

Functional ecology of aquatic phagotrophic protists – Concepts, limitations, and perspectives

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Abstract

Functional ecology is a subdiscipline that aims to enable a mechanistic understanding of patterns and processes from the organismic to the ecosystem level. This paper addresses some main aspects of the process-oriented current knowledge on phagotrophic, i.e. heterotrophic and mixotrophic, protists in aquatic food webs. This is not an exhaustive review; rather, we focus on conceptual issues, in particular on the numerical and functional response of these organisms. We discuss the evolution of concepts and define parameters to evaluate predator–prey dynamics ranging from Lotka–Volterra to the Independent Response Model. Since protists have extremely versatile feeding modes, we explore if there are systematic differences related to their taxonomic affiliation and life strategies. We differentiate between intrinsic factors (nutritional history, acclimatisation) and extrinsic factors (temperature, food, turbulence) affecting feeding, growth, and survival of protist populations. We briefly consider intraspecific variability of some key parameters and constraints inherent in laboratory microcosm experiments. We then upscale the significance of phagotrophic protists in food webs to the ocean level. Finally, we discuss limitations of the mechanistic understanding of protist functional ecology resulting from principal unpredictability of nonlinear dynamics. We conclude by defining open questions and identifying perspectives for future research on functional ecology of aquatic phagotrophic protists.

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Introduction – The Meaning of Functional Ecology and Why It Must Be Applied to Aquatic Protists

What is Functional Ecology – is it a discipline, a concept or a theory? Since there is no formal definition or generally

accepted method of approach, it is not surprising that contrasting views exist on its meaning, nor is it surprising that in the past it was not a generally accepted discipline (Bradshaw 1987; Calow 1987; Grime 1987; Keddy 1992; Kuhn 1996). We consider that functional ecology is both a concept and an emerging major subdiscipline within ecology, closely related to community ecology; the latter studies functional traits* (Table 1) of species and their interactions within the context of abiotic environmental gradients (McGill et al. 2006; Violle et al. 2007). In a broad sense, we define *functional ecology*

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as the branch of ecology that investigates the functions that species have in the community or ecosystem in which they occur. Typical examples of species functions in broad categories (functional guilds^{*1}) are those as primary producers, various consumers (herbivores, carnivores), parasites, and decomposers. A functional guild comprises groups of species that exploit the same resources. This does not mean that all species within a guild occupy the same ecological niche* or ecological role (sensu Grinnell 1917, 1924). For instance, macrophytes and planktonic algae both act as primary producers in aquatic ecosystems with distinct ecological roles. To account for this, diversity ecologists study genetic, morphological, physiological, and life history characteristics of species and their interactions. Within this general context, the cornerstone of *functional ecology* is to enable a *mechanistic understanding of ecological pattern and processes* from the organismic to the ecosystem scale. The organismic level can be broken down further to genes and molecules. Processes and traits represent adaptations to the environment, linking function to fitness* and allowing judgements about their fitness value in different environments (Calow 1987).

Notably, functional ecology is not only concerned with communities as could be considered from our above definition. The link between function and fitness affects, in the first place, the individual organism, determining its survival, fecundity, and development (somatic growth). However, in experimental work with protists growth and ingestion are usually averaged over the population, and numerical and functional responses are presented as average per capita rates vs prey abundance or biomass (see section *Functional community ecology: From microcosms to the ocean* and Conclusions, below).

Irrespective of stochasticity ('noise'), which is inherent in (measurements of) virtually all ecological processes at the population and community level, organism interactions with their biotic and abiotic environment do not result from and do not lead to random patterns and structures; rather, key processes can be represented mathematically, to allow predictions for the evolution of the existing structures. Presumably, the precision and general applicability (robustness) of such model predictions depend on the level of resolution at which the underlying patterns and processes can be studied (*a priori* knowledge). It is at present an open question if a refined level of investigation necessarily yields a better understanding at the ecosystem level. There is increasing awareness that chaotic processes inherent in even seemingly 'simple' systems may principally limit model predictions resulting from mechanistic analyses of processes such as competition for resources and grazing (Becks et al. 2005; Becks and Arndt 2008, 2013; Huisman and Weissing 1999, 2001; see section *Limitations of the mechanistic analysis: When chaos hits*, below).

Although they are of tremendous global and local significance for cycling of matter in the ocean and inland water bodies, aquatic protists have been used less frequently than bacteria and multicellular organisms to investigate conceptual issues. Protists are, as primary producers, predators, food, and parasites structural elements of any aquatic food web; there are many aquatic habitats without macroorganisms, but there is none without bacteria and at least some protist species. This includes extreme environments, such as anaerobic deep sea basins, hydrothermal vents, solar salterns, and extremely acidic lakes (e.g., Alexander et al. 2009; Anderson et al. 2012; Weisse 2014 and references therein). Such extreme habitats are ideal candidates for testing general ecological and evolutionary principles with microbes. For instance, acidic lakes have been used to investigate habitat effects on fitness of protists and provide evidence for their local adaptation (Weisse et al. 2011). Extensive research was performed with rapidly evolving bacteria and some algae to study microevolution* experimentally (Collins and Bell 2004; Cooper et al. 2001; Lenski 2004; Pelletier et al. 2009; Sorhannus et al. 2010). Adaptive radiation of the bacterial prey, *Pseudomonas fluorescens*, in response to grazing by the ciliate *Tetrahymena thermophila* was investigated in laboratory microcosms (Meyer and Kassen 2007). However, overall, the application of theory in microbial ecology is limited (Prosser et al. 2007), and contemporary microbial ecology has been accused of being driven by techniques, neglecting the theoretical ecological framework (Oliver et al. 2012). Recent attempts to apply macroecological* theory to microbes considered almost exclusively prokaryotes (Nemergut et al. 2013; Ogilvie and Hirsch 2012; Prosser et al. 2007; Soininen 2012; but see below). Caron and colleagues (Caron et al. 2009) lamented that in the present 'era of the microbe' single-celled eukaryotic organisms (i.e. the protists) have been largely neglected. This situation is illustrated at recent international microbial ecology meetings where researchers working with protists usually represent an almost exotic minority. Here we emphasise that protists, as the most abundant eukaryotic cells, are ideally suited to test general (macro)ecological and evolutionary concepts (Montagnes et al. 2012; Weisse 2006), revitalising the use of protists as model organisms that started with Gause's classical experiments (Gause 1934) with *Didinium* and *Paramecium* (DeLong et al. 2014; Li and Montagnes 2015; Minter et al. 2011). Since then, microcosm experiments with various protist species have been used to study conceptual issues such as metapopulation dynamics (Holyoak 2000; Holyoak and Lawler 1996), the effect of resources for food web structure (Balčiūnas and Lawler 1995; Diehl and Feissel 2001; Kaunzinger and Morin 1998; Petchey 2000), and food web complexity-stability relations (Petchey 2000) and their sensitivity to global warming (Montagnes et al. 2008a; Petchey et al. 1999). The potential of protist microcosm experiments to investigate general concepts in population biology, community ecology and evolutionary biology has recently been reviewed (Altermatt et al. 2015).

¹ Terms marked by an asterisk are explained in the Glossary.

In many other biological disciplines (e.g., cell biology, biochemistry, molecular genetics) protists have long served as model organisms (Dini and Nyberg 1993; Hausmann and Bradbury 1996; Hedges 2002; Montagnes et al. 2012). This is mainly because protists are easy to cultivate in large cell numbers, have short generation times, and can be manipulated with ease (Montagnes et al. 2012). In addition, many microbial communities can be studied through the combination of field observations and laboratory experiments (Fenchel 1992).

In this article, we address key aspects (numerical and functional response, applicability and limitations of model predictions) of functional ecology for aquatic protists, focusing on heterotrophic and mixotrophic* species. In view of their ecological significance, it may be surprising that this integrative approach is novel since functional ecology as a scientific subdiscipline is approximately 30 years old. The application of molecular techniques for the detection and identification of aquatic protists has fundamentally changed our perception of their biodiversity and, thus, functional ecology over the past two decades (de Vargas et al. 2015; Epstein and Lopez-Garcia 2008; Orsi et al. 2012). However, thus far, there are only a few recent treatises on selected functional groups and taxa available in the literature (Jürgens and Massana 2008; Montagnes 2013; Stoecker et al. 2009; Suzuki and Not 2015).

This paper is based upon five oral contributions presented at the joint VII ECOP/ISOP meeting at Seville, Spain, in September, 2015. We will first describe the conceptual background, then analyse systematic differences of key parameters across taxa and life strategies. In the next step, we will upscale the current knowledge from the laboratory to the ecosystem (ocean) level, including a discussion of problems inherent in this approach. Finally, we will discuss limitations of our approach resulting from principal unpredictability of nonlinear dynamics and identify open questions for future research. The latter is the main goal of this article; we endeavour to encourage our protistological colleagues to take more advantage of their respective pet species for testing general ecological principles.

Evolution of Concepts: From Lotka–Volterra to the Independent Response Model

We begin by outlining two key components of protistan functional biology: how ingestion and growth rates change with prey abundance; i.e., respectively, functional and numerical responses. We then illustrate how together they can be used to assess one fundamental aspect of functional ecology: predator–prey dynamics. Much of that which follows in this section is not new, but is presented here to provide context for the following parts of this paper. However, some of the synthesis is novel and is intended to stimulate researchers

to apply innovative approaches to experimental protistology and functional ecology in general.

The functional response

Ingestion, at the simplest level, can be divided into two steps (Fig. 1): encounter (or searching) and processing (or handling), although these may be separated into a series of mechanistic steps (see Montagnes et al. 2008b). Critical to this analysis, when prey are being processed, encounter ceases; i.e. mechanistically, the consumer stops searching while manipulating captured food. Then, as prey concentration increases, ingestion rate increases, following a rectangular hyperbolic or “Type II” functional response (Fig. 2A, Eq. (1a), Real 1977). In Eq. (1a), I is ingestion rate (prey per predator per time), V is prey (victim) abundance, h is the handling time (prey per time), and a is the affinity between the predator and prey, with dimensions of volume processed per time (Fig. 2A). Eq. (1a) is commonly presented as Eq. (1b), where maximum ingestion rate ($I_{\max}$, prey per predator per time) is $1/h$, and k (prey per volume) is h/a . Note that the initial slope of the curve (a) depicted in Fig. 2a is also the searching rate, and k is the prey concentration that results in $0.5 I_{\max}$ (i.e. the half saturation constant).

$$I = \frac{aV}{1 + haV} \quad (1a)$$

$$I = \frac{I_{\max} V}{k + V} \quad (1b)$$

In this basic, but mechanistic, manner we can obtain predictive functions for how ingestion rate varies with prey abundance. Furthermore, and critical to much of the rest of this paper, we can obtain parameters that may be investigated in terms of their variation due to abiotic and biotic factors. Here, however, we limit the discussion to the effect of prey abundance; clearly, both handling time (h) and searching rate (a) may also vary with prey abundance, altering the shape of the response. The most commonly recognised of these effects is a decrease in searching rate when prey are scarce, presumably to reduce energy expenditure, leading to a “Type III” functional response (Fig. 2A, dashed line). It must be noted, though, that protists often increase their swimming speed at low prey levels (Crawford 1992; Fenchel 1992; Jeong et al. 2004), which will, in fact, increase the searching rate at low levels. Consequently, it is not surprising that Type III responses are rarely observed for protists, with some notable exceptions (e.g. Gismervik 2005).

The rate at which organisms process water can be quantified by the clearance rate (C , Eq. (2)), with dimensions of volume per time (Fenchel 1980; Fenchel 1987):

$$C = \frac{I}{V} \quad (2)$$

From Eq. (2) it is obvious that C changes with prey abundance: as illustrated in Fig. 1, as prey abundance increases

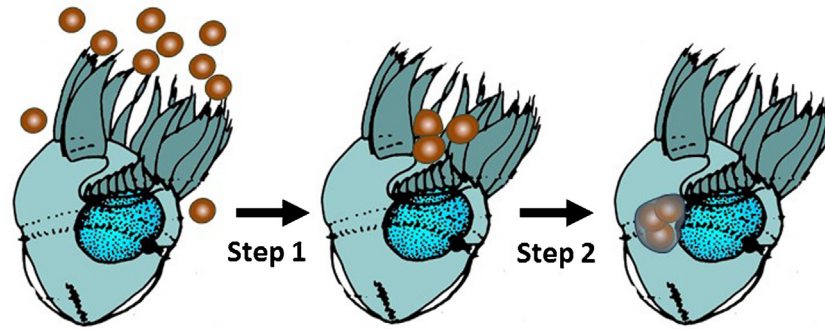


Fig. 1. An illustration of the two steps associated with food capture, using an oligotrich ciliate consuming prey. Step 1 is the *encounter* rate between predator and prey and relies on the swimming speed and the relative size of both predator and prey. Step 2 is *processing* rate, which relies on the predator capturing, manipulating and consuming the prey. Critically, when prey are being processed in Step 2, the predator stops performing Step 1 (swimming may continue but encounters will not lead to capture or consumption).

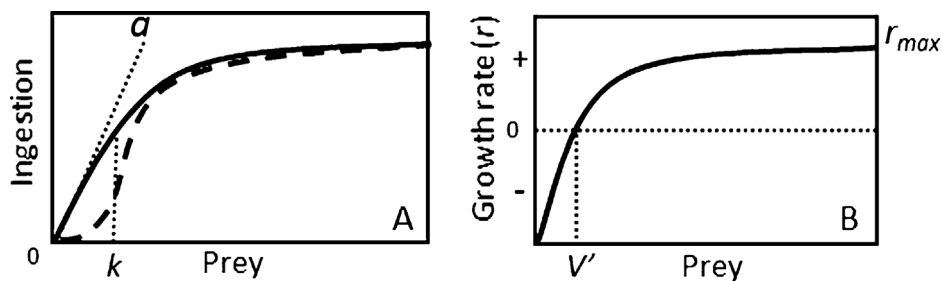


Fig. 2. Cartoons of the (A) functional and (B) numerical responses. For the functional response, the solid line depicts a Type II response (Eqs. (1a), (1b)), and the dashed line is a Type III response (no equation presented); a is the initial slope of ingestion vs. prey abundance and is an indication of the affinity between prey and predator; k is the half saturation constant. For the numerical response $r_{\max}$ is the maximum growth rate, and V' is the threshold concentration (i.e. below which growth is negative).

less time is spent in Step 1 (searching) and more time is spent in Step 2 (handling); consequently, less volume is processed, or “cleared”, as the protist is “busy” dealing with captured food. In Eq. (1a), a , corresponding to maximum clearance rate, is a useful parameter to assess functional ecology, e.g. to compare the performance of different taxa, such as ciliates and flagellates (Hansen 1992; Hansen et al. 1997; Neuer and Cowles 1995; Sherr et al. 1991; see also next Chapter). Functionally a can also be separated into two components: (1) the encounter-area of the predator, which will change with both predator and prey size, and (2) movement, which will change with swimming speed. This allows further assessment of the functional ecology of protists.

Before leaving the functional response, we address the issue of predator interference (the interaction between predators). There is a growing body of literature arguing that not only is ingestion rate prey-dependent, but the number of predators in the system will also influence the ability of the individual predator to ingest prey. Relatively recently, the model predator–prey system of *Didinium-Paramecium* has been used to indicate that increased predator abundance can reduce *per capita* ingestion rate (DeLong and Vasseur 2013). In this case, Eq. (1a) is modified to Eq. (3), where m describes the predator (P denotes predator abundance) dependent decline in searching, independent of prey

abundance. For a wider view on this issue, the reader is directed to (Arditi and Ginzburg 2012).

$$I = \frac{aVP^m}{1 + haVP^m} \quad (3)$$

The numerical response

Based on basic bioenergetics, specific growth rate of the predator (r) should be related to the amount of ingested prey, and thus the numerical response tends to also follow a rectangular hyperbolic function (Fig. 2B); here growth rate approaches an asymptotic level ($r_{\max}$), as prey abundance (V) increases and the initial curvature of the response is described by a constant (k_2) with dimensions of prey abundance. There, however, are two key differences between the functional and numerical responses (cf. Eq. (1b), Eq. (4)). First, at a defined prey abundance the predator obtains, through ingestion, only sufficient energy to maintain itself; there is, therefore, a positive x -intercept (V') to the growth response, below which growth rate is negative (i.e. mortality occurs). Second, as outlined below (see *Conversion Efficiency and Mortality*), the amount of prey that is converted to predators is prey-dependent. Consequently, the shape of the

numerical response will differ from the functional response.

$$r = \frac{r_{\max}(V - V')}{k_2 + (V - V')} \quad (4)$$

A second issue related to predator growth is that predator size (and thus biomass) is also prey-dependent, typically following a rectangular hyperbolic function with a positive y-intercept (Kimmance et al. 2006; Weisse et al. 2002). Changes in the cell volume by a factor of 4–10 occur for marine and freshwater ciliates and athecate dinoflagellates and are affected not only by their nutritional status (Calbet et al. 2013; Fenchel 1987, 1992; Hansen 1992) but also by abiotic factors such as temperature and pH (Kimmance et al. 2006; Weisse and Stadler 2006; Weisse et al. 2002; see also *Functional community ecology: From microcosms to the ocean*, below). Consequently, to assess bioenergetics and biomass flux, predator size (with respect to prey abundance) must be examined; to this end, cell volume is typically measured and then converted to carbon using standard functions (Menden-Deuer and Lessard 2000). Note though that this approach may be limited with protists such as thecate dinoflagellates that remain the same size but become almost empty when starved (P.J. Hansen, pers. observation). To determine the response of predator volume to prey abundance, we have applied Eq. (5), in a purely phenomenological manner, where M is the predator mass, $M_{\max}$ is the asymptotic maximum mass, k_3 is a constant that describes the shape of the curve, and M' is the mass of the predator at zero prey abundance (e.g. Kimmance et al. 2006).

$$M = \frac{M_{\max} V}{k_3 + V} + M' \quad (5)$$

Modelling predator–prey dynamics

Phagotrophic protists act as consumers in both simple models that explore predator–prey theory (Montagnes et al. 2012) and complex food web models that evaluate a range of ecological issues such as climate change and carbon flux (e.g., Blackford et al. 2004; Mitra et al. 2014; Montagnes et al. 2008a). Here, we focus on this fundamental predator–prey link, which almost universally follows a Lotka–Volterra based structure (typically modified to a Rosenzweig–MacArthur model, Turchin 2003), where ingestion rate (the functional response, Eq. (1b)) is parameterised experimentally and predator growth is predicted from ingestion assuming a constant conversion efficiency* (e) and a constant mortality (loss) rate (d). Below, first in words and then using equations, we outline the Rosenzweig–MacArthur model.

Prey population growth = prey logistic growth – predator ingestion

$$\frac{dV}{dt} = \mu V \left(1 - \frac{V}{K}\right) - \frac{I_{\max} V}{k + V} P \quad (6)$$

Here, V and P are prey and predator abundance respectively; μ and K are the prey growth rate and carrying capacity, respectively; and all other terms are described above.

Predator population growth = conversion efficiency $\times$ predator ingestion – loss

$$\frac{dP}{dt} = e \frac{I_{\max} V}{k + V} P - dP \quad (7)$$

Here, all terms are described above, with d representing the per capita mortality rate.

Following the modeller's adage of “rubbish in, rubbish out”, over the last four decades, there has been considerable effort placed on carefully parameterising the functional response of protists. Our recent work on protists has, however, indicated that e and d are both prey-dependent, and including such complexity significantly alters, and improves, a model's predictive ability (Fenton et al. 2010; Li et al. 2013; Li and Montagnes 2015; Minter et al. 2011; Montagnes and Fenton 2012; Yang et al. 2013). Therefore, “rubbish” does not simply concern the quality of the parameters but, critically, the underlying functions. We have developed two model structures to correct for this problem: (1) we have added functions to the existing Rosenzweig–MacArthur structure, providing e - and d -prey dependency (see *Conversion Efficiency and Mortality*, below) and (2) we have strongly recommended that a numerical response is independently determined and used to directly determine predator growth (see *The Independent Response Model*, below).

Conversion efficiency and mortality

Without providing data or details, a “thought experiment” can indicate that e and d should be dependent on prey abundance. Before a protist can divide (i.e. increase in numbers), it must reach a certain size. Reaching this size depends not just on having sufficient resources to survive, but requires further resources to allow it to commit to division. Above a certain threshold level of prey abundance the predators will be able to obtain sufficient resources to divide (creating new individuals). As prey abundance increases more resource is available, and there will be a commensurate increase in e , as a greater amount of prey is allocated towards reproductive growth, ultimately reaching a maximum efficiency. Above this maximum level e may be asymptotic, but processes such as sloppy feeding*, or increased vacuole passage rate may reduce e as prey abundance increases (Fig. 3A). Likewise, in the absence of prey, predator mortality (d) should be maximal (i.e. death is very likely in the absence of food), and as food becomes more available increased fitness of the predator will reduce the likelihood of death (Fig. 3B). Given the, seemingly, obvious nature of these trends, and the universal acceptance in predator–prey models of prey-dependence of prey growth (logistic growth) and predator ingestion rate (functional response) it is rather surprising that prey-dependent e and d functions have failed to be regularly incorporated into food web models.

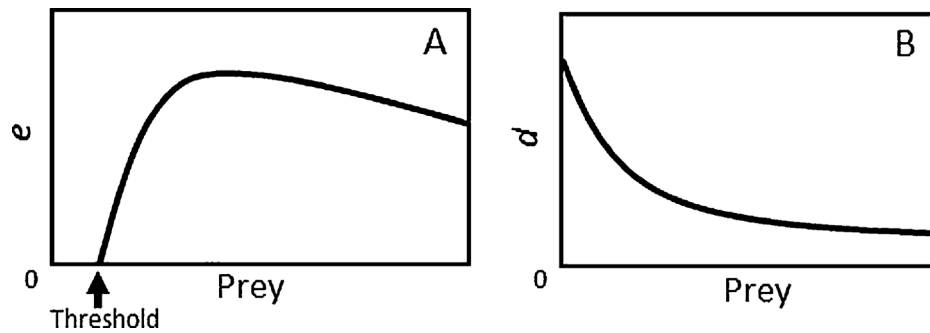


Fig. 3. Conceptual representations of the relation between (A) conversion efficiency (e) and (B) mortality rate (d) and prey abundance. Threshold = the prey abundance where growth is zero (V' , Eq. (4)).

Our work to date has provided functions for prey-dependent e and d (see Li and Montagnes 2015). These may be incorporated into the second equation of the Rosenzweig–MacArthur model (Eq. (7)), so that both e and d are variables (Eq. (8)). We have also outlined how these functions can be experimentally parameterised (Li and Montagnes 2015). However, considerable effort is required to do so, and we argue that there is a simpler approach to take, which we outline in the next section.

$$\frac{dP}{dt} = f(e) \frac{I_{\max} V}{k_1 + V} P - f(d) P \quad (8)$$

The independent response model

Rather than attempting to fully parameterize Eq. (8), which models how the predator population changes over time, it is both pragmatic and parsimonious and also exceedingly easier for protistologists to directly determine predator growth (and mortality) at a range of prey concentrations (i.e. the numerical response, Eq. (4)). Then the second equation in the Rosenzweig–MacArthur model can be replaced by Eq. (9), where all terms have been described above.

$$\frac{dP}{dt} = \frac{r_{\max}(V - V')}{k_2 + (V - V')} P \quad (9)$$

This approach was outlined conceptually by Fenton et al. (2010) and we have used it in several works that explore protistan predator–prey dynamics (e.g., Li and Montagnes 2015; Montagnes et al. 2008b). Furthermore, the Independent Response Model lends itself to allow analysis of how a range of abiotic and biotic factors have different effects on ingestion and growth, providing better understanding of the functional biology of protists and a better ability to predict the outcome of predator–prey dynamics (e.g. strains and temperature: Yang et al. 2013). We, therefore, strongly encourage its use by protistologists who are modelling predator–prey dynamics. For those less interested in modelling but studying the functional biology of protists, we encourage you to examine both the functional and numerical responses. In this way, you will obtain a much better appreciation of their principal abilities.

Systematic Differences of Key Parameters Across Taxa and Life Strategies

Protists, with their diverse life strategies, have adapted to a range of aquatic habitats, exploiting virtually all nutrient resources as bacterivores, herbivores, carnivores, parasites, osmotrophs, detritivores, and histophages* (Fenchel 1987). As outlined in the next section, mixotrophic species using more than one nutritional resource are also widespread across taxa and are, globally, important components of food webs. Accordingly, heterotrophic and mixotrophic species have developed extremely versatile feeding modes, ranging from filter feeding to direct interception and diffusion feeding, and also including passive and active ambush feeding* (reviewed by Fenchel 1987; Kjørboe 2011). Below, we explore the extent to which systematic differences in key parameters of the numerical and functional responses (see previous section) are related to protist life strategies, feeding mode, habitat (marine vs freshwater), and taxonomic affiliation.

Screening the available zooplankton literature (including heterotrophic protists and metazoa), Hansen et al. (1997) analysed maximum growth rates ($r_{\max}$, Eq. (4)), maximum ingestion rate ($I_{\max}$, Eq. (1b)), and maximum clearance rate (a , Eq. (1a)) plotted against cell volume (a proxy for individual cell mass, M , in Eq. (5)). They found that ciliates display maximum ingestion, growth, and clearance rates that exceed those of dinoflagellates by a factor of 2 to 4. Although the average swimming speed of ciliates exceeded that of dinoflagellates, this only partly accounted for the difference in maximum clearance rates (i.e. a , above). In absolute terms, maximum growth rates of ciliates (0.027 – 0.120 h^{-1} at experimental temperatures ranging from 12 to 20°C) were higher than those of similar sized dinoflagellates (0.013 – 0.050 h^{-1}) but lower than those of the smaller nanoflagellates (0.035 – 0.250 h^{-1}). However, if the growth rates of ciliates are extrapolated to the size of small heterotrophic flagellates (excluding dinoflagellates), the derived growth rate would be about three times higher than the rate of flagellates, supporting an earlier analysis (Fenchel 1991).

Hansen et al. (1997) also concluded that gross growth efficiency* or yield (which is associated but not identical to conversion efficiency, described above; see Glossary) is not

Table 1. Glossary.

Term	Definition/Meaning	References
Ambush feeding	Ambush feeders capture prey that comes within their perception range. Passive ambush feeders passively encounter and intercept prey due to the motility of their prey; this includes Fenchel's (1986) diffusion feeding of non-motile grazers. Active ambush feeders passively perceive motile prey and capture these by active attacks.	Fenchel 1986, 1987; Kiørboe 2011
Bet-hedging	Bet-hedging is an evolutionary adaptation that facilitates persistence in the face of fluctuating environmental conditions; it is defined as a strategy that reduces the temporal variance in fitness at the expense of a lowered arithmetic mean fitness. Bet-hedging theory addresses how individuals should optimise fitness in varying and unpredictable environments.	Beaumont et al. 2009; Olofsson et al. 2009; Ripa et al. 2010
Community	An ecological community is a group of actually or potentially interacting species living in the same location. Communities are bound together by a shared environment (habitat) and a network of mutual interactions. The term traces back to Möbius' (1877) biocoenosis.	http://www.nature.com/scitable/knowledge/community-ecology-13228209 ; Möbius 1877
Conversion efficiency	Conversion efficiency (e) is the the fraction of ingested (I) material that is retained within the protist, resulting in new cells (= births in metazoan terms, b), $e = b/I$. This differs from assimilation efficiency (A), which is the fraction of ingested material that is retained within the protist and may be used for "births" and maintenance (i.e. not egested, E), $A = (I - E)/I$	Fenton et al. 2010; Montagnes 2013; this paper
Dilution technique	A method to estimate the grazing impact of microzooplankton on phytoplankton and bacteria. The dilution approach relies on the reduction of encounter rates between prey and predator. Natural water samples are diluted with sterile filtered sea/lake water creating a dilution series, and grazing rate is estimated as the increase in apparent prey growth rate with dilution factor.	Dolan and McKeon 2005; Landry et al. 1984; Landry and Hassett 1982; Weisse 1988
Ecological niche	The ecological niche describes the set of abiotic and biotic conditions where a species can persist. The fundamental niche (FN) is an abstract formalisation that is unique for each species; in reality, due to interspecific competition species are forced to occupy a niche that is narrower than the FN (realised niche).	Elton 1927; Grinnell 1917, 1924; Hutchinson 1957; Wiens 2011
Fitness	The contribution of an allele or genotype to the gene pool of subsequent generations, relative to that of other alleles or genotypes in this population. Individuals that produce the largest number of offspring over many generations have the greatest fitness.	Lampert and Sommer 2007; Šajna and Kušar 2014
Functional group/guild	A group of coexisting species that exploit the same resources in a similar way and, therefore, have the same function in the ecosystem	Blondel 2003; Root 1967; Wilson 1999
Functional trait	A measurable property of organisms, usually measured at the individual level and used comparatively across species, that strongly influences organismal performance; functional traits are also defined as morpho-physiophenological traits which impact fitness indirectly via their effects on growth, reproduction and survival.	McGill et al. 2006; Violle et al. 2007
Gross growth efficiency	Gross Growth Efficiency (GGE) is the fraction of ingested biomass (I) that is converted to growth (r), i.e. births minus deaths ($b - d$); $GGE = r/I$.	Lampert and Sommer 2007; Straile 1997; Welch 1968; this paper
Histophagy, histophages	Histophagy is a specialised type of raptorial feeding; histophages species attack damaged, but still living, other organisms	Fenchel 1987, 1992
Macroecology	The ecological subdiscipline that analyses the relationships between organisms and their environment at large spatial scales to characterise and explain statistical patterns of abundance, distribution and diversity, irrespective of organism size	Brown and Maurer 1989; Gaston and Blackburn 2000

Table 1. (Continued)

Term	Definition/Meaning	References
Microevolution	Microevolution is the change in allele frequencies that occur over time in a population, i.e. it measures adaptation within species	Dobzhansky 1937; Futuyama 2009
Mixotrophy	Use of different sources of energy and carbon for nutrition; in this paper mixotrophy denotes the combination of prey uptake and photosynthesis (i.e. excluding osmotrophy)	Boraas et al. 1988; Calbet et al. 2011; Hansen 2011; Jones 2000
Pallium feeding	A specialised feeding mode of dinoflagellates, defined as the process of attacking and extracellularly digesting prey with a pseudopodial ‘feeding veil’, the pallium	Calbet et al. 2013; Gaines and Taylor 1984; Hansen 1992
Peduncle (tube) feeding	Sucking out the contents of the prey with a feeding tube, the peduncle; another specialised feeding mode of dinoflagellates	Calado and Moestrup 1997; Hansen 1992; Spero 1982
Sloppy feeding	Loss of prey body contents during feeding by an aquatic predator, usually in the form of dissolved organic carbon. Sloppy feeding has been well documented for aquatic microcrustacea; among protists, it may occur in tube and pallium feeding dinoflagellates	Dagg 1974; Lampert 1978
Specific growth rate/intrinsic rate of increase	The rate at which a population increases, assuming exponential growth (in ecological studies typically denoted by r or μ , with dimensions of time ⁻¹). Thus, for a population whose size is represented by n , r can be determined by regressing the natural logarithm ($\ln$) of n vs time (t); e.g. if initial (n_0) and final (n_t) population sizes are known over a set time (t), then $r = \ln(n_t/n_0)/t$. Note that if the population is increasing, then r is positive, while if it is decreasing r is negative.	Banse 1982; Fenchel 1974; Kirchman 2002

different among the different protistan and metazoan taxa, with an average of 0.33. This conclusion was supported by an independent meta-analysis of protozoan and metazoan zooplankton gross growth efficiencies (GGE) published in the same year (Straile 1997), which concluded that mean GGE ranged from 20–30% and hardly differed between taxa. Concerning the uncertainty involved in calculating GGE, the seeming difference between Hansen et al. (1997) and Straile (1997) is minor. However, there are several factors that will result in a change in GGE.

The most obvious factor that will alter GGE is prey abundance, following arguments akin to those for conversion efficiency above; for an example of how GGE may vary with prey abundance (and temperature) see Kimmance et al. (2006). Research has also revealed that varying prey quality (stoichiometric composition) may strongly affect trophic transfer dynamics and, therefore, GGE (Mittra and Flynn 2005, 2007; Sterner and Elser 2002). Similarly, the nutritional history (i.e. past-prey availability) of the grazers can affect both their numerical and functional responses (Li et al. 2013; Calbet et al. 2013) and thus GGE or conversion efficiency. However, it is seldom considered that protists can alter their trophic behaviour according to previous feeding history (Boenigk et al. 2001a; Li et al. 2013; Meunier et al. 2012). Finally, as we have explained above, we now know that conversion efficiency is prey density dependent. It appears that GGE has a maximum of approximately 0.3 under food replete conditions; this is where the food quantity is saturating and food quality is presumably best. The fact that GGE does not increase further implies physiological constraints that should be investigated in more detail for

aquatic phagotropic protists. However, although almost 20 years have passed since these analyses and although more data are accumulating every year, Hansen et al.’s (1997) main conclusions concerning the observed taxonomic differences remain unchallenged.

An intriguing question is, what causes the functional differences between ciliates and similar-sized dinoflagellates? We may seek the answer in their different nuclear structures, feeding modes, and life strategies. Except for a short period during sexual reproduction, the ciliate macronucleus can be continuously transcribed through the (asexual) cell cycle, supporting a higher growth rate than dinoflagellates with their complex dinokaryon can achieve (Cavalier-Smith 1978, 2005a,b). The majority of ciliates considered in the above investigations were (highly efficient) filter feeders, while dinoflagellate feeding behaviour is extremely diverse (Elbrächter 1991; Fenchel 1987; Hansen and Calado 1999; Jacobson and Anderson 1986; Schnepf and Elbrächter 1992). The general rules for pelagic systems of a predator:prey size ratio of ~10:1 (reviewed by Hansen et al. 1994) do not apply for many dinoflagellates. Although there are some ciliate species that can also attack prey that is of similar size or even larger than the ciliate itself (e.g. *Didinium* feeding on *Paramecium*, reviewed by Lynn 2008), a 1:1 or even inverse size ratio between predator and prey is much more common in dinoflagellates. Direct engulfment, pallium feeding* and tube or peduncle feeding* are the three feeding modes of heterotrophic dinoflagellate species (Calado and Moestrup 1997; Calbet et al. 2013; Hansen 1992; Spero 1982). All three feeding modes take longer than food capture of an average (suspension feeding) ciliate, thus increasing the handling

time (h in Eq. (1a)) and reducing maximum ingestion rate ($I_{\max}$ in Eq. (1b)) if all other parameters remain unchanged. Reduced ingestion rates will then tend to result in lower specific growth rates.

Functional differences between ciliates and dinoflagellates are also found at low food concentrations. Aquatic protists typically lead a ‘feast and famine’ existence (Calbet et al. 2013; Fenchel 1987, 1992), i.e. they have to cope with highly variable food conditions in their natural realm. In vast parts of the open ocean and in many oligotrophic lakes food is generally scarce. There are three general adaptive responses to survive food deplete conditions (i.e. those in vicinity or below the threshold prey abundance (V' , Eq. 4; Figs 2B, 3A): (1) to store resources under food replete conditions for use when supply declines; (2) to resist starvation by decreasing metabolic rates and/or formation of dormant stages (e.g. cysts, discussed below); and (3) to increase motility and dispersal to find a new food patch with higher prey levels. Storage has been well documented for nutrient uptake of phytoplankton and bacteria (Droop 1973, 1974; reviewed by Sterner and Elser 2002) and has also been suggested for aquatic herbivores such as *Daphnia* (Sterner and Schwalbach 2001). Among phagotrophic protists, storage has been documented for marine dinoflagellates (Golz et al. 2015; Meunier et al. 2012 and references therein). The second adaptive response to low food conditions, reducing metabolic costs, seems to be common in marine protists (Fenchel 1982; Fenchel 1989; Fenchel and Findlay 1983; Hansen 1992; Khan et al. 2015). However, heterotrophic dinoflagellates tend to sustain starvation better than ciliates, surviving longer at food levels below the critical threshold (reviewed by Sherr and Sherr 2007). The third adaptation to temporarily insufficient food supply is a bet-hedging strategy*; ciliates and some small flagellates may continue dividing several times once starvation has set in. However, the daughter cells produced are significantly smaller, rapidly swimming swarmer cells (Fenchel 1987, 1992), increasing the rate of dispersal and thus the chance to escape the food deplete conditions. Production of swarmers was observed in *Gymnodinium* sp. but the existence of swarmer cells is very rare in heterotrophic dinoflagellates (Jakobsen and Hansen 1997). It appears that this strategy to escape starvation has evolved more often in ciliates than in dinoflagellates.

Cyst formation is not only a response to starvation; it is wide spread among aquatic protists as a general adaptation to survive unfavourable environmental conditions (Corliss and Esser 1974; Foissner 2006; Verni and Rosati 2011). This is another obvious difference between phagotrophic dinoflagellate and ciliates; many more species of the latter are known to form cysts. Cysts may survive in the sediment for several months to many decades (Feifel et al. 2015; Fenchel 1992; Locey 2010; Müller 2002). The ability to encyst may affect the functional ecology of protists. Recently, Weisse et al. (2013) demonstrated experimentally how a ciliate from ephemeral freshwater reservoirs, by forming cysts, can survive in the presence of a superior competitor (with a lower

food threshold, V' , and a higher maximum growth rate, $r_{\max}$, as in Eq. (4)).

The above main differences in the numerical and functional response between planktonic ciliates and dinoflagellates of similar cell size are conceptually summarised in Fig. 4. Overall, the significant differences in their ingestion and growth rates and contrasting sensitivity to starvation suggest that ciliates tend towards r-selective strategies, while dinoflagellates appear to be relatively more K-selected. However, as we have outlined above, there is also a functional difference among heterotrophic dinoflagellates; small dinoflagellates often compete with large ciliates for the same prey size (and may reach similar growth and ingestion rates), but many dinoflagellates prey efficiently on larger prey, which are not available to most ciliates. Those dinoflagellates compete with copepods and other metazooplankton for prey. Evaluating functional and numerical responses across more ciliate and dinoflagellate taxa will decipher their contrasting life strategies more clearly. Overall it is clear that (1) for the same available prey biomass, ciliate grazing pressure on phytoplankton will be significantly higher than that of larger dinoflagellates (see next chapter), and (2) the principle concepts outlined above (Eqs. (1a), (4)) hold for marine dinoflagellates (Hansen 1992; Jeong et al. 2014; Menden-Deuer et al. 2005; Strom and Buskey 1993). This fact provides strong evidence that the relationship between food level and the basic processes of prey capturing, processing, and conversion to predator apply to all aquatic protists, irrespective of their specific feeding mode.

Thus far, we have ignored that there may be systematic differences in the functional ecology of marine and freshwater protists, which have yet to be properly explored. For example, to date, numerical and functional responses of planktonic dinoflagellates have been determined exclusively for marine species. It is clear that there are taxonomic differences; species belonging to foraminifera, radiolarians, tintinnids, the MAST group of uncultured flagellates (Massana et al. 2004; Massana et al. 2014), and the novel ciliate class Caricotrachea (Orsi et al. 2012) are either exclusively marine or predominantly marine. The greater protist diversity in the ocean may result from an overall greater heterogeneity and a higher geological age compared to inland waters. Recent research revealed that species-area relationships (SAR) are comparable between aquatic microbes and macroorganisms (reviewed by Furhman 2009; Soininen 2012), i.e. the number of protist taxa increases with the area or volume investigated. Considering the vast volume of the ocean, relative to that of lakes and rivers (ratio 7500:1; Gleick 1996), the SAR suggests that there are more protist taxa in the ocean than in freshwater. In line with this reasoning, analyses from 18S ribosomal DNA sequences and metagenomics data from the Tara Oceans expedition reported an enormous amount of as yet unknown taxonomic and functional protist diversity (de Vargas et al. 2015; Sunagawa et al. 2015).

Many, if not the majority of bacterial and protist species in the ocean are rare (Caron et al. 2012; Dunthorn et al. 2014;

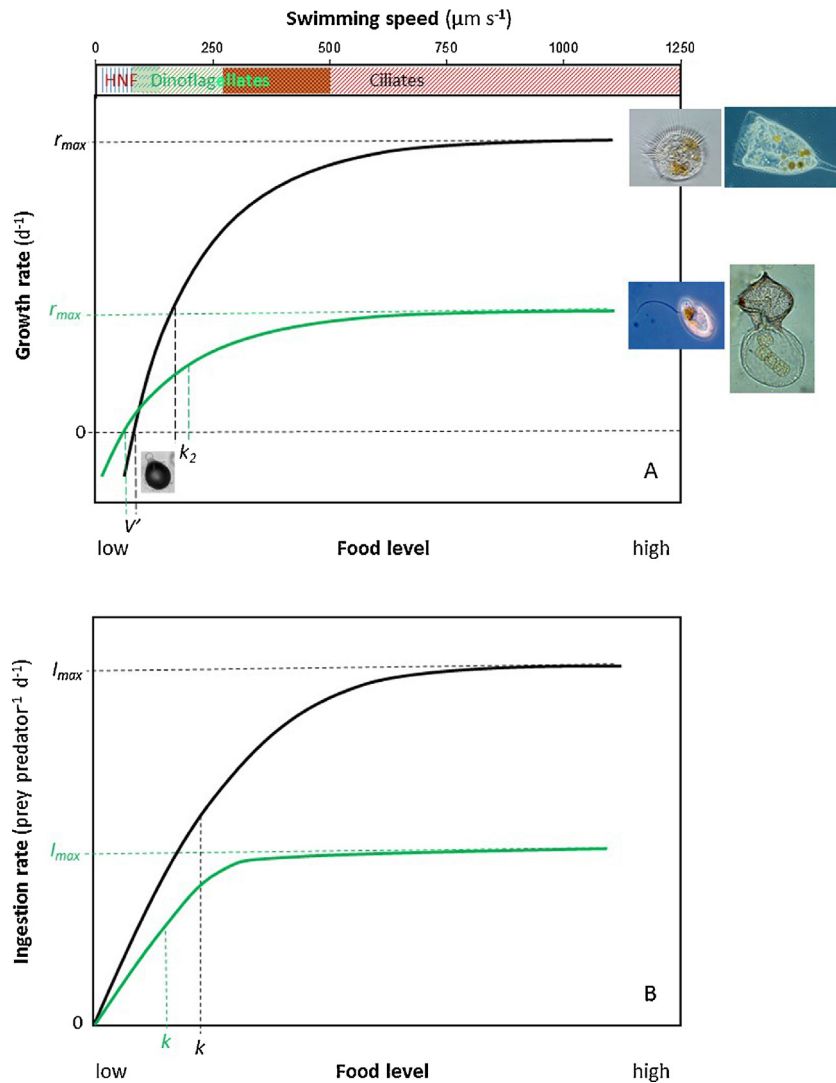


Fig. 4. (A) Conceptual representation of the major differences in swimming speed of three major groups of protists (top) and in the numerical response of ciliates (black curve) and dinoflagellates (green curve). Although the swimming speed of ciliates and dinoflagellates overlap, ciliates reach higher average and maximum values; similarly, the swimming speed of dinoflagellates and the taxonomically diverse group of heterotrophic nanoflagellates (HNF) overlap, but the average swimming speed of dinoflagellates is higher. Concerning numerical response curves, ciliates have steeper initial slopes and reach higher maximum growth rates (r_{max}), have similar or higher x -axis intercepts (V') and lower constants k_2 (where $r = r_{max}/2$) than dinoflagellates of similar size. Ciliates also tend to form more often cysts (insert, bottom left) under food deplete conditions than dinoflagellates. (B) Major differences in the functional response of ciliates (black curve) and dinoflagellates (green curve). Ingestion rate may be expressed as prey cells predator $^{-1}$ time $^{-1}$ (d^{-1} or h^{-1}) or in carbon units predator $^{-1}$ time $^{-1}$. While maximum ingestion rate (I_{max}) is usually higher in ciliates, the half saturation constant of both protist taxa (k , $=0.5 I_{max}$) is more variable and may be even lower in dinoflagellates than in ciliates (e.g. Yoo et al. 2013). The symbols refer to Eq. (4) and Fig. 2 in (A) and to Eq. (1b) in (B).

Sogin et al. 2006; Weisse 2014). Since dominant and rare species in a functional guild are principally similar (see *Introduction*), the rare species may represent ecological redundancy and provide biological buffering capacity allowing relatively stable community functions in spite of taxonomic changes (Caron and Countway 2009). If this assumption is correct, the greater protist diversity in the ocean would not imply altered functional ecology at the community level, relative to freshwater ecosystems. Indeed, with respect to functional ecology, the few cross-system analyses available suggest that there are no systematic differences between marine

and fresh waters other than those related to the taxonomic differences, at least for ciliates and dinoflagellates (Hansen et al. 1997; Straile 1997; Weisse 2006). However, the factors driving population dynamics and selective forces may be different in abundant protist species living in more eutrophic environments such as many freshwater lakes and coastal areas and in rare species dwelling in (ultra)oligotrophic habitats such as the open ocean; e.g., predation and the frequency of sexual reproduction may be reduced, while cooperation in multi-species networks may be more important in the rare biosphere typical of the central ocean gyres (Weisse 2014).

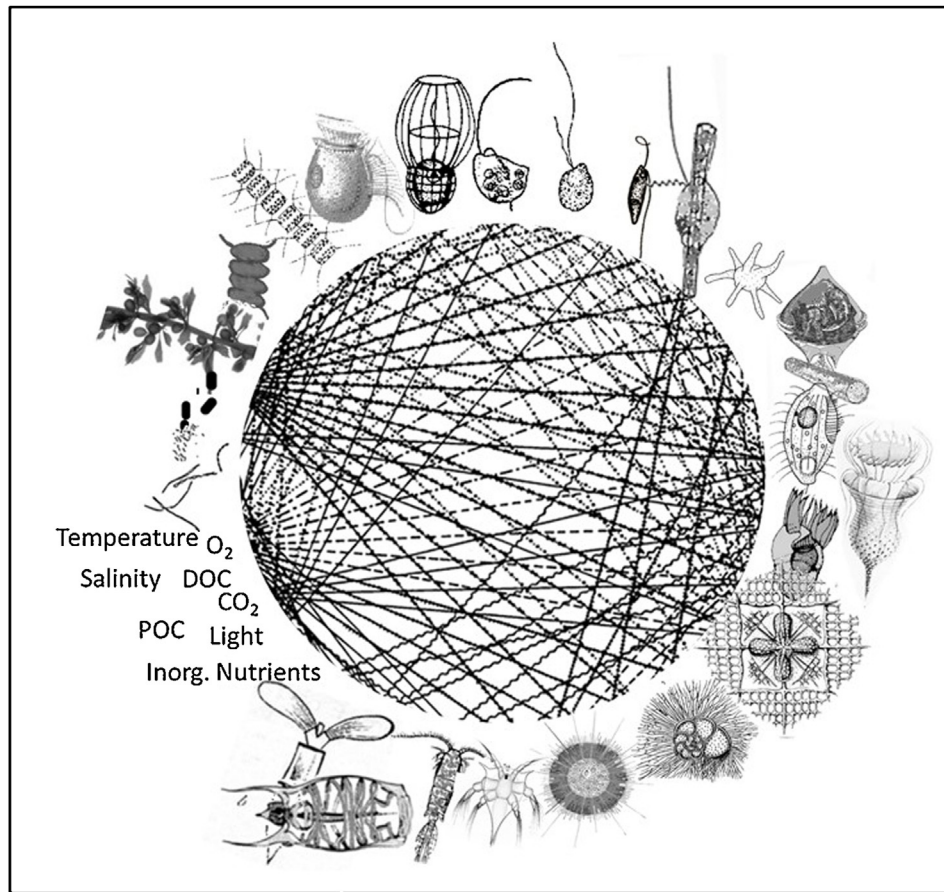


Fig. 5. Potential Interactions of abiotic and biotic parameters in a pelagic food web using a very small number of interacting components only (Arndt unpubl.).

For heterotrophic nano- and microflagellates (HF) the question of taxonomic and ecological dissimilarities between marine and freshwaters is more complex, due to the extreme diversity and the vast array of uncultured, novel flagellate lineages with as yet little known functional ecology (Jürgens and Massana 2008). Arndt et al. (2000) conducted a cross-systems analysis of the dominant taxonomic groups among HF communities within different marine, brackish and freshwater pelagic communities (heterokont taxa, dinoflagellates, choanoflagellates, kathablepharids) and benthic communities (euglenids, bodonids, thaumatomastigids, apusomonads) and concluded that they were surprisingly similar. These authors did not identify systematic differences in the functional diversity of marine vs freshwater HF with respect to their feeding ecology, life strategies, and tolerances to extreme abiotic and biotic conditions. An unequivocal identification of the small HF is often impossible with classical morphological methods in routine samples. With the rapid accumulation of new evidence provided mainly by novel molecular identification of as yet uncultivable strains and species, it has become obvious that HF do not represent a functional entity, but consist of highly diverse organisms with complex, mostly non-linear interactions (Jürgens and Massana 2008; Fig. 5). We will discuss the significance of non-linear networks in more detail

below (section *Diversity of interactions*). The use of video-microscopy allowed the analysis of individual behaviour even of tiny nanoflagellates. Comparative studies revealed that the different phases of the feeding process can be quite different even in very closely related species and might indicate the specific niche of individual flagellate species (for review see Boenigk and Arndt 2002). Unfortunately, only a few species have been analysed yet.

In the following section, we take a new direction and compare the feeding of heterotrophic and mixotrophic protists, here defined as protists capable of obtaining energy and/or nutrients by both phototrophic autotrophy and phagotrophic heterotrophy (Jones 2000; see Glossary).

Functional response, numerical response, and prey selectivity in mixotrophic vs heterotrophic protists

There continues to be a growing recognition that most of planktonic “algal” groups are mixotrophic both in marine (Jeong et al. 2010; Stickney et al. 2000; Stoecker 1999) and freshwaters (Jones 2000). Here we focus only on protists with constitutive photosynthetic organelles, excluding

kleptoplastid protists or heterotrophic protists with photosynthetic symbionts (reviewed by Esteban et al. 2010; Johnson 2011; Stoecker 1999). Rather, we will look at comparative studies on the feeding of mixotrophic and heterotrophic protists, and address if there are wide-ranging (across different phylogenetic groups) differences between the two in their functional and numerical responses and their prey selectivity. The importance of this question lies in the fact that we often treat feeding in mixotrophic protists as equal to that of heterotrophs, and often assume they have an equal impact on their prey community. ‘True’ ecological differences may be blurred by methodological problems. A clear example of this is the commonly used approach of transforming hourly grazing rates, obtained experimentally from *in situ* samples, to daily rates, disregarding any potential influence of light on mixotrophic protist feeding. Since these are in many ways fundamentally different organisms, the validity of these assumptions should be questioned. A recent global modelling approach demonstrated that, because mixotrophs use supplementary resources derived from prey, they can sustain higher levels of photosynthesis for a given supply of limiting inorganic nutrient (Ward and Follows 2016); the net result is a significantly increased trophic transfer efficiency from photo(mixo)trophs to larger heterotrophic organisms.

The existence of autotrophic protists capable of ingesting prey has been known for a long time. However, only within the last three decades we have realised that this is a globally distributed, environmentally relevant nutritional strategy (Hartmann et al. 2012; Sanders and Gast 2012; Unrein et al. 2007; Ward and Follows 2016), present in a wide range of phylogenetically diverse photosynthetic eukaryotes (McKie-Krisberg and Sanders 2014; Unrein et al. 2014). As a consequence, there are considerably fewer studies on functional and numerical responses for mixotrophic protists than for heterotrophs, and for many major protist groups studies are based on only a few representatives. As an example, a large portion of laboratory studies with mixotrophic haptophytes are based on one species, the toxic *Prymnesium parvum* (e.g., Brutemark and Granéli 2011; Carvalho and Granéli 2010; Skovgaard and Hansen 2003). In addition, there are few studies directly comparing the functional and numerical responses of mixotrophic and heterotrophic protists. It is, of course, possible to compare different studies, but for most protist groups we lack a large enough data set to exclude biases caused by, e.g., different culture conditions or prey provided. As an example, two studies with different experimental conditions compared the numerical response of the heterotrophic chrysophyte *Spumella* sp. to two different mixotrophic chrysophytes, respectively *Ochromonas* sp. (Rothhaupt 1996) and *Proterioochromonas malhamensis* (Pålsson and Daniel 2004), and produced completely different results. Rothhaupt observed that *Spumella* sp. consistently showed considerably higher growth rates than *Ochromonas* sp. except at the lowest bacterial concentrations provided. In contrast, Pålsson and Daniel (2004) observed that *P.*

malhamensis showed only slightly lower growth rates than *Spumella*, even at the highest bacterial concentrations, and consistently reached a higher final biomass due to a larger cell size and longer exponential phase. Tittel et al. (2003) combined *in situ* observations on the illuminated surface strata of a lake with laboratory experiments; these authors concluded that mixotrophs (*Ochromonas* sp.) reduced prey abundance (the green alga *Chlamydomonas* sp.) steeply and, as a consequence, grazers from higher trophic levels, consuming both the mixotrophs and their prey, could not persist. How much of the observed difference is due to variations between the mixotrophic species and how much to experimental conditions is hard to tell without any further references. All of these factors are major hurdles to being able to answer the question outlined above, that need to be addressed by an increase in work with cultured mixotrophic protists and the isolation of environmentally relevant strains.

As an exception to the rule, for marine phagotrophic dinoflagellates there are sufficient studies to be able to see some patterns emerge (Hansen 2011; Jeong et al. 2010). One first clear difference is that mixotrophic dinoflagellates are able to grow in the absence of prey, with the exception of obligate mixotrophs. This will also apply to other mixotrophic protists groups, and should provide a competitive advantage in environments with permanently or periodically very low prey concentrations, such as the oligotrophic ocean or during the waning phase of an algal bloom. Maximum ingestion and growth rates, on the other hand, tend to be higher for heterotrophic dinoflagellates, indicating that at high prey concentrations they will have a stronger impact on the prey community (Calbet et al. 2011; Jeong et al. 2010). At intermediate prey concentrations the picture is not yet clear. This is an important point since this usually covers the range of prey abundances normally found *in situ*. Finally, the feeding rates of mixotrophic protists are thought to be more strongly influenced by nutrient and light availability. For a number of species feeding rates are quite low when inorganic nutrients are plentiful leading to only minor increases in growth rates, even if irradiances are low (e.g. Hansen 2011). While a few phytoflagellates may feed to obtain carbon in light limited conditions (Brutemark and Granéli 2011; Skovgaard et al. 2000), for most species light is necessary for fast growth (Berge et al. 2008; Hansen 2011; Li et al. 1999; Li et al. 2000). In this latter case, prey is not necessarily used as a C source, but may instead serve to obtain essential nutrients (N, P, Fe, etc.) required for photosynthesis, with the global consequences for carbon flow discussed above (Ward and Follows 2016).

Overall, these studies provide the general picture that mixotrophic dinoflagellates feed less than heterotrophs, but they are more efficient at very low prey concentrations and are not as dependent on prey availability. We suggest to test rigorously if mixotrophic species have lower $I_{\max}$ (Eq. (1b)), higher a ((Eqs. (1a), (3)) and e (Eqs. (7), (8))), at low food concentrations, and lower V' (Eq. (4)) than their heterotrophic counterparts.

Prey selection is another important aspect to consider when comparing the feeding of heterotrophic and mixotrophic protists. This phenomenon has been studied extensively for heterotrophic protists (Jürgens and Matz 2002; Montagnes et al. 2008b) and has been found to act at all the stages of feeding described earlier in this paper (Fig. 1), from searching for and capturing prey (Matz et al. 2002) through to which prey are handled and assimilated (Boenigk et al. 2001b). Studies are scarcer for mixotrophic protists, with data largely only available for marine dinoflagellates (e.g., Jeong et al. 2005; Jeong et al. 2010; Lee et al. 2014b) but there is no reason to predict that mixotrophs will be any less selective than heterotrophic protists. However, whether their selection patterns and prey ‘preferences’ differ from those of heterotrophs has rarely been addressed, despite indications that this could sometimes be the case. Predator:prey size ratios, for example, appear to be slightly different, with mixotrophic dinoflagellates generally presenting consistently lower optimal prey sizes than heterotrophs (Jeong et al. 2010). Although the opposite also occurs, with some toxin producing and peduncle feeding dinoflagellates inverting the food chain and consuming much larger prey (e.g., Blossom et al. 2012; Hansen 1991). Understanding the different prey selection patterns of mixotrophic and heterotrophic protists will provide a better understanding of how (if at all) they regulate their prey communities, and thereby the processes they control, making this a very interesting field for future studies.

Functional Community Ecology: From Microcosms to the Ocean

In spite of our optimistic notion expressed above (*Evolution of concepts...*), scaling the laboratory work with microcosms to large-scale natural systems (and here we focus on the ocean as the largest ecosystem on Earth) is notoriously problematic. This issue arises most often where ecosystem models, predicting complex large-scale issues are populated by parameters derived experimentally under controlled, but often highly specific conditions. It is clear that the abilities that we observe under laboratory conditions with a few model species may be different for these and other species in the natural environment. However, field studies with protist are rare, relative to multicellular organisms; this fact and the trade-off between idealised laboratory experiments and the complex and often difficult to interpret situation in the field has led to call for a resurgence of field research in aquatic protists (Heger et al. 2014).

Here we will not describe ecosystem models or their limitations, but rather we will focus on several aspects that illustrate issues associated with parameters obtained from laboratory data. We start by describing the limitations of working with model species and then focus on community approaches. Finally, we will present an overview of the global significance of marine protistan grazing on phytoplankton and the sources of variability that make these predictions imprecise.

Species-specific approaches vs manipulation of natural assemblages

Working with mono-specific “model” cultures (see Montagnes et al. 2012) is perhaps the most widely used way to produce data on protistan grazers’ response to environmental factors. The process initiates with isolation from field samples and establishing mono- or poly-clonal cultures under conditions that may not be similar to those experienced in nature including the prey-type (often mono-specific but non-axenic). Once the culture is well established – usually after many generations – is when the experimentation begins, although with many difficult-to-maintain cultures, experiments are rapidly conducted, before clonal decline occurs (see Montagnes et al. 1996).

In the above-described process we already can glimpse some problems that will probably affect the quality of our results. First, the process selects for species adapted to survive under the defined laboratory conditions. This excludes most of the species, including not only rare species but even those that are very abundant in open oceans and thus driving trophodynamics. Second, the use of one single clone/strain of each species overlooks intraspecific responses. For instance, the feeding behaviour of some species of protistan grazers may differ amongst strains (Adolf et al. 2008; Calbet et al. 2013; Weisse 2002; Weisse et al. 2001; Yang et al. 2013). The conclusions of most such studies are based on strains from different locations, which make it plausible to assume that their response to certain environmental factors will differ (Lowe et al. 2005; Yang et al. 2013). However, differences in functional and numerical responses, and even in biochemical composition, are also observed in strains from the same origin (Calbet et al. 2011; Chinain et al. 1997; Lee et al. 2014a). The ecological significance of seemingly minor (10%) clonal differences in growth rates has been demonstrated for freshwater ciliates (Weisse and Rammer 2006).

At times, these remarkable disparities on the performance of the different clones are maintained after many generations of cultivation in the laboratory, suggesting that they are genetically fixed. From knowledge on other groups of organisms (e.g. copepods) we have learned that extended captivity affects feeding rhythms (Calbet et al. 1999) and the overall ingestion and production rates of the organism (Tiselius et al. 1995). Yet, we are far from understanding the changes that protists undergo when raised *in vitro*, most of times in excess of resources and free of the threat of predation. Evidences point towards a diminution in the toxin contents in harmful dinoflagellates (Martins et al. 2004); it is also well known that cyst formation of ciliates declines with time in the laboratory (Corliss and Esser 1974; Foissner 2006). Most aquatic protist species are facultative asexuals. If kept in the laboratory for many generations, one or a few clones may become dominant, thus significantly altering the original population structure. If mating is prevented, clonal decay may lead to declining performance of a species in the

laboratory (Bell 1988; Montagnes 1996). Recent investigations, however, indicated that protists may evolve within a few weeks and traits can change (e.g. terHorst 2010).

The previous problem has the (annoying) solution of continuous isolation of new cultivars. This is, nevertheless, not always feasible because the intermittent or seasonal occurrence of most groups. Therefore, and because of the general constraints inherent in single-species microcosms, it is important to complement experimental studies using model protists with natural assemblages. One such approach is to measure uptake of fluorescently labelled bacteria (FLB; Sherr et al. 1987) or algae (FLA; Rublee and Gallegos 1989) by natural protist assemblages in combination with fluorescence in situ hybridization (FISH; reviewed by Amann et al. 2001); the FISH probes enable detection and identification of the uncultured protist grazers (Jürgens and Massana 2008; Massana et al. 2009; Unrein et al. 2014). The use of FISH to link sequence identity with morphology and even function has recently been reviewed (del Campo et al. 2016). An intermediate step between typical laboratory experiments and manipulations of natural assemblages was taken by del Campo et al. (2013). These authors amended sterile seawater from oligotrophic areas with a mix of natural bacteria collected from the same sampling site and added a single heterotrophic flagellate cell retrieved by serial dilution or by flow cytometry and cell sorting.

There are other obstacles than the culturing bias that can be more easily approached. One of the most obvious is perhaps the need of detailed knowledge on the nutritional history of the grazers (discussed above) and adequate preconditioning to the experimental conditions. In addition to food quantity and quality, a number of studies have documented the influence of different prey characteristics such as size, shape or motility, on protist grazing (e.g. Matz et al. 2002). Further, it is important to consider that even when employing the same predator and prey strains, we can obtain different results depending on the physiological state and past growth conditions of the prey provided (Anderson et al. 2011; Li et al. 2013; Meunier et al. 2012). In conclusion, standardisation in laboratory experiments to derive the parameters needed for ecosystem models is important but will not solve all difficulties inherent in upscaling results from the laboratory to the ocean level. This is also because some natural external drivers are difficult to mimic in the laboratory.

The role of external physical factors

Not only the biotic factors discussed above are at play when conducting *in vitro* experiments with selected species/strains of protistan grazers; also environmental physical factors have to be taken into account as sources of variability. Clearly these need to be controlled for, but recognising they are important allows us to begin to evaluate how these external drivers apply in nature. Below, we briefly outline several important drivers.

Temperature is the most obvious and immediate one, and has received considerable attention (Atkinson et al. 2003; Montagnes et al. 2003; reviewed by Rose and Caron 2007). One of the most striking realisations is that temperature has distinct effects on the functional and numerical responses (e.g., Kimmance et al. 2006; Weisse et al. 2002; Yang et al. 2013). Thus, the underlying mechanisms driving ingestion and growth (Eqs. (1), (3)) seem to respond differently to temperature. By exploring growth and ingestion responses, we can then begin to explore the functional biology of protists.

Light is a major driver of life on our planet and regulates the production of phototrophic organisms. Protist distribution patterns are strongly influenced by its availability and its excess, which can be harmful to sensitive protist species due to exposure to ultraviolet radiation (Sonntag et al. 2011a,b). Light also drives the feeding rhythms of large zooplankton, as has been demonstrated in many laboratory and field studies, both in marine and in freshwater systems (Bollens and Frost 1991; Calbet et al. 1999; Petipa 1958). Regarding protistan grazers, the information is very limited. We have already discussed the general role of light for mixotrophs. For heterotrophs, the few laboratory data available reveal that, contrary to metazoans (e.g. adult copepods), grazing rates are enhanced by light in many species (Jakobsen and Strom 2004; Skovgaard 1996; Strom 2001; Tarangkoon and Hansen 2011; Wikner et al. 1990), although the opposite has been also shown (Chen and Chang 1999; Christaki et al. 2002). The reasons and mechanisms behind these rhythms may be several, and are not within the scope of this article. However, for our objectives it is relevant that the influence of light and the presence of daily feeding rhythms are often not considered when designing experiments with heterotrophs, leading to many of them being carried out in complete darkness or at very low levels of irradiance, $<25 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Gismervik 2005; Verity 1991). Clearly, considering the effect of light and diel feeding rhythms is a challenge for future research when comparing different studies conducted not only with mixotrophs but also with heterotrophs, when integrating protozoan laboratory data into models, or when using laboratory-based grazing rates and field abundances of protist grazers to estimate their role in planktonic food webs.

Turbulence. In our quest for simulating natural conditions in the laboratory we often forget that the organisms are not in steady still conditions in the field, but are exposed to inertial forces (small-scale turbulence, Willkomm et al. 2007). There are very few studies about the effects of small-scale turbulence on protistan grazers. Dolan et al. (2003) observed a negative effect of turbulence on the growth and ingestion rates of the ciliate *Strombidium sulcatum*. Likewise, Havskum (2003), found a negative effect of turbulence on the growth rates of *Oxyrrhis marina*. However, he did not detect significant influences of this variable on ingestion rates. The negative effects of turbulence on protists' growth seem to be quite widespread amongst phototrophic and mixotrophic dinoflagellates (Havskum and Hansen 2005; Sullivan and Swift 2003; Thomas and Gibson 1992). Still, we lack further

solid evidence on how to parameterise this variable for protistan grazers' trophic impacts. Clearly this instance drives home the arguments we have made above (the Independent Response model) that for protists, it is not only practical but essential to measure both functional (feeding) and numerical (growth) responses. In doing so we not only recognise that external drivers, such as turbulence, affect the functional biology differently, we can use these data to begin to mechanistically tease apart their cause. We, therefore, recognise the need for such factors to be controlled in experiments, in spite to the principal difficulty to mirror turbulence realistically in small scale laboratory experiments. However, we also see this “problem” as a great strength of experimental work, allowing future exploration of the functional ecology of protists.

Community approaches

Given single-species laboratory work, by its very nature, ignores biotic interactions, it is logical to consider that community approaches may more closely resemble natural behaviours. We have discussed above (section *Species-specific approaches vs manipulation of natural assemblages*) that it is now possible to combine experimental investigations of natural communities with single-cell observations. Traditional experiments enclosing natural communities in containers of different shapes and sizes are quite common in the literature, and the pros and cons of the various approaches have been discussed extensively in the literature (e.g., Duarte and Vaque 1992; García-Martín et al. 2011; Robinson and Williams 2005) and will not be repeated here.

We will, however, stress the problematic of one basic assumption generally employed to calculate individual (as opposed to community) grazing parameters such as predator ingestion rates: namely, that all study organisms are feeding. This is unlikely to be the case in most scenarios, meaning the use of this assumption can significantly bias our view of the system, especially when it comes to comparing different protist groups (e.g. the *in situ* ingestion rates of heterotrophic vs. mixotrophic protists). As an example, studies using food vacuole dyes have shown significant shifts in the percentage of actively bacterivorous mixotrophic protists with depth (R. Anderson, unpublished data), while González (1999) estimated that the percentage of actively bacterivorous flagellates could range as much as from 7 to 100% in the different study sites analysed. Similarly, Cleven and Weisse (2001) reported from their *in situ* feeding study with the bacterivorous freshwater flagellate *Spumella* sp. that bacterial ingestion varied seasonally, but that the majority of the flagellates did not take up the natural FLB that were offered as food at any time.

Regarding feeding rates of larger herbivorous grazers, the situation is a bit more complicated because of the impossibility to separate grazers from prey simply by size. Even though there have been attempts to add FLA (both dead and alive; Martínez et al. 2014; McManus and Okubo 1991; Rublee and Gallegos 1989), the method more widely used to

quantify microzooplankton grazing in the field is the dilution technique* (Landry and Hassett 1982). The method relies on some specific assumptions, not always met (Calbet and Saiz 2013; Dolan and McKeon 2005; Dolan et al. 2000), and presents some limitations: overestimation of grazing due to the lack of top down predation in the incubations (Schmoker et al. 2013), difficulty in interpreting trophic cascades (Saiz and Calbet 2011), and confounding effects of mixotrophy (Calbet et al. 2012). Particularly, this last aspect, mixotrophy, is seldom addressed in herbivory studies (Calbet et al. 2012; Li et al. 1996); however, there is a burgeoning need for including mixotrophs in ecosystem models (Flynn et al. 2013; Mitra et al. 2014; Ward and Follows 2016). Since many oceanic studies used the dilution technique, its inherent problems affect our current view of the global significance of microzooplankton grazing (Dolan and McKeon 2005) discussed in the next section.

Illustrating the need for protistan functional ecology: global ocean patterns of protist consumers

Despite the constraints described in the previous sections, data indicate that protists are important grazers in the oceans. In a review, Calbet and Landry (2004) concluded protists consume 60 to 70% of the primary production, with this varying amongst marine habitats and regions. Recently, Schmoker et al. (2013) expanded on this, indicating that grazing impacts are variable at different scales, such as biogeographic regions and within regions along the seasonal cycles. Furthermore, there is variability in grazing at much smaller scales both horizontally and vertically (Landry et al. 1995; Landry et al. 2011). Similar patterns in regional and local variability also occur for bacterivorous protists (Jürgens and Massana 2008; Pedros-Alió et al. 2000; Sanders et al. 1992). It is, therefore, clear that we need to recognise that the functional ecology of protists varies, and focus on identifying the major drivers of this variability in natural systems, following procedures outlined above. Then, it will be possible to use field and experimental data to parameterise models and assess both variability and average impacts. To achieve such a task, however, fluent communication and collaboration between modelers and experimentalists is needed; this collaboration between what are sometimes diametrically opposed approaches is one of our largest challenges.

Limitations of the Mechanistic Analysis: When Chaos Hits

The above sections all indicate the complexity behind the functional ecology of protists. The described phenomena of functional and numerical responses, effects of light and turbulences, population dynamics as well as trophic interactions have one feature in common – they are characterised by

nonlinear functions. Combinations of many nonlinear functions reveal principally unpredictable dynamics (e.g. [Turchin 2003](#)). We will illustrate this phenomenon discussing three different aspects regarding limitations of the mechanistic understanding of protist functional ecology.

Diversity of interactions

The potential number of parameters and organisms interacting in each pelagic and benthic environment is extremely high. This is especially true for protists which are genetically and functionally the most diverse component of eukaryote organisms in ecosystems (e.g. [de Vargas et al. 2015](#)). One of the most widely distributed morphotype of heterotrophic flagellates, the kinetoplastid *Neobodo designis*, is probably composed of hundreds of genotypes (and, probably, functionally different ecotypes; [Scheckenbach et al. 2006](#)). The number of interacting protist species comprising all phyla ranges from picoprotists (<2 µm) and nanoflagellates (2–20 µm) to small and large amoebae, small and large ciliates up to large rhizarians comprising many different functional groups. [Fig. 5](#) illustrates the high number of potential relationships including abiotic parameters as well as biotic parameters in protistan communities using the marine pelagic as an example. All (aquatic) organisms are embedded in ecological interactions, in which each species is influenced by multiple other species and abiotic factors. Statistical analyses of co-occurrence networks ([Faust and Raes 2012](#); [Fuhrman 2009](#); [Steele et al. 2011](#); [Worden et al. 2015](#)) are increasingly being used to deduce hypotheses about structure and function of marine microbes (i.e., bacteria, archaea, and protists) from the enormous amount of information gained by high-throughput sequencing (HTS) and various ‘omics’ approaches (metatranscriptome and proteome analyses). For instance, recent sequence similarity network analysis of ciliate data obtained from HTS demonstrated that the extensive novel diversity of environmental ciliates and their tremendous richness of interactions differs in relation to geographic location and habitat ([Forster et al. 2015](#)). Metabarcoding analysis from the Tara Oceans expedition revealed an unsuspected richness of monophyletic groups of heterotrophic protists that cannot survive without endosymbiotic microalgae ([de Vargas et al. 2015](#)). We conclude that the interactions illustrated in [Fig. 5](#) show, at best, a tiny fraction of the multidimensional diversity of protist interactions found in nature. However, if new associations and functions are conjectured, sequencing alone cannot reveal the details of the microbial interactions. Therefore, more functional studies on ecologically relevant model organisms under realistic environmental conditions are urgently needed ([Caron et al. 2013](#); [Worden et al. 2015](#)).

Principal unpredictability of nonlinear dynamics

To illustrate the problem of forecasting protist dynamics and predator–prey interactions, let us add just one additional

prey to the classical predator–prey system mentioned in the section *Modelling Predator–Prey Dynamics*; let us further assume that the preferred prey by the protistan predator is growing faster than the other prey. Under these circumstances the abundance of the protist species can approach equilibrium abundances after a certain time (logistic growth, [Fig. 6C](#)). A slight change in the growth parameters (e.g. growth rate) may switch the dynamics to stable limit cycles (as have been shown for *Didinium-Paramecium* cycles, see above) and deterministic chaos ([Fig. 6D, E](#)). This has been shown by many theoreticians in scenario analyses. One of the first were [Takeuchi and Adachi \(1983\)](#) who found a similar pattern as indicated in [Fig. 6C–E](#) for the system described before. The question is whether this may occur in real food webs. Chemostat systems consisting of two different bacteria prey species and the ciliate *Tetrahymena* showed similar pattern of population dynamics as predicted by Takeuchi and Adachi ([Fig. 6A, B](#); [Becks et al. 2005](#)). Changing dilution rates in the two-bacteria-one-ciliate system switched ciliate dynamics between, e.g., chaotic dynamics ([Fig. 6A](#) left part) and the establishment of equilibrium conditions ([Fig. 6A](#) right part). A time delay reconstruction (graphing actual abundances against the previous abundance) illustrates the switch from a chaotic attractor to a point attractor ([Fig. 6B](#), [Becks and Arndt 2008](#)). This should have dramatic consequences for the functional ecology of the experimental ciliates (*Tetrahymena*). For instance, when chaotic dynamics prevail, the ratio between predator and prey are constantly and unpredictably changing ([Fig. 6A](#)), suggesting that I (Eq. (1)), r (Eq. (4)) and P (Eqs. (7)–(9)) may all be variable at similar prey concentrations.

It has to be considered that the above mentioned scenario and the associated experiments extremely simplify the situations in field communities. Already little temperature shifts forecasted by global change scenarios may change the position of attractors within experimental communities ([Arndt and Monson 2016](#)). It is evident how important the ability of short-term reactions to a changing environment might be even in the “constant” world of a chemostat under chaotically fluctuating prey and predator populations. Thus, at the moment we have to accept a certain extent of fundamental unpredictability of protist dynamics in natural communities. We strongly recommend that the (most likely) occurrence of deterministic chaos in the field and its significance for community estimates derived from applying predator–prey models need to be investigated in future studies. The technical tools to monitor short-term predator–prey dynamics *in situ* are already available. Automated flow cytometry has been applied both in marine and freshwater to study bacterial and protist dynamics at high temporal resolution ([Besmer et al. 2014](#); [Pomati et al. 2013](#); [Thyssen et al. 2008](#)) and has also been used to detect harmful dinoflagellate blooms ([Campbell et al. 2013](#); [Campbell et al. 2010](#)). To our knowledge, such data sets have not yet been analysed for the occurrence of deterministic chaos.

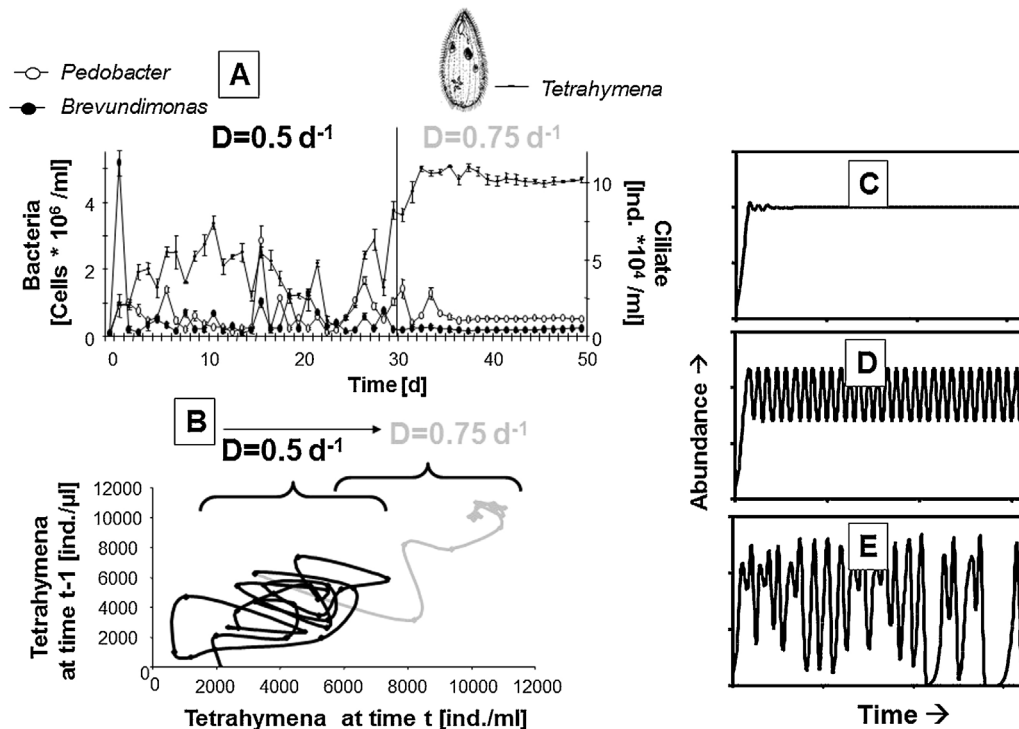


Fig. 6. Population dynamics of the ciliate *Tetrahymena pyriformis* and its two bacterial prey species, *Pedobacter* (preferred food) and *Brevundimonas* (inferior competitor to *Pedobacter*, data from [Becks et al. 2005](#); [Becks and Arndt, 2008](#)). (A) Population dynamics of the two bacteria and the ciliate in chemostat experiments with dilution rates of 0.5/d until day 30 and 0.75/d from day 31 on. (B) Corresponding time delay reconstruction. (C–E) Theoretical populations dynamics showing damped oscillations (C), stable limit cycles (D) and chaotic dynamics (E).

Unpredictability versus forecasting

The aforementioned fundamental unpredictability connected with the nonlinear processes and the enormous diversity of interacting players characterising the functional ecology of protists might disillusion ecologists trying to forecast protist response in complex field populations. However, there are several instances where forecasts should work.

In the early phase of population growth, the dependence on prey concentration etc can well be predicted (see sections before), while it will be very difficult at or close to equilibrium densities. The nearly absence of population records indicating population densities in the field staying at equilibrium concentrations might underline this. On the other hand, theoreticians have shown that the occurrence of deterministic chaos is limited to certain parameter sets (e.g. [Fussmann and Heber 2002](#)), while other sets lead to predictable patterns. In addition, there are several stochastic (non-chaotic) fluctuations of parameters. Little is known about the kinds of dynamic interactions within the “n-dimensional hyperspace” of factors important for the ecological niche of protists and an analysis of at least a few representative examples is a challenge for a better understanding of complexity in the context of functional ecology of protists.

The good news is that even when chaotic dynamics might prevail, knowledge on the boundaries of attractors in which the probability for a certain parameter set is high would offer the chance of prediction at least within certain limits.

Conclusions and Future Perspectives

In the foregoing sections, we have reviewed and built on a mechanistic basis for functional and numerical responses and have then used this structure throughout most of this paper to evaluate the functional ecology of protists. We have identified the following open questions for future research:

1. We emphasise that both functional and numerical responses need to be evaluated to understand the functional ecology of protists and to assess how external drivers affect these independently. In addition, we recommend: (A) The use the Independent Response model to assess functional and numerical responses of aquatic phagotrophic protists, both on theoretical grounds and because of its easier applicability. (B) Properly including protist growth and ingestion rates at (very) low food concentrations, as they are typically met in oligotrophic environments such as the open ocean. The numerical response for heterotrophs, in contrast to the functional response, always has a positive x-axis intercept and mortality occurs below this critical minimum food concentration. The goodness of curve fit is sensitive to data points measured in the vicinity (below, at and just above) the x-axis intercept ([Montagnes and Berges 2004](#)). Similarly, ingestion rates measured at low to medium prey levels are important to differentiate between Type II and Type III functional responses. And (C) studying the issue

of predator dependency on the functional response (interference competition). To our knowledge, there has been no parallel work on the numerical response, and we see this as a field ripe for exploitation.

2. Based upon the existing data on functional and numerical responses of the major aquatic protist taxa, we have identified two key avenues for future studies: (A) The striking differences between ciliates and heterotrophic marine dinoflagellates should be investigated more rigorously, in particular, the reason why dinoflagellates seem to cope better with starvation than ciliates, and (B) the different prey selection patterns of mixotrophic and heterotrophic protists across different taxa need to be systematically explored in order to better understand how they regulate their prey communities. This is imperative to assess the implications of aquatic mixotrophy for the global carbon flow more accurately.
3. Finally, we have demonstrated practical and principal limitations of the mechanistic analysis. Concerning the former, we have identified typical difficulties inherent in laboratory experiments that affect upscaling the results obtained to the ecosystem (or even ocean) level: (A) There is an overall need to obtain and work with environmentally relevant isolates, and characterise their functional and numerical responses, for many phylogenetic groups, including major groups within the mixotrophic protists and the HF. This includes more work with natural multi-species assemblages under quasi *in situ* conditions. (B) Assess how many of the study organisms are actually feeding in a grazing experiment and quantify the resulting bias (i.e. how representative is the per capita ingestion rate of individual, often model, species) for collective estimates of population and community grazing rates. The latter is primarily problematic if the experimental duration is short (minutes to hours) and diel feeding rhythms exist. If this is not the case, the (modelled) community grazing rate may still accurately estimate the grazing pressure on the prey population(s) even if the calculated ‘average’ per capita ingestion rate may not be met by any organism in the predator population. (C) Concerning theoretical and principal limitations, the occurrence of deterministic chaos under natural conditions and the implications of the principal unpredictability of nonlinear dynamics for functional and numerical responses should be studied at the population and community level.

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