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Dissolved Organic Nitrogen Inputs from Wastewater Treatment Plant Effluents Increase Responses of Planktonic Metabolic Rates to Warming

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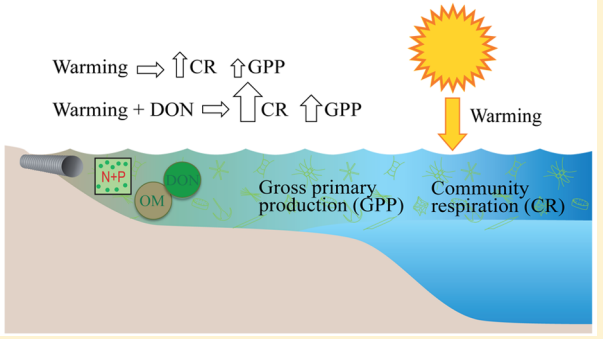
Supporting Information

ABSTRACT: Increased anthropogenic pressures on coastal marine ecosystems in the last century are threatening their biodiversity and functioning. Global warming and increases in nutrient loadings are two major stressors affecting these systems. Global warming is expected to increase both atmospheric and water temperatures and increase precipitation and terrestrial runoff, further increasing organic matter and nutrient inputs to coastal areas. Dissolved organic nitrogen (DON) concentrations frequently exceed those of dissolved inorganic nitrogen in aquatic systems. Many components of the DON pool have been shown to supply nitrogen nutrition to phytoplankton and bacteria. Predictions of how global warming and eutrophication will affect metabolic rates and dissolved oxygen dynamics in the future are needed to elucidate their impacts on biodiversity and ecosystem functioning. Here, we experimentally determine the effects of simultaneous DON additions and warming on planktonic community metabolism in the Baltic Sea, the largest coastal area suffering from eutrophication-driven hypoxia. Both bacterioplankton community composition and metabolic rates changed in relation to temperature. DON additions from wastewater treatment plant effluents significantly increased the activation energies for community respiration and gross primary production. Activation energies for community respiration were higher than those for gross primary production. Results support the prediction that warming of the Baltic Sea will enhance planktonic respiration rates faster than it will for planktonic primary production. Higher increases in respiration rates than in production may lead to the depletion of the oxygen pool, further aggravating hypoxia in the Baltic Sea.



INTRODUCTION

The coastal ocean plays a major role in the global biogeochemical cycles of carbon, nitrogen, and oxygen.[1] The coastal zone, representing about 10% of the ocean surface, supports ~20% of oceanic primary production and ~10% of global primary production.[2] This provides the resource base and habitat for diverse coastal communities. Increasing anthropogenic pressures are threatening the biodiversity and functioning of these coastal ecosystems.[3,4] Ocean warming due to climate change is becoming a critical stressor. Metabolic theory predicts that warming will enhance respiration more than primary production,[5] which could result in a consumption of oxygen. Moreover, warming affects multiple interacting processes that affect oxygen dynamics, such as intensifying stratification, decreasing oxygen solubility, rising sea level, and intensifying coastal upwelling, among others. In all, this points to the risk that warming may generate excessive oxygen consumption, leading to hypoxia (<2 mg O_2/L) or anoxia (undetectable levels of oxygen) if oxygen is not replenished.

Oxygen deficiencies have increased in frequency, duration, and severity in the world's coastal areas during the last decades,[6] and hypoxia is emerging as a major threat to marine coastal biodiversity.[7] The Baltic Sea has the largest area suffering from eutrophication-driven hypoxia,[8] and it has increased ten-fold during the last 115 years.[9] Recently, warming is worsening oxygen conditions in the Baltic Sea due to increased respiration rates with higher temperatures,[9] and temperature is one of the key factors controlling the extent of hypoxia.[10,11] The decline in dissolved oxygen can cause the death of marine organisms and catastrophic changes in ecosystems.[7] Species vulnerable to low oxygen (in particular, fishes and crustaceans) are removed and replaced by tolerant ones. Species diversity and the number of trophic levels are

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reduced. The deleterious effects of oxygen depletion can be further aggravated by ocean warming.[12]

A primary driver of hypoxia is eutrophication. Nutrient loading to rivers and coastal waters have increased over the last century, leading to accelerated primary production, algal blooms, accumulation of organic matter, and excessive oxygen consumption. Managerial efforts to prevent and mitigate hypoxia are focused on reducing inorganic nutrient loading (e.g., Nitrates Directive (91/676/EEC)) and disregard the organic fraction of the nutrient pool. However, the concentration of dissolved organic nitrogen (DON) frequently exceeds that of inorganic nitrogen in both marine and fresh waters.[13,14] Studies indicate that many components of the DON pool are available to phytoplankton and bacteria,[13,15] being a dynamic actor in the North Sea[16] and in the Gulf of Riga.[17,18] For instance, in the Gulf of Riga, DON has been shown to be a major source of nitrogen for phytoplankton.[17] Nevertheless, the possible influence of DON on eutrophication and hypoxia has been neglected in most studies. Climate change may further enhance DON loading to coastal areas by increasing atmospheric deposition, runoff,[19] flooding, and ice melting. How climate change will affect dissolved oxygen dynamics, through temperature effects as well as altered DON loading, remains an open question, with potential consequences to the biodiversity, structure, and function of coastal systems.

Sea-surface temperatures in the Baltic Sea are forecasted to increase between 2 and 4 °C by the end of the century.[20] The primary objective of this study is to assess how metabolic rates (gross primary production (GPP), community respiration (CR), and bacterial production (BP)) respond to warming and how DON loading may modulate that response. Two of sources of DON were used: water from a boreal river and effluents from a wastewater treatment plant (WWTP). The response in metabolic rates was evaluated with and without DON enrichment, both by experimental warming and by seasonal variability.

METHODS

Sampling. A natural marine planktonic community from the Baltic Proper was collected 10 km off of the east coast of Öland, Sweden, at the Linnaeus Microbial Observatory (LMO, 56°55.851 N, 17°03.640 E). Water was sampled from a 2 m depth and filtered through a 150 μm net to remove large grazers.

For DON enrichment, we collected river water from Emån River, which drains into the Baltic Proper, and effluent from the WWTP in Kalmar. The Emån catchment area is dominated by boreal forest (69%), and 2% is peat-land.[21] Samples for DON enrichment were filtered using precombusted (450 °C, 4 h) glass-fiber filters (GF/F Whatman) and 0.2 μm membrane filters and frozen until the start of the experiment. All equipment used for handling the samples was acid-washed.

Warming Experiments. *Treatments.* A total of two warming experiments were performed: one in the summer (beginning of September 2013) and one in the winter (March 2014). Each experiment had two DON treatments, one amended with river water and one amended with WWTP effluent, as well as one control (seawater). These treatments were incubated at four different temperatures (−2, 0, + 2, and +4 °C from the sampling temperature, which was 18 °C in the summer and 4 °C in the winter), leading to a total of 12 treatments (Figure S1). The DON treatments consisted of 1:10 volume/volume of DON source to seawater. An autoclaved sea-salt solution[22] was added with the DON to keep salinity constant across treatments.[22]

Metabolic Rates. Community respiration was assessed by oxygen consumption as measured by Winkler titrations (i.e., CR was calculated as the difference between the initial oxygen concentration and the oxygen concentration after incubation in darkness) and reported in mmol $O_2 \cdot m^{-3} \cdot day^{-1}$. Water was carefully siphoned into 55 mL narrow-mouth Winkler bottles. Bottles for measuring initial oxygen concentration were fixed immediately (nine to ten bottles per treatment). Another five to six bottles for each treatment were incubated in the dark at the four different temperature regimes in controlled temperature chambers. Incubations lasted for 24 and 48 h in the summer experiment and 48 and 72 h in the winter experiment. Dissolved oxygen in the bottles was fixed immediately and analyzed by high-precision Winkler titration, following Carritt and Carpenter,[23] using a precise automated titration system with potentiometric (redox electrode) end point detection (Mettler-Toledo DL28 titrator).[24]

Additional 2 L bottles were incubated to determine nutrient content, bacterial production, and bacterial community composition. Bacterial production was measured for initial samples as well as after 24 and 48 h in the summer experiment and after 48 and 72 h in the winter experiment. BP was estimated by measuring incorporation of ^{3}H-leucine following Smith and Azam.[25] Water samples (1.5 mL, 3 replicates and 1 killed control sample with 5% trichloroacetic acid (TCA)) were incubated 60 min with 98.8 nM of ^{3}H-leucine (13.4 Ci $mmol^{-1}$). The incubation was terminated by adding TCA (5% final concentration). The samples were then centrifuged at 16 000*g* for 10 min, and the bacterial pellet was washed once with 5% TCA and once with 80% ethanol. After the supernatant was discarded, 0.5 mL of scintillation cocktail (Ecoscint A, Kimberly Research) was added and ^{3}H activity measured on a Beckman LS 6500 scintillation counter. BP was calculated assuming a leucine-to-carbon conversion factor of 1.5 kg C mol^{-1} leucine.[26]

Experiments Conducted at in Situ Temperature. *Treatments.* A total of eight experiments were performed during the seasons of summer (three), spring (two), autumn (two), and winter (one). Each experiment consisted of five different treatments, with additions of DON amendments (four experiments with river water and four experiments with WWTP effluents). Of these, one DON treatment consisted of a 1:10 ratio of DON source to seawater, and a second DON treatment had a 1:5 ratio of DON source to seawater. There was also a treatment with the addition of the same concentration of inorganic nutrients (nitrate, nitrite, and phosphate) that were contained in the DON 1:5 treatment (IN). Finally there was a control (C) treatment with only seawater and a diluted control (CD) consisting of seawater diluted with autoclaved Milli-Q water to the same portion of community that the 1:10, 1:5, and IN treatments had (Figure S1). To keep salinity constant in all treatments, we added a salt solution with the amendments and dilutions.

When evaluating the experiments, treatments were grouped into three different types: no addition, including both C and CD; DON addition, including 1:10 and 1:5 treatments; and IN. DON addition treatments were divided into river additions and WWTP effluent additions.

Metabolic Rates. Changes in the dissolved oxygen (DO) in closed bottles were assumed to result from the biological metabolic processes and to represent net community

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production (NCP = GPP − CR). Water from the respective treatments was siphoned carefully into 2.3 L glass bottles sealed with gas-tight stoppers. Bottles were incubated at the in situ temperature in temperature-controlled chambers during 1 week. Oxygen was measured every minute using optical oxygen sensors (optodes) and a ten-channel fiber optic oxygen transmitter (Oxy-10, PreSens).

Incubations were performed under natural light regime, illuminated by artificial light (OSRAM L36W/865 Lumilux Daylight), with a mean photosynthetically active radiation (PAR) intensity of 1373.2 μW/cm^2. Light hours ranged from 7 h 20 m in the autumn experiment (performed in December 2013) to 17 h 10 m in the spring experiment (performed in June 2013). Due to the large difference in light hours between experiments, GPP was standardized by light hours (GPP/light hours).

During the night, changes in DO are produced by respiration because in the absence of light, no photosynthetic production occurs. CR was calculated from the rate of change in oxygen at night, from half an hour after lights went off to half an hour before light went on (NCP in darkness equaled CR). NCP was calculated from the rate of change in DO, at 1 min intervals, accumulated over each 24 h period. Assuming that daytime CR equals that during the night, GPP was estimated as the sum of NCP and CR. To derive daily metabolic rates, we accumulated individual estimates of GPP, NCP, and CR resolved at 1 min intervals over each 24 h period during experiments and reported them in mmol $O_2 \cdot m^{-3} \cdot day^{-1}$. BP was measured on days 0, 1, 3, 5, and 7.

Methods for water chemistry and bacterial community composition are given in the Supporting Information.

Calculation of Activation Energies and Q_{10} values. Arrhenius plots of the natural logarithm of the given metabolic rate against the inverse of the temperature (Kelvin) multiplied by the Boltzmann's constant (8.62×10^{-5} eV k^{-1}) were used to calculate activation energies (E_a). An estimation of the activation energy for GPP, CR, and BP (units of eV) was derived from the slope of the Arrhenius plot. E_a was converted from eV to J mol^{-1} using a conversion factor of 96 486.9.

The Q_{10} (the relative rate of change in a given metabolic rate expected for a 10 °C temperature increase) was calculated by fitting the equation proposed by Raven and Geider:[27]

$$Q_{10} = e^{10E_a/RT^2}$$

where R is the gas constant (8.314472 mol^{-1} K^{-1}), T is the mean absolute temperature across the range over which Q_{10} was measured (K), and E_a is the activation energy (J mol^{-1}).[27]

Statistics. The data from both warming experiments were combined in a single analysis to test for the relationship between metabolic rates (the natural logarithm) and the inverse of temperature ($1/kT$) by a mixed-effects model. To account for temporal pseudoreplication, we used the sampling date as a random effect. We used sampling treatments (addition of DON from rivers (river), from effluent (WWTP), and from the control (seawater)) as fixed factors.

Additionally, linear mixed-effects models were used to test for relationships between temperature and metabolic rates using data from experiments conducted at the in situ temperature. To account for temporal pseudoreplication in the statistical model, we included the sampling dates as random effects and jar identity to account for community pseudoreplication. In addition, DON treatments (addition of DON from rivers (river) and from effluent (WWTP)), no addition (seawater), or inorganic nutrients additions (IN)) were used as fixed factors to check differences between treatments. We used mixed-effects models to test for relationships between temperature and metabolic rates because these models allow accounting for temporal and community pseudoreplication by using sampling dates and jar identity as random effects. Mixed-effects models have been used in similar studies previously.[28−30] We used a posthoc comparison using the generalized linear hypothesis test (glht) with "Tukey" to test for significant differences between treatments. The pseudo-R^2 of the models were calculated following Xu 2003.[31] All statistical tests were made using R statistical software.

RESULTS

Water Chemistry. Nutrients content in seawater and rivers and WWTP effluent are shown in Table S1. Nutrient content in WWTP effluent waters was much higher than in the river water (Table S1). Conversely, dissolved organic carbon (DOC) content in river waters was higher than in WWTP effluents. Nutrient content in seawater tended to be higher during the winter months and decreased in the summer (Table S1).

Warming Experiments. Incubation temperatures in the warming experiments ranged from 1.7 °C (−2 °C treatment in the warming experiment performed in winter) to 22.6 °C (+4 °C treatment in the experiment performed in the summer). Different treatments showed a different dependence with temperature; the mixed-effect model results showed significant differences in the slope ($p < 0.03$) and in the intercept ($p < 0.03$) of the relationship between the natural logarithm of planktonic community respiration and the inverse of the temperature ($1/kT$) for the different treatment types (control, river addition, and WWTP effluent addition). Activation energies for CR derived from the model were 0.82 ± 0.22, 0.81 ± 0.30, and 1.29 ± 0.30 eV for seawater, river addition, and WWTP effluent addition, respectively (Figure 1a and Table 1). The experimental Q_{10} values for CR derived from the mixed-effects model for warming experiments were 3.10 ± 0.94, 3.07 ± 1.29, and 5.95 ± 2.49 for the seawater, river, and WWTP effluent addition, respectively. The posthoc comparison showed that seawater with the WWTP effluent addition had significantly higher activation energy than did the treatment with river addition ($p < 0.05$).

The slope and intercept of the relationship between the natural logarithm of bacterial production (BP) and the inverse of the temperature were significantly different for the different types of DON additions in the mixed effects model ($p < 0.01$ for both slope and intercept). Activation energies derived using the model were 0.92 ± 0.13, 0.70 ± 0.16, and 0.63 ± 0.16 eV for the control, river addition, and WWTP effluent addition, respectively (Figure 1b). The posthoc comparison showed that seawater with WWTP effluent had significantly lower activation energy for BP than for seawater alone ($p < 0.01$).

In the summer warming experiment, there were pronounced differences in the bacterial community composition between treatment extremes, i.e., the −2 compared to +4 °C treatments (Figure 2). The responses to different temperatures varied between amendments. In the river amendment, Gammaproteobacteria became dominant in the −2 and in situ treatments, in contrast to lower abundances of this group and higher abundances of Actinobacteria in the +2 and +4 treatments. In the effluent amendments, Actinobacteria and Bacteroidetes

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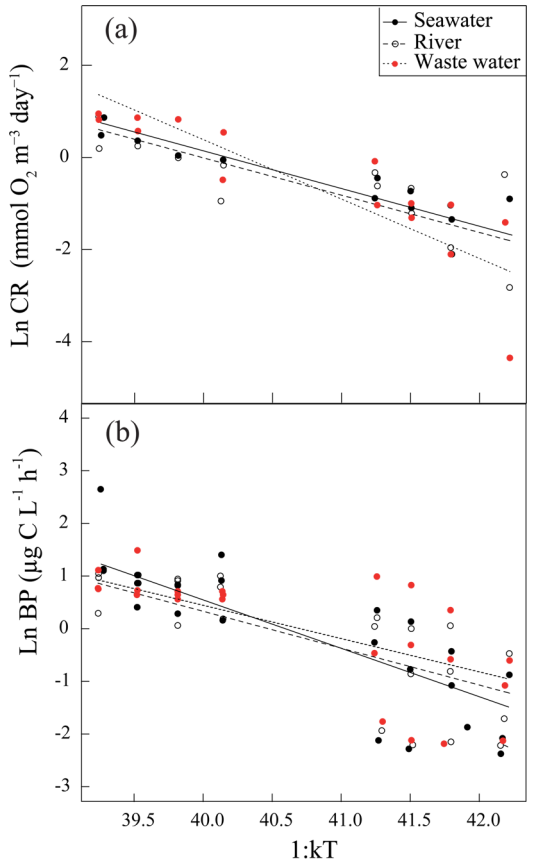

Figure 1. Results of mixed-effects models for warming experiments showing the effect of temperature ($1/kT$) on (a) planktonic respiration and (b) bacterial production. To account for temporal pseudoreplication, the statistical model includes sampling dates as fixed factors. Black dots represent data from seawater, white dots represent samples with DON addition from rivers, and red dots represent samples with DON addition from wastewater treatment plants.

became dominant at −2, while Gammaproteobacteria largely dominated at in situ temperatures and above (Figure 2).

Experiments Conducted at in Situ Temperatures. Incubation temperature ranged from 3.4 °C during the winter experiment with the addition of WWTP effluent in January 2013 to 19.8 °C in the summer experiment with the addition of river water in August 2013. Planktonic community respiration and gross primary production rates ranged over 2 orders of magnitude. The minimum measured CR and GPP were both 0.47 mmol $O_2 \cdot m^{-3} \cdot day^{-1}$, measured in January. The maximum measured CR and GPP rates were 152.17 and 154.08 mmol $O_2 \cdot m^{-3} \cdot day^{-1}$, respectively, measured in December.

Different types of DON additions had significantly different intercept ($p < 0.0003$) and slope ($p < 0.0002$) values for the relationship between the natural logarithm of CR and the inverse of the temperature in the mixed-effects model. Activation energies for planktonic CR derived from the model were 0.28 ± 0.15, 0.36 ± 0.18, 0.63 ± 0.17, and 0.33 ± 0.11 eV for seawater, river addition, WWTP effluent addition, and inorganic nutrient addition, respectively (Figure 3b). The Q_{10} values for CR derived from the activation energies obtained here were 1.48 ± 0.32, 1.65 ± 0.42, 2.38 ± 0.57, and 1.59 ± 0.23 for seawater, river addition, WWTP effluent addition, and inorganic nutrient addition, respectively (Table 1).

The slope and intercept of the relationship between the natural logarithm of GPP/light hours (GPP/lh) and the inverse of the temperature were significantly different for the different DON addition types (mixed-effects model, $p < 0.003$). Activation energies for GPP/lh were 0.15 ± 0.15, 0.34 ± 0.17, 0.41 ± 0.16, and 0.31 ± 0.12 eV for seawater, river addition, WWTP effluent addition, and inorganic nutrients addition, respectively (Figure 3a). The corresponding Q_{10} values for GPP/lh were 1.23 ± 0.25, 1.60 ± 0.38, 1.76 ± 0.39, and 1.53 ± 0.25 for seawater, river addition, WWTP effluent addition, and inorganic nutrients addition, respectively (Table 1). The posthoc comparison showed that WWTP effluent had significantly higher activation energies for CR and GPP/lh than did treatments without additions ($p < 0.03$ and $p < 0.04$, respectively).

The intercept or slope of the relationship between the natural logarithm of BP and the inverse of the temperature did not show significant differences between the different DON types or for treatments with and without DON additions ($p = 0.20$ and $p = 0.18$, respectively). Activation energies were 1.24 ± 0.16, 1.17 ± 0.18, 1.33 ± 0.17, and 1.37 ± 0.14 for seawater, river addition, WWTP effluent addition, and inorganic nutrients addition, respectively (Table 1). The activation energy for BP, calculated using data from all treatments, was 1.25 ± 0.13 eV (Figure 3c). The derived Q_{10} value for BP was 5.63 ± 1.02.

Across the experiments performed at in situ temperatures, temperature was significantly correlated with bacterioplankton community composition; 50% of the variance in community structure could be accounted for by absolute changes in temperature (MANTEL test, Pearson; $r = 0.50$, $p = 0.001$, $n = 101$). Also CR, BP, and Chlorophyll *a* were significantly correlated with community composition but with lower Pearson *r* values (Table S2). When analyzed according to type of amendment, the DON was significantly correlated to shifts in bacterioplankton community structure in the river amendments, whereas GPP, NO_2^-, and PO_4^{3-} were significantly correlated with changes in the bacterial community composition in wastewater. Generally, Bacteroidetes were abundant throughout the in situ experiments. In the summer months, both Verrucomicrobia and Cyanobacteria were more abundant compared to the results from the rest of the experiments performed in other seasons. Betaproteobacteria showed higher relative abundance in winter experiments (Figure 2).

DISCUSSION

The metabolic rates presented here increased with water temperature, as predicted by metabolic theory.[5,32] We found higher activation energies for CR than for GPP when standardized by light hours, supporting a larger increase in respiration rates than that in production rates with warming, as predicted previously.[5,33] The response in CR to temperature was stronger in treatments amended with WWTP effluent than for unamended seawater or seawater amended with river DON for both the in situ experiments and the warming experiments. Activation energies derived using mixed-effects models were higher in warming experiments than in experiments incubated at in situ temperature.

DON from WWTP effluent inputs significantly increased the responses of CR rates to warming. Effects of DON from rivers or inorganic nutrients inputs on the response of metabolic rates to warming were less pronounced. Inputs from both inorganic and organic nutrient inputs increased GPP responses to

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Table 1. Parameters, Their Standard Errors, and Statistics for the Fitted Mixed Effects Models for Arrhenius Plots and Q_{10} Values with Their Associated Standard Error Calculated Using Error Propagation[a]

type of experiment	treatment	metabolic rate	intercept	SE	E_a (eV, negative slope)	SE	Q_{10}	SE[a]	N	AIC	pseudo-R^2
in situ	seawater	CR	14.51	6.33	0.28	0.15	1.48	0.32	555	724.3	0.67
in situ	river	CR	17.61	7.51	0.36	0.18	1.65	0.42	555	724.3	0.67
in situ	effluent	CR	28.34	7.05	0.63	0.17	2.38	0.57	555	724.3	0.67
in situ	IN	CR	16.21	4.37	0.33	0.11	1.59	0.23	555	724.3	0.67
in situ	seawater	GPP	6.60	6.05	0.15	0.15	1.23	0.25	535	791.4	0.79
in situ	river	GPP	14.21	6.95	0.34	0.17	1.60	0.38	535	791.4	0.79
in situ	effluent	GPP	17.02	6.59	0.41	0.16	1.76	0.39	535	791.4	0.79
in situ	IN	GPP	12.76	4.86	0.31	0.12	1.53	0.25	535	791.4	0.79
in situ	seawater	BP	50.65	6.54	1.24	0.16	5.57	1.23	227	334.3	0.93
in situ	river	BP	47.79	7.22	1.17	0.18	5.03	1.23	227	334.3	0.93
in situ	effluent	BP	54.52	6.94	1.33	0.17	6.29	1.47	227	334.3	0.93
in situ	IN	BP	55.82	5.75	1.37	0.14	6.67	1.30	227	334.3	0.93
in situ	all	BP	51.04	5.37	1.25	0.13	5.63	1.02	227	340.7	0.91
warming	seawater	CR	32.88	8.89	0.82	0.22	3.10	0.94	41	95.6	0.79
warming	river	CR	32.41	12.41	0.81	0.30	3.07	1.29	41	95.6	0.79
warming	effluent	CR	18.26	8.51	1.29	0.30	5.95	2.49	41	95.6	0.79
warming	seawater	BP	37.32	5.28	0.92	0.13	3.57	0.64	73	107	0.92
warming	river	BP	28.34	6.62	0.70	0.16	2.64	0.59	73	107	0.92
warming	effluent	BP	25.74	6.62	0.63	0.16	2.40	0.54	73	107	0.92

[a]"In situ", experiments conducted at in situ temperature; river, river water addition; effluent, wastewater treatment plant effluent addition; IN, inorganic nutrient addition; N, number of observations. [a]Standard error.

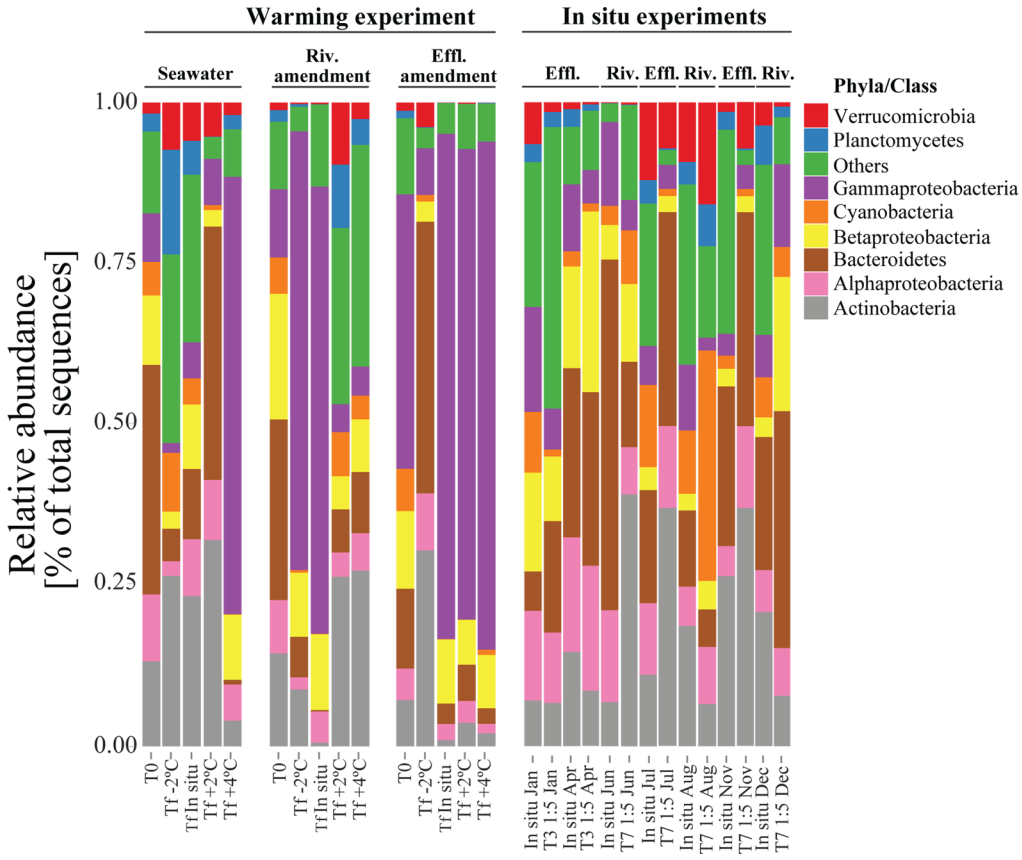

Figure 2. Dynamics in bacterioplankton community composition for the warming and in situ experiments indicated by the relative abundance of major bacterial groups at the phyla and class levels.

warming. This response is steeper for WWTP effluent inputs, followed by river inputs and, finally, inorganic nutrients. WWTP effluent inputs have labile organic matter that can stimulate both primary production and respiration rates with warming. Conversely, river inputs or inorganic nutrient additions did not affect responses of CR rates to warming. Previous studies found a DON lability up to 96% for WWTP effluents[34] and an estuarine bacterial utilization of 30−60% of

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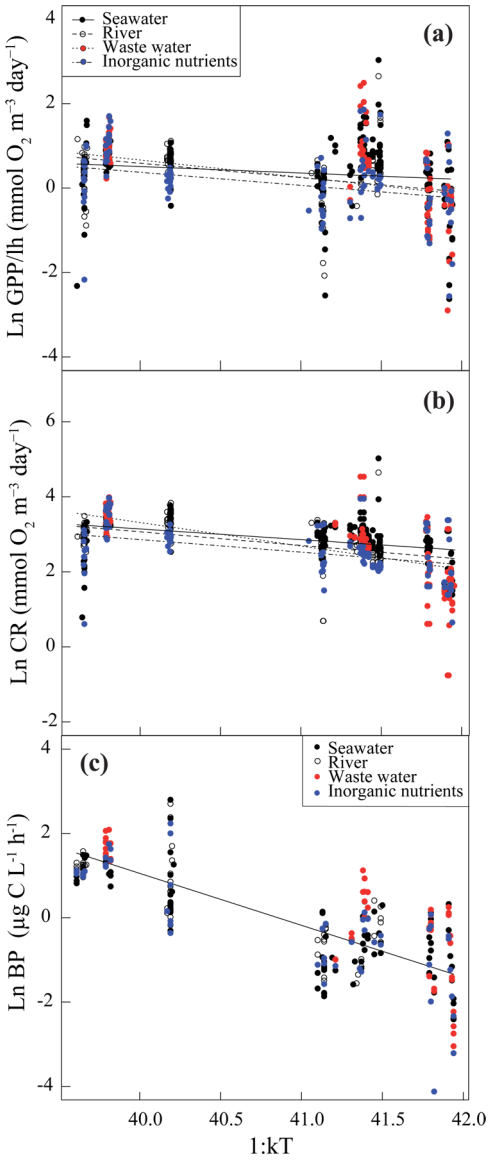

Figure 3. Results of mixed-effects models for experiments performed at the in situ temperature showing the effect of temperature ($1/kT$) on planktonic (a) gross primary production, (b) community respiration, and (c) bacterial production. To account for temporal pseudoreplication, we included in the statistical model (as fixed factors) the sampling dates and jar identity to account for community pseudoreplication. Black dots represent data from seawater, white dots represent samples with DON addition from rivers, red dots represent samples with DON addition from waste water treatment plants, and blue dots represent samples with inorganic nutrient additions.

riverine humic N,[35] regenerating DON and contributing to increased phytoplankton production.[36] The present study adds important insight by demonstrating the synergistic effects of temperature and DON inputs on the stimulation of metabolic rates.

In addition to inorganic and organic nutrients, the amendments may also have carried trace elements that potentially affect the biota. Although such additions may relieve microorganisms from limitation by certain trace elements, we believe that to be unlikely because the water from the Baltic Sea is so heavily influenced by riverine inputs and has high concentrations of, e.g., iron.[37] Amendments may also carry toxic trace elements, such as cadmium (Cd),[38] lead (Pb), or zinc (Zn),[39] that could potentially have restrained the metabolic response to bioavailable DON.

The CR activation energies derived from the warming experiments for natural communities (0.82 ± 0.22 eV) are comparable to the CR activation energy derived from a warming experiment in the Barents Sea (0.85 eV)[40] but lower than that reported for other warming experiments (Table 2). These differences may be related to the computation method. Differences in activation energies are observed when using mixed-effect models compared to the activation energies derived for each individual warming experiment.[28] Indeed, most of the reported activation energies derived as the average of individual experiments had similar values: 1.17 ± 0.42,[28] 1.17 ± 0.06,[41] and 1.19 ± 0.21 eV.[42]

Conversely, experiments incubated at in situ temperature had lower CR activation energies (E_a) for natural communities (0.28 ± 0.15 eV). This E_a can be compared with the activation energy for whole-ecosystem respiration (both benthic and planktonic) for a Mediterranean bay (0.27 ± 0.06 eV[43]) and with the activation energy for both the heterotrophic and the autotrophic respiration rates derived using theoretical models by López-Urrutia el al.[44] (0.28 ± 0.004 eV), but it tended to be lower than most E_a derived using incubations at the in situ temperature or from theoretical models found in the literature (Table 2).

Activation energy for GPP/lh on natural communities was lower than previously reported (Table 2). This could be related to the fact that GPP was standardized by light hours. Previous studies did not standardize by light hours; however, due to the large variation in light hours at higher latitudes in the Baltic Sea, we standardized them to discern between the effect of temperature and illumination. Activation energy for GPP without standardization by light hours was 0.43 ± 0.19 eV. GPP/lh. Q_{10} values derived here for natural communities (1.23 ± 0.25) fall between previously reported ones: 0.2−9.1 for individual warming experiments for Mediterranean coastal communities,[28] 0.69−3.03 for temperate planktonic communities,[41] or 2.7 for temperate microplankton communities.[45]

Differences in activation energies derived here and previously published ones could partly be accounted for by methodological differences on the calculation of activation energies. Also, some of these differences could be related to resource supply and differences in bacterial community composition (Figure 2). Incubation temperatures influence activation energies with a suggested range for communities living at intermediate temperatures proposed to be 0.41−0.74 eV.[5,32] Results derived here when using warming experiments are higher than those proposed for temperate areas, and activation energies derived using in situ temperature incubations had lower activation energies than those previously proposed.

Our results derived from experiments performed at in situ temperature suggest that a future 4 °C warming of surface waters of the Baltic Sea may yield a 15% increment in respiration rates of natural communities, almost double the 8% increment expected for primary production (Figure 4a). Inputs of WWTP effluents would increase this increment to 29%, compared to an increase in GPP of 20% (Figure 4a). Higher increases in respiration rates than in primary production with warming would drive the system further toward net heterotrophy and higher oxygen consumption. These increases would be aggravated with effluent inputs that would provide extra substrate for respiration. Warming experiments yield a higher

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Table 2. Activation Energies for Community Respiration and Gross Primary Production from the Literature[a]

study	area	type of experiment	community	method	metabolic rate	E_a (eV)	SE
this study	Baltic Sea	warming	planktonic	MEM	CR	0.82	0.22
Holding et al., 2013[40]	Barents Sea	warming	planktonic	IE	CR	0.85	–
Vaquer-Sunyer and Duarte 2013[28]	Mediterranean Sea	warming	planktonic	MIE	CR	1.17	0.42
Vaquer-Sunyer and Duarte 2013[28]	Mediterranean Sea	warming	planktonic	MEM	CR	0.34	0.36
Yvon-Durocher et al. 2012[29]	global	warming	estuarine pelagic	MEM	CR	0.59	–
Lefevre et al. 1994[41]	Menai Strait	warming	planktonic	MIE	CR	1.17	0.06
Vaquer-Sunyer et al. 2010[42]	Arctic Ocean	warming	planktonic	MIE	CR	1.19	0.21
García-Corral et al. 2014[58]	Atlantic Ocean	warming	planktonic	MSAE*	CR	1.64	0.36
This study	Baltic Sea	in situ	planktonic	MEM	CR	0.28	0.15
Vaquer-Sunyer et al. 2012[43]	Mediterranean Sea	in situ	benthic and planktonic	IE	CR	0.27	0.06
García-Corral et al. 2014[58]	Atlantic Ocean	in situ	planktonic	IE	CR	1.45	0.6
Mazuecos et al. 2015[59]	South Atlantic and Indian Ocean	in situ	mesopelagic planktonic	IE	CR	0.9	0.29
Regaudie-de-Gioux and Duarte, 2012[60]	global	in situ	planktonic	AP	CR/Chl a	0.66	0.04
López-Urrutia et al. 2006[44]	global	TM	heterotrophic and autotrophic	TM	CR	0.28	0.00
López-Urrutia et al. 2006[44]	global	TM	heterotrophic	TM	CR	0.65	–
This study	Baltic Sea	in situ	planktonic	MEM	GPP/lh	0.15	0.15
García-Corral et al. 2014[58]	Atlantic Ocean	in situ	planktonic	MSAE*	GPP	1.43	0.74
Regaudie-de-Gioux and Duarte, 2012[60]	global	in situ	planktonic	AP	GPP/Chl a	0.32	0.05
López-Urrutia et al. 2006[44]	global	TM	autotrophic	TM	autotrophic processes	0.32	–
López-Urrutia et al. 2006[44]	global	TM	planktonic	TM	NP	0.29	–
Yvon-Durocher et al. 2010[30]	global	Warming	freshwater	MEM	GPP	0.45	–

[a]The asterisk indicates 1 °C temperature-binned CR. Only significant results were used to calculate the mean. MEM, mixed effects model; IE, Individual experiment; MIE, mean of individual experiments; TM, theoretical model; MSAE, mean seasonal activation energy; AP, Arrhenius plot of 1 °C temperature-binned; NP, net production.

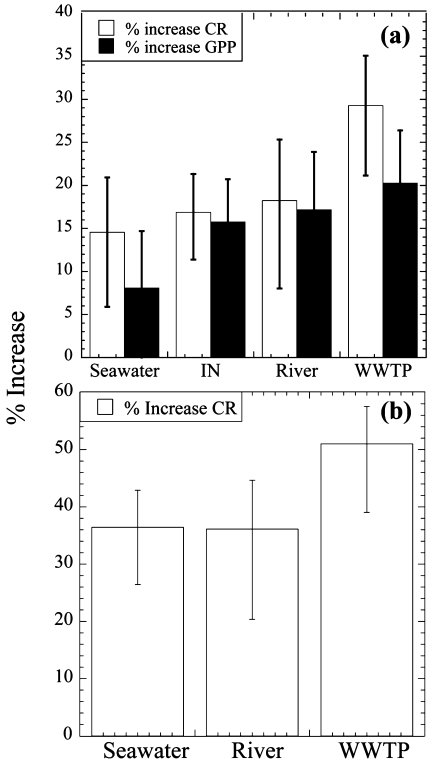

Figure 4. Changes on metabolic rates (gross primary production and community respiration) with a 4 °C water warming calculated from Q_{10} values derived from (a) experiments conducted at the in situ temperature and (b) warming experiments.

increase in CR with 4 °C warming, leading to an increase of CR of 36.4%, and to a 51% increase with the addition of DON from WWTP effluent (Figure 4b).

The differences in activation energies and Q_{10} values between experiments conducted at in situ temperature and experiments with experimental warming may be related to the rate of change in temperature. Whereas warming experiments increase temperature abruptly, not allowing for the acclimation of the organisms, in the experiments performed at in situ temperature, the communities are acclimatized. Part of the differences in activation energies between the different types of experiments could be explained by incubation temperatures. Responses of metabolic rates to warming depend on the range of temperatures assessed.[46] Typically, Q_{10} values are higher at the low end of the natural temperature regime,[47] with winter experiments producing higher Q_{10} values than do experiments performed in the summer. Seasonal variation of metabolic rates is also affected by dynamics in resources, bacterial community composition, and grazers. Experiments performed at in situ temperature may give more realistic responses to seasonal temperature but fail in predicting possible effects of warming because thermal windows of organisms are not exceeded. Using both types of experiments provides a wider overview of the potential effects of warming on metabolic rates and their interactions with DON additions. Gathering different results when using warming experiments compared to those when using data collected at the in situ temperature would provide contrasting results on the effects of warming on marine metabolic rates, depending on the method used. Caution is needed to interpret and extrapolate the forecasts of the effects of future global warming on metabolic rates in the near future.

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Although the rate of change of community respiration and primary production differs with the experimental approach, the pattern is consistent, with respiration increasing faster than production with warming. Because WWTP organic matter inputs will accentuate the increase in metabolic rates with warming, actions to reduce such inputs are needed to avoid worsening the consequences of climate change in the Baltic Sea.

Bacterial production increased faster with warming in seawater than when DON was added in warming experiments. BP in the treatment with WWTP effluent was high at in situ temperature, and increasing the temperature did not increase the rate as quickly as for the seawater treatment. This difference in the response of BP could potentially be explained by the different lability of the organic matter (OM). Whereas WWTP effluent OM appears to be very labile for bacteria, allowing for high BP at the in situ temperature, seawater OM could be less labile, and the increased temperatures could favor its utilization by bacteria. Previous studies have shown divergent lability of wastewater effluents, varying from 1.7%[48] to 96%[34] of the DON removed by microbial communities in biological experiments.

In the summer warming experiment and experiments performed at in situ temperatures, higher temperatures were coupled with higher metabolic rates but also linked with shifts in bacterioplankton community composition. The temperature effect for structuring bacterial community composition occurred across a very wide range of temperatures (3−20 °C) and agrees with previous studies demonstrating the importance of temperature in regulating bacterioplankton community composition.[49,50] The dynamics of bacterioplankton populations in our experiments were consistent with the in situ dynamics at the sampling location, where, e.g., Verrucomicrobia and Cyanobacteria are dominant in the summer.[51]

For experiments conducted at in situ temperature, there were no significant differences between treatments, and an overall Q_{10} value for BP of 5.6 ± 1.0 was derived. This Q_{10} value is higher than that previously reported, with 1.5−2.8 for Arctic communities[52] or 2.5 for coastal Mediterranean communities.[53] The Q_{10} value derived here for BP is higher than that for primary production. A previous study suggested a greater effect of temperature on bacterial carbon demand than on primary production,[54] with more carbon being used by the microbial community. Another study based on a warming mesocosm experiment in the Baltic Sea suggested a change in food web structure with warming, increasing the flow of organic matter through the microbial loop,[55] and a consequent reduction of the transfer to higher trophic levels and of the efficiency of the biological carbon pump in sequestering carbon. Additional mesocosm experiments in the Baltic Sea showed that warming or terrestrial dissolved organic matter (TDOM) inputs increased bacterial production rates and reduced primary production, while a combination of both warming and TDOM inputs amplified these effects.[56] The same study also showed increased zooplankton biomass and fish growth and subsequent food web efficiency when the temperature and TDOM were increased simultaneously.[56] Increased bacterial production and zooplankton and fish biomass with labile carbon additions were also documented in a lake mesocosm experiment.[57] Both studies suggest that bacterial-dominated systems are efficient in transferring carbon to top consumers. Our results support an increased flow of organic matter to bacteria with warming and a possible change to heterotrophic-dominated communities in a warmer world.

The results presented here support the prediction that warming of the Baltic Sea will increase planktonic respiration rates and bacterial production faster than it will increase planktonic primary production. This implies higher biological oxygen consumption than production, which may lead to the depletion of the oxygen pool, further aggravating hypoxia in the Baltic Sea.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00674.

Methods for water chemistry and bacterial community composition determination; tables with information on water chemistry and the MANTEL test of correlations between absolute shifts in bacterioplankton community composition and physicochemical conditions and metabolic rates for the in situ experiments; a scheme of the experimental setup. (PDF)

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Notes

The authors declare no competing financial interest.

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