

CHAPTER 15

Sea ice as a habitat for micrograzers

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15.1 Introduction

Sea ice makes up a physically complex, geographically extensive, but often seasonally ephemeral biome on Earth. Despite the extremely harsh environmental conditions under which it forms and exists for much of the year, sea ice can serve as a suitable, even favourable, habitat for dense assemblages of microorganisms. Our knowledge of the existence of high abundances of microalgae (largely diatoms) in sea ice spans at least a century, but for many years it remained unknown whether these massive accumulations were composed of metabolically active or merely inactive cells brought together through physical processes associated with ice formation. Work began approximately a quarter century ago started to fill this void in our knowledge of sea ice microbiota.

Algal populations and their attendant bacterial assemblages were initially believed to exist largely in the absence of grazing mortality from herbivorous and bacterivorous organisms. Our perception of these microbial assemblages has changed, however, and it is now clear that sea ice supports highly enriched, taxonomically diverse and trophically active microbial consumers in Arctic and Antarctic environments (Laurion et al., 1995; Sime-Ngando et al., 1997a; Thomas & Dieckmann, 2002; Kaartokallio, 2004; Riedel et al., 2007).

Biological aspects of sea ice bacteria and microalgae have been detailed in Chapters 13 and 14, respectively, of this book. The present chapter focuses on the microbial consumers that characterize these unique marine habitats. Within the complex microbial communities of sea ice, the major consumers of bacterial and algal

biomass are single-celled eukaryotic microorganisms displaying heterotrophic ability, usually phagotrophy, which is the engulfment of food particles. The term 'protozoa' has traditionally been employed and is still commonly used to describe these species, but wholesale revision of the evolutionary relationships among eukaryotic taxa throughout the past decade (Adl et al., 2012), and greater understanding of the complex nutritional modes exhibited by single-celled eukaryotes have called the accuracy of this term into question. In particular, the traditional distinction between phototrophic protists (i.e. unicellular algae) and their heterotrophic counterparts (protozoa) presupposes that phototrophy and heterotrophy are mutually exclusive nutritional modes, but they are not. Technically, the word 'protozoa' adequately describes truly heterotrophic protists, but it does not take into account the fact that phagotrophy is a common behaviour among many microscopic algae, nor does it recognize that some apparently photosynthetic species of protists are in fact kleptoplastidic, i.e. heterotrophs that consume photosynthetic prey and retain the chloroplasts in a functional state. Due to the existence of these mixotrophic species, the expression 'phagotrophic protists' provides a more accurate description of the many protistan species employing heterotrophic nutrition, regardless of the presence or absence of chloroplasts. These deficiencies having been noted, the term 'protozoa' will be used synonymously with 'heterotrophic protists' (which includes mixotrophic species) in this text for the sake of brevity.

Scientific understanding of the diversity, abundances and trophic activities of sea ice microconsumers has

only emerged within the past few decades, and much remains to be learned. Although their ecology is still not as thoroughly described or investigated as that of many photosynthetic taxa, bacteria and even some metazoa from polar ecosystems, a basic understanding of the breadth of their abundances and activities now exists. Protozoan populations in sea ice reduce bacterial and algal abundances, remineralize major nutrients contained in their biomass, and constitute additional sources of nutrition for metazoa within or associated with the ice. The available information, complemented by a wealth of knowledge regarding the ecology of heterotrophic microbial eukaryotes from temperate and tropical regions, is beginning to clarify the pivotal role played by protozoa in polar marine ecosystems.

15.2 Origins and fates of sea ice protists

The processes giving rise to sea ice protozoan assemblages presumably are the same for phytoplankton, bacteria and invertebrates. Microbial populations can be either entrained into ice platelets formed in subsurface waters, trapped at the surface during the formation of frazil ice, or invade existing ice through brine channels or fissures (Garrison et al., 1986; Petrich & Eicken, 2015). These processes may selectively concentrate certain microbial taxa because of size selection or other characteristics (e.g. pennate diatoms may attach to ice, while foraminifera possess sticky pseudopodia that may result in their scavenging by ice crystals). Thus, the initial colonizing assemblages in sea ice may represent a 'biased' sampling of microorganisms in the water column (Eicken, 1992).

Microbes that are incorporated into the ice experience chemical and physical characteristics that are vastly different from the surrounding seawater environment. The structure of the ice microbial community is therefore dictated by the growth and mortality of species contained within the initial colonizing assemblage (or invading from the water column), under selective pressures that differ greatly from those in the surrounding water. These selective pressures result in a divergence in community composition from that in the water as the ice ages. For this reason, protistan assemblages in newly formed sea ice have been shown to be similar to assemblages in the water column initially (Gradinger &

Ikavalko, 1998; Garrison et al., 2003), but this similarity decreases as the assemblages adapt to conditions and food availability in the ice (Rózanska et al., 2008).

Demonstrations of different dominant taxa in sea ice and the water column underlying or adjacent to the ice have been documented using traditional and, more recently, genetic techniques (Figure 15.1). DNA fragment analysis, gene sequencing and fluorescent *in situ* hybridization (FISH) approaches have corroborated such differences in drifting and landfast ice, underlying water and overlying snow in Antarctica, the Arctic and the Baltic Sea (Gast et al., 2004, 2006; Bachy et al., 2011; Terrado et al., 2011; Majaneva et al., 2012; Comeau et al., 2013; Piwosz et al., 2013). For example, microbial eukaryotic assemblages in ice samples collected from different sites in the Antarctic were more similar to each other than to the assemblages from water samples collected at the same sites as the ice samples (Gast et al., 2004). Similarly, a microscopical study of sea ice protistan assemblages collected from different locations in the Ross Sea, Antarctica, showed a relatively high degree of similarity in major biomass components, presumably indicating the dominance of specific taxa that were more adapted to existence within the sea ice than in the water (Garrison et al., 2005). Similarities in protistan assemblages within ice from freshwater ecosystems have even been noted between Arctic and Antarctic samples (Jungblut et al., 2012). Heterotrophic protists were observed in all ice samples, often at significant abundances.

These similarities in ice communities notwithstanding, vertical gradients of environmental conditions within sea ice can create microhabitats that differ significantly in their physico-chemical characteristics (Figure 15.2). Snow cover on ice dramatically affects light penetration, an important variable controlling algal biomass within and especially at the bottom of the ice (Grossi & Sullivan, 1985; Gosselin et al., 1986). Gradients of temperature and salinity resulting from seawater infiltration and/or melting of snow and ice produce small-scale spatial heterogeneity in the ice. These factors can lead to dramatic differences in the composition and biomass of the microbial communities growing there. Brine channels, compression ridges, meltwater (slush) at the snow-ice interface, and the bottom of the ice are often microhabitats of exceptionally high microbial biomass, and at times high diversity (Figure 15.2), as are meltwater ponds on sea

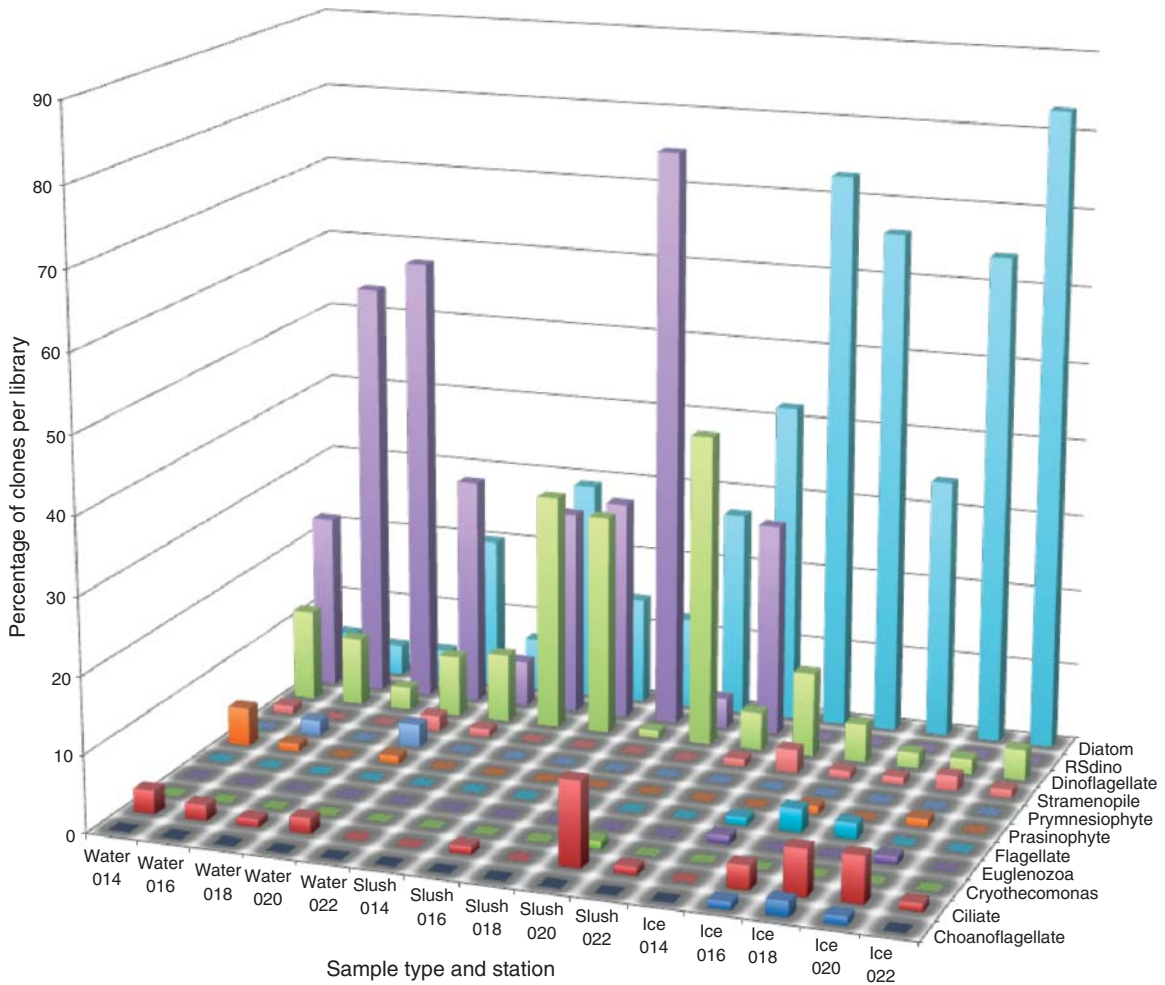


Figure 15.1 Differences in protistan community structure in sea ice, water and meltwater communities (slush) on ice floes from the Ross Sea, Antarctica, as determined from small subunit ribosomal RNA gene sequence libraries. Note the strong dominance of diatoms among the photosynthetic protists within sea ice, but also differences in the abundances of dinoflagellates (including RS dino, which is a kleptoplastidic form) and ciliates within the ice, slush and water.

ice in the Arctic (Figure 15.3) (Vincent, 2010; Mundy et al., 2011). Dramatic differences in the dominant taxa within these microhabitats can exist over small vertical scales due to snow cover, nutrient supply and the presence/absence of specific consumer populations (Figure 15.2g–k). However, we still know very little about the growth and trophic activities of protozoa within the physico-chemical gradients in sea ice (Kaar-tokallio et al., 2007). Even basic information such as which taxa are metabolically active in sea ice is only now becoming available (Majaneva et al., 2012).

Spatial (horizontal) variability in sea ice protozoan communities are influenced by variations in the algal assemblages in the ice (Gosselin et al., 1986; Laurion et al., 1995), which in turn are affected by wind-driven advection of pack ice (Garrison et al., 2003). Vertical variability in sea ice algal communities (Mundy et al., 2011) as well as interannual variability in their biomass have also been reported (Fritsen et al., 2008). Heterotrophic protists often mirror these spatial distributions, or exhibit a lagged response to increases or decreases in the abundance of phototrophic protists and

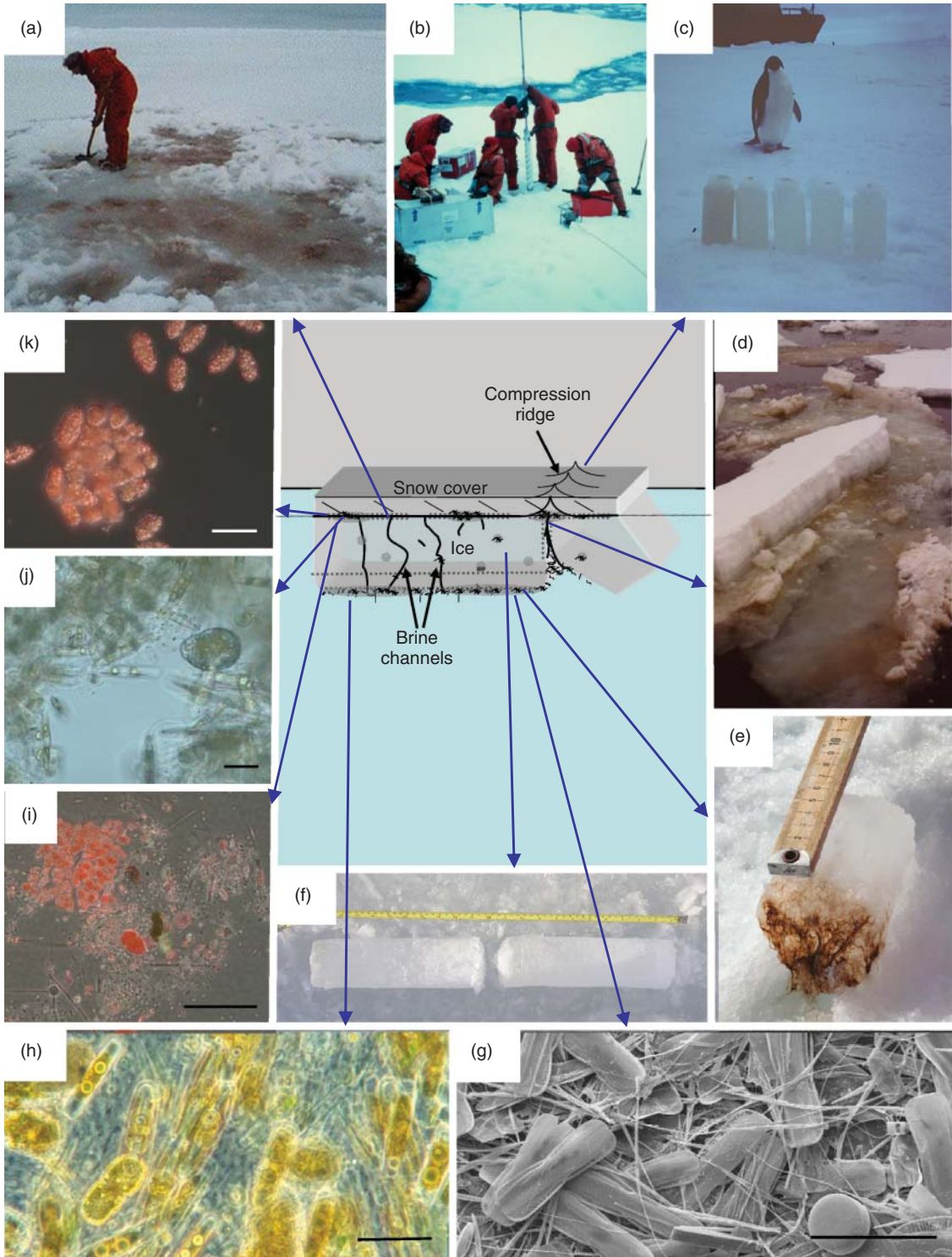


Figure 15.2 A schematic of sea ice microhabitats colonized by protozoa (centre), and the macroscopic manifestations and dominant taxa of these microhabitats (surrounding pictures). (a) Removal of several centimetres of snow reveals dense assemblages of microorganisms (coloured patches) at the tops of brine channels on Antarctic pack ice. (b) Collecting ice core samples from pack ice. (c) Samples of slush (meltwater and infiltration seawater) collected across a ridge compression on pack ice. High concentrations of microorganisms (brown colour in left-hand bottles) found in fissures in the ice decrease rapidly away from the axis of the ridge. (d) Pack ice overturned by a ship reveals densely coloured microbial communities of the snow–ice meltwater microhabitat. (e) Dense mat of diatoms and associated bacteria and protozoa from the bottom of a core of landfast ice in the Ross Sea, Antarctica. (f) A core of pack ice showing low microbial biomass (no visible colour). (g, h) Scanning electron micrograph (g) and transmitted light micrograph (h) of diatoms from the bottom of Antarctic sea ice. (i, j, k) Light micrographs showing the dominance of different taxa in the different meltwater microhabitats. These communities may contain large amounts of phototrophic and heterotrophic populations (bacteria and protozoa) and debris (i), and be dominated by diatoms (j) or phototrophic dinoflagellates (k). The red colour in (i) and (k) is due to chlorophyll fluorescence by phototrophic protists, predominantly dinoflagellates. Scale bars are 100 μm (g, h, i) or 20 μm (j, k).

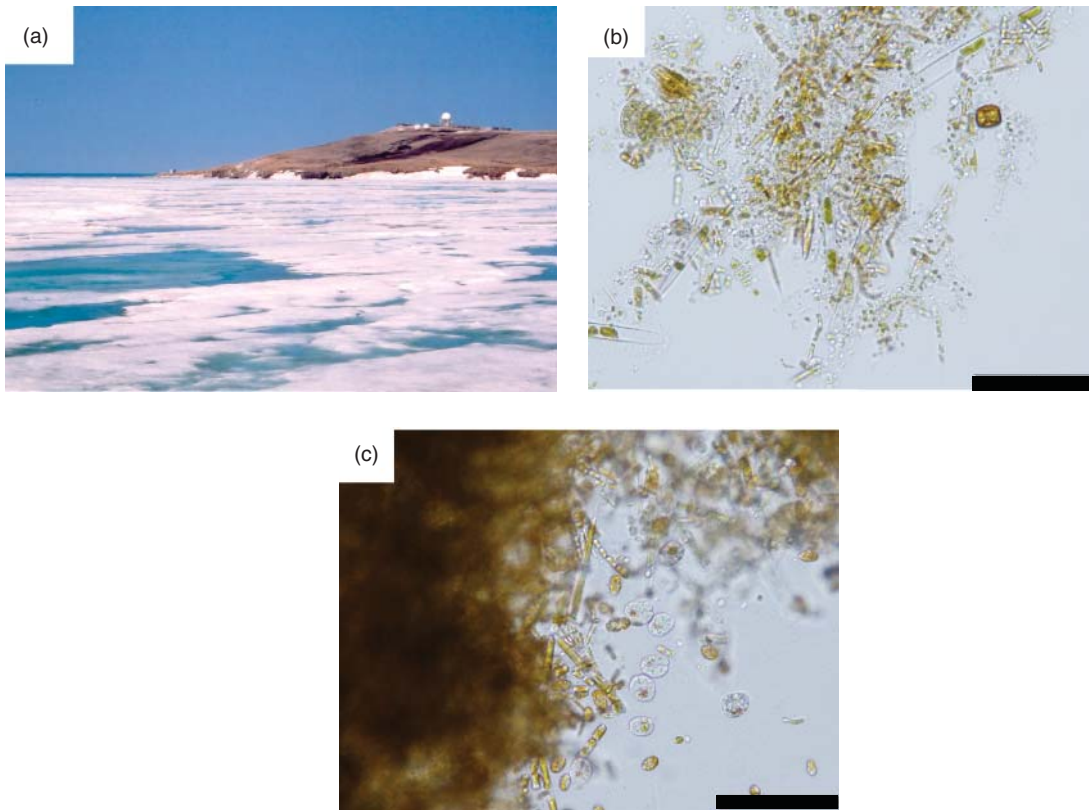


Figure 15.3 Meltwater ponds on sea ice (a) are a common feature of the Arctic during summer (Cape Parry, Northwest Territories, Canada). These ponds allow greater light penetration than snow-covered ice, enhancing primary production in the underlying ice and water. Gross similarities between sea ice microbial communities from the Ross Sea, Antarctica (Figure 2i,j) and sea ice from the Arctic Ocean in the Chukchi Sea (b, c) are exemplified by the complex assortment of photosynthetic and heterotrophic protists, bacteria and detritus. Scale bars in (b) and (c) are 100 μm .

bacteria (Sime-Ngando et al., 1997a, 1999; Riedel et al., 2007; Eddie et al., 2010). Seasonal successions within sea ice appear to be similar to classical patterns observed for water column microbes (Parsons et al., 1984; Strom, 2000), but there are few complete data sets because of the logistical issues of obtaining such time series in polar environments.

The Antarctic Marine Ecosystem Research in the Ice Edge Zone (AMERIEZ) programme remains one of the most complete data sets for examining temporal relationships among sea ice microbial communities (Garrison & Buck, 1989). This study revealed that large quantities of phototrophic protists during spring and early summer gave way to higher abundances of bacteria as organic substrate concentrations increased due to primary production. Heterotrophic protistan abundances increased during summer as prey proliferated (bacteria and algae) and temperature rose. The accumulation of phototrophic biomass observed prior to the build-up of substantial numbers of heterotrophs may be explained in part by the differential effect of temperature on phototrophic and heterotrophic processes (see Section 15.4.2) (Rose & Caron, 2007, 2013).

The physical structure of the ice is presumably a major determinant for the survival and growth of heterotrophic protists, because food acquisition for these species is dependent on movement and prey capture. The physical dimensions of brine channels in sea ice have been examined using water-soluble resin (Weissenberger et al., 1992). These studies indicated a typical diameter of 200 µm for many of the channels, creating highly convoluted, complex microhabitats of narrow passageways and high surface area. These 'ice veins' are probably microhabitats for the survival and growth of sea ice microbes (Price, 2003), and possibly are the reason why many sea ice protozoa display morphological or behavioural features that are more characteristic of protozoa from sediment than from planktonic ecosystems (Figure 15.6d,e,g,i,l and 15.8a,b,g – see later). Active microbes in ice appear to be relegated to the brine, and in liquid water that is maintained through the production of ice-retarding substances (Janech et al., 2006; Uhlig et al., 2015).

The potential influence of the physical structure of sea ice on microbial colonization has been examined experimentally (Krembs et al., 2000). These researchers estimated that 6–41% of the brine channel surface area may be covered by microorganisms. The interstitial

spaces in ice appear to be important determinants of microbial community structure and biomass through the exclusion of some consumers, and controls on the renewal of nutrients or organic substrates.

Sea ice microbiota have been hypothesized to serve as 'seed populations' for spring phytoplankton blooms in polar ecosystems (Kuosa et al., 1992; Michel et al., 1993). A similar role could be hypothesized for protozoa. Sea ice protozoa contribute to microbial grazing pressure in the water column upon release from melting sea ice during spring/summer thaws. As noted above, however, many of the protozoa within sea ice are adapted to consuming prey that are associated with surfaces, or present in microhabitats at highly elevated abundances relative to abundances in the plankton. It is therefore unclear if many protozoa released from ice could capture sufficient prey to support their growth.

Aggregations of microbes in sea ice may also contribute to the vertical flux of carbon and other elements in coastal ecosystems. Sime-Ngando et al (1997a) reported that net secondary production by heterotrophic protists in sea ice was two to four times greater than that reported for sea ice bacteria, but how that carbon is used remains a question. Protozoan biomass in sea ice would be expected to be at its maximum during late summer when ice melt rate is maximal, and thus the contribution of protozoan biomass and protozoan waste materials could be a significant source of sinking particles during that period.

Sea ice protozoa probably serve as prey for metazoa either within the ice or in the surrounding water but we know virtually nothing about this process. In the water column of the south-central polynya of the Ross Sea, *Phaeocystis antarctica* dominates phytoplankton biomass during austral summer (Smith et al., 1998). This alga is a poor food source for some metazoa, but protozoa that consume the alga directly, or consume bacterial biomass supported by the breakdown of *P. antarctica* and other algal detritus, may be important intermediates in the food chain between primary production and zooplankton in this region (Caron et al., 1997). Whether protozoa in sea ice occupy a similar niche between sea ice algae and metazoa is presently unknown.

Some protozoa trapped during ice formation may encyst (flagellates and dinoflagellates) or form resting spores (diatoms) (Garrison & Buck, 1989; Buck et al., 1992; Stoecker et al., 1992, 1997; Ikävalko & Gradinger, 1997; Okolodkov, 1998; Montresor et al., 1999). Many

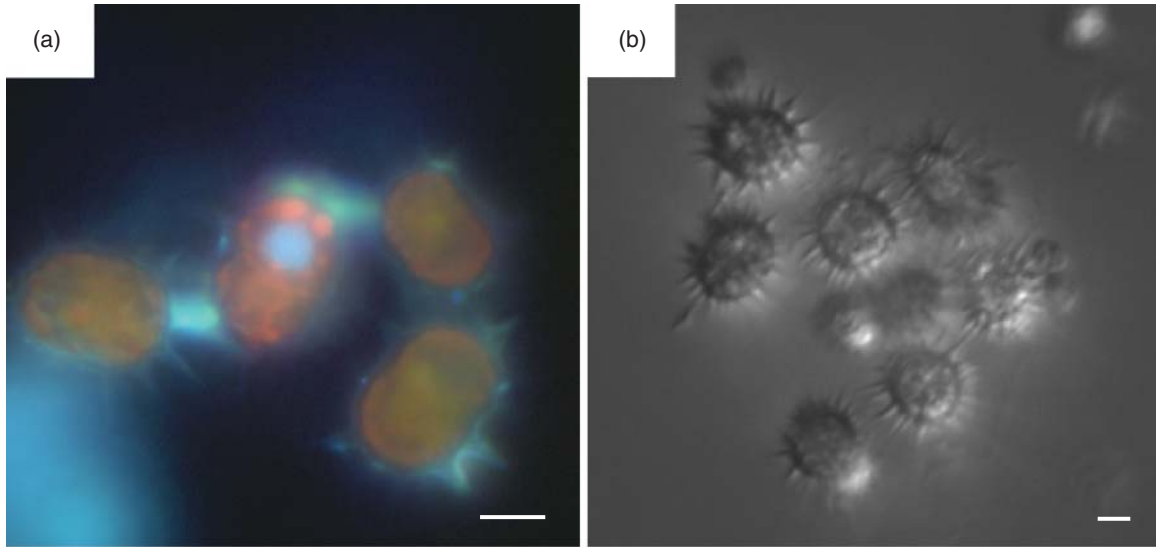


Figure 15.4 Epifluorescence micrograph (a) and transmitted light micrograph (b) of spined cysts of a phototrophic dinoflagellate (probably *Polarella glacialis*) from sea ice of the Ross Sea, Antarctica. The red colour in (a) is due to the chlorophyll fluorescence, and the whitish blue colour is from the fluorochrome DAPI. Scale bars are 10 μm .

photosynthetic protists occur as dormant forms in sea ice (Figure 15.4). The incorporation of dinoflagellate and chrysophyte cysts (i.e. hypnozygotes and statocysts, respectively) into sea ice may allow their retention near surface waters during the winter until daylight is sufficient for vegetative growth. Such a cycle has been documented for some phototrophic protistan taxa in the landfast ice of McMurdo Sound, Antarctica (Stoecker et al., 1997, 1998). Alternatively, cyst formation by some protistan taxa may enable overwintering in sea ice for species whose vegetative life stages take place in the water column during the spring–autumn period.

One additional, potential fate of sea ice protozoa that has recently emerged is infection and lysis by viruses. Viral infection of some heterotrophic protists in pack ice habitats of the Ross Sea has been demonstrated (Gowing, 2003). The high abundances and constrained mobility of microbial eukaryotes may make sea ice a particularly suitable condition for viral infection and transmission.

15.3 Diversity and abundances

The unique physical and chemical conditions of sea ice create a variety of extreme conditions for the microbial

assemblages inhabiting it; salinities can range from highly saline brine to nearly fresh water; exceptionally low temperatures can occur in the upper portions of sea ice to temperatures near those of water just below the ice; light intensities vary vertically from surface melt ponds (Figure 15.3a) to ice bottom, and seasonally from very high and constant during summer to continuous darkness. There is little doubt that the protistan species in sea ice have adapted to these harsh conditions, although it is presently unclear if these adaptations are strain-specific or reflect the presence of large numbers of unique, endemic species in these polar microhabitats.

Sea ice often has conspicuous assemblages of photosynthetic protists, but protozoa also contribute significantly to its communities. Visible discolorations can be the result of high abundances of diatoms, dinoflagellates, *Phaeocystis* (the latter primarily in Antarctic waters) and other taxa. The magnitude of these accumulations on the bottom of some regions of fast ice can be remarkable (Michel et al., 2002) (Figure 15.2e,g,h). These exceptional situations aside, Archer et al. (1996b) noted that sea ice protozoa contributed up to nearly 20% of the total integrated microbial biomass at a coastal Antarctic site and up to approximately 10% of the biomass attached to the bottom of the ice. Similar proportions of phototrophic and heterotrophic biomass in Antarctic

sea ice were observed by Garrison & Buck (1991) and Garrison et al. (2005).

Euglenoid flagellates, heterotrophic dinoflagellates, ciliates and 'nanoheterotrophs' (heterotrophic flagellates 2–20 µm in size) constituted major components of the heterotrophic protistan assemblages in the latter studies, and pack ice and landfast ice assemblages usually differed in species composition. Euglenoid flagellates alone (Figure 15.5d) contributed up to 20% of the biomass in coastal Antarctic sea ice, while heterotrophic dinoflagellates such as *Protoperidinium* (Figure 15.5a) were also abundant (Archer et al., 1996a). In Greenland Sea pack ice, heterotrophic flagellates alone constituted an average of 20% of the total microbial biomass (Gradinger et al., 1999).

These and other comparisons of protists in Arctic and Antarctic sea ice have indicated that relative abundances of heterotrophic and phototrophic species can be comparable (Garrison & Buck, 1989; Buck et al., 1998; Rózanska et al., 2008) but, as noted earlier, the relationship varies with season. Seasonal shifts in the proportion of phototrophic and heterotrophic microbial biomass are also typical of assemblages in the water column of polar ecosystems (Dennett et al., 2001; Lovejoy et al., 2002; Terrado et al., 2011). Absolute abundances of both phototrophic and heterotrophic protists in sea ice (cells per volume of melted sea ice) are often one to two orders of magnitude higher than in the water column (cells per volume of seawater) (Garrison, 1991; Davidson & Marchant, 1992; Garrison et al., 1993; Garrison & Close, 1993; Sime-Ngando et al., 1997a,b; Vaqué et al., 2004; Fonda Umani et al., 2005). These latter studies did not consider the contribution of mixotrophic algae to heterotrophic processes, but one recent study indicates that species known to be mixotrophic were a dominant component of the chloroplast-bearing protists in Arctic sea ice after several months of polar night (Bachy et al., 2011).

Estimates of the total species richness of protozoa in sea ice do not yet exist. Nevertheless, a wide diversity of protozoa has been documented from high-latitude ecosystems and specifically from sea ice (Palmisano & Garrison, 1993; Garrison et al., 2005; Scott & Marchant, 2005; Bluhm et al., 2011; Poulin et al., 2011). Over the last few decades, extensive taxonomic or ecological analyses have been published for specific protozoan groups such as ciliates (Petz et al., 1995) or choanoflagellates (Marchant, 1985; Marchant & Perrin,

1990). These studies, using traditional approaches of microscopy and culture, have concluded that polar ecosystems are characterized by high protozoan diversity (Vørs, 1993; Petz et al., 1995). The description of a number of previously undescribed species of protozoa from sea ice appears to indicate that these microhabitats support unique protozoan assemblages and endemic species (Song & Wilbert, 2000).

Genetic approaches for characterizing and studying protistan species have begun to strongly complement traditional microscopical approaches, and thereby expand our knowledge of the breadth of microbial diversity in sea ice as they have in other ecosystems (Vaulot et al., 2008; Caron et al., 2012). Genetic methods have the advantage that they can characterize species composition across a wide taxonomic range of organisms. Traditional, morphology-based taxonomies of protists are highly taxon-specific, and their application is dependent on considerable expertise. Molecular approaches are able to discriminate small protists that often exhibit few distinctive morphological features (Slapeta et al., 2006; Stoeck et al., 2008), such as the marine stramenopile (MAST) cells (Massana et al., 2004) that were subsequently found in Arctic seas as well (Lovejoy et al., 2006). It is not yet possible to easily distinguish some protozoa (heterotrophic protists) from phototrophic or mixotrophic protists using DNA sequence information alone but, as they improve, genetic analyses will become indispensable for characterizing the phylogenetically diverse protistan assemblages present in sea ice habitats.

15.3.1 Ciliates

Ciliated protozoa constitute a conspicuous component of the protozoan assemblages colonizing sea ice (Figure 15.6). Morphological studies have documented a very wide diversity of taxa from ice habitats of this monophyletic protistan lineage (Stoecker et al., 1993; Petz et al., 1995; Lynn, 2008).

A number of the ciliates associated with sea ice are also common inhabitants of the water column at the same locations (Garrison, 1991). For example, several tintinnid species (Figure 15.6b,c,f,j,k) and strombidiid ciliates are typical filter-feeding 'planktonic' forms whose presence in ice presumably indicates the existence of significant quantities of water within some ice microhabitats. *Mesodinium rubrum*, a kleptoplastidic

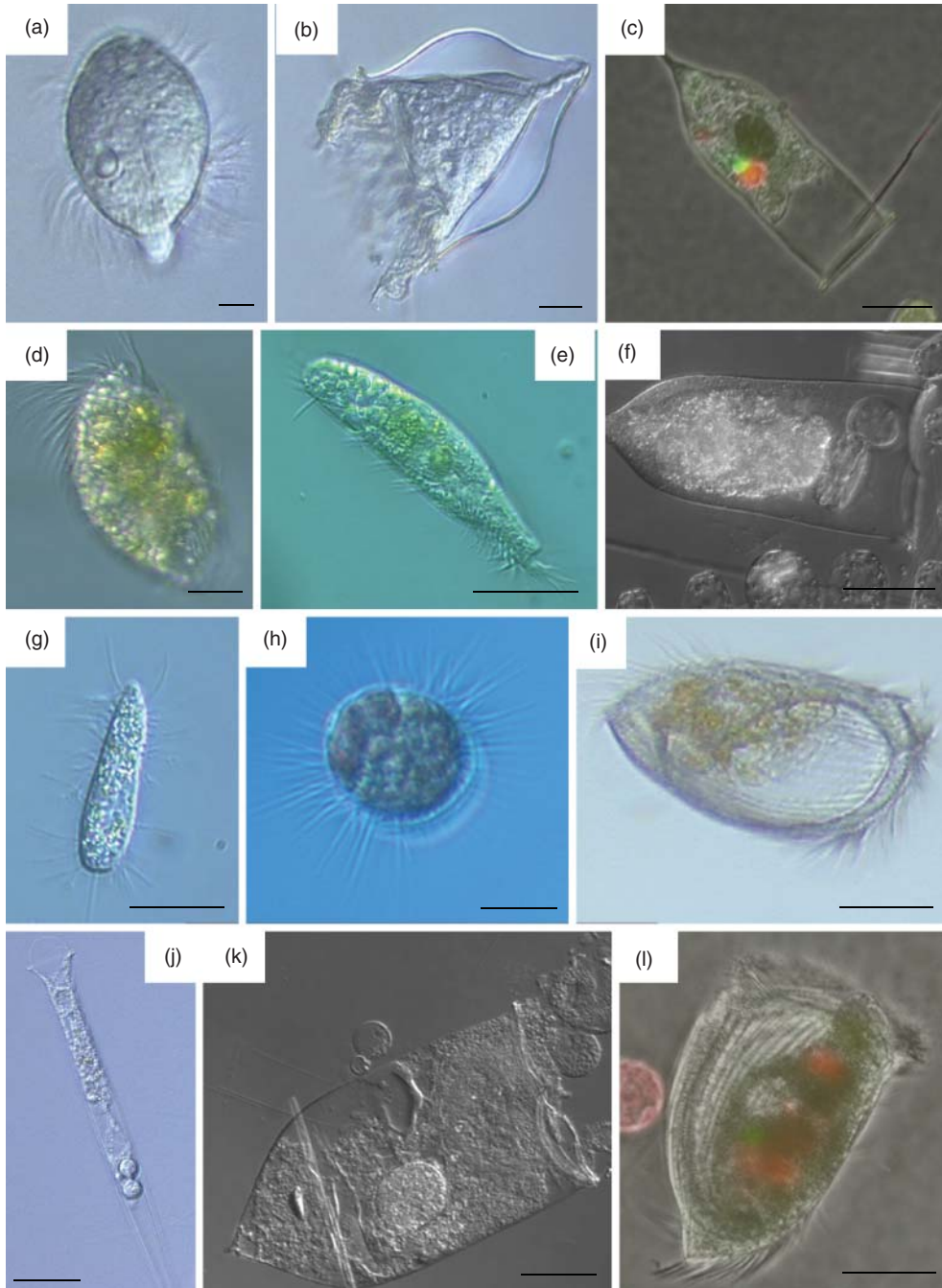


Figure 15.5 Ciliates of sea ice microhabitats in the Ross Sea, Antarctica. (a) *Didinium*, a predatory ciliate. (b, c, f, j, k) Species of tintinnids often encountered in the meltwater assemblages at the ice–snow interface of pack ice are also common in the plankton. (d) An unidentified hypotrich ciliate possessing many food vacuoles filled with algal prey. (e) A heterotrich species with elongated shape characteristic of benthic ciliates. (g) A scuticociliate that displays bacterivory in culture. (h) *Mesodinium* sp., a chloroplast-retaining ciliate. (i, l) Algal prey in food vacuoles in two large ciliates, possibly *Chlamydonella* sp., are evidence of herbivory in sea ice. The red colour in (l) is chlorophyll fluorescence from ingested algae. Scale bars are 10 (a, g), 20 (b, c, d, f, h, k) and 40 μm (e, i, j, l).

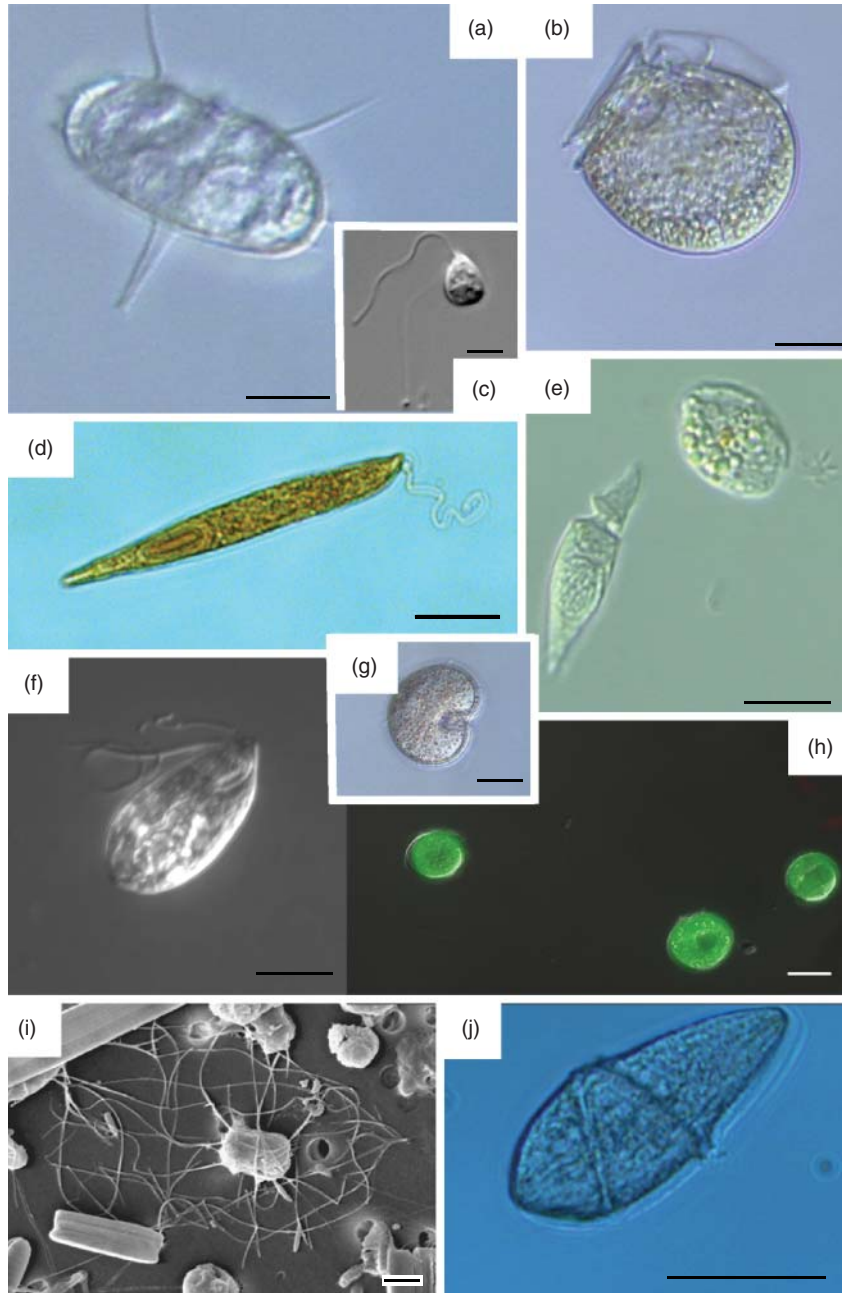


Figure 15.6 Heterotrophic flagellates are taxonomically diverse and often numerically dominant within sea ice. Heterotrophic dinoflagellates (a, b, e, g, h, j) such as *Protoperidinium* (a), *Dinophysis* (b), *Katodinium* (e, left), *Protoperidinium* (g) and *Gyrodinium* (j) may be major consumers of phytoplankton in the ice. (h) An unidentified dinoflagellate displaying a distinctive apple-green fluorescence with blue light excitation. Many heterotrophic dinoflagellates exhibit this fluorescent signal, making them very conspicuous when viewed by epifluorescence microscopy. (c) *Pseudobodo* sp., a small bacterivorous species. (d) A heterotrophic euglenoid flagellate stained with Lugol's iodine to visualize the cell and flagellum. (f) An unidentified flagellate cultured from the Ross Sea, Antarctica. (i) Scanning electron micrograph of the choanoflagellate *Diaphanoeca* sp. showing the cell and the lattice of the lorica (thin structure of bars surrounding the cell). Choanoflagellates are often extremely abundant in the plankton of high-latitude ecosystems, and are also common in sea ice microbial communities. Scale bars are 5 (c, f, i), 20 (a, b, d, e, g, h) or 50 μm (j).

(i.e. chloroplast-retaining) ciliate, can sometimes comprise up to 25% of the ciliate assemblage in sea ice, and over a third of the plankton assemblage in neighbouring waters (Sime-Ngando et al., 1997a). Brine channels, the slush habitats that develop at the ice–snow interface of summer sea ice, and melt ponds (Figure 15.3) may provide environmental conditions that adequately mimic conditions in many planktonic (i.e. non-ice) ecosystems in the ocean.

The global distributions of ciliates appear to vary with species. Several species of tintinnids and strombidiid taxa (e.g. *Strombidium*) are abundant in the marine plankton worldwide (Pierce & Turner, 1992), while other species have only Arctic or Antarctic distributions (Dolan & Pierce, 2012). Petz et al. (2007) conducted taxonomic studies of ciliates from freshwater ecosystems in the Arctic and Antarctic and concluded that at least some of the species (i.e. morphotypes) had bipolar distributions.

In contrast to truly ‘planktonic’ morphotypes, many of the ciliate species described from sea ice are forms that are commonly associated with surfaces, the benthos or eutrophic conditions that are not typical of oceanic plankton (Figure 15.6d,e,g,i,l). For example, species of *Lacrymaria* and *Condylostoma* are large ciliates often observed within dense accumulations of microbial cells and debris. These species possess highly elongated, flexible bodies that facilitate movement and prey capture in confined spaces. Spirotrich, heterotrich and hypotrich ciliate species that possess fused ciliary structures such as membranelles and cirri are mobile along surfaces where they consume surface-associated prey. Such taxa have been commonly observed in the pack ice in the Ross Sea (Figure 15.6d,e,i,l), and in landfast ice of Baffin Bay (Michel et al., 2002), but are rarely observed in the plankton. Many of these latter species show evidence of a herbivorous mode of nutrition (Figure 15.6c,d,e,g,h,i,k,l). Other species, such as *Chlamydonella*, consume fairly large algal prey such as pennate diatoms (Petz et al., 1995).

Oligohymenophorean ciliates comprise a group of predominantly bacterivorous species that are often found in sea ice (Petz et al., 1995; Song & Wilbert, 2000; Scott et al., 2001). *Pseudocohnilembus* sp. isolated from Arctic sea ice preferentially ingested bacteria-sized particles when offered particles of different size (Scott et al., 2001). These species are capable of rapid growth,

but they are best adapted for feeding at relatively high prey (bacteria) concentrations (Figure 15.6g).

Many of the ciliates noted here are rarely encountered in the water column. Prey abundances presumably are not sufficiently dense, or the pelagic habitat not conducive to the development of significant populations of these large, robust ciliates. Thus, it is unclear where the ‘seed’ populations of ciliate assemblages in the ice originate, although it is possible that low numbers rafted on particles through the water column serve as an inoculum but are too rare to appear commonly in water samples. One exception is the existence of substantial ciliate populations that are frequently observed associated with large algal colonies of the prymnesiophyte *P. antarctica* in the Ross Sea. These large (>1 mm) hollow colonies reach extraordinary abundances during austral spring and summer (Smith et al., 1996; Smith & Gordon, 1997), and are often inhabited by heterotrophic flagellates and ciliates living on or within the colonies (Figure 15.7). These large structures may provide a mechanism for inoculating sea ice with particle-associated ciliate taxa.

The morphological and behavioural adaptations of many of the ice ciliates imply that sea ice microhabitats create a ‘false benthos’ in polar marine waters that are analogous to microhabitats created by large detrital aggregates in pelagic environments, so-called ‘marine snow’ (Caron et al., 1986). Marine snow particles are colonized by ciliated protozoa equipped for movement on surfaces and in confined spaces. Similarly, the ciliates of sea ice display morphologies that presumably reflect a strong selection for forms capable of existence in physically complex, nutritionally rich microhabitats.

15.3.2 Heterotrophic and mixotrophic flagellates

Heterotrophic flagellates comprise a phylogenetically diverse group of protistan taxa with representatives from no fewer than six different protistan supergroups (Simpson & Roger, 2004; Burki et al., 2007) (Figure 15.5). Among these disparate taxa, our knowledge regarding species richness is probably most complete for the heterotrophic dinoflagellates because they often display high abundances, large size and distinctive morphologies. Species of *Proto-peridinium*, *Gymnodinium*, *Gyrodinium* and *Polykrikos* are members of sea ice communities (Stoecker et al., 1993; Archer

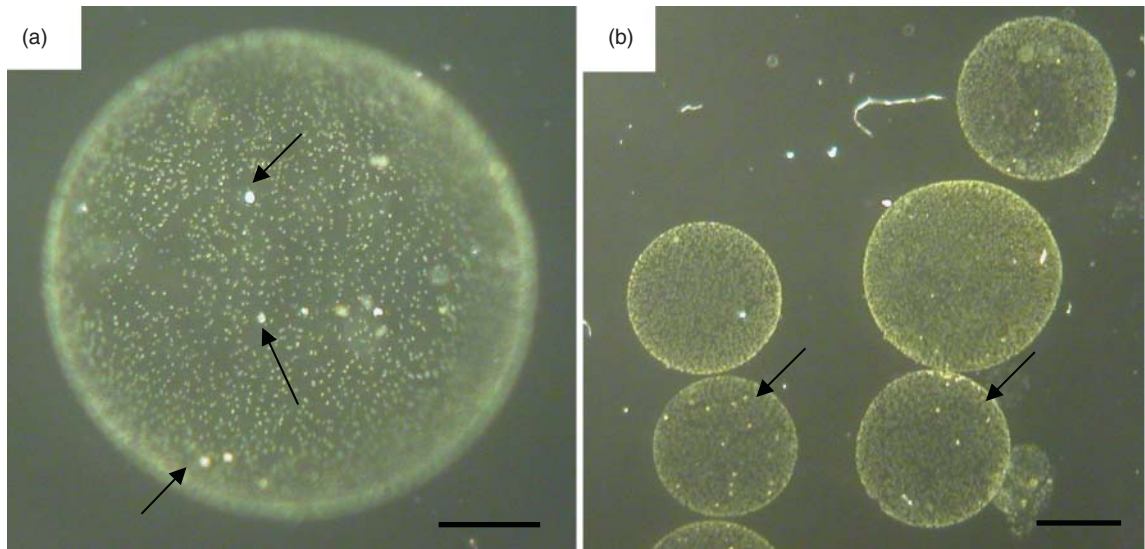


Figure 15.7 Colonization of *Phaeocystis antarctica* colonies by protozoa in the Ross Sea, Antarctica. Individual algal cells are visible in a single colony (a) as minute green dots. Arrows point to several heterotrophic protists associated with the colony. (b) Several colonies from a water sample. The interior of two of the colonies (arrows) are colonized with several heterotrophic protists each (white dots). Scale bars are 200 (a) and 500 (b) μm .

et al., 1996a; Michel et al., 2002), and are large enough to be easily visualized using a dissecting microscope.

Heterotrophic dinoflagellates (including kleptoplastidic taxa) are important herbivores in polar planktonic marine ecosystems (Sherr & Sherr, 2007, 2009; Sherr et al. 2009, 2013). Species inhabiting sea ice presumably exhibit similar trophic activities as their planktonic counterparts, although quantitative estimates of their grazing impact in ice microhabitats are virtually nonexistent. Many of these species feed using delicate pseudopodial projections to surround prey that are often larger than themselves (Jacobson & Anderson, 1986, 1996; Strom, 1991). Because sample handling and preparation for microscopy easily disrupt these feeding structures, it is probable that mixotrophic behaviour by dinoflagellates in sea ice has been severely underestimated. One dominant dinoflagellate from the Ross Sea has been shown to prey on *P. antarctica* and retain its chloroplasts for several months (Gast et al., 2007). Species of *Dinophysis* (Figure 15.5b) also capture and retain chloroplasts from their prey, the ciliate *Mesodinium rubrum*, which in turn have stolen

chloroplasts from its cryptophyte prey (Park et al., 2006).

Choanoflagellates are frequent and abundant components of the heterotrophic plankton of Arctic and Antarctic coastal waters (Buck, 1981; Marchant, 1985; Caron et al., 1997; Sherr et al., 1997; Tong et al., 1997; Lovejoy et al., 2006; Poulin et al., 2011). Blooms of these minute (usually $<10 \mu\text{m}$) bacterivorous species often appear in the upper water column following the summer maxima in bacterial abundances, and are also found in sea ice in the Arctic (Ikävalko & Gradinger, 1997) and Antarctic (Garrison et al., 2005) (Figure 15.5i). Our knowledge of their diversity is aided by the intricate and taxonomically informative skeletal structures produced by these species. Together with *Cryothecomonas*-like cercozoa, choanoflagellates dominated the heterotrophic flagellate assemblages in the ice communities of Saroma-ko lagoon in Japan (Sime-Ngando et al., 1997b).

Cercozoan flagellates of the genus *Cryothecomonas* are also ubiquitous heterotrophic flagellates of Antarctic (Garrison & Buck, 1989; Thomsen et al., 1991; Garrison

& Close, 1993; Stoecker et al., 1993; Gast et al., 2006), and Arctic sea ice (Ikävalko & Gradinger, 1997; Bachy et al., 2011; Thaler & Lovejoy, 2012, 2015). These species appear to be particularly well adapted for survival and growth at extreme temperatures.

Much less information is known regarding the many small, morphologically-nondescript groups of heterotrophic flagellates in sea ice. These species contribute significantly to bacterial mortality in aquatic ecosystems worldwide (Strom, 2000; Sherr & Sherr, 2002), but their overall abundance and diversity are not well known. This is due to the relative absence of taxonomically informative morphological features for most of these species (the choanoflagellates are an exception because they possess distinctive collar structures and loricae) and the increasing tendency to incorporate physiological information into the species concept (Boenigk, 2008). In any event, chrysomonad (especially *Paraphysomonas* spp.), bodonid (Figure 15.5c) and heterotrophic euglenoid flagellates (Figure 15.7d) are commonly observed in enrichment cultures established from samples of sea ice.

15.3.3 Amoeboid forms

Amoeboid protozoan species are a collection of phylogenetically diverse taxa that span a range from some of the least to the most striking protozoan forms observed in sea ice (Figure 15.8). Gymnamoebae or ‘naked’ amoebae

(i.e. lacking skeletal structures) and heliozoa are easily cultured from sea ice samples. However, these species are only occasionally observed directly by microscopy (Garrison & Buck, 1989; Moran et al., 2007) because they are extremely difficult to distinguish when they are entangled with debris and other microbial taxa in natural samples of sea ice communities (Figure 15.8a,b,g). Amoebae are not unique to sea ice but are present in non-ice environments around Antarctica and in marine Arctic waters (Vørs, 1993; Mayes et al., 1997; Tong et al., 1997; Lovejoy et al., 2002).

Larger, much more noticeable amoeboid protozoa in sea ice include the foraminifera, acantharia and radiolaria. The foraminifera bear calcium carbonate tests, and at least one species, *Neogloboquadrina pachyderma*, is a consistent inhabitant of sea ice where it can attain significant abundances (Figure 15.8e). Acantharia also appear in sea ice, but infrequently (Figure 15.8c,d,f,i). Species identifications are based on their distinctive skeletons of strontium sulphate. These structures dissolve rapidly in most preservatives, and can thus be easily overlooked in preserved samples. The taxonomy of these species is difficult, and as a result, their overall diversity and phylogeny are still poorly known (Decelle et al., 2012). Most of these species possess symbiotic algae that are held within the cytoplasm of the host and which supplement the nutrition of these phagotrophic protists. Acantharian host–symbiont

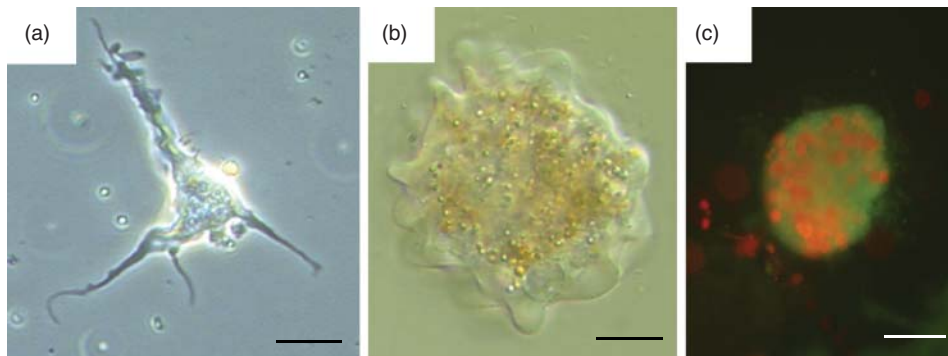


Figure 15.8 Amoeboid protozoa of the plankton and sea ice of the Ross Sea, Antarctica. Gymnamoebae (a, b, g) are inconspicuous, particle-associated protozoa. Acantharia (c, d, f, i) occur in the water column but are also observed in slush microhabitats in apparently good physiological condition. Red-fluorescing structures in a specimen observed by epifluorescence microscopy (c) indicate the presence of endocellular symbiotic algae in the same specimen examined by transmitted light microscopy (f). Endosymbionts are also present as yellowish-brown areas in the light micrographs of specimens in (d) and (i). (e) The ‘planktonic’ foraminifer *Neogloboquadrina pachyderma* is often present in sea ice. (h, j, k) Heliozoa and radiolaria. The unusual heliozoan *Sticholonche* sp. (h) and phaeodarian radiolaria (j, k) are occasionally observed in sea ice, but are more commonly found at the pack ice edge. (k) *Protocystis* sp. Scale bars are 10 (a, b, g, i), 20 (d, h, k), 50 (c, f, j) and 100 μm (e).

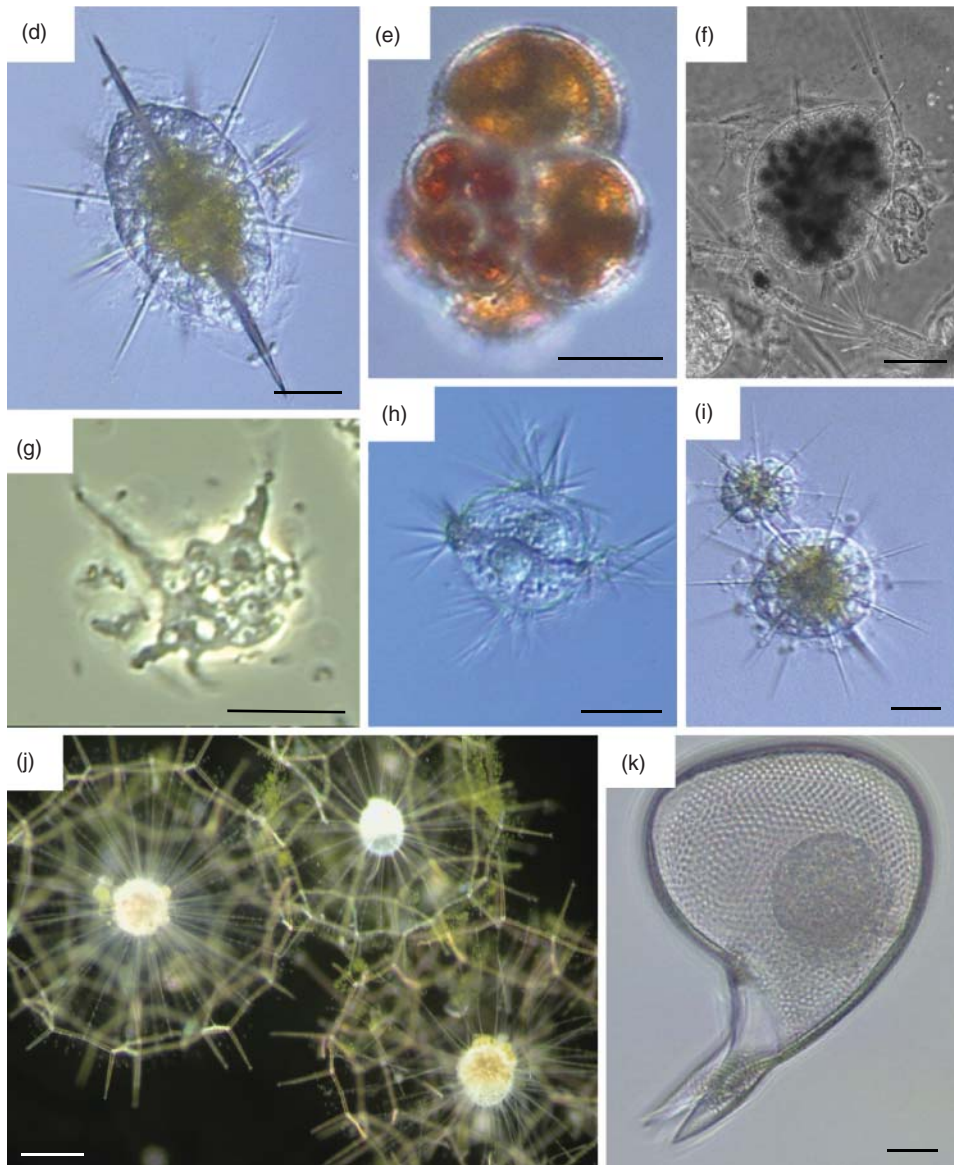


Figure 15.8 (Continued)

associations are abundant in low-latitude ecosystems where the acantharia can contribute significantly to primary productivity and to the vertical distribution of strontium (Michaels, 1991; Caron et al., 1995). It is unclear if the acantharia associated with sea ice habitats are actively growing and reproducing, but specimens in apparently good physiological condition can be recovered from melted sea ice (Caron and Gast, personal observation; Figure 15.8c,d,f,i).

Phaeodarian radiolarians (Figure 15.8j,k) are common in the ice edge regions of Antarctic coastal seas where they can reach abundances comparable to other larger sarcodine protozoa, such as foraminifera and polycystine radiolaria (Gowing, 1989; Gowing & Garrison, 1992). These populations are omnivorous in the water column, consuming prey ranging from bacteria to algae and other protozoa, based on analyses of food vacuole contents. Radiolarians have also been found at

the ice edge in Arctic waters, where juveniles increase in importance as the radiolarian community gets closer to the ice (Swanberg & Eide, 1992). As for other larger amoeboid protozoa, it is not clear whether they are metabolically active within sea ice microhabitats.

15.4 Ecology and biogeochemistry of sea ice protozoa

15.4.1 Trophic behaviours (herbivory, bacterivory and mixotrophy)

Many of the trophic activities ascribed to protozoa in sea ice have been derived from observations of food vacuole contents of recently collected specimens (e.g. Figure 15.6d,i,l). Some information has also been obtained from experimental work conducted on sea ice microbes thawed into seawater (Moorthi et al., 2009), but the results of such studies are difficult to interpret due to possible artifacts of removing microbes from the spatially structured environment of the ice. Observations and experiments performed on samples collected from the water column near sea ice have also been extrapolated to provide information on the probable trophic activity of these species in ice microhabitats. As noted earlier, several species found in the water column may also be encountered in sea ice, so there is reason to believe that many of the same processes take place in both habitats.

Direct measurements of the grazing activities of phagotrophic protists in sea ice typically require the introduction of marker cells for tracing and quantifying consumption, which in turn causes drastic changes in the physical matrix of the ice. Generally, these experiments have been conducted by carefully melting sea ice in a large volume of seawater to minimize osmotic shock when the ice melts, and then applying routine protocols for the measurement of bacterivory or herbivory (Caron, 2000). The physical constraints imposed by sea ice, the very low temperatures that can be experienced in sea ice during winter, and the considerable dilution that microbial populations undergo during thawing and experimental manipulations may alter the rates, and perhaps the very nature, of the trophic interactions between heterotrophic protists and potential prey populations.

As a consequence, there have been very few quantitative measurements of herbivory in sea ice, and virtually

no work on this topic until approximately the last decade. Indeed, some early reports hypothesized that algal growth took place largely in the absence of grazing pressure. This hypothesis is not surprising given that chlorophyll concentrations in excess of $1000 \mu\text{g L}^{-1}$ have been observed (Chapter 14), and primary production by sea ice algae can constitute a significant fraction of total system productivity (Arrigo & Thomas, 2004). However, the high abundances of heterotrophic protists in some samples of sea ice are circumstantial evidence that significant herbivory and/or bacterivory occur there. Strong correlations such as that between ciliate abundances and those of pennate diatoms in Arctic sea ice also imply a predator–prey relationship (Gradinger et al., 1992). Anecdotal information that grazing takes place is further provided by shifts in the proportion of phototrophic to heterotrophic or mixotrophic protists in sea ice during summer and autumn (Gradinger et al., 1992; Stoecker et al., 1993; Garrison et al., 2003, 2005; Bachy et al., 2011).

As noted earlier, heterotrophic dinoflagellates are important herbivores in high-latitude ecosystems (Archer et al., 1996a; Becquevort, 1997; Levinsen & Nielsen, 2002; Sherr & Sherr, 2007). These species are particularly well suited for consuming large diatoms that often dominate coastal polar seas. Dinoflagellate species also reach high abundances in sea ice habitats, and leave tangible evidence of herbivory. Buck et al. (1990) reported an abundant ($>10^5 \text{ L}^{-1}$), heterotrophic dinoflagellate that produced ‘faecal pellets’ containing large numbers of diatom frustules. These faecal pellets are commonly encountered in sea ice samples (Figure 15.9), indicating that the species producing these pellets are probably important consumers of sea ice diatoms (compare figure 15.9 with figure 1E in Nöthig & Bodungen, 1989). As noted above, at least one dinoflagellate common in sea ice also plays a role as a consumer of the prymnesiophyte *P. antarctica*, from which it steals and maintains its chloroplasts (Gast et al., 2007; Torstensson et al., 2015).

Observations of the microbial communities of land-fast ice in McMurdo Sound, Antarctica, indicate the potential importance of flagellate taxa other than dinoflagellates as herbivores in sea ice. Stoecker et al. (1993) noted high numbers of cysts of phototrophic protists in sea ice during austral winter (Figure 15.4), many of which excysted during austral spring as day length became sufficient for vegetative growth.

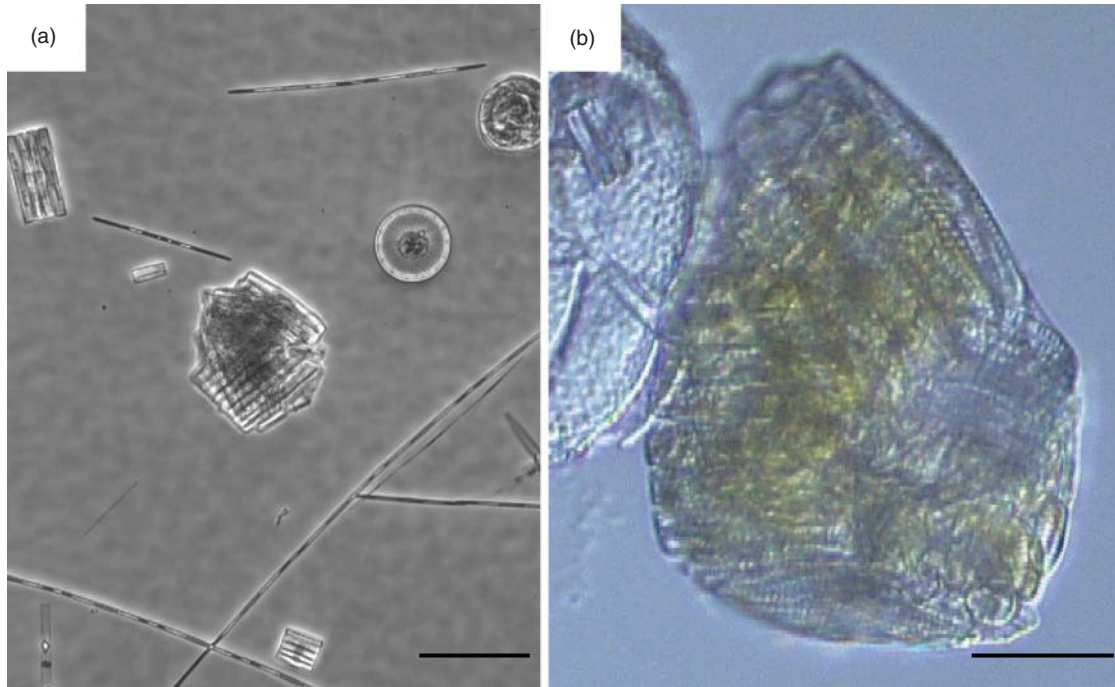


Figure 15.9 Light micrographs of the faecal pellets of heterotrophic dinoflagellates. These membrane-bound pellets are often encountered in samples of sea ice containing high abundances of diatoms. Scale bars are 100 (a) and 50 μm (b).

The appearance of large populations of heterotrophic protists, in particular the cercozoan *Cryothecomonas*, coincided with high abundances of the phototrophic protists that were presumed its prey (Stoecker et al., 1997; Stoecker et al., 1998). *Cryothecomonas* spp. also use pseudopodia to feed on large photosynthetic prey such as diatoms (Schnepf & Kühn, 2000).

Large ciliates are also potentially important herbivores within sea ice (note ingested prey in Figure 15.6), although based on the structure of the oral apparatus, many of these species are better adapted for the consumption of non-diatom prey (Dolan, 2010). For example, many of the species in the dense diatom assemblages at the bottom of landfast ice may not be suitable prey for *Strombidium* spp., which typically consume small algae and bacteria (Michel et al., 2002).

Bacterivory in sea ice is probably dominated by a variety of heterotrophic flagellates (Leakey et al., 1996; Becquevort, 1997; Vaqué et al., 2004). Grading et al. (1992) noted a ratio of bacteria to heterotrophic flagellates in Arctic sea ice of approximately 1000:1, a relationship typical of pelagic ecosystems where these assemblages exhibit strong predator:prey coupling

(Sanders et al., 1992). Choanoflagellates appear to be particularly abundant bacterivores in polar ecosystems (Becquevort, 1997; Caron et al., 1997). However, these minute bacterivores are adapted for feeding on small planktonic bacteria, while many of the bacteria associated with sea ice are attached and filamentous. It is not clear how bacterivores adapted for feeding on free-living bacteria are effective at feeding on sea ice bacteria. Thus, the primary consumers of these ice-attached bacteria are not well known.

Measurements of bacterial ingestion in sea ice are rare. Laurion et al. (1995) and Sime-Ngando et al. (1999) examined bacterivory in Arctic ice and sea ice from a Japanese lagoon, respectively. These studies employed fluorescently labelled bacteria to examine bacterial grazing by small protozoa. Both studies noted that bacterivory was an important trophic activity among small ice protozoa, but further work is needed to better define and quantify this trophic connection.

Omnivory is suspected but unconfirmed in many sea ice protozoa, although some feeding specialists undoubtedly exist. For example, the predatory ciliate *Didinium* (Figure 15.6a) is a predator of other ciliates.

Yet many strains of sea ice protozoa cultured in the laboratory will consume a mixture of algae, bacteria and other small protozoa. Phaeodarian radiolaria are particularly well known for their omnivorous diets (Gowing, 1986, 1989; Nöthig & Gowing, 1991; Gowing & Garrison, 1992). Specimens collected from polar waters often possess numerous food vacuoles that contain the remains of a wide variety of prey. Foraminifera, polycystine radiolaria and acantharia are also known to be feeding generalists in the water column (Caron & Swanberg, 1990; Swanberg & Caron, 1991), but resolving the trophic activities of these larger amoeboid protozoa in sea ice will require further study.

Appreciation of the importance of mixotrophic nutrition by protozoa in Arctic and Antarctic food webs is recent, but expanding rapidly. This broad category of behaviours encompasses phototrophic algae that are capable of ingesting food particles, heterotrophic protists (predominantly ciliates and dinoflagellates) that consume algae and retain the functional chloroplasts of their prey, and symbiont-bearing heterotrophic protists, including many of the radiolaria, acantharia and foraminifera.

The extent of mixotrophic nutrition by phototrophic protists in polar regions is a relatively recent discovery, but is now being recognized as a common ecological strategy in both Arctic and Antarctic ecosystems (Moorthi et al., 2009; Bachy et al., 2011; Sanders & Gast, 2011). A surprising finding of these studies is that very small algae that were previously believed to be exclusively phototrophic appear capable of consuming small bacteria. For example, it has recently been demonstrated that the minute prasinophytes *Pyramimonas gelidicola* and *Micromonas* sp. are both capable of bacterial ingestion (Bell & Laybourn-Parry, 2003; McKie-Krisberg & Sanders, 2014). These species are common in water and sea ice samples from the Antarctic and Arctic, respectively. The contribution of mixotrophic activity by these species to bacterivory and to their own algal nutrition is presently unknown.

Kleptoplastidic ciliates, including *Strombidium* spp. and *Mesodinium rubrum*, have been observed in the brine channels of Antarctic and Arctic sea ice (Stoecker et al., 1993; Archer et al., 1996b; Michel et al., 2002). *M. rubrum* was often the most frequently occurring ciliate species in these studies. These chloroplast-retaining ciliates were also common in such disparate locales as the pack ice of the Ross Sea and Weddell Sea, and in sea

ice of Saroma-ko lagoon (Japan) and Baffin Bay (Garrison & Buck, 1989; Michel et al., 2002; Garrison et al., 2005). The documentation of kleptoplastidy in a novel heterotrophic dinoflagellate of the Ross Sea indicates our rudimentary knowledge regarding the importance of this nutritional strategy in polar microbial ecology (Gast et al., 2007). The potential importance of this behaviour is evidenced by the fact that DNA sequences of this kleptoplastidic, heterotrophic dinoflagellate dominated samples collected from the water column and the microbial communities, as well as by microscopic observation of the dinoflagellate in the slush at the snow–ice interface of Ross Sea pack ice (Figure 15.2i,k).

The presence of symbiont-bearing acantharia in sea ice has been noted previously (Figure 15.8c,d,f,i). These symbiont–host associations are exceptionally abundant in the water column at various tropical and subtropical locations where they can form a significant fraction of the total primary production (Caron et al., 1995). High-latitude regions appear to have substantially smaller contributions of these associations, but this strategy still appears to be important for the autecology of some polar taxa.

15.4.2 Biogeochemical processes and rates

Protozoa play significant biogeochemical roles in all natural ecosystems on Earth. Their trophic activities result in the repackaging of (usually) smaller prey, such as bacteria, small algae and small protozoa, into larger cells that serve as food for other protozoa and metazoa. In the process, a percentage of the prey biomass is released as dissolved and particulate organic compounds that serve as substrates for bacterial growth, or are remineralized completely to inorganic nutrients that fuel primary productivity (Sherr et al., 2007). These species undoubtedly play similar roles in sea ice, but our understanding of the extent of their activities is only beginning to develop, and few models of energy or elemental flow within sea ice communities have thus far been constructed. Vézina et al. (1997) developed a model to examine carbon flow through the sea ice microbial community of Resolute Passage, Canadian High Arctic. The approach used changes in standing stocks of microbial assemblages to infer carbon flow. An important role of herbivory by flagellates and ciliates was indicated by the results of their modelling effort.

While modelling, field observations and experimental studies indicate an important role for protozoa in sea

ice, the fate of carbon and energy entering the microbial food webs in these extremely cold environments is less clear. Temperature directly affects the metabolic rates of microorganisms, and might also change the eventual fate of microbial biomass through its influence on growth rates and growth efficiencies of the microconsumers. There is no question that many species of protozoa can survive and grow at low polar *in situ* temperatures. However, it is probable that most sea ice protozoa do not exhibit true physiological compensation, i.e. maintain constant physiological rates in the face of changing temperature (Peck, 2002). Studies with Antarctic ciliates did not indicate any significant degree of physiological compensation; growth at low temperatures was substantially slower than growth at higher temperatures (Fenchel & Lee, 1972; Lee & Fenchel, 1972). Low temperature has also been reported to greatly depress the growth rates of Antarctic amoebae (Mayes et al., 1997).

Rose and Caron (2007) summarized information on the growth rates of phototrophic and heterotrophic protists from a wide variety of studies, and concluded that the effect of temperature on maximal potential growth rates under optimal conditions (abundant prey or nutrients) is not the same for phototrophic and heterotrophic protistan species. Maximal growth rates for heterotrophic protists (both bacterivorous and herbivorous) decrease more rapidly with decreasing temperature than maximal growth rates for phototrophic protists. Differences between maximal potential growth rates of phototrophs and heterotrophs are most acute at the temperatures experienced by sea ice. Thus, the dominance of total microbial biomass by dense algal accumulations in sea ice during spring may be due, in part, to more rapid algal growth, whereas protozoan growth is constrained by extremely low temperatures. Net growth rates of heterotrophic protists in Antarctic sea ice estimated on changes in abundances in the ice have indicated relatively slow rates, 0.005–0.5 day⁻¹ (Laurion et al., 1995; Archer et al., 1996b; Levinson et al., 2000), in general agreement with the overall constraint of low temperature on metabolic rate.

Studies of the rates of bacterivory and herbivory in sea ice and in coastal polar waters also support the controlling effect of temperature on overall protozoan rate processes. Decreasing water temperature was shown to significantly decrease microzooplankton (predominantly protistan) herbivory when this process

was compared over a large range of environmental temperatures (see summary and references in Caron et al., 2000). Experiments to characterize bacterivory in sea ice (Laurion et al., 1995; Sime-Ngando et al., 1999) also have often yielded low rates (i.e. a few bacteria per protistan consumer per hour). These grazing rates are commensurate with rates expected from other ecosystems with low *in situ* temperatures (Berninger et al., 1991; Leakey et al., 1996; Becquevort, 1997; Caron et al., 1997; Vaqué et al., 2004), but there are presently too few measurements to accurately determine the impact of sample handling in these experiments on the rates of bacterivory in sea ice specifically.

There are conflicting views at present regarding the direction and magnitude of the effect of temperature on the overall fate of microbial biomass consumed by protozoa in polar ecosystems. Growth at low temperature has been reported to increase (Choi & Peters, 1992), dramatically decrease (Mayes et al., 1997) or leave unaffected (Rose et al., 2008) the gross growth efficiencies of protozoa. These differing results are probably due, at least in part, to the fact that measurements of gross efficiency are difficult to make, and are particularly exacerbated by potential artifacts at low environmental temperature (Rose et al., 2013). Nonetheless, the effect of low temperature on protozoan growth efficiency has important implications for energy flow and elemental recycling in sea ice. Low growth efficiencies imply that protozoa act primarily as agents for the decomposition of organic matter and the remineralization of nutrients contained in their microbial prey. On the other hand, high growth efficiencies would suggest a much more important role for protozoa as intermediate steps in the food webs of sea ice biological communities. Resolution of these apparently conflicting observations awaits further study.

15.5 Conclusions and future directions

Studies of sea ice protozoan assemblages during the past few decades provide a basic understanding of the major taxa occurring in sea ice and their contributions to total microbial biomass. Many important trophic interactions involving these species have been observed and characterized, derived from observations of protozoa in sea ice samples, extrapolated from our knowledge

of the activities of the same or similar species in the water column, or established from observations and experimental studies on cultured species.

Major gaps in our knowledge remain, particularly regarding the physiological rates (feeding, growth and mortality rates) of sea ice microbes in intact ice microhabitats. Evidence of feeding activity, such as food vacuole contents and feeding behaviour in laboratory cultures, provide information on the types of prey consumed by protozoa, but it is unclear how this information extrapolates to actual rates of consumption and growth in the ice matrix. Similarly, information on the growth efficiencies of protozoa at environmentally relevant temperatures must be obtained for species that are ecologically important in ice microhabitats in order to establish their impact on decomposition, nutrient remineralization and trophic transfer. These goals are not without significant methodological hurdles and potential artifacts, but this information is key to understanding the overall biogeochemical importance of protozoa to sea ice microbial community structure and function.

Studies on protozoan physiological adaptations, species diversity and abundances within sea ice microhabitats will further improve our understanding of community structure and function in polar ecosystems, and the diverse roles that protozoa play in oceanic biogeochemical cycles. Isolation and culture of these species will provide genetic fodder for biotechnological exploitation of their unique physiological capabilities (Caron et al., 2012). Additionally, information from sea ice protistan species living at the cold extreme of environmental temperature will help to address difficult issues relating to the morphology-based species concept of protists, the existence of cryptic species, and the distribution of these taxa on our planet. Future studies of their diversity and ecology will undoubtedly continue to incorporate genetic approaches, but these findings must be interpreted in conjunction with traditional approaches of microscopy, culture, experimentation and field-based ecological observations.

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