Reservoir, sink, or reactor? Repositioning marine sponges as candidate indicators for coastal antimicrobial resistance monitoring

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Abstract

Antimicrobial resistance (AMR) spreads through the environment, and in coastal areas it is investigated mainly in water and sediment. These matrices capture antimicrobial resistance genes (ARGs) passing through them but may fail to retain that information over time because of the dynamics inherent in these ecosystems. Filter-feeding invertebrates are obvious candidates for the role, and bivalves have already been used for this purpose. Marine sponges (phylum Porifera) surpass bivalves on multiple fronts: they retain submicrometric particles that bivalves let pass, harbor microbial communities orders of magnitude denser than the surrounding water, and are sessile and long-lived in coastal areas receiving anthropogenic waste. Even so, researchers have never evaluated them as AMR indicators because sponge microbiology has focused on bioprospecting. Resistance in sponges almost always appears as incidental antibiogram data, or as annotation in metagenomes generated for other purposes. Here, we argue that the problem is not in collecting samples but in interpreting them: a sponge signal can only be read once an ecological question is resolved. As a reservoir, the holobiont accumulates ARGs and reflects environmental abundance, making it a valid monitoring target. As a sink, it removes ARG-carrying cells without passing them on, decoupling the signal from environmental load. As a reactor, it mobilizes ARGs into the resident symbiont community, so the signal indicates a risk the organism itself generates. This perspective distinguishes each role, identifies factors that confound these observations, and defines the validation strategy required for sponges to serve as indicators in AMR monitoring.

Keywords: Porifera; resistome; AMR ecology; bioindicator; sentinel; horizontal gene transfer; holobiont; One Health

1. Introduction

Antimicrobial resistance (AMR) is a major global public health issue that can evolve, persist, and spread through environmental mechanisms (Larsson & Flach, 2022). The One Health concept applied to AMR posits that antimicrobial resistance genes (ARGs) circulate among human, animal, and environmental compartments, and research has focused on this dynamic: sewage, rivers, coastal waters, soils, and agricultural areas are being studied regarding ARGs and their mobility (UNEP, 2023; Bongiovanni et al., 2025; Liang et al., 2025; Mao et al., 2025; Brown et al., 2026). The coastal marine environment plays a prominent role in this network. This compartment receives the bulk of land-based ARG discharges, particularly from domestic sewage, hospital effluents, aquaculture waste, and agro-industrial runoff, and serves as the point of contact between these discharges and humans, whether through recreational activities or the consumption of fish and seafood (Leonard et al., 2018, 2022; Sala-Comorera et al., 2021). Nevertheless, investigations often focus solely on water and sediment (Cuadrat et al., 2020; Liu et al., 2024; Kong et al., 2025); while these capture the resistome in transit, they do not account for the organisms in which it may be processed and evolve to become more significant.

This reliance represents a technical issue regarding the choice of indicators, not merely a matter of coverage. Grab sampling of water provides an instantaneous value for a compartment where ARG abundance fluctuates with tides, rainfall, and discharge flows, potentially missing critical peak levels (Carney et al., 2019; Jang et al., 2021). What is missing from research aimed at establishing environmental surveillance procedures for AMR, particularly in coastal regions, is a biological integrator: an organism that remains continuously in the water column, concentrates filtered material, and serves as a time-integrated record of the resistome to which it has been exposed. Among potential biological compartments, marine sponges (phylum Porifera) combine a unique set of properties. They are among the most efficient water-filtering organisms known, capable of filtering 20 to 2,000 times their body volume per hour while retaining sub-micrometer particles (Reiswig, 1974; Gerrodette & Flechsig, 1979; Weisz et al., 2008; Mariani et al., 2019; Morganti et al., 2019). They harbor dense, stable, and well-organized microbial consortia, with abundances ranging from 10^6 to 10^9 microbial cells per gram of tissue, levels orders of magnitude higher than those found in the surrounding water (Gloeckner et al., 2014; Moitinho-Silva et al., 2017). Furthermore, they are sessile, long-lived, and

abundant in coastal areas subject to anthropogenic discharges (Van Soest et al., 2012; Stubler et al., 2015).

Here, we argue that marine sponges have been studied from an incomplete perspective, resulting in the loss of a potential indicator of antimicrobial resistance in coastal areas. Our aim is to evaluate the sponge holobiont as a candidate indicator of the coastal resistome and to define what must be measured before interpreting any signal from the sponge. We propose three testable hypotheses: the sponge acting as a reservoir, a sink, or a reactor for antimicrobial resistance. We show that each hypothesis implies a different type of indication, derive the observable prediction that distinguishes each case, and describe the validation strategy capable of differentiating them (Fig. 1).

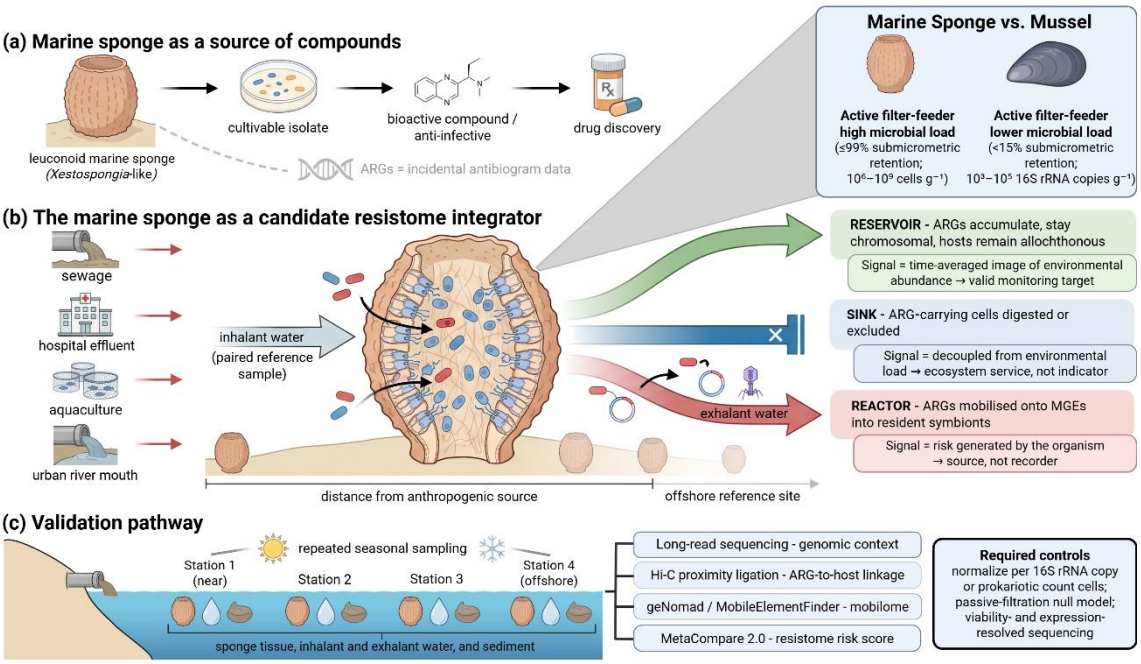

Fig 1. Marine sponges as candidate indicators of the coastal resistome. (*a*) The inherited paradigm: the sponge holobiont has been investigated as a source of bioactive compounds, with ARGs appearing as incidental antibiogram data. (*b*) The sponge as a candidate resistome integrator: filtration delivers allochthonous, ARG-carrying cells from coastal sources into a resident consortium orders of magnitude denser than the surrounding water, retaining the sub-micrometer fraction that bivalves let pass (inset). Three outcomes remain compatible with the available evidence, each assigning a different meaning to the same measured value (indicator semantics, tinted boxes): reservoir, sink, and reactor. Predictions are tested against paired inhalant water and against distance from the anthropogenic source (gradient bar). (*c*) Validation pathway: paired sampling of sponge tissue, inhalant and exhalant water, and sediment along a source-to-offshore

transect, repeated across seasons, with the analytical methods and the controls required to render the signal interpretable. The figure was generated in BioRender (https://www.biorender.com).

2. What indication requires, and why sponges qualify for this role

Based on established frameworks for indicator selection (Carignan & Villard, 2002; McGeoch, 2007; Heink & Kowarik, 2010), we synthesize three conditions that an indicator, such as one for the resistome, must satisfy: (*i*) the organism must integrate the signal over time and space, something a snapshot sample cannot do; (*ii*) the relationship between the signal displayed by the organism and the environmental condition being monitored must be monotonic and easily interpretable; and (*iii*) the measurement must be reproducible across different sites and seasons. Filter feeders naturally meet the first condition; consequently, researchers have used bivalves as sentinels for AMR in coastal and freshwater environments (Bighiu et al., 2019; Grevskott et al., 2017).

The case of bivalves serves as a prime example, as clear data regarding them have already been gathered. Research has shown that zebra mussels concentrated fecal indicator bacteria, such as *Escherichia coli* and *Enterococcus* spp., to levels approximately 132 times higher than those found in the surrounding water, retaining these bacteria for up to two days following a pulse exposure (Bighiu et al., 2019). In field studies, high proportions of antimicrobial-resistant *E. coli* were detected in both mussels and water downstream of a wastewater treatment plant. One-third of the bacteria isolated from mussels exhibited multi-drug resistance, leading the authors to propose these bivalves as an efficient tool capable of time-integrated monitoring to quantify resistant fecal indicators (Bighiu et al., 2019). Similarly, marine bivalve mollusks collected along the Norwegian coast have been proposed as a monitoring tool for antimicrobial-resistant *Enterobacteriaceae* in coastal environments (Grevskott et al., 2017). Currently, the body of literature is sufficiently extensive to support a systematic review and meta-analysis of AMR prevalence in marine bivalves (Albini et al., 2022), and resistome studies have begun to reveal profiles of ARGs and their associations with mobile genetic elements (MGEs) within mussel digestive glands (Fu et al., 2025).

However, marine sponges may surpass bivalves as bioindicators of AMR because of several advantages. Sponges particularly excel regarding the first criterion. Through

filtration, they: (*i*) retain finer particles (sponges retain up to 99% of sub-micrometer particles from the water, compared to <15% for bivalves) (Ward & Kach, 2009; Leys et al., 2018); and (*ii*) maintain these particles within a resident microbial consortium that is far denser than a bivalve's gut microbiome (the mesohyl of high microbial abundance (HMA) sponges harbors approximately 10^6 to 10^9 microbial cells per gram, versus 10^3 to 10^5 copies of 16S rRNA per gram of mussel tissue) (Gloeckner et al., 2014; Moitinho-Silva et al., 2017; Valenzuela et al., 2021). It is worth noting that the <1 μm size fraction is precisely the one carrying the resistome; furthermore, allochthonous bacteria captured by sponges are delivered to a consortium orders of magnitude denser than the one receiving them in bivalves, and it is cell density, along with close contact, that defines the conditions favoring horizontal gene transfer (HGT) (Michaelis & Grohmann, 2023; Djermoun et al., 2025). Therefore, a sponge could, in principle, function as a more efficient resistome integrator than the organism the field has spent decades developing.

Regarding the second criterion, sponges may fail, as might bivalves, though less obviously. Every claim regarding this indicator rests on an unstated assumption: that the resistance signal recovered from the tissue is a concentrated reflection of the resistance present in the surrounding water. This assumption is a hypothesis that remains untested in marine sponges. Until it is, the abundance of ARGs measured in sponge tissue remains a value without a reference point.

3. The inherited paradigm

Scientific interest in the sponge-associated microbiome grew from a bioprospecting perspective (Fig. 1a). Sponges are sessile, soft-bodied, and chemically defended; their symbionts produce these defenses, including antimicrobials with pharmaceutical potential (Van Soest et al., 2012; Hong et al., 2022). This logic was productive: it yielded compounds currently in clinical development and generated a substantial body of literature (Jimenez et al., 2020; Hong et al., 2022; Xia et al., 2024). However, it was also all-encompassing. A major review on sponge-derived antimicrobials frames the entire topic as a search for new anti-infective agents to replenish a depleted clinical pipeline (Devkar et al., 2022): sponges appear as a solution to the problem of resistance, rarely as a site where resistance occurs, and never as a matrix in which resistance could be measured.

The consequence is methodological, not merely rhetorical. Bioprospecting requires isolates, compounds, and biosynthetic gene clusters. Bioprospecting does not need to know an ARG's origin, which host carries it, or whether the ARG is mobilizable; nor does it require a paired environmental reference to calibrate any indicator. When ARGs were described in sponge-associated bacteria, they typically appeared as incidental data from antibiograms of strains isolated for other purposes or as annotations in metagenomes generated for other objectives (Phelan et al., 2011; Versluis et al., 2015; Laport et al., 2016; Costa et al., 2021). Only one study has targeted sponges to search for ARG reservoirs (Versluis et al., 2016), and no body of work has been designed to track ARG flow: there has been no source-to-sink sampling, no analysis of contamination gradients, and no large-scale paired water-tissue comparisons.

This blind spot has already been highlighted within the field: research into sponge microbial biotechnology has focused on secondary metabolites, leaving biomonitoring and pollution indication understudied (De Oliveira et al., 2023).

The clearest illustration of this is technical. Uppal et al. (2022) applied genome-resolved metagenomics to a *Forcepia* sp. sponge and detected a biosynthetic gene cluster with GC content and pentanucleotide frequency vastly different from those of the host symbiont genome, signaling horizontal acquisition. Methods for detecting HGT within a sponge holobiont already exist and have been used; however, they were used to identify an anticancer compound.

4. Why current methods fail to produce an interpretable signal

Five limitations separate existing evidence from the questions a claim of indicator potential must answer.

- **Cultivation.** Sponge symbiont communities are dominated by taxa that do not grow under laboratory conditions; the fraction that can be isolated is small and biased (Dat et al., 2021). Antibiograms derived from this fraction reveal only the phenotypes of the cultivable portion of the sponge microbiome rather than the holobiont’s complete resistome; consequently, they cannot serve as a reliable indicator signal.

- **Gene-centric detection.** Targeted PCR or the mapping of metagenomic reads indicates the presence of an ARG. This presence does not indicate which organism carries the ARG, whether the ARG is located on a chromosome or a plasmid, or whether it is mobilizable. The presence of the ARG can fit three different hypotheses; this is precisely why presence data alone are insufficient.
- **Lack of linkage.** To distinguish a reservoir from a reactor, three elements must be connected: the ARG, the MGE carrying it, and the bacterial host. Short-read assembly of a complex, highly diverse consortium often breaks apart these very regions (e.g., repetitive, mobile, or low-coverage regions), resulting in a loss of linkage between them (Abramova et al., 2024).
- **Denominator/null model of filtration.** Comparing ARG abundance per gram of tissue with abundance per liter of water yields no useful information: tissue biomass exceeds that of seawater by three to six orders of magnitude (Hentschel et al., 2012), making the tissue appear enriched even if the filters were inert. When reported this way, this enrichment factor is a denominator artifact rather than a meaningful signal. Comparisons must be normalized against 16S rRNA gene copies or prokaryotic cell counts, and filtration expectations should be based on pumping rate, retention efficiency, and cell density.
- **Relic DNA in digestive tissue.** Sponges continuously capture and digest prokaryotic cells (de Goeij et al., 2013), so the tissue likely contains relic DNA from lysed cells, a known confounder in similar matrices (Carini et al., 2016). A perfect sink that digests every antimicrobial-resistant cell produces a DNA signature indistinguishable from that of accumulation (reservoir); only sequencing (resolved by viability or expression) can distinguish between them.

The mobility vector, meanwhile, is well-documented. Pascelli et al. (2020) characterized viromes in fifteen individuals across nine sponge species and found assemblages dominated by *Caudovirales* carrying accessory genes for herbicide and heavy-metal resistance; these assemblages varied according to host species and site. Thus, phage-mediated gene flow within the holobiont is not merely hypothetical; it has simply not yet been examined in relation to ARGs.

5. The marine sponge holobiont: reservoir, sink, or reactor?

Conditions favoring HGT are well-characterized in other environmental compartments, such as high cell density and close cell-to-cell contact (Michaelis & Grohmann, 2023; Djermoun et al., 2025), steep chemical gradients, and chronic sub-inhibitory exposure to antimicrobials and biocides (Zhang et al., 2017; Jutkina et al., 2018), and these are precisely the conditions that define hotspots for gene transfer, such as sewage biofilms (Wang et al., 2026). Sponges combine many of these factors: in waters receiving effluent, filtration can deliver allochthonous, resistant bacteria directly to a dense resident consortium maintained within a collagenous matrix.

If transfer can occur within the holobiont, it has already been tested. Cartwright et al. (2020) exposed laboratory cultures of the freshwater sponge *Ephydatia fluviatilis* to donor and recipient strains of *Enterococcus faecalis* in microcosms. Presumed transconjugants increased significantly compared to controls, and fluorescence *in situ* hybridization revealed enterococcal aggregates in direct cell-to-cell contact within the sponge matrix, although the mechanism remains unclear. This may be related to binding to the collagen matrix. This result serves as a proof of concept in a freshwater species under laboratory conditions using a single donor-recipient pair; it has not been replicated in marine sponges, *in situ*, or along a contamination gradient. Consequently, three responses remain consistent with the evidence, each attributing a different significance to the same measured value. Initial expectations are unfavorable: marine sponge symbiont genomes are rich in CRISPR and other defense systems (Horn et al., 2016), which argues for testing H3 rather than accepting it.

H1 – Reservoir. Sponges accumulate and retain ARGs from the water column, but the genes largely remain within their original host organisms. *Prediction:* ARG abundance in the tissue, normalized by prokaryotic cells or 16S rRNA gene copies, exceeds that of paired inhalant water and decreases with distance from anthropogenic sources; ARGs are primarily located on the chromosome; association with MGEs is not higher than in the source water; ARG-carrying taxa reflect allochthonous input. *Indicator semantics:* the tissue signal represents a concentrated, time-averaged image of environmental abundance, making the marine sponge a valid monitoring target.

H2 - Sink. Sponges capture and eliminate ARG-carrying bacterial cells through digestion, host immunity, or competition from resident symbionts, without passing them

on. *Prediction:* normalized ARG levels in the tissue are lower than in the inflow water, or ARGs decrease in viable/expressible fractions compared to total DNA; there is no increase in ARG-carrying MGEs; ARG-carrying taxa appear only transiently and are not resident members of the consortium. *Indicator semantics:* the sponge tissue signal is decoupled from environmental abundance by a removal process that is currently unknown and potentially variable; the sponge functions as an ecosystem service provider rather than an indicator.

H3 - Reactor. The holobiont facilitates ARG transfer from incoming cells to the established symbiont community, increasing resistance frequency. *Prediction:* ARGs are more concentrated in tissue MGEs compared to inflow water; nearly identical ARG sequences appear in phylogenetically distinct hosts within the same holobiont (a signature of recent HGT), with recipients belonging to sponge-specific symbiont lineages rather than allochthonous taxa; the pattern intensifies with proximity to antimicrobial sources; and there is a net export of ARGs or ARG-carrying cells in the outflow water. *Indicator semantics:* the tissue signal reflects a risk generated by the organism itself; the sponge acts as a source of coastal resistance rather than a passive recorder.

When the same ARG appears in phylogenetically distant hosts within a holobiont, it indicates recent HGT. However, this signature does not reveal where the transfer occurred; a marine sponge filtering sewage-impacted water may receive donor-recipient pairs that were already formed upstream. To distinguish between transfer occurring within the holobiont and transfer imported into it, we need two additional constraints on the "reactor" hypothesis. First, ARG sharing must differ among distinct clades, comparing paired tissue and inhaled water samples, rather than looking at the tissue state in isolation. Second, to validate or refute the hypothesis, the recipients' identity must be restricted to established, sponge-specific symbiont lineages rather than allochthonous taxa.

These hypotheses are not mutually exclusive. A marine sponge may function as a sink for some taxa and a reactor for others, with the balance shifting along a source gradient. A mixed regime does not preclude using an indicator but constrains it, as the indicator would need to be defined based on the ARG classes for which the reservoir concept holds and validated separately for each class. The distinction remains valid in all cases. A reservoir is a monitoring target. A sink is an ecosystem service. A reactor is a public health issue that discharges into the same coastal waters used for recreation and the harvesting of fish and seafood (Fig. 1b).

6. What would resolve the issue: a validation pathway

To test the hypotheses raised, appropriate methodological strategies are needed. In this regard, *long-read sequencing* recovers ARGs within their genomic context and indicates whether a gene is located on a chromosome or a plasmid. It also preserves the gene's flanking regions, regions that short-read sequencing tends to fragment (Dai et al., 2022). *Proximity-ligation metagenomics*, or Hi-C, enables physical linkage of ARGs and MGEs to their bacterial hosts without bacterial cultivation (Zhu et al., 2026). The method captures the H3 signature of the same ARG across distinct hosts within a holobiont. This linkage is necessary but insufficient; it must be compared against paired inhalant water to demonstrate contrast. *Mobilome annotation*, performed using software such as geNomad and MobileElementFinder, quantifies the association between ARGs and MGEs (Johansson et al., 2021; Camargo et al., 2024). The *resistome risk score*, calculated via MetaCompare 2.0 or ARG-Ranker, integrates ARG abundance, mobility, and association with pathogens into a single metric (Zhang et al., 2021; Rumi et al., 2024). This metric represents the form that a marine sponge-based index should take to ensure comparability across different sites.

These tools require a gradient design. Marine sponge tissue, ambient water, and sediment are collected together along a transect starting from a defined anthropogenic source, such as an outfall, a hospital effluent discharge, the mouth of an impacted urban river, or an aquaculture site, and extending to open-ocean reference points; sampling occurs during at least two seasons over approximately two to three consecutive years. Comparing tissue and water reveals enrichment or depletion; the gradient assesses dose-dependence (specifically, the indicator's required monotonicity); seasonal repetition checks reproducibility; and Hi-C analysis verifies transfer. Existing resources lower the barrier to entry. The Sponge Microbiome Project collected 3,569 sponge specimens and 370 paired seawater samples using standardized protocols (Moitinho-Silva et al., 2017); however, because the resource relies on 16S rRNA gene amplicons, the samples cannot be re-examined for ARGs. Nevertheless, this database offers a global overview of host-associated community composition and HMA/LMA classification, providing an evidence-based foundation for selecting target species and identifying sites where environmental and tissue communities have already been characterized. To address the

identified scientific and technical gaps, shotgun and long-read sequencing data must be generated anew, taking into account the conditions presented and discussed here.

7. Conclusion

Marine sponges are frequently studied as sources of pharmaceuticals but never as bioindicators of bacterial antimicrobial resistance. They are filter feeders that capture particles more finely and continuously than the bivalves typically used for this purpose in coastal AMR research. In 2020, a study hypothesized that laboratory-reared freshwater sponges could also serve as AMR indicators, acting as a tool to assess it rather than merely being targets of it, though the study did not explore this further. This was not because the question was unanswerable, but rather because it was not the specific question being addressed at the time. Assessing the sponge holobiont as an indicator is cost-effective: the tools already exist, reference databases help select species and sites, and the sampling design is standardized. This approach could yield a new coastal sentinel for AMR, a natural sampler that integrates the resistome of the filtered water, or lead to the discovery of an unmonitored amplification hotspot upstream of recreational areas and seafood harvesting sites. Any of these outcomes would improve AMR monitoring in coastal areas, contributing to the development and implementation of environmental AMR monitoring systems.

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Wellington Felipe Costa: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing, Resources.

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Conflict of interest statement

The author declares no competing financial interests or known personal relationships that could have influenced the work reported in this article.

Statement on the use of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the author used BioRender for the conceptual diagram presented in Fig. 1 and Claude Opus 5 for English grammar review. After using these tools, the author reviewed and edited the content as necessary and assumes full responsibility for the content of the published article.

Data availability

No new data were generated or analyzed in this article. All material discussed is available in the cited published literature.

References

Abramova, A., Karkman, A., & Bengtsson-Palme, J. (2024). Metagenomic assemblies tend to break around antibiotic resistance genes. BMC Genomics, 25(1), 959. https://doi.org/10.1186/s12864-024-10876-0

Albini, E., Orso, M., Cozzolino, F., Sacchini, L., Leoni, F., & Magistrali, C. F. (2022). A systematic review and meta-analysis on antimicrobial resistance in marine bivalves. Frontiers in Microbiology, 13, 1040568. https://doi.org/10.3389/fmicb.2022.1040568

Bighiu, M. A., Norman Haldén, A., Goedkoop, W., & Ottoson, J. (2019). Assessing microbial contamination and antibiotic resistant bacteria using zebra mussels (Dreissena polymorpha). Science of the Total Environment, 650(Pt 2), 2141–2149. https://doi.org/10.1016/j.scitotenv.2018.09.314

Bongiovanni, D., Masson, S., Chialva, M., Fiorilli, V., Votta, C., Lanfranco, L., & Stefanini, I. (2025). Impact of urbanization on antimicrobial resistance in soil microbial communities. Scientific Reports, 15(1), 633. https://doi.org/10.1038/s41598-024-84945-5

Brown, L. P., Marizzi, A., Borrego, C. M., Gionchetta, G., Zhengzheng, Z., Carneiro, R. B., Gago-Ferrero, P., Matamoros, V., & Subirats, J. (2026). Metagenomic assessment of full-scale wastewater treatment plants identifies sentinel antibiotic resistance gene families for monitoring reclaimed wastewater and treated sludge. Environmental Science & Technology, 60(6), 4632–4647. https://doi.org/10.1021/acs.est.5c13541

374 Camargo, A. P., Roux, S., Schulz, F., Babinski, M., Xu, Y., Hu, B., Chain, P. S. G.,
375 Nayfach, S., & Kyrpides, N. C. (2024). Identification of mobile genetic elements with
376 geNomad. Nature Biotechnology, 42(8), 1303–1312. https://doi.org/10.1038/s41587-
377 023-01953-y

378 Carignan, V., & Villard, M. A. (2002). Selecting indicator species to monitor ecological
379 integrity: a review. Environmental Monitoring and Assessment, 78(1), 45–61.
380 https://doi.org/10.1023/a:1016136723584

381 Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., & Fierer, N. (2016).
382 Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nature
383 Microbiology, 2, 16242. https://doi.org/10.1038/nmicrobiol.2016.242

384 Carney, R. L., Labbate, M., Siboni, N., Tagg, K. A., Mitrovic, S. M., & Seymour, J. R.
385 (2019). Urban beaches are environmental hotspots for antibiotic resistance following
386 rainfall. Water Research, 167, 115081. https://doi.org/10.1016/j.watres.2019.115081

387 Cartwright, A., Arnscheidt, J., Conwell, M., Dooley, J.S.G., McGonigle, C., & Naughton,
388 P.J. (2020). Effects of freshwater sponge Ephydatia fluviatilis on conjugative transfer of
389 antimicrobial resistance in Enterococcus faecalis strains in aquatic environments. Letters
390 in Applied Microbiology, 71(1), 39–45. https://doi.org/10.1111/lam.13310

391 Costa, W. F., Giambiagi-deMarval, M., & Laport, M. S. (2021). Antibiotic and heavy
392 metal susceptibility of non-cholera Vibrio isolated from marine sponges and sea urchins:
393 could they pose a potential risk to public health?. Antibiotics, 10(12), 1561.
394 https://doi.org/10.3390/antibiotics10121561

395 Cuadrat, R. R. C., Sorokina, M., Andrade, B. G., Goris, T., & Dávila, A. M. R. (2020).
396 Global ocean resistome revealed: Exploring antibiotic resistance gene abundance and
397 distribution in TARA Oceans samples. GigaScience, 9(5), giaa046.
398 https://doi.org/10.1093/gigascience/giaa046

399 Dai, D., Brown, C., Bürgmann, H., Larsson, D. G. J., Nambi, I., Zhang, T., Flach, C. F.,
400 Pruden, A., & Vikesland, P. J. (2022). Long-read metagenomic sequencing reveals shifts
401 in associations of antibiotic resistance genes with mobile genetic elements from sewage
402 to activated sludge. Microbiome, 10(1), 20. https://doi.org/10.1186/s40168-021-01216-5

403 Dat, T. T. H., Steinert, G., Cuc, N. T. K., Smidt, H., & Sipkema, D. (2021). Bacteria
404 cultivated from sponges and bacteria not yet cultivated from sponges-a review. Frontiers
405 in Microbiology, 12, 737925. https://doi.org/10.3389/fmicb.2021.737925

406 de Goeij, J.M., van Oevelen, D., Vermeij, M.J.A., Osinga, R., Middelburg, J.J., de Goeij,
407 A.F.P.M., & Admiraal, W. (2013). Surviving in a marine desert: the sponge loop retains
408 resources within coral reef ecosystems. Science, 342(6154), 108–110.
409 https://doi.org/10.1126/science.1241981

410 De Oliveira, B.F.R., Freitas-Silva, J., Canellas, A.L.B., Costa, W.F., & Laport, M.S.,
411 (2023). Time for a change! A spotlight on many neglected facets of sponge microbial
412 biotechnology. Current Pharmaceutical Biotechnology, 24(4), 471–485.
413 https://doi.org/10.2174/1389201023666220516103715

414 Devkar, H.U., Thakur, N.L., & Kaur, P. (2022). Marine-derived antimicrobial molecules
415 from the sponges and their associated bacteria. Canadian Journal of Microbiology, 69(1),
416 1–16. https://doi.org/10.1139/cjm-2022-0147

417 Djermoun, S., Rode, D. K. H., Jiménez-Siebert, E., Netter, N., Lesterlin, C., Drescher, K.,
418 & Bigot, S. (2025). Biofilm architecture determines the dissemination of conjugative
419 plasmids. Proceedings of the National Academy of Sciences of the United States of
420 America, 122(17), e2417452122. https://doi.org/10.1073/pnas.2417452122

421 Fu, P., Zhai, J., Yang, X., Gao, J., Ren, Z., Guo, B., & Qi, P. (2025). Distribution and
422 influencing factors of antibiotic resistance genes in two mussel species along the coasts
423 of the East China Sea and the Yellow Sea. Journal of Hazardous Materials, 488, 137399.
424 https://doi.org/10.1016/j.jhazmat.2025.137399

425 Gerrodette, T., & Flechsig, A.O. (1979). Sediment-induced reduction in the pumping rate
426 of the tropical sponge Verongia lacunosa. Marine Biology, 55(2), 103–110.
427 https://doi.org/10.1007/BF00397305

428 Gloeckner, V., Wehrl, M., Moitinho-Silva, L., Gernert, C., Schupp, P., Pawlik, J. R.,
429 Lindquist, N. L., Erpenbeck, D., Wörheide, G., & Hentschel, U. (2014). The HMA-LMA
430 dichotomy revisited: an electron microscopical survey of 56 sponge species. The
431 Biological Bulletin, 227(1), 78–88. https://doi.org/10.1086/BBLv227n1p78

432 Grevskott, D. H., Svanevik, C. S., Sunde, M., Wester, A. L., & Lunestad, B. T. (2017).
433 Marine bivalve mollusks as possible indicators of multidrug-resistant Escherichia coli

434 and other species of the Enterobacteriaceae family. Frontiers in Microbiology, 8, 24.
435 https://doi.org/10.3389/fmicb.2017.00024

436 Heink, U., & Kowarik, I. (2010). What criteria should be used to select biodiversity
437 indicators?. Biodiversity and Conservation 19, 3769–3797.
438 https://doi.org/10.1007/s10531-010-9926-6

439 Hentschel, U., Piel, J., Degnan, S.M., & Taylor, M.W. (2012). Genomic insights into the
440 marine sponge microbiome. Nature Reviews Microbiology, 10(9), 641–654.
441 https://doi.org/10.1038/nrmicro2839

442 Hong, L. L., Ding, Y. F., Zhang, W., & Lin, H. W. (2022). Chemical and biological
443 diversity of new natural products from marine sponges: a review (2009-2018). Marine
444 Life Science & Technology, 4(3), 356–372. https://doi.org/10.1007/s42995-022-00132-3

445 Horn, H., Slaby, B.M., Jahn, M.T., Bayer, K., Moitinho-Silva, L., Förster, F.,
446 Abdelmohsen, U.R., & Hentschel, U. (2016). An enrichment of CRISPR and other
447 defense-related features in marine sponge symbiont genomes. Frontiers in Microbiology,
448 7, 1751. https://doi.org/10.3389/fmicb.2016.01751

449 Jang, J., Kim, M., Baek, S., Shin, J., Shin, J., Shin, S. G., Kim, Y. M., & Cho, K. H.
450 (2021). Hydrometeorological influence on antibiotic-resistance genes (ARGs) and
451 bacterial community at a recreational beach in Korea. Journal of Hazardous Materials,
452 403, 123599. https://doi.org/10.1016/j.jhazmat.2020.123599

453 Jimenez, P. C., Wilke, D. V., Branco, P. C., Bauermeister, A., Rezende-Teixeira, P.,
454 Gaudêncio, S. P., & Costa-Lotufo, L. V. (2020). Enriching cancer pharmacology with
455 drugs of marine origin. British Journal of Pharmacology, 177(1), 3–27.
456 https://doi.org/10.1111/bph.14876

457 Johansson, M. H. K., Bortolaia, V., Tansirichaiya, S., Aarestrup, F. M., Roberts, A. P., &
458 Petersen, T. N. (2021). Detection of mobile genetic elements associated with antibiotic
459 resistance in Salmonella enterica using a newly developed web tool:
460 MobileElementFinder. The Journal of Antimicrobial Chemotherapy, 76(1), 101–109.
461 https://doi.org/10.1093/jac/dkaa390

462 Jutkina, J., Marathe, N. P., Flach, C. F., & Larsson, D. G. J. (2018). Antibiotics and
463 common antibacterial biocides stimulate horizontal transfer of resistance at low

concentrations. The Science of the Total Environment, 616-617, 172–178. https://doi.org/10.1016/j.scitotenv.2017.10.312

Kong, M., Wang, M., Liang, S., Chen, H., Zhang, S., Zheng, M., & Zhang, C. (2025). Relationship between antibiotic resistance genes and microbiome in the Arctic marine sediments. Marine Environmental Research, 210, 107345. https://doi.org/10.1016/j.marenvres.2025.107345

Laport, M. S., Pontes, P. V., Dos Santos, D. S., Santos-Gandelman, J.deF., Muricy, G., Bauwens, M., Giambiagi-deMarval, M., & George, I. (2016). Antibiotic resistance genes detected in the marine sponge Petromica citrina from Brazilian coast. Brazilian Journal of Microbiology, 47(3), 617–620. https://doi.org/10.1016/j.bjm.2016.04.016

Larsson, D. G. J., & Flach, C. F. (2022). Antibiotic resistance in the environment. Nature Reviews in Microbiology, 20(5), 257–269. https://doi.org/10.1038/s41579-021-00649-x

Leonard, A. F. C., Zhang, L., Balfour, A. J., Garside, R., Hawkey, P. M., Murray, A. K., Ukoumunne, O. C., & Gaze, W. H. (2018). Exposure to and colonisation by antibiotic-resistant E. coli in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). Environment International, 114, 326–333. https://doi.org/10.1016/j.envint.2017.11.003

Leonard, A. F., Morris, D., Schmitt, H., & Gaze, W. H. (2022). Natural recreational waters and the risk that exposure to antibiotic resistant bacteria poses to human health. Current Opinion in Microbiology, 65, 40–46. https://doi.org/10.1016/j.mib.2021.10.004

Leys, S. P., Kahn, A. S., Fang, J. K. H., Kutti, T., Bannister, R.J. (2018). Phagocytosis of microbial symbionts balances the carbon and nitrogen budget for the deep-water boreal sponge Geodia barretti. Limnology and Oceanography 63(1), 187–202. https://doi.org/10.1002/lno.10623

Liang, P., Wang, H., Chen, Q., Luo, Q., Cheng, J., Tian, G., He, T., Zhu, W., & Fu, T. (2025). Antibiotic resistance gene transfer characteristic and risk evaluation: The entire process from swine manure to biogas slurry and then to soil. Journal of Environmental Chemical Engineering, 13, 116659. https://doi.org/10.1016/j.jece.2025.116659

Liu, X., Wei, H., Wang, H., Zhang, Y., Song, H. L., & Zhang, S. (2024). A review of spatial distribution of typical antibiotic resistance genes in marine environment

surrounding China. Marine Pollution Bulletin, 203, 116482. https://doi.org/10.1016/j.marpolbul.2024.116482

Mao, X., Yin, X., Yang, Y., Gao, F., Li, S., Shi, X., Deng, Y., Li, L., Leung, K. M. Y., & Zhang, T. (2025). Longitudinal metagenomic analysis on antibiotic resistome, mobilome, and microbiome of river ecosystems in a sub-tropical metropolitan city. Water Research, 274, 123102. https://doi.org/10.1016/j.watres.2025.123102

Mariani, S., Baillie, C., Colosimo, G., & Riesgo, A. (2019). Sponges as natural environmental DNA samplers. Current Biology, 29(11), R401–R402. https://doi.org/10.1016/j.cub.2019.04.031

McGeogh, M.A. (1998), The selection, testing and application of terrestrial insects as bioindicators. Biological Reviews, 73, 181-201. https://doi.org/10.1111/j.1469-185X.1997.tb00029.x

Michaelis, C., & Grohmann, E. (2023). Horizontal gene transfer of antibiotic resistance genes in biofilms. Antibiotics, 12(2), 328. https://doi.org/10.3390/antibiotics12020328

Moitinho-Silva, L., Nielsen, S., Amir, A., Gonzalez, A., Ackermann, G.L., et al. (2017). The sponge microbiome project. GigaScience, 6(10), 1–7. https://doi.org/10.1093/gigascience/gix077

Morganti, T.M., Ribes, M., Yahel, G., & Coma, R. (2019). Size is the major determinant of pumping rates in marine sponges. Frontiers in Physiology, 10, 1474. https://doi.org/10.3389/fphys.2019.01474

Pascelli, C., Laffy, P.W., Botté, E., Kupresanin, M., Rattei, T., et al. (2020). Viral ecogenomics across the Porifera. Microbiome, 8(1), 144. https://doi.org/10.1186/s40168-020-00919-5

Phelan, R. W., Clarke, C., Morrissey, J. P., Dobson, A. D., O'Gara, F., & Barbosa, T. M. (2011). Tetracycline resistance-encoding plasmid from Bacillus sp. strain #24, isolated from the marine sponge Haliclona simulans. Applied and Environmental Microbiology, 77(1), 327–329. https://doi.org/10.1128/AEM.01239-10

Reiswig, H.M. (1974). Water transport, respiration and energetics of three tropical marine sponges. Journal of Experimental Marine Biology and Ecology, 14(3), 231–249. https://doi.org/10.1016/0022-0981(74)90005-7

524 Rumi, M. A., Oh, M., Davis, B. C., Brown, C. L., Juvekar, A., Vikesland, P. J., Pruden,
525 A., & Zhang, L. (2024). MetaCompare 2.0: differential ranking of ecological and human
526 health resistome risks. FEMS Microbiology Ecology, 100(12), fiae155.
527 https://doi.org/10.1093/femsec/fiae155

528 Sala-Comorera, L., Nolan, T. M., Reynolds, L. J., Venkatesh, A., Cheung, L., Martin, N.
529 A., Stephens, J. H., Gitto, A., O'Hare, G. M. P., O'Sullivan, J. J., & Meijer, W. G. (2021).
530 Bacterial and bacteriophage antibiotic resistance in marine bathing waters in relation to
531 rivers and urban streams. Frontiers in Microbiology, 12, 718234.
532 https://doi.org/10.3389/fmicb.2021.718234

533 Stubler, A. D., Duckworth, A. R., & Peterson, B. J. (2015). The effects of coastal
534 development on sponge abundance, diversity, and community composition on Jamaican
535 coral reefs. Marine Pollution Bulletin, 96(1-2), 261–270.
536 https://doi.org/10.1016/j.marpolbul.2015.05.014

537 United Nations Environment Programme – UNEP. (2023). Bracing for Superbugs:
538 Strengthening environmental action in the One Health response to antimicrobial
539 resistance. Geneva, United Nations Environment Programme.
540 https://www.unep.org/resources/superbugs/environmental-action

541 Uppal, S., Metz, J.L., Xavier, R.K.M., Nepal, K.K., Xu, D., Wang, G., & Kwan, J.C.,
542 (2022). Uncovering lasonolide A biosynthesis using genome-resolved metagenomics.
543 mBio, 13(5), e01524-22. https://doi.org/10.1128/mbio.01524-22

544 Valenzuela, T., Rilling, J. I., Larama, G., Acuña, J. J., Campos, M., Inostroza, N. G.,
545 Araya, M., Altamirano, K., Fujiyoshi, S., Yarimizu, K., Maruyama, F., & Jorquera, M. A.
546 (2021). 16S rRNA–based analysis reveals differences in the bacterial community present
547 in tissues of Choromytilus chorus (Mytilidae, Bivalvia) grown in an estuary and a bay in
548 southern Chile. Diversity, 13(5), 209. https://doi.org/10.3390/d13050209

549 Van Soest, R. W., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De
550 Voogd, N. J., Santodomingo, N., Vanhoorne, B., Kelly, M., & Hooper, J. N. (2012). Global
551 diversity of sponges (Porifera). PloS One, 7(4), e35105.
552 https://doi.org/10.1371/journal.pone.0035105

553 Versluis, D., D'Andrea, M. M., Ramiro Garcia, J., Leimena, M. M., Hugenholtz, F.,
554 Zhang, J., Öztürk, B., Nylund, L., Sipkema, D., van Schaik, W., de Vos, W. M.,

555 Kleerebezem, M., Smidt, H., & van Passel, M. W. (2015). Mining microbial
556 metatranscriptomes for expression of antibiotic resistance genes under natural conditions.
557 Scientific Reports, 5, 11981. https://doi.org/10.1038/srep11981

558 Versluis, D., Rodriguez de Evgrafov, M., Sommer, M. O., Sipkema, D., Smidt, H., & van
559 Passel, M. W. (2016). Sponge microbiota are a reservoir of functional antibiotic resistance
560 genes. Frontiers in Microbiology, 7, 1848. https://doi.org/10.3389/fmicb.2016.01848

561 Wang, D., Xu, X., Liu, L., Wang, C., Deng, Y., Polz, M. F., & Zhang, T. (2026). Hi-C
562 sequencing deciphers phage and plasmid host networks in wastewater biofilms.
563 Environmental Science and Ecotechnology, 30, 100683.
564 https://doi.org/10.1016/j.ese.2026.100683

565 Ward, J. E., & Kach, D. J. (2009). Marine aggregates facilitate ingestion of nanoparticles
566 by suspension-feeding bivalves. Marine Environmental Research 68(3), 137–142.
567 https://doi.org/10.1016/10.1016/j.marenvres.2009.05.002

568 Weisz, J.B., Lindquist, N., & Martens, C.S. (2008). Do associated microbial abundances
569 impact marine demosponge pumping rates and tissue densities? Oecologia, 155(2), 367–
570 376. https://doi.org/10.1007/s00442-007-0910-0

571 Xia, J., Chen, X., Li, G., Qiu, P., Wang, W., & Shao, Z. (2024). A review of sponge-
572 derived diterpenes: 2009-2022. Marine Drugs, 22(10), 447.
573 https://doi.org/10.3390/md22100447

574 Zhang, A. N., Gaston, J. M., Dai, C. L., Zhao, S., Poyet, M., Groussin, M., Yin, X., Li, L.
575 G., van Loosdrecht, M. C. M., Topp, E., Gillings, M. R., Hanage, W. P., Tiedje, J. M.,
576 Moniz, K., Alm, E. J., & Zhang, T. (2021). An omics-based framework for assessing the
577 health risk of antimicrobial resistance genes. Nature Communications, 12(1), 4765.
578 https://doi.org/10.1038/s41467-021-25096-3

579 Zhang, Y., Gu, A. Z., He, M., Li, D., & Chen, J. (2017). Subinhibitory concentrations of
580 disinfectants promote the horizontal transfer of multidrug resistance genes within and
581 across genera. Environmental Science & Technology, 51(1), 570–580.
582 https://doi.org/10.1021/acs.est.6b03132

583 Zhong, W., Lin, Z., Schmidt, E. W., & Agarwal, V. (2025). Discovery, biosynthesis, and
584 bioactivities of peptidic natural products from marine sponges and sponge-associated

585 bacteria. Natural Product Reports, 42(12), 2034–2074.
586 https://doi.org/10.1039/d5np00048c

587 Zhu, K., Amirali, A., Auch, B., Babler, K. M., Biswas, P., Bowie, K., Choudhary, S.,
588 Currall, B. B., Grills, G. S., Healy, H. G., Liachko, I., Lucaci, A. G., Mason, C. E.,
589 Sharkey, M., Shigeno Risse-Adams, O., Shukla, B. S., Sisson, Z., Stevenson, M.,
590 Williams, S. L., Zulli, A., Peccia, J., Solo-Gabriele, H. M. (2026). Proof-of-concept of
591 host attribution of antimicrobial resistance genes using wastewater Hi-C metagenome
592 sequencing. Journal of Water and Health, 24(2), 148–159.