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FEATURE ARTICLE

Common temperature–growth dependency and acclimation response in three herbivorous protists

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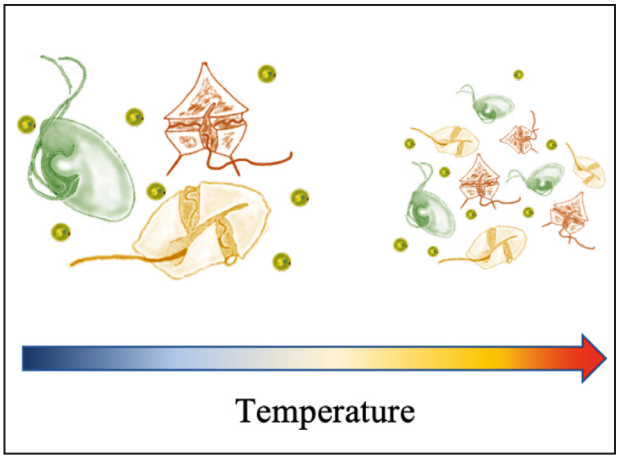
ABSTRACT: Phytoplankton growth dependence on temperature is recognized and has been quantified comprehensively. However, no similar relationship exists for the major phytoplankton predators, the herbivorous protists, especially at low temperatures representing polar and coastal oceans during most seasons. Their acclimation to changing temperatures is also largely unexplored. Here we report acclimated growth and acclimation rates from 0 to 22°C for 3 cosmopolitan herbivorous dinoflagellates. Due to interactive effects between size and temperature, growth increased 40% more rapidly with increasing temperature for production- compared to division-based growth rates (0.043 and 0.062 d^{-1} $°C^{-1}$, respectively). Biomass-based growth rates were 10-fold higher than abundance-based rates at low temperatures, reflecting an average 50% increase in biovolume at ≤2°C. Thus, there was significant biomass accumulation at low temperatures, despite low cell-division rates. Testing different acclimation procedures, we established that acclimated rates emerged after 3 generations. Herbivores required 1.25 d $°C^{-1}$ when acclimating towards higher temperatures and 2.5 d $°C^{-1}$ when transitioning towards lower temperatures. Growth rates increased linearly with temperature, implying a weaker temperature effect on growth than the commonly assumed exponential dependency. A possible consequence is that herbivore growth rates are underestimated at cold and overestimated at warm temperatures. Current and future ocean assessments could thus underestimate trophic transfer rates in polar and cold-water regions and overestimate herbivore growth and thus grazing impact in future ocean predictions. Identifying physiological responses that transcend species-specificity supports cross-biome comparisons of ecosystem structure and function that rely on accurate predictions of matter and energy flow in planktonic food webs.



Temperature-dependent herbivore growth results in increased cell size, and thus biomass, at low temperatures and increased cell division rates at higher temperatures.

Image: Gayantonia Franzè

KEY WORDS: Temperature dependency · Growth rate · Acclimation · Cell size · Protists · Herbivory · Dinoflagellates · Low temperature

1. INTRODUCTION

Herbivorous protists are ubiquitous in marine ecosystems and they have been recognized as key components in planktonic microbial food webs for decades (sensu lato Pomeroy 1974) and, more recently, as the single most important loss factor of phytoplankton production in all seasons and surface ocean habitats (e.g. Calbet & Landry 2004, Modigh & Franzè 2009, Schmoker et al. 2013, Sherr et al. 2013, Morison & Menden-Deuer 2015, Franzè & Lavrentyev 2017, Steinberg & Landry 2017, Lavrentyev et al.

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2019). At the same time, herbivorous protists are a preferred food source for many copepod species (Campbell et al. 2009, Saiz & Calbet 2011) and are critical components of most large-scale, oceanic biogeochemical processes (Menden-Deuer & Kiørboe 2016).

The central role herbivorous protists play in the pelagic food web means that even subtle changes in their abundance, community structure and physiological rates can have large implications for ocean ecosystem functioning (Caron & Hutchins 2013). Yet, the effects of climate change on these organisms at the base of the marine food web are poorly constrained (Falkowski & Oliver 2007). It is essential to quantify the sensitivity of protists to temperature in order to reliably evaluate the effect of climate change on carbon flux, ecosystem productivity and sustainability. Moreover, because temperature is a fundamental driver of biological rates, it directly affects plankton metabolism, and thus organism abundance, and influences interactions among species (Reuman et al. 2014). A recent review revealed that in the past 50 yr, fewer than 30 studies have quantified the growth response of herbivorous protists over a range of temperatures that allow the description of thermal reaction norms (Wang et al. 2019). The scarcity of data on herbivorous protists' growth rate dependence on temperature is particularly pertinent at the lower end of the temperature range, representing much of the highly productive, polar and temperate coastal ocean through much of the year. Understanding temperature sensitivity is critically important to parameterize herbivorous growth dependence in biogeochemical and ecosystem models (Carr et al. 2006, Dunne et al. 2013, Moore et al. 2013) and to gain a predictive understanding of the influence of herbivorous protists on marine primary and export production. Uncertainties and unknown temperature responses of this important group of phytoplankton predators make cross-ecosystem productivity comparisons particularly challenging, as the degree of herbivory is a major determinant in whether primary production is transferred to higher trophic levels or to export (Stock & Dunne 2010).

The scarcity of data on herbivorous protists' temperature sensitivity is remarkable, considering the high degree of diversity among and within single-celled eukaryote species (de Vargas et al. 2015, Worden et al. 2015) and the myriad, often plastic, physiological and behavioral mechanisms that characterize their ecology (e.g. Harvey et al. 2013, Strom et al. 2013, Menden-Deuer & Montalbano 2015). The recognition of this functional diversity has led to the flourishing of ecosystem models that break down larger trophic categories into smaller functional groups (e.g. Yang et al. 2013, D'Alelio et al. 2016, Chen & Laws 2017, Kremer et al. 2017, Michaletz 2018). Thus, taxon-specific thermal sensitivities are needed to enhance our knowledge not only on herbivore-specific responses but also on the role of temperature performance breadths in shaping plankton community structure and function in a changing ocean. For instance, the universal use of cross-taxa approaches that fit the upper envelope of physiological responses of all taxa examined (e.g. Eppley 1972, Rose & Caron 2007, Bissinger et al. 2008) has been questioned, as it might not be a universally suitable descriptor of planktonic population dynamics (Wang et al. 2019).

Here we address 3 important knowledge gaps:

(1) the temperature dependence of herbivorous protists' growth rates over an ecologically important range representing productive regions of the polar and temperate ocean for much of the year (0–22°C);

(2) the importance of temperature-induced cell size changes that may result in deviations between division- and production-based quantifications of growth; and

(3) the potential effect of assuming that plankton metabolism is instantaneously acclimated to target temperatures, when ocean temperatures can in fact fluctuate over short time scales.

First, to measure herbivorous protists' growth and cell-size dependence on temperature, we measured the growth response of 3 cosmopolitan herbivorous dinoflagellates (*Oxyrrhis marina*, *Gyrodinium dominans* and *Protoperidinium bipes*) to an environmentally relevant temperature range (0–22°C). Second, recognizing that temperature affects an organism's physiology on several levels, from cell size to ingestion rate and growth efficiency (e.g. Atkinson et al. 2003, Kimmance et al. 2006, Rose et al. 2009, Forster et al. 2013), we characterized herbivore responses based on both cell division (abundance) and production, i.e. biomass accumulation. By comparing magnitude and shape of the growth response based on these 2 metrics, we wanted to examine the widespread custom of using abundance-based growth rates in carbon flux models, and to what extent interactions between temperature and cell size could lead to discrepancies in abundance, distribution and biomass predictions. Finally, to make these results relevant to a dynamic ocean that can undergo rapid temperature fluctuations of 1–2°C within a few hours or a day, we developed new procedures and investigated the effect of prior acclimation to target temperatures on measurements of population growth and biomass production rates. Understanding how acclimation af-

fects species responses to environmental change is essential for our ability to quantify and predict ocean ecosystem processes. Thus, we explicitly tested different acclimation procedures including short (3 generations), medium (10 d) and long (1 mo) exposures to target conditions. Most importantly, we aimed to identify commonalities among the examined species that transcend species-specific differences, so that herbivorous protists' growth dependence on temperature and ultimately their grazing impact could be parameterized accurately despite intra- and interspecific variations in physiology.

2. MATERIALS AND METHODS

2.1. Culture maintenance

Clonal cultures of *Oxyrrhis marina* (CCMP 3375), *Gyrodinium dominans* (SPMC 103) and *Protoperidinium bipes* were established from single-cell isolation. *P. bipes* was originally isolated from South Korea, while strains of *G. dominans* and *O. marina* are identical to those used by Strom et al. (2013). All cultures were maintained in 0.5 l polycarbonate bottles on a 12:12 h light:dark cycle, with salinity of ~30 psu and light intensity of 10–15 µmol photons m^{-2} s^{-1}. Throughout the experiments, all herbivores were periodically transferred to fresh, 0.2 µm filtered seawater, and all were fed the ~5 µm cryptophyte *Isochrysis galbana* (CCMP 1323) once or twice a week. *I. galbana* was cultured in f/2 enriched seawater minus silicon, at 15°C, 12:12 h light:dark cycle and light intensity of 80–100 µmol photons m^{-2} s^{-1}. *I. galbana* was transferred every 5 d to maintain exponential growth (Guillard 1975). Light intensity and temperature were monitored throughout the experiments with data loggers (HOBO, Onset) placed inside each incubator.

2.2. Acclimation procedure

Herbivorous protists were acclimated to each target temperature before growth rates were measured. Due to the wide temperature range tested (0–22°C), cultures were moved from the initial temperature of 15°C through gradual transitions limited to at most 3°C. Subsequent temperature shifts proceeded only after 3 divisions were completed at any of the intermediate temperatures. The experimental Day 0 (D0) was defined as the first day on which acclimated growth rates were measured. Although acclimation periods have not been reported for herbivorous protists, the number of generations exposed to a new condition has been used to establish acclimated responses (Montagnes & Franklin 2001, Beveridge et al. 2010). In our experimental setup, we defined cultures as being acclimated after they were exposed to target temperatures for at least 3 generations (3 divisions) so that >80% of the population was reared at target temperature.

The validity of our approach and importance of acclimation was further investigated by comparing growth rates of cultures that had been acclimated to target temperature for 3 generations with those that had been continuously incubated at target temperature for 10 d (D10) and 30 d (D30).

2.3. Cell abundance and biomass

To determine predator and prey abundance, samples were preserved in acid Lugol at a 2% final concentration (Menden-Deuer et al. 2001) and enumerated using a Sedgewick-Rafter slide (1 ml volume) and a Nikon Eclipse E800 light microscope equipped with phase contrast. A minimum of 500 cells was counted for each sample, and when abundance was lower than 500 cells ml^{-1}, the whole chamber was counted. Herbivore biovolume was calculated based on linear dimensions obtained from ≥35 cells measured at each time point and temperature treatment using an image analysis system consisting of a high-resolution digital camera (Allied Vision, Stingray F45) and ImageJ software (version 1.5i). The biovolume was determined assuming a prolate spheroid for *O. marina* and *G. dominans*, while the shape of *P. bipes* was calculated according to Jeong et al. (2004). Biovolume was converted to carbon content according to Menden-Deuer & Lessard (2000).

2.4. Growth rate

Growth rates of the 3 herbivorous species were determined at 7 incubation temperatures: 0, 2, 5, 10, 15, 18 and 22°C. Acclimated growth rates were measured after gradual transitions of a maximum 3°C and exposure of 3 generations to the new target temperature. Experiments were conducted using temperature-controlled incubators (I-36LLVL Series, Percival Scientific). Cultures exposed to target temperature were partitioned into triplicate 150 ml bottles after 3 generations and again after 10 and 30 d (see Section

2.2) and fed *I. galbana* at saturating concentration (1.3 to 5 × 10^5 ml^{-1}, Kimmance et al. 2006). Changes in *O. marina*, *G. dominans* and *P. bipes* abundance and biomass were determined over 24 h incubations of the triplicate 150 ml bottles and used to calculate growth rates (μ d^{-1}) assuming exponential growth: $\mu = \ln(N_t/N_0)/t$, where: N_0 and N_t are the initial and final cell abundance (or biomass) respectively, and t is the experiment duration in days.

2.5. Statistical analysis

The combined effect of temperature and incubation duration (D0, D10, D30) on the growth rates of each herbivorous species was examined using a 2-way ANOVA. Differences in division rate and biomass production rate at different temperatures were tested using a 1-way ANOVA. Normality of data distributions and homoscedasticity of variance were verified with a Shapiro-Wilk test. Post hoc tests identified significant factors, and a Bonferroni correction was applied to correct for multiple comparisons and apply a conservative approach to identifying statistical significance. To identify the best model for predicting herbivorous growth response to temperature, we used Akaike's information criterion (AIC) and Bayesian information criterion (BIC) and tested linear and exponential models. The equality of slopes was assessed through analysis of covariance (ANCOVA). All analyses were considered significant at $p < 0.05$, and were conducted using R (version 1.2.1335) and Prism 7.

3. RESULTS

3.1. Acclimation effect

The acclimation response to warming and to 2°C cooling of all 3 herbivorous species was identical (Fig. 1). Furthermore, the rate of acclimation was remarkably similar in the 3 dinoflagellates across the temperature gradient (Fig. 1). Acclimation slowed and cultures took increasingly longer periods of time when the 3 herbivores were exposed to temperatures farther from the original long-term culturing temperature of 15°C (Fig. 1). All 3 species survived temperature transitions at maximally 3°C increase (or decrease), and transitions of 5–7°C resulted in high to total herbivore mortality. In response to warming effects, *Oxyrrhis marina*, *Gyrodinium dominans* and *Protoperidinium bipes* required the same number of

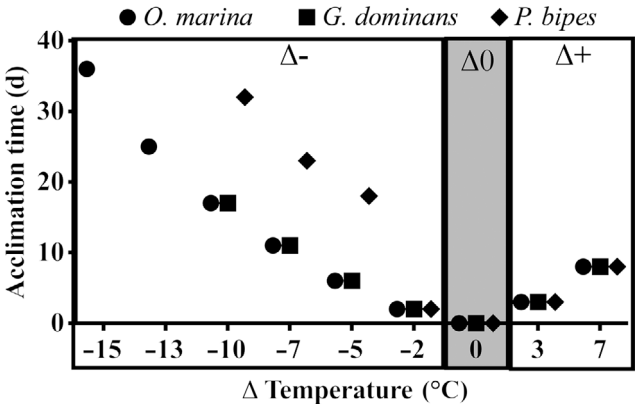

Fig. 1. Cumulative days required by each herbivore species (*Oxyrrhis marina*, *Gyrodinium dominans* and *Protoperidinium bipes*) to achieve acclimated growth through gradual 3°C transitions from the initial isolation temperature (15°C, Δ0) to each target temperature

days to acclimate to higher temperatures, i.e. 3 and 5 d, respectively at 18 and 22°C. In contrast, the time required to acclimate to lower temperatures was different for the 3 species. Overall, *O. marina* needed a cumulative 36 d to be moved from 15 to 0°C. *G. dominans* took 17 d to complete the transition from 15 to 5°C and died at temperatures below 5°C irrespective of acclimation duration, while *P. bipes* required up to 32 d (Fig. 1) to acclimate to the same temperature range (15–5°C) and did not survive at temperatures below 5°C. Nevertheless, the rate of transition, i.e. the slope of the relationship between acclimation time and temperature, obtained for all 3 species were statistically indistinguishable (ANCOVA $F_{2,14} = 1.71$, $p > 0.05$), and a common acclimation rate could be established. To obtain fully acclimated rates, herbivorous protists required 1.25 d $°C^{-1}$ when transitioning to higher temperature and 2.5 d $°C^{-1}$ when transitioning towards lower temperature.

3.2. Acclimated temperature response: abundance- vs. biomass-based growth

Growth rates increased with increasing temperature for all 3 species (Fig. 2). *O. marina* grew at all temperatures tested (0–22°C), presenting the widest performance breadth among the species considered, while *G. dominans* and *P. bipes* reached their temperature minima at 5°C and no rate estimates could be obtained below 5°C. *O. marina* growth rates based on changes in abundance (μ_{ab}) reached a maximum (±SD) of 0.96 ± 0.12 d^{-1} at 22° (Fig. 2a). Positive growth rates were also observed at the 2 lowest temperatures. Within a narrower temperature

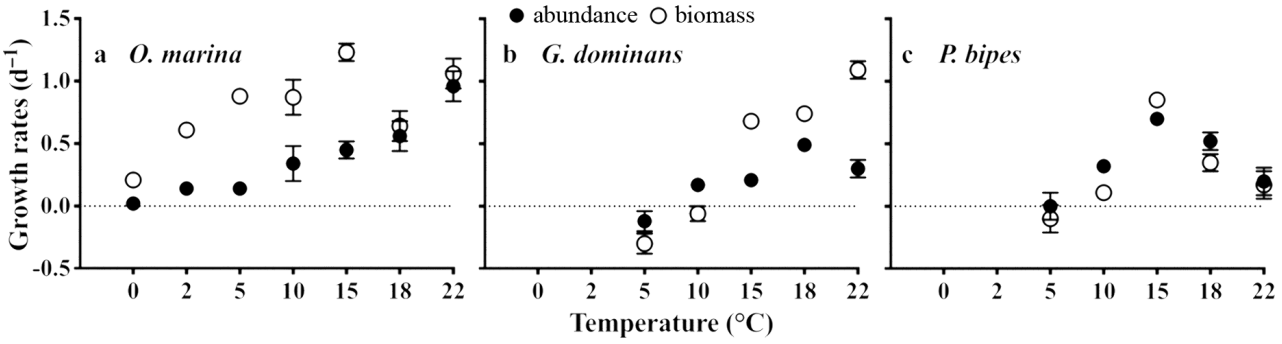

Fig. 2. Temperature dependency of acclimated growth rates based on abundance (cells ml^{-1}, filled circles) and on biomass (µg carbon l^{-1}, open circles) for (a) *Oxyrrhis marina*, (b) *Gyrodinium dominans* and (c) *Protoperidinium bipes* within the temperature range 0–22°C. Due to population mortality, no rate estimates could be obtained below 5°C for *G. dominans* and *P. bipes*. Symbols with error bars indicate means ± SD

range, 5–22°C, *G. dominans* μ_{ab} increased with increasing temperature up to a maximum of 0.49 ± 0.05 d^{-1} measured at 18°C, followed by a decrease in growth at 22°C (Fig. 2b). *G. dominans* was not able to grow at 5°C, where a mortality rate of −0.12 ± 0.08 d^{-1} was observed. Within the same thermal range, *P. bipes* reached its temperature optimum at 15°C, the long-term culturing temperature, with an estimated μ_{ab} of 0.70 ± 0.03 d^{-1}. *P. bipes* μ_{ab} was significantly lower at temperatures above and below the optimal temperature (1-way ANOVA $F_{2,6} = 39.3$, $p < 0.001$, Fig. 2c).

As a result of the temperature effect on cell physiology, division rates at lower temperature decreased and cell size increased. Thus, significant biomass accumulation was still observed at the 2 lowest temperature treatments, because cells became significantly larger, despite the low division rates. Thus, to account for cell size changes due to the mismatch between division and ingestion rate, we measured growth rates based on biomass accumulation. *O. marina* biomass-based growth rates (μ_{bio}) were significantly greater than those based on cell division (2-way ANOVA $F_{6,28} = 15.4$, $p < 0.0001$; Fig. 2a). The difference in magnitude between μ_{bio} and μ_{ab} was greatest at low temperatures, where μ_{bio} was up to 10 times higher than μ_{ab}, reflecting an average 50% increase in biovolume at 0 and 2°C.

G. dominans biovolume also changed significantly with temperature (2-way ANOVA $F_{4,232} = 22.18$, $p < 0.0001$), and the increases in cell volume observed at temperatures above 15°C resulted in μ_{bio} up to 3 times higher than μ_{ab} (Fig. 2b). Contrary to the 2 athecate dinoflagellates, the μ_{bio} of the thecate *P. bipes* was not consistently higher than μ_{ab} (Fig. 2c), reflecting only minor changes in cell volume observed for this species.

3.3. Time dependency of growth rates

The acclimated growth rates were measured on D0 and again on D10 and D30 to estimate changes in growth rate as a function of the duration over which the herbivores were exposed to new target temperatures. The 3 species showed a common response in their ability to reach acclimated growth over 3 generations and sustain this growth for 10 d. The temperature dependence of growth for *O. marina* did not change over time considering either abundance-based (ANCOVA $F_{2,15} = 2.02$, $p > 0.05$, Fig. 3a) or biomass-based rates (ANCOVA $F_{2,15} = 0.62$, $p > 0.05$). A similar result was obtained for *G. dominans*, where growth rates measured at D0, D10 and D30 were all comparable and produced slopes not statistically distinguishable from each other (ANCOVA $F_{2,7} = 1.12$, $p > 0.05$ and $F_{2,7} = 1.97$, $p > 0.05$, based on abundance and biomass, respectively; Fig. 3b). Thus, for the 2 athecate dinoflagellates, a 3 generation exposure to a new target temperature was sufficient to reach consistent growth rates over time and confirms that 3 generations are an appropriate acclimation period. On the other hand, *P. bipes* demonstrated a higher variability. The slight increase in growth rates measured on D10 at 22°C was followed by a significant decline on D30 (Fig. 3c). *P. bipes* growth rates on D30 were significantly lower compared to those observed on D0 and D10 at all temperatures (2-way ANOVA, $F_{8,20} = 67.57$, $p < 0.0001$), indicating a long-term, adverse effect of *P. bipes* exposure to changed temperatures. After the month-long incubation, the only positive growth rate (0.19 ± 0.05 d^{-1}) was measured at 15°C, the original isolation temperature. This indicates that despite the capacity to grow at a range of temperatures, over the long-term *P. bipes* may be unable to sustain growth at altered temperatures and may be more of a temperature specialist.

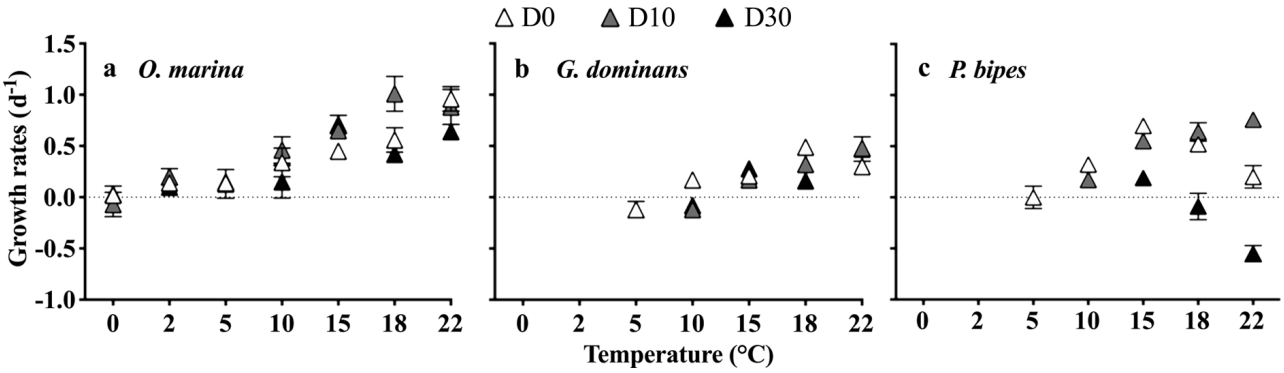

Fig. 3. Fully acclimated abundance-based growth rates of the 3 herbivorous protist species, (a) *Oxyrrhis marina*, (b) *Gyrodinium dominans* and (c) *Protoperidinium bipes*, measured after 3 generation exposure to each target temperature on Day 0 (D0, white), and then again on Days 10 (D10, grey) and 30 (D30, black) within the temperature range 0–22°C. Due to population mortality, no rate estimates could be obtained below 5°C for *G. dominans* and *P. bipes*. Symbols with error bars indicate means ± SD

3.4. Temperature response

Parameterization of maximum growth rates across the range of temperatures 0–22°C (Fig. 4) allowed the identification of comparable responses among the 3 species. Growth rates increased linearly with increasing temperature considering both abundance- and biomass-based rates (Fig. 4, Table 1). Despite the species-specific difference in growth magnitude, highlighted by the difference in intercepts (ANCOVA $F_{2,43} = 43.41$, $p < 0.0001$ and $F_{2,38} = 52.23$, $p < 0.0001$, for abundance- and biomass-based growth rates, respectively), the slopes between maximum growth and incubation temperature obtained for *O. marina*, *G. dominans* and *P. bipes* were not statistically different from each other, either considering abundance-based (ANCOVA $F_{2,41} = 2.59$, $p > 0.05$) or biomass-based rates (ANCOVA $F_{2,36} = 0.38$, $p > 0.05$). Thus, the temperature–growth dependence is best described with a common slope of 0.043 ± 0.002 d^{-1} $°C^{-1}$ and 0.062 ± 0.005 d^{-1} $°C^{-1}$ based on abundance and biomass, respectively (Fig. 4). It is noteworthy that these relationships show an almost 40% faster increase of the biomass-based rates in response to increases in temperature.

3.5. Cell size dependence on temperature

The effect of temperature on cell size was analyzed after cells were exposed for 10 d to target temperatures. The protists were provided with unlimited food, thus the changes in cell size reflected the temperature effect on both growth and prey uptake. Common to all species, mean cell size was larger at cooler temperatures compared to warmer temperatures (Fig. 5). *O. marina* was significantly smaller at 18 and 22°C than at 0°C (1-way ANOVA, $F_{6\ 203} = 48.02$, $p < 0.05$), measuring 21 µm in equivalent spherical diameter (ESD) at the lowest temperature and only 18 µm ESD

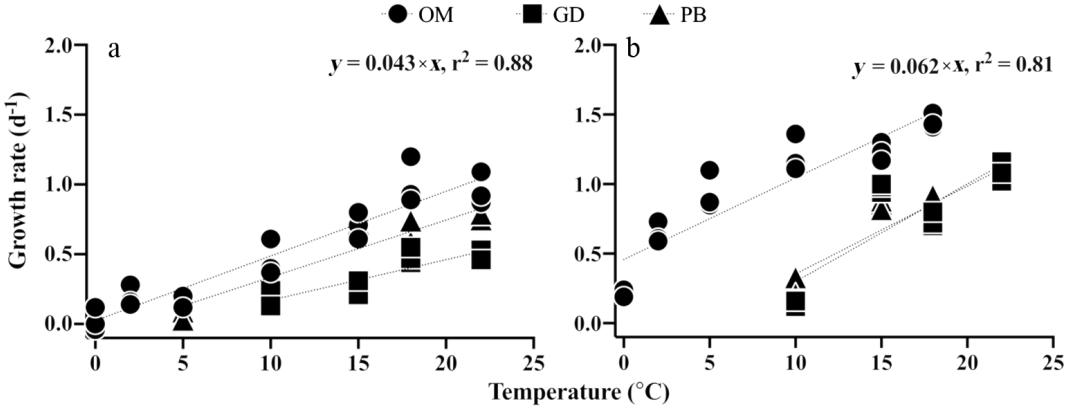

Fig. 4. Linear temperature dependence in growth rates based on (a) maximum abundance-based and (b) biomass-based rates. Equations represent the common linear slope based on the 2 metrics and their explanatory power. OM: *Oxyrrhis marina*; GD: *Gyrodinium dominans*; PB: *Protoperidinium bipes*

Table 1. Results of the comparative analysis between linear and exponential models to best describe growth responses to temperature. The test was performed on both abundance- and biomass-based rates for each species. AIC: Akaike's information criterion; BIC: Bayesian information criterion. The lowest information criteria values and thus the best fits are shown in **bold,** as is the resulting common linear slope describing abundance and biomass growth temperature dependence. Slope values represent means ± SD

Model Species	Slope	Abundance-based growth AIC	 BIC	 df	 R^2	Slope	Biomass-based growth AIC	 BIC	 df	 R^2
Linear										
Oxyrrhis marina	0.046 ± 0.003	**−81.57**	**−72.72**	19	**0.89**	0.059 ± 0.006	**−55.54**	**−57.47**	16	**0.83**
Gyrodinium dominans	0.029 ± 0.004	**−55.57**	**−47.39**	10	**0.78**	0.070 ± 0.010	**−30.36**	**−34.39**	10	**0.71**
Protoperidinium bipes	0.041 ± 0.004	**−57.68**	**−49.82**	10	**0.86**	0.063 ± 0.008	**−42.30**	**−46.33**	10	**0.84**
Common	**0.042 ± 0.002**				**0.88**	**0.062 ± 0.005**				**0.81**
Exponential										
O. marina		−70.84	−63.01	19	0.83		−48.73	−50.66	16	0.75
G. dominans		−52.84	−45.11	10	0.72		−26.89	−30.59	10	0.62
P. bipes		−49.44	−42.76	10	0.74		−37.07	−41.10	10	0.76

at the highest (Fig. 5a). Similarly, the mean cell sizes of *G. dominans* at 18 and 22°C (15 and 14 µm ESD, respectively) were significantly lower than the mean size measured at 10°C (18 µm ESD; 1-way ANOVA, $F_{3,166} = 30.07$, $p < 0.0001$; Fig. 5b). Although the differences in *P. bipes* cell size at each temperature were less pronounced compared to the other 2 dinoflagellates, a decrease in mean ESD from 13 µm measured at 10°C to 10 µm at 15°C was observed (Fig. 5c).

4. DISCUSSION

Temperature is a fundamental driver of physiological rates and impacts biological processes on a global scale. In the ocean, temperature is a descriptor of broad biomes, such as coastal, polar and temperate oceans. However, in such dynamic environments, temperature can fluctuate over short periods of time. Addressing temperature effects on the growth and feeding physiology of unicellular herbivores, key consumers of marine primary production (Steinberg & Landry 2017), is of pivotal importance to accurately quantify and predict cellular processes such as digestion and respiration, as well as relevant ecosystem properties, e.g. predator population abundance and energy transfer through the pelagic microbial food web, and ultimately produce reliable carbon cycle predictions.

Here, we established species-specific growth responses across an important temperature gradient representing polar to temperate oceans for 3 cosmopolitan dinoflagellate species. These data allowed us to observe commonalities among the 3 species that will be useful to predict herbivore responses to short-term temperature fluctuations and long-term warming of marine waters. All species showed: (1) a common rate of increase in growth rate with increasing temperature; (2) a similar rate of acclimation, i.e. slower to cold and faster to warm temperature, that required 3 generations of exposure to the new conditions; and (3) a common amplitude of temperature shift to reach acclimation. At the same time, the observed differences in temperature dependency when considering cell division vs. biomass production rates

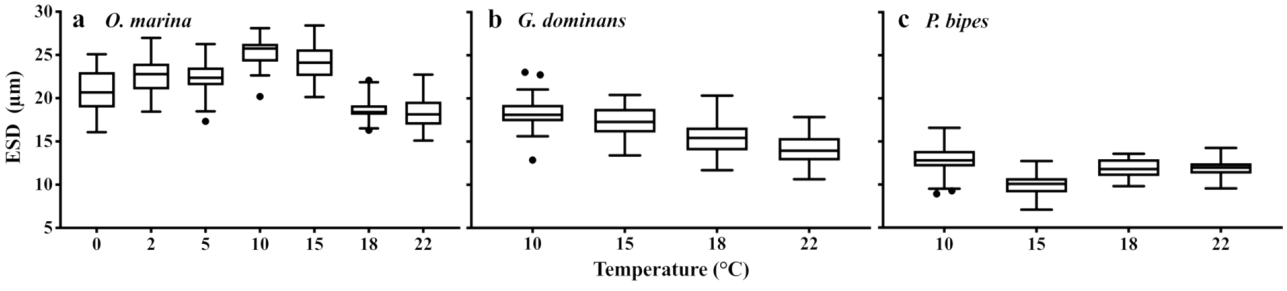

Fig. 5. Cell size expressed as equivalent spherical diameter (ESD), (a) *Oxyrrhis marina*, (b) *Gyrodinium dominans* and (c) *Protoperidinium bipes,* measured after 10 d of exposure to each target temperature at saturated prey concentration. Boxes indicate the lower 25th and 75th percentiles, the line within each box indicates the median, and the whiskers represent the interquartile range, based on the Tukey method. Black dots represent outliers

highlight the need for thoughtful consideration of when abundance-based or biomass-based assessments are most suitable. As shown here and observed previously (e.g. Anderson & Menden-Deuer 2017), the rate at which cells divide can, in fact, be uncoupled from the rate at which biomass accumulates, because feeding status or responses to environmental conditions can affect cell size independently from division rate. Thus, cells not actively dividing but accumulating biomass can function as 'energy packs' for higher trophic levels.

4.1. Acclimation effect

Investigation into herbivorous protist responses to temperature fluctuations poses experimental challenges regarding both the time needed for species to acclimate to a new condition and the amplitude of the temperature shift sustainable in a single step by each species. Information is lacking about both aspects of the acclimation process, particularly with respect to herbivorous protists. For monospecific phytoplankton laboratory cultures, Brand & Guillard (1981) found that in order to achieve stability of a single metric (growth rate), the required acclimation period was 1–3 wk and depended on species. Due to temperature-dependent differences in growth rate, defining temperature acclimation using as a common denominator the number of generations exposed to the new condition instead of the duration in days, might produce more comparable data among temperatures (Montagnes & Franklin 2001). Furthermore, investigations on the amplitude of the temperature shifts that herbivorous protists can survive are completely missing. Shifts in temperature that exceed the maximum temperature change tolerable in a single step could lead to the establishment of inaccurate species' performance breadths, which could affect our understanding of ecosystem structure and function. The approach taken here can point to a best practice in plankton physiology that includes determination of survivable temperature fluctuations and establishment of the time frame of sustained growth, considering exposure to both increasing and decreasing temperature. Such approaches are needed to produce rates suitable for comparisons between studies and/or species and to better represent natural dynamics.

Remarkably, we were able to determine a common amplitude and time frame of temperature shifts at which growth rates can be confidently considered acclimated. The first acclimated rates measured (D0) were either not statistically different or representative of the same trend observed after 1 mo of exposure to the target temperature. The similarity in response in these 3 dinoflagellate species is remarkable, given that other metrics differed in a species-specific manner. While 3 species are insufficient to propose a universal acclimation rate, it appears that once cells were acclimated to target conditions, there were no subsequent intermediate or long-term changes to growth rates. Thus, the data presented here provide a meaningful constraint on the time scale of acclimation. Determination of whether this is the case amongst other dinoflagellates or non-dinoflagellate unicellular herbivores will support ecologically relevant predictions of herbivore responses to fluctuations in environmental temperature and the consequences of these fluctuations on population production.

4.2. Thermal sensitivity

The 3 herbivorous protists investigated in this study, despite being isolated at similar environmental temperature and maintained at a constant temperature over months to years, displayed distinct thermal sensitivities within the thermal range explored. *Oxyrrhis marina* exposed to an environmentally relevant range of temperatures responded as a true thermal generalist, growing at all temperature tested, given enough time to acclimate to the new conditions. Although we did not explore the complete temperature performance curve, the growth rates and the performance breadth measured in our study significantly expanded the unexplored lower temperature range of *O. marina*. The positive rates observed at the 2 lowest temperatures indicate that *O. marina* is able to grow in polar or wintertime conditions. Despite the fact that *O. marina* has been extensively used for experimental studies, some of which have been employed to develop or parameterize mathematical models (Davidson et al. 2011 and literature therein), we found only 1 other study that reported a sufficient thermal range to establish a reaction norm for *O. marina* (Kimmance et al. 2006). This lack of data, even for this well studied species (Montagnes et al. 2011), is remarkable. The growth rates and the performance breadth measured in our study match well with those presented by Kimmance et al. (2006). Combining the data from both studies, *O. marina* grew within a temperature range spanning 0 to 30°C, with the optimum temperature at 25°C, higher than the highest temperature used in our study. *Protoperi-*

dinium bipes and *Gyrodinium dominans* shared the same relatively narrower temperature breadth (5–22°C). However, the long-term exposure tested highlighted possible divergences in the degree of acclimation of these 2 species. The sharp decline in growth on either side of the optimum temperature after a month of exposure to target temperature suggests that *P. bipes* might have a limited ability to withstand large and/or prolonged temperature changes. On the other hand, *G. dominans* was able to withstand changes and acclimate to a range of temperatures, albeit more restricted compared to *O. marina*. The magnitude of growth in *G. dominans* compared well with a *G. dominans* strain isolated from the Ie-shima Islands, Japan, at *in situ* temperature of ~25°C (Nakamura et al. 1992). A performance breadth shifted towards higher temperature is not surprising given the differences in the source environment and the ability of herbivorous protists to adapt to their environment, whether it be a temperature extreme (Franzè & Lavrentyev 2014, Menden-Deuer et al. 2018, Lavrentyev et al. 2019) or characterized by spatially or temporally heterogeneous prey availability (Paffenhöfer et al. 2007, Menden-Deuer & Fredrickson 2010, Anderson & Menden-Deuer 2017).

The difference in thermal responses between species observed in this and previous studies (Chen & Laws 2017, Wang et al. 2019) can determine winners and losers in a changing ocean. Thus, climate can affect ecosystem function directly by imposing species range limits (Gaston 2003), and indirectly through geographically varying competitor abundances and performance (Gross & Price 2000, Price & Kirkpatrick 2009). Characterization of the thermal responses and identification of driving factors (e.g. species specificity, abiotic drivers) affecting the responses of herbivorous protists to temperature will improve our predictive ability of species succession, energy transfer and trophodynamics in a changing ocean.

4.3. Abundance-based vs. biomass-based growth rates

Mean cell sizes of protists change considerably with both temperature and population abundance (Forster et al. 2013). Consequently, the often made assumption that biomass per cell is constant is invalid, because cell size is a commonly used determinant of total cellular biomass (Menden-Deuer & Lessard 2000). Thus, a doubling of cell number does not necessarily result in a doubling in biomass nor does a lack of change in cell abundance imply constant biomass. A system considered static based on low division rates could indeed reveal itself as quite active, with significant ingestion rates and biomass accumulation that can support transfers of energy. Thus, taking into account cellular responses based on division rate or biomass accumulation provides more comprehensive understanding of the systems. Nevertheless, typically no distinction is made between abundance- and biomass-based growth, and the majority of the data available for model integration are abundance-based growth rates. Here we show that the 2 rates are not equivalent, and the fact that the magnitude of the dinoflagellates' thermal response differed significantly whether it was based on cell division or biomass production raises questions about the accuracy of productivity models based on rates that do not represent the potential carbon availability within food webs. Biomass-based rates up to 10 times higher than the abundance-based ones suggest significant biomass accumulation and thus carbon availability also at low rates of cell division. Remarkably, large cell size increases were characteristic of cool temperatures, implying a particular bias of underestimating biomass availability in polar or wintertime regions. The increasing appearance of models that take into account the strategic role of herbivorous plankton in redistributing resources within marine systems and incorporate predator–prey dynamics (Stock et al. 2014, D'Alelio et al. 2015, 2016) show the urgency of recognizing systematic deviations between estimates (e.g. division vs. production) and their causes (e.g. temperature effects on rates vs. cell size changes). Providing common descriptors that transcend species specificity supports these cross-biome comparisons of ecosystem structure and function.

4.4. Temperature response

The maximum growth rates of the 3 herbivorous protists linearly increased with increasing temperature considering both abundance- and biomass-based rates. The major frameworks addressing either species or whole plankton community temperature dependence of growth, often referred to as division rate, all predict that such an increase will follow an exponential trend, whether it is the seminal Eppley curve for phytoplankton (Eppley 1972), the more recent metabolic theory of ecology (Gillooly et al. 2001, Brown et al. 2004) or the widely used Q10

model. A major review of herbivorous protists' species-specific growth rates as a function of temperature also describes the growth–temperature relationship as exponential (Rose & Caron 2007). These approaches combine works based on many species to develop a 'whole community' temperature dependence of growth (e.g. Eppley 1972), which assumes that at any given temperature, some species will grow at their particular maximum rate. Our study was not designed to emulate these large data sets, nor did we aim to quantify the temperature–growth dependence across the entire temperature breadth (norm) of each species (Boyd et al. 2013).

Keeping these limitations in mind, combining our abundance-based data with literature values, we find little support for assuming that growth increases exponentially with increasing temperature. A linear, rather than exponential, dependency of growth on temperature has important quantitative ramifications for resulting predictions of population abundance and food web impacts. Several previous studies have concluded that herbivorous protist growth responds linearly to temperature (i.e. Montagnes & Lessard 1999, Strzepek & Price 2000, Montagnes & Franklin 2001). This hypothesis has been empirically supported by 79 peer-reviewed datasets, which yielded a linear dependence of abundance-based growth on temperature of 0.07 d^{-1} $°C^{-1}$ (Montagnes et al. 2003). Both species-specific and whole community protist growth rates were found to linearly increase with temperature in the Arctic Ocean, particularly at temperatures below 3°C (Lavrentyev et al. 2019). The maximum growth rates based on abundance obtained for the 3 dinoflagellate species examined in this study also showed a linear relationship with temperature, supporting the findings of these previous studies that the response of herbivorous protists to temperature is better represented by a simple linear model (Montagnes et al. 2003, Franzè & Lavrentyev 2014, Lavrentyev et al. 2019). Montagnes et al. (2003) also suggested that the use of the 2-point Q10 model to predict the relationship between herbivorous growth and temperature can introduce a systematic error and inappropriately impose an exponential response to a linear relationship. Scaling the average maximum growth rate for herbivorous protists at 20°C (2.0 d^{-1}, extrapolated from Fig. 6 of Rose & Caron 2007) and applying a linear increase in growth with temperature as observed for abundance-based rates in our study, and widely supported in the literature, the predicted abundance-based growth rates were about 30% lower than the growth rates of herbivores predicted by the commonly used Q10 of 2 or the growth rates of autotrophic protists predicted using Eppley's Q10 (Fig. 6) at temperatures >20°C. A linear temperature dependence of herbivore growth implies rate increases much more modestly with increasing temperature than anticipated by models using an exponential dependency. An important consequence of assuming exponential growth with increasing temperature and greater temperature sensitivity in heterotrophs than autotrophs (Brown et al. 2004) is that predators rapidly outgrow their prey populations, suggesting high trophic transfer rates, low export and possibly food limitation for herbivores. At the same time, the predicted decline in growth at lower temperatures, suggested as one of the mechanisms that allows phytoplankton blooms at high latitudes, is not reflected either in the laboratory observations contributed here, nor in prior *in situ* observations of herbivorous protists growing at their maximum rates at temperatures below 5°C (Franzè & Lavrentyev 2014, Menden-Deuer et al. 2018, Lavrentyev et al. 2019). These discrepancies confirm the inadequacy of the present models to predict much of the ocean ecosystem, and highlight the need to take into account different metrics in order to capture the differential effect that environmental changes might have on cellular physiology. It is necessary to settle these significant questions in order to accurately predict predator–prey dynamics in microbial food webs in a rapidly changing ocean. An important step is to identify statistically supported parameterizations of temperature dependence in physiological rates, as

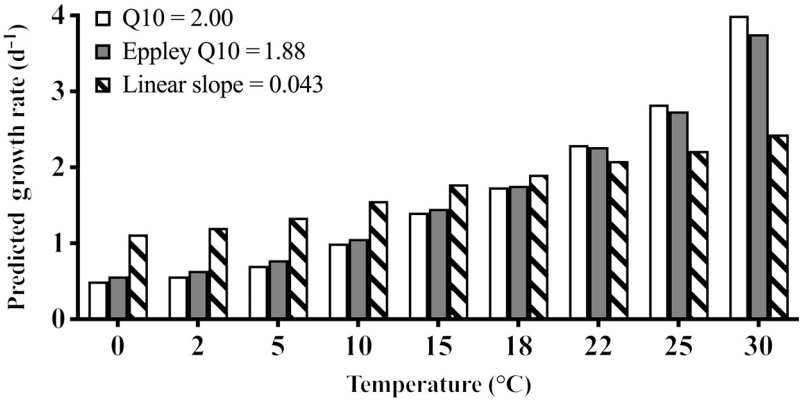

Fig. 6. Comparison between predicted temperature-dependent growth of herbivorous protists based on the Q10 model (white) or the linear model obtained in this study (striped), and that of autotrophic protists following Eppley's (1972) equation (grey)

done here for 3 species, that are valid for many species. Identifying cohesive responses despite the complexity of planktonic systems is critical in producing accurate predictions of ocean ecosystem function, including carbon cycling, export production, species diversity, distribution and responses to environmental change (e.g. Stock & Dunne 2010, Caron & Hutchins 2013, Siegel et al. 2016).

5. CONCLUSIONS

This study provides data on acclimated temperature dependence of 3 cosmopolitan herbivorous dinoflagellates and the required rates of acclimation within an ecologically relevant temperature range, especially at the poorly characterized cool temperatures representative of much of the polar ocean and temperate waters through all but the summer season. The data produced alleviate a fundamental knowledge gap in plankton physiology, improving our understanding of how temperature affects physiological rates, behavior and species interactions that can lead to shifts in community composition and affect spatial and seasonal abundance patterns of these key herbivores. These temperature responses may also aid in anticipating food web function in a warming ocean, particularly the relative rates of increase in the growth rates of phytoplankton vs. herbivorous protists. Through these results, we highlight the importance of applying the appropriate metric, i.e. abundance- or biomass-based growth rates, to produce robust and dependable biogeochemical and ecosystem models. Additionally, the understanding that acclimation is achieved within a few generations will support cross-biome comparisons of growth predictions, helping to constrain future scenarios of ecosystem structure and function.

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