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The long-term persistence of phytoplankton resting stages in aquatic 'seed banks'

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ABSTRACT

In the past decade, research on long-term persistence of phytoplankton resting stages has intensified. Simultaneously, insight into life-cycle variability in the diverse groups of phytoplankton has also increased. Aquatic 'seed banks' have tremendous significance and show many interesting parallels to terrestrial seed beds of vascular plants, but are much less studied. It is therefore timely to review the phenomenon of long-term persistence of aquatic resting stages in sediment seed banks. Herein we compare function, morphology and physiology of phytoplankton resting stages to factors central for persistence of terrestrial seeds. We review the types of resting stages found in different groups of phytoplankton and focus on the groups for which long-term (multi-decadal) persistence has been shown: dinoflagellates, diatoms, green algae and cyanobacteria. We discuss the metabolism of long-term dormancy in phytoplankton resting stages and the ecological, evolutionary and management implications of this important trait. Phytoplankton resting stages exhibiting long-term viability are characterized by thick, often multi-layered walls and accumulation vesicles containing starch, lipids or other materials such as pigments, cyanophycin or unidentified granular materials. They are reported to play central roles in evolutionary resilience and survival of catastrophic events. Promising areas for future research include the role of hormones in mediating dormancy, elucidating the mechanisms behind metabolic shut-down and testing bet-hedging hypotheses.

Key words: cyst, spore, microalgae, dinoflagellate, diatom, long-term viability, sediment record, seed bed.

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I. INTRODUCTION

Terrestrial seed banks are intensively studied, for both research and management purposes, and persistence of seeds over long timescales was recently reviewed by Long *et al.* (2015). Although seeds of vascular plants are also found in lakes (e.g. Li *et al.*, 2008; Bakker *et al.*, 2013), the dominant primary producers in most aquatic systems are algae, and thus aquatic seed banks are predominantly formed by resting stages produced by the phytoplankton, or planktic microalgae. Such seed banks are particularly widespread in coastal marine environments.

Although they are mainly microscopic, marine phytoplankton are arguably the most important primary producers on the planet and are estimated to be responsible for half the global primary production (Field *et al.*, 1998). This is due partly to their rapid division and production rates and partly to the fact that the world's oceans cover approximately 70% of the global surface. As the marine environment is three-dimensional, this corresponds to more than 90% of the biosphere. Phytoplankton form the basis of aquatic food webs and also play important parts in the global biogeochemical cycling of carbon, nitrogen, phosphorus and silicate and are thus of immense ecological importance.

Many phytoplankton species form resting stages as part of their life cycle, and these resting stages in many ways play similar roles in aquatic environments to those of seeds in terrestrial environments. Phytoplankton resting stages are found in such diverse phylogenetic groups as dinoflagellates, diatoms, green algae, cyanobacteria, chrysophytes, haptophytes, cryptophytes, raphidophytes and euglenophytes. Within the phytoplankton there are many different types of resting stages and even within a group, these may play different roles in the life cycle. Some resting stages are linked to sexual reproduction, such as hypnozygotes in dinoflagellates and oospores of some green algae, while other resting stages are asexual and are formed solely in response to environmental cues signalling the end of the growth season, such as akinetes in some cyanobacteria and green algae, and resting spores in some diatoms. However, all these resting stages play central roles in the life cycle, ecology and evolution of phytoplankton.

There are numerous structural, functional and adaptational parallels between seeds of terrestrial plants and phytoplankton resting stages. The main difference is that for terrestrial plants both the seed and the plant are multicellular, whereas for microalgae both the resting stage and the vegetative cell are unicellular. The main similarities include the function in the life cycle, morphological adaptations, roles in ecology and dispersal, and similar survival times. The majority of seeds as well as resting stages are characterized by highly resistant, thick walls and by the capacity to endure physiological dormancy. For many phytoplankton groups, the maximal documented viability is on the order of a century, which is similar to that of seeds of land plants *in situ* [Long *et al.* (2015); although there are reports of much older seeds from archaeological sites (e.g. Sallon *et al.*, 2008)], and aquatic plants, which may likewise persist *in situ* for multi-decadal timescales (Bell & Clarke, 2004).

Over the past years, our knowledge of aquatic seed banks has increased substantially, in parallel with an increased focus on life-cycle studies for several groups of phytoplankton. It is thus timely to review the existing knowledge on these seed banks and their persistence, although they are still much less studied than terrestrial seed banks. Herein we present an overview of the types of resting stages formed in different groups of phytoplankton. We review factors that characterize resting-stage morphology and physiology and focus on groups where long-term viability has been studied. We discuss the roles of resting stages in ecosystem functioning, resilience and adaptation to change as well as in global carbon cycling. We review the literature on long-term viability of aquatic resting stages, and compare and contrast these resting stages with terrestrial seeds with regard to factors influencing persistence, and discuss the parallel roles of these

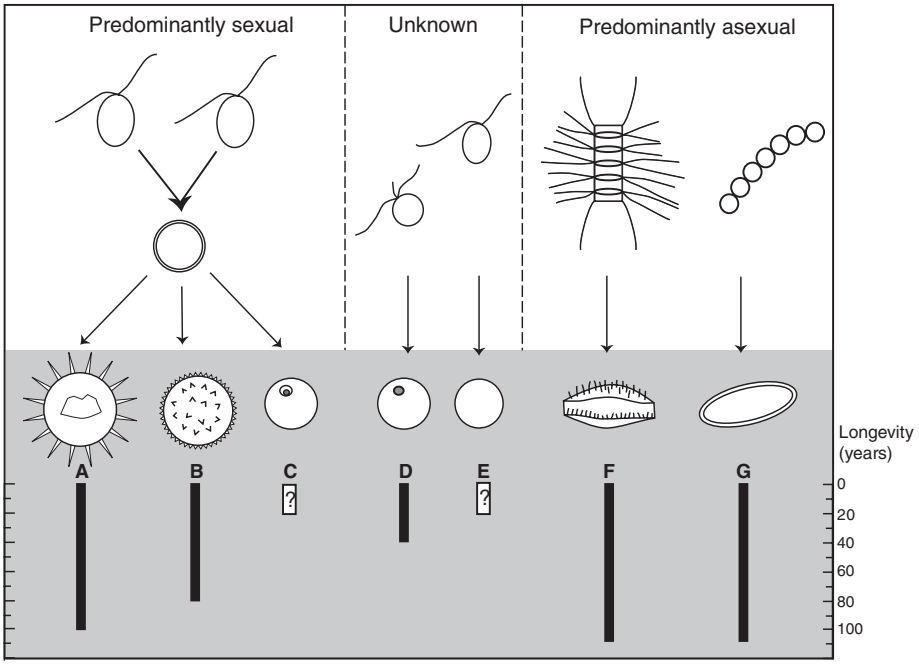

Fig. 1. Simplified scheme illustrating different types of resting stages in the sediment, their documented longevity, and links to metabolically active cells in the water column (plankton). The left panel shows resting stages predominantly produced by sexual reproduction, the right panel asexually produced resting stages and the centre shows groups for which this is unknown. (A) Dinoflagellate hypnozygote (cyst); (B) green alga (Volvocales) hypnozygote; (C) chrysophyte stomatocyst; (D) haptophyte resting stage; (E) cryptophyte, euglenophyte and raphidophyte resting stage; (F) diatom resting spore; (G) cyanobacteria akinete.

two types of seed banks. Our main focus is on marine seed banks, but examples of resting-stage persistence in freshwater systems are also included.

II. PHYTOPLANKTON RESTING STAGES AND RECORDS OF THEIR PERSISTENCE

In marine environments most reports of the persistence of resting stages are for diatoms and dinoflagellates, and in freshwater settings most studies on longevity of resting stages of phytoplankton are for green algae and cyanobacteria, although reports exist on the persistence of freshwater diatom resting stages and oospores of freshwater benthic macroalgae (Charophyceae).

Plankton means 'the wanderer' and plankton are, by definition, free-floating in the water mass. Resting-stage producers more accurately belong to the meroplankton, as the resting stages will typically sink to the sediment and the active stage re-enter the plankton at germination, thus anchoring these organisms to both the plankton and the benthos. Figure 1 shows a generalized and simplified scheme of this connection for different groups of phytoplankton. Table 1 gives an overview of the terminology used here for the different types of resting stages.

In situ records of long-term viability are derived from studies of resting stages germinated from dated sediment core records. Figure 2 illustrates how such records are obtained and shows an example of a core, where structures seen in an X-ray image (Fig. 2C) indicate that the sediment has not been disturbed since deposition. When an undisturbed record is retrieved, analyses going down the core are equivalent to going back in time. Some studies on the long-term viability of phytoplankton resting stages include no information on the age of the sediment layer, but give the depths in the sediment at which resting stages were found. Others estimate the age of sediment layers in which the resting stages are found, e.g. by estimating sedimentation rate or counting yearly varves (visible layers). The most precise chronologies are achieved by radioactive isotope analyses, such as those using ^{210}Pb and ^{137}Cs (for the past 100–120 years) and ^{14}C for older material. In some cases it can be difficult to achieve a reliable chronology, as there are few reliable means of dating accurately in the range 150–500 years before present (BP). In all cases, dating models carry a degree of uncertainty (see e.g. Telford, Heegaard & Birks, 2004; Radzikowski, 2013) that has to be accounted for when studying aquatic sediment records.

Abundances of viable resting stages in surface sediments can vary from none to tens of thousands per g dry sediment, depending both on the productivity of the area and on depositional factors (see e.g. Sandgren, 1983; Lewis, 1988), but calculations show that only a very small proportion of this may be needed to seed a phytoplankton bloom (Lewis, Tett & Dodge, 1985).

Table 1. Commonly used terminology for phytoplankton resting stages

Type of resting stage	Description	Group(s)
Cyst	Generalized term for unspecified resting stage	Many groups
Temporary cyst	General term for short-term stage induced by e.g. division, digestion or stress	Many groups
Hypnozygote	Thick-walled, long-lived zygote (part of the sexual cycle)	Dinoflagellates, some green algae, some chrysophytes
Zygospore	Thick-walled, long-lived zygote (part of the sexual cycle)	Some green algae
Resting spore/hypnospore	Generally non-sexual stage morphologically differentiated from the active stage	Diatoms
Resting cell	Generally non-sexual, not strongly differentiated from the active stage	Diatoms
Akinete	Non-sexual, morphologically differentiated vegetative cell	Cyanobacteria, green algae
Oospore	Type of hypnozygote	Green algae

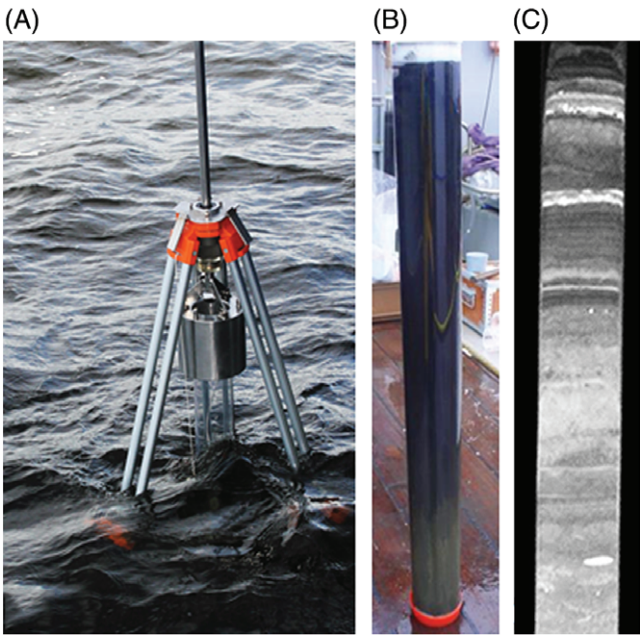

Fig. 2. (A) Super-corer deployed from the side of a ship with empty coring tube entering the water. (B) Coring tube with intact sediment core. (C) X-ray image of an intact sediment core from Koljö Fjord, Sweden.

(1) Phytoplankton groups with resting stages and their documented longevities

(*a*) *Diatoms*

There is a profuse volume of literature on diatom resting stages with the older literature reviewed thoroughly by McQuoid & Hobson (1996). Diatom resting stages can be divided into two (possibly three) different types: resting spores, resting cells and perhaps winter stages (reported from an Antarctic diatom). The resting spores (Fig. 3A), also called hypnospores (Table 1), are morphologically different from the vegetative cells, with thicker frustules (silica walls), a rounder shape and less ornamentation or patterning of the frustule. Resting cells are morphologically similar to the vegetative cells. However, both resting spores and resting cells show physiological and cytoplasmic differences relative to vegetative cells (see Section III.2). Most are not part of the sexual cycle (Edlund & Stoermer, 1997), although there are exceptions, notably resting spores in *Leptocylindrus danicus* (French & Hargraves, 1985). Studies on persistence of diatoms in marine sediments report germination of viable resting stages from sediment layers approximately 30–40 cm below the sediment surface, dated at >55 to >90 years by ^{210}Pb modelling (McQuoid, Godhe & Nordberg, 2002; Härnström *et al.*, 2011; Lundholm *et al.*, 2011; Ellegaard *et al.*, 2013*b*). In fresh water, diatoms have been reported to germinate from down to 40 cm depth in the sediment (Stockner & Lund, 1970; Sicko-Goad, Stoermer & Fahnenstiel, 1986). These studies did not include sediment dating, but estimated age to be 175–275 years, based on estimated sedimentation rates of 1.2–1.5 mm per year (Stockner & Lund, 1970). Species of *Aulacoseira* (as *Melosira*) were generally found viable deepest in the sediment cores. McQuoid *et al.* (2002) found the highest concentration of viable cells of both diatoms and dinoflagellates in the top 15 cm of the sediment core (*ca.* 40 years old).

(*b*) *Dinoflagellates*

Dinoflagellate cyst walls can be preserved in sediments for millions of years (e.g. Evitt, 1985), and there is an immense volume of scientific literature on stratigraphy and palaeoecology of fossil dinoflagellate cysts, which will not be addressed here, as it deals with non-living remains (walls) of the cysts. Of living dinoflagellate species, at least 20% are known to produce resting cysts as part of their life cycle (Head, 1996). This review will focus on those cysts (Fig. 3C), which are long-term resting stages mainly produced as part of sexual reproduction (hypnozygotes; see Table 1 and A in Fig. 1). The classic dinoflagellate life cycle (see e.g. Dale, 1983) includes a haploid, motile, metabolically active flagellate stage. At the initiation of sexual reproduction some

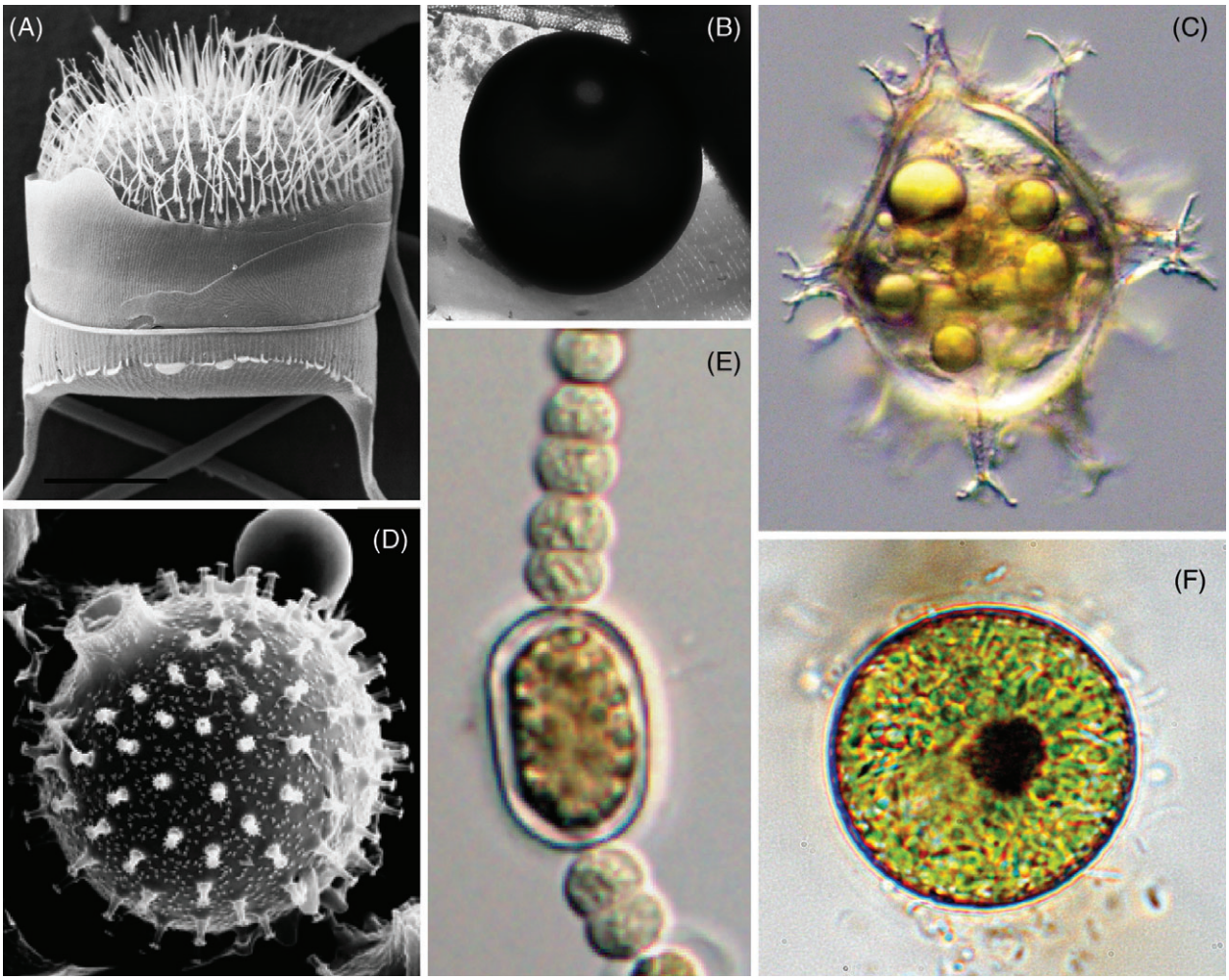

Fig. 3. Photographic images illustrating examples of different types of resting stages. (A) Scanning electron micrograph of a diatom resting spore, *ca.* 15 µm in diameter; (B) transmission electron micrograph of a haptophyte resting stage, *ca.* 5 µm in diameter; (C) light micrograph of a dinoflagellate cyst, *ca.* 50 µm long; (D) scanning electron micrograph of a chrysophyte stomatocyst, *ca.* 13 µm in diameter; (E) light micrograph of a cyanobacteria akinete, *ca.* 20 µm long; (F) light micrograph of a raphidophyte resting stage, *ca.* 40 µm in diameter. A courtesy of Atchaneey Boonprakob, Nina Lundholm and Øjvind Moestrup; D courtesy of Gertrud Cronberg; F courtesy of Karin Rengefors.

cells are differentiated to gametes, which fuse and form a motile planozygote. The thick-walled, immobile resting cyst is formed within this zygote and sinks towards the sea or lake floor. The cyst is usually morphologically distinct from the motile cell. Many dinoflagellates can also form temporary resting stages, which are usually not long-lived and which typically are not part of sexual reproduction. Recent work on the diversity of dinoflagellate life cycles has demonstrated many variations on this general theme, showing that some species can form other types of resting stages in culture, such as sexual cysts that are not long-lived (e.g. Figueroa & Bravo, 2005; Figueroa, Garcés & Bravo, 2007; Ellegaard, Figueroa & Versteegh, 2013*a*). There are also documented cases of resting stages formed without sexual reproduction (Kremp & Parrow, 2006). Dinoflagellate cysts have been germinated from sediment layers dated up to approximately 100 years old at 32–63 cm depth in the sediment (Lundholm *et al.,* 2011; Miyazono *et al.,* 2012; Feifel *et al.,* 2015). In many of these studies, deeper (older) sediment layers were screened without successful germinations, indicating that these ages are likely to represent maximum possible viability in these environmental settings. In studies to date, the number of viable cells generally decreases with sediment depth and age; however some deeper layers had higher numbers (and percentages) of viable cells. Thus Feifel *et al.* (2015) found higher germination percentages of *Alexandrium* and *Scrippsiella* at 30–50 cm depth (13–42 years old) than in surface samples; Miyazono *et al.* (2012) found higher concentrations of intact *Alexandrium tamarense* cysts at 22 cm (*ca.* 50 years old), although the germination percentage fell linearly with depth and exponentially with age. Lundholm *et al.* (2011) found that most species of dinoflagellate cysts had the highest germination percentage in surface samples; however, *Pentapharsodinium dalei* had the highest germination percentage in approximately 28-year-old sediment layers,

coinciding with a time period when this species was most abundant (Harland, Nordberg & Filipsson, 2004).

(*c*) *Haptophytes*

Haptophyte cysts (Fig. 3B) have been reported sporadically since they were first found in the genera *Prymnesium* Massart (Carter, 1937) and *Isochrysis* Parke (Parke, 1949). These cysts were described as small (5–10 μm), spherical to elliptical, smooth to rugose and sometimes with an opening (plugged closed when intact). In these early publications it was not clear whether these cysts were resting stages, but a recent study has confirmed this and reported their survival down to at least approximately 40-year-old sediment layers (Ellegaard *et al.*, 2016).

(*d*) *Green algae*

There are many different types of resting stages within the green algae *sensu lato*, some of which are sexual (see e.g. review by Coleman, 1983). These are called hypnozygotes or zygospores (Table 1), are produced in a similar manner to dinoflagellate cysts, and are likewise morphologically distinct from the flagellate stage. For example, the freshwater algae *Chlamydomonas* Ehrenberg and *Volvox* Linneaus produce such resting stages (e.g. Nozaki, 1996; Aoyama *et al.*, 2014). Some multicellular green algae produce oospores, for example members of the Charales. Other green algae produce asexual akinetes (Coleman, 1983; Table 1). All the above records are from fresh water. In marine settings there have been a few reports of cysts or other resting stages in prasinophyte green algae: a cyst stage has been reported in *Pyramimonas* Schmarda (van den Hoff, Burton & Vesk, 1989) and in *Mantoniella* T.V.Desikachary (Ellegaard *et al.*, 2016). The former was scaled and the latter a small, round, smooth structure. There is one report on long-term persistence of marine prasinophytes in sediment cores (Ellegaard *et al.*, 2016), where they were found down to at least approximately 40-year-old sediment layers. Oospores of charophytes have been reported to be viable for approximately 80 years in freshwater lake sediments (Beltman & Alleggrini, 1997). There is one report that they may be alive after over 300 years (although the authors did not exclude the possibility that they were younger; Stobbe, Gregor & Röpke, 2014). To our knowledge, there are no records of long-term survival *in situ* of chlorophyte hypnozygotes, but they have been reported to remain viable for 24 years in the laboratory (Coleman, 1983).

(*e*) *Cyanobacteria*

Many filamentous cyanobacteria, for example in the genus *Anabaena*, produce akinetes (Fig. 3E; Table 1). These are thick-walled, large cells, produced at the end of the growth season and filled with storage vesicles. Species that form akinetes are mainly found in fresh water, but there are also marine/brackish akinetes (see e.g. Ellegaard *et al.*, 2013*b*). Cyanobacteria akinetes have been germinated from lake sediments of up to 64 years old (Livingstone & Jaworski, 1980) and approximately 120 years old (Wood *et al.*, 2008).

(*f*) *Raphidophytes*

Raphidophyte cysts (Fig. 3F) are generally described as rounded, smaller and thicker walled than the vegetative stage. Cysts are known from the marine genera *Chatonella* B.Biecheler (e.g. Imai, 1989) and *Heterosigma* (Y.Hada) Y.Hada ex Y.Hara & M.Chihara (e.g. Kim *et al.*, 2015). Imai (1989) reported germination of *Chatonella* from sediment samples, and subsequent formation of cysts in culture. Cysts are also known from the invasive freshwater species *Gonyostomum semen* (Ehrenberg) Diesing (Cronberg, 2005). There is a single study reporting cyst DNA from *Gonyostomum semen* in decades-old lake sediments but in this study it was not possible to quantify cyst survival due to low sedimentation rates and dating uncertainties (Johansson *et al.*, 2016).

(*g*) *Chrysophytes, euglenophytes and cryptophytes*

There is an extensive literature on chrysophyte cysts (Fig. 3D), mainly from palaeolimnological studies of the empty walls of stomatocysts found in temporal series in lake sediments (e.g. Piątek *et al.*, 2009). Stomatocysts are small, round or oval, with silicified walls, each with a germination pore. More recent studies on living stomatocysts cover e.g. the production of cysts in culture strains (Holen, 2014) and excystment of stomatocysts in ice (Stoecker *et al.*, 1997). They have been found to persist almost 400 days in storage (Sandgren, 1991). Chrysophyte cysts are found mainly in freshwater environments.

For the euglenophytes, Olli (1996) described cysts of the genus *Eutreptiella* A. da Cunha from the Baltic Sea as having thick and mucilaginous walls, with many paramylon (storage) grains in the cytoplasm. Cysts have also been reported from species of *Euglena* Ehrenberg 1830 and other freshwater genera. Hindák, Woľowski & Hindáková (2000) list three types of cysts formed by *Euglena*: protective, reproductive and temporary. Protective cysts were described as having a ´heavy´ wall.

For cryptophytes, there are a few references to cysts of *Cryptomonas* Ehrenberg, but only in fresh water or snow (e.g. Lichtlé, 1979) where cysts had thick, three-layered walls and lipid and starch droplets in the cytoplasm. The flagella and gullet are reported to disappear during cyst formation (Lichtlé, 1979).

To our knowledge, there are no published studies on the *in situ* longevity of resting stages of chrysophytes, euglenophytes or cryptophytes and these groups are therefore not discussed further below.

III. FACTORS AFFECTING LONG-TERM SURVIVAL OF PHYTOPLANKTON RESTING STAGES

Most research into long-term survival has been carried out on dinoflagellates and diatoms in marine settings, and therefore

we focus mainly on these groups, with some additional examples from green algae and cyanobacteria. In most studies, the entire cyst bank was not targeted, but rather specific groups or particular species. Studies on individual species mainly targeted harmful algal (HAB) species such as the dinoflagellate *Alexandrium* (Miyazono *et al.*, 2012) and the cyanobacterium *Anabaena* (Livingstone & Jaworski, 1980). There is therefore a bias of selectivity in some of the obtained records.

The persistence of phytoplankton resting stages is determined by endogenous and exogenous factors which either prevent early germination (i.e. remaining in the cyst stage), and/or promote the maintenance of cell viability (i.e. survival while encysted). Endogenous factors include the amount of energy available in storage compounds, the thickness and robustness of the cell wall and the mandatory dormancy period. Exogenous factors affecting dormancy and viability *in situ* include light, temperature, and oxygen levels.

Factors affecting germination of algal resting stages will thus to some extent also influence long-term viability (as prevention of germination is an obvious prerequisite for remaining in the resting stage for long periods). These factors were reviewed relatively recently (Agrawal, 2009), including both factors measured *in situ* and in laboratory studies. Below, we consider the main factors known to affect germination *in situ*. We also consider factors reported to be of importance for long-term persistence of seeds, as reviewed by Long *et al.* (2015), to explore potential future research avenues for phytoplankton resting-stage persistence.

(1) Morphological parameters

(*a*) *Size and colour*

In seeds, long-term persistence may be affected by size, wall thickness, mucilage, and colour (Long *et al.*, 2015). A number of studies indicate that smaller seeds persist for longer, although this is not always the case (Long *et al.*, 2015). Colour, mucilage and wall thickness (and hardness) will all influence the likelihood of predation, and can thus affect the propensity of a seed to remain alive (Long *et al.*, 2015). Size, wall characteristics and colour vary among phytoplankton resting stages and are given in Table 2 for dinoflagellate cysts, diatom spores and cyanobacteria akinetes in species where resting stages are known to survive in sediments for many decades. In general, resting stages in these species are colourless, have thick walls and/or a mucilage covering and are relatively small. This pattern of small size, thick walls and a mucilage covering is similar to the features reported for seeds with long survival times (Long *et al.*, 2015). Some of these resting-stage morphological parameters may, however, merely be proxies for other factors determining longevity. For example, in dinoflagellates, cysts of autotrophic species are generally colourless, while cysts of heterotrophic species are typically brown, and it may be this autotrophic/heterotrophic trait, rather than the colour itself, which is critical. Nonetheless, these similar features across taxonomic groups deserve further attention as potential indicators of capacity for long-term survival.

(*b*) *Wall structure*

Characteristics of seed coats have been linked to persistence in terrestrial seeds (Long *et al.*, 2015). Hardness and a mucilage cover offer protection against predation, degradation by microorganisms and imbibition (water intake). Wall structure and mucilage covering have been investigated in several studies on dinoflagellate and diatom resting stages. Bibby & Dodge (1972) found that in the dinoflagellate *Woloszynskia tylota* the wall of the cyst was continuous and twice as thick as the thecal plates of the active stage. Electron microscopy of cysts of several species of the dinoflagellates *Alexandrium* and *Peridinium* Ehrenberg reported a thick, three-layered wall (Dürr, 1979; Fritz, Anderson & Triemer, 1989; Kennaway & Lewis, 2004). Thickening of the wall was also reported for cysts in ice (Buck *et al.*, 1992) and cysts of the dinoflagellate *Scrippsiella* sp. (Gao, Dodge & Lewis, 1989). The composition of the dinoflagellate cyst wall is not yet completely elucidated, but studies on cysts of *Lingulodinium polyedrum* indicate a strongly cross-linked carbohydrate polymer, probably closer to cellulose than algaenan (a resistant polymer in green algae) or sporopollenin (Kokinos *et al.*, 1998; Versteegh *et al.*, 2012). Diatom resting spores are morphologically distinct from the metabolically active cells and most are more heavily silicified (Oku & Kamatani, 1995; McQuoid & Hobson, 1996), although some are rather similar to the vegetative cells (McQuoid & Hobson, 1996). They are found more frequently in marine centric diatoms than in freshwater species (McQuoid & Hobson, 1996) or pennate diatoms (Hargraves & French, 1983). Chlorophyte hypnozygotes likewise have an outer tripartite layer, plus an inner layer of varying thickness, which is crucial for desiccation resistance (Coleman, 1983). Akinetes of both cyanobacteria and green algae are also characterized by thick, impermeable wall layers (Braune, 1980; Coleman, 1983). Thick, multi-layered walls and/or mucilage covering thus seem to be a common trait across phylogenetic groups with the potential for long-term viability.

(2) Physiological parameters

(*a*) *Hormones*

Hormones play a major role in terrestrial seed persistence. The balance of endogenous hormones (e.g. abscisic acid and gibberellic acid) can prevent germination in terrestrial seeds (Long *et al.*, 2015), but their influence has been studied only rarely in phytoplankton. One of the first non-animal species in which melatonin was found was in the dinoflagellate *Lingulodinium polyedrum* (under the name *Gonyaulax polyedra*; Balzer & Hardeland, 1991*b*). Production of this hormone was linked to the formation of temporary cysts (Balzer & Hardeland, 1991*a*). Addition of melatonin mimicked the effects of temporary cyst-induction by photoperiod under otherwise non-inductive conditions. Thus, as in mammals,

Table 2. Morphological characteristics of resting stages found deep (multi-decadal and/or deeper than 20 cm) in the sediment. c, chainforming; m, mucilage covering; s, spines. Data on approximate sizes have, where possible, been obtained from identification keys and similar sources, to obtain ranges. For genera approximate ranges are given (taken from multiple sources)

Diatoms	Approximate diameter (μm)	Wall colour	Wall properties	Other features
Melosira (Aulacoseira) italica (Ehrenberg) Simonsen	4 – 24[a]	None	Medium	c
Melosira (Aulacoseira) islandica (Otto Müller) Simonsen	6 – 18[a]	None	Medium	c
Melosira (Aulacoseira) granulata (Ehrenberg) Simonsen	4 – 17[a]	None	Medium	c
Stephanodiscus hantzschii Grunow in Cleve & Grunow	8 – 15[b]	None	Medium	s
Skeletonema marinoi Sarno & Zingone in Sarno *et al.*	2 – 12[c]	None	Medium	c
Chaetoceros socialis H.S.Lauder	2 – 14[d]	None	Thick	c
Detonula confervacea (Cleve) Gran	6 – 20[d]	None	Thick	c
Dinoflagellates				
Pentapharsodinium dalei Indelicato & Loeblich III	19 – 36[e]	None	Medium	s
Lingulodinium polyedrum (F.Stein) J.D.Dodge	31 – 54[e]	None	Thick	s
Protoceratium reticulatum (Claparède & Lachmann) Bütschli	33 – 48[e]	None	Thick	s
Scrippsiella Balech ex A.R.Loeblich III spp.	*ca.* 25 – 45	None	Calcareous	s
Alexandrium Halim spp.	*ca.* 20 – 50	None	Thin	m
Cyanobacteria				
Anabaena Bory ex Bornet & Flahault spp.	*ca.* 10 – 20	Brownish	Thick	c
Aphanizomenon A.Morren ex É.Bornet & C.Flahault spp.	*ca.* 10 (up to 70 long)	Brownish	Thick	c

[a]westerndiatom.colorado.edu.
[b]craticula.ncl.ac.uk.
[c]Sarno *et al.* (2005).
[d]Hasle & Syvertsen (1997).
[e]Zonneveld & Pospelova (2015).

melatonin was able to mimic short-day treatments (Balzer & Hardeland, 1991*a*). Some evidence suggests that abscisic acid (ABA) has a role in inducing the formation of thin-walled cysts, for example in the green alga, *Haematococcus pluvialis* Flotow where transition into the astaxanthin-rich vegetative cyst stage coincided with a decrease in ABA levels (e.g. Kobayashi, 2003) and in the dinoflagellate *Akashiwo sanguinea* (K.Hirasaka) G.Hansen & Ø.Moestrup in N.Daugbjerg, G.Hansen, J.Larsen, & Ø.Moestrup where it has been speculated to play a role in regulating the formation of thin-walled sexual resting cysts (Deng *et al.,* 2016).

(*b*) *Storage compounds*

The amount and type of storage compounds in plant seeds varies but in most species it is a combination of carbohydrates, proteins and oils (Lüttge, 2013). Storage compounds can affect the persistence of terrestrial seeds, because they determine nutritional quality for predators. Additionally, the balance between different sugars and lipids influences resistance to heat and desiccation damage (Long *et al.,* 2015). Lifespan correlates with degree of saturation of membrane lipids and aging causes changes to the lipid reserves (Long *et al.,* 2015).

In dinoflagellates (e.g. Binder & Anderson, 1990), diatoms (e.g. Hargraves & French, 1983; Kamp *et al.,* 2011), cyanobacteria (e.g. Sukenik *et al.,* 2015) and chlorophytes (Coleman, 1983) changes in cellular content occur during the process of resting stage formation leading to differences in the mature cyst compared to the vegetative stages (Table 3). The resting stages have a higher density of storage vesicles; in dinoflagellates these are mainly starch grains (with some studies also reporting increased lipid levels), in diatoms mainly lipid droplets (sometimes with increased glucose levels), and in cyanobacteria mainly cyanophycin. Other general features include changes in chloroplast structure and/or pigments, increased carbon content and decreased nitrogen levels (Table 3). Most studies indicate that chloroplasts in some form are present in the cyst stage, although there may be structural differences relative to chloroplasts in the active stage. For example, Bibby & Dodge (1972) reported that the thylakoid organization was altered in cysts of *Woloszynskia tylota*, with stacks of 9–10 as well as single thylakoids, in contrast to the stacks of three normally seen in vegetative cells.

Pigment (mainly chlorophyll a; Chla) content also differs in resting stages relative to vegetative cells, but the precise detail varies among species. Anderson (1975) found that Chla content in resting cells of *Amphora coffeaeformis* initially decreased, but after approximately 2 weeks increased again towards levels in vegetative cells. Kuwata, Hama & Takahashi (1993) found in *Chaetoceros pseudocurvisetus* that resting cells had lower Chla levels than vegetative cells whereas in resting spores levels were similar to those in vegetative cells. Persson *et al.* (2016) reported much lower (*ca.* 80%) amounts of pigments per cell in motile zygotes and cysts of *Scrippsiella lachrymosa* than in the vegetative

Table 3. Intracellular and biochemical changes documented in transitions from active stages to the resting stage in phytoplankton. Chla, chlorophyll a

Group	Species	Resting stage	Storage compounds	Chloroplast/ pigments	Other details	References
Dinoflagellate	*Woloszynskia tylota* (H.Mapletoft, M.Montgomery, J.Waters & P.Wells) B.T.Bibby & J.D.Dodge	Hypnozygote	Closely packed lipid droplets	Reduction or disappearance	Appearance of accumulation body and large vacuoles with crystals	Bibby & Dodge (1972)
Dinoflagellate	*Alexandrium* spp.	Hypnozygote	Lipid and starch bodies	Disorganization of chloroplast	Disorganization of Golgi vesicles and mitochondria; appearance of accumulation bodies	Kennaway & Lewis (2004)
Dinoflagellate	*Scrippsiella* sp.	Hypnozygote	Starch bodies	Partial disorganization of chloroplast	Appearance of accumulation bodies; organelles decreased in size and number	Gao *et al.* (1989)
Dinoflagellate	Ice alga (probably *Polarella* M.Montresor, G.Procaccini & D.K.Stoecker)	Cyst	Starch bodies increasing over time; presumed lipid bodies			Buck *et al.* (1992)
Dinoflagellate	*Alexandrium* (as *Gonyaulax*) *tamarense* (Lebour) Balech	Hypnozygote	Lipid and (mainly) starch bodies		Appearance of accumulation body and bodies with concentric structures	Fritz *et al.* (1989)
Dinoflagellate	*Scrippsiella trochoidea*	Hypnozygote			Higher C, lower protein	Binder & Anderson (1990)
Dinoflagellate	*Scrippsiella trochoidea* (Stein) Loeblich III	Hypnozygote	Differences in monosaccharide composition (more glucose)		More cellular phosphorus; less ATP; changes in amino acid composition	Lirdwitayaprasit *et al.* (1990)
Dinoflagellate	*Scrippsiella lachrymosa* J.Lewis	Hypnozygote		Lower Chla; higher β-carotene; activation of xanthophyll cycle	Appearance of accumulation body	Persson *et al.* (2016)
Diatom	*Amphora coffeaeformis* (C.Agardh) Kützing	Resting cell	Lipid droplets; possibly chrysolaminaran reserves		Reorganization; fewer mitochondria; fewer and smaller vacuoles	Anderson (1975)
Diatom	*Chaetoceros pseudocurvisetus Mangin*	Resting spore		Changes in pigment ratios; activation of xanthophyll cycle	Nucleotide content reduced to *ca.* 1/4; less sugar phosphate in spores; changes in ATP and associated compounds	Oku & Kamatani (1995, 1999)

Table 3. continued

Group	Species	Resting stage	Storage compounds	Chloroplast/ pigments	Other details	References
Diatoms	Not specified	Not specified	Large amount of carbon	Higher Chla	Higher C/N; lower respiration; can photosynthesize for one month in the dark	French & Hargraves (1980); cited by Hargraves & French (1983)
Diatom	*Chaetoceros pseudocurvisetus*	Resting cell	Accumulation of neutral lipids	Lower Chla	Lower N	Kuwata *et al.* (1993)
Diatom	*Chaetoceros pseudocurvisetus*	Resting spore	Accumulation of polar lipids/increase of unsaturated fatty acids; large amounts of glucose	Lower Chla	Higher C and Si, lower N	Kuwata *et al.* (1993)
Diatom	*Thalassiosira pseudonana* Hasle & Heimdal	Resting cell	Increase of unsaturated fatty acids			Zhukova (2004)
Diatom	*Chaetoceros pseudocurvisetus*	Resting spore	Accumulation of neutral lipids			Zhukova & Aizdaicher (2001)
Diatom	*Thalassiosira antarctica* Combe	Resting spore	More lipid reserves		Larger mitochondria	Doucette & Fryxell (1985)
Diatom	*Thalassiosira antarctica*	Resting spore		Lower Chla, but increasing over time	Few or no vacuoles	Doucette & Fryxell (1983)
Chlorophyte	*Stigeoclonium tenue* (C.Agardh) Kützing	Akinete	Accumulation of starch and lipids	Disorganization of chloroplast	Reduction of vacuoles	Michetti, Leonardi & Caceres (2002)
Cyanobacteria	*Aphanizomenon ovalisporum* Forti	Akinete	Accumulation of cyanophycin and starch globules	Reduced thylakoids, reduction in phycobili-somes	Genome multiplication	Sukenik *et al.* (2015) and references therein

stage. Interestingly, levels of some pigments were higher in the zygote and cyst stage; β-carotene levels increased significantly and the de-epoxidation ratio (involving the xanthophyll pigments) almost quadrupled.

Studies on changes in carbon pools (mainly storage products) during cyst formation and during the cyst stage have been carried out on the dinoflagellate species *Scrippsiella trochoidea* and the diatom *Chaetoceros curvisetus* Cleve under laboratory conditions (Lirdwitayaprasit *et al.*, 1990; Kuwata *et al.*, 1993). Newly formed cysts of *S. trochoidea* contained an order of magnitude more carbohydrate and significantly less protein and Chla than vegetative growing cells. Lipid content increased, and Chla and carbohydrate content decreased over 141 days in the cyst stage. Just prior to germination, carbohydrate content dropped and protein levels increased; Chla levels increased simultaneously with germination (Binder & Anderson, 1990). High glucose content in the cysts was consistent with carbohydrate storage in the form of starch or some form of glucan (Lirdwitayaprasit *et al.*, 1990). In *C. curvisetus* (and other diatoms) changes in the composition of fatty acids were recorded, mainly consisting of increased amounts of neutral lipids and unsaturated fatty acids (e.g. Kuwata *et al.*, 1993). Some studies report less ATP and associated compounds in resting stages, as well as a general reduction in nucleotide levels (Lirdwitayaprasit *et al.*, 1990; Oku & Kamatani, 1999).

Many studies report the presence of a coloured (red, orange or yellow) accumulation body in dinoflagellate cysts (Table 3). The nature of this structure is still unclear, but it may contain photosynthetic pigments and play a role in reactivation of photosynthesis during or after germination (e.g. Fritz *et al.*, 1989; Persson *et al.*, 2016). The nucleus is reported to be present in most transmission electron micrographs of resting stages; however, it can be somewhat different in appearance than in vegetative cells, e.g. compressed with strongly condensed, granular chromosomes (Kennaway & Lewis, 2004). In the diatom *Chaetoceros pseudocurvisetus*, Oku & Kamatani (1999) found changes in nucleotide content during resting-stage formation. In the cyanobacterium *Aphanizomenon ovalisporum*, massive multiplication of the genome can occur in akinetes; the vegetative cells are also polyploid, but the copy number can be 15-fold higher in akinetes (Sukenik *et al.*, 2012).

Interestingly, many similar modifications of cell morphology, ultrastructure and content are seen in dormant bacterial cells. Lennon & Jones (2011) list nine phenotypic characteristics of dormant microorganisms, many of which apply to resting stages of phytoplankton: physical differentiation, altered quantity and composition of lipids and fatty acids, changes in internal cell structure, accumulation of storage compounds, changes in the stoichiometry of elements, altered composition of proteins or amino acids. Others include reduced DNA and RNA content, and a less-energized cell membrane, which have not yet been investigated in phytoplankton resting stages.

(*c*) *Metabolic activity*

Coleman (1983) stated that both photosynthesis and respiration were not detectable in chlorophyte hypnozygotes. In akinetes of *Aphanizomenon* photosynthesis rates are 7% of those in vegetative cells (Sukenik, Beardall & Hadas, 2007); these authors note that low levels of metabolic activity are reported in other studies of cyanobacteria akinetes. Studies on diatoms reported respiration in resting stages of less than 20% and photosynthesis levels of less than 4% of those in the active stages (Kuwata *et al.*, 1993). In the dinoflagellate *Scrippsiella trochoidea* the respiration rate of cysts was initially approximately 10%, then reducing to about 1.5% of the vegetative rate, largely at the expense of carbohydrate reserves (Binder & Anderson, 1990). If these rates remain constant *Scrippsiella trochoidea* cysts would deplete their carbohydrates after 240 days, thus carbohydrate reserves alone cannot explain the long-term survival reported for cysts of this species (*ca.* 40 years; Lundholm *et al.*, 2011). Kamp *et al.* (2011) showed that diatoms can respire nitrate under dark/anoxic conditions, but this was not explored over longer (months to years) timescales. Rengefors, Anderson & Pettersson (1996) reported uptake of phosphorus by *Scrippsiella* cysts, speculating that accumulating this while in the phosphorus-rich environment of the sediment could give them a competitive advantage upon germination. Lirdwitayaprasit *et al.* (1990) reported higher levels of phosphorus in newly formed cysts than in vegetative cells of *Scrippsiella trochoidea*. By contrast, Oku & Kamatani (1995) reported that cultures with higher proportions of resting spores of *Chaetoceros pseudocurvisetus* had lower levels of most pools of phosphorus per cell.

(*d*) *The metabolism of survival*

As described above, resting stages exhibit different degrees of metabolic suppression, enabling them to persist as more or less inactive stages for prolonged periods. In an historical overview, Withers & Cooper (2010) discussed the characteristics and terminology of different types and degrees of metabolic suppression (mainly providing examples from vertebrates, and a few invertebrates). Here, we discuss how some of these terms may apply to the metabolic status of phytoplankton resting stages. Results from short-term studies of metabolic depression in resting stages fail to explain how these can remain viable for many decades and even for up to a century. For chlorophyte hypnozygotes (Coleman, 1983), akinetes of cyanobacteria (Sukenik *et al.*, 2007), resting spores of *Chaetoceros* diatoms (Kuwata *et al.*, 1993) and cysts of the dinoflagellate *Scrippsiella trochoidea* (Binder & Anderson, 1990), respiration rates are on the order of 10% of the vegetative stages, which would deplete carbohydrate storage within months. Therefore, how can we explain the century-long survival times reported for some species?

Dormancy can last from under a day up to several years. It is pre-emptive and is therefore likely to involve intrinsic controls (Withers & Cooper, 2010), although some authors divide dormancy into quiescence and mandatory dormancy (in some organisms called diapause), the former being exogenously controlled and the latter endogenously (Radzikowski, 2013). Some species may even exhibit secondary or recurrent dormancy. Studies of animals undergoing some type of dormancy showed metabolic rates reduced to 10–60% of normal resting metabolic rates (Withers & Cooper, 2010), thus in the same range as for phytoplankton resting stages. Withers & Cooper (2010) term all types of dormancy 'hypometabolism', and contrast this with 'ametabolism' that is characteristic of cryptobiosis. In ametabolic states, the metabolic rate is depressed to less than 5%, and may approach zero in some species (Withers & Cooper, 2010), although the underlying mechanisms are as yet unclear. One form of cryptobiosis is anoxibiosis, which is characterized by survival at very low oxygen levels. As most aquatic sediments are anoxic, at least below the top few centimetres, it is possible that phytoplankton resting stages enter at some point into anoxibiosis. Clegg (1997) studied encysted embryos of the crustacean *Artemia franciscana* Schlosser and found evidence for some metabolic activity during the first days of anoxia, with no detectable metabolic activity after this for a period of 4 years. Similar processes may occur in phytoplankton resting stages and studies measuring the metabolism of phytoplankton stages kept under anoxic conditions are clearly needed. Whether resting stages go into complete metabolic shut-down or whether they acquire energy from sources other than carbohydrates remains unknown.

(*e*) *Lipid and protein/amino acid composition.*

Seeds of some species exhibit modifications of membranes and lipid composition, which can affect longevity (Long *et al.*, 2015). Changes in fatty acid composition have likewise been reported in diatom resting stages (Table 3). In *Thallasiosira pseudonana*, the proportion of polar lipids increased and this was suggested to constitute an advantage for rapid restoration of photosynthetic and cell membranes upon germination (Zhukova, 2004). In seeds, cellular damage can accumulate during drying which must be repaired upon rehydration; in addition, seeds can suffer oxidative damage. Thus proteins for DNA repair and repair of oxidative damage, such as DNA ligases and heat shock factors may be induced and can be important determinants of seed longevity (Long *et al.*, 2015). In the diatom *Chaetoceros pseudocurvisetus* (Oku & Kamatani,

1999) and in the dinoflagellate *Scrippsiella lachrymosa* (Persson *et al.*, 2016) changes in the xanthophyll cycle, which is also a repair mechanism, were reported in resting stages. In cyanobacteria, upregulation of anti-oxidative machinery [and reductions in reactive oxygen species (ROS) levels] were seen in mature akinetes (Kaplan-Levy, Hadas & Sukenik, 2015). However, the potential presence of repair mechanisms has been little studied in phytoplankton resting stages. Such research could be a fruitful way forward towards understanding the mechanisms behind long-term survival.

According to Long *et al.* (2015) protective sugars (see Section III.2*b*) and secondary metabolites may play also central roles in long-term persistence of terrestrial seeds. To our knowledge, these have not been investigated in phytoplankton resting stages.

(3) Phylogeny

Long *et al.* (2015) discuss whether the capacity for long-term persistence in terrestrial seeds is selectively present in some phylogenetic lineages. This does indeed seem to be the case; however, phylogeny is probably a strong correlate of persistence simply because some factors that are important for seed resistance (e.g. features of seed morphology) are more common within particular families (Long *et al.*, 2015). However, as phylogeny appears to be correlated with persistence we examined phylogenetic patterns in aquatic resting stages of phytoplankton known to have long-term viability. The few data available indicate that long-term viability is indeed concentrated in a few lineages for each group of organisms. This may (at least in the cyanobacteria and centric diatoms) be because only some lineages produce certain types of resting stages, i.e. that long-term viability requires specific types of resting stages, such as akinetes or resting spores, and it is this trait which is phylogenetically determined. Within the diatoms all species shown to exhibit viability of longer than a decade are centric species within the Coscinodiscophyceae (*Aulacoseira*), the Mediophyceae (*Skeletonema*, *Stephanodiscus* and *Detonula*) and Chaetoceraceae (*Chaetoceros*). Within the dinoflagellates, *L. polyedrum*, *P. reticulatum* and *Alexandrium* spp. belong to Gonyaulacales, while *Scrippsiella* and *Pentapharsodinium* belong to the Peridiniales. As the deep nodes of phylogenetic trees in both of these groups are as yet not well supported and topologies differ depending on which genes are studied (e.g. Theriot *et al.*, 2010; Guo *et al.*, 2015) it is not possible at this stage to plot unequivocally the potential for long-term viability onto a phylogeny. However, Hargraves & French (1983) plotted the occurrence of spores onto a then-current phylogeny of diatoms, attributing a common occurrence of spores to be a feature of the families Thalassiosiracea and Melosiraceae. In the cyanobacteria, akinete-producing species belong to the Nostocales and Stigonematales (Kaplan-Levy *et al.*, 2015) and in the Chlorophytes the non-filamentous hypnozygote or akinete-producing species mainly belong to the Volvocales (Coleman, 1983). Several species of diatoms and dinoflagellates known to have century-long persistence in the sediment record (e.g. *Skeletonema marinoi* and *Protoceratium reticulatum*) are cosmopolitan species and it is interesting to speculate whether this capacity for long-term viability is one of the reasons for their ubiquity. The dinoflagellates *Pentapharsodinium dalei* and *Lingulodinium polyedrum* are likewise relatively widespread (Zonneveld *et al.*, 2013) with long-term viability in the sediment.

Long *et al.* (2015) explored whether the capacity for long-term persistence in terrestrial seeds is linked to seed production levels, but failed to establish this. There are very few studies investigating resting stage production levels *in situ*. Dale (1983) presented estimates of ratios of cysts to motile stages for three species of dinoflagellates [*P. reticulatum*, *Protoperidinium oblongum* (Aurivillius) Parke & Dodge in Parke & Dixon, and *Gonyaulax digitalis* (Pouchet) Kofoid]. Of these, only *P. reticulatum* is known to exhibit long-term viability.

(4) Environmental factors

(*a*) *Light*

Due to light attenuation with water depth as well as burial in the sediment, resting stages with long-term viability will *in situ* experience darkness or very low levels of light. A similar caveat applies to seeds buried in soil as light typically only penetrates 1 mm to 1 cm into the soil, depending on soil type (Long *et al.*, 2015). However, before they are buried, the quantity and quality of light can affect germination and dormancy in seeds (Long *et al.*, 2015).

Hargraves & French (1983) found that darkness prolonged survival times of diatom spores in laboratory studies, but most studies on the effects of light describe its central role in the germination of phytoplankton resting stages rather than their longevity. In many studies darkness completely inhibits germination of resting stages (in cyanobacteria, diatoms and green algae) or reduces germination frequency (in cyanobacteria, diatoms, green algae and dinoflagellates; Agrawal, 2009). Some species need a change in light conditions to induce germination (Agrawal, 2009). Anderson, Taylor & Armbrust (1987) showed that light requirements for germination differed among five species of dinoflagellates. *Lingulodinium polyedrum* (*Gonyaulax polyedra*) required light to germinate, three other species, including a *Scrippsiella* species, germinated more rapidly in light and one species germinated at comparable rates in light and dark. Kremp & Anderson (2000) reported reduced germination in the dinoflagellate *Scrippsiella hangoei* (J.Schiller) J. Larsen in the dark. Binder & Anderson (1986) found that *Scrippsiella trochoidea* required light to germinate, although low (non-photosynthetic) levels sufficed, and green light was most effective.

(*b*) *Temperature*

In seeds, temperature and humidity interact and, in general, higher levels and longer durations of both decrease persistence (Long *et al.*, 2015). Temperature cycling and modifications are used to break dormancy in seeds of cultivated plants (reviewed by Tiwari, Tiwari & Prasad, 2016). Temperature plays a central role in life-cycle

transitions in many species of phytoplankton and therefore temperature conditions can influence whether dormancy is maintained. Many phytoplankton species have specific temperature optima for both growth and germination. Outside this germination temperature window, a resting stage will not germinate; the window is, however, very variable and species dependent. Many species require a change in temperature to induce germination (Anderson, 1980; Agrawal, 2009) and will remain in the cyst stage at constant temperatures. Kremp & Anderson (2000) reported suppression of germination in *Scrippsiella hangoei* at non-optimal temperatures.

There are few reports of the effect of temperature on long-term viability. Hargraves & French (1983) reviewed studies of survival times of resting spores of diatoms in laboratory studies and found that they tended to survive for longer at colder temperatures. The same result was found by McQuoid & Hobson (1996). The effect of temperature on the potential for long-term viability *in situ* has not been systematically investigated, but in marine settings, viability of diatoms and dinoflagellates for up to a century has been reported in cold-temperate settings in the Baltic (Härnström *et al.*, 2011; Lundholm *et al.*, 2011), North Washington state (Feifel *et al.*, 2015) and northern Japan (Miyazono *et al.*, 2012). Our own data indicate that the same approximate maximal viability is found in Arctic settings (S. Ribeiro, S. Hardardottir, M. Ellegaard, T. J. Andersen & K. Rengefors, in preparation).

It is common practice in phytoplankton resting stage research to store sediment samples in dark and cold conditions, to suppress degradation and early germination (e.g. Ellegaard *et al.*, 2013*b*).

(*c*) *Oxygen*

Oxygen seems to be a requirement for germination for most species in both seeds and planktic resting stages. Long *et al.* (2015) report that germination of seeds is typically inhibited in hypoxic or anoxic environments; rice is one of the few plants that can germinate under anoxic conditions (Mattana *et al.*, 1996). Starchy seeds are able to germinate at lower partial oxygen pressures than fatty seeds (Al-Ani *et al.*, 1985; Raymond, Al-Ani & Pradet, 1985). Because fermentation is dependent on the availability of carbohydrates and because lipid respiration consumes more oxygen than carbohydrate catabolism (Kolb & Joly, 2010, and references therein), carbohydrates, rather than lipids, would appear to be the most appropriate substrates for anoxic respiration.

Anoxia, or low oxygen levels, seems to play a central role in long-term viability, at least in marine settings. A rapid decline in viability was reported for cysts of the dinoflagellate *Lingulodinium polyedrum* after the sediment core was sliced and the sediment exposed to oxygen. In spite of the cysts having preserved *in situ* viability for approximately 40 years, no germination occurred after 6 months of sediment storage in plastic bags, even though these were kept cold and in the dark (Lundholm *et al.*, 2011; Ellegaard *et al.*, 2013*b*). The possibility that this is an indication of anoxibiosis (see Section III.2*d*) should be explored further. The germination of cysts of several dinoflagellate species has been reported to be completely inhibited by anaerobic conditions, including *Scrippsiella hangoei* (Kremp & Anderson, 2000) and *L. polyedrum* (Anderson *et al.*, 1987).

Kamp *et al.* (2011) suggested that nitrate reduction is used by diatoms to fuel the energy-demanding transition from the vegetative to the resting stage, and linked the ability to store nitrate intracellularly to the long-term survival of diatoms. Rice seeds similarly have been shown to reduce nitrate during germination (Mattana *et al.*, 1996).

Hydrogen sulphide is a highly toxic compound which is often found in anoxic marine sediments (Kasten & Jørgensen, 2000) and results from the bacterial reduction of sulphate, which is available in sea water at much higher concentrations than in fresh water. There is some evidence for differential sensitivity to sulphide among microalgae, e.g. for metabolically active, benthic, pennate diatoms tolerance to sulphide is a factor determining distribution (Admiraal & Peletier, 1979), whereas high sulphide concentrations such as those found in the Danish Mariager Fjord appear not to inhibit long-term survival of *Skeletonema marinoi* resting spores (>80 years; Härnström *et al.*, 2011). By contrast, temporary exposure to high hydrogen sulphide concentrations has been reported permanently to reduce the germination potential of *Scrippsiella hangoei* resting cysts (Kremp & Anderson, 2000).

(*d*) *Predation*

Predators may influence seed distribution through dispersal and selectivity, and passage through the gut may trigger germination (Long *et al.*, 2015). Two studies have tested the effect of passage through invertebrates on the germination and viability of dinoflagellate cysts. Kremp, Shull & Anderson (2003) reported no or a positive effect of passage through polychaete guts on germination of the dinoflagellate *Scrippsiella lachrymosa*. Montresor, Nuzzo & Mazzocchi (2003), in two species of dinoflagellates, *Scrippsiella trochoidea* and *S. ramonii* M. Montresor, and four copepod species, found unchanged germination after passage for one dinoflagellate species and two copepods with no viability for the other combinations. These studies clearly indicate that predation-related factors may either promote or inhibit long-term viability of phytoplankton resting stages, and that these effects are likely to be species-specific.

IV. ECOLOGICAL, EVOLUTIONARY AND MANAGEMENT IMPLICATIONS

(1) Ecology and evolution

(*a*) *Population dynamics and diversity*

The maintenance of a seed (or egg or spore) bed is often hypothesized to constitute a bet-hedging strategy. Long-term resting stages can be viewed as an insurance against an unpredictable future: the sacrifice of short-term fitness when

only a fraction of dormant stages resume development immediately when beneficial conditions are restored is compensated by persistence through longer periods of adverse conditions (e.g. Cáceres & Tessier, 2003). Thus, although the production of resting stages, many of which will never germinate, means loss of genetic material in the short term, it may allow long-term gains. Bet-hedging can thus be viewed as sacrificing mean fitness for reduced variability in fitness over time, and can be viewed as a strategy for risk-spreading and for preparing for an unpredictable future (Childs, Metcalf & Rees, 2010). In plants, bet-hedging has been studied e.g. in desert plants, which inhabit harsh and unpredictable environments (e.g. Phillipi, 1993). Childs *et al.* (2010) reviewed evidence for bet-hedging in a range of plant systems concluding that there is abundant empirical evidence for bet-hedging in simple plant systems, such as annual plants, with seed banks. In aquatic systems, bet-hedging strategies have been proposed mainly for zooplankton. Cáceres & Tessier (2003) in an elegant experiment to test the bet-hedging hypothesis for *Daphnia pulicaria* Forbes resting eggs in lakes, employed reciprocal transplants between lakes and studied hatching series. It proved difficult to disentangle the many factors interacting in germination: genetic factors, maternal effects, environmental conditions, and water depth. García-Roger, Serra & Carmona (2014) reviewed evidence for bet-hedging in rotifer populations. Bet-hedging strategies have not been specifically tested in phytoplankton, but bet-hedging theory (see e.g. Childs *et al.*, 2010; Tielbörger, Petrů & Lampei, 2012) may be applied to phytoplankton populations and studies similar to that of Cáceres & Tessier (2003) could help us to understand the role of seed banks for resting-stage-forming phytoplankton species.

Studies on the temporal dynamics of marine phytoplankton seed banks have shown that these are typically highly diverse (both phenotypically and genetically) and that diversity is generally maintained over several decades (Härnström *et al.*, 2011; Ribeiro *et al.*, 2013; Lundholm *et al.*, 2017). Whether species with resting-stage seed banks show the greatest diversity and resilience is not yet clear, although there is some evidence for this (Kremp *et al.*, 2016; Sundqvist, 2016).

Resting stages are thought to play a central role in seasonal survival. However, the time required for producing, maturing, and depositing dinoflagellate resting cysts and diatom resting spores is too long for these stages to function as very short-term seeding and/or survival strategies (Anderson, 1975; Smayda & Trainer, 2010). This, and the lengthy survival capability discussed above, leads us to consider whether these resting stages function primarily in short-term (seasonal) or long-term (years to decades) survival (see also Radzikowski, 2013). For dinoflagellates, many hyponozygote-producing species also produce asexual temporary resting stages (see e.g. Figueroa & Bravo, 2005), which may be more important as short-term survival stages than the resting cysts. Diatoms similarly produce both resting cells and resting spores (Kuwata *et al.*, 1993). Livingstone & Jaworski (1980) studied long-term survival of cyanobacteria akinetes and concluded that they have an overwintering function, but also contribute to long-term survival. Although nutrient stress is often used as a trigger for cyst formation in cultures, studies on both diatoms (see McQuoid & Hobson, 1996) and dinoflagellates (e.g. Kremp, Rengefors & Montresor, 2009) show that resting stages may be formed during conditions of high food availability, questioning their exclusive role as a stress response and short-term survival strategy. Further, many of these resting stages are also produced by species in tropical and sub-tropical environments (e.g. Härnström *et al.*, 2007), where seasonal shifts in environmental conditions are much less pronounced than in temperate and polar regions. Together, these observations suggest that resting stages have adaptive functions other than seasonal survival, and may have long-term evolutionary significance.

(*b*) *Long-term evolutionary significance*

Resting stages are found in many groups of organisms and have been hypothesized to have a central role in the ancient evolution of eukaryotes (Cavalier-Smith, 2002). Some authors have hypothesized that the life cycle typical of e.g. cyst-forming dinoflagellates, some green algae and chrysophytes, characterized by shifts between a haploid, rapidly dividing, metabolically active stage and a diploid, resistant diploid resting stage, originated very early in the evolution of eukaryotes, and was possibly even a prerequisite for further eukaryote evolution (Walther, 2000; Cavalier-Smith, 2002).

The long-term maintenance of survival and diversity in the resting-stage seed bank has evolutionary implications, with resting stages playing an important role in marine ecosystem resilience and the recovery of populations from abrupt environmental change. Many coastal phytoplankton species form resting stages whereas open ocean groups generally do not (e.g. Dale, 1996); this may be explained by the fact that newly germinated phytoplankton cells are dependent on reaching the photic zone for survival, which is unlikely for resting stages transported into deep ocean sediments. Resting stages have thus been hypothesized as the factor determining the difference between the high survival rate of coastal phytoplankton groups relative to the extinction of many oceanic phytoplankton species at the Cretaceous–Paleogene mass extinction about 65 million years ago (Kitchell, Clark & Gombos, 1986; Ribeiro *et al.*, 2011).

A central role for resting stages in survival and persistence over evolutionary timescales has been argued for both diatoms (Kitchell *et al.*, 1986) and dinoflagellates (Ribeiro *et al.*, 2011). Ribeiro *et al.* (2011) showed that strains germinating from up to 100-year-old cysts had the same capacity for growth as from cysts deposited in surface sediments, i.e. growth capacity was intact even after a century.

Kremp *et al.* (2016) studied the genotypic and phenotypic diversity of the dinoflagellate *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen cyst pools from the Baltic Sea under present and predicted future climate scenarios (temperature and salinity), and concluded that genotypic shifts occurred

rapidly in response to environmental change. This study provided strong experimental evidence that cyst beds aid species survival in the face of environmental and climatic change (Kremp *et al.*, 2016).

(*c*) *Dispersal vectors*

Passive dispersal is by far the most important means of long-distance transport of phytoplankton species. Microalgal resting stages can be dispersed *via* water currents, by wind (see Tesson *et al.*, 2016 for a review on airborne microalgae), or mediated by animals such as humans, insects, fish, mammals, and birds. Aquatic birds may transport resting stages both internally (digestive tract) and externally (feet and feathers) over long distances and are important vectors for the dispersal of freshwater microalgae (e.g. Kristiansen, 1996). Schlichting (1960) studied 205 individuals of waterfowl and found green algae, cyanobacteria, and diatoms (86 species of microalgae on the feet of the birds, 25 on the feathers, and 25 on the beaks). Spores and cysts, as well as cells producing extracellular mucus, proved to be more resistant to desiccation.

Several resting-stage-forming phytoplankton species have expanded their geographic ranges during the past few decades. For harmful species (those associated with toxicity and/or blooms leading to degradation of water quality), this is an alarming trend and the role of resting stages in aiding species dispersals has been investigated. For the toxic marine dinoflagellate *Gymnodinium catenatum* H.W.Graham, transport of cysts in the ballast water tanks of ships appears to be the most likely mechanism for introduction of the species into Australia (Bolch & de Salas, 2007), whereas in the northeast Atlantic (Portugal and Spain), evidence from cyst records suggests that it has undergone a natural range expansion, likely from northwest Africa, associated with sea-surface warming (Ribeiro *et al.*, 2012). The cyst-forming freshwater raphidophyte *Gonyostomum semen* has recently expanded its range and is now considered invasive in Sweden, Norway and Finland (Lebret *et al.*, 2015). Population genetic studies have shown that hydrological transport is not significant in this species; populations are genetically structured and do not follow a simple geographic distance model (Sassenhagen *et al.*, 2015). It is likely that the transport of cysts by animal vectors aids the expansion of this species into new areas, as global temperature increases mean that conditions become more favourable for this raphidophyte (Rengefors, Weyhenmeyer & Bloch, 2012).

(2) Resting stages as carbon sinkers

Phytoplankton resting stages may also provide an important ecosystem service in marine carbon cycling, notably in transport of carbon to the deep sea as carbon sinks and in carbon storage in coastal sediments. Rynearson *et al.* (2013) studied the contribution of *Chaetoceros aff. diadema* (Ehrenberg) Gran cysts to sediment traps deployed at 750 m depth in the North Atlantic, where they constituted 35–95% of the cell flux. Factors such as the weight of diatom spores (due to heavier silicification) and the presence of spines or mucous production (see Table 2) facilitate the formation of aggregates in several species (e.g. Anderson *et al.*, 1985; Smetacek, 1985).

Phytoplankton resting stages are mainly produced by coastal species. Coastal habitats cover less than 20% of the total ocean area, but account for approximately half of the total carbon sequestered in ocean sediments. Aquatic seed banks are therefore likely to represent an important carbon stock in coastal environments, but this remains largely unquantified.

(3) Management implications

For terrestrial seeds there are very clear management interests in studying long-term persistence, notably in the maintenance of human-driven seed banks and issues of weed management in agriculture and conservation (Long *et al.*, 2015). In the case of phytoplankton, long-term viability may also have implications for management. There is the potential for resuspension of resting stages of connection during engineering of harbours and piers as well as during fishery-related disturbances of coastal areas, such as trawling. There are clear parallels with changes in land use in terrestrial ecosystems such as forestry, agriculture and deforestation. Of particular concern is the resuspension of cysts of toxic species. The dinoflagellate genus *Alexandrium* includes many toxic species and is one of the groups with extended survival (e.g. Feifel *et al.*, 2015). Other prominent toxin producers, such as the dinoflagellate *Gymnodinium catenatum* also produce resistant resting cysts. Another exciting research area involving the long-term survival of phytoplankton resting stages is testing their resilience to vacuum and low temperatures to explore their potential use in space travel and planet colonization (e.g. Olsson-Francis *et al.*, 2009, on cyanobacterial akinetes).

V. CONCLUSIONS

(1) Phytoplankton resting stages are characterized by the accumulation of storage products in the form of grains of starch, lipids, or unknown 'electron dense' or 'granular' materials, and have maximal *in situ* survival times on the order of a century. Long-lived resting stages are typically colourless, rounded, with varying ornamentation and mucilage, and thick and multi-layered walls.

(2) Research into factors that are central to seed dormancy and long-term persistence, but have rarely been tested in phytoplankton resting stages may lead to new insights into the regulation of resting-stage dormancy. (*a*) Hormonal balance is important for regulating germination in seeds and some hormones (notably abscisic acid and melatonin) may play a role in life-cycle transitions in phytoplankton resting stages. Further characterization of hormone levels in different life-cycle stages (of different ages) and the effects of these hormones should lead to new insights into phytoplankton resting-stage dormancy and persistence. (*b*) In seeds, long-term persistence is restricted to certain lineages.

Likewise, long-term persistence in diatom, dinoflagellate, cyanobacteria and chlorophyte resting stages appears to be concentrated in a limited number of lineages. This should be explored further by specifically targeting resting stages in other lineages.

(3) Metabolism in the early stages of dormancy is approximately 10% of that of the active stage. This would deplete carbohydrate reserves after about half a year; therefore other mechanisms must be present to allow longer-term dormancy. Future studies should: (*a*) investigate the utilization of alternative sources for respiration, such as lipids or nitrogenous compounds; and (*b*) determine whether these stages enter complete metabolic shut-down or anoxibiosis. Studies on zooplankton egg survival under anoxic conditions may provide a template for such work (Clegg, 1997).

(4) Seeds exhibit structural and metabolic modifications that affect longevity and function as repair mechanisms. In phytoplankton resting stages a few studies indicate that similar mechanisms may be present, but this requires further investigation. Analyses of the regulation of anti-oxidation machinery (such as that known in cyanobacteria), of membranes and lipid composition, and of the xanthophyll cycle are promising avenues for research.

(5) Theory on bet-hedging should be applied to phytoplankton populations and studies similar to those on zooplankton eggs should be applied to resting-stage-forming phytoplankton species. The suggestion that species producing resting stages show the greatest diversity also deserves further attention.

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