

## REPORT

## COASTAL ECOSYSTEMS

# Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates

Joleah B. Lamb,<sup>1\*</sup> Jeroen A. J. M. van de Water,<sup>2,3</sup> David G. Bourne,<sup>2,4</sup> Craig Altier,<sup>5</sup> Margaux Y. Hein,<sup>4</sup> Evan A. Fiorenza,<sup>1</sup> Nur Abu,<sup>6</sup> Jamaluddin Jompa,<sup>6</sup> C. Drew Harvell<sup>1</sup>

Plants are important in urban environments for removing pathogens and improving water quality. Seagrass meadows are the most widespread coastal ecosystem on the planet. Although these plants are known to be associated with natural biocide production, they have not been evaluated for their ability to remove microbiological contamination. Using amplicon sequencing of the 16S ribosomal RNA gene, we found that when seagrass meadows are present, there was a 50% reduction in the relative abundance of potential bacterial pathogens capable of causing disease in humans and marine organisms. Moreover, field surveys of more than 8000 reef-building corals located adjacent to seagrass meadows showed twofold reductions in disease levels compared to corals at paired sites without adjacent seagrass meadows. These results highlight the importance of seagrass ecosystems to the health of humans and other organisms.

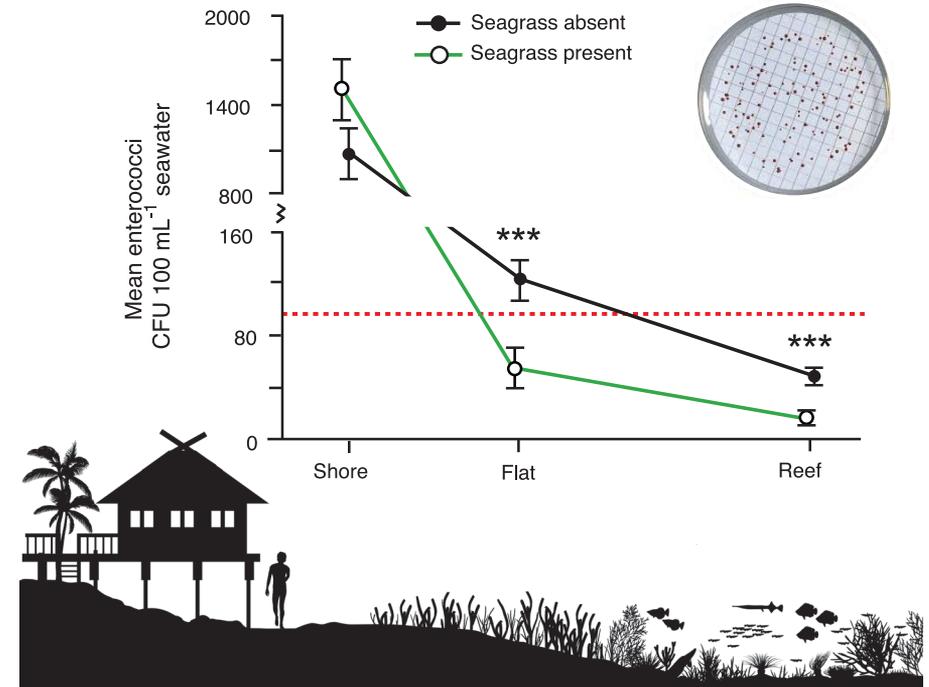
Natural filtration systems are widely used to remove human pathogenic microorganisms from terrestrial effluent. Successful applications range from sewage treatment works to constructed wetlands (1), mangrove forests (2), and restored bivalve reefs (3). Despite being the most widespread coastal ecosystem on the planet (4), seagrass meadows have not been evaluated as a system for pathogen removal or disease mitigation. Plants have been used effectively in many pollution treatment systems (5) because they inactivate pathogens by exposure to natural biocides, biofilm interactions, nutrient removal, and the alteration of soil or water chemistry. In vitro, phytochemicals isolated from seagrass tissues have been shown to kill or inhibit numerous bacterial pathogens that affect humans, fishes, and invertebrates (6).

Here we collected data from a natural field setting (7) to assess the influence of biodiverse seagrass meadows on marine microbial pathogens and disease. We selected four islands located on the midshelf of the Spermonde Archipelago, Indonesia (fig. S1). Each island consists of biogenic sediments from the surrounding reef platforms (8) that enclose areas of intertidal and shallow subtidal flats with expansive areas of

mixed-species seagrass meadows. Human population densities on the islands range between 96 and 325 people per hectare (9). The islands lack

basic sanitation systems, and surface soils are thin and poorly retain wastewater (10). To ensure suitable comparisons, we selected a set of paired sites (7) where seagrass meadows were either present or absent on the intertidal flat surrounding each island (fig. S2 and table S1).

As a standard indicator for enteric wastewater pollution and source of multiple infections in humans and marine organisms (11), we first used *Enterococcus* assays to test whether the presence of seagrass meadows influenced the level of a single bacterial pathogen (7). Our shore seawater samples, collected from all four paired island sites [median colony forming units (CFU) 100 ml<sup>-1</sup> = 1123,  $n = 33$  samples for each status (seagrass present versus absent)], exceeded the U.S. Environmental Protection Agency (EPA)-recommended human health risk exposure levels of *Enterococcus* in recreational water by 10-fold (12) (Fig. 1). Seawater collected in open water between the study islands (7) had low levels of *Enterococcus* (range = 0 to 12 CFU, median = 4 CFU,  $n = 40$  samples), indicating that wastewater pollution was diffuse and likely originated from individual islands. Levels of *Enterococcus* in seawater were reduced when seagrass meadows were present compared with paired sites without seagrass meadows (generalized linear mixed model, estimate  $\pm$  SE<sub>status  $\times$  location</sub> =  $-0.786 \pm 0.202$ ,  $P < 0.001$ ) (Fig. 1 and table S2). On the intertidal flats, *Enterococcus* levels in seawater were threefold lower when seagrass meadows were present ( $n = 82$  samples) compared with the paired



**Fig. 1. Seagrass meadows influence the occurrence of enteric indicator bacteria.** Comparisons of mean ( $\pm$ SE) CFU of enterococci in 100 ml of seawater. Values above the red dashed line exceed the EPA-recommended human health risk exposure levels for a single water sample in recreational waters (12). Differences tested using a generalized linear mixed model followed by pairwise comparisons and Bonferroni correction, where \*\*\* $P < 0.001$  (table S3). Means calculated from paired sites among four study islands. Petri dish exemplifies cultured enterococci.

<sup>1</sup>Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA. <sup>2</sup>Australian Institute of Marine Science, Townsville, Queensland, Australia. <sup>3</sup>Department of Marine Biology, Centre Scientifique de Monaco, Monaco.

<sup>4</sup>College of Science and Engineering, James Cook University, Townsville, Queensland, Australia. <sup>5</sup>Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA. <sup>6</sup>Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Sulawesi, Indonesia.

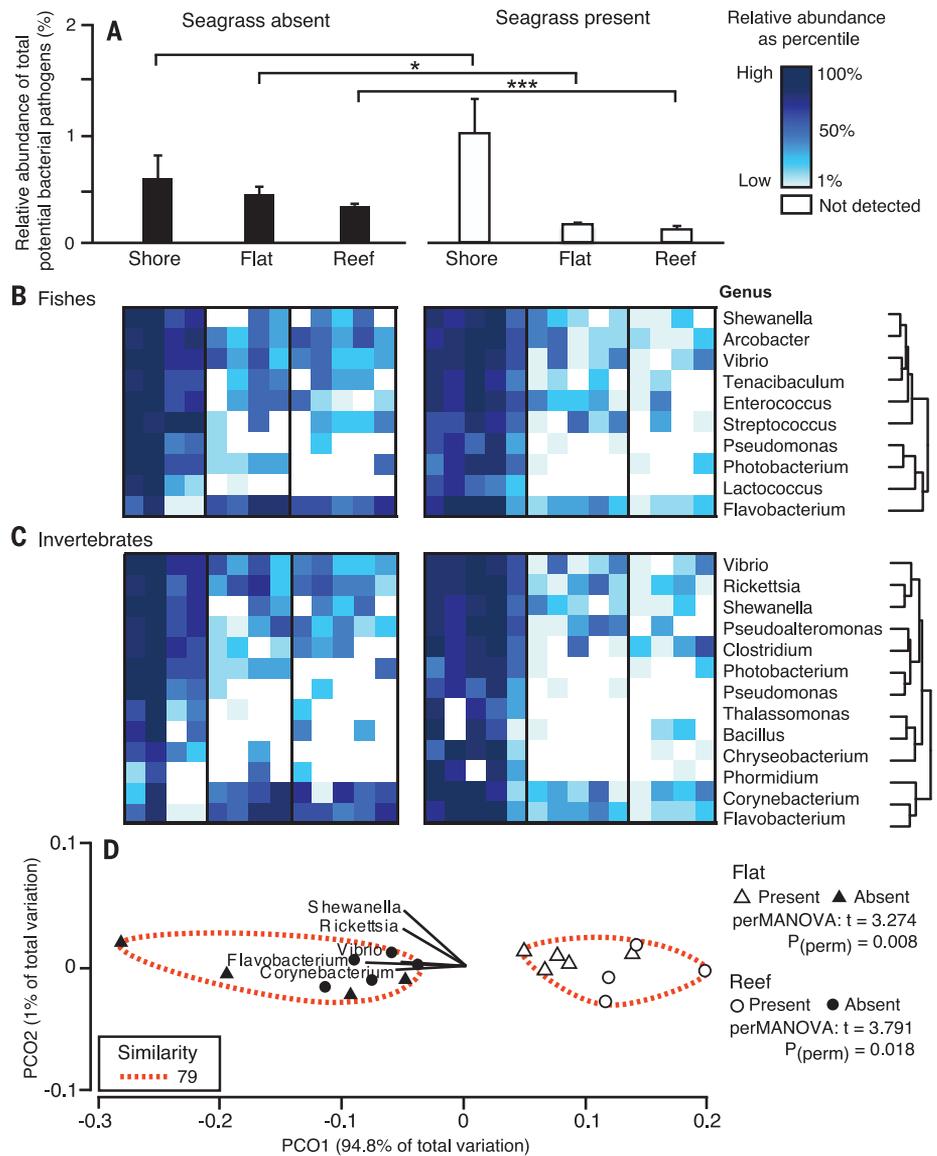
\*Corresponding author. Email: joleah.lamb@cornell.edu

sites ( $n = 72$  samples). Similarly, seawater collected above the coral reef adjacent to seagrass meadows ( $n = 86$  samples) had twofold lower levels of *Enterococcus* than paired sites ( $n = 82$  samples) (generalized linear mixed model, Fig. 1 and table S3).

*Enterococcus* levels often correlate with other human bacterial pathogens found in wastewater (12), suggesting that seagrass meadows could also influence the concentration of pathogens that affect other organisms. A literature review identified bacteria classified as potentially pathogenic to marine fishes, invertebrates, and mammals (42 genera were identified to the lowest common taxonomic level, table S4). High-throughput amplicon sequencing of the 16S ribosomal RNA gene revealed the composition of the microbial organisms in seawater at our paired sites at Bonetambung Island (7). Sequencing of the amplicon library on the Illumina MiSeq platform generated 1,398,719 high-quality reads ( $n = 27$  samples, median 47,592 reads), which were identified as being of bacterial origin, after clustering at 3% divergence (97% similarity) and taxonomy assignment to the most abundant representative sequence (7). Our sequences aligned with 27 of 42 potentially pathogenic genera described in the literature. We removed 9 genera from the final analysis because these taxonomic units were only detected at the shore (table S5), then standardized the relative abundance of bacteria for the remaining 18 genera to the total bacteria enumerated in each seawater sample by performing quantitative polymerase chain reaction (PCR) (7) (fig. S3 and table S6).

Similarly to the findings using *Enterococcus* assays, the relative abundance of potential bacterial pathogens of multiple marine fishes and invertebrates in seawater was lower when seagrass was present compared with the paired site without seagrass (generalized linear model, estimate  $\pm$  SE<sub>status</sub>  $\times$  location =  $-0.790 \pm 0.221$ ,  $P < 0.001$ ) (Fig. 2A and table S7). The relative abundance of bacterial pathogens in seawater from the intertidal flat was 50% lower within the seagrass meadow compared to the paired site (generalized linear model,  $Z = 2.061$ ,  $P = 0.039$ ) and 50% lower in seawater from the coral reef adjacent to the seagrass meadow compared with the paired site (generalized linear model,  $Z = 4.098$ ,  $P < 0.001$ ) (Fig. 2, A to C, and table S8). Assemblages of potential bacterial pathogens (assessed by composition and relative abundance) formed two clear groups (representing 94.8% of the total assemblage variability with 79% similarity among group replicates) and differed significantly between the paired seagrass present and absent sites [permutational multivariate analysis of variance (perMANOVA), Fig. 2D]. The separations between the groups (determined by Pearson correlations  $\geq 0.6$ ) were associated with higher relative abundance of *Flavobacterium*, *Corynebacterium*, *Vibrio*, *Rickettsia*, and *Shewanella* at sites where seagrass meadows were absent (Fig. 2, B to D; fig. S4; and table S9).

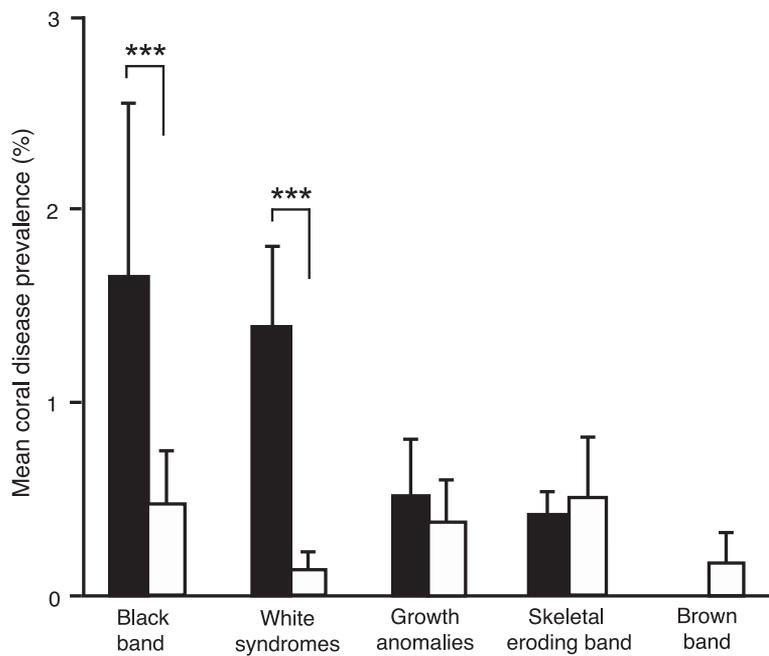
Effective treatment of municipal sewage and a broad range of industrial wastewater treatments using natural ecosystem services have been linked



**Fig. 2. Seagrass meadows moderate multiple potential bacterial pathogens of marine animals.** (A) Total relative abundance (mean  $\pm$  SE) of 18 potential bacterial pathogens of marine (B) fishes, and (C) invertebrates (reviewed in table S1), where  $***P < 0.001$  and  $*P < 0.05$  (generalized linear model, table S8). The heatmap illustrates the relative abundance of potential bacterial pathogens standardized for each genus from the total bacteria quantified in each replicate seawater sample (each column represents a replicate,  $n = 27$ ). Dendrograms represent hierarchical clustering based on similarities among genera. (D) Spatial representation of the relative abundance and composition of bacterial assemblages using a principal coordinate (PCO) analysis. Each point represents an individual sample ( $P_{(\text{perm})}$ , permutation significance level). Vectors superimposed on the ordination signify the strength and direction of genera driving differences between assemblages on axis 1 (PCO1, Pearson correlation  $\geq 0.6$ ). Hierarchical clusters were overlaid using dendrograms based on a Bray-Curtis similarity matrix and denote the percent similarity between samples (range = 0 to 100).

to reductions in preventable waterborne human diseases (13). However, there have been no studies to evaluate this effect for diseases that affect marine organisms. Seagrass meadows and coral reefs are tightly linked habitats (14) and provide an opportunity to assess this effect in situ. We visually examined 8034 reef-building corals for visual signs of tissue loss characteristic of active disease lesions (15) along reefs with adjacent sea-

grass meadows and paired reefs without adjacent seagrass meadows at all four islands (fig. S1 and table S1, benthic area surveyed = 360 m<sup>2</sup>). Overall coral disease prevalence was 50% less on reefs with adjacent seagrass meadows (mean  $\pm$  SE =  $1.6 \pm 0.6\%$ , range = 0 to 2%) than on paired reefs without adjacent seagrass meadows ( $3.9 \pm 1.2\%$ , range = 1 to 7%; generalized linear mixed model,  $Z = -4.213$ ,  $P < 0.001$ ,  $n = 4$  islands) (Fig. 3). Two



**Fig. 3. Diseases of reef-building corals are lower when adjacent to seagrass meadows.** Prevalence of surveyed coral diseases (mean  $\pm$  SE) on reefs with adjacent seagrass meadows (white bars) compared to reefs without seagrass meadows (black bars). Differences were tested using generalized linear mixed models, where  $P < 0.001$  (table S10). A total of 8034 reef-building corals were examined at paired sites among four islands.

of five globally occurring coral diseases, white syndrome and black band disease, as well as signs of coral tissue mortality associated with bleaching and sediment deposition, were significantly less on reefs adjacent to seagrass meadows compared with paired reefs (generalized linear mixed model, Fig. 3 and table S10).

Our results indicated that seagrass meadows significantly reduce bacterial loads and can benefit both humans and other organisms in the environment. Represented on every continental shelf but Antarctica, seagrasses are valued for (16) nutrient cycling, sediment stabilization, reducing the effects of carbon dioxide elevation, and providing nursery habitat for fisheries.

Our observation of reduced levels of coral disease on reefs adjacent to seagrass meadows offers independent support of the benefits that seagrass meadows provide to controlling pathogenic bacteria in marine environments. Although determining the mechanisms involved will require further research, sediment retention by seagrass meadows (16) could play an integral role in ameliorating disease. Sediment-associated bacteria have been

identified as a potential source of coral pathogens from marine and terrestrial substrates (17). Both black band disease (18) and white syndromes (19) have been linked to elevated levels of sediment exposure in situ.

Outbreaks of diseases that affect reef-building corals have recently emerged as a considerable driver of global coral reef degradation (20), with losses in the Caribbean and Indo-Pacific approximating 50 to 80%. For example, one bacterial pathogen isolated from sewage, *Serratia marcescens*, is linked to the decline of two dominant reef-building corals now on the U.S. endangered species list and subsequent ecological phase shifts from coral- to algal-dominated reefs (21). Regardless of the exact mechanisms involved, alleviating coral disease is vital for the well-being and livelihoods of 275 million people living within 30 km of a coral reef (22), as well as being of direct benefit to reef-dwelling species.

#### REFERENCES AND NOTES

1. S. Wu, P. N. Carvalho, J. A. Müller, V. R. Manoj, R. Dong, *Sci. Total Environ.* **541**, 8–22 (2016).

2. Q. Yang *et al.*, *Mar. Pollut. Bull.* **57**, 735–743 (2008).
3. T. K. Graczyk, A. S. Girouard, L. Tamang, S. P. Nappier, K. J. Schwab, *Appl. Environ. Microbiol.* **72**, 3390–3395 (2006).
4. J. L. Olsen *et al.*, *Nature* **530**, 331–335 (2016).
5. U. Stottmeister *et al.*, *Biotechnol. Adv.* **22**, 93–117 (2003).
6. R. R. R. Kannan, R. Arumugam, P. Anantharaman, *Asian Pac. J. Trop. Med.* **3**, 890–893 (2010).
7. Materials and methods are available as supplementary materials.
8. D. E. Hart, P. S. Kench, *Coral Reefs* **26**, 53–68 (2007).
9. K. Schwerdtner Máñez, S. Husain, S. C. A. Ferse, M. Máñez Costa, *Environ. Sci. Policy* **23**, 74–84 (2012).
10. I. White, T. Falkland, *Hydrogeol. J.* **18**, 227–246 (2010).
11. M. N. Byappanahalli, M. B. Nevers, A. Korajkic, Z. R. Staley, V. J. Harwood, *Microbiol. Mol. Biol. Rev.* **76**, 685–706 (2012).
12. U.S. Environmental Protection Agency (EPA), "Recreational Water Quality Criteria" (Publication 820-F-12-058, EPA, 2012).
13. S. B. Grant *et al.*, *Science* **337**, 681–686 (2012).
14. I. Nagelkerken, Ed., *Ecological Connectivity Among Tropical Coastal Ecosystems* (Springer, 2009).
15. J. B. Lamb *et al.*, *Philos. Trans. R. Soc. B* **371**, 20150210 (2016).
16. M. Waycott *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 12377–12381 (2009).
17. G. Hodgson, *Mar. Biol.* **104**, 493–496 (1990).
18. S. Al-Moghrabi, *Coral Reefs* **19**, 330–331 (2001).
19. F. J. Pollock *et al.*, *PLOS ONE* **9**, e102498 (2014).
20. D. Harvell *et al.*, *Oceanography (Wash. D.C.)* **20**, 172–195 (2007).
21. K. P. Sutherland, S. Shaban, J. L. Joyner, J. W. Porter, E. K. Lipp, *PLOS ONE* **6**, e23468 (2011).
22. L. M. Burke, K. Reyter, M. Spalding, A. Perry, *Reefs at Risk Revisited* (World Resources Institute, Washington, DC, 2011).

#### ACKNOWLEDGMENTS

This research was supported by The Nature Conservancy NatureNet Fellowship, Atkinson Center for a Sustainable Future at Cornell University, and the Capturing Coral Reef and Ecosystem Related Services (CCRES) Project funded by the Global Environment Facility and the World Bank (Project ID P123933). We thank R. Yoshioka and A. Tracy, A. Massinai, R. Ambo-Rappe, A. Ahmad, S. Beveridge, and S. Steven for statistical, laboratory, and field assistance. Vector illustrations are credited to T. Saxby of the Integration and Application Network at the University of Maryland Center for Environmental Science and Shutterstock contributors R. A. Hillman (image ID 25377103) and K. Ka (image ID 250306321). We acknowledge village community members for their permission to conduct this study through Hasanuddin University. Sequence data are archived at the National Center for Biotechnology Information (accession no. SRP095480). Data and code are available at the Dryad Digital Repository (doi:10.5061/dryad.51275).

#### SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/355/6326/731/suppl/DC1  
Materials and Methods  
Figs. S1 to S4  
Tables S1 to S10  
References (23–90)

16 October 2016; accepted 23 January 2017  
10.1126/science.aal1956



**Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates**

Joleah B. Lamb, Jeroen A. J. M. van de Water, David G. Bourne, Craig Altier, Margaux Y. Hein, Evan A. Fiorenza, Nur Abu, Jamaluddin Jompa and C. Drew Harvell (February 16, 2017) *Science* **355** (6326), 731-733. [doi: 10.1126/science.aal1956]

Editor's Summary

**Missing meadows fail to mop up microbes**

Seagrass meadows, a prominent feature of most healthy coastal ecosystems, are often also associated with shallow coral reefs. Many plants have bioremediation qualities, and seagrasses, of which there are 60 or so species, produce natural biocides. Lamb *et al.* found that the seagrass meadows of inhabited atolls near Sulawesi, Indonesia, ameliorated seawater pollution from human-origination bacteria. This effect extended to potential pathogens of marine invertebrates and fish: Reefs fringing the seagrass meadows showed significantly less impact from coral and fish disease.

*Science*, this issue p. 731

---

This copy is for your personal, non-commercial use only.

---

- Article Tools** Visit the online version of this article to access the personalization and article tools:  
<http://science.sciencemag.org/content/355/6326/731>
- Permissions** Obtain information about reproducing this article:  
<http://www.sciencemag.org/about/permissions.dtl>

*Science* (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2016 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.