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Zoosporic parasites infecting marine diatoms – A black box that needs to be opened

Bettina SCHOLZ[a,b], Laure GUILLOU[c], Agostina V. MARANO[d], Sigrid NEUHAUSER[e], Brooke K. SULLIVAN[f], Ulf KARSTEN[g], Frithjof C. KÜPPER[h], Frank H. GLEASON[i,*]

[a]BioPol ehf., Einbúastig 2, 545 Skagaströnd, Iceland
[b]Faculty of Natural Resource Sciences, University of Akureyri, Borgir v. Nordurslod, IS 600 Akureyri, Iceland
[c]Sorbonne Universités, Université Pierre et Marie Curie – Paris 6, UMR 7144, Station Biologique de Roscoff, Place Georges Teissier, CS90074, 29688 Roscoff cedex, France
[d]Instituto de Botânica, Núcleo de Pesquisa em Micologia, Av. Miguel Stéfano 3687, 04301-912, São Paulo, SP, Brazil
[e]Institute of Microbiology, University of Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria
[f]Department of Biosciences, University of Melbourne, Parkville, VIC 3010, Australia
[g]Institute of Biological Sciences, Applied Ecology & Phycology, University of Rostock, Albert-Einstein-Strasse 3, 18059 Rostock, Germany
[h]Oceanlab, University of Aberdeen, Main Street, Newburgh AB41 6AA, Scotland, United Kingdom
[i]School of Biological Sciences FO7, University of Sydney, Sydney, NSW 2006, Australia

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ABSTRACT

Living organisms in aquatic ecosystems are almost constantly confronted by pathogens. Nevertheless, very little is known about diseases of marine diatoms, the main primary producers of the oceans. Only a few examples of marine diatoms infected by zoosporic parasites are published, yet these studies suggest that diseases may have significant impacts on the ecology of individual diatom hosts and the composition of communities at both the producer and consumer trophic levels of food webs. Here we summarize available ecological and morphological data on chytrids, aphelids, stramenopiles (including oomycetes, labyrinthuloids, and hyphochytrids), parasitic dinoflagellates, cercozoans and phytomyxids, all of which are known zoosporic parasites of marine diatoms. Difficulties in identification of host and pathogen species and possible effects of environmental parameters on the prevalence of zoosporic parasites are discussed. Based on published data, we conclude that zoosporic parasites are much more abundant in marine ecosystems than the available literature reports, and that, at present, both the diversity and the prevalence of such pathogens are underestimated.

* *Corresponding author*. Tel.: +61 2 9971 2071.
E-mail address: frankjanet@ozemail.com.au (F.H. Gleason).
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Introduction

Zoosporic parasites are facultative or obligate parasites that produce motile spores as their infective propagules. In freshwater ecosystems, zoosporic parasites of diatoms such as chytrids (Chytridiomycota) cause frequent epidemics, which have been studied periodically during the past century (Canter, 1951; Sparrow, 1960; Ibelings et al., 2004; Sime-Ngando, 2012; Gsell et al., 2013a,b; Voigt et al., 2013; Carney and Lane, 2014). In such ecosystems zoosporic parasites play significant roles in controlling population sizes, transferring carbon from relatively inedible substrata at the producer level to higher trophic levels (Kagami et al., 2007; Gleason et al., 2008), and in biodiversity and succession (van Donk and Ringelberg, 1983; van Donk, 1989). In fact, zoosporic parasites are believed to be the major drivers of plankton succession (van Donk, 1989), and as a consequence, infections may alter species composition of diatom hosts in freshwater ecosystems (Canter and Lund, 1951).

In marine ecosystems, published reports of fungal and other zoosporic parasites infecting diatoms have been relatively rare, and thus the ecological roles of these parasites on diatom host taxa are poorly understood (Powell, 1993; Gleason et al., 2011, 2012). From an ecological and biogeochemical point of view diatoms are of crucial importance in marine systems (Allen et al., 2006). Diatoms are among the most cosmopolitan and diverse of photosynthetic algal groups and contribute about 20–25 % of the total global carbon fixation (e.g. Round et al., 1990). Depending on seasons, they can be conspicuously abundant and appear at the bottom of most pelagic and benthic food webs in aquatic ecosystems (Armbrust, 2009). As in fresh water, marine diatoms should represent an abundant resource for zoosporic parasites, infections however have been comparatively poorly reported.

Zoosporic parasites are difficult to identify in environmental samples, because of the lack of morphological characters, and zoospores of diverse taxonomic affiliation all look very much alike (e.g. Figs 1 and 2). For this reason zoosporic parasites are rarely documented in ecological studies, but rather are lost within the ecological pool of (pico- or nano-) flagellates. Thus, these parasites are supposedly much more frequent in marine ecosystems than the literature reports (Gleason et al., 2012). Simultaneous infection of diatom hosts by different pathotypes of one genus or by species in different genera is frequent (Hanic et al., 2009; Peacock et al., 2014), and this further complicates the process of identification.

We describe some examples of chytrids, aphelids, oomycetes, parasitic dinoflagellates, cercozoans and phytomyxids which are all known to be zoosporic parasites of marine diatoms. Of course these are not the only parasites of diatoms. Rather, they are a representative selection of the known zoosporic parasites for which we have some data. This review focuses on eukaryotic parasites of diatoms, thus viruses and bacteria will not be considered here. In addition, we compare the methods of infection and life cycles of these groups of zoosporic parasites and the difficulties in the use of molecular techniques as identification tools for zoosporic parasites in the marine environment.

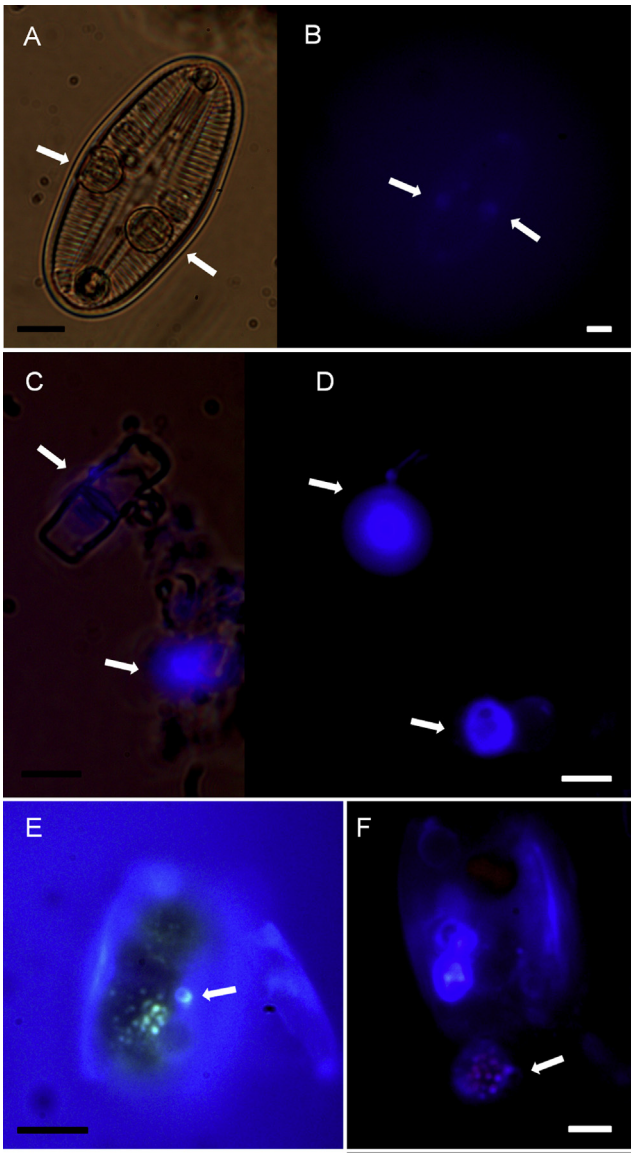

Fig 1 – Epibiotic pathogens infecting marine diatoms in sediment samples collected from the Skagaströnd area (station near the stream, northern Iceland, Scholz and Einarsson, 2015) and culture material. Pathogens were visualised using Calcofluor White stain in combination with transmission light and fluorescence excitation (UV-light, 330–380 nm). (A) Light and (B) fluorescence microscopic picture of an epibiotic pathogen in *Amphora ovalis*. (C–F) Further epibiotic pathogens. Bar: 100 μm.

Diatoms, the hosts

Diatoms (Bacillariophyceae) are unicellular photosynthetic algae that range in size from 5 to 200 μm. However, some groups commonly form colonies (Round et al., 1990; van den Hoek et al., 1997). Free living diatoms are covered by a siliceous skeleton (frustule) composed of SiO_2 and H_2O (van den Hoek et al., 1997). The structure of the frustule (as seen in the

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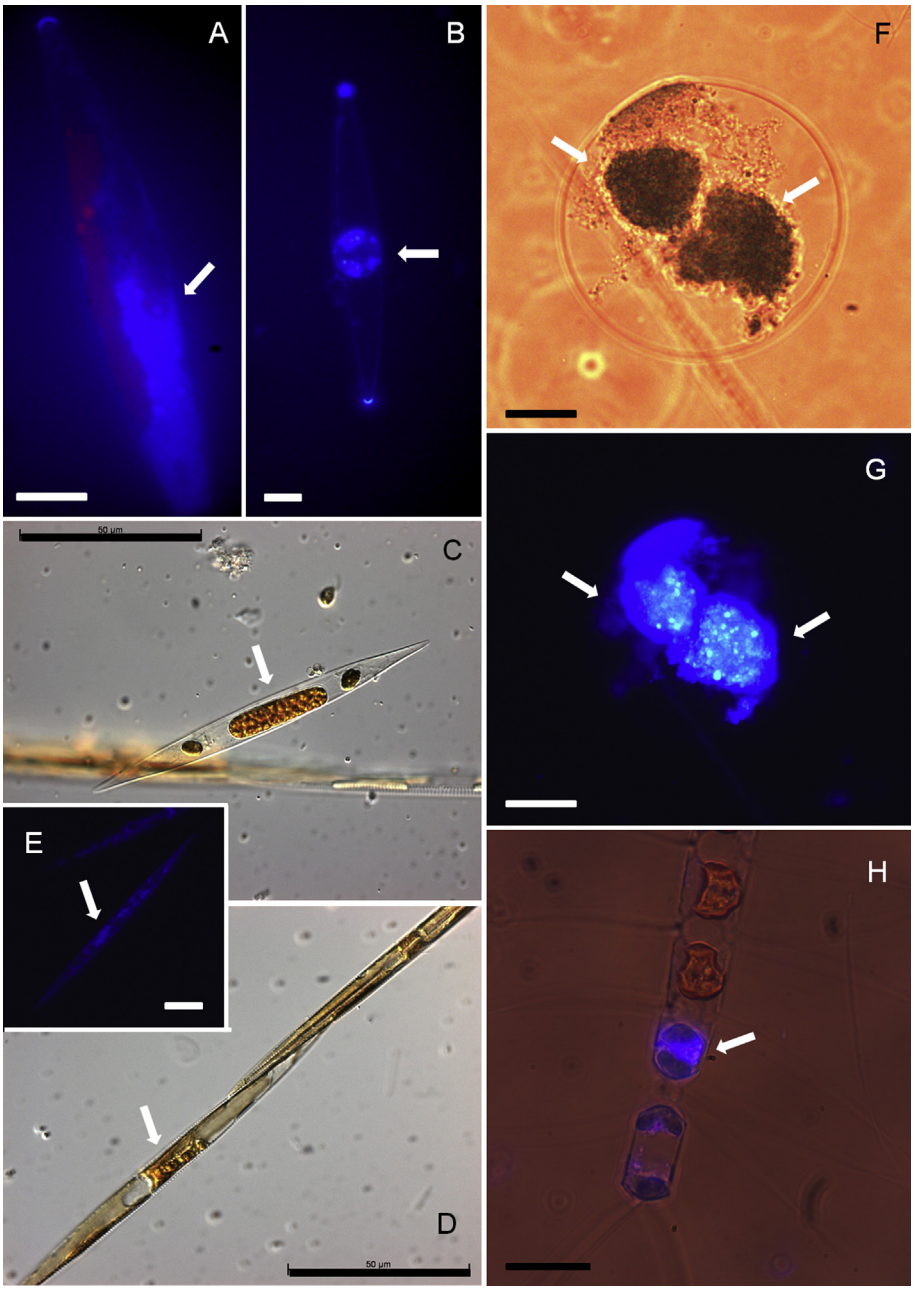

Fig 2 – Examples of endobiotic pathogens infecting marine diatoms. (A–B) Endogen infection of *Pleurosigma* sp. by an unidentified pathogen. (A) The cell is still alive, but the infection has started and (B) shows presumably a resting spore; (C–D) *Pseudo-nitzschia seriata* infected by *Ectrogella* sp. (Oomycota; Lugol-fixed sample; Pictures: Dr. Claire Gachon, Scottish Association for Marine Science). (E) *Pseudo-nitzschia* sp. infected by an unidentified oomycete; (F–G) *Cocinodiscus* sp. infected by *Lagenisma* sp. both views are identical, in (F) transmitted light microscopy and in (G) epifluorescence microscopy of a CFW sample (UV. light, 330–380 nm). (H) *Chaetoceros* sp. infected by an unidentified oomycete. With exception of (C–D), pathogens were visualised using Calcofluor White in combination with usual light and fluorescence excitation. (A–B, E–H) were found in sediment samples collected from the Skagaströnd area (station near the stream, northern Iceland in 2014 and 2015), whereas samples for (C–D) were taken on 30 Sep 08, off the Isle of Ewe, on the West Coast of Scotland. If not otherwise mentioned, bars = 10 µm.

light microscope) is the main feature used to identify species (e.g. Round et al., 1990).

Both benthic and planktonic forms of diatoms are common. Numerous planktonic forms are centric (radial = Coscinodiscophyceae, polar = Mediophyceae). They possess several distinctive types of cell constructions to assist with flotation in the planktonic realm: (i) the bladder type (e.g. *Coscinodiscus*, centric diatom), (ii) the ribbon type (e.g.

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Table 1 – Examples of zoosporic parasites of marine diatoms.

Parasite phylum	Description	Parasite species	Host species	Growth phase	Feeding structure	References of infection
Unikont (opisthokont) zoosporic parasites						
Chytridiomycota	True fungi, characterised by cell walls composed of chitin (with the exception of their zoospores). The most prominent morphological feature of the thallus is the zoosporangium (James et al., 2006).	*Unidentified chytrid species* (*Rhizophydium* (?) *Chytridium* (?))	*Navicula digitoradiata* *N. gregaria* *Achnanthes brevipes* *Diploneis didyma* *Cylindrotheca closterium* *Amphora exigua*	epibiotic	rhizoids	Scholz et al. 2014
		Unidentified chytrid species	*Thalassiosira nordenskioeldii* *Chaetoceros* sp.			Gaertner 1979
		Unidentified chytrid species	*Pseudo-nitzschia pungens*			Hanic et al. 2009
Aphelidae	They have been recently re-classified as Opisthosporidia, a sister group to fungi (Karpov et al., 2014).	*Pseudaphelidium drebesii*	*Thalassiosira punctigera*	endobiotic	infection tube and microfilament	Schweikert and Schnepf 1996, 1997a
Heterokont zoosporic parasites						
Alveolata						
Dinophyta (core dinoflagellates)	The infraphylum Dinozoa is divided into different major groups that included the core Dinophyceae and several basal groups (i.e. Syndinids, Perkinsozoa). Molecular data from ribosomal proteins were used to resolve the deep branching dinoflagellate clades (Bachvaroff et al., 2014). Only a few heterotrophic genera in the core Dinophyceae are parasites of diatoms, and are considered herein. All members of the genus *Paulsenella* Chatton are ectoparasites on marine planktonic diatoms (Drebes and Schnepf, 1988).	*Paulsenella vonstoschii*	*Streptotheca tamesis* (=*Helicotheca tamesis*)	epibiotic	feeding tube, (phagopod)	Drebes and Schnepf 1988
		P. chaetoceratis	*Chaetoceros decipiens*			
		P. kornmannii	*Eucampia zodiacus*			
		Gyrodinium undulans	*Odontella aurita*			Drebes and Schnepf 1998
Stramenopiles						
Oomycota (basal oomycetes)	Cellulosic cell walls (consisting mainly of 1,3-β-glucans, some1,6-β-glucans and 1,4-β-glucans). Sporangia of different taxa within the Oomycetes have diverse morphology. They may be terminal or intercalary (within a hyphal filament), bulbous or not, and if terminal, caducous	*Ectrogella perforans*	*Licmophora hyalina*	endobiotic	infection tube	Raghukumar, 1980 a, b
		Ectrogella sp.	*Pseudo-nitzschia pungens*	endobiotic	infection tube	Hanic et al., 2009
		Lagenisma sp.	*Coscinodiscus radiatus* *Dimeregramma minor* *Gyrosigma peisonis*	endobiotic		Scholz et al., 2014
		Ectrogella sp.	*Coscinodiscus granii*	both	infection tube	Schnepf et al., 1978
		Lagenisma coscinodisci	*Coscinodiscus centralis*	endobiotic	infection tube	Schnepf et al., 1978
			Coscinodiscus granii *Coscinodiscus concinnus*	endobiotic		Wetsteyn and Peperzak, 1991
			Coscinodiscus sp.	endobiotic	infection tube	Drebes, 1966, 1968

	(sporangia detach readily) or not. The basal oomycetes that infect diatoms lack the mycelium and sexual reproduction, commonly found in the crown oomycotes.					
Hyphochytrea (*Pirsonia* clade)	*Pirsonia* is considered as heterotrophic nanoflagellate. According to Kühn et al. (2004) four species of diatom parasites belonging to the genus *Pirsonia*, clustered together in a clade closely related to *Hyphochytrium catenoides*	*Pirsonia punctigerae* *P. verrucosa* *P. mucosa* *P. formosa* *P. eucampiae* *P. diadema* *P. guinardiae*	*Thalassiosira punctigera* *Rhizosolenia delicatula* (=*Guinardia delicatula* *Rhizosolenia shrubsolei* (=*R. imbricata*) *Rhizosolenia setigera* *Eucampia zodiacus* *Coscinodiscus granii* *C. wailesii* *C. concinnus* *Guinardia flaccida*	endobiotic	pseudopodium at surface, pseudopodia inside	Schweikert and Schnepf, 1997b Kühn et al., 1996 Kühn et al., 1996 Kühn, 1998 Schnepf et al., 1990
Labyrinthulomycota (order Thraustochytriida)	Cell wall is made of dictyosome-derived circular scales, arranged in several layers (Darley et al., 1973; Chamberlain, 1980; Moss, 1985). All species (except the thraustochytrid genus *Althornia* (Jones & Alderman) produce a system of ectoplasmic net (EN) elements from one or more points on the cell (Perkins, 1972, 1973; Porter, 1990). Vegetative stages of thraustochytrids consist of single cells which are globose to subglobose (4-20 μm diam.), growing epi-biotically on various substrata (Raghukumar, 1996).	*Schizochytrium* *Ulkenia visurgensis*	*Thalassionema nitzschioides* *Navicula sp.* *Nitzschia* sp. *Coscinodiscus* sp. *Melosira* sp. *Grammatophora* sp.	epibiotic epibiotic	pseudopodia	Gaertner, 1979 Raghukumar, 1986
Bigyra	As far as known for *Labyrinthula* the zoospores may not be the infective agent as it is typical for other parasites described in this review - it infects hosts by direct penetration and osmosis by the vegetative, spindle cells.	*Labyrinthula*	*Nitzschia* sp. *Amphiprora* sp.	both	vegetative, spindle cells	Riemann and Schaumann, 1993

(*continued on next page*)

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Table 1 – (continued)

Parasite phylum	Description	Parasite species	Host species	Growth phase	Feeding structure	References of infection
MAST-3 Rhizaria		*Solenicola setigera*	*Leptocylindrus mediterraneus*			Padmakumar et al., 2012
Cercozoa (*Cryothecomonas* clade)	Large and diverse group of amoebo-flagellates, with tubular mitochondrial cristae, that cluster together in molecular phylogenies inferred mainly from ribosomal gene sequences (small and large subunit rDNA) (Thomsen et al., 1991; Chantangsi et al., 2008). Colourless flagellates, in the free, motile stage, oblong to oval, 9-12 × 4-5 μm, two apically inserted flagella, anteriorly directed flagellum 15 μm long, posteriorly directed flagellum up to 25 μm (biflagellate whiplash flagella)	*Cryothecomonas aestivalis* *C. longipes* *Heteromita globosa* (a related feeding nanoflagellate)	*Guinardia delicatula* *G. flaccida* *Cerataulina bergonii* (=*C. pelagica* *Chaetoceros costatus* *Ch. debilis* *Ch. didymus* *Coscinodiscus granii* *C. radiatus* *Guinardia delicatula* *G. striata* *Leptocylindrus danicus* *Navicula sp.* *Pleurosigma sp.* *Rhizosolenia setigera* *Thalassiosira rotula* *T. punctigera* *Skeletonema costatum*	epibiotic mostly	pseudopodium	Drebes et al., 1996 Peacock et al., 2014 Schnepf and Kühn, 2000 Ohno et al., 2013
Phytomyxea	Including Plasmodiophorida ("plasmodio-phorids") and Phagomyxida are a group of parasitic protists belonging to the Rhizaria (Neuhauser et al., 2010, 2012, 2014; Bulman and Braselton, 2014) that form during their life cycle two types of morphologically very similar, heterokont zoosporic stages (Neuhauser et al., 2011a). Motile stages are heterokont, biflagellate, with one whiplash flagellum	*Phagomyxa bellerocheae* *P. odontellae*	*Bellerochea malleus* (=*B. horologicalis*) *Odontella sinensis*	endobiotic	infection tube ("Stachel and Rohr"); plasmodium	Schnepf et al., 2000

Streptotheca = *Helicotheca*), (iii) the hair type (e.g. *Rhizosolenia*) and (iv) the branching type (e.g. *Chaetoceros*) (van den Hoek et al., 1997). In contrast, benthic diatom populations are usually composed of pennate species, which are either epipsammic or epipelic (e.g. Daehnick et al., 1992; Agatz et al., 1999; Mitbavkar and Anil, 2002). In general, benthic microalgal communities are described as epipelic when motile and epipsammic when attached to sand grains. Epipsammic diatoms are usually araphid (taxa with cells that lack a raphe system, e.g. *Fragilariales*), monoraphid (the raphe is only on one valve; e.g. *Achnanthales*) or centric species (Coscinodiscophyceae). Epipelic forms, on the other hand, are biraphid (a raphe is present on each of both valves, e.g. *Naviculales*, *Thalassiophysales*, *Bacillariales*, *Surirellales*) and move actively through the sediment by means of mucilaginous secretions from their raphes (Round, 1971). However, the difference between epipsammon and epipelon is not absolute, as there are epipsammic diatoms that are capable of movement, though they are generally much slower than epipelic species (Harper, 1969). Furthermore, many diatom genera have representatives in both of these groups (e.g. *Nitzschia*, *Navicula* and *Amphora*: Harper, 1969; Agatz et al., 1999).

Diatoms exhibit an astonishingly high physiological plasticity and flexibility, for example, in terms of photosynthesis (Wilhelm et al., 2006). The underlying mechanisms can be explained by the evolutionary history of their chloroplasts, which derived from a secondary endosymbiosis. This means that an eukaryote acquired the ability to conduct photosynthesis via endosymbiosis of another eukaryotic red alga that already had plastids, which in turn was derived from an endosymbiotic-incorporated cyanobacterium (Archibald, 2009). The resulting organisms are chimaeras with major genomic contributions from two or even more sources (Delwiche, 2007). As a consequence of this genomic mixing, the diatom lineage with specific and often unique physiological and biochemical properties evolved. The emerging picture is that the different species of diatoms are characterized by a complex combination of genes and metabolic pathways acquired from a variety of sources such as red algae, green algae, chlamydial parasites and other bacteria (Armbrust, 2009). The consequences of this genetic mixture are reflected in specific biochemical capabilities. Diatoms, for example, combine an animal-like ability to generate chemical energy from the breakdown of fat with a plant-like ability to generate metabolic intermediates from this catabolic reaction (Armbrust, 2009). Additionally, diatoms possess a complete urea cycle (Armbrust et al., 2004) which serves as a distribution and repackaging hub for inorganic carbon and nitrogen, connecting carbon metabolism and nitrogen fixation/remobilization (Allen et al., 2011). Such a unique combination of metabolic pathways explains the high degree of physiological plasticity. Numerous other examples of this mix-and-match compilation of biochemical and physiological characteristics reiterate the fact that diatoms are neither plant nor animals (Armbrust, 2009).

Zoosporic parasites – who are they?

Zoosporic parasites, as defined in this review, are a heterogeneous group of organisms with several unifying features such as (but not limited to) size and morphology (small, unicellular, flagellated propagation stage), parasitic lifestyle, choice of host (diatoms) and habitat (marine). In contrast, parasites which have non-motile spores, infective amoebae without cell walls or a plasma membrane, or that feed entirely by direct engulfment, are not considered to be zoosporic parasites.

Zoosporic parasites infecting diatoms are divided into two major groups: (i) unikonts or opisthokonts, which includes the chytrids and aphelids and (ii) heterokonts, which includes the SAR supergroup, i.e. Stramenopiles (parasites know within basal oomycetes, Hyphochytrea, Labyrinthulomycota, and MAST-3), Alveolates (core dinoflagellates) and the Rhizaria (Cercozoa, Phytomyxea) (Baldauf, 2008; Adl et al., 2012). Table 1 gives an overview of the main characteristics of the groups, the parasite species and their hosts. All parasitic groups discussed in this review produce zoospores, which are often host-specific, highly infective, extremely virulent propagules (e.g. Gleason et al., 2011; Neuhauser et al., 2011a,b) and with specialized infection structures.

Zoospores – what are they?

Zoospores are single, individual eukaryotic cells with one nucleus and one to several mitochondria, and are released by the process of sporogenesis. Most unikonts produce uniflagellate zoospores with posteriorly directed whiplash flagella, whereas heterokont species are characterized by biflagellate zoospores (Dick, 2001). The ultrastructure of zoospores has become a key feature in the taxonomy of the Chytridiomycota (Barr, 1981; Powell, 1993; Longcore, 1995; Letcher and Powell, 2014), as the morphology of a parasite is often highly variable when physically associated with its host. This is probably true for all eukaryotic lineages. Additionally, due to several morphological transitions during the life histories of the often intracellular, usually holocarpic stages, microscopic identification of the parasitic species is not straightforward. Shape of zoospores has thus a great taxonomic value.

Stramenopiles produce biflagellate zoospores with one anteriorly directed tinsel flagellum with mastigonemes (characteristic tubular, tripartite hairs) and one posteriorly directed whiplash flagellum. Dinoflagellates have two flagella, one transversal providing forward motion and spin, the other, the longitudinal one trailing behind mainly acting as a rudder. Cercozoa may have a very plastic morphology. When flagellated, they may have one anterior and one posterior flagellum. The swimming behaviour of these zoospores generally provides a secure and easy way to classify the parasite. The cell of a zoospore is not surrounded by a cell wall, rather only by a plasma membrane, thus it is possible for zoospores to change shape and in some cases even to show an amoeba-like behaviour by producing pseudopods if the cytoskeleton structure permits (Gleason and Lilje, 2009).

Infection cycles and feeding modes of zoosporic parasites – a comparison

When a zoospore reaches its host or substratum, it loses its flagella. In the case of fungal parasites, the zoospore encysts,

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discharges its flagella and produces a cell wall protecting the cyst from environmental extremes. This cyst then germinates to produce an infection structure which penetrates the host cell (Fig 3). Infection structure of a zoosporic parasite can be a walled infection tube (found e.g. in aphelids and oomycetes, Schweikert and Schnepf, 1996, 1997a; Hanic et al., 2009), a highly modified pseudopod (e.g. *Pirsonia*, Schweikert and Schnepf, 1997b) or another specialized infection structure (e.g. "Rohr and Stachel" of phytomyxids, Kanyuka et al., 2003). This specialized structure penetrates the silica shell of a diatom through the girdle region between the theca, sometimes making a hole. Depending on the species either enzymes or rhizoids are released from the end of the tube allowing it to digest tissue and to grow through the girdle region into the host cell. Other species may use mechanical force, for example if the tube has cell walls, growth in diameter of the tube can wedge open the diatom. Both these mechanisms were first observed in studies on ultrastructure of marine diatoms infected by oomycetes (Raghukumar, 1980a,b) and of freshwater diatoms infected by chytrids (Beakes et al., 1992).

Besides differences in the infection techniques, further development also differs considerably between species and groups of zoosporic parasites (Fig 3). For example, in Aphelids, after penetration, the parasitoid (= any organism whose mode of life is intermediate between a parasite and a predator) becomes an intracellular phagotrophic amoeba, which engulfs the host cytoplasm forming food vacuoles. The parasite continues to grow and forms an endobiotic plasmodium with a residual body as it totally consumes the cytoplasm of the host cell. A multinucleate plasmodium is formed with a large central vacuole and a residual excretion body. The mature plasmodium then divides into a number of uninucleated cells (Karpov et al., 2014). In contrast, in the heterotrophic nanoflagellate *Pirsonia* (hyphochytrids) the pseudopod phagocytises and digests portions of the host diatom protoplast after penetration, and then differentiates into trophosomes. Nutrients are transported from the trophosomes back to the auxosome (the zoospore cyst on the surface) which grows and divides to form more auxosomes which differentiate, separate and eventually become zoospores (Kühn et al., 2004). Drebes et al. (1996) described for the amoebo-flagellate *Cryothecomonas aestivalis* (Cercozoa) that after attachment, the zoospore becomes amoeboid and the entire cell, along with its flagella, squeezes through the diatom frustule in the girdle region. Once the parasite is inside the cell, organic compounds of the diatom are digested by pseudopods. The parasite grows, divides several times and each daughter cell becomes a zoospore. In contrast to the above mentioned parasites, the core dinoflagellate *Paulsenella* sp. (Alveolata) differs from most other parasites by being phagotrophic (not osmotrophic), sucking out the host cytoplasm (Drebes and Schnepf, 1982; Hansen and Calado, 1999). This mode of endocytosis ("myzocytosis") implies that the host plasmalemma is not totally ingested. In a few cases, enough of the host cytoplasm is left to facilitate regeneration of the host cell, and the host is not ultimately killed as it is by most other zoosporic parasites.

The infection cycles and feeding strategies used by parasitic labyrinthulids in relation to their hosts have not been studied carefully until now and only anecdotal evidence suggests that these zoosporic parasites feed directly on marine diatoms (e.g. Gaertner, 1979; Raghukumar, 1980c). While, in general, diatom mucus is well known as an important substratum for thraustochytrid development (Jepps, 1931), it has been also shown that labyrinthulids are able to penetrate and enter the cells of some diatoms (Raghukumar, 2004). However, osmotrophic (extracellular digestion) feeding strategies have also been observed to occur in *Labyrinthula* (a parasite of eelgrass, Young, 1943; Muehlstein, 1992), suggesting variable feeding strategies for these organisms.

Furthermore, molecular surveys in planktonic marine systems have unveiled a large novel diversity of small protists, of which a large part belongs to basal heterotrophic stramenopiles (Massana et al., 2014). In the few groups investigated by metabarcoding approaches, MAST cells were shown to be globally distributed and abundant bacterial grazers, therefore having a putatively large impact on marine ecosystem functioning (Massana et al., 2014). Regarding *Solenicola setigera*, a member of the marine stramenopile clade MAST-3, only few reports pointing to the questionable parasitic nature of this species, because it has so far only been found on empty frustules on the diatom *Leptocylindrus mediterraneus* (Gómez et al., 2011; Skovgaard, 2014).

Two terms are used to describe the reproductive parts of the undifferentiated vegetative cells relative to the substrate: epibiotic and endobiotic. Epibiotic parasites remain on the surface of the host cell. The infection structure releases enzymes inside the host cell that digest the contents of the host cell. Nutrients for growth of the parasite are transported back to the parasite on the surface through the infection structure. The infection cycle is completed, when newly formed zoospores are released and infect another host cell, the infection cycle starts again. In contrast, endobiotic parasites enter the host cell through the infection structure and then obtain their nutrients by, (i) digesting the host cytoplasm using specialised structures and enzymes or, (ii) by altering the metabolism of the host upon infection (Ralph and Short, 2002). The cycle of endobiotic parasites is completed when the zoospores are released from the sutures of the valve. Fig 4 presents a schematic overview of the infection cycle of chytrids and oomycetes as they digest the host diatoms and includes both epi- and endobiotic types of zoosporic parasites.

Host-pathogen interactions

The primary function of zoospores is to seek new uninfected hosts or un-colonized substrata (Sparrow, 1960; Gleason and Lilje, 2009). Most zoospores are believed to be chemotactic, that is, they respond to a chemical cue (or gradient) that guides them towards potential substrata/hosts (Gleason and Lilje, 2009). In the case of chytrid zoospores, it is thought that excretion products of diatoms, such as those related to photosynthesis, trigger parasite-host recognition (Bruning, 1991). It has been shown that zoospores of the marine chytrid *Rhizophydium littoreum* exhibit positive concentration-dependent chemotactic responses, which are elicited by carbohydrates and polysaccharides in the medium (Muehlstein et al., 1988).

Attachment of the zoospore to the host cell wall is the next step in infection. At this point, at least three different responses of the hosts are distinguishable. The first two are:

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(a) the host is susceptible to the eukaryotic pathogen, in which case zoospore encystment and development of a sporangium will follow upon attachment of the zoospore; or (b) the alga is resistant (with no observable response by the zoospore to the host). In context with the latter response, active chemical defence of the host against the attack by a pathogen has been assumed. In fact, marine algae have evolved a variety of defensive mechanisms against grazers (Pohnert et al., 2004; Pohnert, 2005). Activated defences, which involve the rapid conversion of defensive precursors into harmful molecules following cell damage, are found in both macro- and micro-algae. In diatoms, recent reports have clearly demonstrated that chemical defence against grazing also relies on the products of fatty-acid oxidation. Only seconds after diatom cells (e.g. *Asterionella* and *Thalassiossira*) were mechanically wounded, an enzymatic mechanism produced fatty acid derived metabolites, resulting in the release of α,β,γ,δ-unsaturated aldehydes (e.g. Pohnert, 2000; Pohnert et al., 2007). In recent years even halogenated toxic substances such as cyanogen bromide have been reported in benthic diatoms as chemical defences (Vanelslander et al., 2012). In addition, some diatoms produce toxins such as domoic acid (DA, e.g. Trainer et al., 2012) and beta-methylamino L-alanine (BMAA, a non-proteinaceous amino acid, Jiang et al., 2014). The domoic acid group comprises ten potent water-soluble neurotoxins, DA and its isomers, which are responsible for amnesic shellfish poisoning (ASP, Jeffery et al., 2004). These toxins can be bio-accumulated in the food web and are especially recognized during harmful algal blooms (HABs) formed by *Pseudonitzschia* spp. If and to what extant these defensive mechanisms of diatoms are involved in the active defence against parasites is still not proven and is part of actual ongoing studies (Scholz, 2014).

A third response type is the so-called hypersensitive response. The hypersensitive response (HR) is a form of programmed cell death including a burst of superoxide production and the expression of specific defence genes. This response option is widely established in terrestrial plants (e.g. White et al., 2000). Indeed the only case of an HR known so far in an algal system (albeit only microscopically, without any mechanistic details) is from the diatom *Asterionella formosa* infected by *Rhizophydium planktonicum* (Canter and Jaworski, 1979; Wolfe, 2000).

It has to be noted that genetic diversity not only of the host, but also of the pathogen, may be a key feature in all steps of parasite-host interactions described above. For example, Gsell et al. (2013c) have demonstrated for the planktonic freshwater diatom *Asterionella formosa* and its parasite *Zygorhizidium planktonicum*, that host genotypes differed in their overall susceptibility to disease, indicating that they possess different variations in the disease resistance trait. To which extent the variation in biochemical composition of these host genotypes becomes important for the choice of the pathogen in the parasite-host recognition is still unknown.

Impacts of zoosporic parasites on marine diatom host populations – some case studies

In most of the cases of infection of marine diatoms by zoosporic parasites no targeted monitoring experiments have been conducted until recently and for some groups such as phytomyxid parasites of diatoms our knowledge is restricted to a few isolated reports (e.g. Schnepf et al., 2000, Table 1). Nonetheless, there have been reports of diatom epidemics caused by zoosporic parasites, such as the infection of two species of *Guinardia* by *Pirsonia* and *Cryothecomonas* in the North Sea (Tillmann et al., 1999). In the latter case, plankton images were used for identification of *Cryothecomonas aestivalis* infecting *Guinardia delicatula*. These images were collected by Imaging FlowCytobot from 2006 to 2013 at the Martha's Vineyard Coastal Observatory (Massachusetts, USA) and were used to identify and quantify the diatom in environmental samples (Peacock et al., 2014). The results showed events where infection rates exceed 10 % are recurrent on the New England Shelf and suggests that the parasites are an important source of host mortality. Furthermore, Peacock et al. (2014) documented a significant negative relationship between bloom magnitude and parasite infection rate, supporting the hypothesis that the parasites play a major role in controlling phytoplankton blooms.

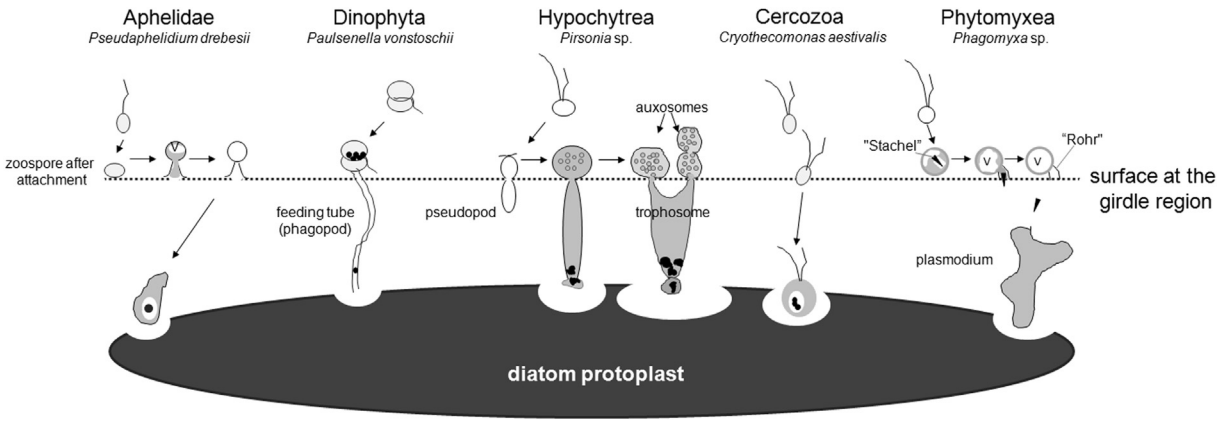

Fig 3 – Examples of infection and feeding modes of some of the zoosporic parasites described in the review. Abbreviations: V: vacuole.

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Further examples of the impact of zoosporic parasites on diatom host populations are two monitoring surveys, using sediment surface samples in combination with Calcofluor White stain and epifluorescence microscopy for the screening of diatoms infected by oomycetes and chytrids (Fig 5). The first monitoring was carried out in the German Wadden Sea area (Scholz et al., 2014, submitted for publication), whereas the second one took place at coastal areas of north-west Iceland (Scholz, 201). During these surveys several marine diatom species were found to be infected by such zoosporic parasites at both sites (Table 1). In all cases, only a small portion of the benthic diatom community was infected during each of the sampling events (only up to 6.3 % and 19.3 % of the total benthic diatom communities in mid-October 2012 and September 2014, respectively, Fig 5H, I), whereas changes in host abundances during the sampling periods were mostly accompanied by increasing numbers of infected diatoms (e.g. *Pinnularia* sp. in May and June 2015, Fig 6A). The majority of infections were caused by chytrids (e.g. 93 % of the infected diatom taxa in the coastal areas of north-west Iceland, Fig 5K). In the case of the Wadden Sea chytrid infections, several morphological features gave evidence for the presence of *Rhizophydium* spp. and *Chytridium* spp. (Scholz et al., 2014), whereas the identification of the chytrids recorded in the coastal areas of north-west Iceland is still in process. Until now, only four species of chytrids (*Rhizophydium littoreum*, *Thalassochytrium gracilariopsis*, *Chytridium polysiphoniae* and *Dinomyces arenysensis*) have been properly identified and partially characterized from brackish and marine ecosystems, and none of these species had been described as pathogens of marine diatoms previously (e.g. Lepelletier et al., 2014). In contrast, the oomycetes *Lagenisma coscinodisci* and *Ectrogella perforans* are especially well known due to earlier studies (e.g. Raghukumar, 1980a,b). In general, representatives of the oomycetes are common in the marine environment and well known to infect several marine macroalgal and seagrass species (Sekimoto et al., 2008a,b; Marano et al., 2012; Sullivan et al., 2013), and planktonic (Drebes, 1966, 1968; Sparrow, 1969; Gotelli, 1971) and benthic diatoms (Scholz et al., 2014, submitted for publication; Scholz, 2014). Regarding the impact of oomycetes on their host population, it was reported, for instance, that an approximate 13 % infection prevalence in a natural population of *Coscinodiscus* was caused by *L. coscinodisci* in the Weser estuary of northern Germany (Raghukumar, 1996). Wetsteyn and Peperzak (1991) showed that during 1985–1990 *Coscinodiscus concinnus* and *Coscinodiscus granii* from the Oosterschelde (The Netherlands) were infected by *L. coscinodisci*. The highest infection percentages varied between 22.2 and 58.3 % in *C. concinnus* and between 7.1 and

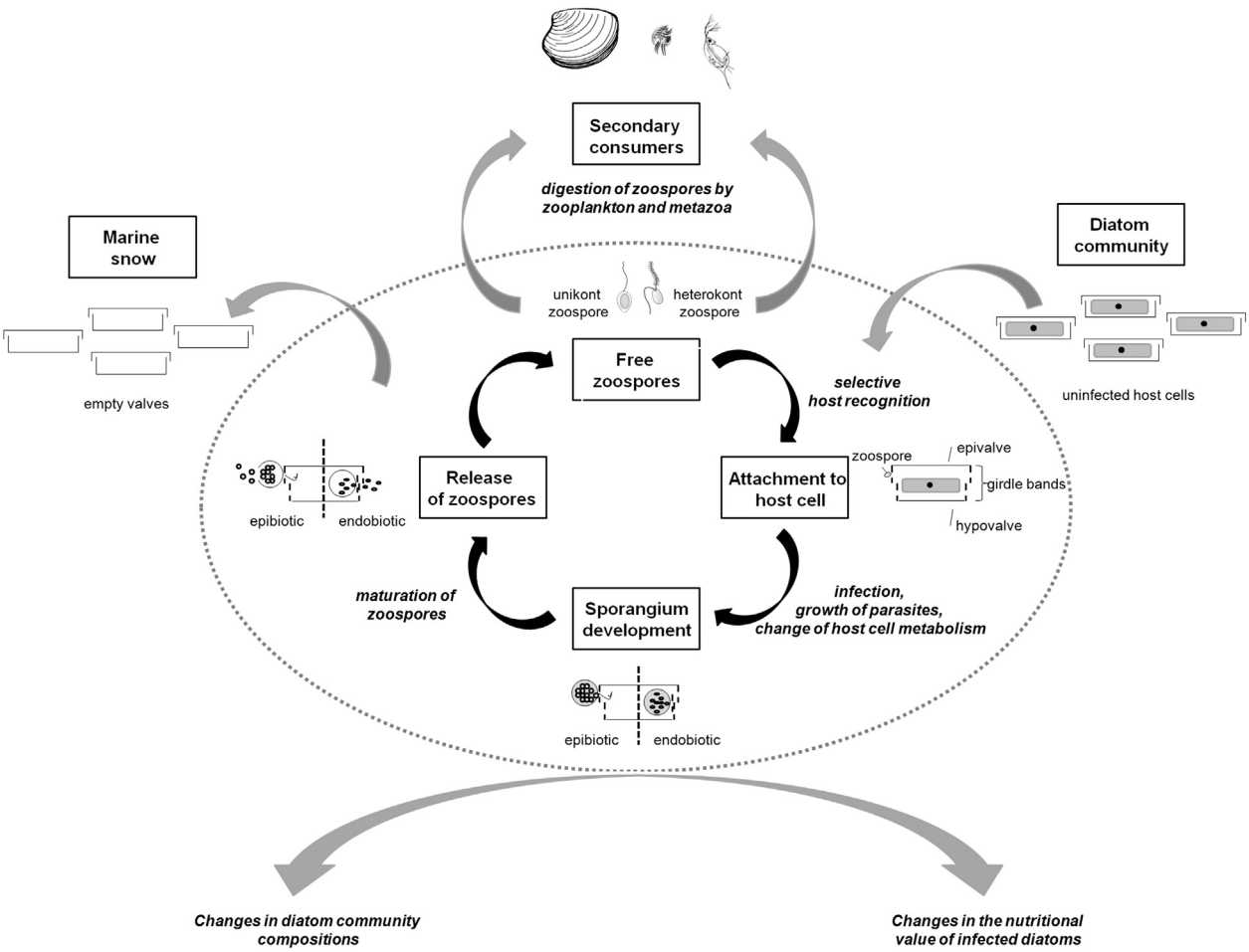

Fig 4 – Schematic life cycle of endo- and epibiotic zoosporic parasites infecting marine diatoms. Besides the main cycle (black arrows), ecological effects on the marine planktonic and benthic community compositions as well as interactions are also depicted (grey arrows).

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41.9 % in *C. granii*. Furthermore, *E. perforans* may cause outbreaks of epidemic proportions in the marine pennate diatom *Licmophora* (e.g. Gotelli, 1971; Raghukumar, 1996).

Influence of environmental factors on the zoosporic parasites prevalence

In general, when conditions are favourable for growth, the asexual life cycles of many zoosporic true fungi, phytomyxids and oomycetes are completed relatively rapidly, resulting in the release of a large number of zoospores into the aqueous environment (also known as zoosporulation) and is an R-strategy ecologically. According to Sparrow (1960), population densities can increase or decrease drastically with changing environmental conditions. Variation in the biotic and abiotic environment can have effects on both, species-level host-parasite interactions but also on host genotype-specific susceptibility to disease (Gsell et al., 2013). According to Lazzaro and Little (2009) the level of host susceptibility to disease often depends on the various environmental parameters, but coevolutionary processes are likely important coplayers. These interrelationships between host, parasite and their environment were first formulated in the disease triangle concept by McNew (1960).

Temperature is one of the most important environmental variables. It is an all-pervasive parameter, affecting metabolism, growth, reproduction and survival of species (e.g. Kingsolver, 2009). Particularly, the specific temperature effects on host-parasite interactions are diverse. Depending on parasite physiology, lower temperatures can increase parasite infectivity (e.g. Schoebel et al., 2011), decrease disease severity (Mitchel et al., 2005) or block infections completely (e.g. Ibelings et al., 2011). For example, in fresh water, Bruning (1991) showed in his experiments that increased temperatures decreased the number of chytrid zoospores produced per sporangium, both under limiting and saturating light conditions for the host. Rising temperatures, also had a strong, negative effect on infective lifetime of the zoospores. The results of the monitoring the marine environment in the temperate Wadden Sea area showed a general increase of infections with decreasing temperatures (Fig 5F), whereas the data obtained from the monitoring of the northern Icelandic sub-arctic intertidal flat demonstrated that the highest infection rates were obtained at temperatures under 10 °C (Fig 5G). Regarding the individual case studies from the northern Icelandic coastal area, it was shown that the highest abundances of *Pinnularia* sp. and *Achnanthes* sp. were recorded in a temperature range between 0.5 and 10 °C (Fig 6). Higher or lower temperatures from this optimum led to decreasing cell numbers in both species (Fig 6B, C). Furthermore, the cell numbers of the parasite of *Pinnularia* sp. showed a similar temperature optimum as its host, despite the fact that *Pinnularia* was also active at temperatures above 10 °C. In contrast, the temperature optimum of the parasitic chytrid of *Achnanthes* sp. was much narrower, ranging between 2.1 and 5.6 °C (Fig 6A, C). In the case of the marine planktonic diatom *Guinardia delicatula*, the infecting stages of *Cryothecomonas aestivalis* were abundant only when water temperature was above 4 °C, while the host itself was observed during all seasons (Peacock et al., 2014).

In the species-specific cases presented in this review, the potential diatom host had the ability to grow under environmental conditions, which were not favourable for the zoosporic parasite. For example, Gsell et al. (2013c) found that the temperature tolerance range of the tested parasite was narrower than that of its host *Asterionella formosa*, providing the host with a "cold" and "hot" thermal refuge of very low or no infection. If host genotypes show different performance ranking orders under abiotic stress than under parasite pressure, then selection in the temperature refuge may also favour a different set of host genotypes (Gsell et al., 2013c). To assume simply that the occurrence of zoosporic parasites follows that of their hosts, does not fully explain the circumstances under which these parasites multiply quickly enough to become epidemic in the field. For marine phytoplankton communities, it has been shown that significant genetic changes occur only in highly seasonal coastal waters but not in the more constant oceanic waters (Brand, 1989). In general, more genetically diverse host populations were shown to be more resistant to disease than genetically poor ones (Altermatt and Ebert, 2008; Whitehorn et al., 2011), as higher host diversity hinders the adaptation of the parasite (De Bruin et al., 2008; Gsell et al., 2012, 2013c). As the global climate changes, and variations in other environmental factors continue (for example periods of lower water temperatures may be shortened or disappear in some regions in the future), it can be assumed that parasite effects on species may increase (such as *Guinardia delicatula*), whereas other parasite-host interrelationships may disappear or change fundamentally (e.g. Ibelings et al., 2011).

Methodological difficulties and recent technological advances

Culture-independent molecular methods based on the amplification, cloning and sequencing of small-subunit (SSU) rRNA genes are a powerful tool to study the diversity of prokaryotic and eukaryotic microorganisms for which morphological features are not conspicuous (Gómez et al., 2011). Traditionally, species description has been based on morphology of host and parasite, which has been extended by discoveries in rDNA sequencing over recent years (Powell and Letcher, 2014; Chambouvet et al., 2015). Zoosporic parasites can show considerable morphological differences caused by nutrient availability or environmental conditions (Hasija and Miller, 1971; Chen and Chien, 1996). Also many species might have been described previously, but their taxonomy has never been resolved or updated to fit our current taxonomic concepts. While DNA barcodes for terrestrial oomycetes are available and widely used (e.g. Robideau et al., 2011), DNA barcodes for most other zoosporic parasites are – despite considerable effort (e.g. Robideau et al., 2011; Schoch et al., 2012; Guillou et al., 2013; Duarte et al., 2015) - missing or not conclusive, and the existing ones rarely allow identification, even to the level of genus (Del Campo et al., 2013; Chambouvet et al., 2015). Further difficulties of DNA-based methods are primer bias within mixed samples going hand in hand with the troublesome establishment and maintenance of 'pure'

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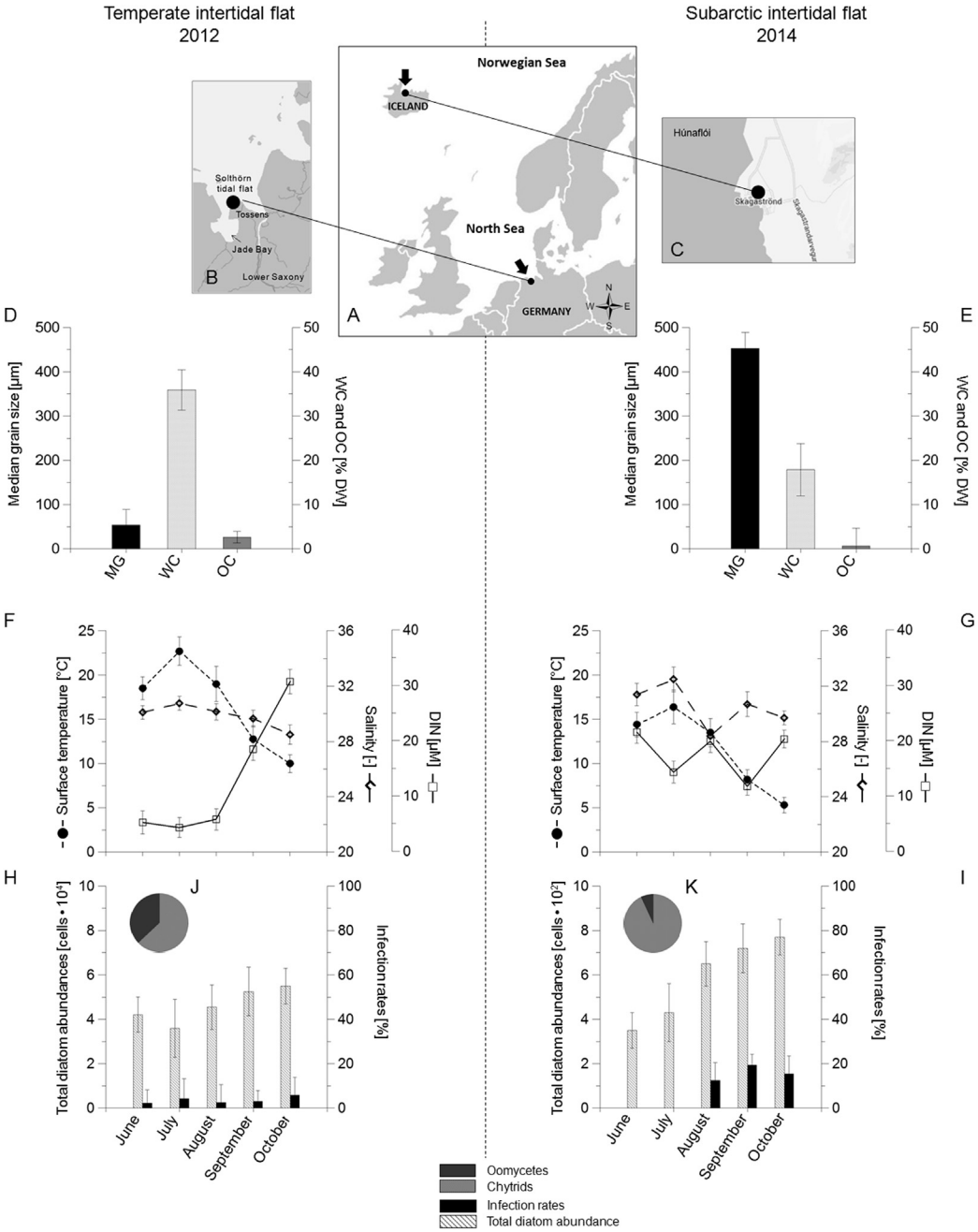

Fig 5 – Comparison of chytrid and oomycete infections of marine benthic diatoms detected in sediment samples collected from the Solthörn tidal flat in 2012 (southern North Sea, Germany, (A, B)) and a subarctic environment in the Húnaflói near Skagaströnd in 2014 (station near the stream, north-west Iceland, (C)). Displayed are the abiotic data of both sampling sides in comparison sediment features (D, E) and surface temperatures, salinity and dissolved organic nitrogen of the overlaying water (F, G) as well as the total diatom abundances and infection rates in the surface sediment samples collected from June to October 2012 (H) and 2014 (I), respectively. In addition total numbers of infections from the different sites are also given (J, K). The percentage of infected cells was calculated by dividing the number of infected cells by the total number of host cells. The mean number of chytrids and oomycetes per cell (host) in the diatom population was also calculated, by dividing the total number of parasites attached to algal cells by the total number of host cells, to normalize the cell density among treatments. This value is referred to as the mean intensity of infection (Holfeld, 2000), reflecting the number of pathogens that succeed in attaching to their host. For identification of eukaryotic parasites the studies of Schenk (1858), Zopf (1884), Sparrow (1960), Johnson and Sparrow (1961), Drebes (1966), Karling (1977) and Letcher and Powell (2012) were used. The diatom identification literature used is listed in Scholz et al. (2014) and Scholz and Einarsson (2015). Sediment samples were collected at bi-weekly intervals at the temperate tidal flat, whereas the sampling in the Skagaströnd area was conducted in monthly intervals. In each case, surface samples of sediment were obtained by inserting 8.5-cm diameter plastic Petri dishes into the sediment to a depth of 1 cm. The sediment samples were prepared as described in Scholz et al. (2014), using ultrasonic pulses of 3 × 2 seconds and density gradient centrifugation of the samples in Ludox-TM (70%). Finally, 150 µl of 10% KOH solution and 150 µl of 0.2% Calcofluor White were added to 1 ml samples in Utermöhl glass counting chambers and diluted to a final volume of 5 ml (incubated for 10 min at room temperature). Abbreviations: DIN: dissolved organic nitrogen; MG: median grain size; WC: water content; OC: organic matter content.

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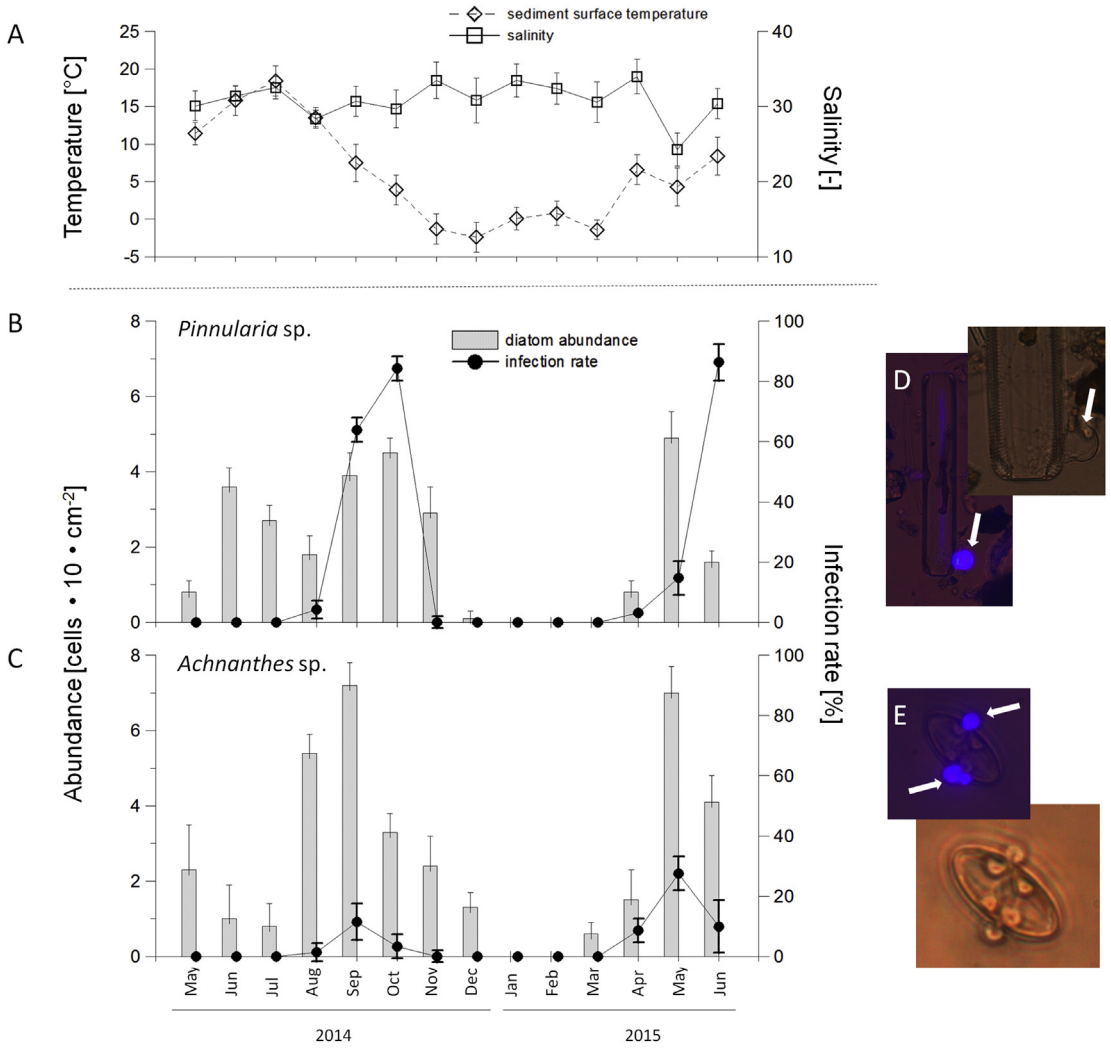

Fig 6 – Case studies of two marine benthic diatoms ((B) *Pinnularia* sp., (C) *Achnanthes* sp.) infected by an unknown chytrid, including abiotic data (A, sediment surface temperature and salinity). Data were recorded during the monitoring in the Húnaflói (Skagaströnd, station at the harbour, northern Iceland) from May 2014 to June 2015, including means ± SD for replicate countings (diatom n = 3; pathogen n = 10). Species identification and the calculation of infection rates were conducted as described in Fig. 5. (D–E) Pictures of the infected cells were obtained by epifluorescence/light microscopy (in combination with Calcofluor White) and usual light microscopy.

dual cultures of host and zoosporic parasite (Chambouvet et al., 2015). Also the genetic diversity within each group of parasites in this review is not known. However, this intraspecific diversity can be considerable in parasites, as shown for e.g. in phytomyxids (Neuhauser et al., 2014) and *Plasmodium* (Nishimoto et al., 2008). In contrast, single cell genomic approaches do exist which allow scientists to circumvent cultivation problems. For example the studies of Ishida et al. (2015) and Ishii et al. (2015) present a PCR-based method to directly analyze genomic DNA of parasites on single infected diatom colonies. This approach could also be used for identifying parasites of marine diatoms and get a better picture of the diversity of diatom-associated parasites in future investigations. However, recent advances in next generation sequencing (NGS) technologies promise to revolutionize the study of such parasites, by providing a comprehensive view of the genome structure and the unexplored reservoir of novel metabolic pathways by studying selected parasite-host pairs in laboratory culture and field experiments (Gerphagnon et al., 2015).

Although, as we have shown, individual examples of the impacts of zoosporic parasites on marine diatoms do exist, research into this subject remains limited at present and further studies are urgently needed to determine the importance of zoosporic parasites in the life cycle of marine diatoms (Worden et al., 2015). Current high throughput sequencing approaches have revealed an unappreciated diversity and abundance of eukaryote parasites in the sunlit, open ocean (De Vargas et al., 2015). Many of those parasites may impact diatom populations, so similar large scale, targeted approaches to sampling during diatom blooms will result in increased knowledge about these parasites. With possible simultaneous infections by different species within diatom blooms in the field, it is difficult to obtain data on prevalence with current research techniques. Since infection by zoosporic parasites may not always significantly affect

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population size of hosts or microbial succession in general, it is important to apply and develop new qualitative and quantitative techniques to study host-parasite interactions. A range of new techniques based on automated sampling and cell sorting (e.g. Lima-Mendez et al., 2015), single cell genomics, and a growing set of reference genomes and transcriptomes (Keeling et al., 2014) will allow informed research into the interactions of zoosporic parasites and their hosts at the genetic level of the organism. Because such methods are becoming more and more available and affordable, it will be possible to account for rapidly changing environmental parameters such as temperature and salinity, day length, nutrient concentrations, tidal cycles, and velocities, as well as grazing activities (e.g. Worden et al., 2015). Additionally, anthropogenic impacts (such as ocean acidification, eutrophication, hypoxia, and over-fishing, etc.) should be considered as potential stressors. Also, in some cases these factors may mask the impacts of the parasite on the overall diatom community composition in the environment. The potential promised by new analytical methods combined with an increasing affordability, and an increased search-ability and public availability of all sorts of 'omics' data (e.g. such as metagenomics, transcriptomic, proteomic, and metabolomics), will allow researchers to answer specific questions and to rapidly identify "new" and "old" zoosporic parasites and their interactions with their hosts.

Conclusions and future prospects

(1) Species of zoosporic parasites from at least seven different phyla have been observed to infect marine diatom hosts (Table 1). There are probably many more species awaiting discovery. Thus we expect that a large number of species with different infection strategies are potential parasites of diatoms.
(2) Some diatoms are considered to be relatively inedible, yet zoosporic parasites appear to have no difficulty accessing and digesting the living cytoplasm inside their silica cell walls, indicating an important role of these parasites for energy transfer within marine food webs (Fig 4).
(3) Zoosporic parasites very likely significantly impact population sizes and species composition of diatom hosts in marine ecosystems through parasitism (Figs 5 and 6).
(4) The effect of climate change on the interactions of parasites and host diatom populations is not known.
(5) More research on diatom-parasite relationships is needed and should be of high priority for research funding, especially with accelerating global climate change.
(6) Very little is known about the interactions between these parasites and their hosts. This area of research remains a black box to be opened.

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