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LETTER

The cycling of glycine betaine and homarine in marine microbial communities: Quantitative flux measurements and the role of competitive uptake inhibition

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Scientific Significance Statement

Dissolved metabolites are the currency for exchanges of carbon, nutrients, chemical structures, and energy among members of marine microbial communities. However, the qualitative and quantitative importance of specific metabolites within these communities is largely unknown, limiting our understanding of the role these compounds play in microbial interactions and biogeochemical cycles. We combine dissolved metabolite concentration measurements with uptake kinetics experiments to quantify the fluxes of two compounds, glycine betaine and homarine, through the dissolved pool across diverse marine environments. Compound fluxes were correlated with their particulate concentrations and equivalent to up to 1% of net primary production. The presence of structurally similar compounds decreased homarine uptake, suggesting uptake competition may be an important factor regulating dissolved metabolite concentrations and cycling in the environment.

Abstract

The flux of carbon through the labile dissolved organic matter (DOM) pool supports marine microbial communities and represents the fate of approximately half of marine net primary production (NPP). However, the behavior of individual chemical structures that make up labile DOM remain largely unknown. We performed 12 uptake kinetics and two uptake competition experiments on the abundant betaine osmolytes glycine betaine (GBT) and homarine. Combining uptake kinetics with dissolved metabolite measurements, we quantified fluxes through the DOM pool. Fluxes were correlated with particulate concentrations and ranged from 0.53 to 41 and 0.003 to 0.54 nmol L^{-1} d^{-1} for GBT and homarine, respectively, equivalent to up to 1.2% of NPP. Turnover times of dissolved GBT and homarine ranged from 1 to 57 d. Betaines and sulfoniums such as dimethylsulfoniopropionate competitively inhibited homarine uptake. Our results quantify GBT and homarine cycling and suggest an important role for uptake competition in regulating dissolved metabolite concentrations and fluxes.

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As a part of the dissolved organic matter (DOM) pool, dissolved metabolites collectively mediate the exchange of nutrients, energy, and chemical structures in marine microbial communities (Moran et al. 2022). The most abundant metabolites measured in marine microbial communities are osmolytes that organisms synthesize in response to osmotic stress (Welsh 2000; Johnson et al. 2020; Boysen et al. 2021; Heal et al. 2021). Among these abundant osmolytes are the betaines glycine betaine (GBT) and homarine, which often are among the 10 most abundant characterized metabolites in marine particulate (GBT: 0.39–73 nmol L^{-1}, homarine: 0.2–2.7 nmol L^{-1}) and dissolved (GBT: 2.8–5.2 nmol L^{-1}, homarine: 0.62–1.7 nmol L^{-1}) metabolomes (Keller et al. 2004; Beale and Airs 2016; Johnson et al. 2020; Boysen et al. 2021; Heal et al. 2021; Sacks et al. 2022; Airs et al. 2023; Dawson et al. 2023) (Fig. 1A). In addition to serving as osmolytes and growth substrates for marine microbes such as SAR11 and roseobacters (McParland et al. 2021; Clifton et al. 2024), GBT and homarine can act as sources of nitrogen, be building blocks for other metabolites (Boysen et al. 2022), serve as methyl donors (Boysen et al. 2022; Sperfeld et al. 2024), facilitate organismal interactions as bioactive molecules and chemotactic signals (Kokoeva et al. 2002; Seymour et al. 2010; Poulin et al. 2018), and serve as precursors to climate active gases such as methane (Jones et al. 2019; Li et al. 2021). Their high concentrations suggest that these compounds play important roles in marine biogeochemical cycles, but quantitative estimates of their fluxes do not exist. Furthermore, the biological and chemical controls on their uptake rates, kinetic profiles, and concentrations are largely unknown.

Uptake studies of GBT and dimethylsulfoniopropionate (DMSP) have provided insights into the rates of and controls on the cycling of betaines and their sulfur analogs, sulfoniums. Marine bacterial communities display high affinities for GBT and DMSP with low half-saturation constants (K_t)

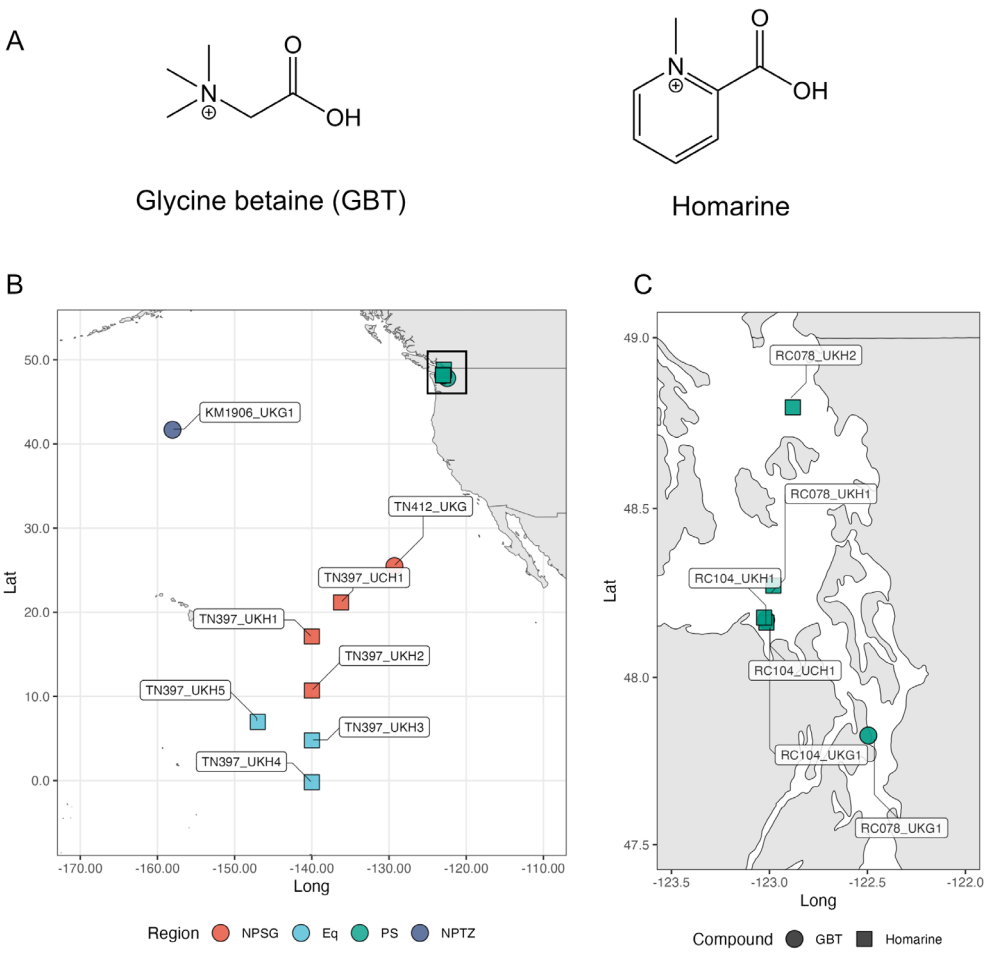

Fig. 1. Structures of glycine betaine (GBT) and homarine (**A**). Map of experiment locations in the North and Equatorial Pacific (**B**) with inset focused on Puget Sound (**C**). The shape of the symbol indicates the compound investigated in the uptake experiment while the color indicates the ocean region (North Pacific Subtropical Gyre (NPSG); equatorial upwelling (Eq); Puget Sound (PS); North Pacific Transition Zone (NPTZ)) where the experiment was performed.

ranging from approximately 1–79 nmol L^{-1} (Kiene et al. 1998; Boysen et al. 2022; Mausz et al. 2022), similar to measured dissolved concentrations in the low nmol L^{-1} range (Kiene and Slezak 2006; Sacks et al. 2022). The correspondence between K_t values and dissolved concentrations led to the hypothesis that the concentration of a compound is controlled by the affinity of the community's transporters for that compound (Moran et al. 2022). However, this interpretation is complicated by the fact that GBT and DMSP are taken up by a wide range of marine microbes including *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and diatoms, which often have higher K_t values than oligotrophic heterotrophic bacteria (Vila-Costa et al. 2006; Petrou and Nielsen 2018; Torstensson et al. 2019). Additionally, GBT, DMSP, and other betaines and sulfoniums act as competitive inhibitors to each other's uptake, suggesting that a compound's cycling is partially controlled by the concentrations of other metabolites present (Kiene and Gerard 1995; Kiene et al. 1998; Vila-Costa et al. 2006; Noell and Giovannoni 2019). Homarine was only recently recognized as an important component of marine metabolomes and to our knowledge, no studies exist describing its transporters or cycling (Heal et al. 2021). Here we characterize community level uptake kinetics of GBT and homarine, identify competitive inhibitors to homarine uptake in marine microbial communities, and quantify the fluxes of these compounds through the DOM pool.

Methods

Study sites and environment characterization

Experiments were performed on five cruises: KM1906 (Spring 2019), TN397 (Fall 2021), and TN412 (Winter 2023) that spanned the equatorial upwelling (Eq), North Pacific Subtropical Gyre (NPSG), North Pacific Transition Zone (NPTZ), and RC078 (Summer 2022) and RC104 (Summer 2023) in Puget Sound (Fig. 1B,C). Samples were collected for particulate organic carbon (POC), particulate nitrogen (PN), chlorophyll *a* (Chl *a*), nitrate and nitrite (N + N), and primary productivity. Locations, dates, and analyses are in supplemental methods and Supplemental Table 1.

Uptake kinetics and competition experiments

For TN397, TN412, RC078, and RC104, seawater was collected through the ship underway flowthrough systems and prefiltered through 100 μm mesh. Triplicate samples were spiked with seven different concentrations of isotopically labeled substrate ($^2H_3$-homarine, $^{13}C_5$,$^{15}N_1$-GBT) ranging from 0 to 1000–5000 nmol L^{-1}, depending on expected in situ concentrations (Supplemental Table 2), and incubated for 30 min in temperature and light-controlled incubators designed to mimic mixed layer conditions (approximately 50% light shading and in situ temperatures). Samples were collected using peristaltic pumps onto PVDF membrane filters, flash frozen in liquid nitrogen, and stored at −80°C. Blanks were collected for each concentration by refiltering the filtrate onto a new filter. For dissolved metabolite analysis, 40 mL of filtrate from the 0 nmol L^{-1} treatments was collected in acid washed 50 mL polypropylene Falcon tubes and frozen at −20°C. The uptake kinetic experiment on KM1906 is detailed in Boysen et al. (2022). Uptake competition experiments (TN397_UCH1, RC104_UCH1) were identical to uptake kinetics experiments except the spike was 50 nmol L^{-1} of $^2H_3$-homarine and 100 nmol L^{-1} of unlabeled competitor (GBT, DMSP, TMAO, trigonelline, or glucose). Additional details are in the supplemental methods.

Metabolite extraction, data acquisition, and quantification

Extraction, analysis, and quantification of KM1906 samples are detailed in Boysen et al. (2022). Other particulate extractions were performed with 40 : 40 : 20 : 0.1 methanol : acetonitrile : water : formic acid solution (Canelas et al. 2009). Dissolved metabolites were extracted using cation-exchange solid phase extraction and quantified as in Sacks et al. (2022). Dissolved and particulate metabolites were measured using liquid chromatography-mass spectrometry (supplemental methods, data available at Sacks 2025). Standard curves were used to quantify labeled GBT and homarine. Blanks showed a linear relationship with spike concentration across all experiments (Supplemental Fig. 1). We used this relationship to calculate the expected concentration of labeled compound on the filter not due to biological uptake and subtracted it from our measured concentrations. Unlabeled particulate metabolites were quantified using internal standards added during reconstitution. One limitation of our approach is that we only measure the concentration of the intact, labeled substrate and therefore may underestimate the uptake of the compound if it is rapidly transformed or catabolized. However, ^{14}C and ^{13}C tracer experiments suggest that catabolism of GBT (and homarine) is minimal after 30 min (Kiene and Williams 1998; Boysen et al. 2022).

Uptake kinetics, flux, and competition calculations

Uptake kinetics parameters were calculated using the Michaelis–Menten (MM) equation (Eq. 1),

$$V_A = \frac{V_{max}(A)}{K_t + S_n + A} \quad (1)$$

where V_A is the uptake rate of the labeled compound, V_{max} is the maximum uptake rate, K_t is the half saturation constant, S_n is the in situ (natural) substrate concentration, and A is the concentration of added labeled compound. These models were fitted using a non-linear least-squares (NLS) approach. Assuming steady state conditions, fluxes through the dissolved phase were calculated as the in situ uptake rate using the MM equation and measured in situ concentrations. Turnover times (TTs) were estimated by dividing the dissolved in situ concentration by the flux. V_{max}, $K_t + S_n$, and TT were also estimated using Wright–Hobbie (WH) plots (Wright and Hobbie 1966). Non-linear least-squares and WH derived MM

parameters were tightly correlated and fell largely on the 1 : 1 line, suggesting our results are robust to changes in model-fitting approach or bias introduced by dissolved concentration measurements (Supplemental Fig. 2). We report the NLS results in the main text. We estimated errors for K_t, V_{max}, and TT using Monte Carlo simulations that sampled the analytical error around each datapoint and calculated the mean and standard deviation of all non-outlier models (Boysen et al. 2022). Uptake inhibition was assessed by comparing the measured uptake rate of $^2H_3$-homarine in the presence of the potential competitor to the uptake rate in the presence of glucose, a "negative control" that we predicted would not impact homarine uptake based on experiments with GBT, and a "no addition" control where only $^2H_3$-homarine added (Kiene 1998; Kiene et al. 1998; Noell and Giovannoni 2019).

Results and discussion

Environmental conditions

Experiments spanned a wide range of biogeochemical gradients (Supplemental Table 1). The oligotrophic NPSG was defined by low biomass (0.073–0.097 mg m^{-3} Chl *a*), low nutrient concentrations (0.017–0.058 μmol L^{-1} N + N), and low productivity (307–456 nmol C L^{-1} d^{-1}). The Eq and NPTZ had intermediate levels of biomass (0.14–0.20 and 0.85 mg m^{-3} Chl *a*, respectively), nutrients (2.9–6.6 and 6.4 μmol L^{-1} N + N, respectively), and productivity (1320–1630 and 675 nmol C L^{-1} d^{-1}, respectively). The coastal/estuarine PS had high but variable biomass (0.73–1.6 mg m^{-3} Chl *a*) and nutrients (1.2–22 μmol L^{-1} N + N), and high productivity (median value of 16,700 nmol C L^{-1} d^{-1}) (Newton and Van Voorhis 2002).

Environmental metabolite concentrations

Particulate concentrations of GBT and homarine were 0.32 ± 0.05–8.9 ± 1.6 nmol L^{-1} and 0.014 ± 0.003–2.4 ± 0.2 nmol L^{-1}, respectively, with the lowest concentrations in the NPSG and the highest concentrations in PS (Table 1; Supplemental Fig. 3). Dissolved concentrations of GBT and homarine were typically higher than the particulate concentrations and ranged from 0.47 ± 0.14–56 ± 9 nmol L^{-1} and 0.06 ± 0.03–13 ± 2 nmol L^{-1} L, respectively (Table 1). Total dissolved concentrations of detected zwitterionic metabolites (betaines, sulfoniums, and trimethylamine N-oxide [TMAO]) ranged from 4.5 ± 1.1 nmol L^{-1} in the NPSG and up to 150 ± 20 nmol L^{-1} in PS and were dominated by DMSP, gonyol, GBT, TMAO, beta-alanine betaine, and homarine (Supplemental Fig. 4; Supplemental Table 3). The measured particulate concentrations are likely underestimates of the total particulate GBT and homarine pools due to the 100 μm prefiltration step. Although intended to remove zooplankton, it also removed larger cells, cell chains, and aggregates. Additionally, some metabolite leakage can occur during filtration, resulting in overestimates of dissolved concentrations and underestimates of particulate concentrations. We collected dissolved samples at the start of filtrations to minimize cell leakage.

Uptake competition

Our uptake competition experiments showed that DMSP, GBT, unlabeled homarine (positive control), and trigonelline significantly inhibited the uptake of $^2H_3$-homarine relative to

Table 1. Environmental concentrations and uptake kinetics parameters of glycine betaine or homarine for each experiment in this study.

Region	Cruise	Experiment	Compound	Particulate concentration (nmol L^{-1}, mean ± standard deviation)	Dissolved concentration (nmol L^{-1}, mean ± standard deviation)	K_t (nmol L^{-1}, mean ± standard deviation)	V_{max} (nmol L^{-1} h^{-1}, mean ± standard deviation)
NPTZ	KM1906	UKG1	GBT	0.46 ± 0.05	7.0 ± 0.5	72 ± 22	0.36 ± 0.03
PS	RC078	UKG1	GBT	8.9 ± 1.6	56 ± 9.0	65 ± 22	3.7 ± 0.2
PS	RC104	UKG1	GBT	0.78 ± 0.10	11 ± 1.0	54 ± 23	0.45 ± 0.03
NPSG	TN412	UKG	GBT	0.32 ± 0.05	0.47 ± 0.15	5.5 ± 1.9	0.28 ± 0.02
PS	RC078	UKH1	Homarine	1.10 ± 0.14	13 ± 2	87 ± 34	0.18 ± 0.02
PS	RC078	UKH2	Homarine	2.40 ± 0.23	12 ± 0.4	490 ± 170	0.49 ± 0.08
PS	RC104	UKH1	Homarine	0.41 ± 0.08	4.1 ± 1.4	180 ± 19	0.30 ± 0.01
NPSG	TN397	UKH1	Homarine	0.014 ± 0.003	0.06 ± 0.03	38 ± 6.1	0.074 ± 0.004
NPSG	TN397	UKH2	Homarine	0.029 ± 0.011	0.19 ± 0.07	35 ± 9.4	0.052 ± 0.005
Eq	TN397	UKH3	Homarine	0.21 ± 0.02	0.39 ± 0.11	230 ± 100	0.17 ± 0.03
Eq	TN397	UKH4	Homarine	0.29 ± 0.04	0.89 ± 0.08	310 ± 41	0.26 ± 0.01
Eq	TN397	UKH5	Homarine	0.26 ± 0.02	0.30 ± 0.04	260 ± 61	0.67 ± 0.08

a glucose control ($p < 0.05$, Dunnett's test) (Fig. 2A). Glucose was not significantly different from a "no addition" treatment in the RC104 experiment suggesting it is an appropriate control. We did not classify TMAO as a competitive inhibitor of homarine due to inconsistent and/or minimal (TN397) inhibition. We synthesized our results with similar uptake experiments from the literature examining competitive inhibition of GBT, DMSP, and choline in natural communities and in cultures of SAR11 to make an inhibition network (Supplemental Fig. 5; Supplemental Table 4). We found that the compounds that inhibited homarine uptake also inhibited the uptake of GBT, DMSP, and choline (Kiene 1998; Kiene et al. 1998; Vila-Costa et al. 2006; Noell and Giovannoni 2019). We hypothesize that other inhibitory compounds from these studies, including dimethylsulfonioacetate (DMSA), beta-alanine betaine, and proline betaine also inhibit the uptake of homarine and vice versa. To understand the uptake dynamics of one of these compounds, the concentration of

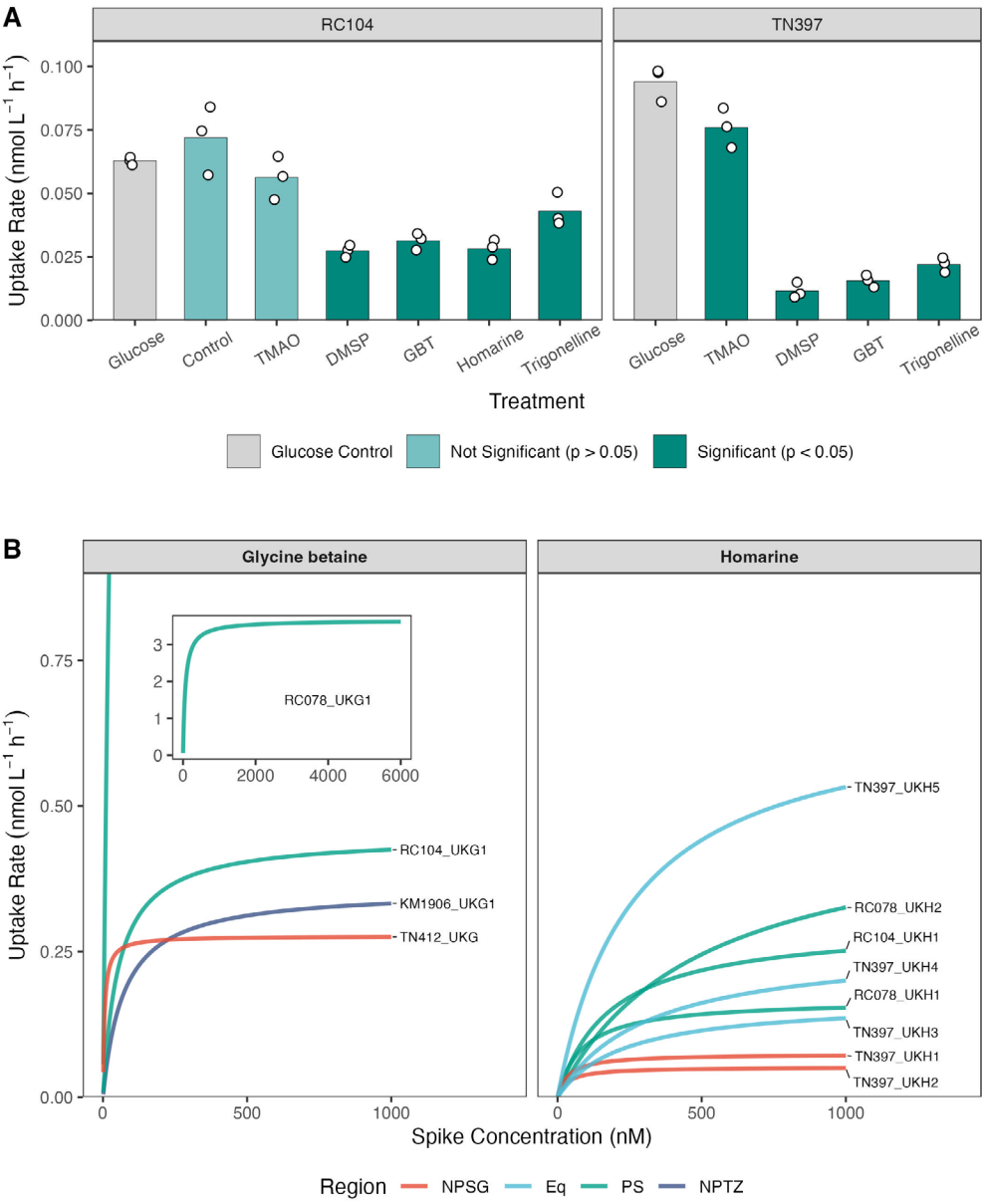

Fig. 2. Results of uptake competition experiment where bars represent the mean uptake rate of a 50 nmol L^{-1} spike of $^{2}H_3$-homarine and the open circles show the uptake rate of each of the three triplicates (**A**). Treatments (a 100 nmol L^{-1} spike of competitor) were considered significantly different from the "negative control" of glucose based on Dunnett's Test (p-value <0.05). "Control" refers to a no competitor control and "Homarine" refers to unlabeled homarine. Michaelis–Menten uptake kinetic profiles for the uptake of labeled GBT and homarine (**B**). Michaelis–Menten parameters for each experiment are detailed in Table 1. Inset shows the full uptake profile for RC078-UKG1. Color indicates experiment region.

the whole pool of betaines and sulfoniums must be considered.

Uptake kinetics

Community K_t measurements reflect the uptake kinetics of all microbial transporters in the community experiencing in situ uptake competition conditions, scaled by the abundance of each of those transporters. Therefore, community K_t and V_{max} measurements reflect community composition, transporter protein expression, total microbial biomass in the system, and in situ dissolved metabolite pools. Community uptake of both homarine and GBT followed MM uptake kinetics. V_{max} values ranged from 0.28 ± 0.02–3.7 ± 0.2 nmol L^{-1} h^{-1} for GBT and 0.052 ± 0.005–0.67 ± 0.08 nmol L^{-1} h^{-1} for homarine (Table 1; Fig. 2B; Supplemental Fig. 6). K_t values ranged from 5.5 ± 1.9–72 ± 22 nmol L^{-1} for GBT and 35 ± 9.4–491 ± 170 nmol L^{-1} for homarine (Table 1). Communities had lower K_t values for GBT than for homarine in the NPSG (5.5 vs. 35–38 nmol L^{-1}) and in PS (55–65 vs. 87–491 nmol L^{-1}), suggesting consistently higher affinity for GBT than for homarine across diverse marine ecosystems. For both GBT and homarine, the oligotrophic NPSG stations had roughly an order of magnitude lower K_t values and lower V_{max} values than the NPTZ, Eq, or PS stations (Table 1). For GBT, the NPSG stations had K_t values closer to the K_t value of SAR11, an oligotrophic bacterium (~1 nmol L^{-1}), while the other regions had K_t values that were more similar to those of diatoms for GBT (189–315 nmol L^{-1}) or DMSP (632 nmol L^{-1}) (Petrou and Nielsen 2018; Noell and Giovannoni 2019; Torstensson et al. 2019). We conclude from these results that the uptake of betaines in oligotrophic environments is dominated by free living oligotrophs, while in eutrophic environments such as PS, phytoplankton and copiotrophic bacteria control the kinetics and cycling of these compounds. This conclusion agrees with results from experiments in cultures and natural systems that show substantial uptake of DMSP and GBT by diatoms and other phytoplankton (Vila-Costa et al. 2006; Petrou and Nielsen 2018; Torstensson et al. 2019; Fernandez et al. 2021; Meyer et al. 2022).

One proposed explanation for the low concentrations of dissolved metabolites in the ocean is that high affinity (low K_t) transporters are able to draw down substrates until their concentration reaches the K_t value of their transporter (Moran et al. 2022). From this we predicted that lower K_t values would correspond to lower in situ dissolved concentrations. However, we did not find a significant correlation between K_t and dissolved GBT ($p = 0.12$, $R^2 = 0.65$) or homarine ($p = 0.20$, $R^2 = 0.14$) individually, or together ($p = 0.51$, $R^2 = 0.05$) (Fig. 3A,B; Supplemental Fig. 7). K_t values were up to 12 and 865 times higher than dissolved concentrations for GBT and homarine, respectively, suggesting substantial decoupling of community K_t and concentration. Additionally, homarine had higher K_t values but lower concentrations than GBT, suggesting that community K_t does not control relative dissolved metabolite concentrations. A subset of extremely high-affinity transporters could draw down concentrations below community K_t, partially explaining these discrepancies. However, GBT and homarine measurements in the NPSG were well below SAR11's 1 nmol L^{-1} K_t for GBT, suggesting high-affinity transporters are insufficient to explain measured dissolved concentrations (Noell and Giovannoni 2019).

One explanation for the observed disconnect between K_t and in situ concentrations for GBT and homarine may be the presence of co-transported compounds (all betaines and sulfoniums). We hypothesize that the total pool size is regulated by the collective K_t of all transporters for these compounds in the community and the relative concentrations of compounds within that pool are controlled by their relative input rates and the affinity of those transporters for each compound. We observed a significant log–log relationship between K_t and the total pool of betaines and sulfoniums ($p = 0.045$, $R^2 = 0.28$), providing some support for this model (Fig. 3C). However, additional kinetics experiments under a range of controlled uptake competition conditions are required to validate this hypothesis.

Turnover times and fluxes

GBT TTs ranged from 21.4 ± 12–220 ± 72 h (0.9–9.2 d) while homarine TTs ranged from 390 ± 120–1400 ± 860 h (16–57 d) (Table 2; Supplemental Table 5). GBT TTs were roughly an order of magnitude shorter than homarine TTs in both the NPSG and PS, suggesting GBT is consistently more rapidly cycled than homarine across diverse environments. Oligotrophic NPSG stations typically had shorter turnover times than NPTZ, Equator, or PS stations for both compounds. These TTs place GBT and homarine in the labile (TTs of hours to days) and semilabile (TTs of weeks to months) DOM categories and suggest that these categorizations are environment dependent.

Fluxes of GBT and homarine through the dissolved pool ranged from 0.53 ± 0.26–41 ± 16 nmol L^{-1} d^{-1} and 0.003 ± 0.001–0.54 ± 0.24 nmol L^{-1} d^{-1}, respectively (Table 2). Assuming steady-state conditions, these daily values correspond to turnover rates of 156–459% (median: 195%) of the measured particulate and 10.9–112% (median: 45%) of the dissolved GBT pools and 3.3–47% (median: 16%) of the measured particulate and 1.8–6.2% (median: 4%) of the dissolved homarine pools (Supplemental Table 6). We note that our particulate values may be underestimates, leading to an overestimate of particulate turnover rates. Our results suggest that the entire measured particulate GBT pool (or more) and up to half of the measured particulate homarine pool move through the dissolved pool every day. These estimates largely agree with estimates of turnover rates from a study in the NPSG showing that osmolytes such as GBT and homarine in particles change by greater than or equal to two-fold over the day-night cycle

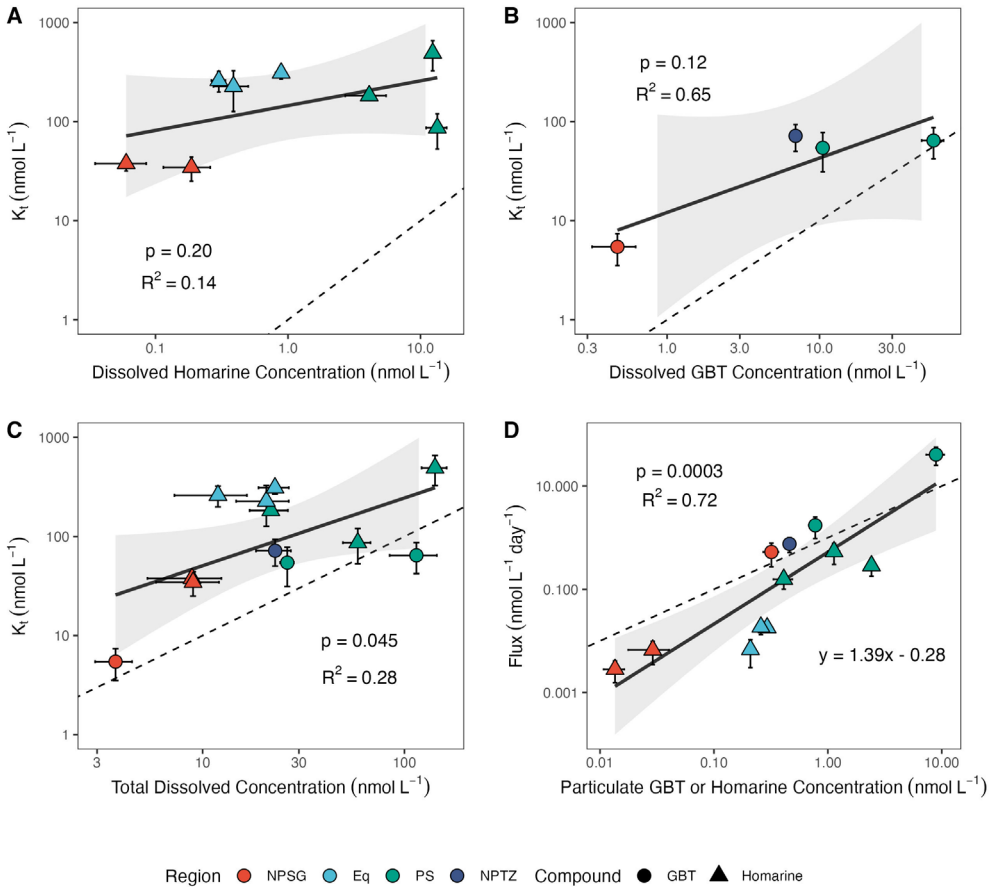

Fig. 3. Relationship between in situ dissolved concentration and K_t for homarine (**A**) and GBT (**B**). Points show mean values for individual experiments. Relationship between K_t for both GBT and homarine and the total dissolved concentration of quantified betaines and sulfoniums (**C**). Relationship between in situ particulate concentration of either GBT or homarine and the daily flux of these compounds through the dissolved pool (**D**). For all panels, solid black lines correspond to linear models and gray shading represents 95% confidence intervals. Symbol color shows region and symbol shape shows compound. Error bars represent one standard deviation. Text shows *p*-values for linear models. Dashed lines are 1 : 1 lines. Note log10 scaling for both axes for all panels.

(Boysen et al. 2021). In this system, living phytoplankton biomass also displayed daily two-fold change oscillations (Boysen et al. 2021). The highest fluxes were observed in PS, reaching daily carbon fluxes of up to 200 ± 79 nmol C L^{-1} d^{-1} for GBT and 3.8 ± 1.6 nmol C L^{-1} d^{-1} for homarine (Table 2). The fluxes of GBT and homarine represent 0.05–1.2% and 0.0049–0.023% of estimated daily primary production, respectively (Supplemental Table 6). The flux of nitrogen in GBT at our NPSG station (0.53 ± 0.26 nmol L^{-1} d^{-1}) is equivalent in magnitude to 29% of mean nitrogen fixation at Station ALOHA (1.84 ± 1.09 nmol N L^{-1} d^{-1}), suggesting that betaines could be a quantitatively important component of the marine nitrogen cycle (Böttjer et al. 2017).

Fluxes of GBT through the dissolved pool were similar to published values for other abundant osmolytes such as taurine (0.2–6.0 nmol L^{-1} d^{-1}) and DMSP (1.7–58.5 nmol L^{-1} d^{-1}) (Kiene and Linn 2000; del Valle et al. 2012, 2015; Clifford et al. 2019; Clifford et al. 2020) (Supplemental Table 7). Homarine fluxes were typically one to three orders of magnitude lower than those of GBT (Table 2). Fluxes of metabolites from the dissolved pool into the particulate pool should not be directly interpreted as catabolism. Significant portions (79–95%) of GBT remain unaltered in the particulate pool for days following uptake, likely reflecting the use of GBT as an osmolyte within the microbial community rather than as a growth substrate for heterotrophic bacteria (Kiene and Williams 1998; Boysen et al. 2022).

Determining fluxes is time and resource intensive. For this reason, we explored if fluxes can be predicted from other parameters. We identified a strong positive log–log linear relationship between particulate concentration and flux for both compounds (Fig. 3D, $p = 0.0003$, $R^2 = 0.72$, slope = 1.39, intercept = −0.28) as well as each compound independently (GBT: $p = 0.0005$, $R^2 = 0.99$, slope = 1.32, intercept = 00.36; homarine: $p = 0.004$, $R^2 = 0.73$, slope = 0.99, intercept = −0.86). For these compounds, particulate concentration is a strong predictor of flux.

Table 2. Turnover times and fluxes of glycine betaine or homarine for each experiment in this study. Flux estimates are expressed as both nanomoles compound L^{-1} d^{-1} and nanomoles carbon L^{-1} d^{-1}.

Region	Cruise	Experiment	Compound	Turnover time (h, mean ± standard deviation)	Metabolite flux (nmol compound L^{-1} d^{-1}, mean ± standard deviation)	Carbon flux (nmol C L^{-1} d^{-1}, mean ± standard deviation)
NPTZ	KM1906	UKG1	GBT	220 ± 72	0.76 ± 0.24	3.8 ± 1.2
PS	RC078	UKG1	GBT	33 ± 14	41 ± 16	200 ± 79
PS	RC104	UKG1	GBT	150 ± 66	1.7 ± 0.8	8.7 ± 3.9
NPSG	TN412	UKG	GBT	21 ± 12	0.53 ± 0.26	2.7 ± 1.3
PS	RC078	UKH1	Homarine	600 ± 280	0.54 ± 0.24	3.80 ± 1.6
PS	RC078	UKH2	Homarine	1000 ± 390	0.29 ± 0.11	2.0 ± 0.8
PS	RC104	UKH1	Homarine	630 ± 310	0.15 ± 0.06	1.1 ± 0.4
NPSG	TN397	UKH1	Homarine	510 ± 310	0.003 ± 0.001	0.02 ± 0.009
NPSG	TN397	UKH2	Homarine	670 ± 420	0.007 ± 0.003	0.047 ± 0.022
Eq	TN397	UKH3	Homarine	1400 ± 860	0.007 ± 0.004	0.048 ± 0.026
Eq	TN397	UKH4	Homarine	1200 ± 230	0.018 ± 0.003	0.13 ± 0.02
Eq	TN397	UKH5	Homarine	390 ± 120	0.019 ± 0.005	0.13 ± 0.04

Comparisons to other studies

Compared to other studies of GBT uptake, our K_t values were slightly higher than reported $K_t + S_n$ values (5.5–72 nmol L^{-1} vs. 1.2–49 nmol L^{-1}), our V_{max} values were lower (0.28–3.7 nmol L^{-1} h^{-1} vs. 0.39–44 nmol L^{-1} h^{-1}), and our TTs were longer (21–220 h vs. 0.2–11 h) (Supplemental Table 7) (Kiene et al. 1998; Mausz et al. 2022). We attribute these differences to differences in experimental design. In previous studies, samples were prefiltered (1.2 μm) to remove phytoplankton and preincubated for 24 h in the dark to allow for bacterial drawdown of in situ dissolved metabolites prior to the start of the experiment. In contrast, we used seawater prefiltered through 100 μm mesh and performed our experiments immediately, minimizing potential alterations to bacterial GBT transporter expression and preserving in situ dissolved metabolite conditions (Vorobev et al. 2018). Previous studies characterized the high affinity transporters of free-living bacteria without competitive uptake inhibition. However, they likely do not reflect in situ cycling rates or the whole community uptake profile. Our results suggest that the cycling rate of GBT may be lower than previously reported and that phytoplankton uptake and competitive inhibition should be considered when measuring uptake kinetics.

Conclusion

We quantified the uptake kinetics and fluxes of two important osmolytes, GBT and homarine, in diverse marine ecosystems, facilitating their incorporation into biogeochemical budgets and informing our understanding of their role in microbial interactions. This work also highlights the potential role of uptake competition in controlling dissolved metabolite concentrations, kinetics, and cycling in the marine environment.

Author Contributions

Joshua S. Sacks and Anitra E. Ingalls designed the study. Joshua S. Sacks, Anitra E. Ingalls, Frank X. Ferrer-González, Katherine R. Heal, Angela K. Boysen, Angelicque E. White, Oscar A. Sosa, and Anna H. Finch performed field work. Joshua S. Sacks, Laura T. Carlson, and Anna H. Finch performed laboratory analyses. Angelicque E. White and David M. Karl provided ancillary data. Joshua S. Sacks analyzed and visualized data. Joshua S. Sacks and Anitra E. Ingalls wrote the manuscript. All authors edited and revised the manuscript.

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Conflicts of Interest

None declared.

Data Availability Statement

All data and code used in analyses and data visualizations are available on GitHub at https://github.com/jssacks/Marine_Betaine_Flux. Contextualizing environmental data used in interpolations from KM1906, TN397, and TN412 are available at Simons CMAP (https://simonscmap.com). Raw mass spectrometry peak area data are available on GitHub and on Zenodo at https://zenodo.org/records/14606227.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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