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Do temperature–food interactions matter? Responses of production and its components in the model heterotrophic flagellate *Oxyrrhis marina*

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ABSTRACT: The consequence of interactions between temperature and food concentration for protistan population dynamics and estimates of aquatic productivity are relatively unknown, primarily because we lack adequate parameters for models. Here, using the heterotrophic flagellate *Oxyrrhis marina* Dujardin, we demonstrate the importance of considering temperature and food concentration in combination, to determine the responses of grazing rate, specific growth rate, cell volume, specific production and yield. Specific growth rate and cell volume responded in different ways to temperature–food concentrations: prey concentration had greatest positive effects on specific growth rate with increasing temperature, and prey concentration had greatest positive effects on cell volume with decreasing temperature. The effect of these contrasting interactions on specific production (=specific growth rate × cell carbon) was a greater response to prey concentration at intermediate temperatures. We also observed that the threshold food concentration for growth increased with increasing temperature, but yield showed no clear thermal response. By applying iterative curve-fitting to data obtained from multiple temperature–food concentration combinations, we produced phenomenological models of grazing rate, specific growth rate, and cell volume. We then compared predictions from a simple predator–prey simulation model that applied either our derived equations or a single exponential (Q_{10}) relationship to the specific growth and ingestion responses at 20°C. Considerable differences in predator and prey abundance were obtained between the 2 models. Our results demonstrate the potentially complex effects of food and temperature in combination on production parameters, and we argue that these should be considered in aquatic ecosystem simulation models.

KEY WORDS: Cell size · Ecosystem model · Functional response · Interaction · Microzooplankton · Numerical response · Prey concentration · Production · Q_{10}

INTRODUCTION

Prey concentration and temperature are significant environmental factors that determine the population dynamics and productivity of many aquatic ectotherms. There is a strong body of literature indicating that prey concentration elicits a rectangular hyperbolic response on parameters such as growth rate, ingestion rate and organism size (e.g. Davidson 1996, Weisse et al. 2002, Gentleman et al. 2003). There is an equally large body of literature indicating that many rate processes increase (e.g. Cossins & Bowler 1987, Montagnes et al. 2003) and organism size decreases (Atkinson et al. 2003) with increasing temperature. However, numeric food web models often treat these 2 variables (temperature, prey concentration) independently, typically imposing one response on the other (see Carlotti et al. 2000).

For instance, a typical scenario in a population model is for growth and grazing rates to be dependent on ambient prey concentration, derived from data collected at a single temperature; then, if temperature were to change in the model, a correction for prey-dependent rates would be imposed, based on a Q_{10}

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response (e.g. Q_{10} = 2.5; Caron et al. 1990). The reason for this methodology is simple: few studies simultaneously examine the effect of both temperature and prey concentration. The concern we raise here is that there may be unaccounted for interaction between temperature and prey concentrations that will alter the outcome of such food web models. Collecting data on prey-dependent responses at many temperatures is arduous work. However, we suggest that predictive responses obtained by fitting equations to data sets that vary both temperature and prey concentration will substantially alter the outcome of food web models.

There are already indications that temperature and prey levels should not be treated independently. Food levels that support growth at low temperatures may be inadequate at high temperatures (Weisse et al. 2002 and references within). Thus, interaction between increased temperature and food resources may produce a counterintuitive decrease in productivity (e.g. Lampert 1977). Furthermore, significant temperature–prey interactions occur for a variety of plankters, including copepods, *Daphnia* spp., rotifers, and ciliates (Orcutt & Porter 1984, Thébault 1985, Achenbach & Lampert 1997, Stelzer 1998, Giebelhausen & Lampert 2001, Weisse et al. 2002). Despite the wealth of information on the impact of temperature and prey concentration on size, and on growth and ingestion rates, the combined effect of these factors has rarely been studied in detail for either metazoans or protozoans. Partially, this lack of data is due to the difficulties of working with organisms with long generation times. Protists, on the other hand, with generation times on the order of hours, are ideal tools to assess the interactive effects of temperature and prey concentration (e.g. Weisse et al. 2002). It is also well recognised that protozoans are important components of many aquatic ecosystems, ranging from the open ocean to sewage systems (Fenchel 1987), all of which can be impacted by both changing prey levels and temperature. Thus, there is a specific need for such studies on protozoans.

The small marine flagellate *Oxyrrhis marina* Dujardin was chosen as a model to examine the combined effect of food concentration and temperature on specific growth rate, ingestion rate, cell size, and production. This protist is likely ubiquitous (Lowe et al. 2005), and has been used as a model to assess responses to prey type and concentration (Flynn & Davidson 1993), nutrient regeneration (Davidson et al. 1995), and salinity (Droop 1959, Buskey et al. 1998). In addition, *O. marina* has been used to model bi- and tritrophic grazing interactions (Wolfe & Steinke 1996), dimethyl sulphide production and grazing selectivity (Wolfe et al. 1997, Wolfe 2000), and the potential control of red-tide organisms (Jeong et al. 2003, Johnson et al. 2003). Thus, further information on this species may not only suggest general relationships but will also build our database on this model organism.

Our aim was first to assess the effects of temperature and food concentration on parameters associated with modelling food webs (e.g. growth rate, ingestion rate, cell size), including the extent of temperature–food concentration interactions. We then briefly used equations that included the combined response to temperature and prey concentration, to illustrate that such equations substantially alter the outputs of resource–consumer population models compared to those that employ independent Q_{10} functions.

MATERIALS AND METHODS

Temperature effects on growth and ingestion of *Oxyrrhis marina*. This study was developed in 4 integrated parts: (1) to visually characterise the interactive effect of temperature and prey concentration on specific growth rate, cell volume, specific production, ingestion, and yield, we examined the effect of prey concentration at several discrete temperatures; (2) to statistically assess the interactions at sub-saturating vs. saturating prey concentrations, we examined the effect of temperature on specific growth rate, cell volume, and production at 3 discrete prey levels established using data from the first set of experiments: zero (0 ml^{-1}), sub-saturating (5 to 70 $\times 10^2$ ml^{-1}), and saturating (1.8 to 2.5 $\times$ 10^5 ml^{-1}); (3) to provide predictive equations that describe the combined effects of temperature and food concentration on growth, ingestion, and cell volume, we fitted equations to response-data from all temperature and prey concentration combinations from the 2 experiments mentioned above; finally (4) to illustrate the impact of using these predictive equations compared with applying a Q_{10}-response to account for temperature effects, we developed a simple population model and compared its outputs. As the population model provided a synthesis of the study, we have reserved its description and evaluation for the 'Discussion'. Methodological details of the other approaches follow.

Culture methods and experimental design of incubations. *Oxyrrhis marina* was isolated from a rock pool ~5 m above mean low tide, (~12°C, 34 ppt) in October 1998 on the Isle of Man (54° 4.06′ N, 04° 44.25′ W); it was maintained on the ~5 µm cryptophyte *Isochrysis galbana* (Culture Collection of the Marine Biological Laboratory, Helsingør, Denmark). Both *O. marina* and *I. galbana* were maintained at 15 ± 0.5°C in f/2 medium (Guillard 1972) and harvested in exponential phase for experiments. *I. galbana* was exposed to 150 µmol photons m^{-2} s^{-1} on a 14:10 h light:dark cycle.

Growth and ingestion rates and cell size were determined for *Oxyrrhis marina* between 8 and 30°C at 20 to 30 *Isochrysis galbana* (prey) concentrations ranging from 10^2 to 2.5×10^5 ml^{-1} (concentrations shown in Figs. 1 to 5). For prey-response experiments at discrete temperatures there was no replication (e.g. see Fig. 1), while for temperature-response experiments at the 3 discrete prey levels each temperature-prey treatment was replicated (n = 3; e.g. see Fig. 2).

The experiments were conducted in temperature-controlled water-baths, in the dark, in 250 ml polycarbonate bottles that were continuously mixed by gentle rotation. Cultures were acclimated to the experimental conditions for at least 1 generation, after which initial counts were made, and were then incubated for 24 h, after which final counts were made. Densities of live-prey and Lugol-fixed (2% final volume) *Oxyrrhis marina* were measured: live-prey densities were determined using a Model II Coulter Counter, and fixed *O. marina* densities were determined using a Sedgewick-Rafter chamber. *O. marina* cell volume was estimated from ≥50 cells (obtained at the end of each 24 h incubation), using length and width measurements and assuming a prolate spheroid shape. All *O. marina* size measurements were made on acid Lugol-fixed material (final concentration 2%) and volume was calculated taking into account fixation-induced shrinkage (Montagnes et al. 1994).

Parameters measured in incubations. Specific growth rate (μ, d^{-1}) of *Oxyrrhis marina* was calculated assuming exponential growth over 24 h: i.e. $\mu = \ln(N_t - N_0)/t$, where N_0 and N_t are initial and final *O. marina* numbers, respectively, and t (d) is the experiment duration. The geometric mean prey concentration was determined in each bottle over the 24 h incubation; although prey numbers decreased during incubation, densities remained sufficiently constant to allow the assumption that, relative to other prey treatments, changes would not alter the assumption of constant exponential growth over 24 h. Ingestion rate (I) by *O. marina* was calculated from prey depletion (using initial and final prey counts) compared to controls, following equations outlined by Heinbokel (1978). *O. marina* carbon content was determined from volumes (0.099 pg μm^{-3}; Menden-Deuer & Lessard 2000). Specific production (Sp) of *O. marina* was calculated after Montagnes & Weisse (2000), modified by converting volume to biomass: $Sp = \mu(v \cdot OC)$, where μ = specific growth rate (d^{-1}); v = *O. marina* cell volume (μm^3); and OC = *O. marina* carbon (pg C μm^{-3}). Yield (=gross growth efficiency, Fenchel 1982) was calculated as: yield = $Sp/(I \cdot pC)$, where I (ingestion) = prey *O. marina*$^{-1}$ d^{-1} and pC = pg carbon *Isochrysis galbana*$^{-1}$, calculated from the temperature–volume and carbon–volume relationships of Montagnes & Franklin (2001).

Response models. To characterise the interactive effect of temperature and prey concentration on specific growth rate, ingestion, cell volume, and specific production, we fitted established response models (e.g. Montagnes & Lessard 1999) to data from experiments conducted at discrete temperatures. Curves were generated by an iterative fitting method, using the Marquardt-Levenberg least squares algorithm (Sigmaplot Version 5, 1999, SPSS); this algorithm is appropriate for describing such biological data sets (Berges et al. 1994). Numerical responses, with non-zero intercepts, were established by fitting Eq. (1) to growth rate data. Functional responses were established by fitting Eq. (2) to ingestion rate data. The response of cell volume to prey concentration was established by fitting Eq. (3) to cell volume data. The response of specific production to prey concentration was established by fitting Eq. (4) to specific production data:

$$\mu = \frac{\mu_{max} \times (p - p')}{k_1 + (p - p')} \quad (1)$$

$$I = \frac{I_{max} \times p}{k_2 + p} \quad (2)$$

$$v = \frac{v_{max} \times p}{k_3 + p} + c \quad (3)$$

$$Sp = \frac{Sp_{max} \times (p - p')}{k_4 + (p - p')} \quad (4)$$

where μ = growth rate (d^{-1}); μ_{max} = maximum growth rate (d^{-1}); p = the geometric mean prey concentration (ml^{-1}); p' = threshold concentration (prey concentration at which μ = 0 or Sp = 0); I = ingestion rate (prey *Oxyrrhis marina*$^{-1}$ h^{-1}); I_{max} = maximum ingestion rate (prey *O. marina*$^{-1}$ h^{-1}); v = cell volume (μm^3); v_{max} = maximum volume (μm^3); c = predicted cell volume at zero prey (μm^3); Sp = specific production (pg C d^{-1}); Sp_{max} = maximum specific production (pg C d^{-1}); k_1, k_2, k_3, k_4 = are constants (ml^{-1}).

Statistical tests for interaction. To assess for interactive effects in the temperature-response experiments conducted at the 3 discrete prey levels (zero, sub-saturating, saturating), analysis of covariance was applied to the specific growth rate and cell volume data. When linear trends with temperature were not apparent, analysis of variance was applied to test for interactive effects.

Cell volume data were further investigated. The relationship between temperature and cell volume in protists has been studied by calculating the slope of the volume–temperature relationship divided by cell volume at a reference temperature (15°C); this parameter, presented as a percent change in volume with temperature (% $°C^{-1}$), is referred to as the relative thermal sensitivity of cell volume (Atkinson et al. 2003).

Following Atkinson et al. (2003), the relative thermal sensitivity of cell volume was calculated at saturating and sub-saturating prey concentrations, and analysis of covariance was applied to test for interactive effects.

Predictive models. Curves were iteratively fit to response-data from all temperatures and prey concentration combinations using the Marquardt-Levenberg least squares algorithm, and parameters were determined. Initially a number of alternative predictive equations were fitted to the data (see Kimmance 2001 for details), and the most appropriate were selected based on their biological suitability (related to Eqs. 1 to 4 above) and best fit to the data; i.e. where regressions were significant, $p < 0.05$, and had the highest adjusted R^2 value.

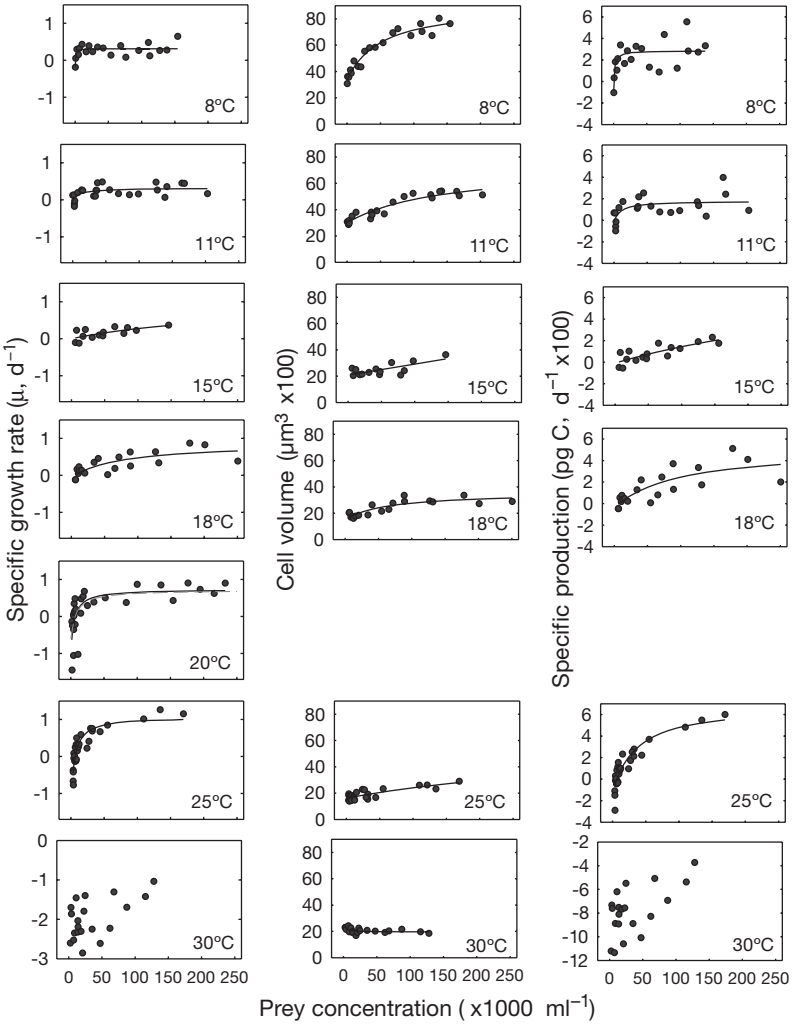

Fig. 1. *Oxyrrhis marina*. Response of specific growth rate, cell volume, and specific production (product of specific growth rate and cell carbon) to prey concentration (*Isochrysis galbana*) at temperatures ranging from 8 to 30°C. Continuous lines are the best fit to the data. Dashed line on the 20°C specific growth rate panel, which is virtually identical to the continuous line was used to assess models (see 'Discussion: Application of interaction between temperature and prey concentration')

RESULTS

Temperature effects on growth and ingestion parameters

Specific growth rate of *Oxyrrhis marina* generally followed a rectangular hyperbolic response with a positive prey-intercept (Fig. 1, Eq. 1). As temperature increased, both the maximum growth rate and threshold food concentration (i.e. the prey concentration where $\mu = 0$) increased (Figs. 1 & 2d), suggesting an interactive effect of temperature and prey concentration on specific growth rate. Further investigation supported this, as the response of the specific growth rate of *O. marina* to temperature differed between the 3 food levels (Fig. 2a). At saturating prey concentrations, mean specific growth rate increased with increasing temperature until 25°C; at 28°C growth decreased. At sub-saturating prey levels, mean specific growth rate also increased with increasing temperature, but the maximum occurred at 18°C. At zero prey concentration, specific growth rate decreased with increasing temperature, becoming negative at 25°C. The apparent (but not significant) positive growth when no food was present (Fig. 2a) may have been due to cells dividing without increasing in size. Analysis of covariance indicated an interaction: for saturating prey (between 8 and 20°C) and sub-saturating prey (between 8 and 18°C), specific growth rate increased linearly with increasing temperature (Fig. 2a), but over these ranges the slopes differed ($p < 0.05$).

Oxyrrhis marina cell volume generally increased with increasing prey concentration and decreased with increasing temperature (Fig. 1). The relationship between *O. marina* cell volume and prey concentration (Eq. 3) changed as a function of experimental temperature (Fig. 1), suggesting an interactive effect of temperature and prey level on predator volume. Further investigation supported this, as the relationship between *O. marina* volume and temperature differed between the 3 prey concentrations (Fig. 2b): analysis of covariance indicated that the slope of cell volume vs. temperature was significantly lower at sub-saturating prey concentrations than at growth-saturating levels ($p < 0.05$). Specifically, at zero prey concentration, the relative thermal sensitivity of *O. marina* cell volume was 2 % °C^{-1}; at sub-saturating prey concentrations it was 1 % °C^{-1}, and at saturating prey concentrations it was 6 % °C^{-1}.

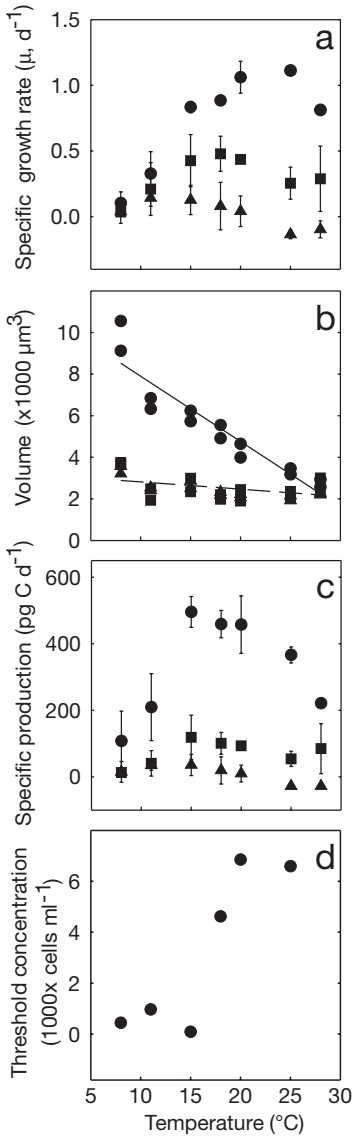

Fig. 2. *Oxyrrhis marina*. Response (mean ± SE) of specific growth rate, cell volume, specific production (product of specific growth rate and cell carbon), and threshold concentration (where μ = 0) to temperature at 3 prey (*Isochrysis galbana*) concentrations: (●) saturating, (■) sub-saturating, and (▲) zero prey; see 'Materials and methods' for details

Specific production (*Sp*) of *Oxyrrhis marina* generally increased with both increasing temperature and increasing prey concentration, following a rectangular hyperbolic response (Eq. 4, Fig. 1). As temperature increased, Sp_{max} increased, and the shape of the hyperbolic function differed between temperatures, suggesting an interaction (Fig. 1). When specific production was examined at the 3 discrete prey concentrations (Fig. 2c), there was further evidence of an interactive effect. Specific growth rate increased and cell volume decreased with increasing temperature (see above), but the product of these (*Sp*) showed a different response. Specific production was virtually zero below 15 to 18°C in the no-prey treatment. Unlike the maximum specific growth rate, which occurred at different temperatures depending on prey level, specific production peaked at ~15°C at both saturating and sub-saturating prey levels. However, production was much lower at sub-saturating concentrations (Fig. 2c).

The ingestion rate of *Oxyrrhis marina* increased with increasing food concentration and temperature, following a rectangular hyperbolic function with an *x*-intercept of zero (Eq. 2, Fig. 3). At lower temperatures ingestion rate tended to increase more linearly with increasing food concentration (Fig. 3), suggesting an interactive effect of food level and temperature on ingestion. The ingestion rates at sub-saturating levels were not ob-

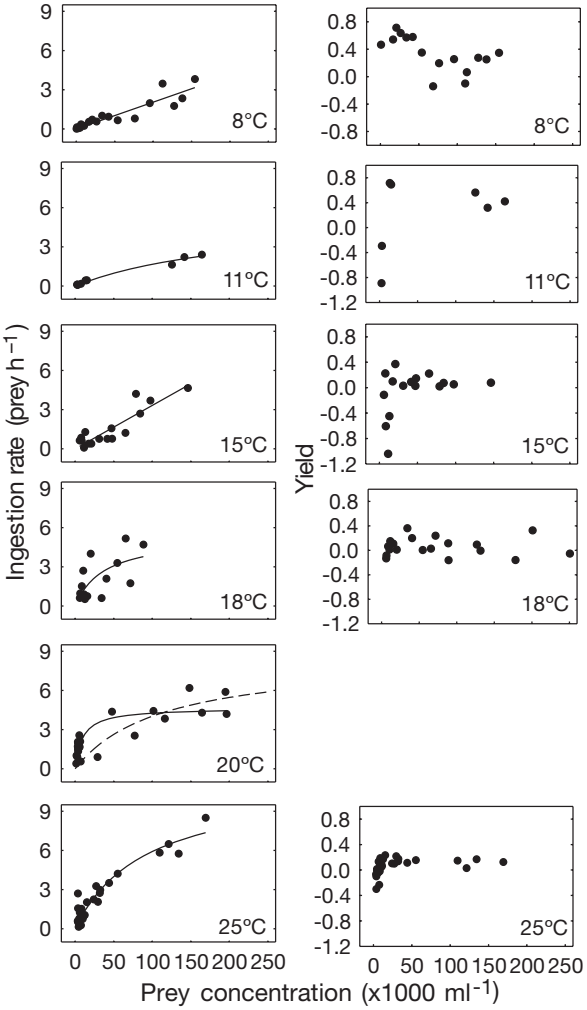

Fig. 3. *Oxyrrhis marina*. Response of specific ingestion rate and yield (=gross growth efficiency) to prey concentration (*Isochrysis galbana*) at temperatures ranging from 8 to 25°C. Continuous lines are the best fit to the data. The dashed line in 20°C ingestion rate panel was used to assess models (see 'Discussion: Application of interaction between temperature and prey concentration')

tained, therefore the interaction was not statistically tested. Ingestion rate at saturating prey levels for growth (see Fig. 1), however, increased with increasing temperature between 8 and 20°C (Fig. 4a), but above 25°C, there was a decline in ingestion rate, and at 28°C it was reduced to nearly the same level as at 8°C (Fig. 4a).

Oxyrrhis marina yield (gross growth efficiency) depended on prey concentration (Fig. 3). A maximum yield of 0.6 to 0.7 occurred between 8 and 11°C, at ~2×10^4 prey ml^{-1}. At 8°C, there appeared to be a decrease in yield with increased prey concentration (Fig. 3). At 11, 15, 18, and 25°C, yield initially increased with increasing prey concentration and then reached an asymptote (Fig. 3). *O. marina* yield at growth-saturating prey levels ranged from 0.04 to 0.43 (Fig. 4b), and exhibited no clear relationship with temperature when data from both sets of experiments were combined. Furthermore, analysis of variance indicated that there was no effect of temperature on yield when only the saturating prey concentrations, from the second set of experiments, was considered ($p > 0.05$).

Predictive models

The response of *Oxyrrhis marina* specific growth rate to the combined effects of temperature and prey concentration was best described by Eq. (5) ($p < 0.05$, adjusted $R^2 = 0.53$, Fig. 5a), where μ = specific growth rate (d^{-1}), p = prey concentration (ml^{-1}), and t = temperature (°C). The response of the ingestion rate to the combined effects of temperature and prey concentration was best described by Eq. (6) ($p < 0.05$, adjusted $R^2 = 0.59$, Fig. 5b), where I = ingestion rate (prey h^{-1}). The response of cell volume to the combined effects of temperature and prey concentration was best described by Eq. (7) ($p < 0.05$, adjusted $R^2 = 0.86$, Fig. 5c), where v = *O. marina* cell volume (μm^3):

$$\mu = \frac{0.94 \times (p - 6393)}{14\,160 + (p - 6393)} \times 0.05t \quad (5)$$

$$I = \frac{23.74 \times p}{112\,010 + p} \times 0.28(t - 7.99)^{0.10} \quad (6)$$

$$v = \left(\frac{54\,000 \times p}{360\,000 + p} + 16\,412 \right) \times (1.44 \times t^{-0.86}) \quad (7)$$

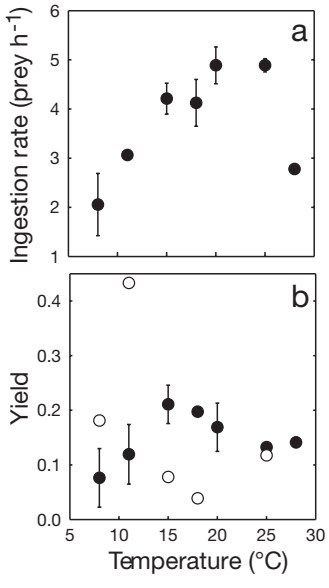

Fig. 4. *Oxyrrhis marina*. Response (mean ± SD) of (a) ingestion rate and (b) yield to temperature at saturating prey (*Isochrysis galbana*) concentration. (●) Data from a set of experiments run only at saturating prey levels (see 'Materials and methods' for details); (○) from asymptotic values obtained from yield data in Fig. 3

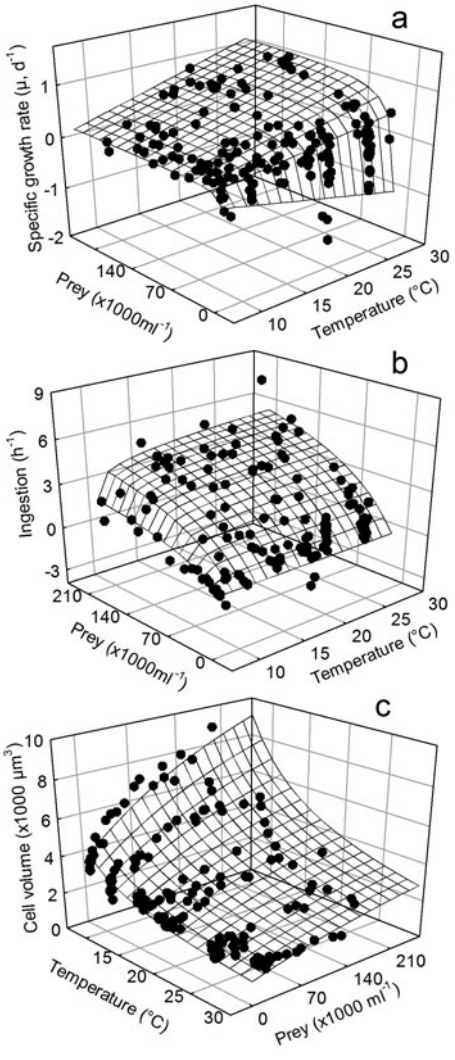

Fig. 5. *Oxyrrhis marina*. Response of specific growth rate, ingestion rate, and cell volume to the combined effects of temperature and prey (*Isochrysis galbana*) concentration; data points obtained from all experiments; fits to data (mesh) in (a), (b) and (c) follow Eqs. (5), (6) & (7), respectively

DISCUSSION

Prey concentration

It is well established that protists typically follow Holling Type II, rectangular hyperbolic, functional and numerical responses, and *Oxyrrhis marina* is no exception. It is possible that some protists may elicit a sigmoidal, Type III, functional or numerical response, but in most studies data are rarely sufficiently precise to assess this, and we were not able to do so here. Similarly, we were not able to detect a threshold level for ingestion rate (i.e. the functional response was best modelled by Eq. 2). In contrast, we were able to reveal that *O. marina* exhibits a threshold prey level, where growth is zero. Such a threshold level for heterotrophic dinoflagellates (to which *O. marina* is related: Lowe et al. 2005) is well supported in the literature, with values typically ranging from 10 to 10^3 µg C l^{-1} (Jacobson 1987, Bjørnsen & Kuparinen 1991, Strom 1991, Hansen 1992, Strom & Buskey 1993, Buskey et al. 1994, Jeong & Latz 1994, Jakobsen & Hansen 1997). Threshold levels can be used to assess whether conditions are adequate to allow a predator to survive; our data suggest that *O. marina* can survive at relatively low prey levels (1 to 80 µg C l^{-1}). Furthermore, establishing threshold levels (and mortality rates below them) may be critical for parameterising food-web models, as they allow predators to die from starvation rather than being consumed by predators (Montagnes 1996).

There is also a tendency for some protists to be deleteriously affected at high prey concentrations (e.g. Montagnes & Lessard 1999), but this did not occur for *Oxyrrhis marina*. Its growth rate reached an asymptote and production and ingestion rate tended to increase, even to $>10^5$ prey ml^{-1}, suggesting that this species can occur and thrive at relatively high prey levels. Cell volume may also increase with increasing prey concentration (e.g. Montagnes & Lessard 1999), and this occurred for *O. marina*, becoming asymptotic at high prey levels. Thus, in general it appears that *O. marina* is able to survive at low prey levels and thrive at high prey levels. In fact, our specific production data indicate that *O. marina* can be extremely productive at temperatures of 18 to 25°C and prey concentrations $>10^5$ ml^{-1}.

Yield (gross growth efficiency) also varied as a function of prey concentration. Basic maintenance requires food, and thus a threshold prey concentration for growth exists. Consequently, at this threshold prey concentration, yield (by definition) is zero, and it will increase above this level to a maximum. This is what occurred for *Oxyrrhis marina*. More interestingly, yield tends to decrease at higher prey levels, suggesting a reduction in efficiency at very high levels, as occasionally seen in other planktonic taxa (Valiela 1995).

Protists are often thought to be efficient feeders, with high yields, and studies typically use protozoan yield >40% to calculate ingestion or growth rates from growth and ingestion rates, respectively (e.g. Caron & Goldman 1990 and references therein, Bjørnsen & Kuparinen 1991, Archer et al. 1996). However, Straile (1997) found that a protist yield of ~20–30% may be more appropriate, decreasing to ~10–20% when food is abundant, which is consistent with at least some of our findings (Fig. 3). Our findings support the application of variable yields, an observation that should be noted by modellers.

Temperature

Both protistan growth rate and cell volume can be modelled as linear functions of temperature (Atkinson et al. 2003, Montagnes et al. 2003), and the data from this study support this, at least over limited ranges. Our data also suggest that ingestion increases linearly with increasing temperature between 8 and 15°C (Fig. 4), but as saturating prey levels were not reached for grazing rate at all temperatures (cf. Figs. 1 & 3), further data collection is still required to assess this trend.

The data (Fig. 2d) suggest that the threshold prey concentration, where growth is zero, increases with increasing temperature. This agrees with our previous work (Weisse et al. 2002) on the freshwater ciliate *Urotricha farcta*, where a step-wise shift from a low threshold at low temperatures to a high threshold at high temperatures also occurred. Possibly this shift reflects a change in physiological state, whereby catabolic processes at higher temperatures dominate, requiring a substantially higher food intake to achieve growth (Atkinson & Sibly 1996).

Finally, yield appeared to show no clear trend with temperature in this study. Yield should change if there is an imbalance in anabolic and catabolic processes with a change in temperature (Angilletta & Dunham 2003): data from single- and multi-species studies suggest that protistan yield may increase (Laybourn & Stewart 1975, Rogerson 1981, Sherr et al. 1983, Straile 1997) or decrease (Rassoulzadegan 1982, Verity 1985, Caron et al. 1990, Straile 1997) with increasing temperature. This diversity of responses to temperature is also seen in various Metazoans (Straile 1997, Angilletta & Dunham 2003). Thus, no clear, single, temperature–yield relationship is evident, which combined with the general adaptation of most organisms to ambient temperature should mean that yield will show no strong temperature-dependence in the field (Straile 1997).

Interaction between temperature and prey concentration

There is clearly an interactive effect between temperature and prey concentration on a variety of the parameters studied. The threshold level for growth seemed to increase stepwise, and specific growth rate and cell volume responded differently under saturating and sub-saturating prey concentrations, depending on temperature (Fig. 2). Furthermore, the shape of the functional, numerical, volume, and production responses differed as a function of temperature (Figs. 1 to 3).

Understanding the physiological basis of temperature–food interactions was not specifically the purpose of this work. However, our data do reveal some potential trends. For instance, the increased effect of food concentration on specific growth rate with increasing temperature (Fig. 2a), which was consistent with the higher threshold prey concentration at increased temperatures (Fig. 2d), is probably caused by higher rates of catabolism necessary for survival (e.g. maintaining ionic gradients, protein turnover: Clarke & Fraser 2004). Furthermore, the range of relative thermal sensitivities of cell volume, from 6% at saturating prey concentrations to 2% at sub-saturating concentrations,

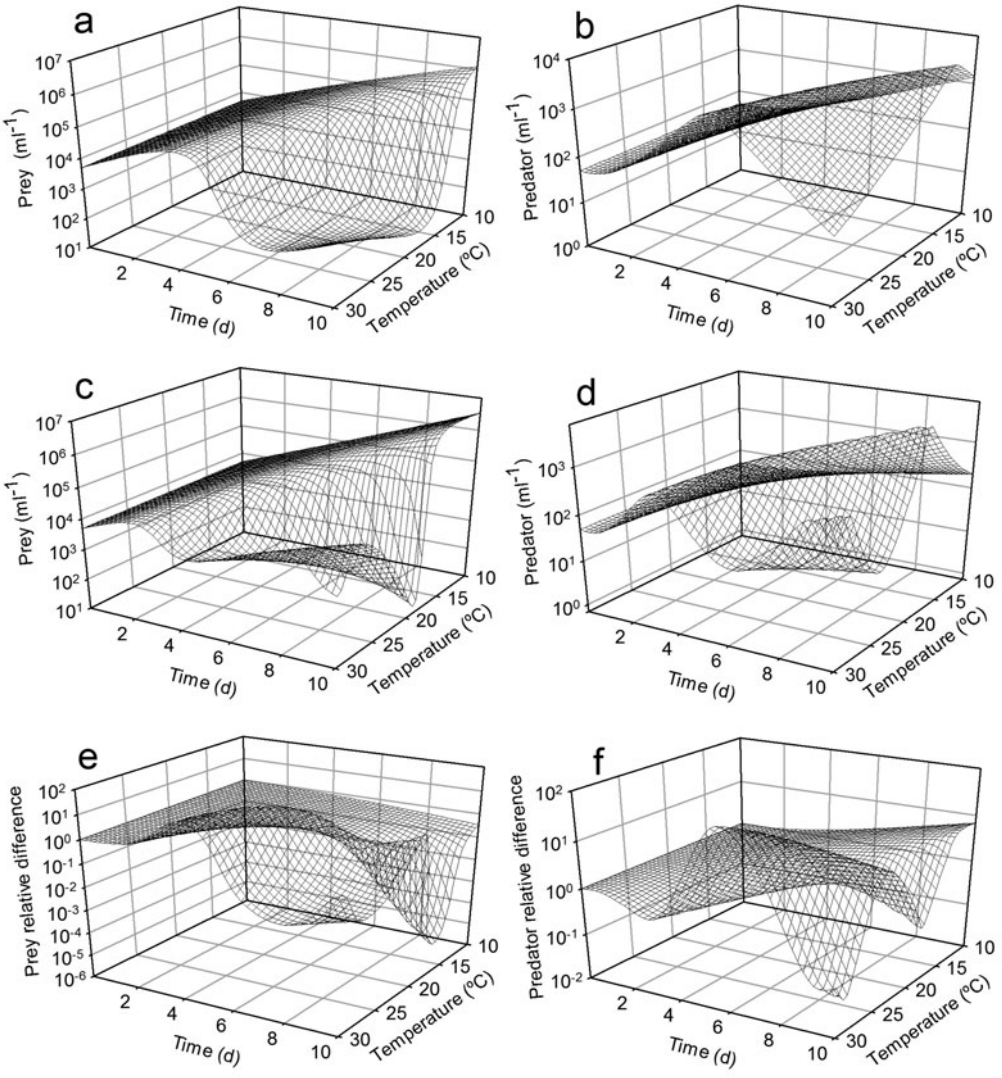

Fig. 6. *Oxyrrhis marina* and *Isochrysis galbana*. Modelled population dynamics of (a,c,e) prey *I. galbana* and (b,d,f) predator *O. marina* over a 10 d simulation. For (a) and (b), specific growth rate and ingestion were determined directly from Eqs. (5) & (6), respectively; thus, in this case the combined effects of ambient temperature and prey concentration were included. For (c) and (d), specific growth rate and ingestion rate were determined only at 20°C from Eqs. (5) & (6), respectively (dashed line at 20°C in Figs. 1 & 3). Subsequently, to determine the response at other temperatures, a Q_{10} of 2.5 was applied to the values obtained at 20°C. (e,f) Relative difference between the 2 scenarios, i.e. the ratio of prey (a:c) and predators (b:d). Predator and prey concentrations are on log-scales

spans the mean value of 2.5 % °C^{-1} observed by Atkinson et al. (2003) for protists, but this significant decrease in slope is due to prey limitation at low temperature, not high temperature as occurred for specific growth rate, suggesting a different mechanism underlying temperature–prey dependency on cell size. Investigating the mechanisms underlying such responses is a goal of our ongoing research, to provide insight into the widespread inverse relationship between temperature and body size in ectotherms (Atkinson & Sibly 1997) and into the scaling of biological rates in general (Brown et al. 2004), as well as helping to provide predictive relationships for ecosystem modelling.

Recognising that interaction occurred, however, we set forth to model these responses, for predictive purposes, by fitting a variety of phenomenological models to the entire data set accumulated from the 2 sets of experiments. The best fits (Eqs. 5 to 7) take into account the effects of temperature–food combinations without adding unjustified complexity of additional interaction terms. Furthermore, they provide both prey concentration- and temperature-dependent components, allowing us to easily manipulate the equations based on their parts. Thus, we have provided predictive equations for future modelling, and a simple example of their implications follows.

Model application of interaction between temperature and prey concentration

As indicated in the 'Introduction', there is a tendency in numeric ecosystem modelling to treat food concentration and temperature independently (Carlotti et al. 2000). Here we use our detailed data on 1 species to illustrate that this may be inappropriate, as temperature and food affect growth and ingestion parameters differently when examined in combination; this may then alter the outcome of food web models. To demonstrate this, *Oxyrrhis marina* growth and grazing responses (Eqs. 5 & 6) were applied to a simple model of predator–prey dynamics. In this illustrative example, volume changes (Eq. 7) were not included. The inclusion of volume would alter the predictions related to food web productivity; clearly all 3 equations would need to be incorporated into ecosystem models that consider carbon flux.

Using STELLA 8 (High Performance Systems), a predator–prey model was simulated, whereby the flagellate *Isochrysis galbana* grows exponentially and is preyed upon by *Oxyrrhis marina*, with no higher level predators consuming the flagellate; thus, *O. marina* mortality is regulated strictly by starvation below threshold levels (i.e. $dP/dt = \mu_p P - IN$ and $dN/dt = \mu_N N$, where P = *I. galbana* abundance; μ_p = temperature-dependent specific growth rate of *I. galbana*; I = temperature- and prey-dependent grazing by *O. marina*; N = *O. marina* abundance; μ_N = temperature- and prey-dependent specific growth rate of *O. marina*). The simulation lasted 10 d, a period sufficiently long, at 20°C, to express 1 predator–prey cycle, given initial conditions of 5×10^3 prey and 50 predators ml^{-1} (the starting condition of all simulations).

In the first application of the model, *Oxyrrhis marina* specific growth rate (μ_N) and ingestion (I) were determined directly from Eqs. (5) & (6), respectively; thus in this case the combined effects of ambient temperature and prey concentration were included. In the second application of the model, *O. marina* specific growth rate and ingestion rate were determined only from the predicted response at 20°C, from Eqs. (5) & (6), respectively (Figs. 1 & 3, dashed lines at 20°C). Then a Q_{10} of 2.5 (see Caron et al. 1990) was applied to the growth and grazing rates at given prey concentrations at 20°C to determine the response at other temperatures. This latter scenario represents the typical way in which many ecosystem models rely on the limited data that are available. In both cases, *Isochrysis galbana* specific growth rate (μ_p) was determined as a linear response to temperature (μ_p = temperature × 0.01 + 0.576), as this best represents the growth rate of *I. galbana* (Montagnes & Franklin 2001).

The model output (Fig. 6) illustrates the difference between applying a full temperature–prey-derived response and simply modifying a single response using a Q_{10} function. At 20°C the 2 responses are identical, as they were derived from identical equations. However, above and below this temperature there is deviation in the responses (Fig. 6e,f). This simulation is not an indication of natural conditions, as the prey have not been bottom-up limited (e.g. nutrients), nor has the predator been top-down limited (e.g. copepod grazing). Nor is the model exhaustive. Still, the output does illustrate our point that inclusion of equations that incorporate the effects of temperature–prey concentration combinations will substantially influence the outcome of food web models. We recommend that the present application of correction factors such as Q_{10} be reconsidered in the future and that continued effort be made to evaluate the effect of interaction between these 2 key variables on plankton dynamics.

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