34) (Table 2-3).

Finally, we evaluate how PLAFOM2.0 (Kretschmer et al. 2018) captures the observed patterns in *N. pachyderma* depth habitat. To this end, we assess the relationship between modelled DH of *N. pachyderma* and modelled SST, SSS, MLD, DCM and chlorophyll concentration for summer months in the geographic area covered by the compilation (Fig.2-1). By comparing modelled with observed ecological patterns, rather than individual observations, we ensure a more meaningful evaluation of the model performance that does not rely in the simulation of individual profiles. Although PLAFOM2.0 simulations also indicate a dominantly subsurface summer depth habitat of *N. pachyderma*, the modelled DH is shallower than observed, with values ranging between 9 and 127 meters (Fig. 2-7). Contrary to observations, the modelled DH shows the highest correlation with the depth of the mixed layer (r = 0.57, p <0.01). Moreover, the observed relationship between the modelled DH and the modelled sea-ice and chlorophyll concentration is lower and of opposite sign to the observations (Figs.2-8a-b).

Table 2-3 *Results of the multiple regression model including sea-ice concentration, chlorophyll concentration at surface, and time since sea-ice break-up as predictors.*

	DH (m)			**log_{10}(DH) (m)**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	110.76	80.37 – 141.15	**<0.001**	2.03	1.89 – 2.18	**<0.001**
Sea-ice (%)	-0.04	-0.08 – -0.00	**0.033**	0	-0.00 – -0.00	**0.021**
Chlorophyll at surface (mg m^{-3})	10.94	-10.82 – 32.71	0.329	0.06	-0.04 – 0.16	0.263
Days after sea-ice break-up	0.71	0.22 – 1.20	**0.007**	0	0.00 – 0.01	**0.005**
Interaction (Chlorophyll and sea-ice break-up timing)	-0.81	-1.25 – -0.37	**0.001**	0	-0.01 – -0.00	**<0.001**
Observations		52			52	
R^2 / adjusted R^2		0.343 / 0.287			0.388 / 0.336	

2.4 Discussion

Previous research indicated the absence of DVM in *N. pachyderma* in the Fram Strait (Manno and Pavlov 2014) but the fact that the sampling was carried out during the midnight sun led the authors to concede that the species still could engage in DVM in the presence of a diurnal light cycle. Indeed, studies on copepods in the Arctic showed that natural patchiness rather than DVM is responsible for shifts in vertical distribution in periods of midnight sun, while in late summer/early autumn, when changes in the diurnal light cycle are apparent, DVM can be observed (Blachowiak-Samolyk et al. 2006; Rabindranath et al. 2011). Our compilation allowed us to assess the behaviour of *N. pachyderma* under changing light condition, but showed no evidence for DVM (Table 2-1). Similarly, a recent investigation on the presence of DVM in planktonic foraminifera from the tropical Atlantic found no evidence for this phenomenon in any of the analysed species (Meilland et al. 2019). Our observations thus add to the existing consensus that planktonic foraminifera are unlikely to participate in DVM. Although we cannot rule out DVM on a very small vertical or geographical scale, we conclude that the observed variability in habitat depth of *N. pachyderma* in our compilation is likely not biased by DVM, allowing us to investigate other potential drivers.

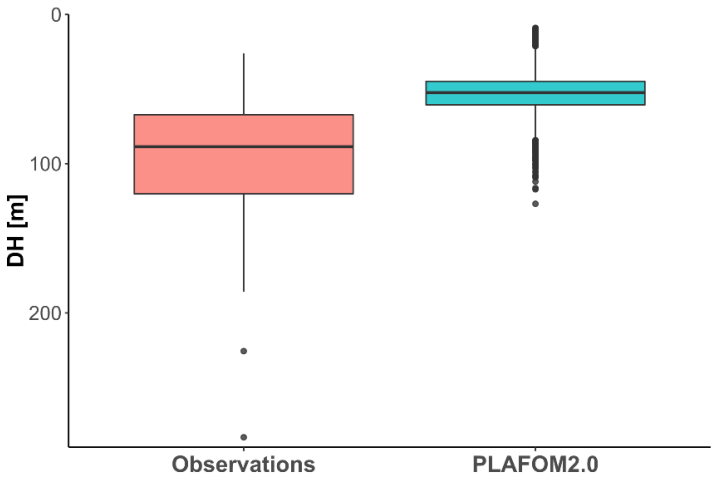

Figure 2-7 *Comparison of observed DH and the PLAFOM2.0 predictions relative to the summer months in the same geographic area covered by our compilation.*

The reproduction of many species of planktonic foraminifera appears synchronized on lunar or semi-lunar cycle (Bijma, Erez, et al., 1990; Jonkers et al., 2015; Rebotim et al., 2017; Schiebel et al., 1997; Spindler et al., 1979), with sexually mature individuals descending towards a deeper habitat to release their gametes (Bijma, Erez, et al., 1990; Erez, 1991). Volkmann (2000) analysed size distribution of *N. pachyderma* in the Arctic and found an indication for a synchronised descent of adult individuals below 60 m during full moon. In our analysis of 104 density profiles, including those from Volkmann (2000), we found no evidence of a systematic shift towards deeper habitat associated with lunar periodicity (Table 2-2). Our analysis cannot resolve whether or not the reproduction in *N. pachyderma* is synchronised nor can we rule out an irregular ontogenetic vertical migration. However, the absence of a systematic relationship between DH and lunar cyclicity in our compilation indicates that a potential ontogenetic vertical migration would likely only contribute a noise component to the DH variability.

Considering all potential sources of noise, including the possibility of an irregular ontogenetic vertical migration, differences in the vertical resolution of the profiles and the counted size fractions, and the large geographical and temporal coverage of the data, it is remarkable that we observe a highly significant relationship between DH and three environmental parameters that collectively explain almost a third of the variance (Table 2-3). This indicates that the vertical habitat of *N. pachyderma* in the Arctic and North Atlantic changes systematically in response to sea-ice and chlorophyll concentration at the surface. The absence of a systematic relationship with any other of the previously considered environmental drivers, like the position of the DCM or thickness of the mixed layer is surprising. It implies that the ecophysiology of the species is not yet completely understood and this lack of understanding is also mirrored in the contrast between the environmental drivers inferred from observations and assumed in PLAFOM2.0 (Fig. 2-8).

There is general consensus that *N. pachyderma* grazes on phytoplankton and it would thus seem reasonable to assume that food availability primarily influences its vertical distribution (Bergami et al., 2009; Carstens et al., 1997; Kohfeld and Fairbanks, 1996; Pados & Spielhagen, 2014; Taylor et al., 2018; Volkmann, 2000). Surprisingly, our analysis yielded no significant correlation between the position of the subsurface chlorophyll maximum and DH. Instead, the DH of the species is always located below DCM and thus most specimens of the population do not appear to be grazing at the DCM. This observation is also in contrast with the modelled relationship between DH and the environmental parameters. As also noted by Kretschmer et al.

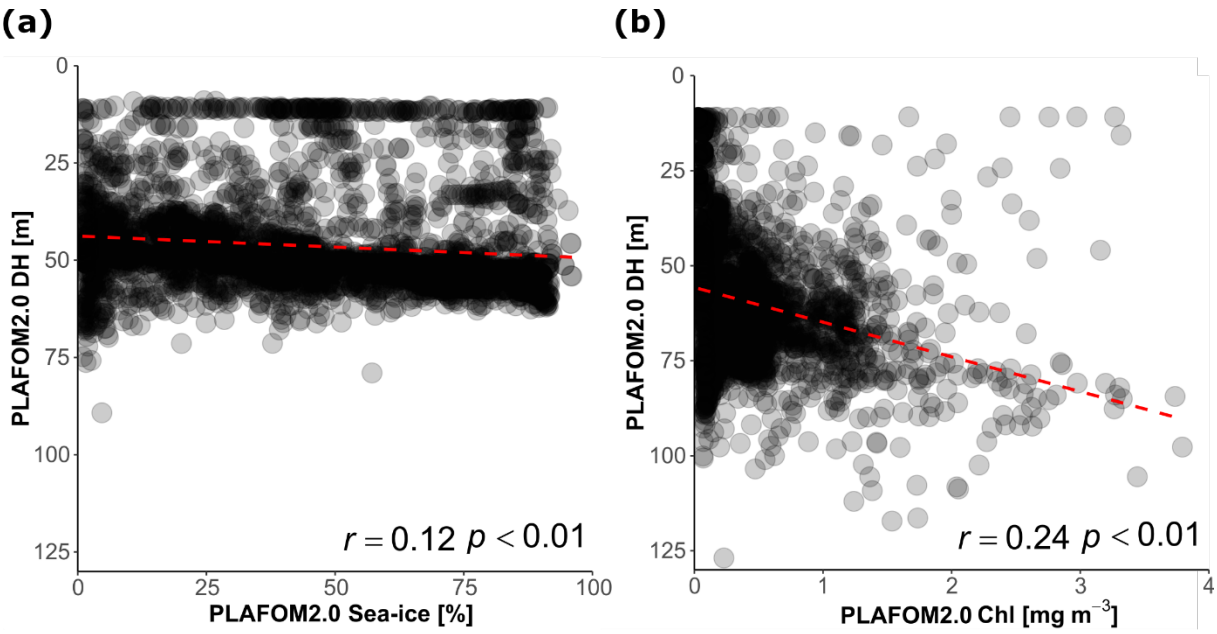

Figure 2-8 *(a) Relationship between the DH predicted by PLAFOM2.0 and (a) sea-ice concentration in the stations covered by sea ice and (b) between DH predicted by PLAFOM2 and chlorophyll concentration at the surface for the sea-ice-free stations (values averaged for the months June, July, August, and September). The dashed red lines show the linear fit.*

(2018), this is because the strong relationship between DH and MLD in the model reflects a strong link between MLD and the position of the subsurface chlorophyll maximum,. This strong link likely results from a bias in the ocean component of the Community Earth System Model (CESM1.2) propagated in PLAFOM2.0. The CESM1.2 model is known to overestimate the mixed layer depth in the Arctic by 20 to 40 meters (Moore et al. 2013). In the model, this overestimation of the MLD affects ocean biogeochemistry and the light regime experienced by the phytoplankton. Specifically, a deeper mixed layer equates to a thicker layer of nutrient depletion, deepening the DCM. Consequently, the simulated depth of the chlorophyll maximum reaches 60 to 95 meters, whereas a recent survey of vertical chlorophyll profiles in the post-bloom period (May- September) in the Arctic indicated that subsurface chlorophyll maxima occur in the top 50 meters (Ardyna et al. 2013), which is also in line with the range of DCM among the studied profiles (Fig.2-9). Clearly, the observed preference of *N. pachyderma* for a habitat below the DCM (Fig.2-9) indicates that the species may not primarily feed on fresh phytoplankton. The possibility of other species of *Neogloboquadrina* feeding on marine snow particles (hence below the DCM) has been recently suggested by Fehrenbacher et al. (2018) and a similar food source, related to degraded organic matter is thus not unlikely for *N. pachyderma*.

Among the other previously considered abiotic drivers of depth habitat of *N. pachyderma*, our analysis provides no evidence for the effect of sea-surface temperature, salinity and stratification (Fig. 2-4). Surface water temperature is the main controller of *N. pachyderma* abundance and it defines its geographic range (Bé & Tolderlund, 1971; Duplessy et al., 1991). Temperature could therefore also be expected to influence the vertical habitat of the species. However, we found no link with surface temperature and *N. pachyderma* depth habitat. This is probably because the temperature range sampled by our compilation remains well within the tolerance limit of the species (Žarić et al. 2005). Thus, temperature does not represent a limiting factor for this species and does not affect its vertical distribution. Previous research has

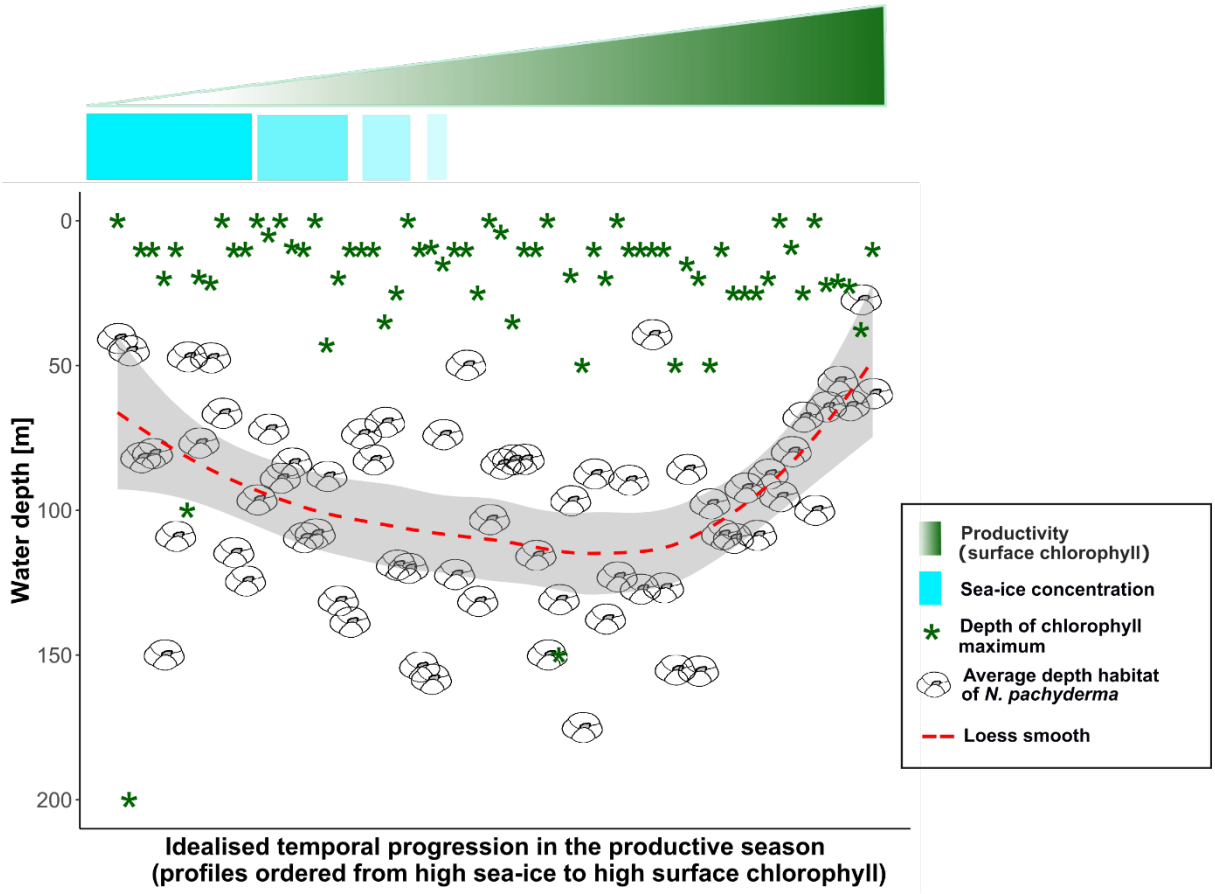

Figure 2-9 *Data-based scheme of the final model: samples are displayed in descending order for sea-ice concentration (light-blue fading bar) and ascending chlorophyll concentration (green fading triangle) to simulate the time dimension. The green star symbols represent the depth of the chlorophyll maximum and the dashed red line shows the smooth fit of the data.*

suggested that *N. pachyderma* may avoid low salinities and preferentially occur deeper in the water column when the surface is fresh (Volkmann, 2000, see also the discussion in Schiebel et al., 2017). Like Carstens and Wefer (1992), we did not find a significant correlation between surface salinity and DH indicating that the inferred response of *N. pachyderma* to surface layer freshening only applies to situations where the salinity reaches values below 30 PSU (below the limit covered by the observations in our compilation). Finally, geochemical analyses of *N. pachyderma* specimens were interpreted as evidence for calcification depth of the species being controlled by the position of the pycnocline (Hillaire-Marcel et al., 2004; Hillaire-Marcel, 2011; Kozdon et al., 2009; Simstich et al., 2003; Xiao et al., 2014). In our data, we found DH always situated below the MLD, within the pycnocline. Thus, our observations confirm that a significant part of the calcification is likely to occur within the pycnocline, but the depth habitat of the species is not reflecting the depth of the local pycnocline.

Our observations indicate that *N. pachyderma* resides closer to the surface when sea ice and/or surface chlorophyll concentrations are high. The DH also increases with time since sea-ice break-up. This suggests that the DH of *N. pachyderma* is controlled by multiple, interacting variables, likely connected in the temporal dimension. The scheme in Fig. 2-9 summarizes our conceptual model: when either sea-ice cover or surface chlorophyll concentrations reach high

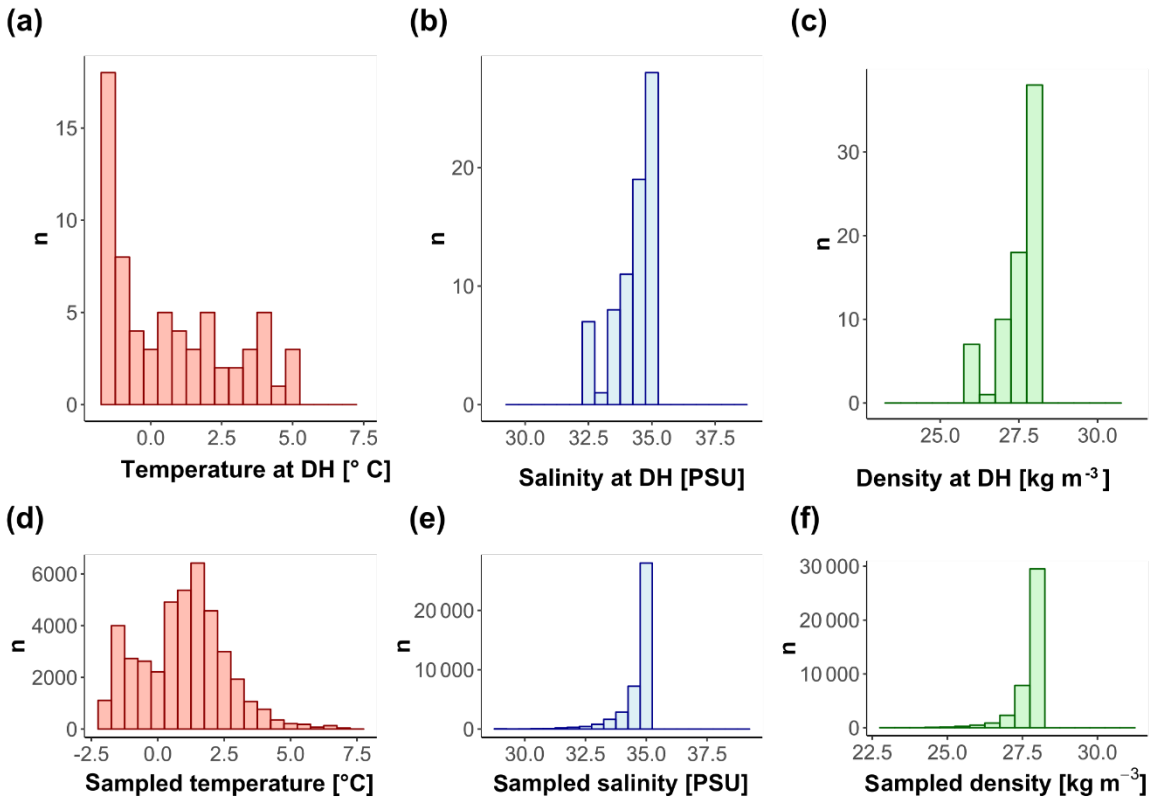

Figure 2-10 *Conditions of (a, d) temperature, (b, e) salinity, and (c, f) density at the DH (a, b, c) and in the first 600 m of the water column (d, e, f) for all the sites with available CTD data.*

values, *N. pachyderma* prefers shallower depths, while in open waters with low productivity levels, it lives deeper. While the relationship with sea-ice has been observed repeatedly (Carstens et al., 1997; Pados & Spielhagen, 2014), the relationship with surface chlorophyll at the surface is unexpected. Intuitively, rather than sea-ice and chlorophyll at the surface, the DH should reflect ambient conditions at depth. The DH does not appear to reflect the DCM (Fig. 2-9), but it could be that the species vertical abundance reflects the local depth at which a specific temperature or salinity optimum occur or where a given density is realised. We have thus extracted data on temperature, salinity and density at the level of DH in all profiles where CTD data were available. The analysis reveals a large variability in all parameters, indicating that the DH is not tracking specific temperature, salinity or density (Fig.2-10). The observation that the subsurface depth habitat of *N. pachyderma* appears to be best predicted by surface parameters is counter-intuitive and points to an indirect relationship to the inferred surface drivers.

A possible link between surface properties and conditions at the DH could be light (or light-related processes). Increasing sea-ice cover and higher chlorophyll at the surface both act to reduce light penetration, potentially explaining why *N. pachyderma* habitat is shallow when either sea-ice or surface chlorophyll are high (Fig. 2-9). The exact mechanism by which the species would respond to light intensity is not clear. So far, there is no evidence that the species would possess photosynthetically active symbionts. On the other hand, a recent molecular study indicated the presence of symbionts in a closely related species *Neogloboquadrina incompta* (Bird et al. 2018), and evidence for potential symbiosis with cyanobacteria in *Globigerina bulloides* (Bird et al. 2017) indicates that the range of symbioses in planktonic foraminifera may be more diverse than previously thought. However, half the observed DH values are > 100 m, indicating that a substantial part of the population of the species inhabits depth where in the

Arctic light for photosynthesis is not available (Ardyna et al. 2013). Alternatively, it could be that the vertical habitat of *N. pachyderma* reflects a compromise between living close to the DCM (finding food) and remaining in darkness (protected from predation). In many places of the ocean, heterotrophic protists are known to be metabolically more active at night (Hu et al. 2018), and predator evasion by remaining in darkness is the leading hypothesis explaining DVM in marine zooplankton (Hays 2003). These hypotheses are at present speculative and more investigations on the diet of *N. pachyderma* are needed for a better understanding of the process regulating its vertical distribution.

2.5 Conclusion

We compiled a dataset of 104 vertically resolved profiles of *N. pachyderma* concentration in the Arctic and North Atlantic and analysed the relationship of the observed depth habitat to a range of potential biotic and abiotic drivers. The analysis confirms that *N. pachyderma* inhabits a wide portion of the water column, but its maximum concentration is typically found in the subsurface. The depth habitat is variable but most of the population is consistently found below the subsurface chlorophyll maximum. This indicates that the species is likely not grazing on fresh phytoplankton. The depth habitat of *N. pachyderma* as recorded by the vertically resolved plankton tow profiles shows no evidence for diel vertical migration or a synchronised change in depth habitat with lunar cycle. Temperature, salinity and density alone (at the surface or at depth) do not show significant relationship with the depth habitat. Instead, sea-ice and chlorophyll concentration at the surface, in combination with the time since sea-ice break up explain almost a third of the variance in the depth habitat data. Most of the population of *N. pachyderma* resides between 50 and 100 m under dense sea-ice coverage and/or high surface chlorophyll concentration. When sea-ice cover is reduced and/or when chlorophyll at the surface is low, the habitat deepens to 75 – 150 m. This pattern reflects a response to an unknown primary driver acting below the DCM and likely reflecting trophic behaviour of the species, which is still poorly constrained. The knowledge gap on the ecological preferences of *N. pachyderma* is reflected in the mismatch in the behaviour of *N. pachyderma* between observations and predictions by the PLAFOM2.0 model. Our findings can serve as a basis to calibrate new ecosystem models and refine paleoclimatic reconstructions based on *N. pachyderma* in the Arctic and its adjacent seas. Our analysis rejects the hypothesis that the vertical habitat of the species is tied to the DCM and the existence of a significant relationship with sea ice and surface chlorophyll allows us to derive a model that can predict the depth habitat of the species across the Arctic realm.

2.6 Acknowledgements

The master and crew of the F.S Maria S. Merian are gratefully acknowledged for support of the work during the MSM09/2 cruise. This project was supported by the Deutsche Forschungsgemeinschaft (DFG) through the International Research Training Group “Processes and impacts of climate change in the North Atlantic Ocean and the Canadian Arctic” (IRTG 1904 ArcTrain).

Chapter 3

SINGLE-CELL METABARCODING REVEALS BIOTIC INTERACTIONS OF THE ARCTIC CALCIFIER *Neogloboquadrina pachyderma* WITH THE EUKARYOTIC PELAGIC COMMUNITY

This manuscript is in preparation for submission.

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Data availability: CTD data will be available on PANGAEA after publication. DNA sequences will be available at the European Nucleotide Archive. The code and scripts used to analyse the data will be made available on GitHub once the manuscript is accepted for publication.

Abstract

Isotopic and trace-element signals in the calcite shells of the planktonic foraminifera *Neogloboquadrina pachyderma* represent key proxies to reconstruct past climatic conditions in northern high latitudes. A correct interpretation of these chemical signals requires knowledge of the habitat and trophic interactions of the species. Direct observations on the biological interactions of *N. pachyderma* in polar environments are lacking and to date no consensus exists on the trophic behaviour of this species. Here we use single-cell metabarcoding to characterise the interactions of 39 specimens of *N. pachyderma* from two locations in the Baffin Bay with the local eukaryotic pelagic community. From our analyses, the eukaryotic interactome of the foraminifera is dominated by diatoms, accounting for > 50% of the assigned amplicons in 17 of the samples, but other groups such as Crustacea and Syndiniales can also be abundant. The high abundance of amplicon sequence variants (ASVs) belonging to Syndiniales in some of the analysed specimens suggests that these known protistan parasites appear to also infect *N. pachyderma* and may play an important role in its population dynamics. PERMANOVA tests showed a marked difference (*p-value* = 0.001, R^2= 32%) in interactome composition between specimens collected from different sites, and the same difference was reflected in the composition of the ambient (mainly algal) pelagic community sampled by bulk water filtration. A secondary weaker signal in the composition of the interactomes indicates small but systematic difference between specimens sampled at the surface and in the subsurface (*p-value* < 0.05, R^2= 6%). The strong but taxonomically non-specific association with algae, existing irrespective of depth and occurring below the photic zone, indicates that opportunistically grazed diatom-fuelled marine aggregates likely represent the main interaction substrate of *N. pachyderma*.

3.1 Introduction

Neogloboquadrina pachyderma is the dominant planktonic foraminifera species in high latitudes where it makes up to 90% of the total assemblage (Volkmann 2000; Schiebel et al. 2017). Paleoceanographers use the geochemical signal preserved in the calcite shells of this species to investigate past states of the Arctic Ocean and reconstruct past circulation, sea ice formation and glacier melt water events (e.g., Hillaire-Marcel et al., 2008; Knies & Vogt, 2003; Stein et al., 1994). The correct interpretation of the paleo-reconstructions relies on a thorough understanding of species-specific ecology of living planktonic foraminifera in the water column (Ezard et al., 2015; Jonkers & Kucera, 2015). In particular, constraining the biological interactions (diet, presence of symbionts/parasites), can dramatically improve our understanding of the mechanisms and the context of how the environmental signal is recorded in foraminifera shells (Fehrenbacher et al. 2018; Morard et al. 2019). In addition, understanding the biotic interactions of the species can help develop more accurate numerical models that predict the response of *N. pachyderma* to future climate change (Roy et al. 2015; Kretschmer et al. 2018).

Despite being widely applied in palaeoceanography, the ecology of *N. pachyderma* in the Arctic remains elusive (Xiao et al. 2014). A recent pan-Arctic investigation on the distribution of this species highlighted the necessity to disentangle its biological interactions as abiotic factors alone could only explain a fraction of the observed variability (Greco et al. 2019). Besides

various speculations based on cytoplasm pigmentation (Kohfeld and Fairbanks 1996; Stangeew 2001), no direct observations currently exist on the diet of *N. pachyderma* in the Arctic Ocean (Volkmann 2000; Bjorbækmo et al. 2020). Most authors consider this species as herbivorous (Kohfeld and Fairbanks, 1996; Manno & Pavlov, 2014; Pados & Spielhagen, 2014; Schiebel et al., 2017), other as omnivorous (Stangeew 2001), while in culturing experiments the species is known to survive when fed with *Artemia*, and therefore could be regarded as carnivorous (Manno et al. 2012). Next to the food source, other biotic interactions of *N. pachyderma* have not been investigated, and nothing is known about interactions such as presence of symbionts or parasites (Bjorbækmo et al. 2020). Such observations could be relevant for the understanding and prediction of a range of pelagic processes, including the carbon cycle in the Arctic Ocean. For example, by increasing physiological stress on and possibly killing *N. pachyderma*, parasite activity could result in an increase of the release of particulate organic matter (POM), fuelling in turn the microbial loop (Skovgaard 2014).

Gaining insights on Arctic protistan trophic interactions has been historically challenging as it used to require well-developed culturing protocols and time-consuming microscope observations. The advent of high-throughput sequencing presents the opportunity to overcome such limits (Lovejoy 2014). In this study, we use single-cell metabarcoding to constrain trophic interactions between *N. pachyderma* and the eukaryotic pelagic community in the Baffin Bay. To this end, we identify the taxonomic composition of eukaryomes extracted from 39 *N. pachyderma* specimens collected at two different depths from two sites representing distinct oceanographic settings (Fig. 3-1). To identify the interacting pelagic community, we use classical metabarcoding approach to sequence bulk DNA extracted from contextual seawater samples. We use the resulting dataset to test the specificity of *N. pachyderma* interactions with the eukaryotic community and their ecological significance by comparing (i) the data derived from single-cell metabarcoding and from water samples (ii) the taxonomic composition in specimens collected at different depths, and (iii) the taxonomic composition in specimens sampled in different environmental conditions.

3.2 Material and Methods

Sampling

During the MSM66 cruise in the Baffin Bay on the R/V Maria S. Merian, planktonic foraminifera were sampled at different depths using a Multi-net (HydroBios 92B, Kiel, Germany, equipped with five net bags with 100 µm mesh diameter). Samples for metabarcoding were taken from the sites GeoB22333-2 and GeoB22365-3 on the 7th and on the 20th of August 2017, respectively (Fig.3-1). Individual specimens were isolated from the plankton sample and stored on micropaleontological slides at a temperature of -80 °C. In parallel, seawater (1L) from Niskin bottles was sampled at surface (~5 m) and at depth (200 m) and filtered through 0.2 µm cellulose filters. The filters were then stored in buffer (1.8 mL of 50 mM Tris–HCl, 0.75 M sucrose and 40 mM EDTA; pH 8.3) at a temperature of -80 °C.

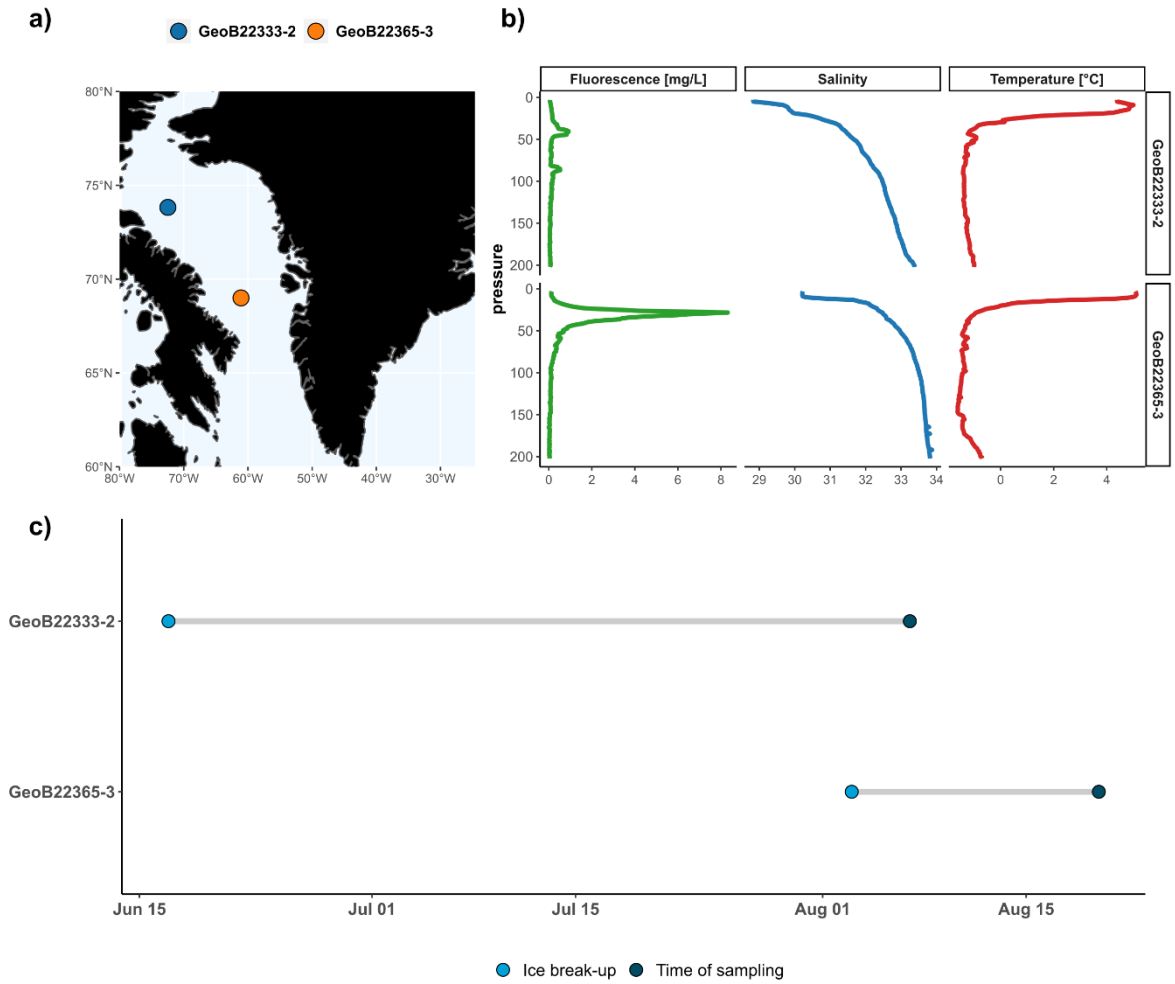

Figure 3-1 *a) Map showing sampling site locations. b) CTD vertical derived data at the two stations showing Fluorescence (productivity), Salinity and Temperature profiles. c) Time between ice break-up timing and sampling date at the two studied sites.*

Environmental data

Before each plankton tow, a conductivity– temperature–depth (CTD) device equipped with a fluorescence sensor (WETLabs ECOFLNTU (RT)D) was deployed to obtain vertical profiles of physical properties and algae pigment concentrations (Dorschel et al. 2017). The time of sea-ice break-up at the two stations was determined by extracting in situ sea-ice concentration from 25 km×25 km resolution passive microwave satellite raster imagery obtained from the Sea Ice Index Version 3.0 product of the National Snow and Ice Data Centre (Fetterer et al. 2017).

DNA Extraction, Amplification and Sequencing

Specimens of *N. pachyderma* were transferred from the slides and total DNA was extracted from each specimen following the GITC* protocol (Weiner et al., 2016). Total DNA from filters was extracted using E.Z.N.A. kit following manufacture instructions including blank extractions to control for (cross-) contamination events. DNA extractions were then amplified in triplicates using the Eukaryotic V4 tagged primers TAReuk454FWD1 (5'-CCAGCA(G/C)C(C/T)GCGG-TAATTCC-3') and TAReukREV3 (5'-

ACTTTCGTTCTTGAT(C/T)(A/G)A- 3'). Each tagged PCR primer consists of a unique tag sequence of 8 nucleotide appended to the 5'end of the common amplification primer sequence.

Each PCR was performed in a total volume of 25 µl, including 0.02 U/µl of Taq DNA polymerase (Phusion), 1.03 umol/µl of 5 × Green Buffer (Phusion), 0.2 mM of each dNTP, 0.41 umol/µl of each primer, 2.56 umol/µl of $MgCl_2$ and 1 µl of DNA extract. The conditions for the first amplification consisted of a pre-denaturation step at 98 °C for 30 s to melt the complex genomic DNA mixture, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 52 °C for 30 s and extension at 72 °C for 45 s, followed by a final extension step at 72 °C for 10 min.

Positive triplicates were successively purified using the QIAquick PCR purification Kit (Qiagen) and the DNA content quantitated using a QUANTUS fluorometer (Promega). Samples were finally loaded in 4 different pools. Purified products from the water samples were pooled in double concentration to get a higher sequencing depth. Pools were sent to the University of Geneva for sequencing (Illumina Miseq).

Sequence data pre-processing and taxonomic assignment

In total, amplicon sequencing produced 21,586,952 raw paired-end (PE) reads. Raw reads were demultiplexed with Cutadapt 2.7 (Martin 2013) using the Combinatorial Dual Indexing option allowing for 2 bp errors in the barcode sequence and no indels. Reads with the valid barcode combinations were selected for the following steps and reads containing ambiguous bases (Ns) were removed using DADA2 1.14.1 (Callahan et al. 2016) in R 3.6.0 (R Core Team 2017). Primers were removed from the reads using the "linked" adapter option in Cutadapt 2.7. At most 2 errors were allowed during filtering, while 20 bp were trimmed from the end of the forward and 50 bp from the end of the reverse reads to remove barcodes and primers. Reads were further processed with the DADA2 pipeline in R 3.6.0. The minimum allowed read length was set to 175 bp. After dereplicating forward and reverse reads, the DADA2 pipeline was used to identify amplicon sequence variants (ASV) in the dataset. The forward and reverse reads were merged and chimeras were identified and removed based on matches with combinations of 3'- and 5'-segments of different sequences. The ASVs were then taxonomically classified with the naïve Bayesian classifier method implemented in DADA2 based on the PR^2 database (Guillou et al., 2013).

Statistical Analyses

The ASVs belonging to land taxa were considered as contaminants and removed. Triplicates were merged and the ASV table was rarefied to 24000 sequences per sample using the phyloseq package in R (McMurdie and Holmes 2013). The differences in the taxa composition in the foraminifera samples were firstly explored using a multivariate approach. The ASVs abundance were binned at genus level and transformed in relative proportions. From the resulting dataset, we calculated Bray Curtis dissimilarity among the different samples which were represented by two dimensional Principal Coordinates Analysis (PCoA). Next, in order to test whether collection depth or sampling site significantly affected the taxa composition of the samples, we performed a permutation multivariate analysis of variance (PERMANOVA) using the adonis function in the vegan R package (Oksanen et al. 2018). Finally, to understand the differences in the composition between groups, we carried out the Analysis of Composition of Microbiomes (ANCOM) using the ANCOM package in R (Mandal et al. 2015; Kaul et al. 2017). This test performs a differential abundance analysis on the ASV table (not binned and not transformed)

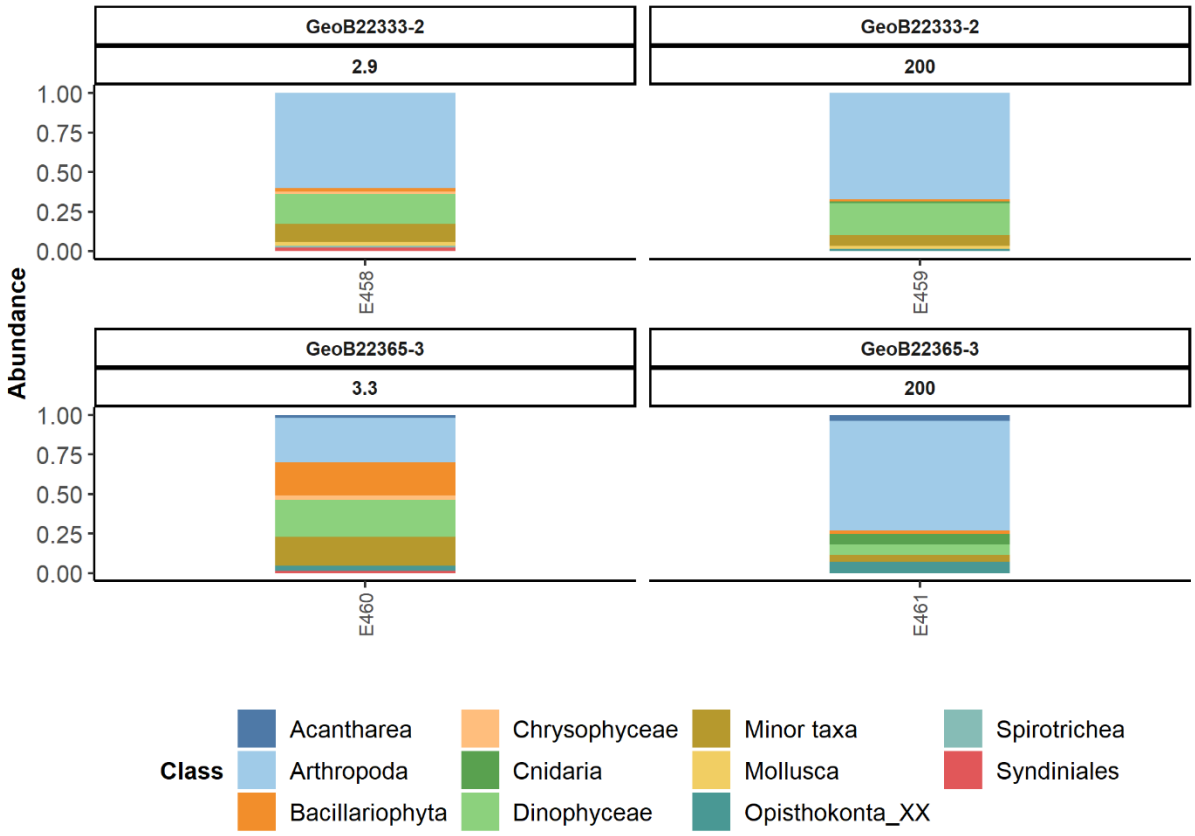

Figure 3-2 *Stacked bar plot showing taxonomic composition of the amplicons obtained from DNA extracts retrieved from filtered ambient water samples at the surface and subsurface. Colours represent different Classes (Taxonomic groups occurring with a frequency below 0.01 were condensed in the category "Minor Taxa").*

to detect differentially abundant ASVs across different experimental group allowing to control for False Discovery Rate (FDR); we opted for a high cut-off (0.9) for a conservative interpretation of the results (smaller FDR).

3.3 Results

The two sampling sites presented quite similar thermohaline profiles but differed in terms of productivity (Fig. 3-1). This was reflected in the taxonomic signal of the water samples showing a higher proportion of ASVs belonging to diatoms (Class Bacillariophyta) in the GeoB22365-3 station, especially in the surface (Fig. 3-2). Otherwise, the overall taxonomic composition in the water samples of both sites was quite similar, with Crustaceans (Class Arthropoda) and Dinoflagellates (Class Dinophyceae) representing the majority of reads (Fig. 3-2).

In the foraminifera samples, diatom ASVs dominated in most of the analysed specimens reaching abundance above 50% in 17 specimens. Arthropoda and Syndiniales ASVs were also abundant in some samples (Fig. 3-3). The tree maps in figure 3-4 show that, within the Bacillariophyta, the genus *Chaetoceros* was the most abundant in both water and foraminifera samples especially from site GeoB22333, represented by ASVs belonging to the cold-water species *Chaetoceros gelidus* and *Chaetoceros socialis*. In the *N. pachyderma* specimens from

the GeoB22365 site, a higher proportion of the Raphid-pennate diatoms of the genus *Fragilariopsis* was present. The algal composition in the water sample from the same site was more diverse with abundant ASVs from the genera *Pseudo-nitzchia* and *Thalassiosira*. Furthermore, the foraminifera collected at station GeoB22365 yielded abundant ASVs of Syndiniales (Fig. 3-3).

The Principal Coordinate Analysis (PCoA) of the foraminifera eukaryomes indicated systematic differences in composition by site and also by depth (Fig. 3-5). The majority of the variance (50.8 %) was explained by the first component separating eukaryomes from the two stations irrespective of sampling depth. Individual specimens collected at the same depth differed considerably in the composition of the most abundant genera indicating a high inter-individual variability (Fig. 3-5). The PERMANOVA analyses confirmed that the differences in community linked to sampling site was statistically significant (*p-value* = 0.001, R^2= 32%). A much lower, but still significant (*p-value* < 0.05, R^2= 6%), portion of variability in the composition was explained when collection depth was tested as grouping factor (Table 3-1).

The ANCOM analysis indicated that ASVs belonging to diatoms (*Chaetoceros*), Syndiniales (*Dino Group I*) and Acantharians (*Chaunacanthida*) were significantly different in abundance across the two sampling sites (Fig. 3-6). On the other hand, no significant difference in ASV abundance was found between specimens collected at different depth, when the same FDR threshold was used. Relaxing the threshold to 0.7 resulted in the identification of two ASVs of the genus *Chaetoceros* as being significantly more abundant in the shallower *N. pachyderma* specimens.

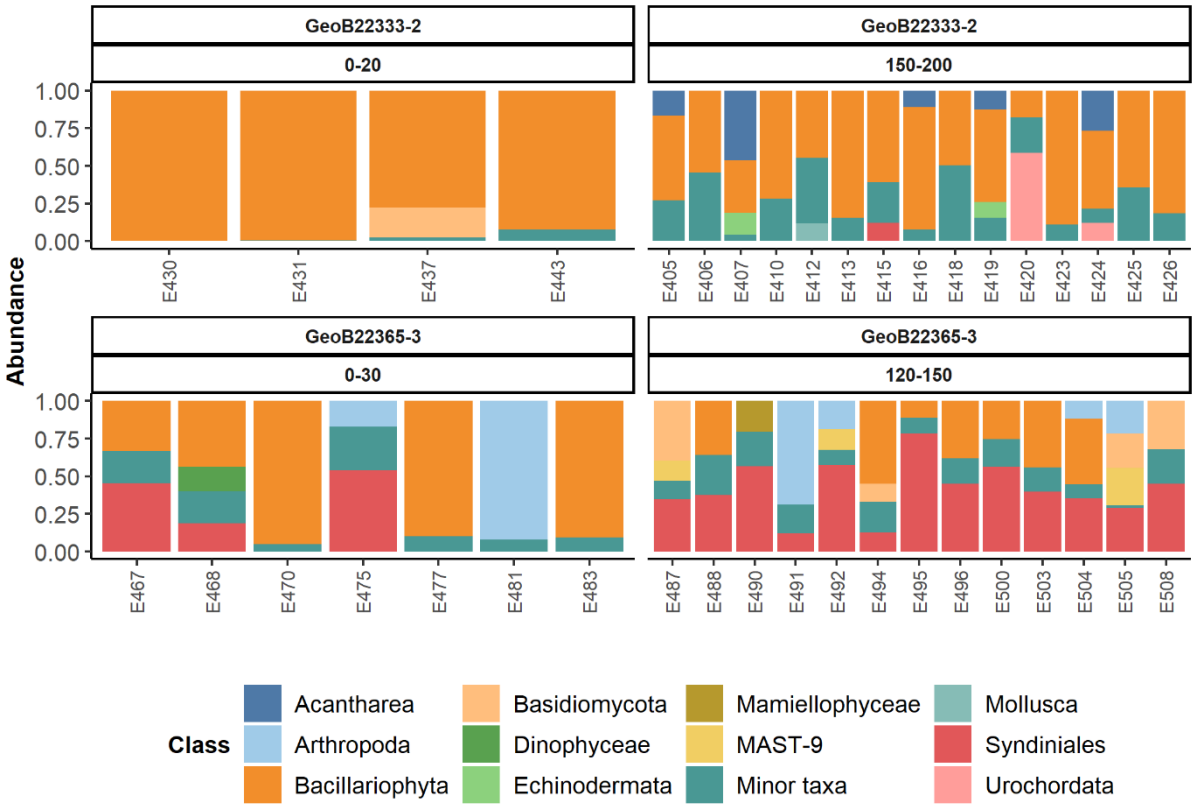

Figure 3-3 *Stacked bar plot showing taxonomic composition of the amplicons retrieved from single-cell extractions from N. pachyderma sampled at different depths. Colours represent different taxonomic groups (Taxonomic groups occurring with a frequency below 0.01 were condensed in the category "Minor Taxa").*

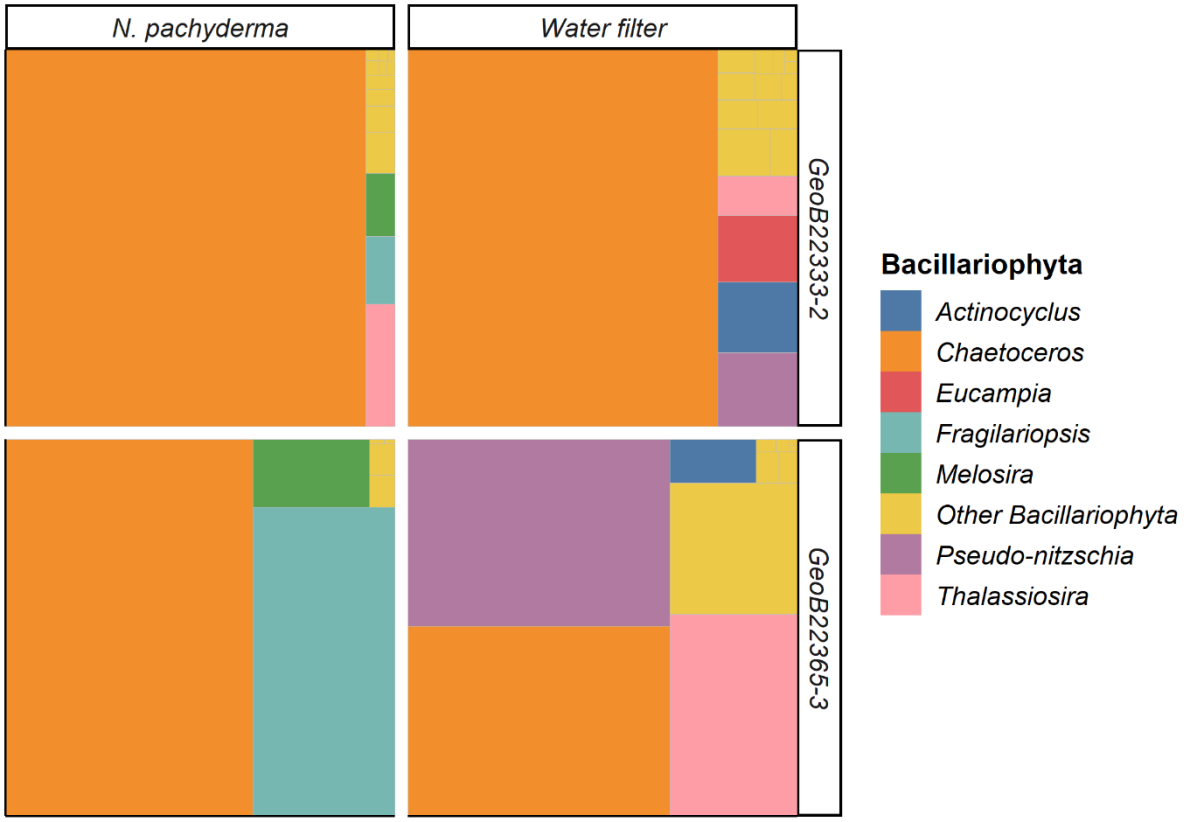

Figure 3-4 *Treemaps showing the average Bacillariophyta composition in foraminifera and in the ambient water samples averaged throughout the water column at the two sites. Colours represent the different identified genera (Taxonomic groups occurring with a frequency below 0.01 were condensed in the category "Other Bacillariophyta").*

3.4 Discussion

The pelagic community signal recovered from the water samples appeared homogenous in terms of the main taxonomic groups represented (Fig. 3-2), a deeper look at the algal composition reveals a signature of the different ecological conditions at the two sites at the time of collection (Fig. 3-4). At station GeoB22333, most of the Bacillariophyta ASVs in both water and foraminifera samples were assigned to the two closely related species *C. gelidus* and *C. socialis*, known to be the most abundant centric diatoms in the Baffin Bay during summer (Chamnansinp et al. 2013; De Luca et al. 2019). In the water samples from site GeoB22365, along with *Chaetoceros*, another centric diatom, *Thalassiosira* is highly represented. Blooms of diatoms of the genus *Thalassiosira* have been described as intense and transient, and are usually rapidly replaced by *Chaetoceros* spp. blooms (Booth et al. 2002; Lafond et al. 2019). Next to *Chaetoceros* and *Thalassiosira*, diatoms of the genus *Pseudo-nitzchia* are the most abundant in water samples from station GeoB22365. This pennate diatom taxon is generally observed in locations where sea ice cover is present (Poulin et al. 2011) and usually precedes *Thalassiosira* and *Chaetoceros* in the algal bloom succession after sea-ice break up (Lafond et al. 2019). In *N. pachyderma* specimens collected at the same station, the most abundant Bacillariophyta ASVs belonged to sea-ice diatom *Fragilariopsis* (Mock et al. 2017) and

Melosira arctica, a diatom that grows filaments anchored on the underside of the sea-ice (Boetius et al. 2013; Poulin et al. 2014).

The algal taxonomic composition of both water and foraminifera datasets combined with CTD and sea-ice data (Fig. 3-1) indicate that sampling at the two sites occurred at different stages of the local algal bloom succession. Site GeoB22365 was sampled shortly after sea-ice break with a mixed algal structure transitioning from sea-ice associated taxa to centric diatoms dominated pelagic community. The situation for the sampling site GeoB22333 was different as the sea ice melted long before the sampling date (Fig. 3-1c) and the algal community displayed a more homogenous structure dominated by diatoms of the genus *Chaetoceros* that are able to maintain their populations at low nutrient levels (Booth et al. 2002).

Table 3-1 *PERMANOVA results based on Bray-Curtis dissimilarities using genus abundance in the foraminifera samples in relation to compartment for a) Sampling site and b) depth of collection (p-values based on 999 permutations).*

	Df	Sum Sq	Mean Sq	F model	R^2	*p-value*
a) Station	1	3.221286	3.221286	17.42954	0.320222	0.001
Residuals	37	6.838252	0.184818		0.679778	
Total	38	10.05954			1	
b) Depth	1	0.65178	0.65178	2.563399	0.064792	0.044
Residuals	37	9.407759	0.254264		0.935208	
Total	38	10.05954			1	

The differences in the algal community structure between the two sites significantly affected the taxonomical structure of the eukaryomes in the foraminifera samples as confirmed by the PCoA and PERMANOVA results (Fig. 3-5, Table 3-1). This was expected as diatom ASVs were extremely abundant in most of *N. pachyderma* specimens. The ANCOM outcome is also consistent with a change in the algal community since the ASVs from the genus *Chaetoceros* were recognised as significantly more abundant in the specimens collected at station GeoB22333. The weaker depth-related signal of relative genus abundance detected in the PERMANOVA in the foraminifera samples, is also likely related to the diatoms with ASVs of the genus *Chaetoceros* showing a reduced abundance in deeper waters as revealed in the "relaxed "ANCOM test. These results indicate that diatoms are the main interaction party of *N. pachyderma* and their presence in the specimens follows abundance and structure in the surrounding pelagic community (Figs 3-2, 3-3 and 3-4). Contrary to benthic foraminifera, diatoms endosymbionts have not been observed in planktonic species (Bjorbækmo et al. 2020). In particular, the most represented genera (*Chaetoceros* and *Fragilariopsis*) in *N. pachyderma* specimens are never known to be symbiotic. Moreover, a recent survey on photosymbiosis in planktonic foraminifera, has shown that the species *N. pachyderma* possesses chlorophyll but does not carry out photosynthesis (Takagi et al. 2019). Given also the low specificity nature of the interaction between *N. pachyderma* and the diatoms, we deduce that algae represent the main food source of this species.

Diatoms of the genus *Chaetoceros* generally from chain-like structures which in presence of high levels of biomass, tend to cluster together into larger colonies forming aggregates and

producing abundant exopolymeric gels that lead to high carbon export (Booth et al. 2002; Chamnansinp et al. 2013; Duret et al. 2020). This is particularly true in the Baffin Bay, where it has been estimated that in July cells of *C. gelidus* can contribute up to 91 and 49% of total phytoplankton abundance and carbon respectively (Booth et al. 2002).

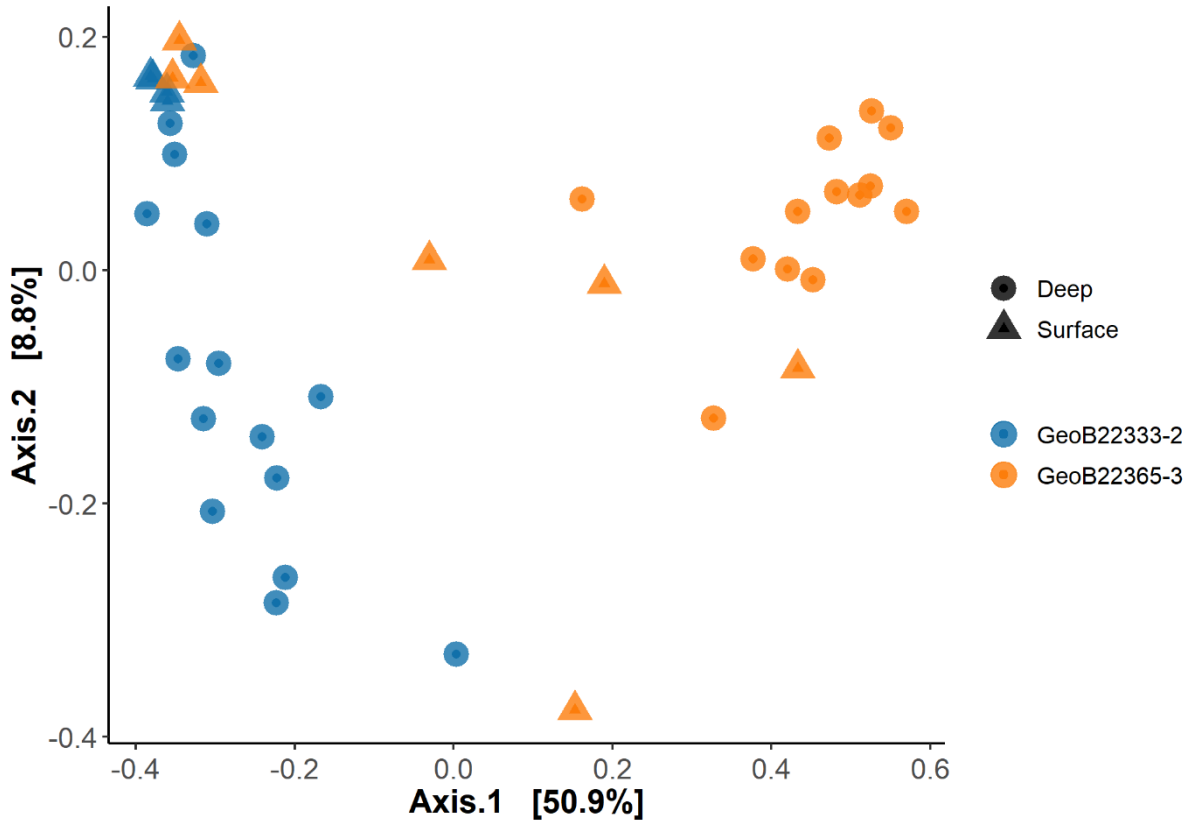

Figure 3-5. *PCoA of Bray-Curtis dissimilarity score calculated on genus relative frequency of each N. pachyderma sample. Colours represent sampling site and shapes the depth of collection.*

We speculate that these diatom-fuelled aggregates can represent the principal microhabitat of *N. pachyderma*. From geochemical data, Fehrenbacher et al. (2018) deduced that non-spinose planktonic foraminifera species like *N. pachyderma* may calcify within organic aggregates, a microenvironment that allows them to adopt a benthic-like lifestyle in the water column. Analogously, we propose that aggregates represent the main interaction substrate of *N. pachyderma* with the pelagic community. This can explain why the sea-ice associated diatoms are present in the foraminifera. Their bloom is over in the water column, but the aggregates are still left over and grazed on by *N. pachyderma*, thus revealing the bloom structure as it occurred in the water column some weeks earlier.

The fact that we did not observe strong differences in taxonomic composition in specimens of *N. pachyderma* collected at different depth might very well be the result of this particular behaviour (Table 3-1). The non-diatom ASVs present in the foraminifera samples (Fig. 3-3) can also easily be explained when the aggregate microhabitat hypothesis is considered. Crustaceans and soft-bodied Urochordata are known to actively or passively participate in the formation of marine aggregates (Duret et al. 2020). These organisms present in the aggregates

are also likely to be part of *N. pachyderma* diet as evidence from culturing experiments suggests and observed in other non-spinose species (Hemleben et al. 1989; Manno et al. 2012).

The higher abundance of Acantharians ASVs at site GeoB22333 is also likely associated with the sinking diatom fuelled aggregates as *Chaetoceros* is also distinctively abundant in the same site (Fig. 3-6). Research on Acantharians has shown that *Chaunacanthida* cysts participate in organic carbon export to depth (Decelle et al. 2013), potentially explaining why their ASVs are more abundant in the deeper specimens of *N. pachyderma* (Fig. 3-3).

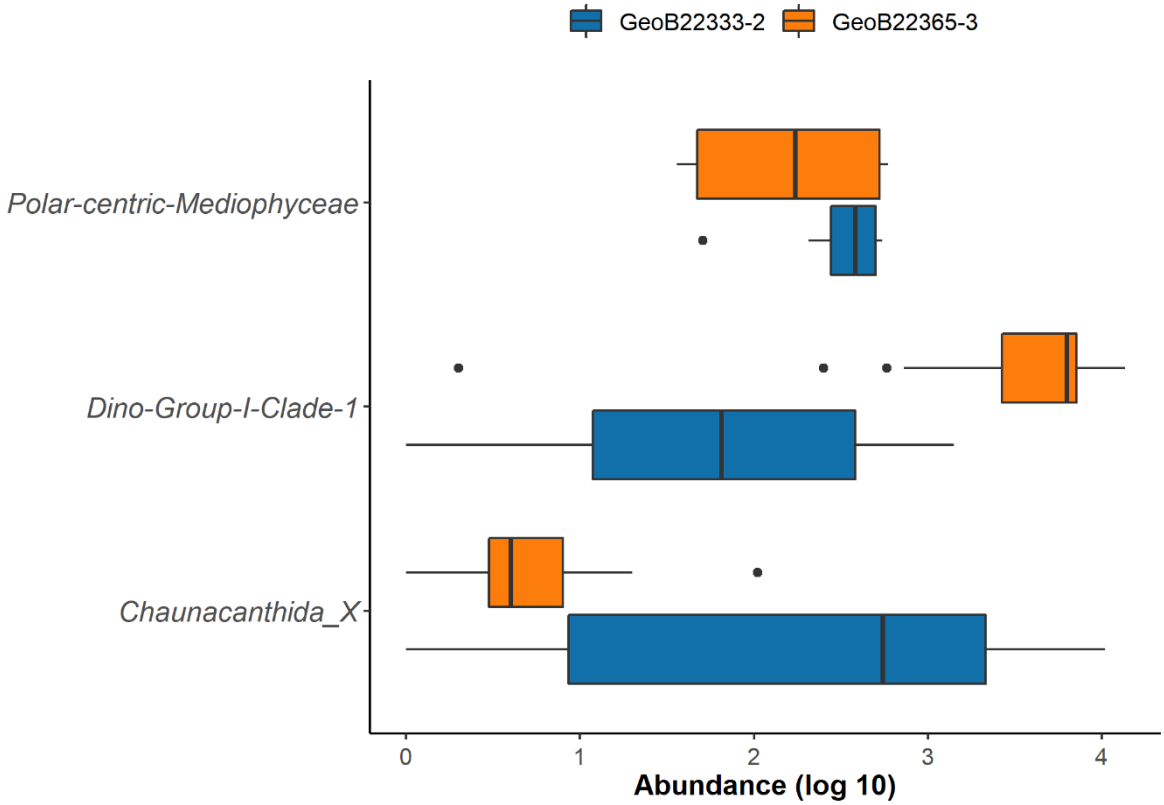

Figure 3-6 *ASVs found in the ANCOM analysis (abundance is log transformed) identified 3 taxa to be associated with differences between the two sampling sites.*

Next to diatoms, Syndiniales Group I ASVs also constituted a large portion of the reads in *N. pachyderma* samples (Fig. 3-3), especially in the ones collected at Station GeoB22365 as confirmed by the ANCOM results (Fig. 3-6). Syndiniales are a monophyletic lineage at the base of the dinoflagellate clade, widely distributed in the world oceans (de Vargas et al., 2015; Guillou et al., 2008). In recent marine 18S surveys, the group I is has been observed to occur in high abundance in polar oceans and in particular in near the sea-ice edge and in correspondence with algal blooms (Bachy et al., 2011; Clarke et al., 2019; Cleary & Durbin, 2016) as we observed at sampling site GeoB22365 (Fig. 3-1). As all the Syndiniales, dinoflagellates of Group I are parasitoids that can infect distantly related hosts like other protists (dinoflagellates, cercozoans, radiolarians) or metazoans (copepods, fish eggs) and release free-living dinospores following host death (Clarke et al., 2019; Guillou et al., 2008). Given that Syndiniales of this group have been frequently observed in other Rhizaria (Bjorbækmo et al.

2020), it is likely that *N. pachyderma* could also represent a possible host of this ubiquitous parasites implying that parasitism could play a role in regulating the population dynamics of *N. pachyderma* in the Arctic. To our knowledge, this is the first time that a parasite interaction with Syndiniales is reported for planktonic foraminifera. With our dataset, we cannot definitively resolve the putative parasite–host associations, more investigations employing culturing experiments and the development of fluorescent in situ hybridisation (FISH) probes are needed to establish the interactions between these two groups. Moreover, our data is based on the analysis specimens larger than 100 μm, so interactions and lifestyle of *N. pachyderma* juveniles remain undetermined.

3.5 Conclusions

In this study, we used single-cell metabarcoding to constrain biological interactions between the planktonic foraminifera *N. pachyderma* and the eukaryotic pelagic community in the Baffin Bay. Diatoms (Bacillariophyta) were highly represented in most of the foraminifera samples with differences in composition that reflected the algal assemblage in the water column at the sampling site. Marked weaker, but significant difference in taxonomic composition of the eukaryome was also observed among *N. pachyderma* specimens sampled at different depths. The non-specific relationship with the pelagic diatoms along with the presence of DNA from groups as Crustaceans and Urochordata in the foraminifera specimens, suggest that *N. pachyderma* lives and opportunistically feeds in association with organic aggregates. In addition, our data indicate that *N. pachyderma* could be infected by Syndiniales parasites of Group I. These results advance our knowledge on the ecology of *N. pachyderma* placing it in the context of the multilevel trophic system of the Arctic pelagic realm and can improve interpretations of paleoclimatic signals preserved in its shells. Our findings showcase the potential of single-cell metabarcoding as a powerful tool that can significantly improve our understanding of planktonic microbial ecology.

3.6 Acknowledgements

The master and crew of the F.S. Maria S. Merian are gratefully acknowledged for support of the work during the MSM66 cruise. This research has been supported by the Deutsche Forschungsgemeinschaft (DFG) through the International Research Training Group “Processes and impacts of climate change in the North Atlantic Ocean and the Canadian Arctic” (IRTG 1904 ArcTrain).

Chapter 4

THE EFFECT OF AN EXPERIMENTAL DECREASE IN SALINITY ON THE VIABILITY OF THE SUBARCTIC PLANKTONIC FORAMINIFERA *Neogloboquadrina incompta*

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Data availability: Observations and data in support of the findings are available on figshare at https://doi.org/10.6084/m9.figshare.11309627.v1

Abstract

Chemical signatures in the calcite of shells of polar and subpolar planktonic foraminifera have been frequently used to trace and quantify past meltwater discharge events. The assumption of this approach is that the foraminifera can tolerate low salinity under extended periods. To obtain a first experimental constraint on salinity tolerance of subarctic foraminifera, we carried out a culturing experiment with specimens of the subpolar species *Neogloboquadrina incompta* collected in the northern Norwegian Sea off Tromsø in October 2018. The foraminifera were exposed to a gradient of salinities between 35 and 25 PSU. Survival was monitored over 26 days by measuring the extent of the rhizopodial network. Although chamber growth only occurred in one of the observed specimens, likely due to the largely unknown dietary preference of the species, we observed a strong differential rhizopodial activity pattern along the gradient. The highest rhizopodial activity occurred at salinity between 35 and 31 PSU. The species is clearly able to survive long-term exposure to salinities as low as 28, but no rhizopodial activity and signs of cytoplasm degradation were observed in all specimens exposed to 25 PSU. These preliminary observations provide the first direct evidence for the salinity tolerance of *N. incompta*, indicating a range of salinity that could be plausibly expected to be recorded in the chemistry of fossil shells of the species.

4.1 Introduction

The chemical and isotopic composition of fossil shells of planktonic foraminifera is a well-established approach to investigate the past state of the ocean (e.g., Ravelo and Hillaire-Marcel 2007; Pearson 2012). For example, the oxygen isotopic signature ($\delta^{18}O$) in shells of *Neogloboquadrina incompta* has been used to infer the presence of meltwater injected into the surface ocean by icebergs (McManus et al. 1999; Came et al. 2007; Rashid and Boyle 2007; Voelker et al. 2009). The reconstructions are based on the assumption that the calcification of the shell, and thus the incorporation of the chemical signal, occurred within the water layer affected by the discharged meltwater. This is particularly relevant in situations where the properties of the target water layer may be modified to a degree that is too hostile for the survival of the foraminifera. In this scenario, specimens of the species could be largely excluded from surface low-salinities habitat and the oxygen-isotope composition of the remaining specimens dwelling deeper would be recording conditions below the meltwater layer, thus systematically underestimating the surface salinity anomaly. Indeed, past meltwater injections in the North Atlantic likely had a magnitude sufficient to modify surface salinity below the range of naturally occurring values in the present ocean (Hemming 2004). Among the species of planktonic foraminifera occurring in the north Atlantic during these events, especially in the more distal part of the iceberg discharge plume, is *N. incompta* (Dickson et al., 2008; Voelker et al., 2009).

However, to date, no experimental data are available to constrain the range of salinities under which *N. incompta* survives and which it thus could potentially record.

Most existing experiments in which planktonic foraminifera were exposed to a gradient of environmental parameters have been carried out on tropical to temperate species (Bé et al., 1977; Bertlich et al., 2018; Bijma et al., 1990; Davis et al., 2017; Fehrenbacher et al., 2018; Lea et al., 1999; LeKieffre et al., 2018; McCrea, 1950). High-latitude planktonic foraminifera have been rarely kept in culture (Manno et al. 2012) and standardized culturing protocols have not been established for the cultivation of these species under cold conditions (Kozdon et al., 2009; Schiebel et al., 2018). Here we present the results from a preliminary laboratory experiment on the subarctic planktonic foraminifera *N. incompta* with the purpose to constrain the salinity tolerance of the species. With this experiment, we aim to provide a first insight into the changes in the physiology and viability of *N. incompta* in response to different salinity conditions and introduce a novel way of monitoring its physiology, applicable in the absence of growth, by measuring the extent of its rhizopodial network.

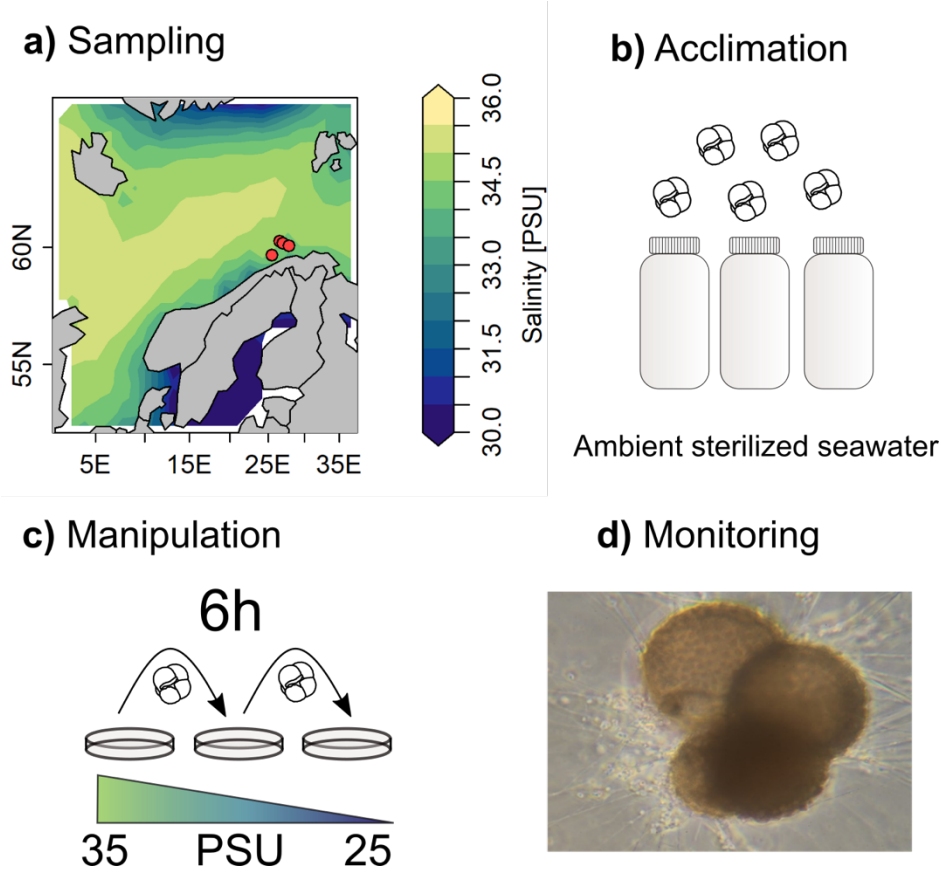
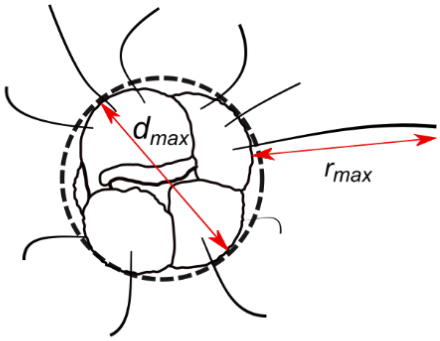


Figure 4-1 *Schematic representation of the different phases of the culturing procedure, from the location of the sampling (a) to monitoring (d). Salinity values in (a) refer to the monthly average surface salinity in October, taken from World Ocean Atlas 2018* (Zweng et al. 2019).

4.2 Material and Methods

Sampling

The experiment and the microscope observations were performed in a cold room in one of the facilities of the Arctic University of Norway in Tromsø.



$$\text{rhizopodial activity} = \frac{r_{max}}{d_{max}}$$

Figure 4-2 *Scheme illustrating the method used to derive the rhizopodial activity index in this study.*

Specimens of *N. incompta* were collected during a cruise on the *R/V Helmer Hansen* in October 2018 to the shelf and slope of northern Norway off Tromsø (Håkjerringdjupet). In the sampling area, surface water temperature ranged between 6 °C and 10 °C. The encountered community of planktonic foraminifera was dominated by *N. incompta*, which gave us the opportunity to study the salinity tolerance of this species at the lower end of its thermal range, under conditions that can be expected to resemble those of past meltwater injections, with cold temperatures due to iceberg melting. Specimens were sampled from a water depth between zero and 100 m, using a WP2 plankton net (63 µm mesh size) that was towed vertically. The retrieved specimens were picked on board and incubated in jars containing seawater at 35 PSU previously filtered through a 0.22 µm nitrate cellulose filter (Fig. 4-1) and allowed to recover for ~16 hours at treatment temperature (6 °C).

Culture methods

Onshore, cytoplasm-bearing *N. incompta* specimens were transferred from the collection flasks into Petri dishes and after 6 hours, a fraction of the specimens was transferred into new Petri dishes with a salinity lowered by 3-4 PSU to avoid osmotic shock (acclimation step). This procedure was repeated from the Petri dish with lowered salinity at intervals of 6 hours until the minimum tested salinity of 25 PSU was reached. The tested range of salinities was chosen to reach below 30 PSU, which is the lower limit of salinity estimates in the Heinrich meltwater layers (Maslin et al. 1995; De Vernal and Hillaire-Marcel 2000) The culturing medium for the treatments (35 PSU/Control, 31 PSU, 28 PSU, and 25 PSU) was obtained by consecutive dilutions of ambient seawater with MilliQ water. Salinity was measured by means of a digital refractometer. From the treatment series, cytoplasm-bearing specimens of *N. incompta* were removed and cultured individually under treatment salinity in 75 mL Falcon flasks and constant temperature of 6 °C in a cold room under 8 hours light cycles (intensity of 150 µmol photons m^{-2} s^{-1} , Manno et al. 2012). They were fed daily with 30 µL autoclaved marine microalgae *Nannochloropsis* food mix (30µL *Nannochloropsis* concentrate: 200 mL filtered seawater), attempting to simulate a diet involving phytoplankton detritus. A population of 16 specimens in the size range of 95-203 µm was initially selected for the experiment. A larger population number was not possible given the sampled population size and the effort associated with individual monitoring. After the introduction of the treatment gradient, one specimen was left for the individual culturing in the control (ambient) treatment (35 PSU), three for 31 PSU, three for 28 PSU and two for 25 PSU

Analyses

The response of the individual specimens to the treatment was monitored until cytoplasm decay was observed (Fig. 4-3d). Cytoplasm-bearing specimens that did not display any rhizopodial

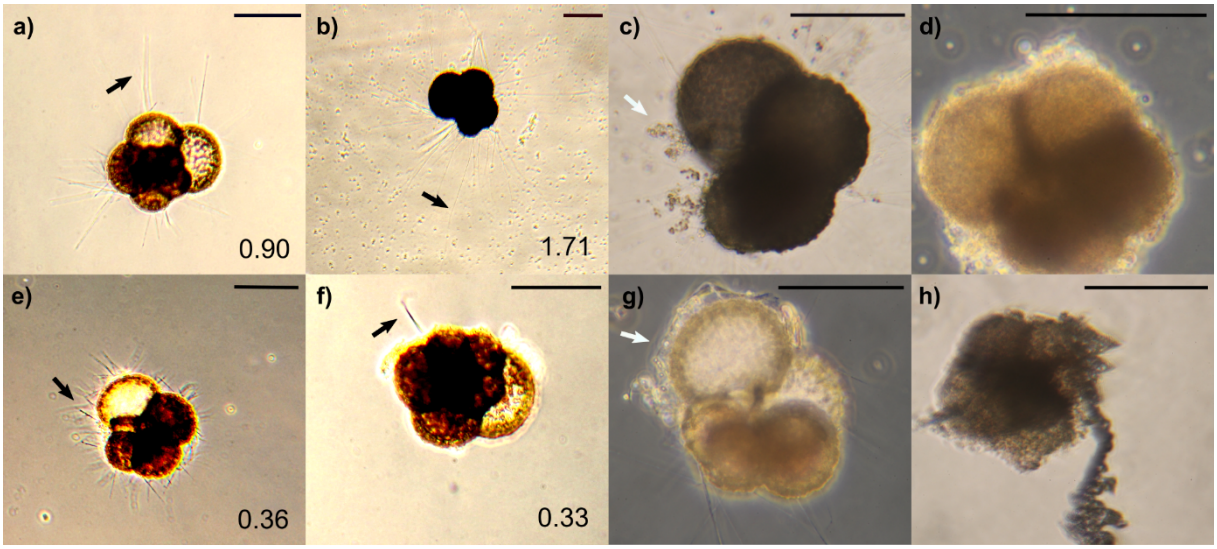


Figure 4-2 *Cultured specimens of N. incompta. Panels (a), (b), (e) and (f) show specimens displaying different levels of rhizopodial activity. Black arrows indicate the rmax used to derive the rhizopodial activity. Contrast in the pictures has been artificially enhanced to visualise the rhizopodia. White arrows in the panels (c) and (g) indicate respectively specimen feeding on Nannochloropsis and another one producing a feeding cyst. Panel (d) shows cytoplasm decaying in a specimen from the 25 PSU treatment, later followed by partial dissolution of the shell (h). (Scale bar: 100µm). In (a) specimen from Salinity treatment (S)= 31 PSU, at day (D)= 7; (b) S= Ctrl, D= 23, (c) S= Ctrl, D= 26; (d) S= 25 PSU, D= 12; (e) S= 31 PSU, D= 8; (f) S= 28 PSU, D= 4; (g) S= 31 PSU, D= 9; (h) S= 25 PSU, D= 14.*

net for 18 days from the start of the experiment were re-checked after day 22. The foraminifera were photographed using a digital camera attached to an inverted microscope and the state (colour) of the cytoplasm was reported. The software ImageJ (version 1.8.0) (Schneider et al. 2012) was used to measure the rhizopodial activity of each specimen calculated as the ratio between the maximum shell diameter and the maximum extension of the rhizopods (Fig. 4-2). This parameter was chosen because both measurements are largely invariant to rotation on a plane (the specimens were not floating during observations) and because estimating the number of extended rhizopodial is difficult and more ambiguous than a determination of the maximum extension length. Repeated measurements on selected specimens indicate that the uncertainty on determination of the maximum shell diameter is 3% and assuming similar uncertainty on the rhizopodial extension, the resulting uncertainty on the index should be about 6%. After the experiment, the cultured specimens were photographed using a scanning electron microscope (SEM) at the University of Bremen.

Given the small scale of our experiment, we decided to refrain from statistical analyses.

4.3 Results

In the control treatment, the viable *N. incompta* specimen survived for the entire duration of the experiment, displaying the highest rhizopodial activity registered (1.71; Fig. 4-4). In the 31 PSU treatment, one specimen survived until day 3, formed a kummerform final chamber and showing signs of shell thickening (Fig. 4-4). The remaining two specimens showed rhizopodial

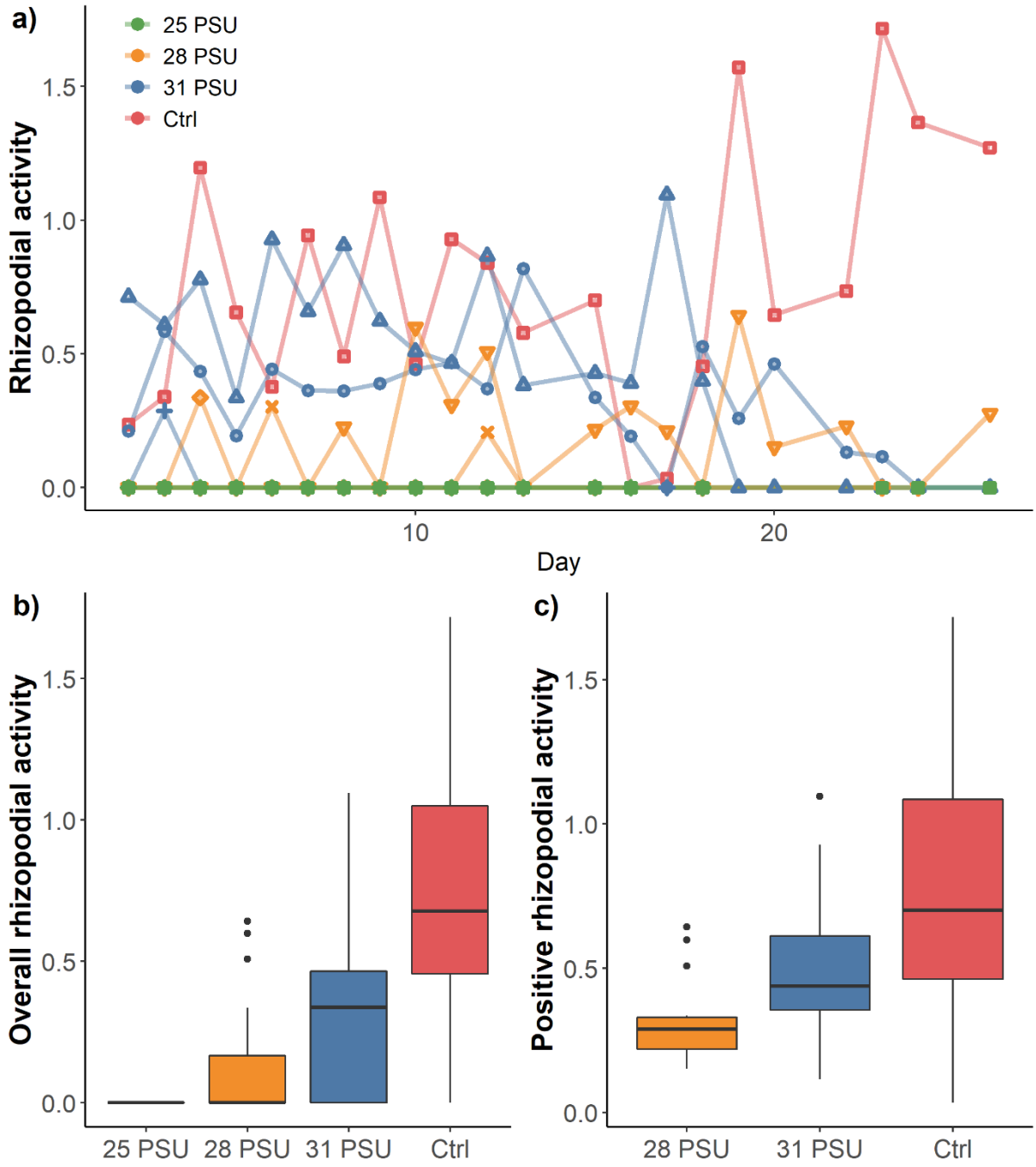


Figure 4-3 *Individual (a) and overall (b, c) rhizopodial activity observed during the experiment in the different treatments. Symbols in (a) refer to the different specimens, colour indicates the salinity treatment.*

activity until days 18 and 24, respectively. The overall average rhizopodial activity was lower in this treatment than in the control (Fig. 4-4b). The same applies to the average activity for all observations when extended rhizopodia were observed (Fig. 4-4c). At 28 PSU, two of the specimens stopped displaying rhizopodial activity after day 12 and later showed signs of cytoplasm decay. Only one specimen survived until the end of the experiment. The overall average rhizopodial activity, as well as the average activity for all observations when extended rhizopodia were observed, was the lowest (Fig. 4-4). None of the specimens cultured at 25 PSU

showed rhizopodial activity during the experiment and both specimens showed signs of cytoplasm decay after day 15 (Fig. 4-3d).

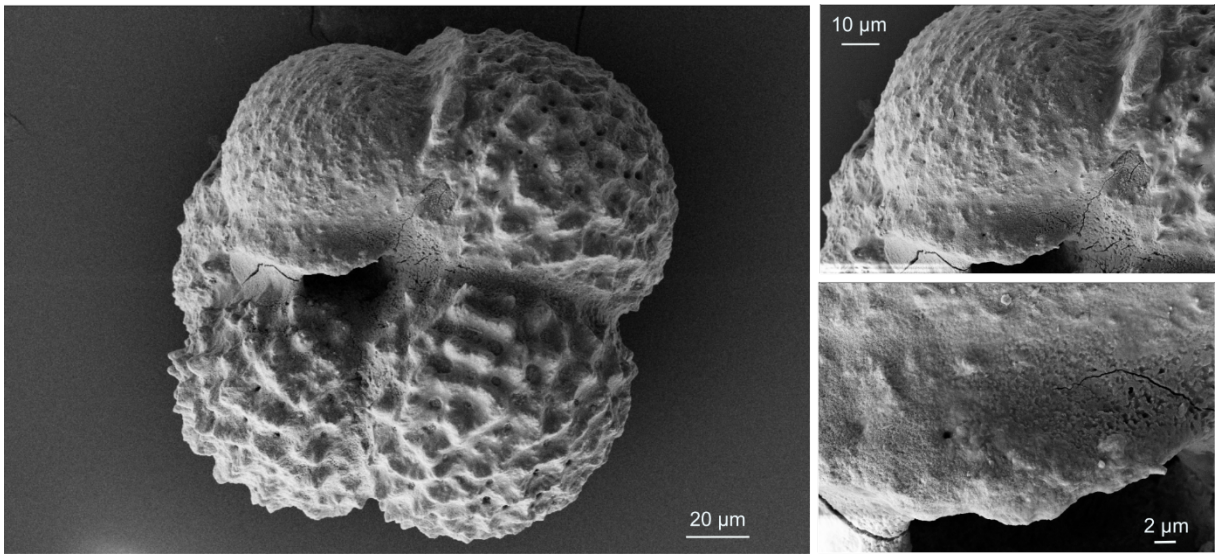


Figure 4-4 *SEM images of the cultured specimen of N. incompta from the 31 PSU treatment that formed a kummerform chamber and died (presumably due to completion of its life cycle) after 4 days in culture.*

4.4 Discussion

Our observations indicate that *N. incompta* rhizopodial activity decreases on exposure to salinity from 35 to 28 PSU, but survival under an extended period of time (weeks) is possible within this salinity range, whereas it appears that extended exposure to 25 PSU is lethal. There are no earlier experimental observations on the salinity tolerance of this species, and ambient salinities in the modern ocean where planktonic foraminifera occur, even in the Arctic, where the lowest salinity conditions are expected, are always >29 PSU (Greco et al. 2019). However, Bijma et al. 1990 presented data on salinity limits of the related species *Neogloboquadrina dutertrei*. Although these authors used a different definition of viability based on growth, they observed that the vital processes of the tested specimens of *N. dutertrei* were completely inhibited at 25 PSU. This observation is in agreement with our results on *N. incompta*. It is important to note that the ability of *N. incompta* to survive under reduced salinities under laboratory conditions does not necessarily mean that it will inhabit a similarly low-saline meltwater lens in the natural environment. Indeed, laboratory experiments can only constrain the maximum range of salinities under which survival in the field may occur.

In the present experiments, one of the cultured specimens showed signs of chamber formation and thickening under the light microscope. As no calcification label was added to the culture seawater, we confirmed the observation by subsequent analyses of the recovered shell using SEM. This revealed the addition of a kummerform chamber and of shell-thickening by secondary calcification (Fig. 4-5). Both observations are consistent with the normal behaviour prior to gametogenesis in planktonic foraminifera (Hemleben et al. 1989). This indicates that

the laboratory conditions in our experiment did not preclude growth or calcification and, therefore, the termination of its natural life cycle explains why this specimen died so early despite exposure to the non-lethal salinity level of 31 PSU. In the light of this observation, it remains unclear why the remaining specimens in our experiment survived but did not grow.

A possible explanation may be due to the low cultivation temperature of 6 °C. Indeed, the few previous culturing studies on *N. incompta* grown under different temperatures reported no growth in specimens cultured at 6 °C, but growth occurred at 9 °C (Von Langen et al. 2005; Davis et al. 2017b). Unfortunately, both culturing studies were carried out in the Pacific, which is inhabited by a different cryptic species of *N. incompta* (Darling et al. 2006), making it difficult to directly transfer these observations on the North Atlantic species. Alternatively, it could be that the autoclaved *Nannochloropsis* used for feeding the cultured *N. incompta* does not represent a suitable food source for this species. In previous experiments, cultured *N. incompta* specimens were fed with freshly killed *Artemia* (Von Langen et al. 2005; Davis et al. 2017b), but recent molecular investigations revealed that this species may feed on bacteria (Bird et al. 2018). With the food preference of this species unknown (*Artemia* cannot be the natural prey and has been taken as a substitute for marine copepods), we opted for autoclaved *Nannochloropsis* assuming that it emulates the likely available food found below the sunlit layer (*N. incompta* is a subsurface species, Rebotim et al. 2017) and considering that it was found to be accepted by other foraminifera (Schmidt et al. 2015). We observed that the autoclaved *Nannochloropsis* was accepted by *N. incompta* and collected by its rhizopodial network (Fig. 4-3c and g), forming a feeding cyst (Spindler et al. 1984; Hemleben et al. 1989; Heinz et al. 2005; Bird et al. 2018), but it is possible that either the quantity or quality of the food was insufficient to facilitate shell growth.

4.5 Conclusions

Our study provides first experimental and preliminary evidence for physiological stress in *Neogloboquadrina incompta* with decreasing salinity under "polar" conditions. We show that the species survives extended chronic exposure from 35 to 28 PSU and we interpret the complete absence of extended rhizopods at 25 PSU as evidence for physiologically lethal conditions. Our experiment indicates that quantification of the extent of rhizopodial activity may be an effective measure of physiological health, which can be used even in situations and at time-scales where no shell growth occurs. Because of the low number of specimens investigated, these conclusions require validation by further experiments, but the preliminary results provide a context for assessing the salinity tolerance of this species and can serve as a basis to better interpret the paleoclimatic reconstructions based on fossil shells of *N. incompta*.

4.6 Acknowledgements

The captain and crew of the R/V Helmer Hansen and engineers B.R. Olsen and T. Holm are gratefully acknowledged for support of the work during the sample collection. We also thank

the Department of Geosciences at the Arctic University of Norway, Tromsø for providing logistical support. Samples were collected during a teaching cruise for courses GEO-3111 and GEO-3122 and financially supported by the Department of Geosciences, UiT the Arctic University of Norway, Tromsø, Norway.

Chapter 5

DECADAL TRENDS OF PLANKTON COMMUNITY CHANGES AND HABITAT SHOALING IN THE ARCTIC GATEWAY RECORDED BY PLANKTONIC FORAMINIFERA

This manuscript is in preparation for submission.

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Data availability*:* All new data on which this study is based will be deposited on the public repository PANGAEA. The data as used in this study, including the environmental data, will be provided on ZENODO. The code and scripts used to analyse the data will be made available on GitHub once the manuscript is accepted for publication.

Abstract

The Fram Strait plays a crucial role in regulating the heat and sea-ice dynamics in the Arctic. The marine biota of this Arctic gateway is experiencing significant changes with increasing advection of Atlantic species. The footprint of this "Atlantification" of marine diversity is based largely on fragmented observations of species occurrences representing different parts of the plankton community. Multi-decadal investigations on how regional climate change facilitates the invasion of Atlantic species and affects the ecology of the resident species are lacking. Here we use planktonic foraminifera as a proxy for recent changes in the pelagic community in the Fram Strait in three dimensions. Our analysis is based on 51 species-resolved stratified population profiles collected during seven surveys between 1985 and 2015. The analysis of the data reveals an ongoing shift towards an Atlantic component, occurring independently of changes in local environmental conditions, thus reflecting higher production of the Atlantic species in their "source" region. At the same time, the ongoing extensive sea-ice export from the Arctic proper and associated cooling in the Fram Strait negatively affected the resident species *T. quinqueloba* that showed declining density and habitat shoaling. Since the resident *N. pachyderma* persists, the planktonic foraminifera community shifts to a new state, where the thriving cold-adapted resident is confronted with increasing advection of Atlantic expatriates. Our results show that both remote forcing of the Atlantic invaders and local climatic changes acting on the resident species induce rapid response of community structure as well as vertical distribution of planktonic foraminifera in the Fram Strait. When the strong summer export of Arctic sea-ice will decrease, the Arctic gateway will likely experience rapid restructuring of the pelagic community even in the absence of further warming. Such a large change in the gateway region will likely propagate into the Arctic proper.

5.1 Introduction

Over the last decades, the Arctic has experienced warming and sea ice decline of "unprecedented" extent, shifting to a climatic state not experienced throughout the 20th Century (Box et al. 2019). A key region for the heat budget and sea ice dynamics of the Arctic is the Fram Strait. This narrow passage accommodates the only deep-water connection with the Atlantic, facilitating inflow of a large portion of the warm and saline Atlantic Water, the export of sea ice from the Arctic (Beszczynska-Möller et al. 2012), and exchange of marine biota between the polar Arctic mediterranean and the subarctic North Atlantic (Bluhm et al., 2015; Kosobokova & Hirche, 2009; Wassmann et al., 2015). The Atlantic Water (AW) is transported through the eastern part of the strait by the West Spitsbergen Current (WSC), while on the western part, the East Greenland Current (EGC) carries polar water and sea ice from the Nordic Seas to the south (Fig. 5-1). The AW inflow to the Arctic has warmed over the last decades (Beszczynska-Möller et al., 2012; Polyakov et al., 2012; Wassmann et al., 2015) constituting one of the main drivers of the current changes in the Arctic marine environment (Onarheim et al. 2014).

The apparent increase in the advection of AW and the resulting changes in the Arctic environment are reflected in the "Atlantification" of the marine community in the Fram Strait (Kraft et al. 2013; Gluchowska et al. 2016; Andrews et al. 2018; Schröter et al. 2019). A long-term record of planktonic foraminifera shells in a marine sediment core from the Fram Strait indicates that the recent changes are unparalleled over the last two millennia (Spielhagen et al. 2011). The ongoing Atlantification of the Arctic gateway contrasts with the changes in the physical environment of the upper ocean in the region. Unlike the rest of the Arctic realm, in the summers between 1985 and 2015, the Fram Strait has been cooling at the surface (~ 0.5 °C), and sea ice has expanded along the east coast of Greenland and Svalbard (Fig. 5-1). This seemingly counterintuitive trend is reflecting the increasing summer sea ice reduction in the Arctic and the associated export of Arctic sea ice into the Greenland Sea (Wang et al. 2019). Net changes in SST in the Fram Strait are therefore the result of the combined effect of increasing advection and warming of northward-flowing AW and increased sea-ice export in the EGC flowing southward. The higher export of Arctic sea ice and its melting in the Greenland Sea also contribute to a large-scale surface freshening, which suppresses oceanic mixing and facilitates cooling of the surface waters (Kwok et al. 2005) that are overlying the warm Atlantic inflow in the subsurface.

Thus, the environmental conditions in the Fram Strait, taken alone, should not facilitate Atlantification of the marine biota. Indeed, the observed increase in abundance of subpolar species and associated community changes have been interpreted as a consequence of warming in the North Atlantic "source" region and intensification of the AW inflow carrying the subpolar biota into the Fram Strait (Wassmann et al., 2015). In this scenario, the increasing proportion of Atlantic biota should occur independently of the local conditions in the Fram Strait and the Atlantification process should be associated with a re-arrangement of the vertical structure of the pelagic communities.

Here, we analyse three decades of changes in population structure and vertical distribution of planktonic foraminifera, a distinctive group of Arctic unicellular zooplankton, in the Fram Strait recorded by 51 species-resolved standing stock vertical profiles sampled between 1985 and 2015. Planktonic foraminifera species show a precise biogeographic distribution controlled by temperature (Bé & Tolderlund, 1971; Fenton et al., 2016; Morey et al., 2005) and species-specific depth habitats, which vary with changing environmental conditions (Rebotim et al. 2017; Greco et al. 2019). Their sedimentary record preserved on the seafloor indicates that they are sensitive indicators of climate change (Spielhagen et al. 2011) and their populations have been shifting globally in the ocean in line with recent global change (Jonkers et al., 2019), making them ideal sentinels of Fram Strait Atlantification and changes in vertical habitat structure. In combination with in-situ and regional environmental descriptors, the standing stock data were analysed to assess the extent and environmental determinants of recent changes in (i) planktonic foraminifera community structure, (ii) species density and (iii) shifts in species vertical distribution.

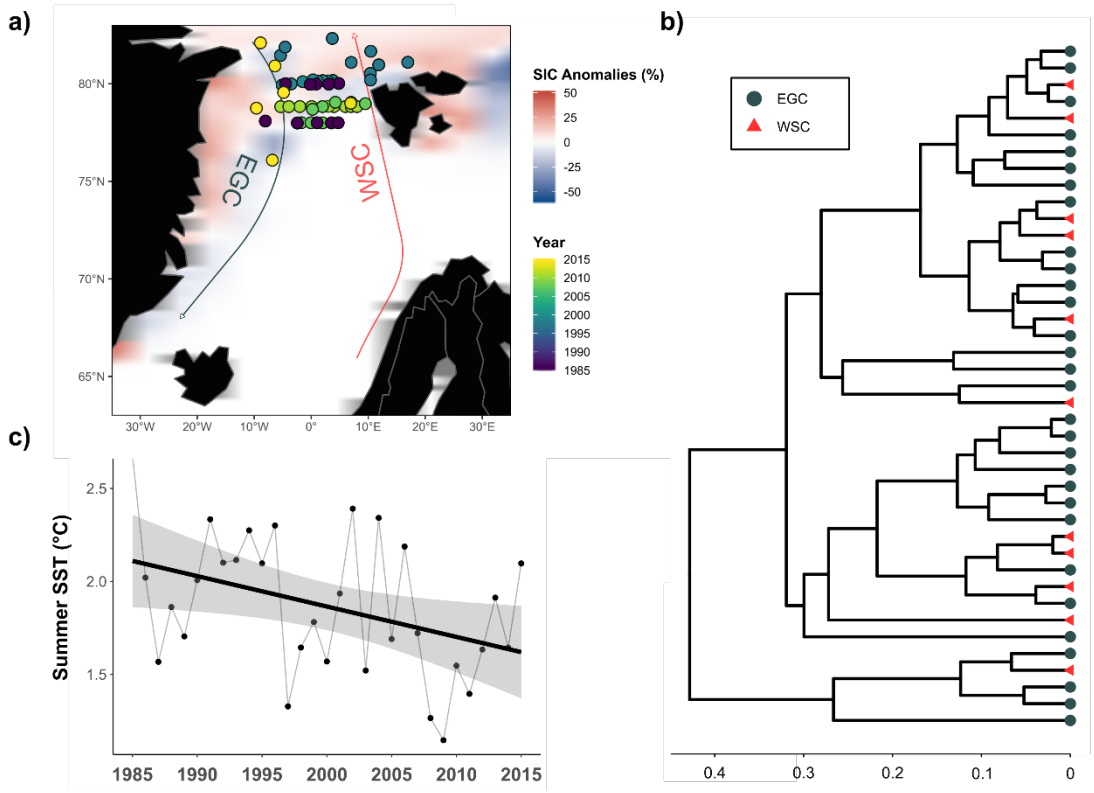

Figure 5-1 *a) Plankton net stations with vertically resolved planktonic foraminifera counts used in this study color-coded by year of sampling. Background colour indicates Sea Ice Anomalies in the Fram Strait calculated for the period 1985-2015. Data from Sea Ice Index Version 3.0 (Fetterer et al., 2017). Arrows indicate the two main water masses present in the Fram Strait. b) Hierarchical cluster analysis showing the similarity of foraminiferal assemblages, symbols show the water mass identified with the position of the station. c) Summer SST in the sampling area in the period 1985-2015. Data from NOAA Optimum Interpolation Sea Surface Temperature V2 [weekly resolution] (Reynolds et al., 2002).*

5.2 Material and Methods

Biological data

Over the last four decades, the plankton community of the Fram Strait has been sampled heavily with replicate vertical profiles available for most sampling years. Among the collected zooplankton, the planktonic foraminifera have been the most frequently quantified and reported at species level, allowing us to compile a dataset of five surveys of planktonic foraminifera repeated in virtually the same location and same time of year in the Fram Strait between 1985 and 2011 containing a total of 45 density profiles. In order to extend the length of the time series, we generated new data from one profile taken in July 2014 and a survey with five profiles sampled in July 2015. In 2014, water was sampled from three different depth intervals (0-50, 50-200, and 200-600) by the means of a bongo net with aperture 0.25 m^2 and mesh size of 63 µm during an oceanographic cruise on the R/V Helmer Hansen. The following year, sampling was carried out on the R/V Polarstern using a multiple closing plankton net (Hydro-Bios, Kiel) with an opening of 0.25 m^2 and equipped with 5 nets each with a mesh size of 55 µm.

Samples from both expeditions were sieved through 250 and 63 µm sieves and stained with Rose Bengal/ethanol mixture after collection to facilitate the distinction between cytoplasm-bearing and empty shells. The samples were processed at the University of Bremen and at the Arctic University of Norway in Tromsø, where planktonic foraminifera were picked under a binocular microscope and air-dried. All specimens in the fraction above 63 µm were counted and identified to species level following the taxonomy of Brummer and Kroon (1988) and Hemleben (1989). Concentrations of the resident (*Neogloboquadrina pachyderma* and *Turborotalita quinqueloba*) and of the Atlantic species (*Globigerina glutinata*, *Globigerina bulloides*, *Neogloboquadrina incompta*, *Globigerinita uvula*, and *Orcadia ridelii*) were derived from counts by using the volume of filtered water determined from the product of towed interval height and the net opening.

The new data and the literature data had to be first harmonised to the same taxonomy. As a result, counts of *N. pachyderma* and the Atlantic species from the ARK III/3 cruise could not be used in the analyses due to the different taxonomical resolution of the original study (Carstens et al. 1997). In their paper, the authors did not distinguish between *N. pachyderma* and *N. incompta*, previously considered coiling varieties of the same species but now known to be genetically distinct (Darling et al. 2006). Data on the polar species *T. quinqueloba* collected during the same expedition were included in the analyses. Because of their consistently low density and variable species composition, the concentrations of all non-resident (Atlantic) species were lumped into one category for the downstream analyses. For samples collected in 2008, the density of the Atlantic species was assumed to be 2% of the total assemblage as stated by the authors of the original study (Manno and Pavlov 2014). Because of the taxonomic lumping, the vertical habitat of the Atlantic species could not be evaluated. Since the distinction between cytoplasm-bearing and empty shells has not been done consistently, the analysis is based on the concentration of all shells. Greco et al. (2019) have shown that this treatment causes a slight but consistent overestimation of the vertical habitat depth, but since the vast majority of the collected specimens in the plankton are cytoplasm-bearing, the effect on standing stock estimates is likely negligible. However, the different surveys have used different vertical sampling schemes and resolutions. Therefore, the individual vertical density profiles were converted to a common vertical scheme resolving standing stock at three depths (0–50, 50–100, 100–200) using a custom script in R (R Core Team, 2017). This scheme was chosen to avoid extrapolation and reflects the most shared position of depth-interval boundaries among the sampling schemes. We derived total species density as the sum of the concentrations within the different intervals and for the two polar species, *N. pachyderma* and *T. quinqueloba*, the depth habitat was calculated as in Greco et al. (2019).

Environmental parameters

The habitat of planktonic foraminifera is reflecting the vertical structure of physical and biological properties of the surface ocean layer. Therefore, next to the consideration of the temporal trends, to understand why the population densities, species composition and vertical habitat have been shifting, we have tested models explaining the observed variability with physical properties of the environment. The main parameter affecting planktonic foraminifera species composition appears to be temperature (Jonkers et al., 2019; Morey et al., 2005) in Arctic polar waters in combination with sea ice concentration (Carstens et al., 1997; Pados &

Spielhagen, 2014) and in the Fram Strait an important parameters is also the depth of the Atlantic layer (Pados et al., 2015; Simstich et al., 2003). Salinity, within the range of typical open marine conditions, has been shown not to affect planktonic foraminifera (Greco et al. 2019).

In-situ temperature profiles were retrieved from CTD data from the respective expeditions. Data deposited in PANGAEA were accessed using the R package "pangaear" (version 08.2) (Simpson and Chamberlain 2018). For nine stations from the iAOOS and HH14 cruises, CTD data were obtained from the original investigators. The CTD temperature profiles were used to extract Sea Surface Temperature (SST), here defined as the average temperature in the first 6 meters from the sea surface, and the minimum depth of the Atlantic Water layer (AWz), defined as the depth where temperature rises above 2 °C (Beszczynska-Möller et al. 2012). As no CTD data were collected during the ARK III/3 cruise (Carstens et al. 1997), for these stations we extracted the SST and AWz from the NOAA Optimum Interpolation Sea Surface Temperature V2 [weekly resolution] (Reynolds et al. 2002) and the Hadley Centre EN4 dataset (Good et al. 2013), respectively. For all stations, in situ sea ice concentration and the distance from the ice margin at the time of sampling were extracted from 25 km×25 km resolution passive microwave satellite raster imagery obtained from the Sea Ice Index Version 3.0 product of the National Snow and Ice Data Centre (Fetterer et al. 2017) using a custom function in R.

The foraminifera assemblage captured in the net is the result of growth over several weeks (Carstens and Wefer 1992) and to a certain degree the composition thus reflects processes acting throughout the habitat traversed by the plankton before being intercepted by the net. To account for the effect of these processes, next to the in-situ parameters, we also analyse two descriptors of the overall oceanographic state of the sampling area (spatial polygon including all the sampling locations present in our compilation) at the time of sampling. These include the average SST of the sampling area from the NOAA Optimum Interpolation Sea Surface Temperature V2 (Reynolds et al. 2002) and the average ice extent of the sampling area from the Sea Ice Index Version 3.0 (Fetterer et al. 2017). In addition to the physical environment, the foraminifera population also likely reflects the trophic structure of their habitat. This is often highly correlated with the physical parameters of the environment (sea ice extent, distance from sea-ice edge), but could also act independently. Unfortunately, neither in-situ observations, nor satellite image data are available throughout the sampling period to generate representative and robust estimates of productivity.

Statistical analyses

We used the obtained dataset to investigate the effect of the environmental parameters and time (sampling year, since all years the sampling took place in summer) on the composition, abundance and depth habitat of planktonic foraminifera species in the Fram Strait. First, we had to rule out the potential influence of the longitudinal gradient of physical properties in the Fram Strait (Fig. 5-1) on the monitored parameters. Since in most years, the geographical extent of the sampling straddled this gradient, the presence of different hydrographic regimes in the east and in the west Fram Strait (WSC and EGC respectively) could potentially be the dominant factor influencing the planktonic foraminifera community. To test the effect of the longitudinal gradient, we performed a hierarchical cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) based on the Bray–Curtis dissimilarity index on species

density data using the *hclust* function in the package "vegan" (version 2.5-6) (Oksanen et al. 2018) in R and observed the clustering of sample sites assigned to the two hydrographic regimes in the region defined as in Fadeev et al., (2018). This analysis revealed no preferential clustering of samples according to the region (Fig. 5-1b), indicating that the observed variability is due to factors other than the sampling location.

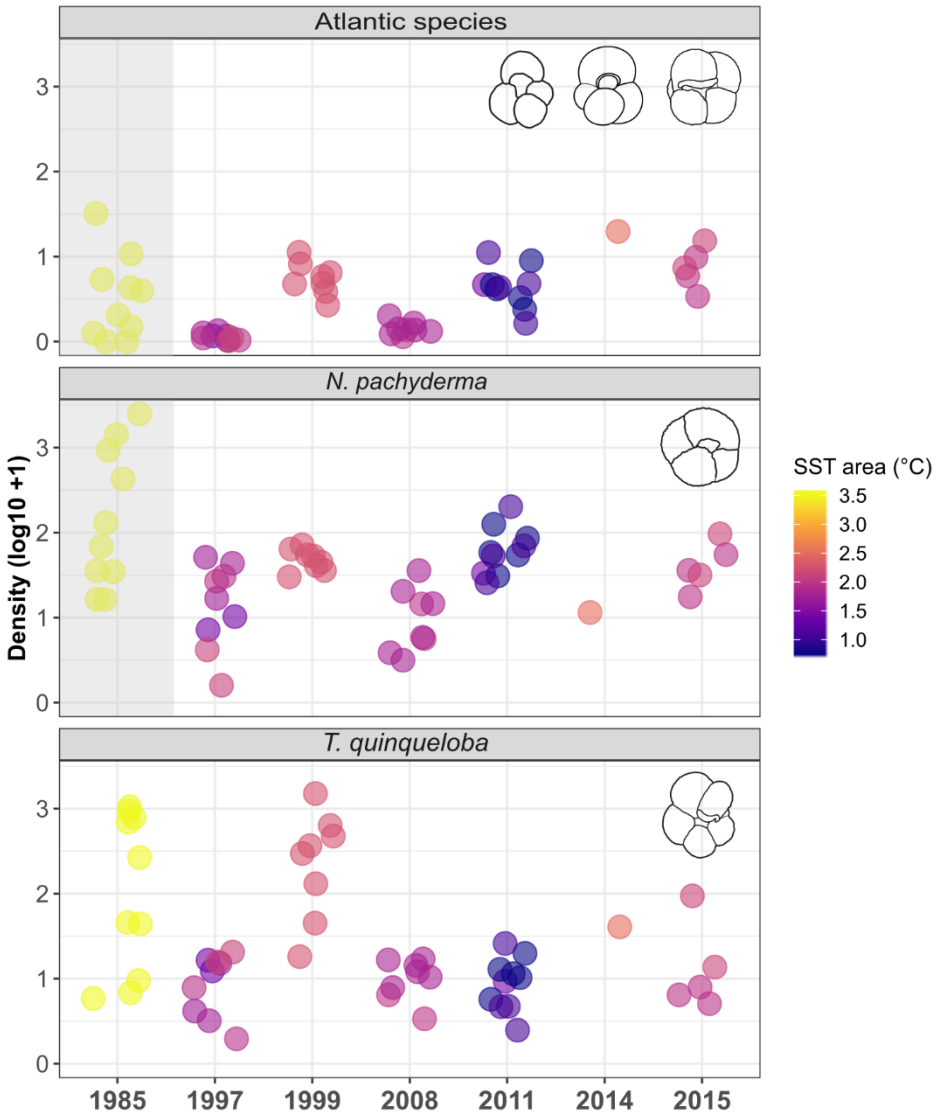

Figure 5-2 *Density of the planktonic foraminifera species plotted against year. Colour indicates temperature of the area at the time of sampling. The grey-shaded area indicates the samples excluded from the analyses for taxonomical incongruences (See Material and Methods).*

Changes in the community structure of planktonic foraminifera were then analysed using a multivariate approach. We used nonmetric multidimensional scaling (NMDS) to visualize the similarities of assemblages observed across the stations using the *metaMDS* function from the R package "vegan" (version 2.5-6) (Oksanen et al. 2018). For the NMDS, data by Carstens (1997) was not included in order to eliminate potential biases due to the taxonomic ambiguity in the counts (*N. pachyderma* and *N. incompta* not distinguished). The obtained ordination was used to assess the individual effects of the tested environmental variables on the foraminifera community by performing BIOENV analysis (Clarke and Ainsworth 1993). This test allows the identification of variables that best explain the variance in the biological community by

calculating a correlation coefficient that is then subjected to a permutation test to determine its significance. Prior to this step, we checked for the presence of collinearity between the environmental variables using the variance inflation factor (VIF) with the *vifstep* function from the R package "usdm" (version 1.1-18). The function calculates the VIF for a set of variables and excludes the highly correlated variables (VIF > 5) (Fenton et al. 2016) from the set through a stepwise procedure. The remaining environmental variables were included in the BIOENV analysis using the *envfit* function from the R package "vegan" with 999 permutations.

Next, generalized linear models (GLM) were applied to assess the effects of time and environmental drivers on the individual abundances of *N. pachyderma*, *T. quinqueloba* and of the Atlantic species. As we analysed count data, we used the *floor* function in R to derive discrete values from the total concentrations of the three taxonomic group as prior step (Zuur et al., 2007) and explored relationship with the potential predictors with bivariate GLM using the *glm* function in R indicating a quasipoisson error distribution with log as link function. For the three groups, *N. pachyderma*, *T. quinqueloba* and Atlantic species, the total concentration (dependent variable) was regressed against the sampling year (time), longitude (as a proxy for the two hydrographic regimes), SST, average SST of the sampling area, ice-concentration, distance from ice-margin, AWz, and average sea-ice extent of the sampling area as independent variables. Trait variance explained by individual parameters was calculated using pseudo-R^2 for Poisson GLMs as 100*(model null deviance- model deviance)/model null deviance (Dobson 2002). Where more than one predictor displayed a significant effect on the abundance, VIF was calculated among the variable, variables identified as causing variance inflation were dropped, and the GLMs were re-applied allowing for interactions among the remaining variables.

The relationship between environmental and temporal controllers on the depth habitat (DH) of the resident species *N. pachyderma* and *T. quinqueloba* were investigated through bivariate correlation (Pearson *r*). Square root transformation was performed on *T. quinqueloba* data to obtain symmetric distribution. Multiple linear models were applied to species depth habitat and variables that displayed a significant correlation. The normality of the residuals was checked after the linear model was applied (Zuur et al. 2009). As for the abundance, in case of more than one predictor displayed a significant correlation with DH, we proceeded to calculate the VIF between the concurrent variables and re-applied the linear model for the remaining variables allowing interactions. Results from models that explained most of the variance (higher pseudo-R^2 and R^2) are presented and discussed.

5.3 Results

Species composition

All samples contained an assemblage typical for the polar environment of the Fram Strait, dominated by *N. pachyderma*, which represented 56% of the total assemblage in our compilation. Not all the stations presented the same species proportions: *T. quinqueloba* was the most abundant species in 17 stations. The abundance of the North Atlantic also varied greatly in our compilation ranging from total absence to 27 % of the total assemblage in the

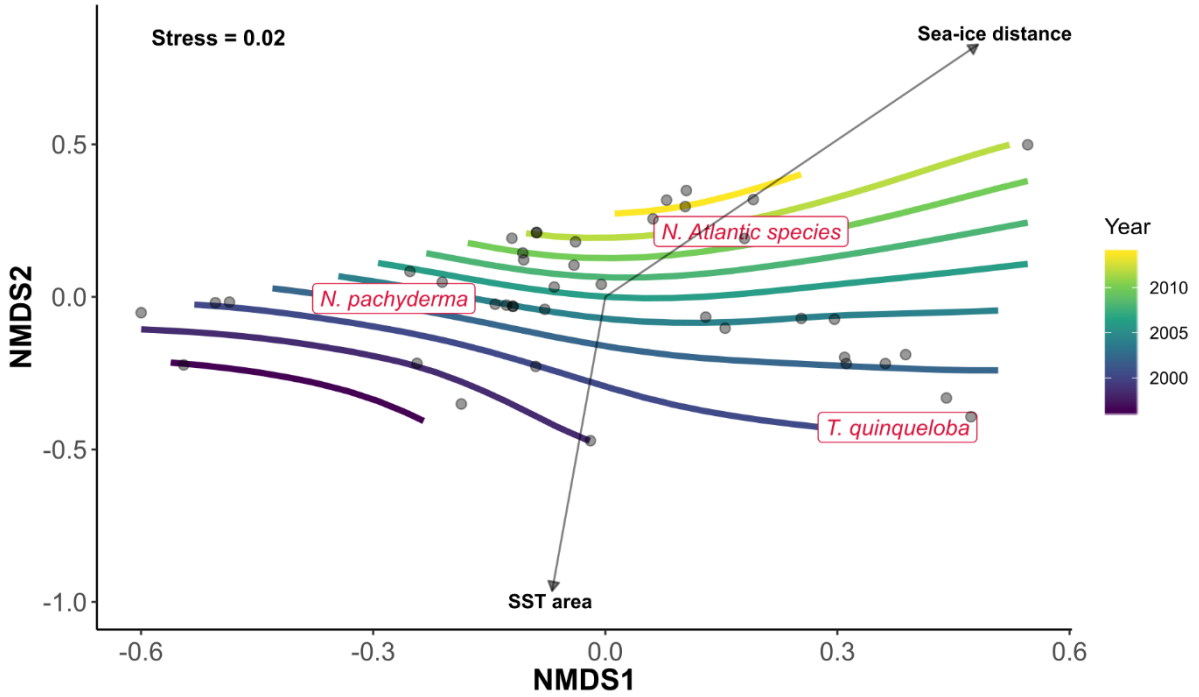

Figure 5-3 *NMDS ordination based on Bray-Curtis similarities Index of planktonic foraminifera abundances with fitted environmental vectors. Contour lines were derived from surface fitting (GAM) of the variable sampling year.*

samples taken in 2014. The BIOENV analysis revealed that three of the tested variables correlate significantly with the obtained ordination without variance inflation: Year ($R^2 = 0.46$, p-value = 0.001), SST of the sampling area ($R^2 = 0.36$, p-value = 0.008), and distance from the sea-ice margin ($R^2 = 0.23$, p-value = 0.023) (Fig. 5-3 and Fig. 5-4b). This indicates that the assemblage composition has changed through time and that at least part of the change can be attributed to changes in the physical environment.

Species density

The time series of population density reveal a considerable amount of variance within each sampling period, the presence of years with unusually high density for some species, and an apparent trend of increasing density of Atlantic species (Fig. 5-2). Potential predictors of the observed trends in population density of the three taxonomic groups were thus investigated using generalised linear model and the results are summarised in Table 5-1 and in Figure 5-4. The two best predictors of *N. pachyderma*'s density were longitude (Pseudo-$R^2 = 0.18$, p-value = 0.01) and the SST of the sampling area (Pseudo-$R^2 = 0.14$, p-value = 0.002), both showing a negative relationship with the concentration of *N. pachyderma* (Fig 5-4a). The final model including the summing effects of the two predictors explained 38% of the total variance. The SST of the sampling area was also negatively associated with the density of *T. quinqueloba* (Pseudo-$R^2 = 0.14$, p-value = 0.002), but the variable was removed due to high collinearity (VIF > 5). The remaining two predictors, year of sampling and sea-ice extent had a VIF < 2 and were included in the final model along with their interactions explaining 51% of the observed

variance. Only the year of sampling alone was identified as a significant predictor of the total density of the Atlantic species (Pseudo-R^2 = 0.17, p-value = 0.02).

Depth habitat

The species *N. pachyderma* and *T. quinqueloba* displayed a similar vertical distribution in the water column with average living depths 37-140 m and 44-142 m, respectively. Factors controlling the variability in the observed depth habitat of the two species were investigated by a linear model. This revealed that sea ice extent in the sampling area alone was the only significant predictor of the depth habitat of *N. pachyderma* (Adj. R^2 = 0.091, p-value = 0.03). In contrast, all the variables investigated showed a significant correlation with the variability of depth habitat of *T. quinqueloba* (Table 5-1) and only the overall SST in the area had to be excluded because of variance inflation. A combined linear model with interactions still identified three variables as significantly and independently affecting the depth habitat of the species. Longitude, sea-ice extent and sampling year explain together 47 % of the observed variance in the depth habitat of *T. quinqueloba*.

5.4 Discussion

The results of the BIOENV analysis indicate a steady rise in the Atlantic species concentration throughout the observational period (Fig. 5-3). The same pattern emerged from the GLM (Table 5-1, Fig. 5-4) with the year of sampling significantly correlated with the density of the Atlantic species and explaining 18% of the variance in our observations. The declining trend in SST in the Fram Strait suggests that the observed increase in the abundance of Atlantic species in the region cannot be the result of habitat tracking. Since none of the tested environmental factors was a significant predictor of the density of Atlantic species in the region either, their rising abundance must reflect changes in the "source" region in the Nordic Seas from where the species are advected to the North with the AW. An increase in density or a change in phenology of these species in the Nordic Seas would result in higher density in the Fram Strait region even without changes in the intensity of AW inflow. Indeed, evidence from moorings has shown that the variability observed in the advection of "Atlantic" copepods in the Fram Strait reflects their phenology and not the intensity of the AW inflow (Basedow et al. 2018). The invoked changes in Atlantic species population dynamics in the "source" region is consistent with the increasing abundance of planktonic foraminifera in the North Atlantic recorded in CPR observations (Beaugrand et al., 2013).

In contrast to the rising abundance of the Atlantic expatriates, the subpolar resident species *T. quinqueloba* shows decreasing population density through time (Figures 5-2, 5-3 and 5-4) leading to lower proportions in the planktonic foraminifera community (Fig. 5-3). Contrary to the non-resident, advected Atlantic species, the Fram Strait region is the primary habitat of *T. quinqueloba* (Schiebel et al. 2017) and its abundance in the Fram Strait is not reflecting AW inflow. However, this species is known to prefer warmer, subpolar, waters and is largely absent from the Arctic proper (Carstens et al., 1997; Manno & Pavlov, 2014; Pados & Spielhagen, 2014; Volkmann, 2000). Therefore, the increasing sea-ice export and decreasing SST in the

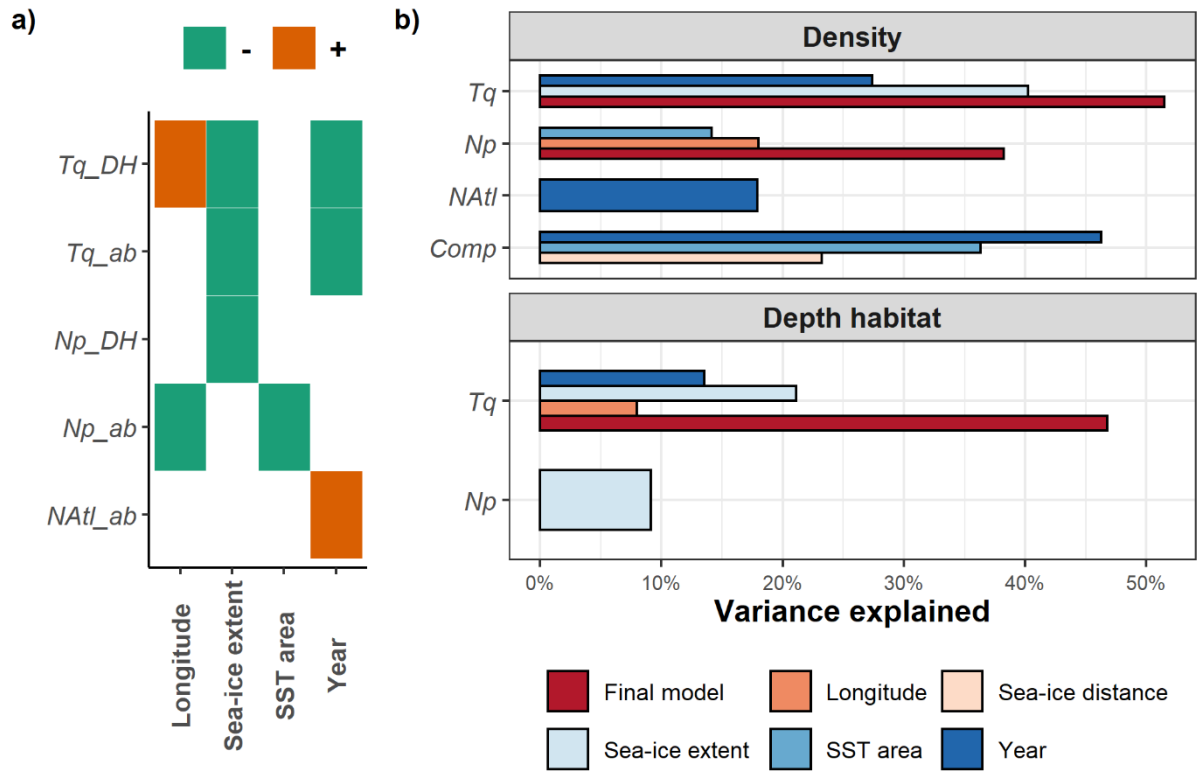

Figure 5-4 *a) Heat-map showing the direction of the relationship with tested environmental variables and modelled responses. b) Bar plot showing amount of variance explained by the singular predictor and the final model. (Abbreviations: Np= Neogloboquadrina pachyderma, Tq= Turborotalita quinqueloba, Atl= Atlantic species, DH= depth habitat, ab= abundance).*

Fram Strait make the region less suitable for this species. Indeed, next to time, a significant component of the variability in the abundance of this species can be explained by local properties in the Fram Strait, in a direction consistent with the above hypothesis: the species is less abundant where/when sea-ice cover is more extensive (Table 5-1, Fig. 5-4). Since the local conditions in the Fram Strait are highly variable, a single observation like that by Manno & Pavlov (2014), would easily appear to indicate an opposing trend, highlighting the necessity and merit of the long-term replicated data series presented in this study.

Thus, the changing abundance of *T. quinqueloba* appears to be consistent with habitat tracking, responding to the temporal evolution of local conditions in the Fram Strait. This conclusion is further supported by the observed concomitant shallowing of the vertical habitat of this species through time (Fig. 5-4), which is reflecting the shallower habitat of the species in the presence of sea ice (Table 5-1). Previous observations on *T. quinqueloba* in the Fram Strait also showed shallower habitat in the presence of sea ice (Carstens et al. 1997; Volkmann 2000). The current increase in sea ice in the Fram Strait thus acts to reduce the population density of this species and shoal its vertical habitat, both occurring in the direction consistent with habitat tracking.

Consistently with the increasing sea ice extent and decreasing temperature, the habitat of the Fram Strait remains suitable for the polar species *N. pachyderma*, which shows no significant temporal trend in its abundance or vertical habitat (Fig. 5-2). Instead, the variability in these parameters can be explained by local parameters with higher density and shallower habitat

occurring when and where sea-ice covered is more extensive (Table 5-1, Fig. 5-4). Peaks in *N. pachyderma* density in cold polar waters were observed in previous studies (Volkmann 2000; Manno and Pavlov 2014) as well as its high occurrence along the sea ice margin considered, where higher primary production by diatoms represents a major food source for this species (Carstens et al., 1997; Pados & Spielhagen, 2014). The habitat shoaling towards sea-ice is entirely consistent with a recent analysis of factors affecting the vertical habitat of this species (Greco et al., 2019).

Table 5-1 *Results of the Generalised linear models and mixed linear models.*

	p - value	**Pseudo / Adj. R^2**
***N. pachyderma* Density**		
Longitude	0.01	18.03
SST area	0.02	14.12
SST area + Longitude		38.23
***T. quinqueloba* Density**		
Year	0	27.42
SST area	0	29.06
Sea-ice extent	0	40.26
Year * Sea-ice extent		51.5
N. Atlantic species Density		
Year	0.02	17.91
***N. pachyderma* Depth habitat**		
Sea-ice extent	0.03	9.13
***T. quinqueloba* Depth habitat**		
Longitude	0.03	7.96
Latitude	0.02	8.53
Year	0	13.54
SST	0.02	8.4
SST area	0	19.14
Sea-ice concentration	0.01	12.5
Sea-ice distance	0	13.54
Sea-ice extent	0	21.11
Longitude * Sea-ice extent * Year	0	46.74

Our in-situ vertically resolved observations of three decades of plankton change in the Fram Strait provide direct evidence that trends in population density are associated with significant shifts in the vertical position of the involved species. This observation is significant, as it could

not have been derived from CPR or sediment trap devices or remote sensing of the ocean surface. The existence of systematic vertical shifts in plankton populations has significant consequences for biogeochemical cycling in the upper ocean (Bianchi et al. 2013). In addition, the changes in plankton vertical habitat in the Fram Strait may affect species interactions with other resident or immigrant Atlantic species, as vertical niche partitioning among closely related species of zooplankton is an important mechanism of adaptation to the Arctic environment (Kosobokova et al., 2011). In light of these observations, we postulate that the assessment of future changes in the marine biota in the Arctic gateway must also consider the vertical dimension of the pelagic habitat (Gluchowska et al., 2017; Knutsen et al., 2017; Kosobokova et al., 2011).

Overall, we thus show that plankton in the Arctic gateway is assuming an unusual composition, with the resident species shifting towards more polar taxa and shallower habitat, tracking local environmental change, being confronted with increasing abundance of Atlantic expatriates, rising due to processes favouring their growth in the Nordic Seas. Since there is no reason to believe that this observation based on planktonic foraminifera should not apply to other plankton, this shift in community composition likely alters the diversity of planktonic communities, in turn affecting the established food webs of the involved species (Kortsch et al. 2015; Griffith et al. 2019). At present, the increased sea-ice export in the Fram Strait compensates the overall regional warming in the Arctic, muting the changes in plankton communities in the region. Once this process is not acting, the planktonic community of the Fram Strait will likely abruptly shift to a completely different state with more Atlantic and more non-sea-ice species. Acting as the gateway to the Arctic, this rapid shift will likely propagate into the Arctic proper.

5.5 Acknowledgements

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Chapter 6

GENERAL CONCLUSIONS

In this thesis, the ecological niche of Arctic planktonic foraminifera species was investigated with a focus on the biotic and abiotic processes regulating their distribution and abundance in both space and time. To this end, a combination of different approaches was used and the hypotheses presented in Chapter one were tested.

> h_1: Environmental factors are the main drivers of *N. pachyderma* vertical distribution in the Arctic

The results in Chapter two showed that seawater temperature, salinity, and density do not display a significant relationship with the depth habitat of *N. pachyderma*, while sea-ice and chlorophyll concentrations at the surface, in combination with the time since the sea-ice break-up, explained almost a third of the variance in the data. The species showed a shallower depth habitat (between 50 and 100 m) under dense sea-ice coverage and/or high surface chlorophyll concentration. When sea-ice cover is reduced and/or when chlorophyll at the surface is low, the depth habitat deepens to 75 – 150 m. The observed pattern underlines the presence of unknown primary driver(s) acting below the position of the deep chlorophyll maximum and possibly reflecting the trophic behaviour of the species. Thus, h_1 is rejected in the context of the environmental variables included in the analysis and previously proposed as the main drivers of *N. pachyderma* vertical distribution.

> h_2*: N. pachyderma* is herbivorous and feeds on diatoms

The metabarcoding dataset in Chapter three offered insights into the biotic interactions of *N. pachyderma* in the water column. The analyses showed a non-specific relationship between pelagic algae and *N. pachyderma*. Moreover, the presence of DNA from groups as Crustaceans and Urochordata indicated that diatom-fuelled marine aggregates likely represent the main interaction substrate of *N. pachyderma* suggesting that the species is omnivorous and grazes opportunistically on degraded organic matter. Hence, h_2 is rejected.

> h_3: *Neogloboquadrina incompta* can survive in low salinity conditions

The study in Chapter four reported on the first experimental evidence of physiological stress in *Neogloboquadrina incompta* with decreasing salinity under polar conditions. The species was exposed to a range of salinities chosen to reach below 30 PSU, which is the lower limit of surface salinity estimates during past meltwater events. Results suggested that the species can survive extended chronic exposure from 35 to 28 PSU. The working hypothesis h_3 is therefore accepted.

> h_4: Changing Arctic climate is already affecting polar planktonic foraminifera diversity and distribution

Results in Chapter five were based on an analysis of 51 species-resolved stratified population profiles collected in the Fram Strait during seven surveys between 1985 and 2015. The data revealed ongoing Atlantification of the planktonic foraminifera community, occurring independently of changes in local environmental conditions, thus reflecting higher production of the Atlantic species in their "source" region. At the same time, a different response of the resident species *T. quinqueloba* and *N. pachyderma* was observed. The ongoing extensive sea-ice export from the Arctic proper and associated cooling in the Fram Strait negatively affected *T. quinqueloba* that showed declining abundance and habitat shoaling while the cold-adapted *N. pachyderma* persists. Thus, h_4 is accepted.

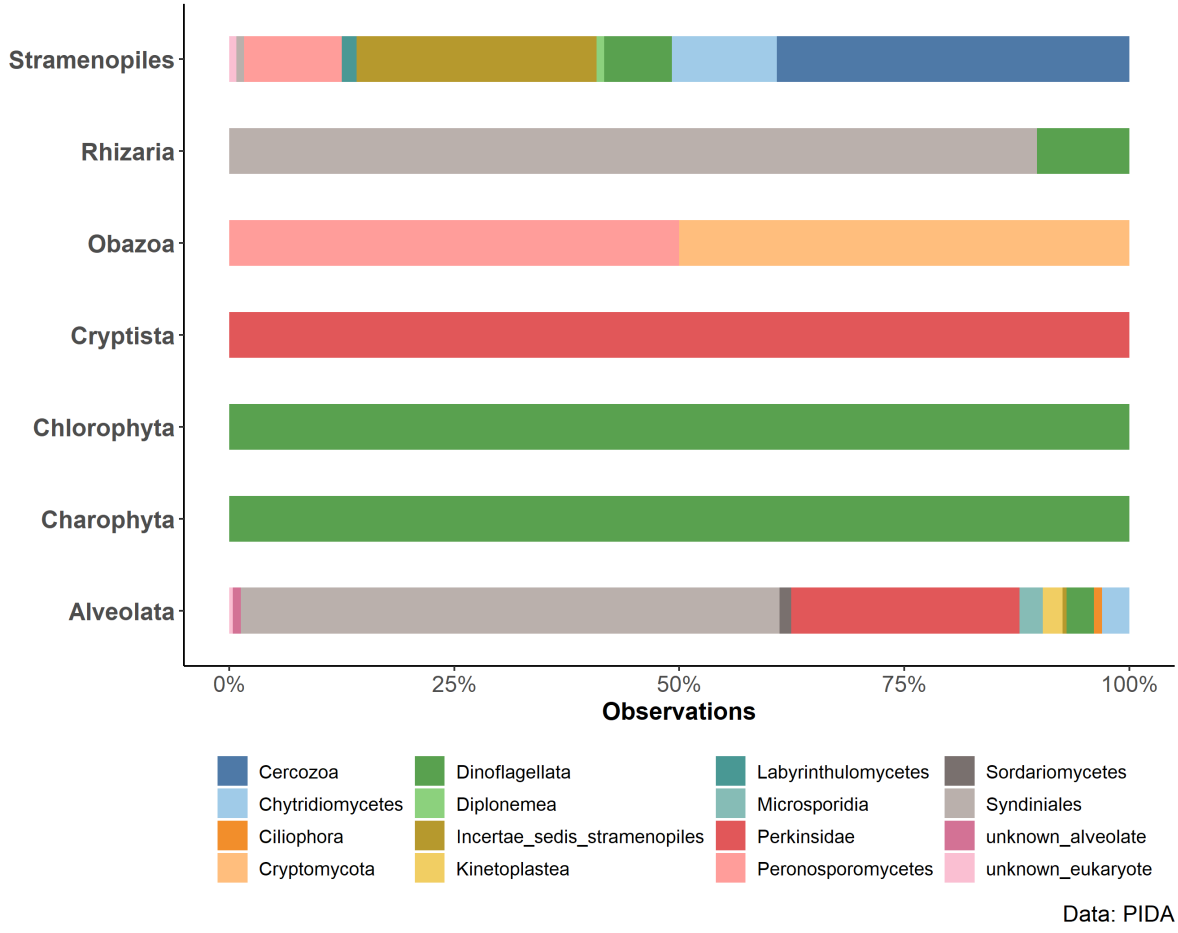

Figure 6-1 *Reported observations of parasites interactions for each eukaryotic Supergroup. Data from PIDA (Bjorbækmo et al., 2020). Own visualisation.*

6.1 Implications and Outlook

Collectively, the results of this thesis improve our understanding of the abiotic and biotic processes regulating the ecology of planktonic foraminifera in the Arctic Ocean. This work benefited from the synthesis of published and new observations from multiple expeditions that magnified the power of the analyses presented, offering a more complete understanding of the

role and contribution of the environmental variables in shaping planktonic foraminifera abundance, diversity, and distribution patterns (Chapters 2 and 5). Importantly, temperature was not found to be the main driver influencing planktonic foraminifera species composition (Chapter 5) and did not affect the vertical distribution of the species investigated (Chapters 2 and 5). This has relevant implications for paleo reconstructions since transfer functions use past assemblage compositions to reconstruct temperatures, relying on the assumption of direct causality (Juggins et al. 2015). Instead, three decades of observations revealed that planktonic foraminifera diversity is highly influenced also by other processes such as changes in sea ice coverage. This confirms high responsiveness of this planktonic group to climatic transformations but also imply that reconstructions of past temperatures based on census counts of planktonic foraminifera may not directly reflect that parameter, but rather processes indirectly related to it. Furthermore, the species-specific shifts observed in the vertical distribution through time, showcase the value of time-series and the importance of including vertical dimension in plankton monitoring programs to obtain a three-dimensional assessment of climate change effects on the marine species community (Jorda et al. 2020). Further analyses of time-series of planktonic foraminifera vertically resolved population profiles could clarify the controllers on species depth habitat, and, if coupled with flux observations, also help to understand shifts in species phenology driven by further climate change. Furthermore, such investigations could help to disentangle the (seasonally and vertically) integrated nature of the environmental signal in sediment assemblage data (Lessa et al. 2019).

Meta-analysis is a great tool to derive robust ecological generalizations and to identify new relevant research questions. It also offers the potential to formulate and test new hypotheses on planktonic foraminifera ecology not limited to the impact of climate change, but related to species biology and population dynamics. Indeed, the pattern that emerged from the meta-analysis in Chapter 2 highlighted a knowledge gap in the trophic interactions of the species *N. pachyderma* that could play a major role in regulating its vertical distribution, previously assumed to be associated to the depth of the chlorophyll maximum because the species was presumed to feed on fresh phytoplankton (Kohfeld and Fairbanks 1996). To date, few studies on planktonic foraminifera interactions exist and only recently molecular investigations began to shed light on the trophic interactions of some species (Bird et al. 2017, 2018). The single-cell metabarcoding approach used in Chapter three has the potential to provide insights on the interactome of planktonic foraminifera and to bridge the gap between single-cell biology and species population dynamics. For instance, in Chapter 3, sinking marine aggregates emerge as the main interaction platform of *N. pachyderma* but could also have some role in its vertical dispersal as has been already shown for prokaryotes (Mestre et al. 2018). More research on planktonic foraminifera interactions including also smaller (juvenile) specimens could furtherly improve our understanding of the dispersal strategy in *N. pachyderma* and other species that live in association with marine aggregates. This behaviour has further implications in the usage of the chemical signal in the shells of *N. pachyderma* to reconstruct water column conditions. Indeed, if the species lives and calcifies in association with marine aggregates, the chemical signal in its shell may yield information on the chemistry of past aggregate microenvironment rather than of the water column (Fehrenbacher et al. 2018). Beyond the food source, the metabarcoding analysis sheds light on other relevant biotic interactions of *N. pachyderma*, indicating that this species could potentially be infected by Syndiniales, the most common

eukaryotic parasites observed in Rhizaria (Fig. 6-1). This observation needs to be validated by further research, but if confirmed, it might imply that this parasite group can potentially infect other planktonic foraminifera species, influencing their abundance and diversity. Ecological knowledge of this kind could be implemented in eco-physiological models (Lombard et al. 2011; Kretschmer et al. 2018) improving predictions of planktonic foraminifera response to future climate change.

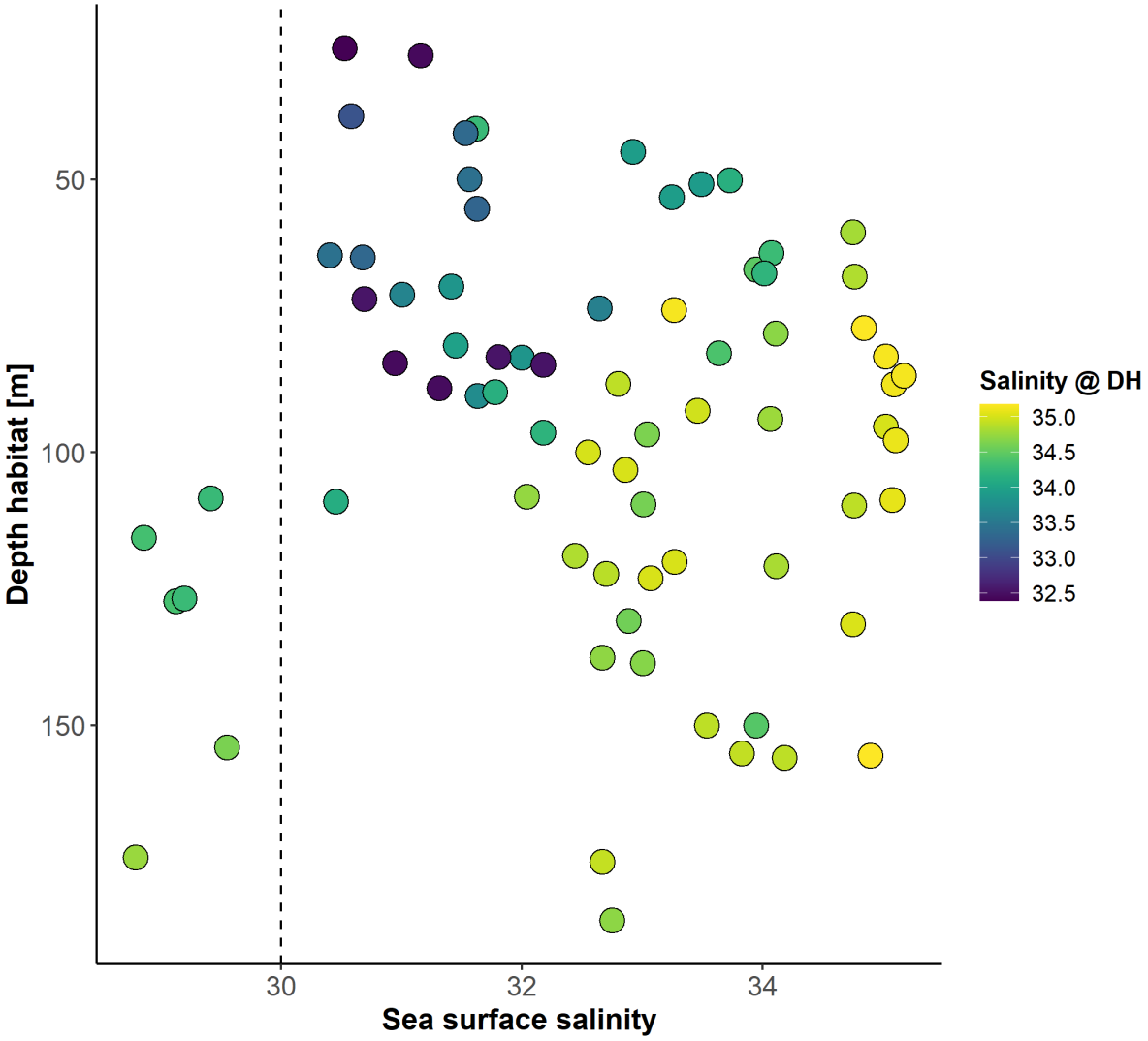

Figure 6-2 *Relationship between depth habitat (DH) of N. pachyderma and sea surface salinity. The colour of the data points represents the salinity at the depth habitat. Data from Greco et al. (2019).*

The results reported in Chapter 4, also present significant insights of planktonic foraminifera physiology. Specimens of *N. incompta* showed no rhizopodial network when exposed to a salinity of 25 PSU. Thus, 25 PSU was interpreted as the lower salinity limit of the species. These observations, even if preliminary, are in agreement with previous evidence from experiments on other Neogloboquadrinids (Bijma et al., 1990) and, the observed salinity limit may apply to all species in the clade, including the polar *Neogloboquadrina pachyderma*. Indeed, in the data presented in Chapter 2, it is possible to observe that *N. pachyderma* displays

a deeper and more saline habitat when surface salinity levels drop below 30 (Fig. 6-2). Therefore, the ability of a planktonic foraminifera species to survive reduced salinity levels under laboratory conditions might not necessarily imply that they will inhabit a similarly low-saline meltwater lens in the natural environment, specimens could instead record more suitable conditions below the surface. If this behaviour is confirmed by further investigations and experiments, it will imply that reconstructions based on the shells of *N. incompta* and *N. pachyderma* underestimate past salinity anomalies, possibly resulting in an overestimation of the strength of the Atlantic meridional overturning circulation during past meltwater events.

Next to proxy calibration, the culturing protocol presented in Chapter 4 could also lay the basis of more sophisticated experiments involving transcriptomic technique and aimed to gain a deeper understanding of the physiology of planktonic foraminifera. This could provide new biological and ecological knowledge that would not only improve the interpretation of the fossil record but also constrain the role of these marine protists in the ocean microbiome.

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Appendix

Versicherung an Eides Statt / Affirmation in lieu of an oath

Versicherung an Eides Statt / *Affirmation in lieu of an oath*

gem. § 5 Abs. 5 der Promotionsordnung vom 18.06.2018 /
according to § 5 (5) of the Doctoral Degree Rules and Regulations of 18 June, 2018

Ich / I, _____

(Vorname / First *Name*, Name / *Name*, Anschrift / *Address*, ggf. Matr.-Nr. / *student ID no., if applicable)*

versichere an Eides Statt durch meine Unterschrift, dass ich die vorliegende Dissertation selbständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe und die zu Prüfungszwecken beigelegte elektronische Version (PDF) der Dissertation mit der abgegebenen gedruckten Version identisch ist. / *With my signature I affirm in lieu of an oath that I prepared the submitted dissertation independently and without illicit assistance from third parties, that I appropriately referenced any text or content from other sources, that I used only literature and resources listed in the dissertation, and that the electronic (PDF) and printed versions of the dissertation are identical.*

Ich versichere an Eides Statt, dass ich die vorgenannten Angaben nach bestem Wissen und Gewissen gemacht habe und dass die Angaben der Wahrheit entsprechen und ich nichts verschwiegen habe. / *I affirm in lieu of an oath that the information provided herein to the best of my knowledge is true and complete.*

Die Strafbarkeit einer falschen eidesstattlichen Versicherung ist mir bekannt, namentlich die Strafandrohung gemäß § 156 StGB bis zu drei Jahren Freiheitsstrafe oder Geldstrafe bei vorsätzlicher Begehung der Tat bzw. gemäß § 161 Abs. 1 StGB bis zu einem Jahr Freiheitsstrafe oder Geldstrafe bei fahrlässiger Begehung. / *I am aware that a false affidavit is a criminal offence which is punishable by law in accordance with § 156 of the German Criminal Code (StGB) with up to three years imprisonment or a fine in case of intention, or in accordance with § 161 (1) of the German Criminal Code with up to one year imprisonment or a fine in case of negligence.*

_____ _____

Ort / *Place*, Datum / *Date* Unterschrift / *Signature*