

SUPPLEMENTARY INFORMATION

Lessons from the equator: a window on the future of vibriosis in a warming Earth

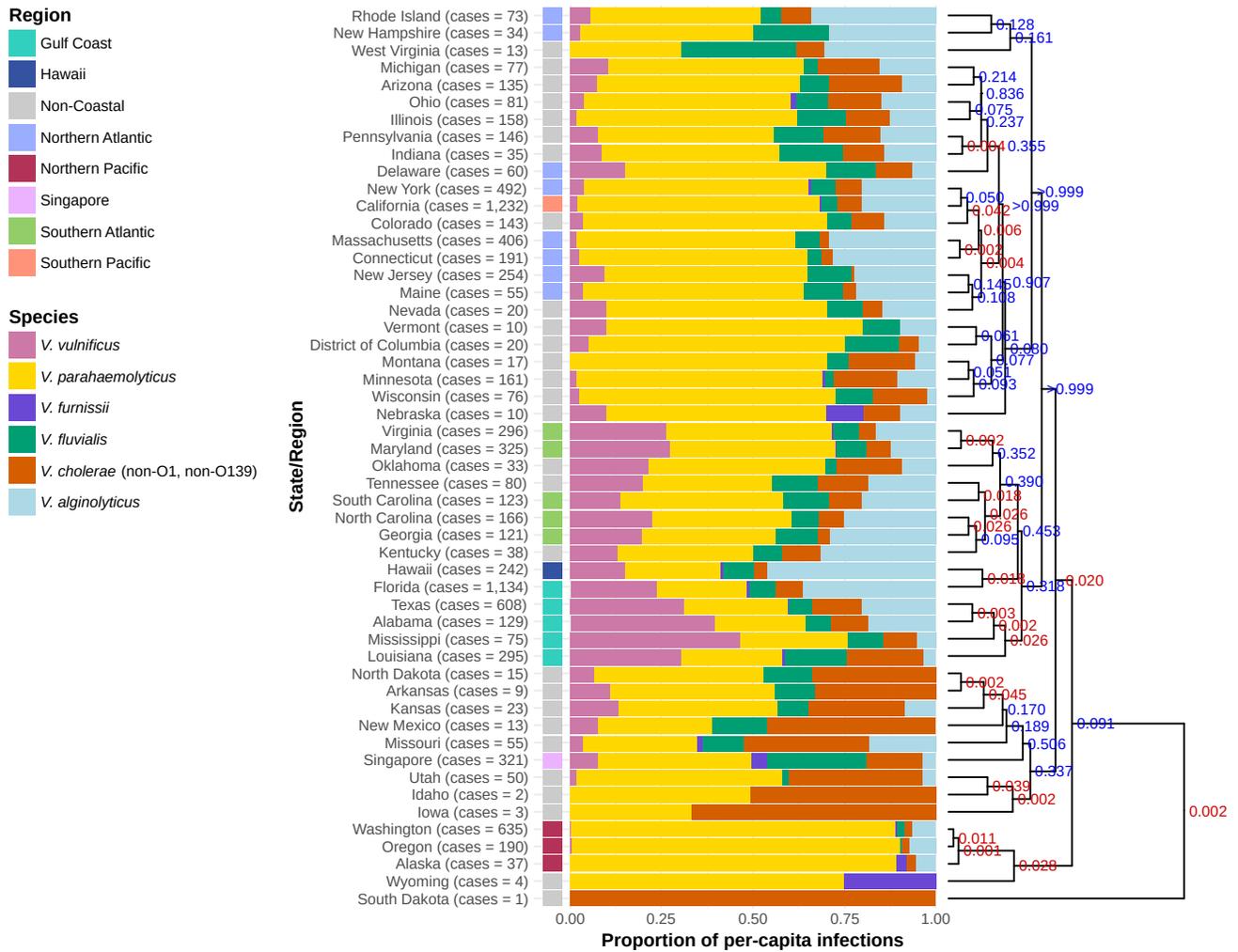
Christopher Colin Neoh, Eric Dubois Hill, Fabini D. Orata, Craig Baker-Austin, Marisa Hast, Michael J. Hughes, Bryan M.H. Keng, Patrick Martin, Tan Yen Ee, Crystal Shie Lyeen Wong, Chew Ka Lip, Su Gin Douglas Chan, Chen Yihui, Timothy Barkham, Swapnil Mishra, Jaime Martinez-Urtaza, Alison E. Mather, Christine Lee, and Yann Felix Boucher*

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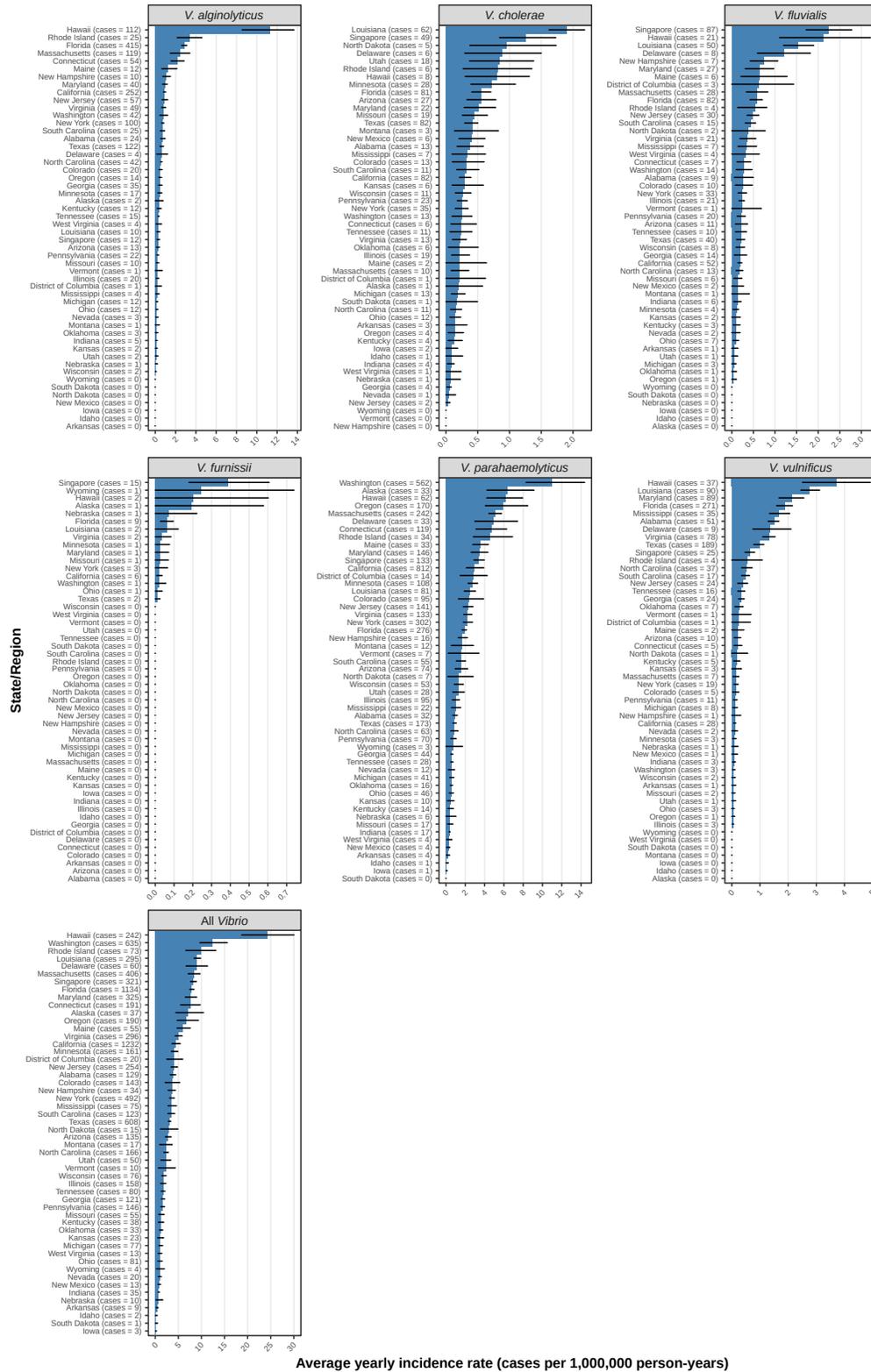
This supplementary file includes:

- Supplementary Figures 1–7
- Supplementary Methods
- References

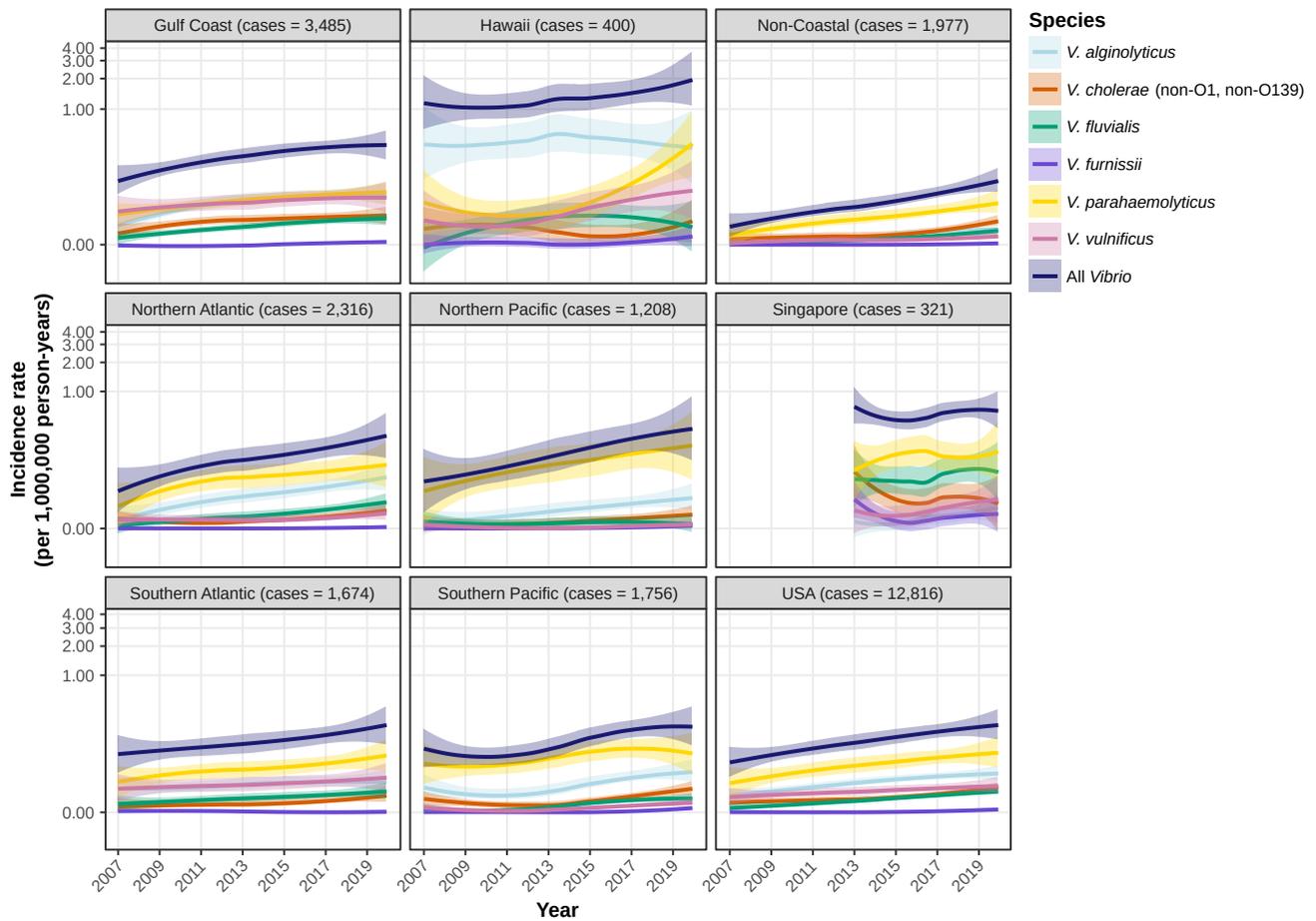
SUPPLEMENTARY FIGURES



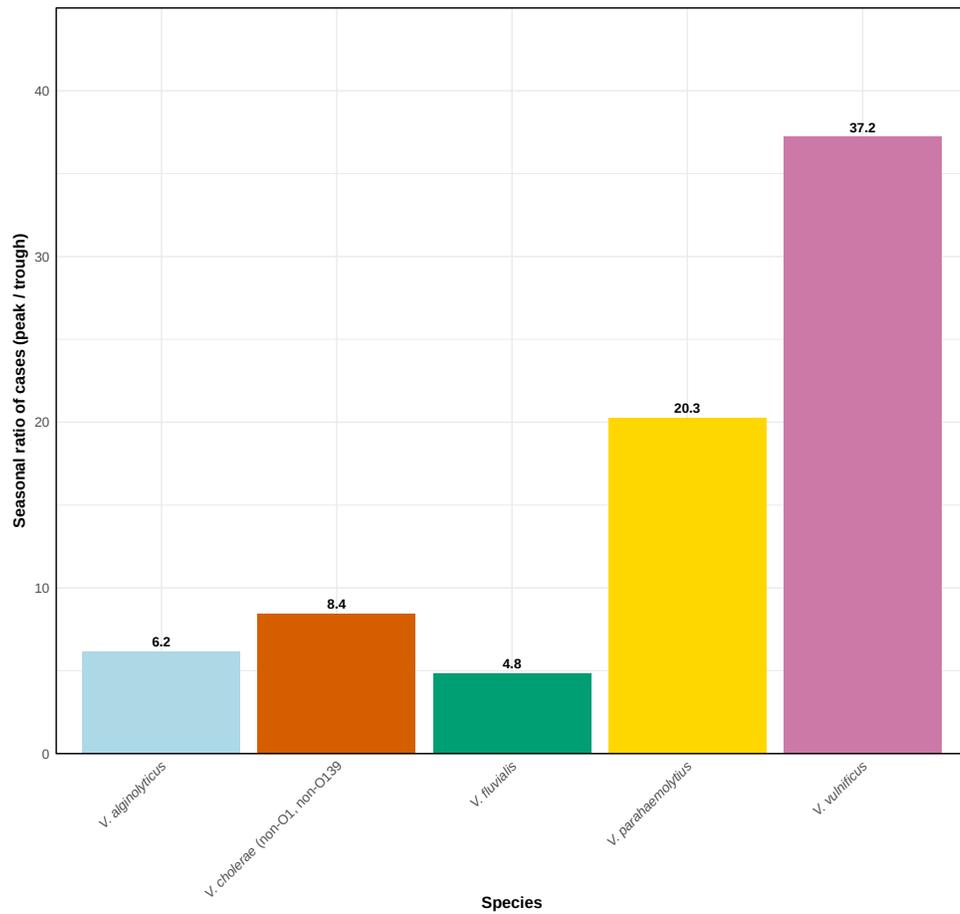
Supplementary Fig. 1. Relative proportion of *Vibrio* infections by causative species across US states and Singapore. US states and Singapore were grouped with agglomerative hierarchical clustering based on Bray–Curtis dissimilarities of their species-level relative-abundance profiles, using the average-linkage (UPGMA) method to merge clusters. The p -values test the null hypothesis that the cluster does not exist, with red ($p \leq 0.05$) denoting statistically significant clusters and blue ($p > 0.05$) indicating non-significant groupings.



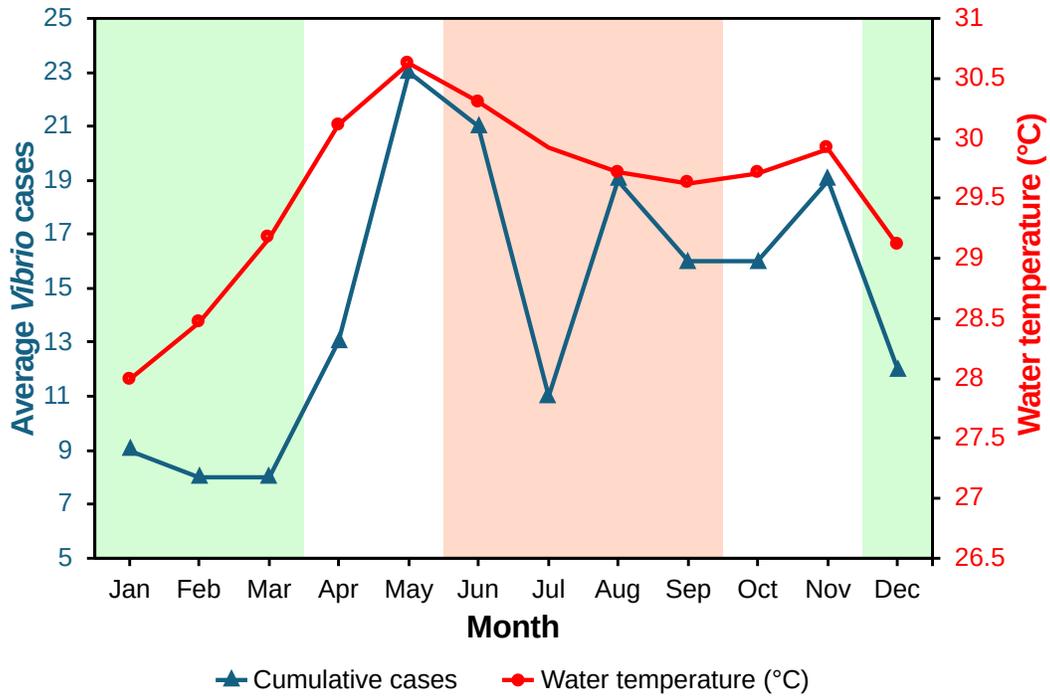
Supplementary Fig. 2. Incidence rates of vibriosis by causative species across US states and Singapore. Incidence rates were calculated for 2013–2019 from the US Cholera and Other Vibrio Illness Surveillance (COVIS) database and records from five major public hospitals (covering 57% of national bed capacity) for Singapore. Error bars represent the 95% confidence intervals for the mean incidence rate per 1,000,000 person-years, calculated using bootstrapping with 10,000 replicates.



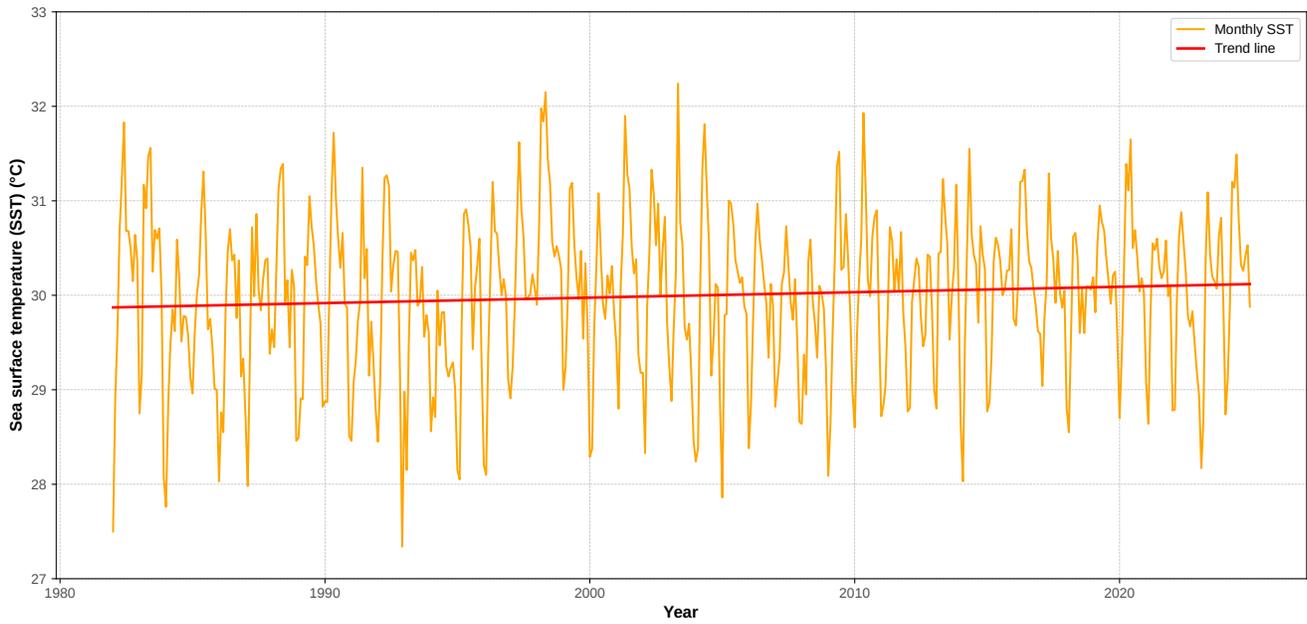
Supplementary Fig. 3. Incidence rates of vibriosis in different US regions and Singapore. Smoothed mean lines were calculated using LOESS regression (span = 1.0), with shaded areas showing the 95% confidence intervals.



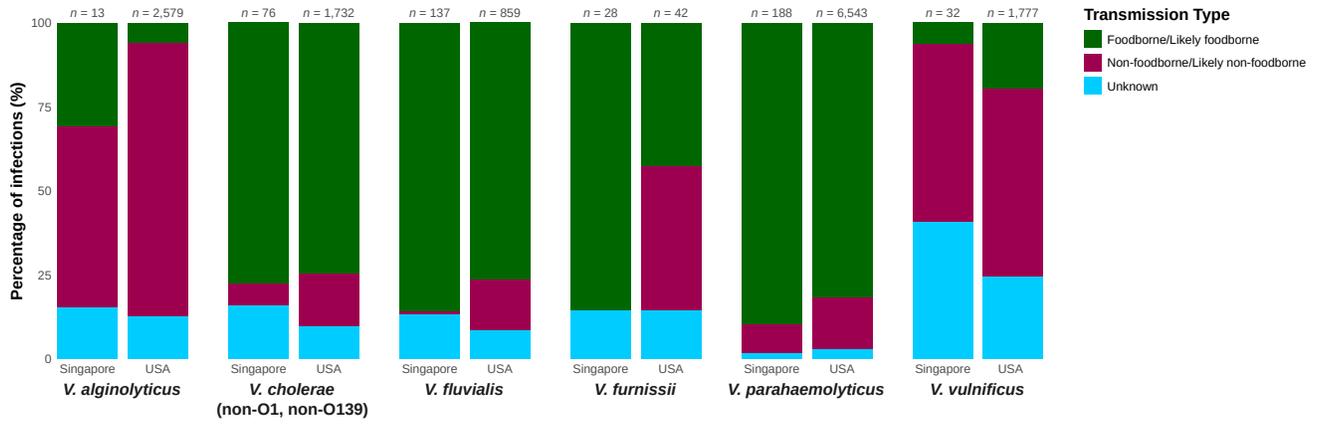
Supplementary Fig. 4. Seasonal ratio of Vibrio infections between high and low seasons. Ratios were calculated by comparing mean monthly incidence during the high season (summer/peak) with that during the low season (winter/trough).



Supplementary Fig. 5. Monthly variation of vibriosis cases versus water temperature in Singapore. Vibriosis case totals and water temperature across the months of January to December. Shaded sections indicate the Southwest monsoon (June–September) and Northeast monsoon (December–March).



Supplementary Fig. 6. Long-term trend in average sea surface temperature (SST) in Singapore (1982–2024). Monthly SST near Singapore from NOAA’s $\frac{1}{4}^\circ$ Daily OISST v2.1 monthly product, 1982–2024. The orange curve shows monthly SST; the red line represents the linear trend. The fitted slope is equivalent to $\sim 0.058^\circ\text{C}$ per decade, implying $\sim 0.25^\circ\text{C}$ warming over 1982–2024. Warmer coastal SSTs are associated in the literature with increased environmental suitability for marine pathogens and related infection risk.



Supplementary Fig. 7. Modes of transmission for vibriosis by species in the United States and Singapore. Bar graphs show the proportion of infections attributed to different transmission pathways (i.e., foodborne/likely foodborne, non-foodborne/likely non-foodborne, and unknown) for each *Vibrio* species.

SUPPLEMENTARY METHODS

Vibriosis case definitions

One consideration with vibriosis is that not all infections are symptomatic. For most *Vibrio* species, many infections are mild, self-limited illnesses that often go undiagnosed, with the exception of *Vibrio vulnificus*, which typically presents with more severe symptoms.¹ In the United States, reporting classifications are based on the Centers for Disease Control and Prevention (CDC) case definitions for probable and confirmed cases.² Currently, culture-independent diagnostic tests (CIDTs) are sufficient to meet the criteria for 'probable cases.' Culture confirmation is required for a case to be classified as 'confirmed,' which is the definition used in this study. If two or more *Vibrio* species are detected, each is considered a separate infection.² This approach was chosen to minimize the effects of CIDT introduction (in 2017) on apparent increases in diagnosis.

Vibrio species reported to the US COVIS database are based on results from state and local laboratories, which conduct culture confirmation using various methods such as API 20E or MALDI-TOF, both of which have limitations in sensitivity and specificity.³ Whole genome sequencing-based identification was also available nationwide through PulseNet, the national molecular surveillance system for food- and waterborne pathogens, providing an additional layer of quality assurance for species identification.⁴ For consistency in identification and reporting in this study, these CDC definitions were also applied to the Singapore dataset.

Statistical analysis

Monthly case counts were analyzed using a log-linked negative-binomial regression model to account for overdispersion and to express incidence rates on a per capita basis.⁵ The model included main effects for species and region, as well as their interaction, a linear time term (months since the first observation) and its interactions with both species and region, and an offset term of $\log(\text{Population})$. From this model, we obtained marginal time slopes (annual changes in incidence) for each species within each region; these were reported as rate ratios with Wald 95% confidence intervals. Resulting *p*-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate procedure.⁶

REFERENCES

1. Scallan, E. *et al.* Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* **17**, 7-15, doi:10.3201/eid1701.p111101 (2011).
2. US Centers for Disease Control and Prevention. *Vibriosis (any species of the family Vibrionaceae, other than toxigenic Vibrio cholerae O1 or O139) 2017 case definition*, <<https://ndc.services.cdc.gov/case-definitions/vibriosis-2017>> (April 16, 2021).
3. O'Hara, C. M., Sowers, E. G., Bopp, C. A., Duda, S. B. & Strockbine, N. A. Accuracy of six commercially available systems for identification of members of the family *Vibrionaceae*. *J Clin Microbiol* **41**, 5654-5659, doi:10.1128/JCM.41.12.5654-5659.2003 (2003).
4. Swaminathan, B., Barrett, T. J., Hunter, S. B., Tauxe, R. V. & Force, C. D. C. P. T. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerg Infect Dis* **7**, 382-389, doi:10.3201/eid0703.010303 (2001).
5. Linden, A. & Mäntyniemi, S. Using the negative binomial distribution to model overdispersion in ecological count data. *Ecology* **92**, 1414-1421, doi:10.1890/10-1831.1 (2011).
6. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol* **57**, 289-300, doi:10.1111/j.2517-6161.1995.tb02031.x (2018).