

Zoosporic true fungi in marine ecosystems: a review

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Abstract. Although many species of zoosporic true fungi have been frequently observed and studied in freshwater and soil ecosystems, only three species have been properly identified and partially characterised from brackish and marine ecosystems, namely *Rhizophydium littoreum* Amon, *Thalassochytrium gracilariopsis* Nyvall, Pedersén et Longcore and *Chytridium polysiphoniae* Cohn. These species are either facultative or obligate parasites of marine macroalgae and invertebrates. Also, some species of *Olpidium* and *Rhizophydium* are parasites of small marine green algae and diatoms. Although the physiological effects of these pathogens on the growth and metabolism of their hosts are poorly understood, parasitism by *C. polysiphoniae* possibly affects the rates of photosynthesis and patterns of growth in infected communities of brown algae. Saprobic ecotypes of *R. littoreum* can also colonise dead-plant and animal substrates. Zoospores from zoosporic true fungi and other groups of microbes possibly provide important food resources for grazing and filter-feeding zooplankton and metazoans in marine ecosystems where the prevalence of disease is high or where accumulated detritus enhances biodiversity in food webs. However, quantitative studies have not yet been attempted. Recently, environmental sampling with molecular techniques has revealed unknown clades of zoosporic true fungi in extreme marine ecosystems. These fungi have been grossly under-sampled and under-studied in marine environments.

Additional keywords: *Chytridiomycota*, *Chytridium*, chytrids, food webs, marine algae, *Olpidium*, *Rhizophydium*, salinity, *Thalassochytrium*.

Introduction

Most of the commonly known species of true fungi belong to the zygomycetes, ascomycetes and basidiomycetes. These species produce non-motile airborne spores and have adapted to terrestrial ecosystems. However, many species of ascomycetes have adapted to freshwater and marine ecosystems and produce waterborne spores (Shearer *et al.* 2007). Zoosporic true fungi (commonly called chytrids) include a very large and diverse group of fungi that produce motile spores (zoospores) and require water to complete their life cycles. These fungi have frequently been observed growing as saprobes or parasites in many freshwater and soil ecosystems (Sparrow 1960; Powell 1993; Barr 2001; Shearer *et al.* 2007).

In contrast, only a small number of species of zoosporic true fungi have been reported in brackish and marine ecosystems

(Sparrow 1960; Johnson and Sparrow 1961; Porter and Kirk 1987; Shearer *et al.* 2007). In particular, a few species belonging to the genera *Rhizophydium* and *Olpidium* and one species in the genus *Chytridium* have been characterised tentatively as marine ecotypes by Sparrow (1960) and Johnson and Sparrow (1961). Many other species in these genera are very common inhabitants of freshwater and soil ecosystems (Sparrow 1960). More recently, Nyvall *et al.* (1999) described a new marine species of *Thalassochytrium*. Also, there are several brief reports of other estuarine and marine species that have not been properly characterised.

The zoospores of most true fungi are characterised by a single posteriorly directed whiplash flagellum (Barr 2001). At present, most genera of the zoosporic true fungi are placed into three phyla, the Blastocladiomycota, Chytridiomycota and

Neocallimastigomycota, whereas some genera such as *Olpidium* and *Rozella* have not yet been assigned to any phyla (James *et al.* 2006b). The orders within the Chytridiomycota have undergone considerable reorganisation recently, on the basis of sequences of rRNA genes and the ultrastructure of zoospores (Letcher *et al.* 2008a, 2008b; Mozley-Standridge *et al.* 2009; Wakefield *et al.* 2010).

The mode of nutrition is considered to be an important ecological aspect of microbes, i.e. whether they are parasites, saprobes or mutualists. In freshwater and soil ecosystems, most species of zoosporic true fungi are thought to be saprobes (Powell 1993; Shearer *et al.* 2007; Gleason *et al.* 2008) and also a large number of species are parasites of phytoplankton (Sparrow 1960; Kagami *et al.* 2007; Gutman *et al.* 2009). Some species are parasites of higher plants (Sparrow 1960; Powell 1993), invertebrate and vertebrate animals (Barron 2004; Gleason *et al.* 2010) and other fungi (Sparrow 1960; Powell 1993). Recently, there has been much research on the pathology of *Batrachochytrium dendrobatidis* which has been implicated in the recent decline in populations of numerous amphibians worldwide (Hyatt *et al.* 2007; Goka *et al.* 2009). Finally, some species are mutualists in the anaerobic digestive systems of vertebrate animals (Trinci *et al.* 1994; Mackie *et al.* 2004; Rezaeian *et al.* 2004; Liggenstoffer *et al.* 2010).

In general, not much is known about zoosporic true fungi living in brackish and marine environments. Only three ecotypes of marine zoosporic true fungi have been properly identified and characterised. These are species of *Rhizophyidium* (Amon 1984), *Thalassochytrium* (Nyvall *et al.* 1999) and *Chytridium* (Küpper and Müller 1999; Müller *et al.* 1999), which are either facultative or obligate parasites of macro-algae and invertebrates. Preliminary data suggest that some species of *Olpidium* and *Rhizophyidium* are parasites of marine green algae and diatoms (Raghukumar 1987; Elbrächter and Schnepf 1998; Hanic *et al.* 2009). All four genera have been placed in the phylum Chytridiomycota and are discussed in the present review (Table 1). The phylogenetic relationships of these ecotypes to other members of this phylum are not fully understood because of the lack of molecular data.

In contrast, many species of heterotrophic stramenopiles (Oomycota, Hyphochytriomycota and Labyrinthulomycota) and higher fungi have been reported frequently in marine ecosystems, and it is widely assumed that some of these microbes play ecological roles in the marine environment

similar to those of zoosporic true fungi and higher fungi in freshwater and soil ecosystems (Sparrow 1960; Johnson and Sparrow 1961; Amon and Yei 1982; Raghukumar 2002; Shearer *et al.* 2007; Strittmatter *et al.* 2008). Research on the ecology of the zoosporic true fungi, which are found in marine environments, lags far behind the research on other groups of true fungi and heterotrophic stramenopiles.

Andrews (1976), in his review of the pathology of marine algae, listed many species of heterotrophic stramenopiles and higher fungi but no zoosporic true fungi that are known to be parasites of marine algae. However, more recent reports of the widespread occurrence of zoosporic true fungi as parasites of some common marine algae have suggested that these fungi are much more frequent in marine environments than was previously thought (Küpper and Müller 1999; Müller *et al.* 1999). Furthermore, environmental sampling with molecular techniques has revealed unknown clades of zoosporic true fungi in extreme marine ecosystems (Le Calvez *et al.* 2009). Because of the lack of research on zoosporic true fungi, we conclude that these fungi have been grossly under-sampled in marine environments.

The present review discusses some of the recent research on zoosporic true fungi and their ecological roles in marine ecosystems. Our current knowledge of molecular phylogeny and the general characteristics of the genera of zoosporic true fungi observed in marine and estuarine ecosystems are summarised. Because a clear distinction between freshwater, estuarine and marine ecotypes is difficult, we have included a discussion of the effects of salinity on growth rates in some isolates that have been studied in the laboratory. The evidence that zoosporic true fungi play significant roles in estuarine and marine ecosystems, as they do in freshwater ecosystems, is growing, and will be discussed; however, a full appreciation of the impacts of these microbes awaits further investigation.

Molecular phylogeny

Although there has been much recent work devoted to reconstructing the molecular phylogeny of extant zoosporic true fungi isolated from soil and freshwater ecosystems, there is still very little data on the placement and distribution of marine taxa. To understand the difficulty in characterising the evolutionary relationships of zoosporic true fungi, it is necessary to consider both the historical and modern practices of classifying these microbes.

Table 1. Species of zoosporic true fungi from estuarine and marine ecosystems

Species name	Ecotype	Mode of nutrition	Host genus	Ref.
<i>Rhizophyidium littoreum</i>	Estuarine	Facultative parasite	<i>Bryopsis</i> , <i>Codium</i>	Amon (1984)
<i>Rhizophyidium littoreum</i>	Marine	Facultative parasite	<i>Cancer</i>	Shields (1990)
<i>Thalassochytrium gracilariopsis</i>	Marine	Probable biotroph	<i>Gracilariopsis</i>	Nyvall <i>et al.</i> (1999)
<i>Chytridium polysiphoniae</i>	Marine	Probable biotroph	<i>Pyaiella</i> , other genera	Küpper and Müller (1999)
<i>Olpidium rostriferum</i> , <i>Olpidium</i> sp.	Marine	Probable biotroph	<i>Cladophora</i> , <i>Pseudo-nitzschia</i>	Raghukumar (1987), Elbrächter and Schnepf (1998)
<i>Rhizophyidium</i> sp.	Marine	Probable biotroph	<i>Pseudo-nitzschia</i> , <i>Bellerochea</i> , <i>Cylindrotheca</i>	Hanic <i>et al.</i> (2009), Elbrächter and Schnepf (1998)
Unknown (obligate anaerobes)	Marine	Mutualist	<i>Endocardium</i> , <i>Amblyrhynchus</i>	Thorsen (1999), Mackie <i>et al.</i> (2004)

Classically, zoosporic true fungi (i.e. the Chytridiomycetes before James *et al.* 2006b) were described according to modes of reproduction, thallus development and ecology (reviewed in Blackwell *et al.* 2006) and, most importantly, morphological characteristics of the thallus. Among the morphological characters used to diagnose species were size, shape and ornamentation of the sporangia, presence or absence of an operculum, and rhizoidal arrangement. Most of the described species were circumscribed primarily on the basis of morphology, often in the absence of thallus development data (Sparrow 1960). However, numerous culturing studies have demonstrated that the thalli of these fungi exhibit morphological plasticity that can be influenced or induced by substrate, light intensity and pH (Haskins and Weston 1950; Paterson 1963; Miller 1976; Powell and Koch 1977a, 1977b). These findings, coupled with the often-scant records accompanying descriptions of marine species, cast doubt on the validity of taxonomic groupings and placement of some delineated marine taxa. Further complicating early efforts to resolve the systematic and taxonomic affinities of true marine species was the use of 'chytrid' as an umbrella term encompassing diverse and divergent organisms possessing similar morphologies and lifestyles (Sparrow 1960).

The introduction of electron microscopy to the study of the ultrastructure of zoospores established a new suite of ostensibly stable ultrastructural characters, both quantitative and qualitative, by which these fungi could be classified and their relationships predicted. Preliminary steps towards revising systematics using subcellular architecture of the zoospore focussed on terrestrial (Spizellomycetales: Barr 1980) and freshwater taxa. Because culturing is necessary to amass the volume of zoospores required for ultrastructural analysis, few marine taxa are available for study. However, for marine zoosporic true fungi that can be successfully maintained in culture, ultrastructural analysis has proven to be a powerful tool for taxonomic reassessment. For example, examination of the zoospores of two marine isolates preliminarily assigned to the genus *Phlyctochytrium* suggested that these isolates were likely to be conspecifics, with ultrastructural characteristics previously observed in members of the genus *Rhizophydium*. These isolates were subsequently described as a new species, *Rhizophydium littoreum* (Kazama 1972a; Amon 1984). Ultrastructural analysis of another marine species, *Thalassiochytrium gracilariopsisidis*, revealed a zoospore architecture that has yet to be observed in any other taxa (Nyvall *et al.* 1999).

Although comparative ultrastructure provides a foundation for higher-order groupings of chytrid taxa, zoosporic characters have limited utility in distinguishing among taxa at the generic and species levels. With the advent of molecular phylogenetic methods, however, it is possible to reconstruct the evolutionary relationships of extant taxa with increasing confidence and resolution. In addition to allying zoosporic lineages to the higher fungi (Förster *et al.* 1990; Bowman *et al.* 1992), molecular analyses have also demonstrated the breadth of diversity within the zoosporic true fungi. During the past decade, most of the once taxonomically problematic zoosporic true fungi have been resolved into the following three phyla: Blastocladiomycota (James *et al.* 2006b), Neocallimastigomycota and Chytridiomycota (Hibbett *et al.* 2007). Combined molecular and ultrastructural analyses have resulted in the erection of the following four new orders within the 'core chytrid clade' (James *et al.* 2006a,

2006b) of the Chytridiomycota: Rhizophydiales (Letcher *et al.* 2006), Rhizophlyctidales (Letcher *et al.* 2008a), Lobulomyceales (Simmons *et al.* 2009) and Cladochytriales (Mozley-Standridge *et al.* 2009). Additionally, two provisional orders ('Polychytriales' and 'Synchytriales') are currently undergoing taxonomic revision.

Despite increased efforts to resolve the phylogeny and phylogenetic diversity of this group of microbes, very few marine taxa have been included in previous studies. The relative absence of marine representatives from published phylogenies is again a consequence of both the difficulty inherent in culturing these organisms and the lack of marine surveys of their biodiversity. The only circumscribed marine zoosporic true fungi whose phylogenetic placement has been determined are *Rhizophydium littoreum* (Letcher *et al.* 2006, 2008b) and *Chytridium polysiphoniae* (James *et al.* 2006a; Küpper *et al.* 2006; Simmons *et al.* 2009).

Marine and estuarine ecotypes

Rhizophydium littoreum (estuarine ecotype)

Rhizophydium littoreum is a monocentric species that has been observed growing on the surface of two common genera of siphonous green algae, *Bryopsis* and *Codium* (Chlorophyta), in estuaries. Four variants or morphotypes of this fungus have been isolated into pure culture from different locations; however, they were subsequently placed into the same species (Kazama 1972b; Amon 1976; Porter and Smiley 1980; Amon 1984). Many other species in the genus *Rhizophydium* have been placed recently into new genera by Letcher *et al.* (2008b) in their extensive revision of the order Rhizophydiales. Although ecotypes of *R. littoreum* have been associated with *Bryopsis* and *Codium*, very little is known about their pathology. Furthermore, they have never been found growing on other algae. Because they grow very well in pure culture on chemically defined media, they are probably very weak, facultative parasites or more likely saprobes. Large numbers of zoospores (2–4 µm in diameter) are produced by these fungi when growing on rich media (Amon 1984). Sequence analysis of two rDNA genes confirmed that *R. littoreum* belongs in the order Rhizophydiales (GenBank Accession numbers DQ485540 and 485604) (Letcher *et al.* 2006).

Rhizophydium littoreum (marine ecotype)

Rhizophydium littoreum was observed growing primarily as a saprobe on dead eggs from the yellow rock crab, *Cancer anthonyi* (Shields 1990). However, this fungus also attacks and kills live eggs, although significantly more dead eggs than live eggs are infected. Prevalence of infection ranged from 14 to 52% throughout the year. This fungus grows well in pure culture. For these reasons, it is probably a facultative parasite. The life cycle and pathology were investigated by Shields (1990). This fungus produces large numbers of zoospores, which range in size from 3 to 4 µm in diameter. The yellow rock crab is subtidal so that this ecotype must be able to tolerate undiluted seawater. No mention was made of the presence of siphonous green algae growing in the area where the crabs were collected and which might act as a reservoir for this parasite. However, siphonous green algae are commonly found along this part of the coast of central California.

Thalassochytrium gracilariopsis

Thalassochytrium gracilariopsis is a marine, endosymbiotic, polycentric species that infects cultures of *Gracilariopsis* sp. (Rhodophyta) (Nyvall *et al.* 1999). A yellow discoloration of seawater is caused by a massive release of oval zoospores (~3 µm in diameter). Intensely orange and large endobiotic sporangia (50–300 µm in diameter) are embedded in algal tissues, with only the tips of the discharge tubes protruding. Multinucleate, septate hyphae penetrate between the cells in the tissues of the algal host and form haustoria that penetrate mainly medullary cells. Thalli of the host were not killed by the fungal infection. Thylakoid membranes became disorganised and starch reserves were degraded in infected medullary cells during sporulation of the fungus. Whether this is a true parasite or a mutualist has not been determined; however, the relationship appears to be species-specific. This fungus has never been grown in pure culture, and molecular data are not yet available.

Chytridium polysiphoniae

Chytridium polysiphoniae is a marine, monocentric species that infects *Pylaiella littoralis* and many other species of brown algae (Phaeophyta) (Raghukumar 1987; Küpper and Müller 1999; Müller *et al.* 1999; Küpper *et al.* 2006). The sporangia sit on the surface of the host cells whereas the haustoria penetrate into the cytoplasm of individual host cells (Fig. 1). *C. polysiphoniae* has not been grown in pure culture yet; however, it can be grown in co-culture with *P. littoralis* (Müller *et al.* 1999). Therefore, it is considered to be an obligate parasite or biotroph. Zoospores of *C. polysiphoniae* attack the distal uniseriate parts of polistichous thalli and the filamentous macrothalli of brown algae with heteromorphic life cycles. In culture, individual infected host cells become depleted and eventually die, whereas the host alga will continue to grow. This fungus prefers to infect actively growing uniseriate filaments. Infection experiments were attempted with 48 species of brown algae in culture, and 23 were susceptible to infection by *C. polysiphoniae* (Müller *et al.* 1999). Because this fungus can infect a large number of species from many parts of the world, it may be considered a ubiquitous pathogen (even though this is currently not supported by field records outside the European North Atlantic). Massive epidemics have been observed along the European coast. The prevalence can often exceed 10% of *Pylaiella* thalli at sites in Shetland (Küpper and Müller 1999). Molecular studies have shown that this algal pathogen probably should not be placed within the genus *Chytridium* (Küpper *et al.* 2006). Instead, its 18S rDNA sequence clusters with that of environmental, uncultured, morphologically unknown taxa in a novel clade of the Chytridiomycota (GenBank Accession number AY032608). The zoospores of *C. polysiphoniae* are 3–4 µm in diameter. Interestingly, Martin (1922) reported a similar fungus as a parasite on the red alga *Callithamnion*.

Rumen fungi

Some evidence for the presence of anaerobic, zoosporic true fungi in marine environments has been published by Thorsen (1999) and Mackie *et al.* (2004). The digestive systems of many groups of animals contain populations of microorganisms that contribute to the absorption of nutrients through the gut.

Gut-living microorganisms are known to play significant roles in digestion by producing enzymes that hydrolyse carbohydrates. One group of zoosporic true fungi, the rumen fungi or Neocallimastigomycota, are commonly found in the digestive systems of herbivorous mammals and reptiles and are obligately anaerobic, i.e. they do not grow in the presence of oxygen (Trinci *et al.* 1994; Rezaeian *et al.* 2004; Liggenstoffer *et al.* 2010).

In the sea urchin, *Echinocardium cordatum*, zoosporic true fungi have been observed to make up part of the microflora in the anterior caecum, intestinal caecum and coelomic fluid (Thorsen 1999). Some parts of the digestive system are thought to be relatively anoxic and the microflora to be obligately anaerobic. This species of sea urchin digs and feeds in anaerobic muddy substrates in marine environments. Sea urchins are herbivores and feed primarily on marine algae. Whether the zoosporic true fungi observed in *E. cordatum* are phylogenetically related to rumen fungi found in herbivorous mammals is not yet known.

Mackie *et al.* (2004) reported anaerobic zoosporic true fungi in the digestive systems of the marine iguana *Amblyrhynchus cristatus*. These herbivorous reptiles feed on marine algae and have fermentative digestion processes similar to those in herbivorous mammals. Because the diet of these animals comes exclusively from marine environments, these fungi may be able to grow in seawater.

Olpidium

Raghukumar (1987) recorded *Olpidium rostriferum* as a parasite of *Cladophora* in marine environments in southern India. Elbrächter and Schnepf (1998) reported an unidentified *Olpidium* species as a parasite of the marine diatom *Pseudo-nitzschia*. Several species of *Olpidium* are ubiquitous obligate parasites of the roots of flowering plants (Herrera-Vásquez *et al.* 2009; Hartwright *et al.* 2010) and invertebrates, especially rotifers and nematodes in soil and freshwater (Gorbunov and Kosova 2001; Barron 2004). The species of *Olpidium* found on green algae, diatoms and invertebrates have not yet been properly characterised by molecular methods, and the relationships to the species infecting plant roots are not known.

Other Rhizophyidium species

Elbrächter and Schnepf (1998) reported unidentified *Rhizophyidium* species as parasites of the diatoms *Bellerophon malleus* and *Cylindrotheca closterium*. Hanic *et al.* (2009) reported an unidentified zoosporic true fungus as a parasite of the bloom-forming diatom *Pseudo-nitzschia* in marine environments near Prince Edward Island, Canada. This parasite is probably a *Rhizophyidium* species but it has not been properly characterised. However, many other species of zoosporic true fungi are common parasites of diatoms and other species of phytoplankton in freshwater ecosystems (Kagami *et al.* 2007).

Unknown genera

DNA sequences attributed to zoosporic true fungi have been detected in samples that were collected from various sites in the environment surrounding deep-sea hydrothermal vents in the Mid-Atlantic Ridge and the East Pacific Rise (Le Calvez *et al.* 2009). No species of zoosporic true fungi were isolated into pure

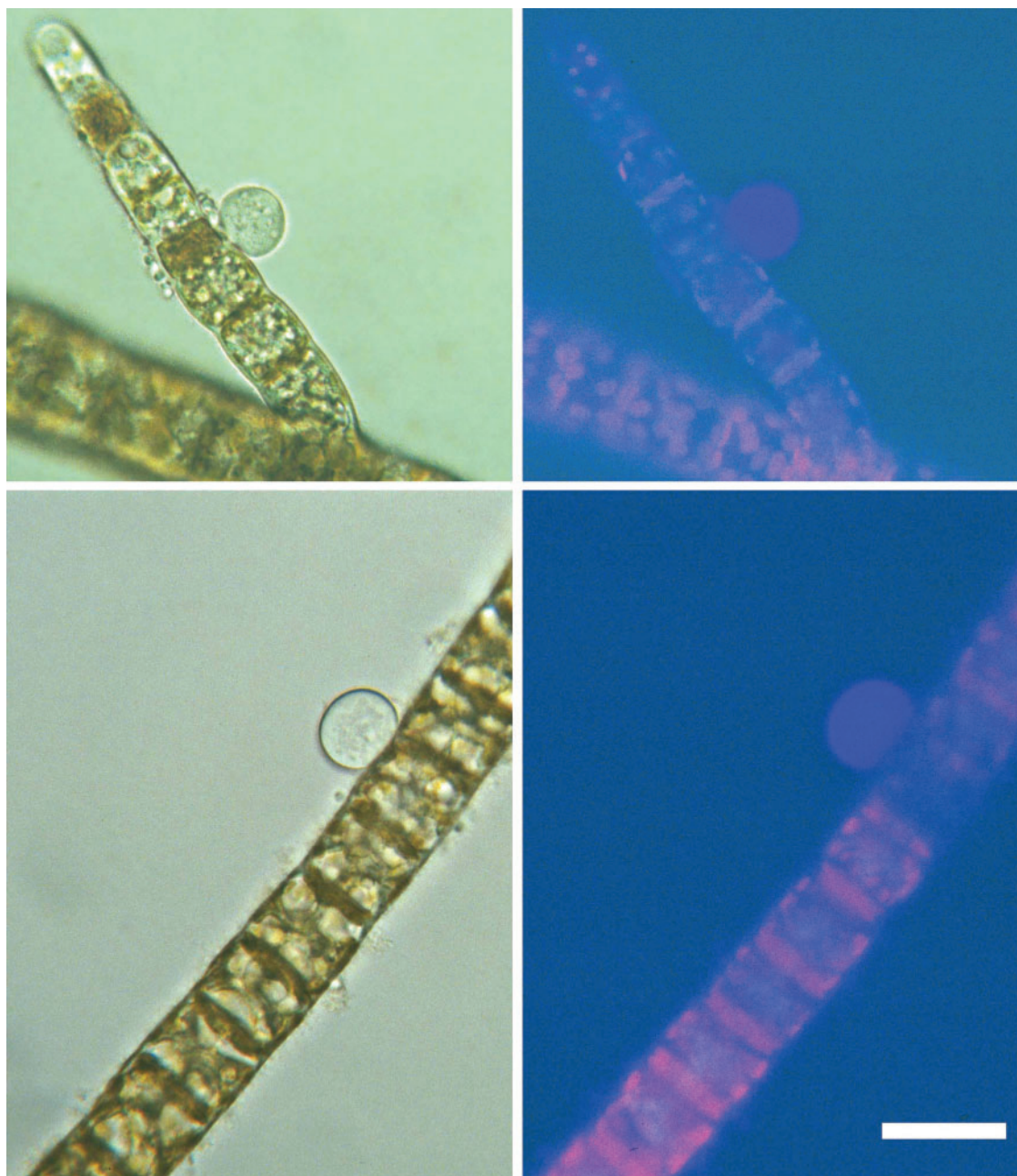


Fig. 1. *Chytridium polysiphoniae* from Shetland infecting *Pylaiella littoralis* Pyl IR (CCAP 1330/3) in culture, observed by epifluorescence microscopy. Left, brightfield; right, UV excitation. The images show *Chytridium* sporangia on the surface of *Pylaiella* filaments. The infection of this holocarpic pathogen is limited to single algal cells. Scale bar = 50 μ m.

culture by Le Calvez *et al.* (2009), although members of other groups of fungi were. Previously unknown clades of zoosporic true fungi were detected in the extreme environments. In addition, Nagano *et al.* (2010) collected samples of deep-sea sediments from five sites off the Japanese islands. They detected DNA sequences attributed to zoosporic true fungi from one site in the Izu-Ogasawara Trench. Rarefaction curves obtained on rDNA libraries show that increasing the sequencing effort would increase the number of phylotypes identified, implying that

many more unknown species of zoosporic true fungi occur in marine ecosystems (Le Calvez *et al.* 2009).

Some physiological properties of marine, estuarine and freshwater zoosporic true fungi

Salinity

Some physiological properties of *Rhizophydium littoreum* have been studied in the laboratory. One study investigated the

tolerance of an ecotype of *R. littoreum* isolated from an estuarine ecosystem to sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) ions (Amon and Arthur 1981). This ecotype requires higher concentrations of Na, Mg and Ca ions than those in freshwater but lower than those in seawater. The composition of seawater is ~470 mM Na, 10 mM K, 54 mM Mg and 10 mM Ca (Sverdrup *et al.* 1942). This ecotype also appears to tolerate a wide range of salinities. It will grow in media supplemented with Na ions at concentrations from close to 0 to more than 560 mM; however, the maximum growth rate was obtained at 237 mM, approximately one-half the concentration of seawater (Amon 1976). Therefore, pure seawater is not optimal for the growth of this estuarine ecotype. In contrast, the subtidal ecotype of *R. littoreum* isolated from crab eggs by Shields (1990) will grow well on solid media with full-strength seawater.

There is an absolute requirement for Na in the growth medium for the estuarine ecotype of *R. littoreum* (Amon 1976; Amon and Arthur 1981). Amon (1976) measured the internal Na concentration by atomic absorption spectroscopy. This ecotype may absorb Na from the environment or excrete Na back into the environment to regulate internal Na concentrations and presumably to attain osmotic equilibrium. Microbes that live in estuaries must adapt to fluctuating salinities with changes in the tides. Furthermore, microbes living on the surface of substrates in the intertidal zone, such as *Bryopsis* and *Codium*, will experience increased salinity when water evaporates in the sun and decreased salinity during rain or freshwater runoff at low tide.

Amon and Yei (1982) investigated the effect of salinity on the growth of isolates of both *R. littoreum* (Chytridiomycota) and *Thraustochytrium striatum* (Labyrinthulomycota) inoculated together in mixed cultures, using pine pollen grains as a substrate. They measured the number of thalli of each species on pollen grains immersed in water with different Na concentrations. These studies suggested that this ecotype of *R. littoreum* is better adapted to estuarine conditions rather than to full-strength seawater, whereas the ecotype of *T. striatum* tolerates higher concentrations of NaCl. These two microbes differed in their response to salinity at least when competition is a factor.

Other studies on the growth of zoosporic true fungi at different salt concentrations have investigated ecotypes of various genera isolated from soil or freshwater. One objective of these studies was to determine the tolerance of various concentrations of NaCl. The osmotic potentials of soil and vernal pools change during cycles of wetting and drying, and so we would expect that ecotypes in soil and freshwater should be somewhat tolerant to salinity. Several different genera of zoosporic true fungi isolated from soil and freshwater have been tested for growth at different salinities. Gleason *et al.* (2006) tested 20 isolates of zoosporic fungi in the orders Blastocladiales, Chytridiales, Cladochytriales, Rhizophydiales, Rhizophlyctidales and Spizellomycetales for their ability to grow on media with high osmotic potentials including NaCl. All of these fungi grew on complex solid media supplemented with 170 mM but not with 340 mM NaCl. Nielsen (1982) observed growth of nine isolates of *Allomyces* in complex growth media supplemented with 175 mM and some isolates in media with up to but not over 275 mM NaCl. Booth (1971) observed growth of most isolates of zoosporic fungi from his culture collection in media

with seawater diluted to 170 mM and some isolates to 255 mM (approximate osmolarity).

Most of the previous research has focussed on the maximum salinity that would allow growth, whereas possible inhibition of growth at low salinities has not been measured. An experiment with one isolate from cropping soil, *Gaertneriomyces semiglobifer* Mar C/C2, was designed to address this issue (F. H. Gleason, unpubl. data). Growth was measured in complex liquid PYG media, with and without addition of 170 mM NaCl. The composition of the medium (g L^{-1}) was glucose 3.0, yeast extract 1.25, peptone 1.25, KH_2PO_4 1.36, Na_2HPO_4 0.71 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.12. A volume of 0.2 mL of zoospore suspension was added to 100 mL screw-cap bottles containing 25 mL of PYG liquid growth medium. The bottles were placed on a rotary shaker (RatekTM, model EOM5 orbital mixer, Melbourne, Vic., Australia) at 20 rpm. After growth for 7 days at 20°C, the fungi were harvested on Whatman filter paper, dried overnight at 70°C and then weighed to 0.1 mg. There were five replicates at each concentration. The means and standard deviations for dry weights (mg) were 27.5 ± 1.0 for the control without supplemental NaCl and 24.9 ± 1.5 for the growth medium with 170 mM NaCl. The total growth (dry weight) for 170 mM NaCl was slightly less than that for the control ($P < 0.05$). Therefore, the growth of this fungus is inhibited slightly (~9%) by addition of 170 mM NaCl to the growth medium.

From the studies discussed in the present paper, we would predict that most zoosporic fungi isolated from freshwater or soil environments would be unable to grow in pure seawater (approximately equivalent in total osmolarity to 600 mM NaCl). Furthermore, although different procedures were used in these studies, the growth of ecotypes from soil and freshwater appear to be strongly inhibited by concentrations of NaCl that are optimum for the estuarine ecotypes, and in some cases by much lower concentrations. However, some soil and freshwater isolates can remain dormant in hypersaline conditions and then resume growth later when conditions become more favourable (Gleason *et al.* 2006). For this reason, these isolates can be considered to be tolerant to high salinity, i.e. halotolerant. In contrast, marine ecotypes would be expected to grow in pure seawater and not in freshwater. Unfortunately, this has not been tested in the laboratory.

In many ecosystems, salinity is variable. For example, when soils or vernal pools dry during the summer, the salinity will increase. During low tide in the intertidal zone, the salinity will increase in the sun on a hot day and decrease during rain on a cold day. Each ecotype must adapt to a range in salinity. Therefore, the range in addition to the optimum needs to be determined for each ecotype.

Phototaxis

Another interesting physiological property of *Rhizophyidium littoreum* is the behaviour of zoospores in a light gradient (phototaxis). Zoospores of *R. littoreum* respond positively and strongly to low intensities of blue light (Kazama 1972a; Muehlstein *et al.* 1987). Very high intensities of sunlight, such as within 1 cm of the surface of water, appear to kill zoospores. A positive response to light gradients may influence vertical migration of zoospores and cause zoospores to remain within

euphotic environments where hosts and substrates are available. Zoospores of the marine species *Thalassochytrium gracilariopsis* (Nyvall *et al.* 1999) and one species of the freshwater genus *Allomyces* also responded positively to light gradients (Robertson 1972). The light-sensitive pigment that guides the zoospores of *Allomyces* is rhodopsin (Saranak and Foster 1997).

Production of zoospores

We would expect zoosporic fungal parasites of algae to produce large numbers of zoospores periodically to ensure survival in their natural environments. There are almost always strong local currents in the intertidal and subtidal zones where their marine algal hosts grow. Zoospores of true fungi cannot swim very rapidly and are motile only for short periods of time before they run out of energy reserves and before they encyst (Gleason and Lilje 2009). Although many zoospores have been shown to be chemotactic (Muehlstein *et al.* 1988), they must find and attach to a readily utilisable substrate quickly. Most zoospores are probably quickly washed away from their hosts by the currents. In culture with their hosts, massive zoospore discharge has been

observed from *T. gracilariopsis* by Nyvall *et al.* (1999) and large numbers of zoospores are released by *R. littoreum* (Amon 1984; Shields 1990); however, no quantitative data are available from studies in the field with either of these fungi. Amon and Yei (1982) have observed zoospore discharge from *R. littoreum* colonising pine pollen grains in axenic culture as well.

Food webs

All of the zoosporic true fungi discussed above are considered to be heterotrophic flagellates (HF), because they produce zoospores which are less than or equal to $\sim 5 \mu\text{m}$ in diameter and which lack chlorophyll (Sime-Ngando *et al.* 2011). Zoospores of all groups of HF provide valuable food resources for grazing and filter-feeding zooplankton and metazoan invertebrates in freshwater environments (Kagami *et al.* 2007, 2011; Gleason *et al.* 2008; Sime-Ngando *et al.* 2011). Similarly, we would expect that the large numbers of zoospores released by HF parasites of algae possibly provide important food resources in coastal marine environments where algae form dense communities and where the prevalence of infection is high (Fig. 2).

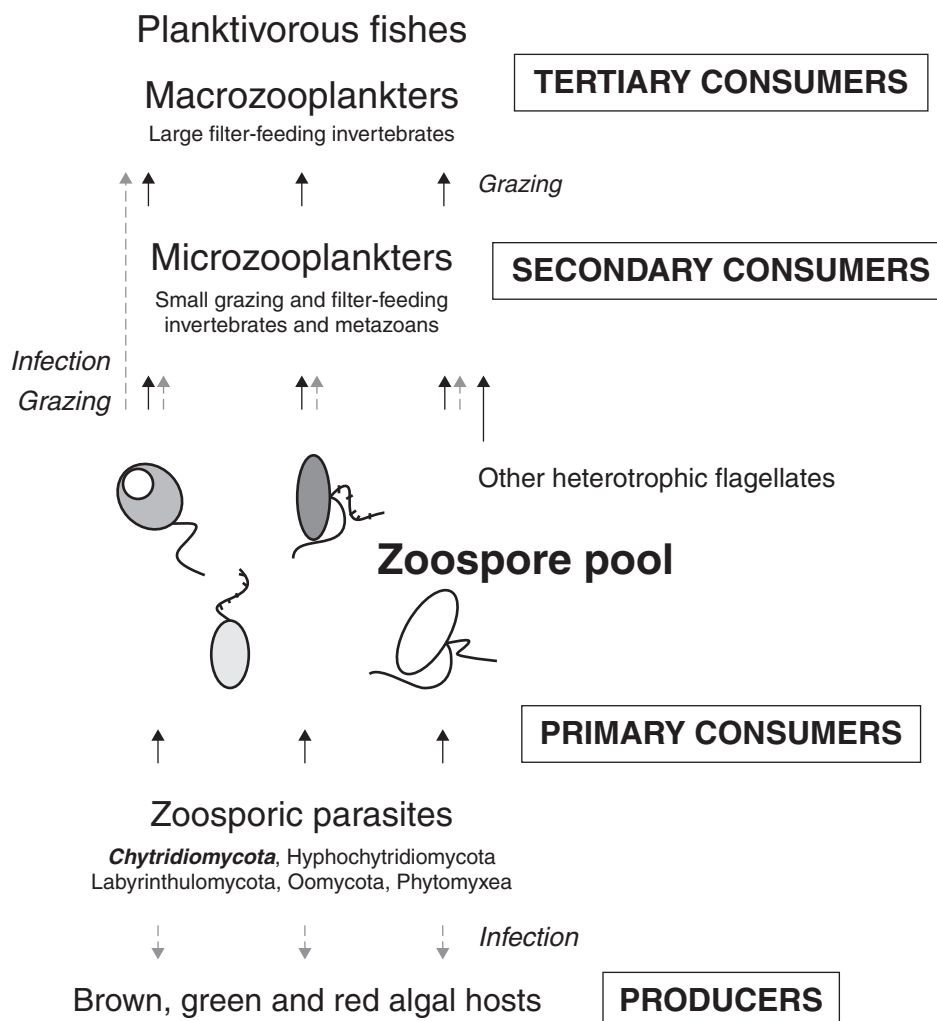


Fig. 2. Possible contributions of zoosporic parasites to the food web in a marine community.

Zoospores are also released from HF saprobes colonising detritus (Sime-Ngando *et al.* 2011). Several common groups of HF parasites of marine algae have been identified. These include Chytridiomycota, Hyphochytriomycota, Labyrinthulomycota, Oomycota and Phytomyxea (Andrews 1976; Raghukumar 1987; Küpper and Müller 1999; Parodi *et al.* 2010).

Furthermore, we would expect parasites of marine algae (primary consumers) to transfer significant amounts of matter and energy from their algal hosts (primary producers) to their predators (secondary consumers). If the food chains involving parasites were added to the present marine food-web models, it would greatly increase their complexity. However, quantitative data on energetics in marine food webs with zoosporic fungal parasites are not yet available. One quantitative study estimated the amount of carbon transferred from host to parasite by *Amoebophyra* spp., a dinoflagellate (Salomon *et al.* 2009). This food chain involves transfer from *Ceratium falciforme* (host) to *Amoebophyra* spp. (parasite) to tintinnid ciliates (predators). In this particular case, the authors estimated that up to 6.5% of the carbon in the decaying host population became available to grazers through the parasite spores. While the relative proportion of zoosporic true fungi among other marine HF remains unknown, the contribution of pathogen spores to carbon fluxes has hitherto been systematically ignored in marine microbial food-web dynamics (Sime-Ngando *et al.* 2011). Even though zoosporic fungal pathogens are widespread, their total biomass could be very low and therefore their total contributions to the marine food webs may not be as great as those of some of the other groups of HF.

Effect of parasitism on photosynthesis

Gachon *et al.* (2006) used spatially resolved (imaging) microscopic measurements of chlorophyll-fluorescence kinetics to follow the fate of individual cells of *Pylaiella littoralis* when infected by *Chytridium polysiphoniae*. This work revealed strong differences among individual parameters of the inhibition of photosynthesis, unravelling mechanistic details about the impact of infection on host photosynthesis. These results indicated that the infection strongly affects the regulation of energy dissipation from the algal antenna apparatus. The impact of reduced rates of photosynthesis on growth and development of populations of brown algae in the field is unknown.

Discussion, conclusions and future perspectives

In general, the placement of many genera of zoosporic true fungi into orders based on higher-level phylogenetic classification (James *et al.* 2006a; Hibbett *et al.* 2007) is not yet possible for the following two reasons: the molecular data for these genera are not yet available and the rearrangement of genera into orders is still in progress (see Letcher *et al.* 2008a, 2008b; Mozley-Standridge *et al.* 2009; Wakefield *et al.* 2010). Several orders such as the Cladochytriales, Rhizophydiales, Rhizophlyctidales and Spizellomycetales have been intensively investigated, whereas research on others has not yet been undertaken or completed. The orders Rhizophydiales and Spizellomycetales include large groups of fungi observed in soil and freshwater ecosystems which vary considerably in morphological and physiological properties.

In contrast, only three genera of marine zoosporic fungi have been studied in detail, and molecular data are available for only one genotype of *Rhizophyidium littoreum* (Letcher *et al.* 2006) and one genotype of *Chytridium polysiphoniae* (Küpper *et al.* 2006). (No data are available for *Thalassochytrium gracilariopsis*.) The data for *C. polysiphoniae* include only the sequence of the 18S rRNA gene. James *et al.* (2006a) included sequence data for six genes in their phylogenetic study. Therefore, it has not been possible to place *C. polysiphoniae* and *T. gracilariopsis* into orders yet. *R. littoreum* belongs to the order Rhizophydiales (Letcher *et al.* 2006). Furthermore, no data are available on genetic variability within the marine genera.

Future work on resolving the phylogenetic placement of marine species – both known and as-yet undescribed – will likely be driven by environmental sampling studies as outlined by Le Calvez *et al.* (2009). Recent environmental DNA sampling of deep-sea vents has detected as-yet undescribed sequences of marine zoosporic fungi (Le Calvez *et al.* 2009), suggesting that there may be entire lineages of these fungi yet to be catalogued. However, although these studies provide us with a fuller understanding of the genetic diversity in marine environments, they do so at the expense of our understanding of morphological, ultrastructural and ecological variation. Without intensive sampling and culturing surveys aimed at assessing the species richness in marine environments and the ecological roles they play therein, our picture of biodiversity will remain incomplete.

The effects of facultative and obligate zoosporic true fungal parasites on the growth and metabolism of macro-algae in the marine environment are not yet fully understood. Some studies have suggested that parasitism by *Chytridium polysiphoniae* may affect the rates of photosynthesis (Gachon *et al.* 2006) and possibly patterns of growth and development in communities of brown algae (Müller *et al.* 1999). Because brown algal communities predominate in many marine coastal environments, especially in colder parts of the world, potential hosts for *C. polysiphoniae* are common. Despite the paucity of records and the lack of quantitative data, *C. polysiphoniae* may be an important parasite globally. Species in three other genera of zoosporic true fungi (*Synchytrium*, *Olpidium* and *Physoderma*) are common parasites of flowering plants in cropping soils worldwide (Powell 1993).

The discovery of zoosporic fungal parasites of marine diatoms by Elbrächter and Schnepf (1998) and Hanic *et al.* (2009) suggests that these parasites could have roles in the control of algal blooms. Population dynamics of zoosporic true fungal pathogens have been studied intensively in freshwater ecosystems (Kagami *et al.* 2007, 2011) but not in marine ecosystems. Overall, more attention should be devoted to the impact of pathogens on the physiology of their hosts, the structuring of communities, and ultimately on global processes (Gachon *et al.* 2010). Baseline data on the impact of these parasites are necessary as part of the research designed to estimate the effects of climate change and environmental deterioration on marine communities.

Another important research topic that needs investigation is the interaction among different species of parasites. Zoospores of different species of parasites may compete for the same sites on the same host. Zoospores of many saprobic and parasitic species of zoosporic true fungi and stramenopiles are known to

be chemotactic (Muehlstein *et al.* 1988; Fan *et al.* 2002; Gleason and Lilje 2009). We would expect most zoospores to sense a gradient of small organic molecules diffusing away from utilizable substrates. In particular, chemotaxis has been studied in both *Rhizophyidium littoreum* (Muehlstein *et al.* 1988) and in *Thraustochytrium striatum* (Fan *et al.* 2002). Amon and Yei (1982) noted that salinity affected the relative frequency of colonisation of pine pollen grains by isolates of these two microorganisms in the laboratory. Quantitative differences in the responses of zoospores of the two species to a variety of small organic molecules diffusing from the pollen grains may be an important factor, as well as salinity. If environmental conditions differentially affect zoospores of different species, competition could be reduced.

The marine–freshwater boundary is characterised by an extreme change in salinity and is difficult for many microbes to cross (Logares *et al.* 2009). Therefore, changes in the composition of communities along a salinity gradient would be expected (Logares *et al.* 2009). Increasing salinity in both soil and aquatic environments is a continuous problem in many parts of the world such as in the dry climates of Australia and the cold climates of Canada and in agricultural regions worldwide. The distribution of zoosporic true fungi along the gradients between oligotrophic freshwater and full-strength seawater awaits investigation. Because the community composition of zoosporic true fungi could change with salinity, this could result in significant alteration of the structure of and the ecological processes within the entire ecosystem.

Furthermore, many phylogenetic studies on unicellular eukaryotes show a clear evolutionary separation between marine and freshwater lineages (Logares *et al.* 2009). This hypothesis cannot be tested yet with zoosporic true fungi because so few marine and estuarine isolates have been studied. However, we believe that many unknown species of zoosporic true fungi including saprobes, parasites and mutualists are present in marine environments. This is suggested by recent data from environmental surveys using molecular techniques (Le Calvez *et al.* 2009). Zoosporic true fungi are very difficult to identify with the light microscope without fluorescent-staining techniques and without expert knowledge of their morphology and life cycles. With methods currently available, these fungi are difficult to isolate into pure culture and maintained on defined media in the laboratory. Furthermore, biotrophs must be grown with their hosts in two-membered cultures. Once quantitative data have become available, a better understanding of the relative importance of zoosporic true fungi in marine, estuarine and freshwater ecosystems will be possible. Molecular investigations, in particular when empowered by next-generation sequencing technologies, represent the most promising avenue to increase our knowledge on the diversity and functions of microorganisms in the near future.

Martin (1922) stated ‘The morphology and distribution of the fungi parasitising marine plants are still so imperfectly known that additional facts concerning any of them seem worth placing on record.’ Unfortunately, this is still true today. Intensive research is necessary worldwide before we can begin to appreciate the roles of zoosporic true fungi in marine environments. We hope that the synopsis of information provided in the present review will stimulate future research.

Acknowledgements

The authors thank Professor John A. West, Dr Sigrid Neuhauser and Professor Paul Boon for their constructive criticisms and suggested revisions for this manuscript during the review process and Professor Andrew Boulton for his encouragement, suggestions for revision and attention to detail during the final editing process.

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Manuscript received 23 November 2010, accepted 13 February 2011