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Thermally adaptive tradeoffs in closely related marine bacterial strains

Cheuk-Man Yung,[1] Marissa K. Vereen,[1] Amy Herbert,[1] Katherine M. Davis,[1] Jiayu Yang,[1] Agata Kantorowska,[1] Christopher S. Ward,[1,2] Jennifer J. Wernegreen,[1] Zackary I. Johnson[1,3] and Dana E. Hunt[1,3]*

[1]*Nicholas School of the Environment,* [2]*Integrated Toxicology and Environmental Health Program, and* [3]*Biology Department, Duke University, Durham, NC 27708, USA.*

Summary

Time series studies have shown that some bacterial taxa occur only at specific times of the year while others are ubiquitous in spite of seasonal shifts in environmental variables. Here, we ask if these ubiquitous clades are generalists that grow over a wide range of environmental conditions, or clusters of strain-level environmental specialists. To answer this question, vibrio strains isolated at a coastal time series were phylogenetically and physiologically characterized revealing three dominant strategies within the vibrio: mesophiles, psychrophiles and apparently generalist broad thermal range clades. Thermal performance curves from laboratory growth rate experiments help explain field observations of relative abundances: the mesophilic clade grows optimally at temperatures 16°C higher than the psychrophilic clade. Strains in the broad thermal range clade all have similar optimal growth temperatures but also exhibit temperature-related tradeoffs with faster growth rates for warm temperature strains and broader growth ranges for strains from cool temperatures. Moreover, the mechanisms of thermal adaptation apparently differ based on evolutionary time scales: shifts in the temperature of maximal growth occur between deeply branching clades but thermal performance curve shape changes on shorter time scales. Thus, apparently ubiquitous clades are likely not generalists, but contain subclusters with distinct environmental preferences.

Received 20 September, 2014; revised 7 November, 2014; accepted 8 November, 2014. *For correspondence. E-mail dana.hunt@duke.edu; Tel. (+252) 504 7542; Fax 252-504-7648.

Introduction

Repeated observations of microbial communities from a single location (i.e. time series) or discrete samples across environmental gradients are often used to identify environmental factors and organismal interactions that structure the diversity of aquatic microbial populations (Johnson *et al.*, 2006; Steele *et al.*, 2011; Gilbert *et al.*, 2012). However, observational techniques are limited in their ability to identify the proximal drivers of microbial diversity due to a number of confounding factors: strong correlations between environmental variables (Gilbert *et al.*, 2012), top down control by predation or viruses, associations with particles and other microscale features (Hunt *et al.*, 2010), or unmeasured environmental variables. Moreover, a taxon observed in a given environment does not imply adaptation to current environmental conditions due to spatial or temporal uncoupling of the environment and populations (Jones and Lennon, 2010; Caporaso *et al.*, 2011; Hunt *et al.*, 2013). Therefore, observation-based predictions of environmental niche specialization are often complemented by laboratory studies of culturable organisms, which can confirm hypotheses and provide additional mechanistic insight. Nevertheless, despite these limitations, field sampling of marine and freshwater microbial communities remains one of the fundamental approaches to study the ecology of uncultivated microbial populations.

In observational studies, temperature is consistently one of the most important variables structuring microbial communities and populations across many different environments (Selje *et al.*, 2004; Fuhrman *et al.*, 2006; 2008; Johnson *et al.*, 2006; Sikorski and Nevo, 2007; Shade *et al.*, 2010), although other variables may be more important globally (Lozupone and Knight, 2007). In addition to ease of measurement (and therefore extensive datasets), two characteristics of temperature may drive taxa–temperature relationships in aquatic environments. First, the high specific heat of water means that large changes in temperatures occur on a time scale longer than the average generation time of microbial populations. Thus, resident microbes are likely to be adapted to *in situ* temperature conditions as selection persists over time scales long enough to induce changes in microbial population abundances. In contrast, other environmental variables

such as nutrients often exhibit pulsed or patchy inputs relative to the generation time of resident microbes (Johnson *et al*., 2013; Yawata *et al*., 2014). Second, genomic specificity to temperature may be distinct from adaptation to other environmental variables. For environmental factors such as nutrients or carbon sources, a horizontally transferred gene or operon could allow for utilization of new resources. Whereas, temperature adaptation likely involves genome-wide changes as all enzymatic rates are affected by temperature, and growth in a new thermal environment requires that all essential cell processes must function at that temperature. Thus, the evolution of thermal preferences likely differs from that involved in the use of other resources. Despite these distinct properties of temperature, using observational techniques, it remains difficult to distinguish the effects of temperature from those of other seasonally cycling variables (e.g. light) and phenomena (e.g. seasonal coastal upwelling).

Warming of marine microbial communities generally increases microbial metabolism presumably due to enhanced enzyme kinetics (Fuhrman and Azam, 1983; Kirchman *et al*., 2005; López-Urrutia and Morán, 2007). Both *in situ* and laboratory studies have shown that progressive warming alters bacterial specific growth rates (White *et al*., 1991), secondary production (Rivkin *et al*., 1996) and respiration rates (Vazquez-Dominguez *et al*., 2007). In cultures, growth rates increase with temperature, but only within a certain range, above which growth rates generally decrease rapidly (Izem and Kingsolver, 2005; Chen and Shakhnovich, 2010). Laboratory-derived relationships between temperature and growth rate also predict evolutionary tradeoffs where increased fitness at a specific temperature decreases fitness at other temperatures (Bennett and Lenski, 2007; Hall *et al*., 2010; Chang *et al*., 2013), although exceptions have been noted (Bennett and Lenski, 2007; Knies *et al*., 2009). Given the temperature sensitivity of bacterial growth and metabolism, it is not surprising that small temperature changes correspond to dramatic shifts in some environmental bacterial populations (Johnson *et al*., 2006). Yet, in many time series studies, dominant marine microbial populations are present year-round, even though their relative abundance varies (Gilbert *et al*., 2012; Chow *et al*., 2013). The ubiquity of dominant taxa suggests these clades may be eurythermic environmental generalists, rather than thermal specialists. Alternately, ubiquitous taxa may be composed of multiple temperature-specialized subtypes that cannot be resolved using standard measures of uncultured microbial diversity [e.g. 16S rRNA gene based operational taxonomic units (OTUs)] as was recently suggested for the marine group SAR11 (Eren *et al*., 2013). Thus whether ubiquitous taxa are thermal generalists or can be subdivided into specialists remains an open question. Moreover, with growing concerns about climate change-induced alterations in the oceans, understanding the mechanisms of bacterial thermal adaptation is essential to predicting microbial responses to sea surface temperature changes.

In this study, we explore seasonal partitioning of closely related bacterioplankton focusing how temperature modulates community structure. We have selected the bacterial family *Vibrionaceae* for further investigation due to their ease of culturability, ubiquity in coastal environments and status as a model system for marine bacteria (Polz *et al*., 2006). Vibrio are metabolically versatile heterotrophs known for their metabolism of chitin (Hunt *et al*., 2008a), associations with zooplankton (Turner *et al*., 2009) and the inclusion of a number of facultative marine pathogens (e.g. *Vibrio cholerae*). In the environment, vibrio abundance is driven by changes in environmental parameters including temperature, salinity, chlorophyll and zooplankton (Kaneko and Colwell, 1973; Randa *et al*., 2004; Turner *et al*., 2009; Johnson *et al*., 2012). Here, we examine vibrio community dynamics at a temperate, coastal site with a large (~20°C) seasonal variation in temperature (Johnson *et al*., 2013). Field observations are corroborated with laboratory-based growth rate studies revealing temperature tradeoffs in growth rates and ranges.

Results and discussion

Environmental drivers of seasonal vibrio population dynamics

In order to examine seasonal distributions of closely related bacterioplankton, roughly 100 vibrio isolates were obtained every 2 weeks over 18 months (August 2011–January 2013) in conjunction with the Pivers Island Coastal Observatory (PICO) sampling, a well studied time series located at the Beaufort Inlet (Beaufort, NC, USA; Fig. S1). This coastal ecosystem is a highly dynamic environment with strong seasonal patterns in variables such as temperature and pH (Johnson *et al*., 2013). After streaking to isolation, strains were phylogenetically characterized by sequencing a housekeeping gene (*hsp60*) that offers enhanced resolution over 16S rRNA gene sequencing (Goh *et al*., 1996; Thompson *et al*., 2005). This analysis revealed a diverse array of *Vibrio* and *Photobacteria* taxa including *Vibrio fischeri*, *Vibrio logei* and *Vibrio splendidus*. Similar to other temperate locations, we observed seasonal variability with distinct vibrio clades present at different times of the year (Kaneko and Colwell, 1973; Thompson *et al*., 2005). In order to identify the environmental drivers of vibrio community diversity, a constrained set of environmental variables was identified using forward stepwise selection. Environmental variables statistically associated with vibrio community

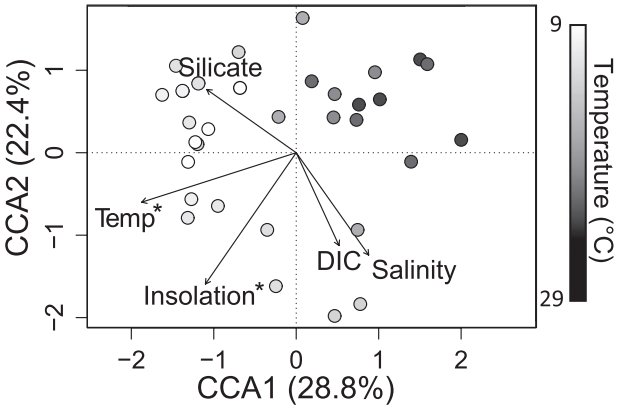

Fig. 1. Canonical correspondence analysis (CCA) ordination diagram of axes one and two of the isolated vibrio community at each sampled time point from the Pivers Island Coastal Observatory (PICO) time series. The percent of variation in the vibrio community explained by each axis is in parenthesis after the axis. The constrained set of environmental variables analysed are indicated as vectors: temperature (Temp); projected no-sky daily insolation (Insolation), Silicate, dissolved inorganic carbon (DIC) and Salinity. The vibrio community profiles from each time point are represented as circles; the grey scale of circles' colouring indicates water temperature. Environmental variables marked with asterisks are statistically significant when assessed by the marginal effect of the terms ($P < 0.05$).

structure ($P < 0.05$) by forward selection using Akaike's information criterion and 999 permutation tests are daily insolation, water temperature, salinity, silicate and dissolved inorganic carbon (Table S1). These variables were used as the constrained variable set to identify drivers of vibrio diversity using canonical correspondence analysis (CCA: Fig. 1). Among these variables, temperature is the largest driver of vibrio diversity ($P = 0.010$) followed by insolation ($P = 0.016$) (Fig. 1). Not surprisingly, these two variables are correlated (Pearson's $r = 0.739$), but the solar maxima precedes thermal maxima by ~1 month at this location (Johnson *et al.*, 2013) and both variables independently impact vibrio community structure. Whereas temperature is predicted to directly affect bacterial physiology through enzyme kinetics, light likely indirectly influences vibrio community structure by stimulating primary production. Although the time series is located at the mouth of an estuary and vibrio physiology is linked to salinity (Randa *et al.*, 2004; Materna *et al.*, 2012), salinity did not contribute significantly to vibrio community structure, likely due to the high average value (32 ppt) and low variability (28–34 ppt) at the study site.

The significance of temperature on vibrio community structure led us to focus on thermal specialization within this group of bacterioplankton. In order to visualize the phylogenetic relationships of the vibrio isolates and their distribution across temperature environments, a maximum likelihood phylogenetic tree based on partial *hsp60* gene sequences was constructed (Fig. 2A). Mapping the water temperature from which the strains were isolated onto the phylogenetic tree revealed that temperature was visibly non-randomly distributed on the vibrio phylogeny (Fig. 2A, outer ring). However, to gain a more quantitative understanding of the thermal distributions of specific clades, we employed AdaptML (Hunt *et al.*, 2008b) to identify potential thermal niches. Isolate sequences were assigned to 3°C temperature bins based on water temperature, and distinct thermal 'habitats' were identified statistically as structured distributions across these temperature bins ($P < 0.0001$). AdaptML identifies the bounds of thermally constrained groups on the phylogenetic tree by assigning clades to 'habitats' or projections of the realized thermal niche onto these temperature bins (Fig. 2A, coloured dots on branches), allowing identification of differences in temperature-adaptive strategies employed in the vibrio. This approach avoids any *a priori* assumptions about the thermal or phylogenetic bounds at which organisms differentiate but does not take into account potential confounding effects between temperature and light or other environmental parameters. This approach robustly (≥ 98% of the time) identified four thermal habitats corresponding to a mesophilic (H_A), a more generalist (H_B) and two psychrophilic (H_C and H_D) distributions. Between the two psychrophiles, H_D has a stronger affiliation with water temperatures < 15°C. Although temperature was previously observed to significantly affect vibrio community structure (Fig. 1), this analysis indicates a range of distinct, thermal strategies within the vibrio community in response to seasonal water temperature changes.

In contrast to the apparent ubiquity of many taxa in previous time series studies, we observed that clades partition largely into mesophilic or psychrophilic thermal distributions across the sampled time points (H_A and $H_C + H_D$, respectively, in Fig. 2). However, it is worth noting that clades which are truly randomly distributed by temperature would not be assigned to a novel habitat by AdaptML. We refer to groups found at a range of environmental temperatures as 'broad thermal range' clades and their existence argues against adaptation of clades to a narrow temperature window. To better visualize clade thermal distributions without the assignment of AdaptML 'habitats' and the potential resultant bias against even temperature distributions, vibrio isolates were also clustered into 97% identity *hsp60* OTUs (Fig. S3). As in the AdaptML analysis, we observe psychrophiles, mesophiles and broad thermal range clades (Fig. 3); however, the prevalence of broad thermal range clades appears higher with this approach. Broad thermal range clades are observed at environmental temperatures intermediate between mesophiles and psychrophiles and occupy a broader environmental niche (Fig. 3). While thermal specialization supports the concept of temperature as a key

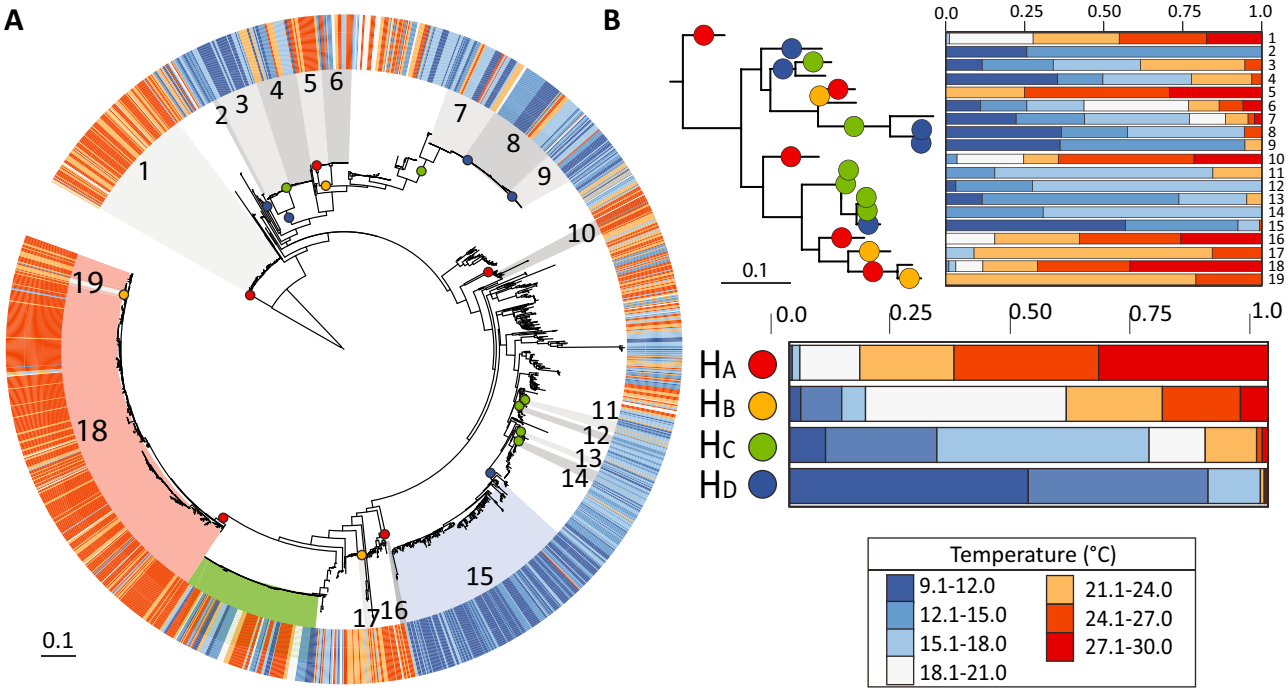

Fig. 2. Thermal distributions and habitat predictions mapped onto *Vibrionaceae* isolate phylogeny inferred by maximum likelihood analysis of partial *hsp60* gene sequences. Projected habitats are identified by coloured circles at the parent nodes. A. Maximum likelihood tree of isolate sequences with *Escherichia coli* K-12 outgroup. The ring of colour surrounding the tree indicates the water temperature from which each strain was isolated. Ecological populations predicted by AdaptML are indicated by shaded and numbered clusters if they pass an empirical confidence threshold of 99.99%. Bootstrap confidence values are shown in Fig. S2. Exemplar mesophilic, psychrophilic and broad thermal range clades are indicated by red, blue and green shading of clusters respectively.
B. Tree summarizing habitat-associated populations identified by the model and the distribution of each population among 3°C temperature bins. The habitat legend matches the coloured circles in (A) and (B) with the habitat distribution inferred by AdaptML. Habitat distributions (legend) are normalized by the total number of counts in each temperature bin to reduce the effects of uneven sampling.

environmental factor that structures microbial populations and communities, the presence of apparent thermal generalists (broad thermal range clades) suggests that thermal adaptive tradeoffs may not occur in environmental bacteria (Chang *et al.*, 2013). Thus, based on environmental data alone, it is unclear whether clades observed over a wide range of temperatures are thermal generalists or if other factors such as microdiverse ecological specialization drive observed environmental patterns in these groups.

Thermal performance curves for vibrio isolates

To investigate the thermal performance of these strains relative to their environmental distributions and to explore potential thermal adaptive tradeoffs, we characterized the growth rates of a number of representative isolates over a range of temperatures. We chose clades for growth rate analysis which exemplify the three strategies (mesophile, psychrophile and broad thermal range) identified previously (Table 1). Specifically, the mesophile clade (Fig. 2A: clade 18, red shading), most similar by 16S rRNA gene sequence comparisons to *Vibrio harveyi* ATCC BAA-1116

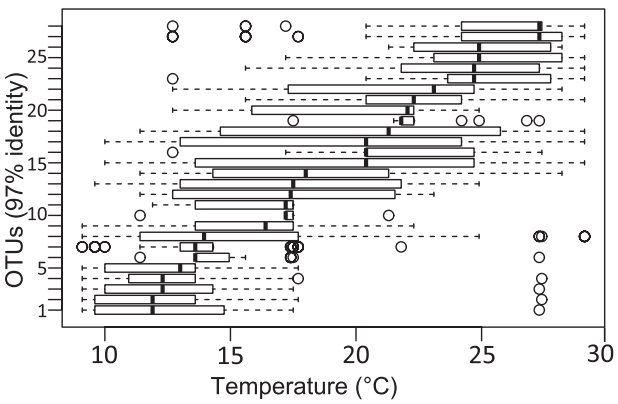

Fig. 3. Box and whiskers plot depicting the observed thermal distribution of vibrio isolate 97% sequence identity *hsp60* operational taxonomic units (OTUs) containing at least 10 members across sampled temperatures (phylogenetic tree of these OTUs in Fig. S3). For each OTU, the thick bar represents the median of the water temperatures when this group was isolated. The box indicates the first quartile (Q1) and third quartile (Q3), and the whiskers indicate the range from Q1 – 1.5 (Q3 – Q1) to Q3 + 1.5 (Q3 – Q1), but not exceeding the range of measured values. Outlier observations beyond the whiskers are indicated by open circles. For ease of interpretation, clades are ordered by the median observed environmental temperature.

Table 1. Details of the strains selected for growth rate experiments.

Strain	Clade[a]	Date of isolation[b]	Water temperature at isolation (°C)
Mesophile 1	Clade 18	30/08/2011	27.3
Mesophile 2	Clade 18	26/06/2012	27.6
Mesophile 3	Clade 18	29/08/2012	26.8
Psychrophile 1	Clade 15	16/12/2012	13.6
Psychrophile 2	Clade 15	28/02/2012	12.3
Psychrophile 3	Clade 15	05/12/2012	14.3
Warm 1	Board thermal range	13/09/2011	27.3
Warm 2	Board thermal range	13/09/2011	27.3
Intermediate 3	Board thermal range	01/11/2011	15.6
Intermediate 4	Board thermal range	01/11/2011	15.6
Cool 5	Board thermal range	03/01/2012	10
Cool 6	Board thermal range	03/01/2012	10

a. Clades correspond to designations in Fig. 2.
b. day/month/year.

among the sequenced genomes, was isolated from an environmental temperature range of 21.3–27.3°C (average = 24.3°C). The psychrophilic clade (Fig. 2A: clade 15, blue shading) was identified as *V. splendidus* by 16S rRNA gene sequence comparison with sequenced genomes and was isolated at temperatures between 9.1°C and 15.6°C (average = 11.6°C). However, this observed fidelity to specific temperature ranges is not found in all clades. A number of clades were assigned to the thermally mixed habitat (H_B) by AdaptML or were not assigned to a habitat at all, indicating a statistically random distribution across temperature bins. This later group includes the green-shaded clade (Fig. 2A) which was identified as most similar to the sequenced genome strain of *Vibrio parahaemolyticus* RIMD 2210633 and was isolated from water temperatures between 10°C and 29°C (average = 20°C). Prior to growth rate studies, we selected at least three isolates per clade and confirmed that the *hsp60* phylogeny reflected that of other housekeeping genes (*adk*, *mdh*). This analysis revealed high within-clade similarity at all three loci with an average nucleotide identity ≥ 99.8% (Fig. S4). To confirm the thermal preferences of the three categories of clades (mesophile, psychrophile, broad thermal range), we examine their thermal performance curves in culture. To evaluate the growth potential of these strains at a range of temperatures, the maximal growth rate of isolates was established in glucose minimal media batch culture over a temperature range from 4°C to 40°C (Fig. 4). Although no strain was shown to grow at temperatures greater than 40°C, several cultures had measurable growth rates at 4°C, indicating that their true thermal minima is below this temperature range. Thus, the upper but not lower limits of growth were established for these culturing conditions. These laboratory culture-based experiments can be used to test field-based predictions of vibrio physiology.

In comparing the thermal performance curves of these putatively temperature-differentiated clades, a number of features are readily apparent including differences in the optimum growth temperature (T_{opt}), shape or skewness of the thermal performance curve, temperature range and growth rate (Fig. 4). First, similar to previous studies, laboratory measured T_{opt} is higher than the observed environmental distributions of strains likely due to enhanced kinetics (Materna *et al.*, 2012). However, the psychrophilic and mesophilic clades exhibit a 16°C shift in T_{opt} that indicates that observed thermal partitioning in the

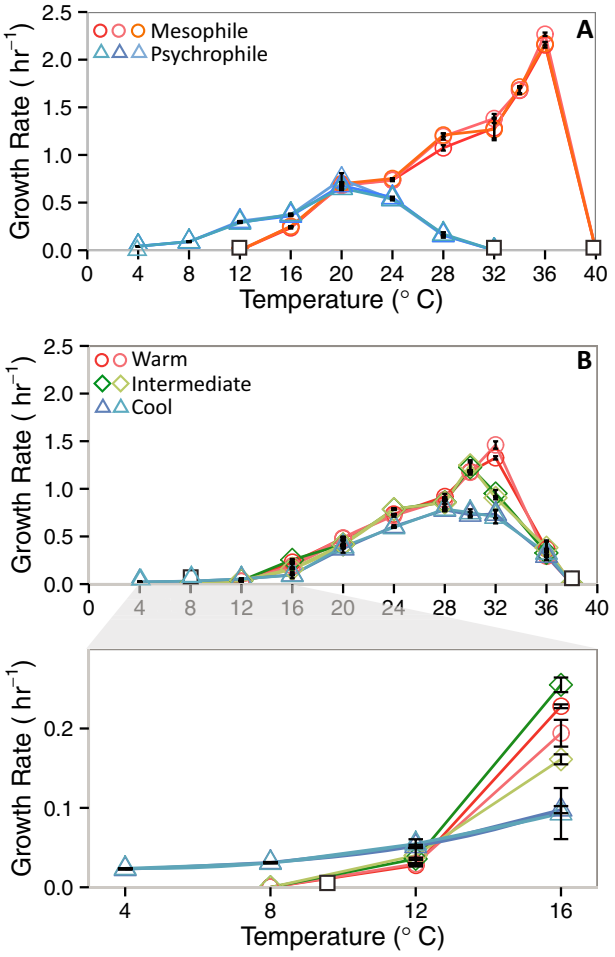

Fig. 4. Maximum growth rate (h^{-1}) of vibrio strains in glucose minimal media batch culture over a range of temperatures. Coloured shapes indicate the average maximal observed growth rate at that temperature for three biological replicates per strain; error bars indicate one standard deviation of measured growth rate for these replicates. Black open squares indicate the strain does not grow at that temperature.
A. Three strains each from exemplar mesophilic (red circles) and psychrophilic clades (blue triangles), which were the highlighted red and blue clades, respectively, in Fig. 2.
B. Six strains from the exemplar broad thermal range clade (green in Fig. 2) two each isolated from warm (red circles), intermediate (green diamonds) and cool (blue triangles) temperatures with inset showing growth rates from 4°C to 16°C.

environment is also evident in laboratory-characterized physiology (Fig. 4A). Second, we noted differences in the shape of the thermal performance curves. The mesophile clade displays a classic ectothermic growth curve with a sharp decline in growth rate above T_{opt} and a long tail at temperatures $< T_{opt}$ (Izem and Kingsolver, 2005; Chen and Shakhnovich, 2010). In contrast, the psychrophilic strains exhibit thermal performance curves with a roughly symmetric distribution of growth range above and below T_{opt} and have a wider growth range at temperatures greater than T_{opt} compared with the mesophilic clade. Third, we observed differences in the bounds of the temperature range, as the psychrophile clade grows at lower temperatures (< 4°C) than the mesophile clade but not at temperatures >28°C where the mesophile grows optimally. However, the upper bounds of the psychrophile clade's growth were higher in culture than in the environment, where it was not observed at temperatures above 16°C. Optimal growth at 20°C suggests that this group should more appropriately be termed psychrotolerant, but we preserve the term psychrophile for clarity of discussion. Differences in field and laboratory thermal growth range highlight the more permissive growth conditions in rich media as well as the lack of resource competition in culture. Finally, we observed differences in growth rate; the psychrophilic clade has a lower maximal growth rate than the mesophilic clade at their respective T_{opt}s, but below 20°C, psychrophiles exhibit a higher growth rate than the mesophiles (Fig. 4A). This suggests a potential 'cost' or tradeoff associated with growth at low temperatures that limits either growth range or maximal growth rate. Although each of these clades is represented by three isolates taken from non-sequential sampling points (e.g. ≥ 1 month apart), there is remarkable within-clade similarity in growth rate for isolates (one-way analysis of variance, $P > 0.05$), suggesting that strains within these clades behave similarly with regards to temperature.

In contrast to the thermal fidelity observed in the mesophile and psychrophile clades, the broad thermal range clade exhibits distinct thermal performance curves for isolates obtained from different water temperatures (Fig. 4B). However, all strains within this clade have similar T_{opt}s ~28–32°C, intermediate between that of the mesophile and psychrophile clades. Despite this similarity in T_{opt}, there is a temperature-related trend similar to that observed previously: strains isolated from warmer water temperatures (Table 1) grow faster at their T_{opt}. Further, strains isolated from cooler water temperatures display a lower maximal growth rate, but they grow at non-permissive temperatures for strains isolated at warm and intermediate temperatures (i.e. ≤ 8°C; Fig. 4B). Thus, these thermal performance curves show that 'broad thermal range' clades are in fact not thermal generalists but rather exhibit thermal specialization at very fine phylogenetic scale that is not resolved by sequencing multiple housekeeping genes (Fig. S4). However, clades with optimal growth intermediate between mesophiles and psychrophiles may be genetically poised to fine tune their thermal niche compared with strains with more extreme T_{opt} values.

In addition to shifts in T_{opt}, the shape of the thermal performance curve growth range and growth rate, an interesting temperature-related phenomenon was observed for strains from the psychrophile clade and the cool strains within the broad thermal range clade (strains 5 and 6; Table 1): at temperatures ≤ 20°C, these strains formed visible aggregates, suggesting temperature-mediated lifestyle differentiation. Attachment to nutrient sources, including chitin for vibrios, has previously been shown to protect against thermal cold stress (Amako *et al.*, 1987) and biofilm formation provides many bacteria resistance to environmental stressors. This temperature-dependent aggregation suggests that lifestyle differentiation, in addition to enzymatic temperature optima, may be a mechanism that vibrio use to buffer the effects of temperature stress. Here, temperature is shown to dictate the environmental range of bacteria. However, thermal adaptation has a cost with apparent tradeoffs even within very closely related strains which argues against the evolution of thermal generalists (Chang *et al.*, 2013).

Conclusions

This study suggests that there are multiple mechanisms of thermal adaptation within the vibrio community encompassing changes in T_{opt}, the shape of the thermal performance curve, the bounds of the growth range, growth rate and attachment. These findings argue against thermal generalism in ubiquitous clades both at the genus level (vibrios) and at the strain level; rather clades observed under a range of temperature conditions (broad thermal range clades) exhibited thermal subspecialization at very fine-scale phylogenetic resolution. It remains to be seen if this exquisite thermal adaptation observed in vibrio can be generalized to other prevalent microbial taxa or environmental variables. While thermal adaptive tradeoffs have been explored in *Escherichia coli* which inhabits a host's relatively constant thermal environment (Bennett and Lenski, 2007; Chang *et al.*, 2013), little information is available for environmental bacteria which are subject to large seasonal temperature changes. These results suggest that there is a physiological cost to adaption to specific temperatures (particularly cooler temperatures) and that temperature specialization can occur among closely related strains (Fig. 4 and Fig. S4). Further, the observed thermal specialization over relatively short evolutionary time scales indicates that few genes or cellular processes may limit

expansion to a different thermal niche. Characterizing the mechanisms of thermal adaptation is essential to understanding seasonal succession as well as potential microbial responses to larger scale processes like climate change-induced ocean warming.

Experimental procedures

Sample collection

Water samples were collected as part of the PICO time series in Beaufort, North Carolina. This site (34°71.81'N, 76°67.08'W) is situated on Pivers Island at the Beaufort Inlet (Fig. S1). Sampling for vibrio isolates was performed biweekly from 30 August 2011 to 2 January 2013. Time series measurements were conducted as previously described (Johnson *et al.*, 2013). Briefly, water was sampled at 10:30 AM local time using a Niskin bottle centred at 1 m. All measurements are replicate samples. Nutrients (NO_2, NO_3, PO_4, $SiOH_4$) were measured in duplicate on 0.22 μm filtered samples stored at –80°C until later analysis using an Astoria-Pacific A2 autoanalyser. Salinity was measured using a refractometer (Atago). Spectrophotometric pH measurements using a pH-sensitive dye were collected on a UV-Vis-NIR spectrophotometer (Cary 4000, Varian). Chlorophyll pigment samples were extracted in 100% methanol and measured using a calibrated Turner 10-AU fluorometer. Oxygen was measured optically using an *in situ*, calibrated probe (YSI ProODO). Turbidity was measured on discrete samples using a calibrated handheld turbidimeter (Orion AQ4500). Dissolved inorganic carbon was quantified as carbon dioxide using Apollo Scitech DIC analyser AS-C3. Tidal height and wind speed were obtained from NOAA station #8656483 (Beaufort, NC, USA). Incoming no-sky solar radiation was estimated using the USGS AIR_SEA toolbox for Matlab.

Vibrio culturing and sequencing

To obtain vibrio isolates, seawater samples were concentrated on a 0.2 μm pore size polyethersulfone membrane filter (Pall Supor-200). Filters were then placed on Thiosulfate Citrate Bile Sucrose media (Difco) supplemented with 10 g l^{-1} of NaCl (TCBS) and incubated at room temperature. Colonies were isolated by streaking three times, alternately on Luria-Bertani with 20 g l^{-1} of NaCl and TCBS (Thompson *et al.*, 2005). Cultures were preserved in glycerol at –80°C and DNA was extracted using Lyse-N-Go (Thermo Scientific) and stored at –20°C. Isolates were screened by sequencing the *hsp60* gene as previously described (Goh *et al.*, 1996; Hunt *et al.*, 2008b). For selected isolates, the housekeeping genes *adk* and *mdh* were sequenced using modified primers as described previously (Preheim *et al.*, 2011). Amplicons were sequenced on an ABI automated sequencer at the Duke Center for Genomic and Computational Biology. Sequences were manually edited using Sequencher (Genecodes) and manually aligned using Bioedit. This analysis yielded unambiguous alignments (no gaps) for *hsp60*, *adk* and *mdh* of 500, 440 and 426 bp respectively.

Statistical analyses

After editing, *hsp60* sequences were obtained for 1803 isolates (average sequences/sample = 59.3, minimum = 35, maximum = 84). We used the furthest neighbour algorithm to cluster *hsp60* sequences into 171 OTUs with > 97% identity. We removed 68 OTUs that were observed a single time from data used for the CCA so that the importance of these low abundance OTUs was not overestimated. CCA was used to identify the environmental factors associated with differences between vibrio community composition at different time points (ter Braak and Verdonschot, 1995). Prior to the CCA, the 'step' function in the R package vegan (Oksanen *et al.*, 2007) was used to automatically select constraints in the model using forward stepwise selection using Akaike's information selection criterion with 999 permutation tests at each step. Only the terms that are statistically significant in the stepwise selection were used as the constrained set of parameters to relate the environmental variables to vibrio community composition using the 'cca' function in vegan (Oksanen *et al.*, 2007). Statistical significance of the individual environmental variables was assessed using the marginal effect of the terms.

We used MEGA 6 (Tamura *et al.*, 2013) to find the best-fit substitution model and transition/transversion ratio to construct the maximum likelihood tree based on *hsp60* gene sequences using using PhyML 3.0 (Guindon *et al.*, 2010). The following parameter settings were used: DNA substitution was modelled using the GTK parameter; estimated proportion of invariable nucleotide sites was 0.48; the gamma distribution parameter was set to 0.6; five gamma rate categories were used and a BIONJ tree was initially used and improved using NNI. Isolates were partitioned into ecologically cohesive clades according to genetic and ecological similarity using AdaptML (Hunt *et al.*, 2008b) and visualized using the Interactive Tree of Life tool (Letunic and Bork, 2007).

Vibrio physiology

Closely related isolates from distinct water temperatures with high similarity among the three sequenced housekeeping genes were analysed further using thermal performance curves.

For clusters of closely related isolates, the growth rate of triplicate biological replicates was established in glucose minimal media (Tibbles and Rawlings, 1994) supplemented with 10 mM ammonium, F/2 trace metals and F/2 vitamins (Guillard, 1975) over a range of temperatures (~4°C intervals spanning 4–40°C). Cultures were incubated at the designated temperature with constant agitation until entering mid-exponential phase. These cultures were then diluted 1:100 in fresh media and incubated at the designated temperature with constant shaking at 120 r.p.m. The growth rate of each isolate was measured spectrophotometrically using the optical density at 600 nm. Cultures were determined not to grow at a given temperature if the optical density remained < 0.05 after 2 weeks. For cultures with visible aggregates, subsamples were sonicated at 5 W for 3 s using a Microson Ultrasonic cell disruptor XL-2000 immediately prior to reading the optical density; this treatment was

experimentally shown not to change the optical density of non-aggregated samples.

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Data deposition

Vibrio partial 16S rRNA, *hsp60, mdh* and *adk* gene sequences are deposited in GenBank under Accession No. KM573823–KM575707. Environmental sampling data are available as part of the Pivers Island Coastal Observatory (PICO) database available through BCO-DMO Project #2281.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Map showing location of Pivers Island Coastal Observatory (PICO) time series station part of the Albemarle-Pamlico Sound system. (A) Map depicting the United States Eastern Seaboard. (B) Inset depicts detail of the study site at the Beaufort Inlet, which is indicated by an arrow.

Fig. S2. Bootstrap, maximum likelihood phylogenetic tree of *Vibrionaceae* isolates based on partial *hsp60* gene sequences with *Escherichia coli* K-12 outgroup. The ring of colour surrounding the tree indicates the water temperature from which each strain was isolated. Bootstrap values greater than 80% are indicated by a grey circle at the node.

Fig. S3. Maximum likelihood phylogenetic tree of *Vibrionaceae* isolates based on partial *hsp60* gene sequences with *Escherichia coli* K-12 outgroup. Green circles at the node indicate 97% identity operational taxonomic units containing at least ten members which are used for the box and whiskers plot (Fig. 4).

Fig. S4. Maximum likelihood, concatenated partial gene tree (*hsp60, adk, mdh*) for selected *Vibrionaceae* isolates used in growth rate studies. Grey dots indicate a bootstrap value greater than 80%. *Escherichia coli* K-12 was used as the outgroup.

Table S1. Complete set of environmental variables used for principle components analysis for the Pivers Island Coastal Observatory time series. Environmental variables marked with asterisks were the constraint set of variables used in canonical correspondence analysis.