Shape and evolution of the fundamental niche in marine *Vibrio*
Supporting Information

Temperature gradient apparatus

Stable and reproducible temperature gradients were established using a custom made device (Fig. S1). The temperature-conducting top parts of two ECHOtherm IC25 chilling/heating devices (Torrey Pines Scientific, San Marcos, CA) were each connected to a 248 x 72 x 10 mm copper sheet, acting as a temperature conductor. A single 352 x 248 x 8 mm brass sheet on top of the copper conductors connected both chilling/heating devices, with a thin layer of thermal ceramic grease separating the copper conductors from the brass top sheet. Grooves, 3 mm wide and 3 mm deep, specifically designed to accommodate the pedestals of the bio-assay dishes, were edged into the top sheet. This aligned the bio-assay dish, the underlying copper conductors and the subjacent chilling/heating devices, allowing a 10 mm overlap between the edges of the bio-assay dish and the copper conductors. Materials were chosen based on their specific thermal conductivity to enable lateral temperature uniformity at each point along the temperature gradient: The insulating layer of thermal ceramic grease (specific thermal conductivity ~3 $W \cdot m^{-1} \cdot K^{-1}$) between the highly conductive copper conductors (specific thermal conductivity 401 $W \cdot m^{-1} \cdot K^{-1}$) and the less conductive brass top sheet (specific thermal conductivity 121 $W \cdot m^{-1} \cdot K^{-1}$) acts as a barrier, which enables lateral temperature equilibration in the copper conductors, an effect that is further enhanced by the reduced thermal conductivity of the top sheet relative to the copper conductors.

After establishment of a salinity gradient, the bio-assay dish was installed in its position on the brass top sheet with the axis of the salinity gradient perpendicular to the chilling/heating devices. To avoid insulating effects from residual air trapped between the bottom of the salinity gradient dish and the surface of the temperature gradient top sheet, a thin film of water was applied between both surfaces. Evaporation was postponed by sealing the gap between dish and top sheet with 2% w/v agarose gel. Finally, a custom made layer of insulating foam minimized heat loss to the surrounding air by encasing the entire temperature gradient setup, with exception of the bio-assay dish. The uninsulated bio-assay dish thus acted as heat sink enabling efficient conduction of the temperature gradient into the solid medium salinity gradient.

2D-gradients: accuracy and reproducibility

Individual temperature measurements had an average standard deviation of ~0.6 ˚C. In addition, the reproducibility of temperature gradients was high as indicated by low standard deviations when measuring at identical sites in independent experiments. On average the standard deviation between experiments was 0.71 ˚C (max. 0.94 ˚C) for the colder settings and 1.00 ˚C (max. 1.37˚C) for the warmer settings (Fig. S3).

Salinity gradients were established by allowing wedges of solid media supplemented with either 0 M or 2.2 M NaCl to equilibrate by diffusion. Gradients equilibrated within 40 hours and remained essentially constant throughout the duration of the experiment (an additional 48 hours; Fig. S4). Due to spatial heterogeneity (up to 3 ppt across a gradient could be detected; Fig. S4D), the actual values for each culture spot along the ZNGI were obtained by destructive sampling

and refractometry (core samples yielded sufficient material for duplicate measurements for each core). We observed low standard deviations (on average 0.56 ppt = 0.021 M NaCl) and a tight distribution (R2=0.9954) of salinity values measured along multiple hypothetical salinity isoclines at different time points during the experimental 48 h time window (Fig. S4B, C).

Neutral model of niche parameter evolution

Although niche shapes differ substantially between groups, and niches would seem likely to be under evolutionary pressure in the wild, we found that the patterns of niche shape parameters did not differ significantly from those expected under a neutral, Brownian motion evolutionary model. In the Brownian motion model, trait values (e.g. optimal temperature) change randomly over time, in an unbiased manner (i.e. traits are as likely to increase as to decrease.), and at a constant rate. When a speciation event occurs, the derived taxa initially have identical trait values, but tend to drift apart as time progresses, resulting in more closely related organisms having more similar trait values.

This neutral Brownian motion model could not be rejected for the evolution of M, T_{Max} and S_{Max} (Table S1, Materials and Methods). However, this is not positive proof that niche shape in not under selection. It is possible that selections is weak at the limited range of phylogenetic and environmental differentiation examined in this study, but intensifies at larger scales. Alternatively, the observed niche shapes could be under selection, to which they respond rapidly, however the direction of the selection pressure itself is random (e.g., because of fluctuating environmental conditions.). Distinguishing these scenarios requires a more comprehensive study, involving a larger number of more diverse organisms and environments.

Figure Legends

Figure S1 – 2D gradient apparatus
Upper Panel: Illustration of two wedged shape media layers containing LB-S medium with high (red) and low (blue) concentrations of NaCl. The bottom medium layer is poured into a Nunc BioAssay dish after controlled slanting of the dish. After solidification of the bottom layer, the medium dish is returned form a slanted into a horizontal position, and the top medium layer is added.
Mid Panel: Schematic model of the Thermogradient device (top view with lateral view below). Thermal conductivities for brass (yellow), thermal grease (white), and copper (brown) are shown on the side. For heating/cooling, base units without tube adapters of Torrey-Pines heating/chilling dry-bathes (ECHOthermTM Model IC20XT Digital, Electronic Chilling/Heating Plate) are used. The cooling and a heating dry-bath units (the overlap of dry bath surface with the top plate is shown as (cold) blue dashed outline/solid blue, and (hot) red dashed outline/solid red) conduct temperature through copper sheets (brown hatched lines/solid brown) and a thin layer of ceramic conductive grease (white intersection) to a brass top sheet (yellow) with groves fitting the pedestales of the Nunc BioAssay Dishes. The higher thermal conductivity of the copper sheets allow for an even lateral distribution of temperature before conduction through the thin insulating layer of thermal grease to the brass top sheet. Adjusting the two dry-baths controls the temperature range of the temperature gradient.
Lower Panel: To ensure that the medium acts as the major heat sink, the entire device was wrapped into custom fitted insulating foam, shown in green.

Figure S2 - Calibration of salinity measurements
Salinity was measured by destructive sampling along 2-dimensional salinity temperature gradients. The liquid phase of medium samples was extracted as described in the Materials and Methods section of the main paper, and analyzed using a salinity refractometer. To normalize for light diffraction caused by medium compounds or salts other than NaCl a standard curve was measured. Duplicate measurements of 8 LB-S agar samples of known NaCl concentration were obtained. Linear regression of the averaged results revealed the following function $f(x) = 0.0338x - 0.2834$, which converts salinity measured in ppt units in the medium into mol NaCl per l. A regression coefficient of $R^2 = 0.9989$ confirms a tight fitting of the linear function to the measurements.

Figure S3 - Temperature gradient - profile and reproducibility
(A) Temperature gradient across a medium dish at either the cold (blue) or warm setting (red). In both cases the gradient increases mostly linearly, only leveling off slightly towards the edges of the medium dish. The upper error bars, demonstrating the error of the temperature measurements, show the average standard deviation obtained at 22 different sites over all analyzed gradients. The lower error bars, demonstrating the reproducible establishment of temperature gradients, show the standard deviation of the mean temperature averaged over all experiments for 22 different positions along the gradients.
(B) Lateral homogeneity of the temperature gradient. 22 measurements taken along 6 different isoclines revealed minimal variation of generally $\leq 0.5^{\circ}C$ (error bars) along isoclines.

Figure S4 - Salinity gradient - profile and reproducibility

(A) Diffusion driven formation of the salinity gradient over time, measured at 4 different time point over 90 hours. After 40 h of gradient formation the diffusion driven process reaches an equilibrium that remains stable for at least 50 h.
(B) Stability of the salinity gradient during the time window in which growth experiments were conducted. Multiple salinity measurements taken along the gradient axis at different time points during a 50 h time window fit tightly (R^2>0.99) to a cubic function.
(C) The tight fit and low standard deviations between measurements taken along salinity isoclines at the different time points further emphasize the stability over time of the gradient, and suggest a high reproducibility of salinity measurements. A moderate lateral inhomogeneity along hypothetical salinity isoclines, requires the salinity to be measured directly for individual culture spot along the ZNGI.
(D) For better illustration, only the 3 largest observed standard deviations resulting from multiple measurements along 10 different salinity isoclines on a gradient are shown.

Figure S5 - Modeling the error of salinity and temperature measurements resulting from the culture spot intervals on 2D gradients
(A) Spotting cells in a grid pattern onto the 2D salinity-temperature gradients introduced an additional uncertainty into ZNGI estimates. The true ZNGI may occur anywhere between the most extreme point that showed growth at the position i and the adjacent grid point that inhibited growth (spot interval). The maximum error introduced by this uncertainty can then be described by the difference in temperature (ΔT) or salinity (ΔS) between both points plus/minus the standard deviation (σ) from the actually measured value at position i. Since σT was independent of the position i on a gradient (data not shown), the average standard deviation of all temperature measurements obtained in the respective set of experiments (cold or warm temperature setting) was used instead. Salinity measurements could only be performed in duplicates. Therefore the error of individual measurements was not obtained.
(B) Maximum error resulting from the interval between two spots predicted for each spotting position along a salinity gradient.
(C and D) Maximum error potentially resulting from the interval between two spots predicted for each spotting position along a temperature gradient. Blue color (Panel C) indicates the cold, red color (Panel D) the hot temperature gradient setup. Whiskers represent the average standard deviation obtained for all temperature measurements.

Figure S6 – Observed ZNGIs
Zero Net Growth Isoclines (ZNGI), as obtained from growth on 2-dimensional (2-D) gradients, approximating the partial shapes of the fundamental salinity-temperature niche for 17 *Vibrio* strains. The ZNGI plots are listed by their morphology index (M values) in decreasing order from top to bottom, and from left to right. M values were obtained for the high temperature/high salinity quadrant (highlighted in red) as described in the Materials and Methods section of the main paper. To illustrate the reproducibility of the growth experiments, replicates were performed for *V. splendidus* 12B1 and for *V. fischeri* MJ11. The deviations between replicates in the high temperature/high salinity quadrant are within the predicted maximum experimental error resulting from measurement inaccuracy and the “blind” intervals that originate from the grid-like culture spot pattern of 2-D gradients (See also Fig. 2 and SI 4).

Figure S7 – ZNGIs of all strains at the low temperature/high salinity quadrant, colored by M values.
Classes of niche shapes in the low temperature/high salinity quadrant are consistent with those of the high temperature/high salinity quadrant (compare Fig. 3A of main text).

Figure S8 – Independent contrasts analysis
Relation between M contrast and branch length. For traits whose evolution follows a brownian motion model there should be no relation between contrasts and branch lengths. Similar analysis was performed for all other shape parameters (data not shown).
(A) All contrasts. Contrast that were outliers with respect to any parameter were excluded (red crosses). Linear ordinary least squares fit (red), does not show significant trend (Table S1), supporting the brownian motion model.
(B) Contrasts included in the analysis. The distribution of these contrasts was tested against a normal distribution using the Shapiro-Wilk test (Table S1).