MODELING THE METABOLIC EVOLUTION OF MIXOTROPHIC PHYTOPLANKTON IN RESPONSE TO RISING OCEAN SURFACE TEMPERATURES

Running Title: Mixotroph evolution and climate change

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ABSTRACT

Background: Climate change is expected to lead to warming in ocean surface temperatures which will have unequal effects on the rates of photosynthesis and heterotrophy. As a result of this changing metabolic landscape, directional phenotypic evolution will occur, with implications that cascade up to the ecosystem level. While mixotrophic phytoplankton, organisms that combine photosynthesis and heterotrophy to meet their energetic and nutritional needs, are expected to become more heterotrophic with warmer temperatures due to heterotrophy increasing at a faster rate than photosynthesis, it is unclear how evolution will influence the response of these to warmer temperatures. In this study, we used adaptive dynamics to model the consequences of temperature-mediated increases in metabolic rates for the evolution of mixotrophic phytoplankton.

Results: We find that bacterivorous mixotrophs tend to evolve to become more reliant on heterotrophy as temperatures rise, leading to reduced prey abundance through higher grazing rates. However if prey abundance becomes too low, evolution favors greater reliance on photosynthesis. These responses depend upon the tradeoff that mixotrophs experience between investing in photosynthesis and heterotrophy. Mixotrophs with a concave tradeoff maintain mixotrophy over the greatest range of temperatures; evolution in these generalist mixotrophs exacerbates carbon cycle impacts, with evolving mixotrophs exhibiting increased sensitivity to rising temperature.

Conclusions: Our results show that mixotrophic phytoplankton may respond more strongly to climate change than predicted by phenotypic plasticity alone due to evolutionary

shifts in metabolic investment. However, the type of metabolic tradeoff experienced by mixotrophs as well as ecological feedback on prey abundance may ultimately limit the extent of evolution along the heterotrophy-phototrophy spectrum.

KEYWORDS: heterotrophy, photosynthesis, adaptive dynamics, metabolic scaling, carbon cycle, eco-evolutionary feedback

BACKGROUND

Anthropogenic climate change is expected to increase oceanic surface temperatures [1], with cascading effects on ocean ecosystems. An important outcome of warmer temperatures will be faster metabolic rates due to the temperature sensitivity of chemical reactions [2,3] This thermal acceleration will influence carbon cycling processes that depend on metabolic rates, including ecosystem respiration and gross primary production [4] Since respiration responds more strongly to temperature than photosynthesis [5,6] carbon cycling in some regions may shift to favor increased CO_2 production, driving a positive feedback loop with implications for the future of the biosphere and Earth's climate [7].

In addition to the direct effects of temperature on their metabolic rates, organisms, especially microorganisms with their relatively fast generation times, will likely evolve in response to warmer temperatures. These evolutionary responses may either exacerbate or mitigate short-term plastic responses [8]. For example, phytoplankton, primary producers which account for nearly half the planet's photosynthetic net primary production [9], may evolve by shifting their optimal growth temperature [10] or decreasing rates of both photosynthesis and respiration to compensate for temperature-dependent changes in metabolism [11]. A better

understanding of microbial evolutionary adaptations to warming, and their consequences for the carbon cycle, will likely increase the quality of long-term climate forecasts.

Today, many microbial primary producers are known to be mixotrophic [12]: they combine photosynthesis and heterotrophy to meet their energetic and nutritional needs. Thus, these lineages may act as carbon sinks (via photosynthesis) or sources (via heterotrophy) depending on the environmental context. Mixotrophs are increasingly recognized as important components of surface ocean ecosystems, where they may dominate bacterivory [13] and regulate carbon export via the biological pump [14]. Because mixotrophs can continue to obtain nutrients through heterotrophy when inorganic nutrient concentrations are low, they are expected to dominate late-season and oligotrophic ecosystems [15]. These nutrient-limited, mixotroph-favoring conditions are expected to become more widespread in a warmer, more stratified ocean [16], so accurate predictions of mixotroph responses to warming temperatures are needed. However, mixotrophs' dual metabolism complicates predicted plastic and evolutionary responses because both sets of metabolic processes are to some extent competing for finite cellular resources and differ in their temperature dependencies.

Here, we focus on constitutive mixotrophs, single-celled planktonic eukaryotes that contain chloroplasts yet retain the capacity for feeding, typically on bacteria [15,17]. The different thermal scaling of heterotrophy and photosynthesis [5] has led to the prediction that these mixotrophs (like ecosystems) should become more heterotrophic at warmer temperatures [18]. This prediction has mixed empirical support however: In short-term studies of phenotypic plasticity, Wilken et al. (2013) [18] found that freshwater mixotrophs from the genus *Ochromonas* became more heterotrophic at warmer temperatures, while Princiotta et al. (2016) [19] found that mixotrophs from the genus *Dinobryon* maintained photosynthesis even as

temperatures warmed. Further, it is unclear how mixotrophs will respond over evolutionary timescales due to the costs that mixotrophs experience from maintaining two fundamentally different forms of metabolism [20-22].

Eco-evolutionary theory, which accounts for the interplay between evolutionary and ecological processes [23,24], can be a useful framework for understanding the evolution of organisms in the context of their environments. Previous work applying eco-evolutionary theory to model mixotroph evolution has shed light on the factors contributing to metabolic specialization in mixotroph lineages by focusing on cell size [25] and the costs and benefits of heterotrophic and photosynthetic metabolism [26]. We applied adaptive dynamics [27,28] to explore how eco-evolutionary feedback influences mixotrophic metabolism in response to temperature, assuming a tradeoff exists between investment in heterotrophic and photosynthetic metabolism. Our model predicts that mixotrophs may evolve to become more dependent on heterotrophy with rising temperatures, but that this investment is influenced by the type of tradeoff experienced between photosynthesis and heterotrophy as well as ecological feedback on prey density, which together influence the contribution of photosynthesis and heterotrophy to mixotrophic growth.

METHODS

We model the interaction between a mixotroph M and its bacterial prey B living in a well-mixed water column. An individual mixotroph grows through photosynthesis $P(\theta, z, I, T, M)$ and grazing $G(\theta, T, B)$, both of which depend on temperature T, and has a constant per capita mortality rate l. Thus, the change in mixotroph population density over time is given by:

$$\frac{dM}{dt} = [P(\theta, z, I, T, M) + G(\theta, T, B) - l]M \qquad (1)$$

Both photosynthetic rates and grazing rates are dependent on a combination of environmental factors—e.g., mixotroph (M) and bacteria (B) density, temperature (T), and light (I)—and physiological factors—e.g., mixotroph investment in heterotrophy (θ) and the tradeoff shape parameter (z) (Figure 1, Table 1).

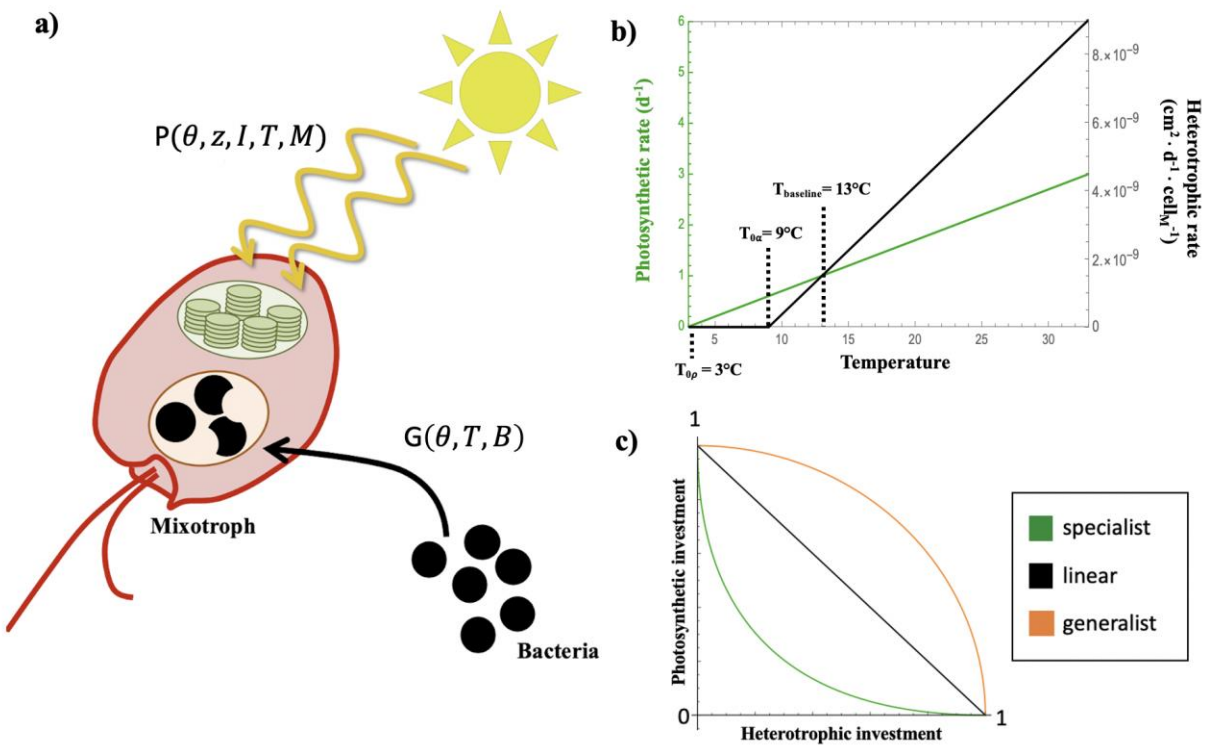

Figure 1. Diagram of model components. (a) Mixotrophs grow by combining photosynthesis (harvest of sunlight, yellow lines, using a chloroplast, green oval) and heterotrophy (consumption of bacteria, black circles). The rates of both photosynthesis and heterotrophy depend upon temperature T and mixotroph investment θ. (b) Both heterotrophy and photosynthesis depend on temperature. We assume that, over the temperature range of interest, thermal responses can be approximated as linear, with heterotrophy (black line) more sensitive to increases in temperature than photosynthesis (green line). (c) Mixotroph investment in photosynthesis and heterotrophy is constrained by a tradeoff: Assuming that metabolic resources are finite, a mixotroph cannot simultaneously achieve maximum rates of both forms of metabolism. We model three different types of tradeoffs: concave (orange), which produces generalist-type mixotrophs that tend to maintain both forms of metabolism; convex (green), which produces specialist-type mixotrophs that tend to invest fully in either photosynthesis or heterotrophy; and linear (black).

Table 1. Model parameters with units and values used in simulations.

Parameters:	**Units**	**Simulation values**
$\boldsymbol{\rho_{max}}$: maximum carbon uptake rate	d^{-1}	1
$\boldsymbol{\alpha_{max}}$: attack rate of mixotroph on bacteria	$cm^2 \cdot d^{-1} \cdot cell_M^{-1}$	$1.5 \cdot 10^{-9}$
$\boldsymbol{b}$: conversion rate of bacteria to mixotroph	$cell_M \cdot cell_B^{-1}$	0.15
$\boldsymbol{K_B}$: carrying capacity for bacteria	$cell_B \cdot cm^{-2}$	$1 \cdot 10^8 – 3 \cdot 10^8$
$\boldsymbol{r}$: growth rate of bacteria	d^{-1}	0.3
$\boldsymbol{h}$: half saturation constant for photosynthesis	µmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$	250
$\boldsymbol{I}$: incident light	µmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$	100,150
$\boldsymbol{k}$: mixotroph light absorbance constant	$cm^2 \cdot cell_M^{-1}$	$5 \cdot 10^{-7}$
$\boldsymbol{l}$: mixotroph mortality rate	d^{-1}	0.05
$\boldsymbol{m_\rho}$: photosynthetic temperature sensitivity coefficient	$^\circ C^{-1}$	0.1
$\boldsymbol{m_a}$: heterotrophic temperature sensitivity coefficient	$^\circ C^{-1}$	0.25
$\boldsymbol{T_{0\rho}}$: minimum temperature for photosynthesis	$^\circ C$	3
$\boldsymbol{T_{0\alpha}}$: minimum temperature for heterotrophy	$^\circ C$	9
Variables:		
$\boldsymbol{M}$: mixotroph	$cell_B \cdot cm^{-2}$	
$\boldsymbol{B}$: bacterial prey	$cell_M \cdot cm^{-2}$	
$\boldsymbol{\theta}$: heterotrophic investment	--	
$\boldsymbol{z}$: tradeoff parameter	--	
$\boldsymbol{T}$: temperature	$^\circ C$	
$\boldsymbol{t}$: time	d	

Photosynthetic growth follows the model developed by Huisman & Weissing (1994) [29]. Huisman and Weissing's model begins with the assumption that photosynthesis is a saturating function of light. The photosynthetic rate at a given irradiance $\rho(I)$ is a function of the maximum photosynthetic rate ρ_{max}, the irradiance I, and the light level at which photosynthesis reaches half of its maximum rate h:

$$\rho(I) = \rho_{max} \frac{I}{h+I} \qquad (2)$$

For a population of mixotrophs M living in a well-mixed water column, the light environment I is a function of depth. In particular, light is assumed to attenuate from a surface input irradiance of I_{in} with depth due to absorption by photosynthetic cells which have a per-cell

absorptivity k. Huisman and Weissing computed the average photosynthetic rate across depth, and obtained the per capita photosynthetic growth rate, defined here as $P(\theta, z, I, T, M)$:

$$P(\theta, z, I, T, M) = \frac{\rho(\theta,z,T)}{kM} ln\left(\frac{h+I}{h+I \cdot exp(-kM)}\right) \qquad (3)$$

Mixotrophs also obtain resources by grazing on bacteria. Therefore, we included a grazing term (sensu Moeller et al. 2019 [30]) assuming that, at relevant bacterial population densities, mixotroph grazing on bacteria at density B can be approximated using a Type I functional response [31], where $\alpha(\theta, T)$ represents the mixotroph attack rate and b represents the conversion efficiency from bacterial to mixotroph biomass:

$$G(\theta, T, B) = b\alpha(\theta, T)B \qquad (4)$$

Metabolic rates are known to scale with temperature [3]. To simplify our model, we assume that photosynthetic and grazing rates are linear functions of temperature (Figure 1B):

$$\alpha(\theta, T) = \alpha_{max}\theta(max(0,\ m_\alpha(T - T_{0\alpha})) \qquad (5)$$

$$\rho(\theta, z, T) = \rho_0(\theta, z)(max(0\ , m_\rho(T - T_{0\rho}))) \qquad (6)$$

Here, θ is an investment parameter, ranging between 0 and 1, that represents the proportion of metabolic resources the mixotroph dedicates to heterotrophy (with the remainder of these resources allocated to photosynthesis). T_{0a} and $T_{0\rho}$ represent the temperatures at which the rates of heterotrophy and photosynthesis reach zero, respectively. The temperature coefficients m_ρ and m_a were estimated by calculating the Q_{10}-values from the activation energies of carbon fixation and grazing previously measured for *Ochromonas* sp. [18,32]. Although in reality, thermal reaction norms are typically unimodal nonlinear functions of temperature [33], in our case assuming a linear function allows us to avoid introducing an underlying thermal nonlinearity into our system. This simplifies our interpretation of modeled non-linear evolutionary responses by allowing us to attribute these non-linearities to ecological feedback

rather than underlying assumptions about the shape of the thermal response. The use of an exponential function did not lead to qualitatively different results (Additional File 1: Figure S1). For similar reasons, we chose to fix bacterial growth rates rather than making them increasing functions of temperature. Again, this simplification allows us to isolate the role of mixotroph thermal responses in driving evolution.

We introduced a tradeoff between photosynthesis and heterotrophy by allowing the photosynthetic rate to vary as a function of heterotrophic investment θ and a shape parameter z.

$$\rho_0(\theta, z) = \rho_{max}(1 - \theta^{2^z})^{2^{-z}} \qquad (7)$$

The shape parameter z determines the photosynthesis-heterotrophy tradeoff strategy for mixotrophs (Figure 1C). Mixotrophs with shape parameters of $z < 0$ are defined as specialists because investment in one metabolic strategy comes at a large cost in investment in the alternative. In contrast, mixotrophs with shape parameters of $z > 0$ are defined as generalists because they are capable of maintaining relatively high levels of photosynthesis and heterotrophy simultaneously. When $z = 0$, there is a linear tradeoff between heterotrophy and photosynthesis.

Assembling these components, mixotroph population dynamics are governed by the following equation:

$$\frac{dM}{dt} = [\frac{\rho(\theta,z,T)}{kM} ln(\frac{h+I}{h+Iexp(-kM)}) + b\alpha(\theta,T)B - l]M \qquad (8)$$

Bacterial prey populations are assumed to grow logistically, with a growth rate r and carrying capacity K_B, and are grazed on by mixotrophs M:

$$\frac{dB}{dt} = [r\left(1 - \frac{B}{K_B}\right) - \alpha(\theta,T)M]B \qquad (9)$$

ANALYSIS AND RESULTS

We applied an adaptive dynamics framework to our ecological model to track the evolution of mixotrophic growth strategies (heterotrophic and photosynthetic investment) as a function of temperature (Additional file 1: Section S2). In effect, we assume that ecological dynamics are based on the resident mixotroph allele and come to equilibrium before new mutations altering the mixotroph strategy arise. When a new mixotroph allele appears, the mutant allele may be lost when rare or begin to spread in the population. If a mutant allele spreads when rare, it will continue to fixation unless frequency dependence causes the relative fitness of the resident allele to increase above that of the mutant allele. When ecological dynamics occur on a fast time-scale relative to mutation, the evolutionary trajectory can be modeled as a series of replacement events [34-36], So long as we consider small changes in the mixotroph strategy, if a mutant invades the resident and the resident cannot invade the mutant, then the mutant will replace the resident [37,38]. If we observe mutual invasibility, on the other hand, that is sufficient to show that neither allele represents an evolutionary stable point, but not sufficient to show that coexistence or adaptive dynamic branching will occur. Based on these considerations, we model the evolution of mixotroph strategies as a trait-substitution sequence and note that alternative formulations of evolution within the model are likely to generate the same conclusion [39].

Mutant mixotrophs with investment strategy θ_{mut} arise by mutation as rare genotypes within the resident mixotrophic population with investment strategy θ_{res}. The mutant growth rate is dependent on the equilibrium densities of the prey and resident mixotrophs, giving the following per-capita growth rate equation for a mutant, defined as the invasion fitness [27]:

$$\lambda(\theta_{mut}, \theta_{res}) = \frac{\rho(\theta_{mut}, z, T)}{kM^*(\theta_{res})} ln\left(\frac{h+I}{h+Iexp\,[-kM^*(\theta_{res})]}\right) + b\alpha(\theta_{mut}, T)B^*(\theta_{res}) - l \quad (10)$$

where $M^*(\theta_{res})$ and $B^*(\theta_{res})$ represent the population densities of resident mixotrophs and bacteria at equilibrium. When $\lambda(\theta_{mut}, \theta_{res}) > 0$ and $\lambda(\theta_{res}, \theta_{mut}) < 0$ (fitness of a single resident invading a population of mutants at equilibrium) the mutant invades and replaces the resident mixotroph population. This invasion-replacement process occurs until an investment strategy that is unbeatable is reached (θ_{ESS}), resulting in an evolutionarily stable state where the system is locally stable to further invasion. We used our model to predict the evolutionarily stable heterotrophic investment strategies for mixotrophs as a function of temperature, $\theta_{ESS}(T)$. Since $M^*(\theta_{res})$ and $B^*(\theta_{res})$ do not have closed-form solutions, $\theta_{ESS}(T)$ was solved numerically. We considered a temperature range from 9°C to 33°C at varying light levels and maximum prey capacities.

Assessing mixotroph invasibility across heterotrophic investment space

Analyzing invasion fitness for all combinations of θ_{mut} and θ_{res} and identifying the regions where $\lambda(\theta_{mut}, \theta_{res}) > 0$ using pairwise invasibility plots (PIPs) provides a useful way to visualize the global evolutionary behavior of the model [27]. PIPs were plotted for mixotrophs with specialist, linear, and generalist tradeoffs at 13°C, 18°C, and 23°C (Figure 2).

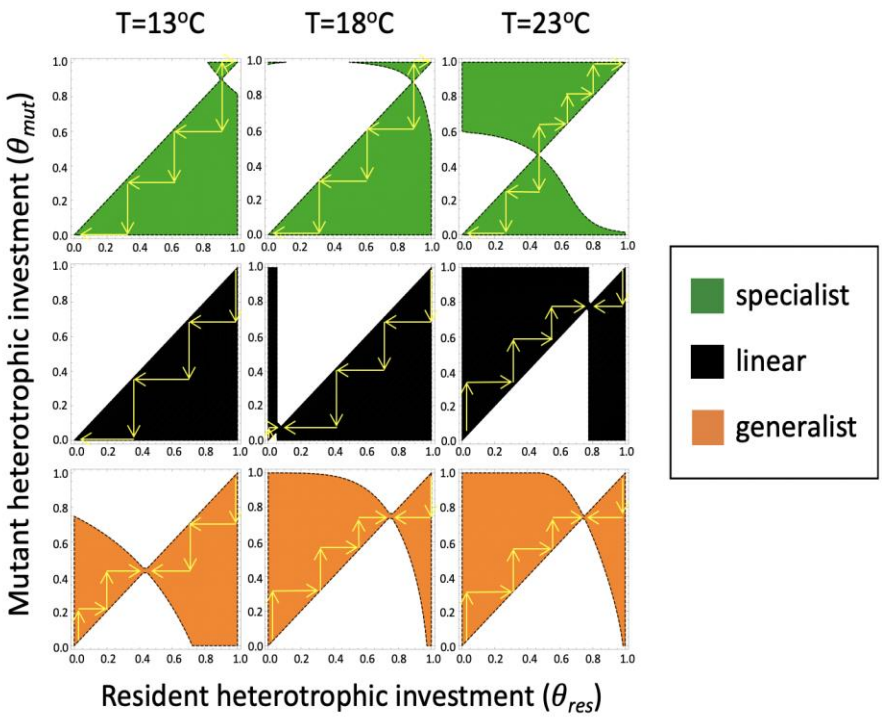

Figure 2. Pairwise invasibility plots for mixotrophs of varied tradeoff functions. Shaded areas indicate the mutant strategies that can successfully invade residents with a particular strategy indicated on the x-axis. Arrows indicate the general direction of evolution and should not be interpreted as a specific path through trait space. Top row: Specialist mixotrophs ($z = -1$) evolve to become completely specialized (i.e., $\theta_{ESS} = 1$ or $\theta_{ESS} = 0$ depending on the starting metabolic investment) regardless of temperature. Middle row: Mixotrophs with linear tradeoffs ($z = 0$) evolve to invest more in heterotrophy as temperatures increase. Bottom row: Generalist mixotrophs ($z = 1$) are functionally mixotrophic ($0 < \theta_{ESS} < 1$) across a wider range of temperatures compared to mixotrophs with linear tradeoffs. Evolutionary branching was not found to occur for specialist mixotrophs over this temperature range. $K_B = 1 \cdot 10^8$ $cell_B \cdot cm^{-2}$ and I_{in} = 100 µmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$ used to generate plots.

For specialist mixotrophs, the evolutionarily singular strategy was a repeller point under most simulated conditions, leading to extreme investment in either photosynthesis or heterotrophy depending on the investment strategy of the initial resident mixotrophs. Specialist mixotrophs were unable to converge on intermediate θ_{ESS} values between 0 and 1 while the sole evolutionarily singular strategy was a repeller point. At extreme temperatures, an additional evolutionarily singular strategy arises for specialist mixotrophs that is always a branching point

(Additional file 1: Section S3). θ_{ESS} for linear tradeoff mixotrophs varied with temperature, converging on values at or near total photosynthetic specialization at low temperature but increasing towards higher heterotrophic investment at higher temperatures. The trend for generalist mixotrophs was similar to that of linear tradeoff mixotrophs, but favored an intermediate θ_{ESS} across a larger range of temperatures. This reduced sensitivity of θ_{ESS} to temperature suggests that generalist mixotrophs experience less selective pressure with respect to metabolic strategy and are able to remain functionally mixotrophic with changing temperature more easily than mixotrophs with linear or specialist tradeoffs. Across all tradeoff types, evolution to θ_{ESS} produced equilibrium mixotroph densities that were lower than the maximum achievable density (Additional file 1: Section S4).

θ_{ESS} varies with temperature and prey availability

We computed $\theta_{ESS}(T)$ as a function of the light and prey resource landscape (Figure 3).

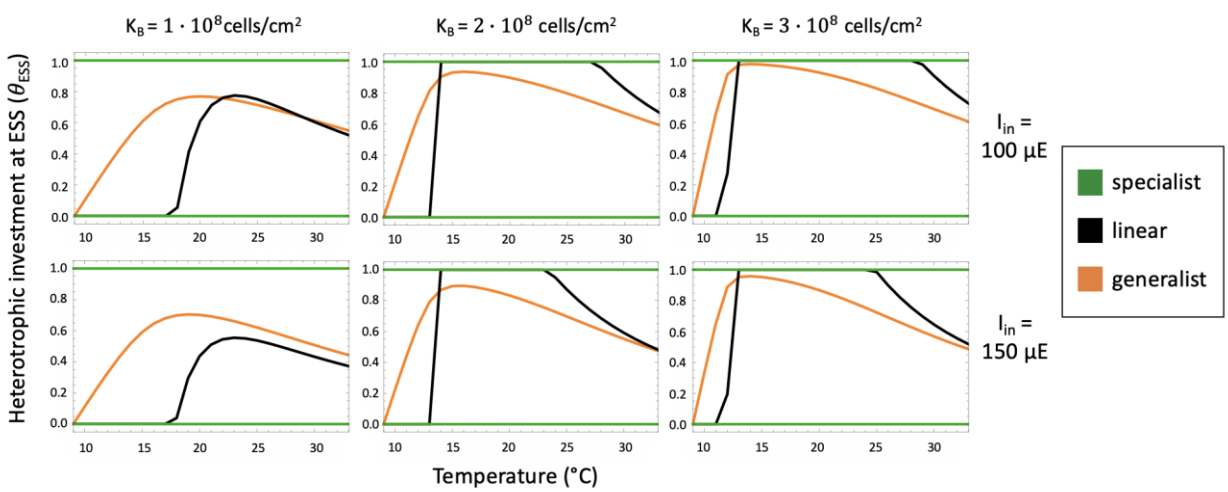

Figure 3. θ_{ESS} as a function of temperature, light, and prey carrying capacity for specialist (green), generalist (orange), and linear (black) tradeoff mixotrophs. Specialist mixotrophs exhibited evolutionary bistability at $\theta_{ESS} = 0$ and $\theta_{ESS} = 1$ across the temperature range simulated (parallel green lines at 0 and 1).

In the case of linear and generalist tradeoff mixotrophs, θ_{ESS} initially increases with rising temperatures. However, at much higher temperatures, θ_{ESS} begins to decrease, indicating that photosynthetic investment eventually returns to favorability (Additional file 1: Section S5). Mixotrophs with a specialist tradeoff function were found to exhibit bistability with $\theta_{ESS} = 0$ (fully photosynthetic) or $\theta_{ESS} = 1$ (fully heterotrophic) as evolutionarily stable strategies. This is consistent with the concavity of the specialist tradeoff function, which penalizes investment in more than one form of metabolism. Specialist tradeoffs were found to be the only tradeoff type that lead to evolutionary branching (Additional file 1: Section S3), in which evolution converges towards an evolutionarily singular strategy before diverging into coexisting lineages that reach different evolutionarily stable states [27]. In our model this only occurred at exceptionally high temperatures, under which our assumption about linearly increasing metabolic rates with temperature would likely not apply. As a result, we did not focus on conditions and outcomes of evolutionary branching. Increasing prey carrying capacity pushed mixotrophs with generalist and linear tradeoffs towards higher levels of heterotrophy while increased light availability caused mixotrophs with linear and generalist tradeoffs to rely more on photosynthesis. Specialist-tradeoff mixotrophs maintained evolutionary bistability over the range of temperatures simulated.

Mixotrophic contribution to carbon cycling with rising temperatures

In order to gain insight into how the evolution of mixotroph strategies in response to rising temperatures would influence carbon cycling, we compared model behavior for genetically static mixotrophs (θ_{ESS} was fixed at its baseline value from 13°C) to model behavior for evolving mixotrophs. This allowed us to contrast strictly thermal responses of mixotrophs separately from

their coupled thermal and evolutionary responses. Specifically, we analyzed differences in both mixotroph and bacteria population densities as well as population-level growth rate components (growth contributions from photosynthesis $P(\theta, z, I, T, M) \cdot M$ and heterotrophy $G(\theta, T, B) \cdot M$) for mixotrophs at equilibrium (Figure 4). These population level growth rate components were used as proxies for mixotroph carbon fixation and remineralization.

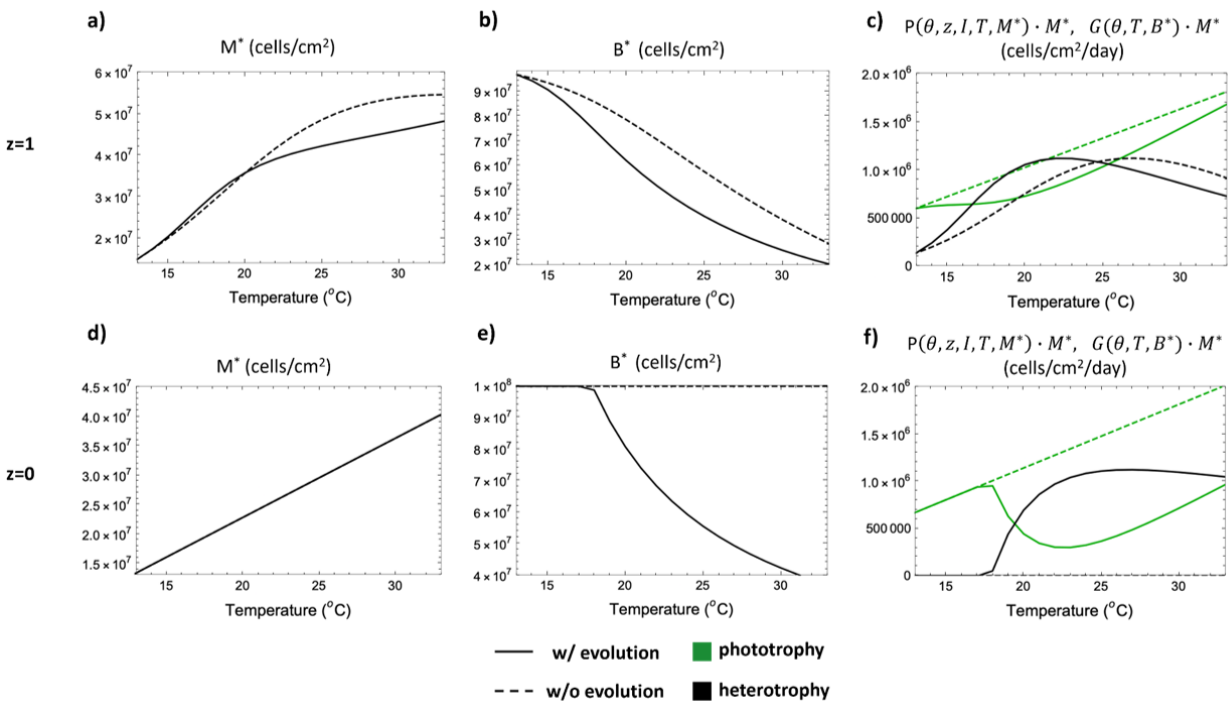

Figure 4. Comparing results of mixotroph-bacterial ecosystems involving either evolving (solid) or unevolving (dashed) mixotrophs as a function of temperature for generalist-tradeoff (top row) and linear-tradeoff (bottom row) mixotrophs. (a,d) Mixotroph population densities at equilibrium (M^*) vs temperature. (b,e) Bacterial prey population densities at equilibrium (B^*) vs temperature. (c,f) Photosynthetic and heterotrophic mixotrophic population growth rate components at equilibrium, $P(\theta, z, I, T, M^*) \cdot M^*$ and $G(\theta, T, B^*) \cdot M^*$ vs temperature. $K_B = 1 \cdot 10^8$ $cell_B \cdot cm^{-2}$ and I_{in} = 100 µmol quanta $\cdot m^{-2} \cdot s^{-1}$ used to generate plots.

Over a twenty-degree temperature range, generalist mixotrophs had the greatest impact on carbon cycling (Figure 4). As temperature increased, evolving generalist mixotrophs tended to have larger population sizes than genetically static populations (Figure 4A), while bacterial

population sizes tended to be smaller with mixotroph evolution (Figure 4B). Generalist mixotroph contributions to ecosystem carbon fixation and remineralization were more complex. Overall, evolution reduced generalist mixotroph contributions to community primary production (Figure 4C). Below 23°C, evolved mixotroph populations contributed more to carbon remineralization through grazing than unevolved lineages (Figure 4C), but at higher temperatures reduced bacterial population density (which feeds back to reduce mixotroph investment in heterotrophy; Figure 3) constrain mixotroph heterotrophy. Thus at high temperatures, evolved generalist mixotrophs actually contributed less to heterotrophy. In contrast, mixotrophs with linear tradeoff functions had increasing population sizes with temperature that did not vary with evolutionary history (Figure 4D). This is because, at the reference temperature of 13°C, the linear mixotroph's $\theta_{ESS}$was 0 (fully photosynthetic). For this mixotroph type, the selection gradient dictates that, at θ_{ESS}, the mixotroph's equilibrium population size is the same as a mixotroph with $\theta = 0$ (Additional file 1: Section S6). However, bacterial populations decreased sharply when these mixotrophs could mount an evolutionary response (Figure 4E) due to increased rates of heterotrophy above 18°C (Figure 4F). Because of the tradeoff between investments in heterotrophy and photosynthesis, photosynthetic contributions decreased (Figure 4F). For mixotrophs with specialist tradeoff functions, θ_{ESS} did not change with temperature, so evolution did not impact ecosystem-level dynamics.

DISCUSSION

The evolutionary response of mixotrophs to rising temperatures has implications for both the survival and abundance of these taxa, and for the cycling of carbon through aquatic ecosystems. The rate of aerobic respiration typically responds stronger to temperature than the

rate of photosynthesis [5, 6], leading to the hypothesis that mixotrophs should be more heterotrophic at warmer temperatures. Our eco-evolutionary models indicate that over sufficiently long timescales, mixotroph evolution may compound this prediction, with mixotrophs relying increasingly heavily on heterotrophy as oceanic temperatures rise. However, our findings are complicated by the interplay of physiology (e.g., internal tradeoffs between energetic investment in heterotrophy and photosynthesis) and ecology (e.g., the availability of bacterial prey and extent of self-shading) with evolution. While empirical tests of our model's predictions (i.e., evolution experiments testing mixotroph responses to increased temperatures) are, to our knowledge, not yet available, our findings are consistent with broader experimental data on planktonic evolution. Organisms typically exhibit some capacity for adaptive response to new conditions, but these responses are modulated by environmental conditions [10,40].

Our model predicts that mixotroph responses to temperature are non-monotonic. As temperature increases, mixotrophs on average increase their reliance on heterotrophy. However, at sufficiently high temperatures, investment in heterotrophy counterintuitively decreases (Figure 3, Figure 4). This is because mixotroph responses are shaped by the dynamic tension between internal cellular metabolism and external ecological factors. As mixotrophs become more heterotrophic, they deplete their supply of bacterial prey (an ecological feedback), reducing the favorability of grazing until it no longer becomes beneficial to increase heterotrophic investment (Additional file 1: Section S5). Thus, the direction of selection shifts towards increased investment in photosynthesis. These findings illustrate the complexity of accounting for evolution in the context of ecological feedback. In the absence of these eco-evolutionary feedback (e.g., if prey never became limiting) and assuming a more realistic nonlinear temperature-metabolic rate relationship used in our model, we might expect more heterotrophy

than is actually observed at the high temperatures simulated in our model. Although we chose to use a linear approximation of the thermal responses of heterotrophy and photosynthesis to temperature, no metabolic reactions can realistically increase indefinitely as a function of temperature. At some point, at a maximum critical temperature, enzymes will denature, and the metabolic process will halt. Indeed, previous studies have shown that evolved responses to warmer temperatures are especially constrained at this upper thermal maximum [10]. However, in our model, the use of a linear thermal responses is a good approximation for metabolic rates over relatively small temperature increases and allows us to isolate the ecological feedback which drive non-linearities in the mixotrophs' evolutionarily stable strategy from non-linearities imposed by the thermal responses themselves.

Tradeoff type had a significant impact on the evolutionary response of mixotrophs to warmer temperatures. In our model, mixotrophs with a generalist tradeoff function exhibited mixotrophy (i.e., invested in both photosynthesis and heterotrophy simultaneously, $0 < \theta_{ESS} < 1$) over a wider range of temperature than mixotrophs with linear tradeoff functions or, especially, specialist tradeoff functions. Indeed, consistent with the nomenclature of a specialist tradeoff type, these mixotrophs tend to specialize by completely investing in either photosynthesis ($\theta_{ESS} = 0$) or heterotrophy ($\theta_{ESS} = 1$). Other studies have found that evolutionary outcomes depend upon tradeoff functions, with concavity having a large effect on the outcome. While we focused on three different tradeoff types, including linear tradeoffs between heterotrophic and photosynthetic metabolism, truly linear tradeoffs are likely rare [41] and mixotrophs with such a tradeoff between photosynthetic and heterotrophic metabolism may be uncommon.

Our results have implications for the relative frequency of different mixotroph tradeoff types in nature. Mixotrophs with a specialist tradeoff had two evolutionarily stable states under most conditions: both extremes of either $\theta_{ESS} = 0$ or $\theta_{ESS} = 1$ depending on the ancestral investment strategy. Thus, our model implies that specialist tradeoff structures are unlikely to lead to the evolution of a mixotrophic lifestyle, and thus that mixotrophs with specialist tradeoffs may be rare in nature. Of course, other factors not considered by our model, such as environmental fluctuations (e.g., diurnal light cycles, and seasonal prey and nutrient availability), could impact the fitness of different mixotroph tradeoff types. While mixotrophs with linear tradeoffs were found to have intermediate evolutionarily stable investment strategies under a limited range of temperatures, under most cases they converged towards extreme values. In addition to suggesting that mixotrophs experiencing linear tradeoffs are less successful at maintaining a mixotrophic growth strategy relative to generalists, the fact that linear-tradeoff mixotrophs were found to be mixotrophic under some conditions at the baseline temperature suggests that linear-tradeoff mixotrophs may be prone to evolving towards metabolic specialization given enough pressure from elevated temperatures. Metabolic specialization arising from mixotrophs is supported theoretically and observationally (42; de 25; 26; 22), and long-term temperature shifts which could potentially promote such specialization are known to have taken place throughout Earth's history [43]. On the other hand, we found that generalist mixotrophs maintained mixotrophic metabolism (i.e., invest in both photosynthesis and heterotrophy) over a larger temperature range and rarely evolved to extreme investment in our model. This suggests that generalist mixotrophs are less prone to metabolic specialization, and therefore generalist tradeoffs between heterotrophy and photosynthesis may be more common among extant mixotrophs.

Over temperature ranges in which scaling of metabolic rates with temperature can be assumed to be monotonic, our model predicted that evolution initially exacerbates increases in heterotrophic rates with respect to temperature while suppressing increases in photosynthetic rates, suggesting that the evolution of mixotroph metabolic strategies under warming ocean surface scenarios will cause a shift towards greater net heterotrophy beyond that predicted from the temperature dependence of metabolic rates alone. The evolution of increased heterotrophy at the expense of photosynthesis in mixotrophs would have important implications for the future of ocean ecosystems and carbon cycling. A reduced capacity for photosynthesis would lower overall primary production, limiting the level of inorganic carbon that can be fixed and used to build biomass. Higher levels of heterotrophy could add to this effect, increasing respiration and leading to more rapid CO_2 production as well as a potential reduction in concentration of POC in the water column, which would reduce sinking flux via the biological carbon pump [44]. Such effects may lead to a shift from net sinks to net sources of carbon in ocean ecosystems where mixotrophs play a major role. By increasing the rate of biological carbon dioxide flux to the atmosphere, this could create a positive feedback accelerating warming [7].

CONCLUSIONS

In our model, mixotrophs typically responded by evolving greater investment in heterotrophy at the expense of photosynthesis as temperature rises. These results suggest that evolution will lead mixotrophs to contribute increasingly to atmospheric CO_2 flux if ocean temperatures continue to warm several or more degrees as predicted by climate models. However, our results are sensitive to ecological feedback on prey density as well as the intrinsic tradeoff between heterotrophy and photosynthesis experienced by mixotrophs. Our results

highlight the importance of taking evolution into account in addition to phenotypic plasticity for predicting organismal response to climate change. Although our model focused on the metabolic response of mixotrophs to increasing temperatures, the effects of climate change span many dimensions. Future work should take into account other factors including changes in ocean pH, pCO_2 and nutrient concentrations and how these factors will synergistically affect aspects of mixotrophic physiology including cell size and prey preference. Theoretical studies predict that mixotrophs have an important effect on the marine carbon cycle (Ward and Follows 2016), but more field and experimental studies will be required to provide support for these predictions. To better understand the influence of mixotrophs on global biogeochemistry and how it will change as they evolve, it will be necessary to better characterize the abundance and metabolic heterogeneity of mixotrophic phytoplankton across different regions of the ocean.

DECLARATIONS

Ethics approval and consent to participate

No ethical guidance or approval was required for this study. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the GitHub repository, https://github.com/lg11235/mixotroph-adaptive-dynamics

Competing interests

The authors declare that they have no competing interests

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Author's contributions

LMG designed the research, with input from SRP and HVM. LMG and SRP conducted the research, and all authors wrote and revised the paper.

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