Arctic Ocean Biodiversity and DNA Barcoding – A Climate Change Perspective

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Abstract

Global changes are initiating a cascade of complex processes, which result, among other things, in global climate warming. Effects of global climate change are most pronounced in the Arctic, where the associate processes are progressing at a more rapid pace than in the rest of the world. Intensified transport of warmer water masses into the Arctic is causing shifts in species distributions and efforts to understand and track these change are currently intensified. However, Arctic marine fauna is the result of different recurring colonization events by Atlantic and Pacific Ocean populations, producing a very confounding evolutionary signal and making species identification by traditional morphological taxonomic analysis extremely challenging. In addition, many marine species are too small or too similar to identify reliably, even with profound taxonomic expertise. Nevertheless, the majority of current research focusing on artic marine communities still relies on the analysis of samples with traditional taxonomic methods, which tends to lack the necessary taxonomic, spatial and temporal resolution needed to understand the drastic ecosystem shifts underway. However, molecular methods are providing new opportunities to the field and their continuous development can accelerate and facilitate ecological research in the Arctic. Here, we discuss molecular methods currently available to study marine Arctic biodiversity, encouraging the DNA barcoding for improved descriptions, inventory and providing examples of DNA barcoding utilization in Arctic diversity research and investigations into ecosystem drivers.

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Biodiversity of the Arctic Ocean

Today's Artic marine biodiversity is highly impacted by newly formed current systems that bring warmer waters and their boreal inhabitants from the Atlantic and Pacific Oceans through the Fram and Bering Straits, respectively (Piepenburg et al. 2011). In the past, the resident diversity was primarily shaped by recurrent invasions, habitat fragmentation, and processes associated with glacial and interglacial periods, like bathymetric changes (e.g., Hewitt 2000, 2004; Ronowicz et al. 2015; Weydmann et al. 2017).

The Quaternary glaciation and deglaciation events were associated with global sea level fluctuations often exceeding 100 m, which lead to recurrent eradication of shelf biota and favored the survival of bathyal species and those thriving in isolated refugia, with subsequent recolonizations from the Atlantic and Pacific Oceans (Golikov and Scarlato 1989). In addition, the presence of ice sheets covering the open ocean further limited the dispersal of planktonic organisms (including larval stages of the benthic fauna) in the transarctic perspective (Hardy et al. 2011). The relatively recent, dynamic glacial history of the area have created complex evolutionary patterns, often blurring species delineations and hampering traditional morphological taxonomic methods, whereby, e.g., cryptic taxa can be easily overlooked (Hardy et al. 2011). Evidence for the underlying processes can also be gleaned from paleoceanographic data (Gladenkov and Gladenkov 2004). The geology of the Bering Strait, for example, revealed that, since its first opening at the Miocene-Pliocene boundary, this gateway between the Pacific and Arctic Oceans has been opened and closed repeatedly, providing opportunities for multiple invasions (Gladenkov and Gladenkov 2004; Hardy et al. 2011) from both sides (during the first 0.9–1.0 Ma after opening the prevailing currents flowed southward; Haug and Tiedemann 1998).

The five oceanic basins of the Arctic Ocean (Canada, Makarov, Amundsen, Nansen and Eurasian Basin) are separated by mid-oceanic ridges that limit dispersal of the

deep-sea species within the Arctic (Bluhm et al. 2011a), but also their inflow of waters from the adjoining oceanic regions (Carmack and Wassmann 2006). These dispersal barriers, together with the glacial history of the area, have resulted in isolated assemblages of distinctive marine biota, while maintaining the close relatedness to species found in neighboring oceanic regions (Bucklin et al. 2010).

Once thought to be relatively poor, the biodiversity of the Arctic Ocean is now considered to be at an intermediate level (Hardy et al. 2011), with the number of extant species estimated to about 8000 (Bluhm et al. 2011b). However, this number is dynamically increasing, with new taxa described ever more frequently (see e.g. Matsuyama et al. 2017) and estimates of several thousand yet undescribed species (Bluhm et al. 2011b; Appeltans et al. 2012). The ecologically harsh, but diverse setting of the Arctic Ocean underlies the local biodiversity (see Table 1). Sea ice, for example, aside from aforementioned dispersal limitation, constitutes a unique ecosystem where sympagic (ice-associated) organisms thrive (Bluhm et al. 2009a). This group includes many endemic taxa and those of panarctic distribution (Bluhm et al. 2009a), but remains largely unstudied with many taxa still awaiting descriptions (see Piraino et al. 2008).

The diversity level of each Artic marine ecological group is also tightly coupled with the highly specific ecosystem functioning of the Arctic. Seasonality, with light and dark periods lasting for large parts of the year (polar day and night, respectively), and the variable sea ice extent, govern the phenology of the whole ecosystem. Algal blooms, as main energy source for secondary producers and thus higher trophic levels, follow a two-part succession. The first ice algae bloom appears towards the end of winter, which is succeeded by a second bloom of planktonic algae, once the sea-ice melts (Leu et al. 2015). Both phases are significantly restricted in duration, due to light availability and water stratification (Sakshaug 2004). When the sea ice melts, surface waters warm up and, together with the presence of the fresh melt water, limit water mixing and consequently the amount of nutrients available to autotrophs, thus terminating the bloom (Sakshaug 2004). In spite of limited primary pro-

Table 1 Species diversity of marine Arctic biota of different ecological groups

Ecological group	Number of species
Unicellular eukaryotes	2106 (1027 sympagic; 1875 planktonic)
Sea ice fauna	At least 50
Zooplankton	354
Seaweeds	c. 160
Zoobenthos	c. 4600
Fish	243
Seabirds	64
Marine mammals	16

Modified after Bluhm et al. (2011b)

duction, the trophic web of the marine Arctic is relatively rich and diverse. It can probably be explained by lower metabolic rates of organisms from higher trophic levels, resulting from permanently low temperatures in the Arctic Ocean (Bluhm et al. 2011b).

Most of the primary production is spatially restricted to shelves, and thus the most diverse community of consumers can be found there (Piepenburg et al. 2011; Wei et al. 2010). Availability of concentrated organic matter attracts primary consumers (zooplankton), which later become easy prey for secondary consumers (e.g. macrozooplankton, fish, sea birds) at shallow depths. Ungrazed organic matter, metabolic products and remains of the organisms sink to the bottom, where they fuel the complex benthic community. This concentration of biomass in the shelf regions draws the attention of top predators, like sea birds and marine mammals, for whom the Arctic shelves constitute the main forage areas (Wei et al. 2010).

The tight coupling between the functioning of the diverse marine Arctic ecosystems and environmental drivers renders them particularly susceptible to changes. The most detrimental anthropogenic impacts affecting the state of the Arctic Ocean usually include enterprises like shipping (including tourism), oil and gas exploration and fisheries related damages (ACIA 2004). However, the factor with the most obvious impact on the future of the marine Arctic is clearly climate change (IPCC 2014). An increase in sea surface temperatures reduces the geographic extent and thickness of the sea-ice cover directly, inducing a habitat loss for sympagic organisms, but also initiating regional shifts in species distributions or declines in primary production on a larger scale (Bluhm et al. 2011a; IPCC 2014).

In spite of insufficient amounts of decadal biodiversity studies encompassing the broad range of Arctic ecosystems, rapid (year-to-year) changes in different aspects of species biology have already been detected. On the autecological scale, these changes included e.g., biomass, diet or fitness (see review by Wassmann et al. 2011). On a broader view, the climate change driven modifications in Arctic communities are leading to a northward extension of the distribution ranges of boreal species (see examples in Hegseth and Sundfjord (2008) for phytoplankton; Weydmann et al. (2014) for zooplankton; Bluhm et al. (2009b) for zoobenthos; Mueter and Litzow (2008) for fish; Piatt and Kitaysky (2002) and CAFF (2010) for sea birds; Moore (2008) for marine mammals), replacing the long-lived and slow growing Arctic organisms with their smaller and short-lived boreal counterparts (e.g., Berge et al. 2005; Węsławski et al. 2010), while population of more susceptible, and usually less plastic species decline (e.g., Gilchrist and Mallory 2005).

DNA Barcoding

Biodiversity studies represent the first step to provide a baseline for detecting the effect of climate change on marine biota. A precise identification of all ecosystem components will allow to analyze interspecific interactions and will enable to determine factors, which influence its functioning. Until recently, most of the biodiversity research has been based on morphological analyses, which have many limitations, what might result in underestimation of diversity. In the marine environment, cryptic speciation is common, resulting in genetically differentiated lineages that are undistinguishable morphologically (Bickford et al. 2006). Nonetheless, their recognition is important, as they can have different functions in ecosystems (Fišer et al. 2015). Similarly, the identification of very small organisms or early life stages may be problematic, resulting in identification restricted to the phylum or family level.

A promising auxiliary approach is the use of molecular methods for identification and discrimination of species, known as DNA barcoding, which enables not only the assignment of unknown species, but it also enhances the discovery of new species (Bucklin et al. 2011), by matching their genetic fingerprint to a known barcode reference. Its development in recent years enabled more accurate species identification (Hebert et al. 2003), and the effectiveness of this approach has been established for several large groups of organisms (Bucklin et al. 2011), due to contribution of big, international projects, like Barcode of Life (www.barcode-oflife.org). Here, species identification is achieved by the analysis of a short DNA sequence from a specific gene region, called "the barcode", by comparing it with the library of reference barcode sequences derived from species of known identity (Hajibabaei et al. 2007). The method is based on the assumption that genetic differences between sequences within a species (intraspecific variability) are smaller than genetic differences among species (interspecific variability), reflected in the so-called "barcoding gap" (a min. % difference between intra- and interspecific variability), can be used to match the specimen's barcode in the database, if an appropriate reference sequence is available. The presence, extent, and "position" of the barcoding gap differs between species, and hence there is a need to use different markers for different groups of organisms. One of the most commonly used markers in animals is a 648-base fragment at the 5′ end of mitochondrial gene cytochrome *c* oxidase I (COI), as it has no introns (in some groups of animals), limited recombination and many copies per cell (Hajibabaei et al. 2007). Other popular markers include the genomic ITS (internal transcribed spacer I and II), 18S and the mitochondrial 16S rDNA. The number of sequences in databases like GenBank or BOLD are constantly increasing at a very fast rate. Hajibabaei et al. (2007) summarized the number of available

sequences in public databases, and in only few years these numbers have increased several times. Information on popular markers used for DNA barcoding and the corresponding number of available sequences per organism group are presented in Table 2.

Like all identification methods, DNA barcoding has its flaws, as it requires a reference sequence in the database based on accurately identified organisms. Even though the development of Gen Bank is very dynamic – new sequences are submitted every day – sequences from many organisms are lacking whilst other sequences may be present under a wrongly identified species name. Nevertheless, molecular methods may have advantages over morphological methods in species identification as there is a lack of unique diagnostic morphological or morphometric characteristics separating species, but it can also be performed by a person without specialized taxonomic knowledge. An integrative approach using both molecular and morphological analyses, has been shown to strengthen species identification in previous polar taxonomic studies and provided the most reliable taxonomic resolution (Heimeier et al. 2010) as compared to using either method alone.

Indeed, identification of organisms based on nucleotide sequences it is not always 100% accurate, which has led to the use of the term Operational Taxonomic Unit (OTU) or – in case of barcoding – Molecular Operational Taxonomic Unit (MOTU), instead of "species". Studies have been carried out where the function of particular organisms in the ecosystem have been attributed to MOTUs (Ryberg 2015).

In the following sections we will provide examples to illustrate the use of DNA barcoding in Arctic diversity research and how can it be useful for detecting and monitoring of different processes in several important groups of marine organisms.

Plankton

Plankton is a very diverse group, containing very small organisms like viruses, heterotrophic single-cell organisms (bacterioplankton), autotrophic organisms (phytoplankton) and bigger animals (zooplankton). The diverse planktonic communities encompass both the tiniest autotrophs, like unicellular algae *Synechoccocus* and *Prochloroccocus,* which are responsible for the production of approximately 60% of the atmospheric oxygen, as well as the siphonophores, which can grow to about 40 m in length (Robison 1995). Yet another important component of the plankton are pelagic copepod crustaceans, which in many regions of the World's Ocean are the key species of the pelagic food webs, constituting up to 70% of the whole plankton biomass (Søreide et al. 2008). Their relatively short life cycles, high reproductive outputs, lack of direct antropogenic pressure and distributions depen-

Table 2 Common molecular markers. Numbers of available sequences in GenBank on 01.02.2017

Marker	Region	Numbers of sequences			
		Animals	Plants	Protists	Fungi
COI	Mitochondrial	2,219,762	30,511	1162	2043
18S	Genomic	161,263	25,130	9264	583,384
16S	Mitochondrial	345,915	4072	5221	382,418
ITS1	Genomic	47,842	82,880	33,235	481,840
ITS2	Genomic	61,956	88,157	14,535	236,705
CYTB	Mitochondrial	413,039	619		15,090
rbcL	Plastid	–	45,737	31,463	–

dent on the local hydrography make the plankton ideal for monitoring climate related changes in biodiversity (Hays et al. 2005). However, uncertainty in the taxonomic identification impedes further reasoning on climate-driven alterations of pelagic ecosystems.

Arctic zooplankton is characterized by a high seasonality and a strong spatial diversification resulting from distinct biogeographic origins of species (Błachowiak-Samołyk et al. 2008; Weydmann et al. 2014). A good example of such structuring of the plankton, comes from the analysis of the *Calanus* species complex. Three species of *Calanus* copepods coexist in the European Arctic: *C. finmarchicus*, *C. glacialis* and *C. hyperboreus*. In spite of similarities in their morphology and life cycles, there are some striking differences such as the type of lipids that characterize these congenerics, what should be taken into account, as they play a role in the lipid-based energy flux in the Arctic (Falk-Petersen et al. 2008). So far, *C. finmarchicus* was considered a boreal species, *C. glacialis* a typical Arctic shelf species, and *C. hyperboreus* the Arctic open-water species (Falk-Petersen et al. 2008). Their distribution ranges were clearly established, and in areas where they coexisted, species identification just followed the size criterion (Unstad and Tande 1991). However, the accuracy of this method, has been questioned, because of the potential interspecific hybridization and growth plasticity (Gabrielsen et al. 2012; Nielsen et al. 2014), which already has been documented by Parent et al. (2012) in the Arctic and Northwest Atlantic.

Hence, the distribution records of these three key planktonic species may have to be revised whilst knowledge on exact distribution ranges is crucial for the understanding of ecosystem functioning. In the Arctic, little auks (*Alle alle*), an ecologically important sea bird species, mainly feed on *Calanus glacialis*. With the observable increase of Atlantic water inflow to the Arctic (Polyakov et al. 2011), the distribution of this Arctic copepod is predicted to decline, while a northward range expansion is expected for its boreal sister-species *C. finmarchicus*. This comparatively much smaller Atlantic counterpart, *C. finmarchicus,* is an undesirable food source for little auks since it is not as energy rich as *C. glacialis,* and thus capture of a sufficient amount of *C. finmarchicus* comes with more energy expenses

(Wojczulanis-Jakubas et al. 2013). In order to validate the hypothesis of distribution shifts between those two species, Lindeque et al. (2004) employed both morphological (based on the prosome length) and molecular (barcoding of the 16S rDNA gene) methods for species identification. Results obtained by molecular techniques proved that *Calanus* species co-occur and have wider distribution than it was established based on morphological analysis.

Another example illustrating the efficiency of molecular methods for plankton species identification is a study on pandeid hydromedusae. Four morphologically similar genera are currently co-existing in the Arctic: *Catablema, Halitholus, Leuckartiara* and *Neoturris*. The taxonomic features used for species delineation are often inconspicuous and in some cases assumed to be growth-dependent, and thus variable within the species (see comments in Schuchert 2007). Besides the need to thoroughly re-examine the life cycle of some of these species, molecular methods can be a solution for the identification problems. In the case of Hydrozoa, the use of 16S rDNA as barcode marker has certain advantages over COI (Lindsay et al. 2015), and therefore initiatives aiming at supplementing sequence data, using this particular gene should be encouraged (see project HYPNO, Dr. Aino Hosia, https://artsdatabanken.no/Pages/168312).

Microorganisms

Microorganisms, are particularly important as primary producers for the functioning of marine ecosystems, but they also play an important role in all biogeochemical processes (Sogin et al. 2006). Nonetheless, knowledge is limited due to the difficulties associated with the investigation of small organisms like pico- (0.2–2 μm), and nanoplankton (2–20 μm). Previous research in the Arctic has shown strong seasonal variations in microorganism communities, related to changes in irradiation. However the development of molecular techniques in recent years enabled further investigation of their diversity (Marquardt et al. 2016). Genetic analyses proved that microorganisms in Arctic waters are of greater importance than previously believed. Furthermore, they are also widely spread during polar night: in fjords and

open ocean, deep and shallow water (Vader et al. 2015), which is particularly interesting as our knowledge regarding processes during the dark season was limited for a long time due to logistic difficulties with conducting research in winter. It should be taken into account that temperature increase and decrease in sea ice cover may influence the community structure of microorganisms and this effect has the potential to be translated to all upper trophic levels (Berge et al. 2015).

One of the most common methods used in the analysis of microorganisms, is barcoding based on the comparison of DNA and RNA derived OTU. While DNA is a very stable molecule and able to persist outside of the source organism for a long time, RNA is less stable and degrades rapidly. RNA analysis is therefore useful in informing about the current situation in the water column. In Svalbard waters, 4000 OTUs were differentiated based on DNA and only 2000 OTUs based on RNA (Marquardt et al. 2016). Differences can be explained by the fact that DNA is stable and may be present in the water column even after the death of an organism, but may also be caused by the high number copies of rRNA genes (Gong et al. 2013). The result of this research based on molecular data, has shown a high activity of heterotrophic groups during the polar night. It also revealed that species considered as autotrophic can become mixotrophic during winter. Based on a seasonal analysis of DNA and RNA, a succession of different microbial groups was demonstrated and their presence explained by particular environmental preferences, which may suggest that increasing temperatures will significantly influence community composition (Marquardt et al. 2016). Another study, in which microorganism communities were compared before and after the Record Sea Ice Minimum in the Arctic in 2007 (next were observed in 2012 and 2016), the genetic diversity of microorganisms appeared to be much lower (Comeau et al. 2011). This may be the result of particular adaptations to the sea-ice environment, as some are known to belong to the sympagic community. Differences in the community composition of Bacteria and Archaea, responsible for carbon and nutrient cycles, may influence productivity, but also the release of CO_2 from the Arctic Ocean (Legendre and Le Fèvre 1995). These findings underline the importance of future research focusing on the ecology and functions of microorganisms to predict consequences of forthcoming changes.

Benthos

Some areas of the Arctic Ocean, especially the continental shelves, are well-recognized for their tight bentho-pelagic coupling, inferred from the high amount of carbon fixed near the oceans' surface that sinks ungrazed to the seafloor, where it fuels benthic communities (Ambrose Jr. and Renaud 1995; Renaud et al. 2008). In the Arctic, biogenic sedimentation is far greater than at lower latitudes, thus explaining the high biomass of benthos thriving there (Petersen and Curtis 1980;

Ambrose Jr. and Renaud 1995). Even with winter-limited primary production these benthic communities are relatively stable (Dunton et al. 2005).

The Arctic benthos is composed of a relatively young community that acquired lot of its current form during Quaternary glaciations (Zenkevitch 1963). The ice-mediated inflow and -outflow of mature organisms and their offspring, the isolation in refugia, and species extinctions led to the present day state of the Arctic benthic biodiversity (Hardy et al. 2011; Ronowicz et al. 2015). Although much is known about the current state of the Arctic benthos, a higher spatial and taxonomic resolution for biodiversity data is needed for an improved inferring of its future.

High phenotypic plasticity (e.g., in body pigmentation) further impedes species identification and hence the understanding of environmentally-dependent spatial diversification of benthic communities (Hardy et al. 2011). Bottom-dwelling polychaetes of the Arctic properly portray this trend. Until recently, this speciose group was perceived as lacking geographic structure on the global scale (Fauchald 1984). However, the use of molecular methods revealed numerous phenotypically indistinctive sibling species whilst it confirmed the presumed cosmopolitism of others (Carr et al. 2011). Hence, morphology-based taxonomy coupled with COI barcoding better resolved the diversity of Arctic polychaetes, showing that almost 25% of the over 300 "species" examined, were in fact complexes of two or more divergent lineages (Carr et al. 2011). Using COI sequences, Carr et al. (2011), were also able to retrace possible historical changes in distribution ranges of polychaetes found on Canadian coast of the Arctic, suggesting the Pleistocene glaciation as the main factor responsible of the increased diversification observed in this taxon.

Similar studies were conducted on echinoderms of the Canadian Arctic (Layton et al. 2016). Out of 141 taxa examined, 118 constituted morphologically distinctive species, while the remaining 23 were taxa assigned to different genera but not representing recognized species (Layton et al. 2016). It may suggest that in this area 23 morphologically indistinctive species new to science, or new for this region, may exist. Interestingly, with the sole usage of COI sequences, these authors also discussed various aspects of the phylogeography of echinoderms. For example, they pointed out that all species, where no pronounced spatial genetic structure could be observed between specimens collected in two or three oceanic regions of Canada, possessed a planktonic larval stage, which may justify the high levels of gene flow (Layton et al. 2016).

The above examples illustrate the utility of barcoding in delineation of the species composing the benthic communities of shallow shelf areas of the Arctic. Unfortunately, similar studies, focusing on the deep ocean assemblage remain uncommon, mostly because of the obvious difficulties of sampling below certain depths (Layton et al. 2016). One of

the few examples of such studies, is Song et al. (2016), who used combined morphological and molecular approach to investigate the collections of Chinese National Arctic Research Expeditions in the Bering Sea. By means of 16S rDNA sequences, a new species, *Sertularia xuelongi*, was described and the potential biogeographic origin of this species discussed. By comparing 16 sequences of *S. xuelongi* and of other congeneric species from the northwest of France, Iceland, and the Chukchi Sea, they suggested that these species are of Pacific origin, but may in fact constitute a significant part of the deep-sea benthic fauna of the Arctic (Song et al. 2016).

As mentioned earlier, important factors in shaping nowadays Arctic diversity were glaciation processes, during which species were forced into refugia in order to survive, what caused long-term isolation and thus differentiation of the species. After glaciation ceased, some of the expanding species went in secondary contact, however, undergoing processes were much more complicated (Maggs et al. 2008). One of the interesting examples is blue mussel, *Mytilus edulis,* which was gone for a long time from Svalbard waters, however warming of the Arctic enabled its re-appearance (Berge et al. 2005). It has been proven that blue mussels can create hybrids with other species, like *Mytilus trossulus* and *Mytilus galloprovancialis* in different Arctic regions, what leads to local adaptations (Mathiesen et al. 2017). This topic has not been investigated well yet, nonetheless it requires more insight as *Mytilus* spp. are ecosystem engineers and global warming opens new paths for invasions of boreal species in the Arctic.

Nekton

The benthic and planktonic organisms discussed above constitute food sources for higher trophic levels, which in the Arctic are primarily nektonic vertebrates. Aside from marine mammals and sea birds, this group is represented by a speciose community of fish. In the Arctic, there are 243 species of fish (Bluhm et al. 2011a), comprising several key species like polar cod and capelin (Hop and Gjøsæter 2013) as well as species with unique traits including the longest living vertebrate, the Greenland shark (Nielsen et al. 2016).

The biogeography of this ecologically and economically important group remained, unfortunately, largely unknown. Only recently, Mecklenburg et al. (2011) have improved the taxonomic identification of all Arctic species, thereby improving the resolution available for the spatial structure of their diversity. COI barcoding, combined with morphological analyses, allowed them to revise the biogeographic origin of species, showing that some of the past fish records from Arctic waters were misidentified. They found that a majority Arctic fish species (59%) are cosmopolitan species with boreal distribution, while the remaining 41% are Arctic, mainly-Arctic, and boreo-Arctic species (Mecklenburg et al. 2011). Such detailed knowledge on the biodiversity is required to trace the climate-change derived alteration of, for example, species distribution. The study also shows the hidden potential of the simultaneous morphological-molecular approach to taxonomy. In this particular case, it could be used to resolve the cod mother identity, or to acquire data of unprecedented species-resolution (Carr and Marshall 2008). For some fish species like *Arctogadus glacialis, Boreogadus saida* complete genomes are available (Breines et al. 2008). There is a high interest in postglacial colonization of fishes like *Salvelinus fontinalis* (Pilgrim et al. 2012), *Coregonus nasus* (Harris and Taylor 2010) or *Coregonu lavaretus* (Østbye et al. 2006). We can also find lots of studies about genetic diversity of different species (Kai et al. 2011; Kovpak et al. 2011), as in the future it might be crucial for adaptations to a changing environment.

The overall low number of species and distinctive morphology allow a relatively easy acquisition of high-resolution data on marine mammal diversity by means of classic taxonomic methods. Furthermore, such approaches have already revealed pronounced modifications in species ecology and biology, by detecting shifts in distribution ranges, decrease in body size and size of the separate populations, as well as alterations of food migrations (Kovacs et al. 2010). All of these changes might affect marine mammal species populations. Even though molecular research does not focus on biodiversity, it may cover a wide range of other aspects, like evolution, population genetics or phylogeography.

Future Perspectives

The Arctic Ocean is warming three times faster than the global average (IPCC 2014), thus further changes in species composition and entire ecosystem functioning are inevitable. Temperature has an impact on many aspects of physiological processes and it can affect reproduction, growth, and survival. Changes in single species distributions can effect entire ecosystems through all trophic levels, as was shown for the case of a potential mismatch between phytoplankton blooms and reproduction of *Calanus glacialis* in Arctic waters (Søreide et al. 2010). Hence, using only traditional methods might not be enough to timely observe what is going on in this fragile ecosystem. Kędra et al. (2015) emphasized the lack of biodiversity research in some Arctic areas, especially in the deep-sea region, but also the lack of research predicting direction of changes in species distribution resulting from global change.

DNA barcoding has been proven a useful tool in biodiversity assessment, however, the evolution of molecular methods is very fast, including the development of new approaches such as metabarcoding. This method involves the extraction

of DNA from an entire sample, without the need of picking out single individuals, like larvae or other targeted groups of mesozooplankton. It is based on the New Generation Sequencing (NGS) technology, where millions of short sequences (reads) are produced allowing to screen entire genomes or transcriptomes in order to obtain a higher resolution of spatio-temporal patterns of species distribution (Bucklin et al. 2016). This technique is becoming increasingly available as sequencing is getting cheaper. Commonly used genetic markers for metabarcoding are 16S, 18S and 28S, while COI is not often used as it requires specific primers (Deagle et al. 2014). So far, metabarcoding has mainly been used for microorganism research, however, it might also be used for monitoring of zooplankton for which the dynamic changes may not be detected with other tools. It is now also possible to obtain DNA from environmental samples (environmental DNA, eDNA), like water or soil, without prior isolation of target organisms, as they continuously expel DNA into their surroundings from where it can be collected (Thomsen and Willerslev 2015). This approach can provide information about the presence and type of organisms which were in a particular location in the recent past, like fishes or whales (Sigsgaard et al. 2016). Metagenomics represent an even more advanced method, for which entire genomes present in environmental samples are analyzed. Yet it is mostly applied on microorganisms, since not enough reference genomes exist for metazoans (Wooley et al. 2010). Nevertheless, as mentioned at the beginning of this chapter, databases are growing at an enormous speed and new genomes are published every day, what means that analyses of metagenomes of different ecological groups will become possible in the nearest future. Metagenomics significantly exceeds beyond species identification, in biodiversity research it allows for investigation of uncultured microbial populations. It is a very powerful tool, which enables exploration of metabolic diversity, isolation and identification of enzymes and it may be an effective way to produce novel bioresources (Kodzius and Gojobori 2015).

Currently, the analysis of high-throughput sequence (NGS) data requires an in-depth knowledge in bioinformatics. Moreover, the obtained results are rather qualitative than quantitative, e.g. based on presence/absence of DNA in a sample, however, this is currently being improved. Nonetheless, until these methods are not optimized for converting number of sequences into abundances of organisms in the field, the best method remains the integrative taxonomic approach, which combines molecular with morphological data.

In conclusion, molecular data are a promising tool for detecting the influence and consequences of global warming on different communities. Standard molecular methods have been successfully applied in Arctic research and their fast development will render analysis even more feasible and cost-effective. The use of DNA barcoding should be emphasized for long-time monitoring studies. Considering the opportunity of acquiring fast results, however, caution should be taken with regard to the choice of an adequate molecular marker, a careful analysis of the data and if possible, the application of an integrative approach by supporting these results with morphological analyses.

Knowledge on the existing biodiversity is the baseline for many studies, e.g. on ecological and physiological aspects. In order to investigate the future of the Arctic ecosystems, further research should focus on combining data obtained from biodiversity assessments with modelling and experiments, in which molecular tools can be used as well.

Appendix

This article is related to the YOUMARES 8 conference session no. 8: "Polar Ecosystems in the Age of Climate Change". The original Call for Abstracts and the abstracts of the presentations within this session can be found in the appendix "Conference Sessions and Abstracts", chapter "9 Polar Ecosystems in the Age of Climate Change", of this book.

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