Short-term temperature change may impact freshwater carbon flux: a microbial perspective

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Abstract

Small freshwater bodies are abundant and economically and ecologically important on a global scale. Within these, protozoa play an important role in structuring planktonic food webs and sequestering CO2. We hypothesized that short-term (~20 days) fluctuations, of 2–10 °C, will significantly alter carbon flux associated with predator–prey interactions within the microbial planktonic food web. We examined the model ciliate, Urotricha farcta, which is abundant and common; it was fed the autotrophic flagellate Cryptomonas sp., which is also common. Laboratory experiments were conducted over relevant ranges: 8–24 °C; 0–2 × 10^5 prey mL^-1. Mechanistic-phenomenological multiple regressions were developed and fit to the data to obtain relationships for (1) growth rate and volume changes of the flagellate vs. temperature and (2) growth rates, grazing, and cell volume change of the ciliate vs. temperature and prey concentration. Responses revealed interaction between temperature and prey levels on all ciliate parameters, indicating it is inappropriate to apply simple temperature corrections (e.g. Q_10) to such functions. The potential impact of such temperature changes on carbon flux was illustrated using a simple ciliate–flagellate predator–prey model, with and without the top grazer, Daphnia, added. The model indicated that predator–prey pulses occurred over 20 days, with the ciliate controlling the prey population. For ciliates and prey, carbon production peaked at 20 °C and rapidly decreased above and below this maximum; differences between minimum and maximum were approximately fourfold, for both prey and ciliate, with low levels at 25–30 °C and 10–15 °C. Including literature data to parameterize, the influence of the grazer Daphnia did not alter the prediction that the ciliate may control short-term flagellate pulses and temperature will influence these in a nonintuitive fashion.

Keywords: episodic shift, microbial food web plankton

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Introduction

Because of their high productivity, freshwaters, globally and locally, can play a significant role in processing and sequestering atmospheric carbon (e.g. Schindler, 1978; O’Sullivan & Reynolds, 2003). Specifically, in these systems, the pelagic food web influences carbon flux by fixing and releasing water column CO2 and mediating carbon losses through sedimentation and respiration (Flanagan et al., 2006). Such freshwaters represent ~3% of the terrestrial surface (Downing et al., 2006), with much higher contributions in some regions (e.g. northern temperate North America and Europe), and it is noteworthy that >7.2 million of these water bodies are small, having a surface of 0.01–0.1 km² (Reynolds, 2003). As many of these will have substantial economic and social importance and are an intimate link to wetland and terrestrial ecosystems (Moss, 1998; O’Sullivan & Reynolds, 2003), there is strong impetus to study them.

Climate change will raise the average temperature of small water bodies, following predicted long-term increases (3–6 °C over the next century; Houghton, 2005), but of more immediate impact is the predicted increase in intensity and frequency of short-term varia-
tions, due to global warming (Houghton, 2005). Furthermore, short-term warming events caused by other global processes such as the North Atlantic Oscillation will alter the temperature of small water bodies (e.g. Gerten & Adrian, 2000). Thus, large lakes are likely to change slowly, but for small water bodies and the productive shallow regions of larger lakes, temperatures will respond rapidly to air temperature (Carpenter et al., 1992), and episodic changes in water temperature by >5°C over 1–2 weeks may be more common (see McKee et al., 2000 for an example of the present prevalence of >5°C pulses in temperate small water bodies). In response to this prediction, we have hypothesized that short-term fluctuations, on the order of 2–10°C, will substantially influence population dynamics and carbon flux in a major component of the biota of small water bodies: predator–prey interactions within the microbial planktonic food web.

Freshwater ecosystem studies have tended to focus on the classical food web: the link between primary production and zooplankton, such as Daphnia (Jürgens, 1994). There is, however, a growing recognition that the microbial food web, often dominated by protozooplankton grazers, plays a significant role in these systems (e.g. Riemann & Christoffersen, 1993; Jürgens, 1994; Weisse, 2003); through it much of the organic matter produced by phytoplankton passes to flagellates and ciliates (Azam et al., 1983; Porter et al., 1988), with ciliates having a key role in shaping food web structure (Zingel et al., 2007). Unlike most metazooplankton, ciliates have inherently rapid growth rates, often exceeding those of their prey (Weisse, 2006). There is good evidence that many of the planktonic ciliates respond rapidly to increases in prey abundance (e.g. Montagnes, 1996), and short-term pulses of a few similar species (or virtually monospecific pulses) can occur, when prey become abundant (e.g. Müller et al., 1991; Wilson, 2002, see ‘Discussion’). Such rapid increases may occur when prey populations are stimulated to grow (e.g. by brief increases in temperature). This component of the microbial food web may then be linked to the classical food web through zooplankton grazers such as Daphnia (Jürgens, 1994), as localized increases in protozoa may act as discrete regions of improved nutritional resource.

As growth of the protistan component of food webs will respond more rapidly than the metazoan components to temperature change, it seems prudent to consider localized protistan predator–prey dynamics when assessing temperature-induced changes in aquatic carbon flux, especially if these temperature effects are extreme and sufficiently short-term to not propagate up the food web. In general, we know temperature has a number of pronounced effects on protists. Their cell size (and presumably carbon content; see Menden-Deuer & Lessard, 2000) decreases with increasing temperature by ~2.5% of their size at 15°C for an increase of 1°C (i.e. cell carbon content may decrease by 25% over 10°C; Atkinson et al., 2003). Temperature and prey concentrations also have unexpected, interactive effects on the growth, production, and population dynamics of protozoa and their prey (Weisse et al., 2002; Kimmance et al., 2006), placing into question the typical application of Q10 as a parameter to independently predict thermal sensitivities (Montagnes & Lessard, 1999; Montagnes et al., 2003; Kimmance et al., 2006). Here, we extend these works by taking an inductive approach and establish a series of carefully determined responses for a single highly relevant species that is a ‘typical’ freshwater protozoan (Foissner et al., 1999). Then to illustrate the potential importance of these monospecific pulses, following the example of others (e.g. Davidson, 1996; Shertzer et al., 2002; Fulton et al., 2003), we apply our responses to an exploration of predator–prey dynamics, using a focused, minimized model. Clearly, the impact of these changes may eventually propagate through the food web and alter population and community dynamics and carbon flux. However, constructing a full food web model is not our intent, instead we apply a predator–prey model to emphasize, at a general level, that including these dynamics will significantly alter estimates of carbon flux; then we discuss the use and limitations of our responses as a predictive tool. Such integration of laboratory work and fundamental population modelling is uncommon in a single study; here we illustrate how it can be an important, iterative step before developing more complex food web models.

Specifically, in the laboratory we examined responses of the model ciliate, Uroticina, which occurs throughout the year, and is abundant, in oligotrophic temperate, boreal, and subtropical ponds, lakes, and rivers (Foissner et al., 1999; Weisse et al., 2001). The ciliate was raised on the flagellate Cryptomonas sp., which is also common in a multitude of freshwater environments (Sommer, 1986; Dokulil, 1988; Pedros-Alió et al., 1995) and is used as a standard food in experiments with ciliates (Müller & Geller, 1993; Weisse & Montagnes, 1998; Müller & Schlegel, 1999; Montagnes & Weisse, 2000). Using these laboratory data we first develop and evaluate responses of how temperature and prey concentration influence the grazing, growth, and cell volume of the ciliate and then we use these responses to indicate how temperature may alter short-term (20 days) carbon flux in a ciliate–flagellate predator–prey model. Finally, recognizing that both Uroticina and Cryptomonas fit into the classical food web, and may be exposed to top-down control by metazooplankton (Weisse, 2003, 2006), we use literature parameters to explore the influence of adding the grazer.
Daphnia to our model. Therefore, we have focused on an energetically important model system to provide insights by highlighting the temperature sensitivity of predator-prey dynamics within the microbial food web.

Materials and methods

Study organisms

The prostomatid ciliate Urotricha farcta (~25 µm) was isolated from the mesotrophic Lake Schöhssee, Germany (Weisse & Montagnes, 1998). The prey flagellate Cryptomonas sp. strain 26.80 (~10 µm) was obtained from the Culture Collection of Algae in Göttingen (Germany). Both the ciliate and prey were maintained in modified Woods Hole medium (MWC medium, Guillard & Lorenzen, 1972) at 15±1°C, throughout all experiments. Cultures were not axenic, but U. farcta does not feed on bacteria if suitable flagellates are abundant (Weisse et al., 2001). For all experiments, both ciliate and flagellate cultures were harvested in exponential phase.

Phytoplankton (prey) response to temperature

To determine how temperature affected the prey-specific growth rate and cell volume, batch cultures were maintained at 12 temperatures (see Fig. 1) at a continuous irradiance of ~55 µmol photons m^{-2} s^{-1}; cultures were suspended in double glazed, cooled or heated water baths that maintained temperatures ±1°C. Before experiments, cultures were acclimated to the experimental conditions for eight generations. Then specific growth rate (µp, day^{-1}) was determined during exponential growth phase from measurements made over 3–5 days, from the slope of ln abundance vs. time; note population decline at low temperatures was assumed to be a per capita rate (i.e. in these cases µp<0; see Montagnes, 1996). Abundance was determined once per day from Lugol’s fixed samples (2% v/v), enumerated in a Sedgewick-Rafter chamber. Cell volume was determined from length and width measurements of 30 live, exponential-phase cells, assuming a prolate spheroid shape. Prey cell volumes were converted to carbon (C) following \( C = 0.216V^{0.939} \), where \( V \) is cell live-volume (µm³) (Menden-Deuer & Lessard, 2000).

Ciliate response to prey and temperature

Ciliate stock cultures were maintained at 15°C. Over 5–7 days, ciliates and prey were step-wise (up to 3°C day^{-1}) acclimated to experimental prey levels and six temperatures (see Fig. 2) at 70 µmol photons m^{-2} s^{-1}; these experiments were run in incubators that maintained temperatures ±0.5°C. Prey levels were monitored with an electronic particle counter during the acclimation period, and ciliates were regularly fed to maintain food levels. Prey levels were maintained in exponential phase under the same temperature conditions as the ciliates.

After acclimation, ciliates were inoculated into flasks containing acclimated prey at concentrations ranging from \( 1.0 \times 10^{4} \) to \( 2.5 \times 10^{5} \) mL^{-1} at 70 µmol photons m^{-2} s^{-1}. Initial concentrations of ciliates ranged from \( 3.0 \times 10^{2} \) to \( 7.4 \times 10^{3} \) mL^{-1} but in most experiments were initiated at \( 5.0 \times 10^{2} \)–\( 1.0 \times 10^{3} \) mL^{-1}. Controls for prey growth, without ciliates, were run at prey and light levels identical to the ciliate–prey treatments. All cultures were maintained for 24 h at each temperature. After 12 and 24 h, prey numbers were adjusted with temperature-acclimated prey or medium alone if they deviated from the target levels by >20%. The experimental incubation began immediately after this re-adjustment of prey concentrations and lasted 24 h. Each treatment was run with three to five replicates.
Samples were taken at 6–12 h intervals and fixed: for flow cytometric analyses samples were fixed with formalin (2% v/v); for microscopic analyses samples were fixed with acid Lugol’s (as above). Prey and ciliate numbers were determined by flow cytometry according to our published protocols (Lindström et al., 2002; Weisse et al., 2002) or counted microscopically (as above).

Ciliate volumes were determined from length and width measurements, assuming a prolate spheroid shape, on >50 exponential phase ciliates obtained at the end of the experiment, from each treatment. Ciliate size measurements were made on Lugol’s fixed material, which underestimates ciliate live volume by ~30% (Jerome et al., 1993), and this was corrected for. Ciliate volumes were converted to carbon units using the conversion of Menden-Deuer & Lessard (2000); see above.

Ciliate grazing rate (prey ciliate\(^{-1}\) h\(^{-1}\)) was determined from changes in growth rate of prey observed in controls, without ciliates, minus prey growth rate measured in the experimental containers with predators present, following methods outlined in Weisse et al. (2001).

**Developing response equations**

For modelling purposes, we established functions that could be used to predict predator ingestion rate, specific growth rate, and cell volume in response to varying temperature and prey concentrations. We also established responses for prey-specific growth rate and volume changes to temperature alone. These responses are presented here and further evaluated in the ‘Discussion’.

Predator (ciliate) ingestion rate (\(I_c\), prey predator\(^{-1}\) h\(^{-1}\)) was assumed to vary with prey concentration (\(P, \text{mL}^{-1}\)), following the mechanistic, Holling Type II equation [Holling, 1959, Eqn (1)], where \(a\) and \(b\) are constants. Predator-specific growth rate (\(\mu_c\), day\(^{-1}\)) was assumed to follow a similar rectangular hyperbolic response, but with a nonzero intercept (\(P'_c, \text{mL}^{-1}\)), determined by the predator’s basal metabolic rate (Montagnes, 1996), where \(c\) and \(d\) are constants [Eqn (2)]. Predator cell volume (\(V_c, \mu m^3\)) was also assumed to

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**Fig. 2** The combined effect of temperature and prey (Cryptomonas sp.) abundance on Urotricha farcta: (a) ingestion, (b) specific growth, and (c) cell volume; note the temperature axis on the last panel (c) is reversed to adequately display the shape of this response. The grids are the fits of Eqns (5)–(7) to the data (see text for details); see Table 2 for the parameters of these fits and estimates of their error.
follow a rectangular hyperbolic response, but with a positive value at zero prey, assuming a minimal cell size \( [V'] \), Montagnes & Lessard, 1999, Eqn (3)], where \( e \) and \( f \) are constants. Temperature \( (T, ^\circ C) \)-dependent responses \( (R) \) for predator and prey growth rate \( (\mu_c \text{ and } \mu_p, \text{ respectively}) \), predator ingestion rate \( (I_c) \), and predator volume \( (V) \) were assumed to increase exponentially to a maximum, plateau, and then decrease with changing temperatures, following a phenomenological model [Flinn, 1991, Eqn (4)], where \( g, h, \text{ and } i \) are constants (see Appendix A). Flinn & Hagstrum (2002) and Menon et al. (2002) combined a Type II functional response with their temperature response, and we have followed this approach for ingestion, growth, and volume responses [Eqns (5)–(7)], where lower case, italicized letters represent constants, and \( a \) [Eqn (6)] has a value of unity and dimensions of \( T^{-1} \). Prey volume was assumed to decrease linearly with temperature, following predictions of Atkinson et al. (2003).

\[
I_c = \frac{aP}{1 + bP} \tag{1}
\]

\[
\mu_c = \frac{c(P - P')}{1 + d(P - P')} \tag{2}
\]

\[
V = \frac{eP}{1 + fP} + V' \tag{3}
\]

\[
R = \frac{1}{1 + (g - hT + iT^2)} \tag{4}
\]

\[
I_c = \frac{jP}{[1 + (k - lT + mT^2)]P} \tag{5}
\]

\[
\mu_c = \frac{n[P - TzP']}{[1 + (a - qT + rT^2)][P - TzP']} \tag{6}
\]

\[
V = \frac{uP}{[1 + (v - wT + xT^2)]P} + V'. \tag{7}
\]

Ciliate growth, ingestion, and volume responses were related to the treatment temperature and average prey concentration \((P)\); note, average \( P \) over incubations was determined following methods described by Frost (1972). The above equations [Eqns (5)–(7)] were, respectively, fit to the temperature-influenced numerical, functional, and volume response data using the Marquardt–Levenberg algorithm (SIGMAPLOT, SPSS Inc., Chicago, IL, USA); this algorithm is appropriate for describing such biological datasets (Berges et al., 1994). Adjusted \( R^2 \) values for the responses and standard errors of the estimates were determined, using SIGMAPLOT, as indications of their goodness of fit.

Modelling

To illustrate the extent to which temperature change may impact carbon flux in this predator–prey system, a model was constructed using our experimental flagellate (prey) and ciliate responses (see 'Results') and responses for \( Daphnia \) from the literature (see below).

In this system, flagellates grew exponentially (with no carrying capacity as maximal prey levels were always predator controlled) and were preyed upon strictly by ciliates or by both ciliates and \( Daphnia \) (predators); ciliate growth rate was prey dependent, and ciliate mortality was regulated either strictly by starvation below threshold levels or also by top-down control by the grazer (\( Daphnia \)); the following couplet of differential equations [Eqns (8) and (9)] describe the model:

\[
\frac{dP}{dt} = \mu_P P - I_cC - I_{dp}D \tag{8}
\]

\[
\frac{dC}{dt} = \mu_C C - I_{ca}D, \tag{9}
\]

where \( P \) is the flagellate abundance; \( \mu_P \) is the temperature-dependent specific growth rate of the flagellate; \( I_c \) and \( \mu_c \) are the temperature and the prey-dependent grazing and growth rates, respectively, of the ciliate; \( C \) is the ciliate abundance; \( I_{dp} \) and \( I_{ca} \) are the temperature and prey-dependent grazing rates of \( Daphnia \) on flagellates and ciliates, respectively; and \( D \) is \( Daphnia \) abundance. Flagellate and ciliate numbers were then converted to volumes using our predictive equations (see 'Results') and then to carbon, following the conversion of Menden-Deuer & Lessard (2000) (see above).

\( Daphnia \) grazing pressure was based on assuming: (1) a constant abundance \((0.05 \text{ mL}^{-1}) \) of generic, \( Daphnia \) \((\sim 0.8 \text{ mm long}) \) being maintained over the simulations (Gilbert, 1988; Gliwicz, 2003); (2) specific filtration rates on the ciliate and prey being the same (Jürgens, 1994); and (3) the influence of temperature on filtration rate \((F, \text{ mL}^{-1} \text{ individual day}^{-1}) \) followed Eqn (10), derived by Mourelatos & Lacroix (1990):

\[
\log F = 2.07 \log L + 0.126T - 0.0024T^2 - 0.628, \tag{10}
\]

where \( L \) is \( Daphnia \) length and \( T \) is temperature (Fig. 50).

To assess the impact of the temperature-induced responses on ciliate–prey pulses, a 20-day simulation was used to represent the maximum period over which a single warming event might influence a small water body, without the influence of other abiotic (e.g. nutrient change) or biotic (e.g. \( Daphnia \) population growth)
factors substantially altering the predator–prey system. Furthermore, this period was sufficiently long to express one predator–prey cycle, given initial conditions of 4 × 10^3 prey and 10 predators mL^{-1} (the starting condition of all simulations of population dynamics); these initial abundances represent typical levels that occur in nature (Weisse et al., 1990). Note, a range of initial concentrations of ciliate and prey, similar to those found in nature, produce cycles over 5–20 days (data not shown); we chose the above single set of initial levels as realistic examples, which is in concordance with the aims of our analysis. However, to illustrate the robustness of our analysis, we also assessed and present the impact of varying both initial flagellate (200–8 × 10^3 mL^{-1}) and ciliate (1–100 mL^{-1}) abundances on the respective total production over the 20-day simulation. All simulations were run for temperatures ranging from 8 to 30 °C, a range over which the prey and ciliate might be found (e.g. Foissner et al., 1999).

Results

Phytoplankton (prey) response to temperature

Specific growth rate of Cryptomonas sp. increased from its lower temperature limit (cells did not survive at <8 °C; data not shown) to a maximum at ~20 °C and then subsequently decreased (Fig. 1a). A response was fit to the specific growth data following Eqn (4), where in this case \( R \) is prey-specific growth rate (\( \mu_p, \text{day}^{-1} \)) (Table 1). Prey volume decreased linearly with temperature (Fig. 1b, Table 1).

Ciliate response to prey and temperature

Ciliate ingestion rate increased with increasing prey concentration; it also increased with increasing temperature to a maximum at ~22 °C and then decreased (Fig. 2a). A response was fit to the ingestion data following Eqn (5) (Table 2). Ciliate-specific growth rate increased with both increasing prey concentration and temperature (Fig. 2b). A response was fit to the growth data following Eqn (6) (Table 2). Ciliate volume increased with increasing prey concentration; it first increased with increasing temperature to a maximum at ~10–12 °C and then decreased (Fig. 2c). A response was fit to the volume data following Eqn (7) (Table 2).

The impact of temperature change on population dynamics and carbon flux

For the flagellate–ciliate simulations without top-down control by Daphnia, there was one prey and one predator peak over almost the entire range of temperatures examined (Fig. 3). An example, at 20 °C, illustrates the change in biomass of prey and predator, over time (Fig. 3a). A synthesis of the entire set of simulations is presented as predator and prey density plots, to indicate population dynamics over time, across the examined temperature range (Fig. 3b and c). Simulations indicated that the prey populations increased over several days, always peaked before that of the predator and then rapidly decreased, as they were grazed by the growing predator population. The predator population increased rapidly, once prey were abundant, and then decreased more gradually than that of the prey, as the former died due to starvation; this decrease became more rapid at higher temperatures, reflecting higher mortality rates at low prey concentrations and higher temperatures (Fig. 2b). Finally, at high and low temperatures, pulses were not pronounced and occurred near the end of the 20-day simulation, while at 20 °C the pulses reached the highest numbers (cf. Fig. 3a) and occurred early, between days 4 and 7 (Fig. 3a-c).

The biomass produced by the prey and predator (ciliate) (Fig. 3d and e) equates to the sequestered carbon from atmospheric CO₂, through primary production of the prey and its transfer to the consumer, the predator. Production of both predator and prey was greatest when simulations were initiated with few predators and prey (Fig. 4); in these cases reduced grazing pressure initially allowed the prey population to rise, but then there was a subsequent bloom of the ciliates, resulting in high production of both (population cycle, data not shown). In all cases, carbon production peaked at or near 20 °C and rapidly decreased above and below this maximum (Figs 3 and 4); this consistency of the distribution of this measurement of production illustrate the robustness of the model output over a range of prey and ciliate levels (Fig. 4). Differences between minimum and maximum production were on the order of 5–10-fold, for both prey and predator (Fig. 3d and e).

![Table 1](https://example.com/table1.png)

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<td>( h )</td>
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<td>( \mu^3 )</td>
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When Daphnia were introduced to the system, there is a clear indication of its grazing impact, but ciliate–phytoplankton pulses still occur over a limited range of temperatures (Fig. 5a–c), following patterns described above, indicating that the direct microbial link can remain active, even when a higher level predator exists, at typical levels. Maximum flagellate and ciliate production (at $C_2^201^1C$) over the 20-day period was altered compared with when Daphnia was absent, but not to a great extent; however, there was a pronounced effects of temperature on production at the upper and lower limits of the range (cf Figs 3b, c and 5b, c). The amount of carbon transferred to Daphnia, by grazing, was also influenced by temperature (Fig. 5g and h), indicating that temperature change could have larger scale impacts on more complex food web dynamics.

**Discussion**

**Temperature response functions**

There is a continuing need to parameterize pelagic ecosystem models (Anderson, 2005), especially components of the microbial food web, which can be pivotal in terms of freshwater pelagic carbon flux (Weisse et al., 1990; Straile, 1998; Zingel et al., 2007). Food levels, grazing pressures, and growth rates have typically been considered to be the primary influencing factors in planktonic ecosystem dynamics, and the last 20 years have seen a focus on establishing functional and numerical responses for protozooplankton species (e.g. Jonsson, 1986; Montagnes, 1996; Jürgens & Šimek, 2000; Gismervik, 2005), using methods and approaches similar to ours. However, with the recognition that

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<td>$x$</td>
<td>$4.89 \times 10^{-6}$</td>
<td>$C_0^{-2}$</td>
<td>$1.01 \times 10^{-6}$</td>
<td></td>
</tr>
</tbody>
</table>

When Daphnia were introduced to the system, there is a clear indication of its grazing impact, but ciliate–phytoplankton pulses still occur over a limited range of temperatures (Fig. 5a–c), following patterns described above, indicating that the direct microbial link can remain active, even when a higher level predator exists, at typical levels. Maximum flagellate and ciliate production (at $\sim 20^C$) over the 20-day period was altered compared with when Daphnia was absent, but not to a great extent; however, there was a pronounced effects of temperature on production at the upper and lower limits of the range (cf Figs 3b, c and 5b, c). The amount of carbon transferred to Daphnia, by grazing, was also influenced by temperature (Fig. 5g and h), indicating that temperature change could have larger scale impacts on more complex food web dynamics.
climate change will both increase ambient temperature and change the intensity and frequency of warming events, there has been a commensurate effort to consider temperature responses in models of protozoan feeding and growth. Generally, the approach has been to impose a thermal function on existing protozoan rate processes (e.g. a simple or modified $Q_{10}$ function; Blackford et al., 2004), but this simplistic approach is likely inappropriate (e.g. Montagnes et al., 2003; Kimmance et al., 2006). The present study provides, to our knowledge, the first indication of how more complex, empirically derived temperature relationships can alter short-term carbon flux, influenced by predator–prey dynamics, within the freshwater microbial food web.

Our responses [Eqns (4)–(7), Tables 1 and 2] are based on a combination of mechanistic and phenomenological equations. There is good indication that functional [Eqn (1)] and numerical [Eqn (2)] responses are at least semimechanistic (Holling, 1959; Fenchel, 1986). In contrast, the quadratic function [Eqn (4)] employed by Flinn (1991) is a phenomenological model. We have compared this model to a number of mechanistic and phenomenological models using information theory (Burnham & Anderson, 2002), and it has proven consistently to be one of the best to describe thermal responses (Appendix A). We recognize that more complex, mechanistic functions may explain temperature responses (e.g. Schoolfield et al., 1981), but to provide simple, predictive functions for modelling, we have followed the approach of others (e.g. Flinn & Hagstrum, 2002; Kimmance et al., 2006) to combine mechanistic and phenomenological functions, to yield parsimonious equations.
Our main alteration to the model of Flinn & Hagstrum (2002) was to impose a temperature dependency on the threshold concentration \( P_0 \); Eqn (6); \( P_0 \) represents the prey concentration where sufficient food is available to allow the predator population to survive but not increase or decrease (Montagnes, 1996). The value of this parameter typically increases with temperature (Weisse et al., 2002 and references within; Kimmance et al., 2006), presumably as increased temperature raises metabolic needs. Including this modification provided a better fit to the data than responses that lacked the interaction (data not shown). We suggest that our response equations [Eqns (5)–(7)], if not their specific parameters (Tables 1 and 2), are a good method of assessing the protozoan contribution to carbon flux in models that examine thermal impacts; we illustrate this below.

**How might temperature alter population dynamics and carbon flux?**

Using a specific example, we indicate that temperature changes of 2–10°C can impact protistan population dynamics and carbon flux (Figs 3–5). Assuming that ambient temperatures in temperate freshwaters is generally <20°C, the model suggests that a rise towards 20°C would increase the magnitude and occurrence of predator–prey pulses. Such increases in temperature on the order of 5°C are not uncommon in small ~3000 L, temperate water bodies (see Mckee et al., 2000), but as indicated above, climate change is predicted to increase the prevalence and the severity of these fluctuations (Carpenter et al., 1992). The ensuing increase in pulses of ciliates and prey may then alter food web dynamics on a larger scale. However, because of their brief nature and likely local occurrence, it is, and will be, difficult to observe short-term pulses in routine sampling programs, and this may be why they are rarely carefully considered.

Our model output is, however, comparable to *in vitro* incubations: using the same taxa as we have examined, Weisse et al. (2001) presented time series data for predator–prey abundances, at 15 and 20°C, over ~20 days; their data exhibit distinct predator–prey pulses over 10–15 days, which occurred earlier at the warmer temperature, supporting our arguments for the importance of this short-term phenomenon and the influence of temperature on the dynamics. A second approach to assess if predator–prey pulses occur is to regularly monitor communities in microcosms (i.e. large tanks populated with seminatural assemblages, simulating *in situ* conditions; e.g. McKee et al., 2000). Although there have been several such studies to examine the impact of global warming on freshwater pelagic

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**Fig. 4** The influence of initial ciliate and prey abundance on carbon production over a 20-day simulation, at temperatures ranging from 8 to 30°C. (a–c) *Urotricha farcta* production; (d–f) *Cryptomonas* sp. production. Parts (a, d) were initiated with 1 *U. farcta* mL⁻¹; parts (b, d) were initiated with 10 *U. farcta* mL⁻¹; parts (c, f) were initiated with 100 *U. farcta* mL⁻¹. Values denoted by shading in bottom panels apply to all above panels and are in units of µg C mL⁻¹ 20 day⁻¹.

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ecosystems (tanks maintained 3–5 °C above ambient levels; e.g. McKee et al., 2002; Strecker et al., 2004; Christoffersen et al., 2006), to our knowledge only one has focused on protozooplankton, and specifically ciliates (Montagnes et al., 2002; Wilson, 2002); in this study, there was an increased occurrence of ciliate species blooming when temperatures were raised 3 °C above ambient levels, and fish were included to remove crustacean grazers. Similarly, but less apparent, blooms of *Urotricha* spp. and other small ciliates occur in field observations (e.g. in Lake Constance in the summer when crustaceans, such as *Daphnia*, are not abundant; Müller et al., 1991). These laboratory, microcosm, and field data suggest that our modelled predator–prey pulses, although difficult to observe, may be common in nature and suggest that warming may increase such pulses, increasing carbon flux through populations within the microbial food web. However, it is also instructive to note that we predict, in our specific case, an increase in temperature (≥20 °C) will reduce the magnitude and period of the predator–prey dynamics and carbon flux.

**Fig. 5** Results from the model of predator (*Urotricha farcta*) and prey (*Cryptomonas* sp.) population dynamics over 20 days with the addition of *Daphnia* as a grazer (see ‘Materials and methods’ for details of the model). Predator and prey are presented as μg C mL⁻¹. (a) An indication of the population dynamics over 20 days at one discrete temperature: 20 °C; prey (solid line), predator (dashed line). (b, c) Density plots of prey and predator population dynamics, respectively, at temperatures ranging from 8 to 30 °C, over 20 days. (d, e) An indication of the carbon produced by the prey and predator, respectively, over the 20 days; note this represents the total amount made, regardless of its fate. (f) An illustration of the response of *Daphnia* filtration rate to changing temperature (see ‘Materials and methods’ for details of this response). (g, h) An indication of the prey and predator carbon, respectively, consumed by the upper level grazer, *Daphnia*, over the 20 days; note this represents the total amount grazed, regardless of its fate.
Rarely, however, do simple predator–prey models, such as ours, accurately predict in situ or in vitro population dynamics, and this is not necessarily their intention, rather it is to explore and test hypotheses (see Turchin, 2003). A combination of uncharacterized stochasticity, under-parameterization of responses, and omission of deterministic variables can be attributed to the cause of the mismatch (Turchin, 2003). However, it is well accepted that although such models are rarely explicitly predictive, they reveal qualitative effects (e.g. Shertzer et al., 2002; Fulton et al., 2003). Thus, we propose that our fundamental premise that small, episodic, temperature changes, induced by climate change, will upset food web dynamics in an unexpected manner is well supported by our model results, and our analysis of production (Fig. 4) reveals that our predictions are robustly supported over a range of predator and prey levels. Furthermore, the analysis suggests that our responses [Eqns (5)–(7)] should provide researchers with data to further parameterize temperature effects on ciliates in existing pelagic ecosystem models. Possibly of greater impact though is our illustration that predators and prey will often have different thermal sensitivities, and these produce unintuitive, nonlinear results (May, 1986). Recently, it has been argued that planktonic autotrophs are less sensitive to temperature than planktonic heterotrophs (Rose & Caron, 2007), but our data suggest that the change in dynamics above and below ~20 °C are at least in part due to the rapid positive and negative autotrophic-prey response near 20 °C (Fig. 1), suggesting that in this case the thermal sensitivity of Cryptomonas sp. is driving the strong patterns in the system. These findings emphasize the more general recognition that different temperature dependencies of autotrophy and heterotrophy may significantly decouple existing plankton dynamics on local and global scales (e.g. López-Urrutia et al., 2006).

Application, integration, and extension of this work

There are some caveats to our conclusions that are worth noting. First, our temperature responses (Fig. 2) are for genera that are ubiquitous in temperate freshwaters, but U. farcta strains can have different thermal sensitivities, as do different species within the genus Urotricha (Weisse & Montagnes, 1998). This is not surprising, as regional adaptations of taxa to a variety of environmental variables, including temperature should be expected (e.g. Mitchell & Lampert, 2000) and was recently demonstrated for freshwater ciliates (Gächter & Weisse, 2006). Still, meta-analyses regarding protistan growth and volume responses to temperature (e.g. Atkinson et al., 2003; Montagnes et al., 2003; Bissinger et al., 2008) suggest predictable similarities across taxa, and aggregation of species into functional groups is advocated for large-scale ecosystem models (Fulton et al., 2003). Thus, although the specifics of our model output might not be repeated, the trends of population pulses and carbon flow might be similar, regardless of the taxa; as a case in point, our own study on a marine, heterotrophic flagellate supports our arguments for the temperature sensitivity of population dynamics (Kimmance et al., 2006).

Second, like most ecophysiological responses, our discrete measurements (i.e. points on Figs 1 and 2) were determined under constant temperatures. In shallow freshwater environments, ambient temperature will not be constant, possibly fluctuating hourly or daily by several degrees, and fluctuating ambient temperatures (on a daily regime) can alter Urotricha growth rates (both up or down) compared with constant temperatures (Montagnes & Weisse, 2000); such transient behaviour requires further parameterization before it is incorporated into models. These concerns, however, do not invalidate our parsimonious approach towards examining trends (which reflects that of most ecosystem and many population models) or the potential applicability of the responses provided, but they do indicate that a further degree of sensitivity analysis, related to the temperature optima of taxa and their response to fluctuating temperatures, would be appropriate in more complex modelling efforts.

Given the limitations of the study, how then does the model predict general patterns of carbon uptake and sequestration? At 20 °C, the phytoplankton prey produce ~7 µg C mL⁻¹ over 20 days (Fig. 3). However, some of this production is transferred to the ciliate, little of it remains phytoplankton biomass, and by the end of the 20 days, in virtually all cases, the ciliate standing stock biomass is negligible (i.e. almost all ciliates die), indicating that this biomass is not sequestered; similar responses occur when the upper-level grazer, Daphnia, is added. Likely, this small protozoan, which has no hard parts, if not grazed, would die, rupture, and become part of the dissolved organic carbon pool; the biomass would then cycle through the microbial loop (Azam et al., 1983; Montagnes, 1996). Consequently, little to none of the carbon would be sequestered on a seasonal or longer scale (e.g. in sediment), as might be the case when crustacean predators are present (see Flanagan et al., 2006). Recognizing, and incorporating, this potential source of recycling will undoubtedly alter estimates of carbon sequestering in freshwater models; that is, the impact of short-term protozoa–phytoplankton pulses could potentially substantially reduce estimates of freshwater carbon sequestration.

The first scenario we have modelled (Fig. 3) may occur in freshwaters, when upper level zooplankton consumers are not abundant (e.g. when Daphnia are
consumed by fish; McKee et al., 2002). However, when the keystone grazer Daphnia is present, it effectively consumes ciliates and their prey (Jürgens, 1994). The model also reveals a clear impact of Daphnia grazing on the system, but pronounced ciliate–phytoplankton pulses still occur over a range of temperatures (Fig. 5b and c), suggesting that the direct microbial link may remain active. Support for this conclusion was recently provided by a long-term data analysis from the spring to the clear-water phase development in Lake Constance (Tirok & Gaedke, 2006): although ciliates remained the most important algal grazers in spring, Daphnia benefited from increased temperature by 1–2°C, substantially enhancing their grazing on small, prey such as Cryptomonas spp.

In our model, production over the 20-day period is also altered when Daphnia are added, but there is a more pronounced effect of temperature on the change in production (cf Figs 3b, c and 5b, c). The amount of carbon produced that is transferred to in production (cf Figs 3b, c and 5b, c). The amount of temperature could have larger scale impacts on food web dynamics (Orcutt & Porter, 1984). We do, however, emphasize that our inclusion of Daphnia is based on realistic but limited information. Both prey level and temperature have nonlinear effects on Daphnia growth and grazing parameters (Kibby, 1971; Orcutt & Porter, 1984; Mourelatos & Lacroix, 1990; McKee, 1995; Giebelhausen & Lampert, 2001), and studies exist on interactive impacts of food and temperature on Daphnia feeding attributes (e.g. Orcutt & Porter, 1984; Giebelhausen & Lampert, 2001). However, none of the published functional responses provide sufficient detail to appropriately parameterize models (i.e. produce functions similar to those illustrated in Fig. 2a–c). These past works have focused on a few discrete temperatures and prey concentrations, replicating to test for treatment effects. We have taken a different approach in this work and others (e.g. Montagnes et al., 2001; Kimmance et al., 2006): to rigorously establish nonlinear responses appropriate for application in models, focus must be placed on spreading measurements along the independent variable(s), rather than replicating discrete treatments (Montagnes & Berges, 2004); this approach provides key information on the shape of responses. Given the importance of Daphnia in freshwater food webs, we propose that future work focuses on carefully parameterizing such temperature–prey responses.

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References


Appendix A

Determining the most likely model to describe the growth rate of Cryptomonas sp. as a function of temperature: an information-theoretic approach

Seven candidate models were identified that might describe the response of Cryptomonas sp. growth rate to temperature (Fig. A1, Table A1). From these the most likely was selected using an information-theoretic approach (Burnham & Anderson, 2002). The major advantage of this approach, as compared with simple measures of fit, such as $R^2$, is that it accounts for the model’s complexity, that is the number of parameters (Angilletta, 2006). The first step in this procedure is to calculate the Akaike information criterion (AIC) for each candidate model. This provides an estimate of the relative distance between the fitted model and the unknown true mechanism; the most likely model is that with the lowest AIC, relative to those in the set of models (Burnham & Anderson, 2002).
In this study, the candidate models (Table A1) were the four best performing thermal responses assessed by Angilletta (2006) (i.e. Gaussian, quadratic, modified Gaussian, Weibull) and three models that describe thermal rate responses of ectotherms (see Flinn, 1991; Hinshelwood, 1947; Schoolfield et al., 1981). Of these models, only those presented by Schoolfield et al. (1981) and Hinshelwood (1947) have a mechanistic basis; the others are phenomenological.

Models were iteratively fit to response data using the Marquardt–Levenberg least squares algorithm (SIGMA-Plot), and parameters were determined. Then, using the residual sum of squares (RSS) from each fit, the maximized log-likelihood value \( L \) of the model [Eqn (A.1)] was determined (Burnham & Anderson, 2002); this was used to calculate the \( \text{AIC}_c \) (AIC corrected for small sample size) for each model [Eqn (A.2)]:

\[
L = \log \left( \frac{\text{RSS}}{N} \right) - \frac{N}{2} \quad \text{(A.1)}
\]

\[
\text{AIC}_c = -2L + 2K + \frac{2K(K + 1)}{N - K - 1} \quad \text{(A.2)}
\]

where \( N \) is the sample size and \( K \) is the number of parameters (including the error term). The Akaike weights [Eqn (A.4)] were calculated from the likelihood of each model [Eqn (A.3)], as a proportion of the total

\[
\omega_i = \frac{\exp \left( \frac{L_i - \max L}{2} \right)}{\sum \exp \left( \frac{L_i - \max L}{2} \right)}
\quad \text{(A.3)}
\]

\[
\text{AIC}_c, \text{ Akaike information criterion, } K \text{ is the number of parameters in the model (including the error term), } \Delta_i \text{ is the difference between a given model’s } \text{AIC}_c \text{ and that of the lowest } \text{AIC}_c, \text{ and } \omega_i \text{ is the Akaike weight that is the normalized likelihood that the model is the best one in the set. In the Schoolfield et al. (1981) model, } a \text{ is the growth rate at temperature } T (K), b \text{ is the enthalpy of the activation of the reaction that is catalyzed by the limiting enzyme, } c \text{ is the change in enthalpy associated with high-temperature inactivation of the limiting enzyme, } d \text{ is the temperature (K) at which the enzyme is half active, and } R \text{ is the universal gas constant (i.e. the Boltzmann constant, } 8.617343 \times 10^{-5} \text{ when expressed in eV). In the Hinshelwood (1947) model, } a \text{ and } b \text{ are the pre-exponential factors, } c \text{ and } d \text{ are activation energies, and } R \text{ is the universal gas constant (i.e. the Boltzmann constant).}

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likelihoods of all the candidate models.

\[
\ell(g_i|x) \propto e^{-\frac{1}{2} \Delta_i} \quad (A.3)
\]

\[
\omega_i = \frac{e^{-\frac{1}{2} \Delta_i}}{\sum_{r=1}^{R} e^{-\frac{1}{2} \Delta_r}} \quad (A.4)
\]

where \(\ell(g_i|x)\) is the likelihood of the model \(g_i\), given the data \(x\); \(\Delta_i\) is the difference between a given model’s AICc and that of the lowest AICc; and \(R\) is the set of candidate models.

Thermal response curves typically exhibit a left-sided skewness that plateaus at the optimal temperature, and then declines (e.g. as described in Montagnes et al., 2003). Furthermore, the skewed portion of the response generally follows an exponential relationship (e.g. Eppley, 1972; Bissinger et al., 2008). From the candidate models fitted to the Cryptomonas sp. data (Table A1), the quadratic model was unable to describe this initial exponential response (Fig. A1), and was therefore rejected. Of the remaining functions, the phenomenological model of Flinn (1991) had both the lowest AICc and highest Akaike weight. Thus, it was the most likely function of this set of models and was used to provide a predictive response in our modelling work.

A further assessment of this type of curve fitting on 39 phytoplankton thermal response datasets revealed that the Gaussian and quadratic functions were the most likely models (Fig. A2), with the Flinn (1991) function the third best performing model. However, as noted above, the quadratic function does not describe the exponential portion of the response; so for consistency we have used the Flinn (1991) function (Table A1) to structure all our temperature responses in this work on modelling the effects of temperature on protistan population dynamics.

Appendix references


