Oxyrrhis marina-based models as a tool to interpret protozoan population dynamics

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Oxyrrhis marina-based experiments have frequently been used to underpin the construction and, or, parameterization of protozoan mathematical models. Initially, we examine the suitability and limitations of O. marina for this task. Subsequently, we summarize the range of aut- and synecological modelling studies based on O. marina, examining the questions asked and conclusions drawn from these, along with the range of processes and functions employed within the models. Finally, we discuss future modelling directions based on studies of O. marina.

KEYWORDS: dinoflagellate; experimental design; Oxyrrhis marina; models

INTRODUCTION

With improved understanding of the pivotal role that protozoa play within microbial food webs (Azam et al., 1983; Pomeroy et al., 2007), an increasing body of experimental work has investigated their response to a range of environmental conditions. Knowledge of the functional relationships that underpin protozoan growth and grazing, in turn, allows us to derive mathematical models that represent their behaviour. Such protozoa-specific modelling studies provide a means of understanding predator–prey interactions than could not be achieved from observation alone. Furthermore, the specific inclusion of protozoa within more general population and ecosystem models allows us to assess their role in the natural environment. Finally, as protozoa exhibit rapid generation times and are easily manipulated, they are an excellent tool for population dynamic studies and model parameter generation in general. Protozoa have, therefore, for a considerable time, been used as the basis for mathematical models of population growth (e.g. Gause et al., 1936; Painting et al., 1993; Fenton et al., 2010). Inevitably, such models are derived for the species that we can grow in the laboratory, and for planktonic protozoa these have proven to be few. This paper is about one such species, Oxyrrhis marina, that can act as a model for others and the insights that have been obtained from mathematical models based on its study.

The heterotrophic flagellate O. marina is an ideal candidate organism for the experimental study and modelling of the natural and theoretical population dynamics of protozoan predators. It is easy to find, isolate, maintain in culture and manipulate in the laboratory and has been maintained in culture for over 50 years in a number of culture collections (see Montagnes et al., this issue-a). Oxyrrhis marina is, therefore, often a natural choice as a model organism and is extensively used for experimental studies, some of which have been employed to develop or parameterize mathematical models. Within this paper, we review the literature to: (i) examine the limitation of using O. marina as a model organism; (ii) indicate the breadth of responses and functions that are available for its use, and thus facilitates mathematical model development; (iii) summarize modelling studies that have been conducted with O. marina, and briefly review the questions asked and conclusions drawn from these and finally (iv) discuss continued directions of research for modelling studies using O. marina.


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TO WHAT EXTENT IS OXYRRHIS MARINA A REPRESENTATIVE MODEL ORGANISM?

Meta analysis studies (e.g. Hansen et al., 1997) suggest that *O. marina* is representative of the dinoflagellates. However, phagotrophic protozoa are diverse and abundant organisms in aquatic environments, including taxa typically with a size range of 2–200 μm (Montagnes et al., 2008a). Hence, no single species or even genus will be representative of the functional group, and championing *O. marina* as a representative of the heterotrophic dinoflagellates or even heterotrophic protists en masse raises some reservations. Therefore, we first consider factors that may limit the general applicability of *O. marina*-based results to phagotrophic protozoa.

Mode of nutrition

*Oxyrrhis marina* is a raptorial feeder that directly engulfs its prey. Although protozoa exhibit a range of nutritional modes (Montagnes et al., 2008a), many, and possibly most, of the protozoa in aquatic pelagic ecosystems (e.g. ciliates, flagellates) also engulf their prey, and thus *O. marina* might be considered directly comparable to these. Furthermore, anecdotal data suggest that *O. marina* ingests prey between 1 and 12 μm, indicating that its predator:prey size ratio includes, but also exceeds, the approximate 10:1 ratio predicted by others (e.g. Azam et al., 1983). Thus, as a first approximation, we support the use of *O. marina* as a model organism in this sense.

Habitat

*Oxyrrhis marina* is rarely seen in pelagic samples, although “red-tide” blooms occur in large bays, reaching up to 10² cells mL⁻¹, and it can regularly be found in some estuaries at abundances of 10–100 mL⁻¹ (Johnson et al., 2003; Begun et al., 2004; Jeong et al., 2004). In contrast, *O. marina* is typically found in shallow waters associated with the shoreline, such as splash pools and tide pools (Johnson, 2000; Kimmance et al., 2006). Still, *O. marina* is planktonic, not benthic, and in mixed cultures remains well distributed (Davidson, Montagnes, unpublished results), although it may accumulate at mid-water column interfaces (Menden-Deuer and Grünbaum, 2006). Thus, again, in this sense, it seems an appropriate model organism for planktonic processes. Furthermore, using protists associated with very shallow waters to model planktonic systems is not uncommon; much of the earlier work on protozoa, used to obtain rate processes and conversion factors for pelagic ecosystem models, has been obtained from semi-benthic species (e.g. Capriulo, 1990). Examples of such an approach include the semi-benthic rock pool dwelling *Stombidium sulcatum* (S. inclinatum; see Modeo et al., 2003) that has been extensively used to represent planktonic ciliates and the frequently studied mixotrophic chrysophyte *Ochromonas danica*, which was originally isolated from an acidic moor (Pringsheim, 1955). There is, thus, considerable precedence for using taxa like *O. marina* as model pelagic organisms, mainly because they are easy to grow, maintain and collect, as indicated above. We, therefore again, support the past and continued use of the *O. marina*, with the codicil that it is not necessarily typical of open water taxa and should, ultimately, be compared to them.

Taxonomy

*Oxyrrhis marina* is unlikely to be a single species, and there are strain-differences in eco-physiological responses (Lowe et al., 2005a, 2010). There are serious implications regarding this point, related to population studies. For instance, the growth response of *O. marina* strains differs based on responses to: salinity (Lowe et al., 2005a), prey concentration and type, and temperature (Montagnes, unpublished results). However, such strain-specific responses are far from unique to *O. marina*; e.g. similar strain-specific differences occur in a model freshwater ciliate, *Urotrichia* (Weisse and Montagnes, 1998). Thus, modellers must simply be aware of these differences and consider them when interpreting results. In fact, as strain differences are becoming topical in ecological research (see Weisse and Montagnes, 1998), this “problem” can become an asset, and modellers will undoubtedly begin to use the responses of the various strains to examine potential strain-succession, as we are at present doing (Yang et al., this issue). Finally, modelling studies based on *O. marina* typically use defined strains, and we are exceptionally fortunate with *O. marina* that several commercial and personal culture collections have maintained these (Lowe et al., this issue). Hence, notwithstanding the caveats highlighted above, and the recognition that further comparative studies of the behaviour of *O. marina* and other planktonic protozoa are required, *O. marina* seems fit for purpose as a representative protozoan, from which mathematical models can be derived.

OXYRRHIS MARINA-BASED MATHEMATICAL MODELS

We, therefore, now turn to those studies that have derived or parameterized mathematical models based on *O. marina*. Broadly, these fall into two categories:
Table I: Studies that determine or apply O. marina-based equations and their functional forms

<table>
<thead>
<tr>
<th>Equation type and number</th>
<th>Equation (see caption for symbols)</th>
<th>Selected works that employ the function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Functional response</td>
<td>( l = \frac{a}{k + p} )</td>
<td>Kimmance et al. (2006); Strom (1993)</td>
</tr>
<tr>
<td>2. Numerical response</td>
<td>( r = \frac{a}{k + p} )</td>
<td>Jeong et al. (2008)</td>
</tr>
<tr>
<td>3. Numerical response</td>
<td>( l = \frac{a}{k + p} )</td>
<td>Kimmance et al. (2006); Strom (1993)</td>
</tr>
<tr>
<td>4. Functional response</td>
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<td>Kimmance et al. (2006)</td>
</tr>
<tr>
<td>5. Numerical response</td>
<td>( l = \frac{a}{k + p} )</td>
<td>Kimmance et al. (2006)</td>
</tr>
<tr>
<td>6. O. marina volume as a function of prey abundance</td>
<td>( v = \frac{a}{k + p} )</td>
<td>Kimmance et al. (2006)</td>
</tr>
<tr>
<td>7. O. marina volume response modulated by temperature</td>
<td>( v = \frac{a}{k + p} )</td>
<td>Kimmance et al. (2006)</td>
</tr>
<tr>
<td>8. Difference equation for O. marina increase in abundance</td>
<td>( N_{t+1} = \frac{r \times N_t}{1 + a \times N_t} )</td>
<td>Johnson (2000)</td>
</tr>
<tr>
<td>9. Ingestion of detrital particles modulated by a selection factor (SF)</td>
<td>( Re = \frac{I \times \left[ \frac{D}{\sigma + SF} \right]}{\frac{D}{\sigma + SF}} )</td>
<td>Strom (1993)</td>
</tr>
<tr>
<td>10. distance a predator has to travel to encounter a volume of prey equal to its own volume</td>
<td>( \vartheta = \frac{N_{t+1} \times \alpha \times (S_p \times p)^{-1}}{a} )</td>
<td>Flynn et al. (1996)</td>
</tr>
<tr>
<td>11. Functional response modified (decreased) by the influence of inhibitor (a) obtained from ingestion of non optimal prey</td>
<td>( l = \frac{a}{k + p} )</td>
<td>Davidson et al. (1995a); Mitra et al. (2003); Mitra and Flynn (2005)</td>
</tr>
<tr>
<td>12. Ratio-based selectivity function for multiple prey</td>
<td>( l = \frac{a}{k + p} )</td>
<td>Fasham et al. (1990); Mitra and Flynn (2006)</td>
</tr>
<tr>
<td>13. Rate of prey capture related to a selectivity function ( f ) that defines the relationship with a specific prey (p) concentration</td>
<td></td>
<td>Mitra and Flynn (2006)</td>
</tr>
<tr>
<td>14. Nitrogen regeneration as a function of predator N content and protozoan C:N ratio</td>
<td></td>
<td>Davidson et al. (1995b)</td>
</tr>
<tr>
<td>15. Nitrogen regeneration as a function of respiration, gross growth efficiency and prey and predator C:N ratios</td>
<td></td>
<td>Caron and Goldman (1988); Davidson et al. (1995b); Davidson et al. (2005)</td>
</tr>
<tr>
<td>16. Nitrogen regeneration as a stepwise function of protozoan C:N ratio</td>
<td></td>
<td>Davidson et al. (2005)</td>
</tr>
</tbody>
</table>

\( a, b, c \) are constants; \( I \) the ingestion rate; \( l_{\text{max}} \) the maximum ingestion rate; \( p \) the prey abundance; \( k \) the half saturation constant of the ingestion curve; \( r \) the specific growth rate; \( f_{\text{max}} \) the maximum specific growth constant; \( p' \) the threshold prey abundance (at which \( r = 0 \)); \( k_0 \) a constant (\( k_0 \) = half saturation constant of the growth curve); \( T \) the temperature; \( v \) the volume; \( v_{\text{max}} \) the maximum volume; \( k_0 \) the half saturation constant of the ingestion curve; \( V \) the volume at zero food abundance; \( N \) the O. marina concentration; \( Re \) the re-ingestion of faecal particles; \( D \) the concentration of faecal particles; \( SF \) the selection factor; \( N_{\text{max}} \) the number of prey cells equivalent to one predator in terms of biomass; \( \alpha \) the variable related to the swimming speed of prey and predator; \( S_p \) the cross-section of encounter party of predator and a given prey species; \( \eta \) the preference for different prey types; \( C_i \) the capture rate of specific prey species; \( f \) the capture rate parameter; \( E \) the nitrogen excretion rate; \( n \) the O. marina nitrogen content; \( \eta \) the C:N ratio of prey or predator; \( R \) the respiration rate; \( S \) the gross growth efficiency.

(i) autecological studies that specifically simulate the response of O. marina to a set of physical, biological or chemical conditions and (ii) synecological studies that embed O. marina-based responses within food web simulations, to study the wider role of ecosystem processes. These models are reviewed (grouped in relation to the hypotheses tested) below with the mathematical responses that underpin them summarized (Table I).

**Autecological models**

**Functional and numerical responses**

Functional and numerical response relationships are often central to population models (Turchin, 2003), typically characterized by a rectangular hyperbola or a “type II Holling” response. Such rectangular hyperbolic responses are used extensively to characterize the behaviour of protozoa (e.g. Taylor, 1978; Montagnes, 1996; Jeong et al., 2004; Fenton et al., 2010). Their suitability to simulate ingestion and growth of O. marina has been supported by a number of studies [Table I, equations (1–2)] on a range of prey species (for further details beyond the scope of this modelling-based review, see Lowe et al., this issue; Montagnes et al., this issue-2; Roberts et al., this issue). Modifications of these two responses (Table I) are also fundamental to a number of the mathematical models that we review; e.g. equation (3) (Table I) is a modified version of equation (2) (Table I), where the numerical response is recognized to
be negative below a threshold \( p' \) abundance of prey (Fenton et al., 2010). It is also important to note that, to our knowledge, for \( O. marina \) there are no data that suggest inhibition of growth or grazing rates at elevated prey concentrations, as has been indicated for other protozoa (e.g. Montagnes and Lessard, 1999), although see Prey inhibition of grazing, below.

The influence of abiotic factors
Various abiotic factors will modify protist (and specifically \( O. marina \)) population dynamics; e.g. salinity (Droop, 1959; Samuelsson et al., 2006), turbulence (Peters and Marrase, 2000), temperature (Montagnes et al., 2003; Kimmance et al., 2006) and pH (Droop, 1959; Pedersen and Hansen, 2003). Two \( O. marina \) modelling-based studies have specifically sought to investigate the role of such abiotic factors.

Temperature
Trends of increasing water temperature have the potential to influence the productivity and biodiversity of marine phytoplankton (Bresnan et al., 2009). Understanding the temperature response of protozoa is equally important, as any temperature-induced mismatch between predators and prey in pelagic communities could have significant implications for trophic transfer (Montagnes et al., 2008a; Koeller et al., 2009). Using \( O. marina \), Kimmance et al. (Kimmance et al., 2006) demonstrated the need for an adequate representation of temperature response by making a range of rate parameters within an \( O. marina \) model a (experimentally determined) function of temperature and prey density [Table I, equations (4) and (5)]. Furthermore, recognizing that both prey abundance and temperature will alter cell volume, Kimmance et al. (Kimmance et al., 2006) established a relationship between these and \( O. marina \) volume [Table I, equation (6)], allowing models to determine the production in terms of carbon (assuming a relation between volume and carbon content; Menden-Deuer and Lessard, 2000). Application of these functions within a mathematical model demonstrated different dynamics when the full temperature-prey response was incorporated, in comparison to the more commonly used \( Q_{10} \) based function (see Montagnes et al., 2003). The potential of these functions to improve model predictions suggests that they should now be incorporated into larger ecosystem models.

Physical influences on the distribution of protozoan population
Protozoa may be locally and globally distributed by abiotic factors such as currents and wind driven aerosols. Models that assess such factors in governing distributions are potentially useful not only for protozoa but also for small metazoans, such as invertebrate larvae.

An example of abiotic influence is that of tidal action on coastal \( O. marina \) populations. Johnson (Johnson, 2000) developed a simple mathematical model to study this phenomenon and specifically to test the hypothesis that \( O. marina \) has a competitive advantage that makes it prevalent in rock pool shoreline environments. The model simulated cell abundance in rock pools using a simple difference equation [Table I, equation (8)] to determine population size, based on the intrinsic population growth rate and the carrying capacity of the environment. The influence of flushing and the effects of extreme conditions on the upper shore were included in the model by making growth rate a function of pool location in relation to tidal height. The model predicted that \( O. marina \) distribution was influenced by both rock pool height on the shore and tidal cycle and that it differs from other protozoa in the pools, in that it is more stress tolerant. The stress tolerance of \( O. marina \) is consistent with its success in the rock pool habitat and its success in the (presumed somewhat stressful) conditions of laboratory culture. However, it also indicates that mathematical models based on \( O. marina \) are most appropriate for other stress tolerant protozoa, and observations of this species should be viewed in this light.

Other abiotic factors
Clearly, there is scope to extend modelling work on \( O. marina \) to examine other physical factors. There are data in the literature that would potentially allow a relationship between growth rate and salinity to be established (Droop, 1959; Lowe et al., 2005a); possibly assuming growth rate is a quadratic function of salinity (Lowe et al., 2005b). Similarly, the data of Droop (Droop, 1959) and Pedersen and Hansen (Pedersen and Hansen, 2003) could be used to establish a growth response to pH, which superficially appears to be either sigmoidal or rectangular hyperbolic in shape. We have also conducted preliminary experiments to parameterize the influence of turbulence on growth of \( O. marina \) (Montagnes, unpublished results), following similar work on the autotrophic flagellate Isochrysis galbana (Downes-Tettmar and Montagnes, 2008), and it appears that only the level of turbulence generated by heavy wave action in rock pools will reduce \( O. marina \) growth rate. We, therefore, recommend that (i) further experimental data are collected on these physical parameters and (ii) existing data are used to parameterize new functions for incorporation into models.
Swimming behaviour and aggregation

Study of *O. marina* to characterize the swimming behaviour of protists is dealt with in a separate study (Boakes et al., this issue), and representation of *O. marina* searching trajectories using Lévy walk or other similar encounter statistics is discussed by Bartumeus et al. (Bartumeus et al., 2003) and Reynolds (Reynolds, 2008). In a more general sense, modelling-based interpretation of *O. marina* foraging in response to prey aggregations was conducted by Menden-Deuer and Grünbaum (Menden-Deuer and Grünbaum, 2006) who characterized the availability of patchy prey by means of the Frost number, a composite parameter based on forager speed, turning interval, distance between prey patches and patch longevity. In addition, *O. marina* has also been used in models that assess mesozooplankton swimming and feeding (e.g. Mariani et al., 2008). Thus, we see the potential for this species to be incorporated into multilevel behavioural models in the future.

Feeding behaviour

Protozoa can discriminate between prey types, with selective grazing, on the basis of prey quantity or quality; now being recognized as a key issue in the functioning of microbial food webs (Montagnes et al., 2008a). A range of factors such as morphology, chemical defence and nutritional quality may govern the selectivity of prey items by protozoa (Montagnes et al., 2008a). The *O. marina*-based studies that have experimentally addressed these factors are not germane to our work, but the interested reader is directed to (Roberts et al., this issue); here we specifically review how models have addressed feeding behaviour.

Modelling re-ingestion of faecal material

Coprophagy is a well-recognized process in planktonic systems that may have considerable impact on food web dynamics. For instance, low chlorophyll:phaeopigment ratios have been proposed to indicate high levels of mesozooplankton coprophagy, but given the importance of microzooplankton in food webs (e.g. Azam et al., 1983; Davidson, 1996), it may be that they too are important in this process. To this end, Strom (Strom, 1993) developed a mathematical model to test the hypothesis that re-ingestion of faecal material by protozoa could account for the observed variation in the conversion of chlorophyll to phaeopigment. In this model, which was applied to her experiments on *Strombidium* and *Gymnodinium* and to the *O. marina*-based data of Klein et al. (Klein et al., 1986), protozoa preyed on phytoplankton and faecal particles (with which phaeopigment was associated). Protozoan growth was made a function of phytoplankton prey [Table I, equation (1)], with a selection factor for phytoplankton cells or faecal particles that allowed the determination of an ingestion rate for faecal particles [Table I, equation (9)] and hence, by subtraction from the functional response, an ingestion rate for phytoplankton cells. Although the model did not lend support to the notion that re-ingestion governed phaeopigment distributions, it demonstrated that the re-ingestion of faecal material by protozoa was a plausible trophic pathway that may have significant implications for energy flow within the microbial loop.

Multi prey selectivity

Following Strom’s (Strom, 1993) model-based demonstration of discrimination between live and dead food by protozoa, the selection of alternative live prey by *O. marina* was addressed by Flynn et al. (Flynn et al., 1996) who presented a theoretical relationship for the distance travelled by a raptorial predator to encounter a volume of prey equal its own volume [δ, Table I, equation (10)]. To illustrate how such simulations are developed, below we use this model as an example.

The model was developed by first determining that the cross-sectional area (S_p) of the encounter path for the predator and a given prey (p) species is S_p = π(r_{pred} + r_p)^2, where r_{pred} and r_p are the radius of the predator and prey cells, respectively. The rate of prey encounters per predator (E) was then given by E = S_p \cdot N_p \cdot \sqrt{C_p^2 + C_{pred}^2}, where speeds of predator and prey are C_{pred} and C_p respectively, and N_p is the number of prey. The number of prey cells equivalent, in terms of biovolume, to one predator (N_eq) was then determined from N_{eq} = V_{pred} \cdot V_p^{-1}, where V_{pred} and V_p are mean predator and prey cell volumes, respectively. This allows the calculation of the encounter distance δ = N_{eq} \times \alpha \times (S_p \times N_p)^{-1}, where

\[
\alpha = C_{pred} \cdot \left( \sqrt{C_p^2 + C_{pred}^2} \right)^{-1}.
\]

If predation of a particular prey type continues when δ is greater than that of alternative prey, then the predator is deemed to select the former item. This concept was applied to a set of laboratory experiments in which *O. marina* ingested three differently sized prey species. The analysis demonstrated the occurrence of selection of live cells and suggested that selective grazing in microbial communities may be complex and dependent on both prey size and prey quality, both of which may change with time, rather than simple random encounter. Clearly, in this case, *O. marina* acted as a model organism to test general issues associated with selection.


Prey inhibition of grazing
The role of selective grazing in governing the temporal changes of both prey and predator was further studied by Davidson et al. (Davidson et al., 1995a) through simulation of two independent data sets: the I. galbana–O. marina study of Flynn and Davidson (Flynn and Davidson, 1993b) and the multi-prey–O. marina experiments of Flynn et al. (Flynn et al., 1996).

Flynn and Davidson (Flynn and Davidson, 1993b) suggested that O. marina initially ingested but then rejected the flagellate prey, but Davidson et al. (Davidson et al., 1995a) found that no parameterization of a standard type II functional response [Table I, equation (1)] generated adequate simulations of their data. Instead, qualitatively better simulations were achieved if the maximum ingestion rate was made to decrease with continued prey ingestion [Table I, equation (11)], a factor that was attributed to the build up of an inhibitor within O. marina through the ingestion of the prey. The same model structure was also able to simulate the O. marina–multiple prey data of Flynn et al. (Flynn et al., 1996). Thus, this O. marina-based model quantitatively demonstrated that the quality of a prey item as well as its abundance or size may govern its suitability as a prey item for protozoa. Given the tractability of using O. marina for grazing experiments (e.g. Kimmance et al., 2006), it would now seem appropriate to test these model predictions with empirical data.

Prey quality governing predator functional response
The role of prey quality was further explored by Mitra et al. (Mitra et al., 2003) who hypothesized that it could influence predation through modulation of either (i) the rate of ingestion [Table I, equation (11)], with “α” in equation (11) being a variable rather than a constant or (ii) the efficiency of assimilation of this ingested material. The study examined the relative importance of these processes through a model that related both maximum rate of predation and assimilation efficiency to prey quality (defined as its C:N ratio) in a range of different functional forms. By making the maximum predation rate a function of prey quality, the model simulated experimentally observed phenomena exhibited by O. marina of “surge feeding” (Opik and Flynn, 1989) and prey rejection (Flynn et al., 1996). In particular, this study indicated that while different functional formulations for ingestion and assimilation of prey caused the model to predicted similar trophic transfer of carbon, this occurred on very different timescales, demonstrating that such physiological responses of protozoa could influence the temporal availability of organic matter for trophic transfer. However, it is important to note that this response has yet to be documented for O. marina, and again we suggest that experiments in this direction are needed.

Prey quantity governing predator assimilation response
While Mitra et al. (Mitra et al., 2003) focused on the effect of prey quality on assimilation rate, Fenton et al. (Fenton et al., 2010) have explored the relationship between prey abundance and protozoan assimilation efficiency; they indicate that many protozoa, including O. marina, exhibit a decreasing assimilation efficiency with increasing prey concentration. Then, by comparing simple Rosenzweig–MacArthur-based predator–prey models (using O. marina and I. galbana parameters, derived from Kimmance et al., 2006) with either a constant or variable assimilation efficiency, Fenton et al. (Fenton et al., 2010) indicate that prey carbon production may be increased by >65% when a variable assimilation efficiency is applied. Thus, using O. marina as a model, they conclude that, from an applied perspective, such as examining biomass productivity for food web dynamics or examining the recycling of nutrients within an ecosystem, including prey abundance–dependent assimilation efficiency, leads to very different quantitative predictions from those given following commonly applied models.

Stoichiometry and selectivity
Mitra and Flynn (Mitra and Flynn, 2005) continued to study the influence of stoichiometrically driven ingestion and assimilation, using a model that was optimized by fitting to the I. galbana–O. marina-based data of Flynn and Davidson (Flynn and Davidson, 1993b). The authors reached similar conclusions to Davidson et al. (Davidson et al., 1995a), with best simulations being obtained with “negative modulation” of ingestion; i.e. a decrease in ingestion rate on the flagellate in response to non-optimal quality of this prey [Table I, equation (11)]. However, when this analysis was extended to mesozooplankton-based data sets (Jones et al., 2002), simulation required assimilation of non-optimal prey to also be linked to the prey quality. This difference was related to O. marina’s (and other protists’) lack of a gut and hence the greater likelihood of modulation at the point of capture and ingestion rather than digestion in protozoa.

Mitra (Mitra, 2006) extended the above work through the derivation of a generic multi-nutrient zooplankton model that included specific representation of both ingestion and assimilation, both of which were functions of prey nutrient stoichiometry, which she termed “stoichiometric modulation of predation”. This model was again fitted to the experimental data of Flynn and
Davidson (Flynn and Davidson, 1993b), with similar conclusions to those reached above, i.e. it is necessary to decrease ingestion rate for poor quality prey to obtain a fit of the model to the data.

In the most recent of their suite of O. marina-related publications, Mitra and Flynn (Mitra and Flynn, 2006) studied the influence of two alternative modelling formulations to represent predator selectivity. They incorporated either a ratio-based function [Fasham et al., 1990, Table I, equation (12)] governed by the relative abundance of different prey types or made prey capture a function of prey availability and a capture rate parameter [Table I, equation (13)], that could take a range of functional forms based on prey quality (or other factors). Again, the model response was compared to the data of Flynn et al. (Flynn et al., 1996) and Flynn and Davidson (Flynn and Davidson, 1993b). The new prey capture function [equation (13)] was found to be most appropriate, albeit with the caveat that further modulation of ingestion based on prey quantity and quality was necessary to optimize the fit, a finding that is consistent with the models above.

The quantitative importance of nutrient regeneration and cycling

While mathematical models are often used in predictive mode, an equally important application is the analysis of processes that cannot be easily understood by simple observation. One of the most important of these for microbial community population dynamics is the regeneration of inorganic nutrients by protozoa, and their subsequent use by phytoplankton (Goldman et al., 1987). This process maintains the stoichiometric balance of nutrients within the predator and fuels further growth of the prey (Caron, 1991).

To this end, Davidson et al. (Davidson et al., 1995b) studied nutrient regeneration using a predator–prey model that included O. marina and I. galbana, to assess the response of different nitrogen (N) regeneration equations. The model incorporated the phytoplankton growth model of Davidson et al. (Davidson et al., 1993) that simulates both carbon (C) and N dynamics of I. galbana during unbalanced growth, allowing simulation of experiments in which prey exhibit active growth. Again, as an example of how such models are developed, we provide the relevant equations.

The equation for the rate of change of I. galbana N is give by

\[
\frac{dI_N}{dt} = -E_l \cdot I_s - \left( \frac{I_N}{I_s} \right) \cdot I \cdot O_N
\]

and the rate of change of O. marina N was simulated by

\[
\frac{dO_N}{dt} = \left( \frac{I_N}{E} \right) \cdot I \cdot O_N - E_N \cdot O_N,
\]

where O, I, O_N and I_N represent the number and N-content of O. marina and I. galbana, respectively; I is the ingestion rate of O. marina; and E_I and E_N are the N-regeneration rates of I. galbana and O. marina, respectively. Within the equations, N-regeneration by O. marina was simulated either as a constant or on the basis of an “optimal” O. marina C:N ratio, using a range of N regeneration models.

The study demonstrated a need to include N-regeneration in protozoan-based models to adequately simulate experimental data. This model observation is consistent with many mathematical models of microbial communities. However, such models often simulate this process in an unsophisticated manner, making regeneration a constant amount of nutrient, independent of prey or predator physiology or composition (Davidson, 1996). Using O. marina, Davidson et al’s (Davidson et al., 1995b) model lent support to arguments about protozoa made by Goldman et al. (Goldman et al., 1985) by indicating that a dynamic [Table I, equation (14) and (15)], rather than constant, regeneration rate must be applied to protozoan nutrient cycling, indicating the utility of an O. marina-based model to provide more general insights. It may now be possible to use developing methods, such as stable isotope labelling, to assess nutrient cycling and assess empirically if O. marina behaves according to model predictions.

Summary of autecological work

Four main insights are clear from the above review of autecological models: (i) O. marina is useful as a “model organism”; (ii) there are O. marina experimental data in the literature that could be used to more fully parameterize its numerical and functional response; (iii) notwithstanding the previous point, we need to collect more data to extend and improve upon the responses that need parameterization (e.g. given concerns of ocean acidification, to pH); and (iv) we need to extend our empirical testing of predictions that have been obtained from O. marina-based models.

Synecological Oxyrrhis marina-based models

Above, our review has revealed an extensive range of autecological models associated with O. marina; these provide an understanding of its behaviour and how it
can be used to assess key ecological processes. Therefore, it may be surprising that application of such O. marina-based models to study the influence of protozoa in food webs is, to date, relatively limited. To indicate how O. marina might be incorporated into larger models, we examine case studies, below.

**Parameterizing microplankton models**

Lee et al. (Lee et al., 2003) developed a carbon–nitrogen-based model of the phytoplankton, bacterial and protozoan components of a planktonic food web, which was embedded in a three layer physical framework. The protozoan model was developed as a single compartment with constant C:N ratio consistent with experimental and modelling results discussed above. O. marina parameters, from Fuller (Fuller, 1990), were used to parameterize the protozoan component of the model. Given the substantial increase in parameter estimates for O. marina over the past 15 years (Table I) since Fuller (Fuller, 1990), it may be appropriate to revisit such models.

**Control of toxic dinoflagellate blooms by microzooplankton and parasites**

Montagnes et al. (Montagnes et al., 2008b) incorporated O. marina parameters into a model that examined the relative role of microzooplankton grazing (by large ciliates) and protozoan parasites in the control of dinoflagellate blooms. O. marina was included as a representative grazer of nanoflagellates and zoospores, the dispersal stage of the parasite. The conclusion of this model was that parasites, not microzooplankton, could control dinoflagellate blooms, even when the dispersal stages can be reduced by top-down control from O. marina-like predators.

**The influence of N regeneration on a food web**

The role of protozoan N-regeneration was assessed in a microbial food web model (Davidson et al., 2005); this was an extension of the model by Fasham et al. (Fasham et al., 1990) that included a multiple currency of C and N, and hence variation in the C:N ratio of both phytoplankton and their protozoan predators. The protozoan compartment of the model was parameterized from laboratory experiments on O. marina. Nutrient regeneration was related to the relative C:N ratios of prey and predator using three alternative functions all capable of representing, to some degree, the N regeneration efficiency of O. marina: a constant nitrogen regeneration efficiency (NRE); a stepwise switching function between low and high NRE, termed threshold elemental ratio [Table I, equation (16)]; and a dynamic nutrient regeneration equation; Caron and Goldman, 1988; Table I, equation (15)].

Simulations demonstrated quantitative differences between the output generated by the different models, particularly between the switching and dynamic models and the constant NRE model in terms of the density of phytoplankton blooms. Differences in C:N ratio of model components were also evident with only the dynamic model predicting a stoichiometrically balanced zooplankton C:N ratio close to 6.6, the Redfield value, similar to the values that experimental estimates suggest that protozoa, including O. marina, maintain (Goldman and Dennett, 1992; Nakano, 1994; Davidson et al., 1995a). Such O. marina-derived results have important implications for the formulation of the multiple functional type models that are now being formulated to better understand the global C cycle (e.g. le Quéré et al., 2005).

**Summary of synecological work**

Considering the importance of protozoa within marine ecosystems and the relatively wealth of data, response relationships and models based on O. marina (Table I), it is unclear why so few synecological modelling studies have drawn on this resource, to date. Clearly, considerable scope exists to develop and improve existing models, and to produce alternative formulation for comparison, as illustrated above (The influence of N regeneration on a food web). Moreover, the now recognized diversity of O. marina (e.g. Lowe et al., 2005a, 2010) offers the potential to produce data sets that will allow an “organism sensitivity analysis” to better quantify biologically reasonable ranges of model parameter values. It may also be possible to examine spatial distributions using the framework established by Johnson (Johnson, 2000) and the physical–biological functions outlined above; perhaps allowing the assessment of large scale patterns.

**FUTURE DIRECTIONS OF O. MARINA-BASED MODELLING**

**Better parameterization**

Response relationships such as those presented in Table I are increasingly being derived for O. marina, and these studies provide a valuable resource for model construction. Modellers need to be made aware of the existing data sets for O. marina, which have yet to be fully exploited for model parameterization (e.g. Fuller, 1990; Jeong et al., 2001, 2004; Kimmance et al., 2006). Hopefully this paper has helped fulfil that role.
However, many of the existing studies have been related to the grazing impact on harmful or aquaculture-relevant prey species (e.g. Jeong et al., 2001), and we suggest that further study of cosmopolitan and benign prey and the role of abiotic factors in modulating these relationships are required.

Independent time-series for comparison

Models also require test data, independent to the observations on which they are derived. Considering the number of studies conducted using O. marina, surprisingly few have proved to be amenable for this purpose. For example, studies that analyse the selective grazing properties of O. marina are particularly prominent in the works we have reviewed. However, a disproportionate high fraction of these have applied their model to the data of Flynn and Davidson (Flynn and Davidson, 1993b). Further time course experiments following O. marina and one, or more, prey items in differing environmental conditions are, therefore, required to better validate these and future models. As guidance, we present an example of such a data set for model comparison (Fig. 1). This represents a subset of numerous time course experiments beginning at many initial predator and prey concentrations. Such an approach, while generating time-course data, may minimize the bottle effects (e.g. accumulation of toxins and fouling on surfaces) that can bias long-term incubations. Population models, independently derived from O. marina functional and numerical response and prey growth data may be tested using the resultant phase-plot data.

One of the reasons that the O. marina–I. galbana data set of Flynn and Davidson (Flynn and Davidson, 1993b) has been so often used as a comparison with simulations is the availability of a robust model that predicts prey growth in non-steady state conditions (Davidson et al., 1993; Davidson and Cunningham, 1996). Hence, the experimental study and modelling of O. marina, in particular, and protozoa in general must be

![Fig. 1. An indication of how further population dynamics might be obtained for comparison with model output: abundance of Oxyrrhis marina and the prey flagellate Dunaliella primolecta, grown at 16°C in 32 PSU seawater enriched with f/2 media (Sigma). (a)–(c) are 18-day time-course incubations of predator (open circle) and prey (closed circle) in triplicate flasks. (d) and (e) are phase plots: (d) is a plot of the three 18-day time-course incubations (a–c); (e) is a plot of a series of short (5–11 days) incubations, indicating a semblance of population cycling.](http://plankt.oxfordjournals.org)
conducted in parallel with that of their prey, to allow both trophic levels to be simulated to the same level of complexity. This requires experimental studies to measure a sufficient array of parameters including numbers, biomass, ingestion, grazing, respiration and nutrient cycling rates to allow appropriate model parameterization and testing, to minimize the need to “fit” free model parameters.

Improving model structure, using *O. marina*

Doney (Doney, 1999) highlighted the need for succinct but realistic mathematical models capable of simulating the cycling of multiple nutrients within microbial food webs. These are necessary to simulate the transfer of production to higher trophic levels and the export flux of C to the ocean floor. Within this context parameterization of zooplankton or microzooplankton response in a range of different model structures is increasingly a topic of debate. To this end, below, we provide an indication of how *O. marina* might be used in a range of models to help resolve this debate.

Nutrient—phytoplankton—zooplankton models

In general, the classical nutrient—phytoplankton—zooplankton (NPZ) models (e.g. the widely used model of Fasham et al., 1990) use a simple closure term to represent grazing. The potential deficiency in this approach was highlighted by Mitra (Mitra, 2009) who discussed the difference between the theoretical response of NPZ models that employ generic “closure” functions and those that include specific representation of carnivory and cannibalism, finding that these generated differences in simulated primary production and f-ratio. In a similar vein, Gentleman et al. (Gentleman et al., 2005) comprehensively reviews the mathematical formulation and use of a range of different multiple resource functional response relationships for zooplankton. Again, her comparison of response was based on theoretical simulations. Hence, while studies such as these highlight the potential pitfalls for modellers from an erroneous choice of functional response, experimental verification of the most appropriate functions is still required.

Considering the relative wealth of information on *O. marina* revealed in this review, it seems a very suitable organism with which to test the suitability of alternative functional relationships to represent grazing processes. For example, *Isochrysis galbana*, the prey species on which much of the *O. marina*-based predator—prey modelling is based, becomes smaller during N depravation (Davidson et al., 1992; Flynn et al., 1994). However, other phytoplankters, e.g. *Nannochloropsis oculata*, increase in size under such conditions (Flynn et al., 1993). Understanding and modelling the response of microzooplankton grazers to alternative prey following simple changes in environmental conditions will be a necessary step to the development of robust models in the future.

Plankton functional type models

Plankton functional type (PFT) models are increasingly being employed in ocean biogeochemistry, and here again using *O. marina* may be instructive. The use of PFT models is somewhat controversial, with some authors (e.g. Anderson, 2005) suggesting that their application may be premature. However, notwithstanding this debate, there is consensus that better model parameterization is required. This is particularly pertinent at the microzooplankton level. For example, the dynamic green ocean model (Le Quéré et al., 2005) contains five separate autotrophic functional types, but only a single composite, protozooplankton compartment to represent heterotrophic flagellates and ciliates. While this is understandable in terms of model tractability, the parameterization of the equations used to represent this “functional group” requires deeper consideration in the light of the wealth of behaviour that different species and genera are capable of (Montagnes et al., 2008a) and observations of temporal succession of different microzooplankton groups (Davidson et al., 2007).

Analysis of the functional response of *O. marina* in comparison with other heterotrophic marine micro- or dinoflagellates, expanding on initial studies such as those of Jeong et al. (Jeong et al., 2008), and with multiple prey items (John and Davidson, 2001), would add confidence to single functional group parameterization, or provide definitive evidence that multiple microzooplankton functional groups are required in models. The application of developing techniques such as lectin labelling (Wootton et al., 2007), flow cytometric separation of prey and predators (Montagnes et al., 2008a), the analysis of stable isotope signatures (Flynn and Davidson, 1993a) or stable isotope probing (Radajewski et al., 2000), will hopefully provide the data sets from which to progress this field.

Individual-based models

Individual-based models (IBMs) provide an alternative modelling strategy to those that seek to represent the ecosystem as a whole, and *O. marina* is an ideal candidate for these. IBMs calculate biological variables while following individual (or meta-) particles in space. These models may then be of particular use for the study of advective populations and/or species that form only a small fraction of the biomass of a trophic level but are important for other reasons. A number of important
biodiversity producing phytoplankton species such as the advective *Dinophysis* spp. (Hart et al., 2007) or the low-biomass high-toxicity dinoflagellate *Alexandrium tamarense* (Touzet et al., 2010) fit these criteria. As *O. marina* ingests bioxenon producing dinoflagellates (Jeong et al., 2001, 2003), it may be a suitable candidate organism for developing grazing terms within such models. Furthermore, in rare cases, it forms large blooms of up to 10^7 cells mL^-1 (Begun et al., 2004) and thus its own may be important in short-term rapid fluxes of nutrients in some ecosystems. Thus, *O. marina*-IBM may too be justified in the future.

**SUMMARY**

What has *O. marina* modelling delivered to the scientific community? Of the autecological models reviewed, the majority deal with some aspect of prey selectivity; the combined body of work in this area is particularly useful in demonstrating that prey selectivity by microheterotrophs is, indeed, capable of influencing the trophic transfer of phytoplankton biomass (e.g. Strom, 1993; Flynn et al., 1996; Davidson et al., 1995a, b) and that functional form used to simulate this selectivity will influence model results (Mitra et al., 2003; Mitra, 2006; Mitra and Flynn, 2006). In this light, there is a somewhat surprising relative lack of more basic combined experimental-modelling studies based around *O. marina* that specifically seek to model the response to particular environmental drivers (an exception being the temperature based study of Kimmance et al., 2006). This is an obvious area for further fruitful study. The relative lack of synchronostatic studies employing *O. marina*-based model parameterization perhaps reflects this need, with the more sophisticated models of prey selectivity requiring a fundamental underpinning prior to their wider application.

In conclusion, as *O. marina* is not often abundant in open water samples, it might not be the organism of first choice to parameterize the protozoan component of such models. However, as indicated above, available evidence suggests its use is appropriate, and the relative wealth of *O. marina* studies makes it a pragmatic choice. Furthermore, there are ecosystems where *O. marina* may be abundant, and in these regions using *O. marina*-derived parameters would be entirely appropriate. Therefore, we support its continued use as a model organism to parameterize simple and more complex population models.

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