



Effects of Temperature on the Bioenergetics of the Marine Protozoans *Gyrodinium dominans* and *Oxyrrhis marina*

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We exposed *Gyrodinium dominans* and two strains of *Oxyrrhis marina* to temperatures well above (25°C) and below (12°C) their maintenance temperature (18°C) to study the mechanisms controlling the overall physiological response to thermal stress. As variables, we measured growth, ingestion, and respiration rates (this latter with and without food). The growth rates of *O. marina* strains plotted as a function of temperature showed maximum values at the maintenance temperature, as expected in a typical unimodal functional response. However, *G. dominans* showed similar growth rates at 12 and 18°C, and even a marked decrease in growth rates at 25°C, happened to be not significant. *G. dominans* ingestion rates were not significantly different at all temperatures (although apparently decreased at 25°C), whereas both *O. marina* strains showed higher ingestion rates at 18°C. The respiration rates of *G. dominans* were unaffected by temperature, but those of *O. marina* strains increased with temperature. The specific dynamic action (respiration increase produced by feeding activity) ranged from 2 to 20% of the daily carbon ingestion for all organisms investigated. The calculated energetic budget indicated that the responses to temperature were diverse, even between in strains of the same species. *G. dominans* maintained similar growth at all temperatures by balancing metabolic gains and losses. In *O. marina* strains, on the other hand, the decrease in growth rates at the lowest temperature was driven mainly by reduced ingestion rates. However, increased respiration seemed the primary factor affecting the decrease in growth rates at the highest temperature. These results are discussed in the light of previous studies and on its suitability to understand the response of wild organisms to fluctuations in temperature.

Keywords: growth, grazing, respiration, specific dynamic action, energetic budget, temperature, *Gyrodinium dominans*, *Oxyrrhis marina*

INTRODUCTION

Temperature is a key variable affecting most physiological rates of all organisms (Huey and Kingsolver, 1989; Alcaraz et al., 2014; Sinclair et al., 2016). Thus, climate-driven distribution shifts of microplankton, copepod species, and fish have been related to their different thermal sensitivities (Helaouët and Beaugrand, 2007; Takasuka et al., 2008; Wilson et al., 2015). Usually, the changes in

survival and physiological rates of an organism due to temperature are represented by a unimodal function (Huey and Kingsolver, 1989; Schulte et al., 2011; Alcaraz et al., 2014; Sinclair et al., 2016), commonly shaped by a progressive increase up to a peak (optimum temperature) from where the decline is more or less abrupt (Van Der Have, 2002; Angilletta, 2006; Sinclair et al., 2016). This graphical representation of the changes in an organism physiology with temperature is the result of the coupling/uncoupling among the different thermal sensitivities of key ecophysiological activities (e.g., feeding, respiration, assimilation, excretion, molting, growth; Alcaraz et al., 2014; Saiz et al., 2022). Thus, understanding how the uncoupling between physiological rates ultimately translates into growth and survival is crucial in predicting organisms' responses to temperature (Pörtner, 2002; Pörtner and Farrell, 2008; Alcaraz et al., 2014).

For marine protozoans, while the effects of temperature on vital rates are well studied (Hansen et al., 1997; Montagnes et al., 2003; Rose and Caron, 2007), the subjacent processes behind this response are poorly investigated (Kimmance et al., 2006). Marine protozoans, also referred to as microzooplankton, are key components of the marine pelagic food webs because of their role as the main link between primary producers (daily consuming ca. 75% of the oceans' overall phytoplankton primary production) and higher trophic levels (Calbet, 2001; Calbet and Saiz, 2005; Schmoker et al., 2013), and because their relevance as remineralizers of inorganic nutrients, supporting regenerated primary production (Ikeda et al., 1982; Alcaraz et al., 1994; Alcaraz et al., 1998). Because of these functions in the marine ecosystem, and considering the threat of global change, it is crucial to gather a deeper understanding of how temperature affects the different physiological rates. Only with a better knowledge of these processes we will be able to conduct accurate predictions about the performance, fate, and role in the ecosystem of the different species that assemble marine microzooplankton. In this respect, it will be under thermal stress where the different process rates, with different Arrhenius breakpoints (Alcaraz et al., 2014), may become uncoupled, resulting in overall detrimental effects on growth rates. We can hypothesize that the mechanisms acting at each side of the equilibrium temperature, at which species are adapted, may be different. Given the exponential nature of the relationship between temperature and respiration activity (Q_{10} concept; e.g., Caron et al., 1986; López-Urrutia et al., 2006) and the physiological upper bounds for ingestion (Aelion and Chisholm, 1985; Kimmance et al., 2006), we can hypothesize that respiration rates will mostly drive the resulting growth rates at high temperatures. At lower temperatures, on the other hand, both respiration and ingestion rates find zero at their lower bound, and it will be the different Q_{10} of both rates the ones depicting the overall growth response.

To demonstrate this hypothesis, we designed a series of experiments that simultaneously compared the physiological responses of three marine heterotrophic protozoans (*Gyrodinium dominans*, and two strains of *Oxyrrhis marina* originated from different locations) at their maintenance

temperature (18°C) with those at 12 and 25°C. We chose these temperatures to be apart enough to theoretically show different physiological responses (Calbet and Saiz, 2022), being within reasonable limits of the temperature fluctuation likely experienced by the species in nature (Estrada et al., 1993; Calbet et al., 2015; Hobday et al., 2016; Oliver et al., 2018).

METHODS

The Cultures

As experimental organisms we used one strain of *Gyrodinium dominans* isolated by H.H. Jakobsen in 1996 from the northern part of Øresund (Denmark; Hansen and Daugbjerg, 2004), one strain of *Oxyrrhis marina* MED isolated by A. Calbet off Barcelona coast (NW Mediterranean) in 1996, and one strain *O. marina* CRB (purchased from the SCCAP Denmark; code SCCAP-0592) that originates from the Great Bay Salt Pond in St Maarten (Dutch Caribbean). All were kept in autoclaved 0.2- μ m filtered seawater with added trace metals and EDTA (0.003 mL off/2 metal stock solution per liter of suspension, Guillard 1975), at 18°C and 38 salinity, under dim light and fed with *Rhodomonas salina*. The strains that originated from places with different temperatures and salinity from our laboratory standard conditions (Mediterranean waters) had been gradually adjusted for months. The prey, *R. salina* strain K-0294 (RHO; range ESD: 6.3-6.5 μ m), was obtained from the Scandinavian Culture Collection of Algae and Protozoa at the University of Copenhagen, and it has been growing in our laboratory for years. The prey were kept in f/2 medium (Guillard, 1975), under exponential growth conditions, at 18°C, 38 salinity, 100-200 μ E $m^{-2} s^{-1}$, and a light:dark cycle of 12h.

The Experimental Setup

We concurrently estimated the protozoan growth, ingestion, and respiration rates from the same incubation bottles incubated at 12, 18, and 25°C, being 18°C the maintenance temperature for all the cultures. Prior to the experiments, the grazers were kept in 2-4 L Pyrex glass bottles fed ad libitum with *R. salina* at their maintenance temperature (18°C). We calculated the amount of prey offered on the last day of feeding to be mostly depleted after 24h. Then, we gently concentrated the dinoflagellates by gravity filtration through a 3- μ m polycarbonate filter to remove the excess of bacteria and detritus, re-suspended them in 0.2- μ m filtered seawater, and pre-conditioned them (without food) for 24 hours to the experimental temperature (12, 18 and 25°C). All incubations were conducted in quadruplicate 130 mL Pyrex glass bottles, in darkness, at 16, 18, and 25°C (in temperature-controlled incubator baths), and lasted for 24 hours. Dinoflagellate density in the incubations was ca. 3000-4000 ind mL^{-1} . In the treatments with food, *R. salina* was added at a concentration of ca. 100000 cells mL^{-1} to ensure saturated food conditions (Calbet et al., 2013). In parallel, we set suspensions only with the prey at the same concentration as the previous one (grazer + prey) to serve as controls for the

growth of the algae. We added 10 mL of f/2 per liter of suspension (i.e., a final nutrient concentration equivalent to f/200) to override microzooplankton excretion effects on the algae. The concentrations and cellular volumes of prey and grazer were estimated with a Multisizer III particle counter. To calculate grazing rates and average prey concentrations, we used Frost's equations (Frost, 1972), and *per capita* values were calculated using the average concentration of grazers in each replicate (Heinbokel, 1978). The corresponding volume to carbon (C) equivalence was obtained from Calbet et al. (2013), which provides elemental content/volume for the same strains in similar degree of starvation as in our treatments (Table 5 of Calbet et al., 2013).

Oxygen concentration was monitored using optical oxygen sensors (Oxygen Dipping Probe DP-PSt3, Presens®), at a measuring frequency of 15 min, with a setup similar to the one described in Almeda et al. (2011). The respiration activity of the three dinoflagellates was measured in the presence and in the absence of food. The previously described control bottles with only *R. salina* were used to account for changes in oxygen concentration due to the presence of prey alone, whereas an additional set of bottles filled with only 0.2 μm filtered seawater served as background control. In the case of both the prey and the grazers incubated alone in filtered seawater, oxygen consumption rates (μmol O₂ d⁻¹) were estimated as the slopes of the linear regression equations relating incubation time and dissolved oxygen concentration, after correcting for any changes in dissolved O₂ observed in the extra bottles with only filtered seawater. Cell-specific respiration rates (μmol O₂ cell⁻¹ d⁻¹) were then calculated by dividing the experimental oxygen consumption rates per bottle by the average cell concentration during the incubation.

In the case of the grazers incubated with prey, the oxygen consumption observed in the bottles resulted from the combined respiration of the grazers (heterotrophic dinoflagellates) and the prey. For this reason, before calculating the actual per capita consumption by the feeding dinoflagellates, we had to subtract the decline in oxygen concentration (incubations were in darkness) due to the presence of *R. salina*. To do so, we first determined the *R. salina* growth rates based on the initial and final cell concentrations using an exponential growth model; then, we estimated the average cell concentrations at each oxygen-measurement time interval. Next, we applied the cell-specific *R. salina* respiration rates from the incubations in filtered seawater to the actual *R. salina* concentration in the grazing bottles, estimated at each measuring time interval, and computed the expected oxygen concentration decrease in the incubations with feeding grazers due to the presence of *R. salina*. We then subtracted the oxygen consumption by *R. salina* from the time series of oxygen concentrations in the incubation bottles with the mixture of predator and prey, and estimated the oxygen respiration rates of the feeding dinoflagellates from the slope of changes in the remaining dissolved oxygen through time. The per capita respiration rate of the dinoflagellates under the presence of food was calculated taking into account the mean number of dinoflagellates incubated assuming exponential growth.

To calculate Q₁₀ coefficients, we first conducted a simple linear regression analysis between the inverse of temperature (1/T, in Kelvin degrees) and the natural logarithm of the carbon-specific physiological rate (i.e., the Arrhenius plot). Then, the activation energy was calculated from the slopes of the linear fit as $E_a = -\text{slope} \times R$, where R is the universal gas constant (8.3145 J mol⁻¹ K⁻¹). Finally, Q₁₀ coefficients were calculated from E_a as $Q_{10} = \exp(E_a/R \times 10/T_m^2)$, where T_m is the mean for the range of temperatures over which the organisms have been exposed (i.e., between 12 and 25°C) (Raven and Geider, 1988).

The oxygen consumption rates were converted into C losses using a respiratory quotient of 0.97 (Omori and Ikeda, 1984). Gross-growth efficiency (GGE) was calculated as in Kjørboe et al. (1985), dividing the growth rates by the ingestion rates in specific C units and expressed as percentage. Assimilation efficiency (AE) was calculated as $AE = 100 \times (\mu_C + R_C)/I_C$ (where μ_C and I_C are the C-specific growth and ingestion rates, respectively, and R_C is the respiratory carbon losses also expressed as C specific; Kjørboe et al., 1985). Specific dynamic action (SDA; the increase of respiration rates produced in the presence of food) was calculated as the difference between C-specific respiratory losses between feeding and non-feeding grazers, and expressed as % of C ingested daily.

RESULTS

Growth and Ingestion Rates, and Gross-Growth Efficiency

The growth rates of *G. dominans* were not significantly different at any of the temperatures tested (ANOVA and Tukey's test), although the rates dropped to less than half at 25°C (**Figure 1A**). *O. marina* MED showed growth rates higher at 18°C, and reduced at 12°C (albeit not significantly different from 18°C, Tukey's test) and 25°C (p<0.01, **Figure 1A**). In the case of *O. marina* CRB, 12°C drastically decreased the growth rates to negative values (mortality); on the other hand, at 18°C the growth rates were reasonably high (more than one doubling per day), while they dropped significantly at 25°C (ANOVA, Tukey's test, p<0.01; **Figure 1A**).

All the strains studied showed a similar pattern of ingestion rates, with peaks at 18°C and lower rates at 12 and 25°C (**Figure 1B**). However, only *O. marina* strains showed significant differences in ingestion rates between temperatures (ANOVA, Tukey's tests, p<0.01). Regarding GGE, there was a tendency to be inversely related to temperature for *G. dominans* and *O. marina* MED, but it was not statistically significant due to the high associated error (**Figure 1C**). There were significant differences for *O. marina* CRB, between the GGE at 12°C (zero because of the lack of growth at this temperature) and the rest of the values at higher temperatures (ANOVA, Tukey's tests, p<0.01; **Figure 1C**).

Respiratory Activity

The respiration rates of *G. dominans* were not significantly different at the temperatures investigated, neither for starved

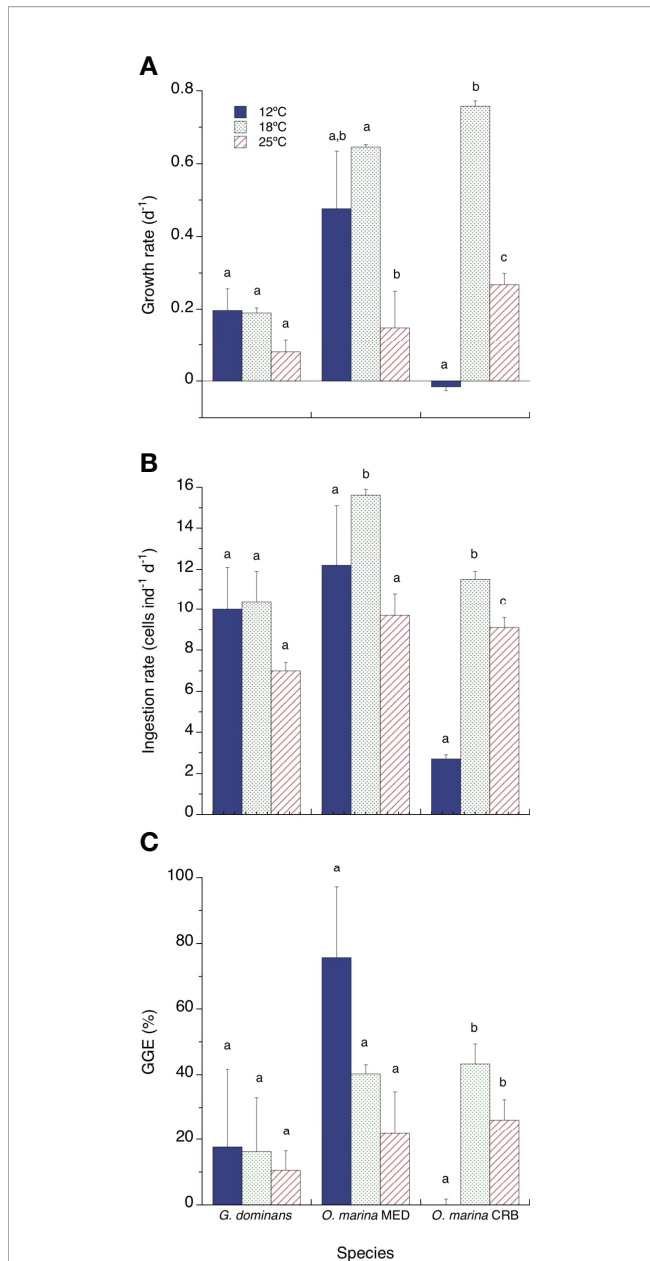


FIGURE 1 | Growth rates (A, d⁻¹), ingestion rates (B, cells ind⁻¹ d⁻¹) and growth gross efficiencies (C, GGE %) of the three protozoans investigated at 12, 18 and 25°C. Statistical differences (p < 0.05) within each species are indicated by different letters. The error bars are SE. For GGE, the SE have been calculated using the square root formula for error propagation.

nor for fed organisms (Figure 2A; Two-way ANOVA, Tukey's tests). However, respiration rates of this species with food were always significantly higher than those without food (Figure 2A; Two-way ANOVA, p < 0.001). *O. marina* MED respiration rates were significantly different between starved and fed organisms, and the rates at 12 and 18°C were different from those at 25°C, both in starved and fed treatments (Two-way ANOVA, Tukey's test, p < 0.001; Figure 2B). The respiration rates for starving and

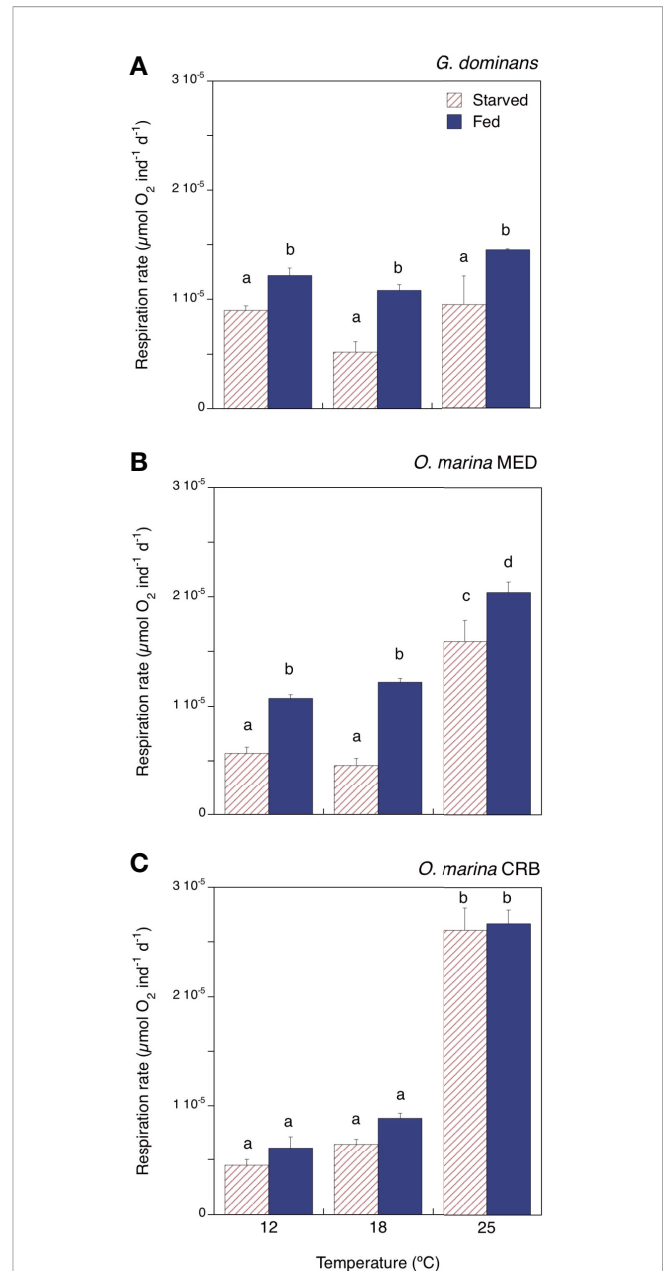


FIGURE 2 | Respiration rates (μmol O₂ ind⁻¹ d⁻¹) of starved and fed (A) *G. dominans*, (B) *O. marina* MED, and (C) *O. marina* CRB at the different temperatures investigated. Statistical differences (p < 0.05) within each species are indicated by different letters. The error bars are SE.

fed *O. marina* CRB were not statistically different (Two-way ANOVA; Figure 2B), and within treatment, the rates were different between 12 and 25°C and between 18 and 25°C (Tukey's test, p < 0.001; Figure 2C).

The respiratory activity of the grazers under starving conditions rendered 8 to 82% of the C biomass respired daily, the highest for *O. marina* CRB at 25°C and the lowest for *O. marina* MED at 12°C (Table 1). The metabolic cost associated with the feeding process (SDA) ranged from 2 to 20% of the C ingested, with the maximum

for *G. dominans* at 25°C, and the minimum for *O. marina* CRB at the same temperature (Table 1). *G. dominans* showed an increase of SDA with temperature, whereas in *O. marina* strains this pattern was not evident. In the case of *O. marina* CRB, we provide SDA values in Table 1, even if the presence of food during the incubations did not significantly enhance respiratory activity. The Q_{10} values of the respiration rates between 12 and 25°C for starved and fed organisms are also provided in Table 1. *G. dominans* was the species showing the lowest Q_{10} coefficients; *O. marina* MED showed intermedium values, with Q_{10} higher for starved organisms than for fed ones (Table 1). Overall, the maximum Q_{10} found in the study corresponded to *O. marina* CRB starved, followed by fed organisms.

Energetic Budget

The allocation of the C ingested per organism and temperature is presented in Table 2. Approximately half of the C ingested (46–69%, depending on temperature) was assimilated by *G. dominans*. From the C biomass incorporated per day, roughly 2/3 were respired at 12 and 18°C (85% at 25°C), and 15–36% was converted into somatic growth at all temperatures (Table 2). *O. marina* MED assimilated nearly all C ingested at the highest and lowest temperatures (approx. 60% at 18°C; Table 2), incorporating an equivalent to about its

biomass per day at 18°C and half of it at the highest and lowest temperatures. Only 25–35% of the C assimilated was respired at 12 and 18°C, and 73% at 25°C. The remaining C was allocated to growth (Table 2). Finally, the combination of ingestion rates and assimilation efficiencies of *O. marina* CRB resulted in daily C incorporations equivalent to, and even slightly more than, its C biomass at 18 and 25°C, but this quantity reduced to 16% of its biomass at 12°C. The low amount of C ingested at 12°C by this protozoan was respired completely, about 1/3 was respired at 18°C, and a about 82% at 25°C. The C used for growth was lower at 25°C compared to 18°C (Table 2).

To stress the relative effects of temperature on the different physiological rates, we show in Figure 3 a graphical summary of the results presented in Figures 1, 2. This figure does not intend to report the effects of temperature on the different rates again, but to provide a snapshot of the magnitude and sign of the effects of low and high temperatures on the different physiological variables measured, compared to those at 18°C. This analysis will be instrumental to understand each variable's weight in explaining the effects on growth rates due to temperature changes. *G. dominans* was the species showing the lowest effects of temperature, these being only evident (although not significant) at 25°C. *O. marina* CRB is the species showing larger variations in the different physiological rates with temperature variations, particularly at 25°C (Figure 3C). In general, ingestion rates are always negatively affected by higher and lower temperatures, compared to the control at 18°C, and respiration only clearly increases at 25°C (Figure 3). Regarding these two last variables, the effect of an increase of temperature on respiration rates is always more acute than that on ingestion rates for *O. marina* strains (Figures 3B, C); at the lowest temperature, the effect seems to be the opposite (Figures 3B, C). Finally, AEs are often increased when the temperature is higher and lower than 18°C.

With knowledge of the different physiological parameters of all species pooled together, we attempted to find relationships between specific ingestion rates, respiration rates, and growth rates (d^{-1}). We only found significant relationship for specific ingestion rates (ING) and growth rates (GR). The linear regression of this variable with growth rates explained 62% of the variance on growth rates ($GR = 0.431 \times ING - 0.126$).

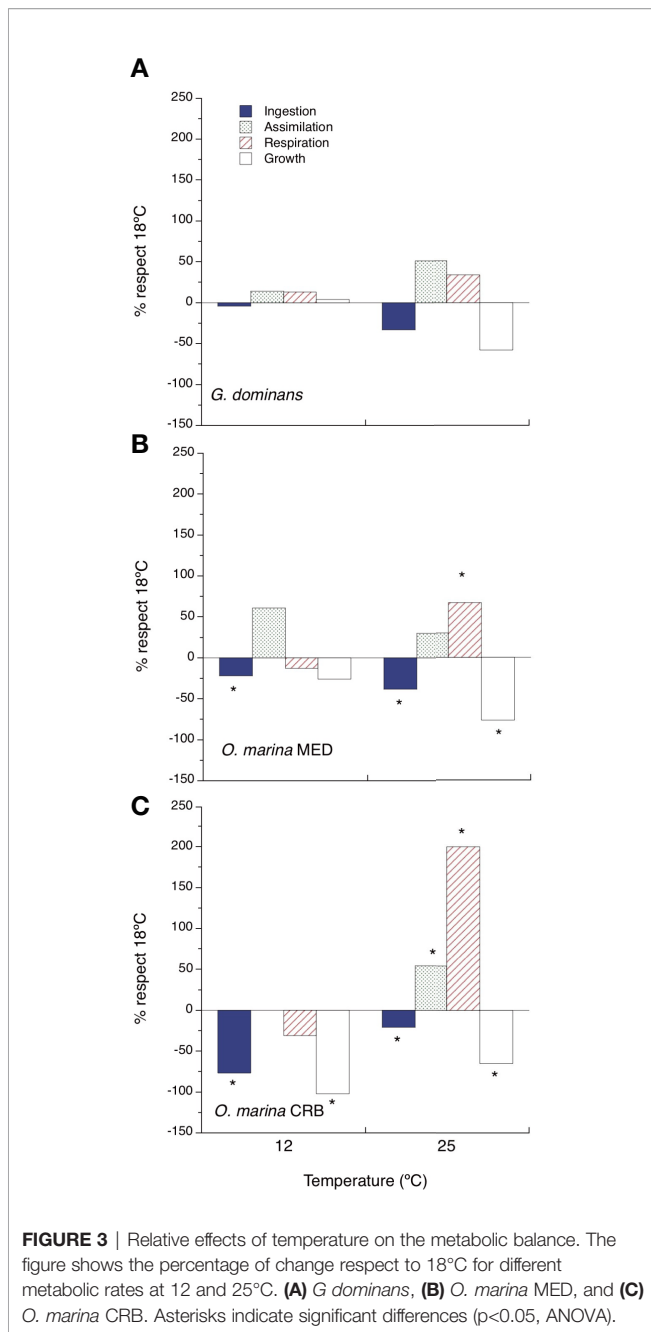
TABLE 1 | Basal respiration of starved organisms as % of the cell carbon, specific dynamic action (SDA) as % of the carbon ingested, and Q_{10} for starved and fed organisms at the three temperatures investigated.

Species	Temperature (°C)	Basal respiration (% cell C)	SDA (% C ing)	Q_{10} starved	Q_{10} fed
<i>G. dominans</i>	12	28.5	9.0	1.1	1.2
	18	16.4	15.2		
	25	29.4	20.1		
<i>O. marina</i> MED	12	8.3	11.5	2.3	1.7
	18	13.2	13.8		
	25	31.3	13.0		
<i>O. marina</i> CRB	12	11.5	17.2	3.9	3.1
	18	27.7	6.0		
	25	82.4	1.7		

TABLE 2 | Metabolic balance of the three protozoans studied at the three selected temperatures.

Species	Temp. (°C)	Ingested (% body C d^{-1})	Assimilation efficiency (% ingested C)	C Assimilated (% body C d^{-1})	Respired (% assimilated C d^{-1})	Growth (% assimilated C d^{-1})
<i>G. dominans</i>	12	112.1	52.0	58.2	66.2	33.8
	18	116.7	45.5	53.1	64.2	35.8
	25	77.3	68.6	53.0	84.8	15.2
<i>O. marina</i> MED	12	63.0	100.0	63.0	24.6	75.4
	18	160.6	62.2	99.9	35.4	64.6
	25	68.2	80.8	55.1	72.9	27.1
<i>O. marina</i> CRB	12	24.1	64.9	15.7	100.0	0.0
	18	175.0	65.1	113.9	33.5	66.5
	25	102.1	100.0	102.1	82.4	26.2

We provide data on specific ingestion rates, assimilation efficiency of the ingested carbon, amount of carbon assimilated, % of carbon respired out of the one assimilated, and % of the carbon assimilated allocated to growth.



DISCUSSION

Unveiling the Processes Behind Growth at Different Temperatures

The main objective of this work was to shed light on the different metabolic processes acting under temperature stress in marine protozoans. To do so, we chose some representative species and exposed them to temperatures above and below that of maintenance. Overall, it seems the direct effects of temperature on ingestion rates are the major driver of growth in the species tested, but a closer look at the effects of temperature on both sides

of the maintenance temperature gives some clues on the actual mechanisms taking place there.

G. dominans was the species less responsive to temperature changes. This protozoan was able to balance the variations in ingestion rates by modifying the assimilation efficiency (AE) and kept the respiration relatively stable despite the variations of temperature, balancing the metabolic gains and losses to maintain roughly similar growth at all the temperatures. For the *O. marina* strains, the adverse effects of both higher and lower temperatures were too strong to be dampened by increasing AE. In these strains, the decrease in growth rates at the lowest temperature was driven mainly by reduced ingesta. However, increased respiration was the primary factor affecting growth rates at the highest temperature. Interestingly, the growth of the Caribbean strain of *O. marina* (CRB) was more impaired by lower temperatures, whereas the Mediterranean one (MED) was more affected by higher temperatures. Also, Calbet et al. (2013) stressed the phenotypic differences between these strains in terms of feeding rates, gross growth efficiency (GGE), and biochemical composition. Our results point towards deeply rooted adaptations at each environment inhabited by the different strains of *O. marina* that were not modified by the long-term conditioning to our laboratory conditions. It can, then, be concluded that diversity among strains, originating from different habitats, can be as significant as species themselves when responding to temperature changes. Several authors also reached a similar conclusion for other variables and found significant differences in behavior, toxic capacity, and biochemical composition among different strains of dinoflagellates (Loret et al., 2002; Adolf et al., 2008; Calbet et al., 2011).

One variable that we did not consider relevant when placing our hypotheses was AE. We had previous knowledge that AE depended on ingestion rates; higher ingestions tended to lower the AE and the other way around (Kiørboe et al., 1985; Straile, 1997; Schmoker et al., 2011). We did not expect this variable to depend on temperature (Rogerson, 1981; Caron et al., 1986). We should keep in mind, however, that if AE is not directly measured but calculated based on ingestion, respiration, and growth rates, and these rates are dependent on temperature, a relationship between AE and temperature could theoretically arise. This was not our case, likely because of the opposite effects of ingestion and respiration on growth.

Specific Dynamic Action (SDA) was another important variable of our study for which we did not find a relationship with temperature, except for *G. dominans*. It was surprising that this energetic expenditure was not related to feeding because, theoretically, it results from feeding activity (Kiørboe et al., 1985). However, given the large number of physiological processes involved in the SDA energetic budget: feeding, digestion, absorption, biomass formation, etc. (Krebs and Allison, 1964; Bayne and Scullard, 1977; Tandler and Beamish, 1979), all of them with its particular response to temperature, it may well be any relationship between SDA and ingestion rates being masked by the effects of temperature. Actually, Kiørboe et al. (1985) suggested for marine copepods that SDA is largely

related to biosynthesis and transport, and that other processes, such as feeding, gut activity, amino acid oxidation, and excretion are of minor importance. SDA, therefore, represents the 'cost of growth' rather than the 'cost of feeding'.

Previous Studies and a Caution Note on the Use of Biomass for Calculations

To our knowledge, there is no previous report including the many physiological rates for several protozoan species at various temperatures shown here. However, there are references to particular data to compare with ours. For instance, Calbet et al. (2013) reported comparable growth, grazing, and respiration rates at 18°C for the same species studied here. Likewise, they found similar AEs and GGEs. On the other hand, on a latter study with the same species adapted to 16, 19, and 22°C, Calbet and Saiz (2022) found slightly higher growth and ingestion rates. Schmoker et al. (2011) calculated the metabolic balance of *G. dominans* at 17°C under different food conditions. The growth, ingestion, and respiration rates found in that study were somehow higher than ours, but their estimated GGEs and AEs are within the range found here. Kimmance et al. (2006) exposed *O. marina* to different concentrations of prey (*Isochrysis galbana*) and temperatures. For well-fed organisms, they observed a bell-shaped response for specific growth rates, although with maximum around 25°C; this maximum shifted towards lower temperatures when food was not so abundant. Overall, their reported specific growth and ingestion rates were slightly higher than ours. Finally, the Q_{10} values we report for respiration are within the average for marine protozoa (Verity, 1985; Caron et al., 1986). We did not find literature on SDA of protozoans, but our data fit well within the limits found for ectotherms (Kjørboe et al., 1985; Secor, 2009).

When comparing our results with previous reports, we should consider the distinct methodologies, strains, and previous history of the organisms used in the different experiments, which may introduce variability and explain some of the differences between studies. Therefore, perfect matches are not expected. Another source of variability between studies is found in the biomass assessment. For such purpose, it is common to use previously published volume-to-C relationships (e.g., Menden-Deuer and Lessard, 2000) or even simply take the average C contents per organism from other studies. As stated in the methods section, our calculations on metabolic budgets are based on volumetric conversion factors obtained for the same strains in our laboratory (Calbet et al., 2013). The selection of the conversion factors will undoubtedly affect the final data. Therefore, it is always advisable to conduct specific elemental content analyses for each particular experiment. We must be aware, however, of another important aspect seldom considered that relates to the C-conversion factors. Protozoans that show a certain degree of body plasticity tend to increase their volume when feeding (Calbet et al., 2013). This augmentation of the cellular volume could be misinterpreted as growth, but in fact it is produced by an accumulation of undigested prey in the cell vacuoles and not by an actual increase in the biomass of the grazer. For growth calculations, then, it is important to avoid using the volume of fed

organisms, and rely only on cell abundance. If conversion to biomass is required, our advice is to use the biomass of the protozoans that have had time to empty the cell vacuoles (Calbet et al., 2013; Calbet and Saiz, 2022).

Concluding Remarks: Are Our Results of Any Ecological or Predictive Use?

We have shown how the response of the different physiological rates to temperature is species- and strain-dependent. Despite so, some overall conclusions can be reached. It seems temperature-mediated effects on ingestion rates are relatively well translated into growth in marine protozooplankton. In this regard, ingestion rates seem to be driving the growth rates at lower-than-equilibrium temperatures. However, respiration rates appeared largely uncoupled at higher temperatures and are likely an important factor responsible for the observed decrease in growth rates.

Notwithstanding the effect we observed of high temperatures on the performance of protozooplankton, it is important to stress that our intention was not to address the effects of climate change on physiological rates of this group of organisms. For such purpose, long-term adaptations and narrower ranges of temperatures are advised (Calbet and Saiz, 2022). We limited our study to a narrow range of temperatures that the organisms could naturally experience. Oscillations of 5-6°C below and above a given temperature can be found in nature. For one side, in temperate and tropical seas, the differences between upper and lower layers of the thermocline can encompass (and surpass) such thermal gradient (Estrada et al., 1993; Calbet et al., 2015); vertical water masses displacements, eddies, and even vertical migration in some species may produce, then, these thermal exposures in short periods. Moreover, temperature rises of 1-5°C and durations of at least five consecutive days are expected during heatwave episodes (Hobday et al., 2016; Oliver et al., 2018). In the Mediterranean Sea, the increases are exceptionally high, 2-4°C, and are expected to increase in amplitude along with the water temperature rise due to climate change (Oliver et al., 2019). Therefore, even if not our original intention, the data presented in this study may help answering particular questions about the future effects of climate change on marine planktonic ecosystems.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: <https://doi.org/10.20350/digitalCSIC/14561>.

AUTHOR CONTRIBUTIONS

AC and RM contributed to the conception and design of the study. RM conducted the experiments. All the authors contributed to the data analysis and interpretation of the results. AC wrote the manuscript and all the authors

contributed to the manuscript revision. All authors contributed to the article and approved the submitted version.

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