The definitive publisher version is available online at 10.1016/j.tree.2022.04.008

Disentangling compartment functions in sessile marine invertebrates

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Abstract

Sessile invertebrates are frequently sampled and processed whole for downstream analyses. However, their apparent structural simplicity is deceptive as these organisms often harbour discrete compartments. These compartments have physico-chemical conditions that differ markedly from neighbouring tissues, and that have likely evolved to support specific functions. Here, we argue that such compartments should be specifically targeted when characterising sessile invertebrate biology and we use the coral gastrovascular cavity to support our argument. This complex compartment displays steep and dynamic chemical gradients, harbours distinct microorganisms and presumably plays a key role in coral biology. Disentangling the functions played by (and amongst) compartments will likely provide transformative insight into the biology of sessile invertebrates and their future under environmental change.

The need to focus on compartments

Many sessile invertebrates such as corals, sponges, and oysters, are key to the trophic foundation and habitat structure of marine ecosystems. They are also threatened by climate change and other anthropogenic stressors. In the last two decades, omics platforms have increasingly improved our capacity to resolve the molecular, metabolic, cellular, and microbial properties that likely govern the fitness of these organisms [1]. However, the amount of biomass typically required by most omics approaches, combined with our perception of sessile invertebrates as structurally simple, often results in the organism being sampled whole and homogenised during sample processing. Homogenisation dismantles the spatial structure of these organisms, blends cells, microorganisms and chemicals that are naturally separated, increasing the risk of overlooking or misinterpreting key aspects of their biology [2,3].

Sessile invertebrates harbour discrete compartments resulting in a variety of micro-environments within an individual that can differ in their physico-chemical composition, creating unique niches for microbiomes of distinctive identity and activity [4–7]. Compartments are involved in specific functions and play key roles in the biology of these organisms, but remain largely unstudied at appropriate scales. Among sessile invertebrates, one compartment where chemical and biological properties have been studied at fine-scales is the **gastrovascular cavity** (GVC, see Glossary) of reef-building corals (order: Scleractinia), yielding novel insight into their biology and ecophysiology. We propose that akin to the gut of many terrestrial animal models, GVC function is likely central to coral health, stress response and disease susceptibility - outlining why targeted exploration of compartments is critical to understand the biology of other sessile invertebrates.

Structural characteristics of the coral gastrovascular cavity

Scleractinians are colonial organisms, with numerous inter-connected **coral polyps** anchored collectively to a calcium carbonate skeleton. Like other cnidarians (e.g., sea anemones and jellyfish), coral polyps are tube-shaped with a mouth at one end surrounded by tentacles that opens up via the actinopharynx to the GVC. Despite a relatively simple structure, the GVC performs roles equivalent to the oral cavity, stomach, intestine and reproductive system of vertebrates.

Corals are diploblastic, i.e., their body is composed of two cell layers, the ecto- and endo-derm, with an acellular mesoglea in between that functions as a hydrostatic skeleton to maintain tissue structure [8] (Fig. 1). The cells are ciliated, and together with muscular tissue contractions, the beating of these cilia drives fluid circulation within and in between polyps [9], and over their external tissue surface [10,11]. Within the GVC, endodermal tissue (**gastrodermis**) forms multiple folds known as mesenteries that run from the apical to the basal end of the polyp, dividing the GVC into sections (Fig. 1). Much like the folds of vertebrate intestines, mesenteries increase the surface area of the GVC to enhance nutrient absorption [12]. The GVC also contains mesenterial filaments (Fig. 1) that contain **nematocysts**, zones of **phagocytosis** and at least three types of secretory cells [13] (Fig 1).

The degree of physiological integration between polyps of a coral colony largely depends upon the specific architecture of the gastrovascular system [13]. **Imperforate** taxa (such as *Caulastrea* and *Galaxea*) possess a solid skeleton where adjacent polyps are connected by only a thin tissue layer (coenosarc, Fig. 1**)** [14]. The coenosarc contains **gastrovascular canals** that enable sharing of liquid, food particles and nutrients, but only when polyps are fully expanded [9]. Conversely, **perforate** species (such as *Acropora* and *Porites*) contains numerous voids in their skeletal matrix (coenosteum)

allowing tissue to form continuous connections between all polyps via a complex subsurface gastrovascular canal system [13,15].

Extreme chemical environment

Profiling of the GVC with microsensors (Box 1) has revealed the presence of pronounced vertical chemical gradients over microscale distances. In the daytime, photosynthesis dominates metabolism in the uppermost GVC regions, resulting in strongly elevated levels of O_2 and pH (up to 400% air saturation and pH 9.7, respectively) [4]. Conditions become progressively deoxygenated and acidic with increasing depth as respiration overtakes photosynthesis as the dominant metabolic process. Lowermost GVC regions exhibit constantly low pH (down to ~7.5) and O_2 conditions (in some cases reaching anoxia) [4,16,17]. Presence of both oxic and anoxic zones, together with low pH indicates that conditions in the GVC are similar to those found in the more-complex digestive tracts of other animals [18]. Distinct gradients of Ca^{2+} and CO_3^{2-} in the GVC have also been observed in some corals [19–21], however the internal environments of <1% of scleractinian species have been explored with microsensors. Our understanding of how taxonomy, morphology or behaviour affects the GVC chemical microenvironment is limited. It also remains largely unknown whether zones of anoxia and low pH are permanent features of this compartment, or are transiently disrupted during mixing of fluid in the polyp.

Extreme chemistry in the GVC is not restricted to O_2 and pH, but also nutrients, where concentrations can be markedly elevated compared to surrounding seawater; for example, 100- and 30-fold greater concentrations of nitrate and vitamin B12, respectively [4,22]. Whether such nutrients originate from recycling of coral-produced organic matter (e.g., mucus), remineralisation of ingested organic/food particles and/or *de-novo* synthesis by GVC-specific microbial partners is unknown. Combining

75 microsensor analysis of multiple chemical species with microsampling and low-volume omics analyses
76 (Box 1) could prove instrumental in resolving chemical and microbial dynamics within the GVC, and
77 likewise analogous compartments in other sessile invertebrates.

78 Functional roles of the GVC

79 The GVC mediates a number of functions central to coral health, and provides a crucial link between
80 the coral organism and reef ecosystem.

81 Digestion

82 The GVC hosts the coral digestive system that processes ingested food such as plankton and particulate
83 organic matter. Parallels exist between coral and vertebrate digestive systems, including structures to
84 maximise absorptive surface area (i.e., mesenteries), mesenterial filaments with specialised cells
85 secreting chymotrypsinogen - a precursor of the digestive enzyme chymotrypsin widely found in
86 vertebrate pancreases [23], and presence of both cilia and mucus-secreting cells [12]. Mesenterial
87 filaments can even be extruded through the polyp mouth or perforations in the polyp wall to digest prey
88 externally, taking over the function of the tentacles (Fig. 1) [24,25]. Importantly, the GVC allows corals
89 to supplement autotrophic carbon fixation with heterotrophic acquisition of N, P and other nutrients,
90 meeting 15-35% of metabolic demand [26]. Such trophic flexibility allows corals to meet energetic
91 demands during periods of environmental stress [27,28], especially during **coral bleaching**, when loss
92 of photosynthetic endosymbionts (**Symbiodiniaceae**) leaves the coral without its primary energy source
93 [29].

94 Circulation

95 Scleractinians lack discrete circulatory, respiratory and excretory organs [12], so the GVC and associated
96 gastrovascular canals (collectively termed the gastrovascular system) perform the function of a simple

circulatory system, moving nutrients, O_2 and waste products within the colony. Circulation capacity is enhanced in perforate corals, where continuous connections between polyps permits homogenous distribution of resources across the colony. Circulatory flow is facilitated by cilia that line the gastrovascular system and drive fluid transport at velocities up to ~300 μm s^{-1} in *Acropora cervicornis* [9]. While the direction of flow is unknown in scleractinians, more recent studies of octocorals have shown capacity for simultaneous, bi-directional fluid exchange between polyps [30]. Greater polyp connectivity is proposed to confer perforate coral species enhanced resistance to bleaching (e.g., [16]), thus understanding connectivity and exchange between compartments in other sessile invertebrates could prove critical in resolving their resistance to environmental stress.

Checkpoint of entry and departure for endosymbionts

The GVC plays a pivotal role in establishment of symbiosis between coral and photosynthetic endosymbionts. It serves as the entry point for Symbiodiniaceae that are ingested and enter the gastrodermis via phagocytosis, yet evade digestion by suppressing innate immune response from the host, ultimately preventing formation of microbicidal **phagolysosomes** [31,32]. Ingested Symbiodiniaceae instead persist in an organelle-like **symbiosome** vacuole [33] facilitating metabolic coupling between coral and symbiont [34]. It remains unknown whether and how the chemical microenvironment in the GVC modulates symbiont acquisition.

Coral response to environmental stress might also involve interactions between the tissue, chemistry and microbes present in the GVC. Indeed, altered nutrient cycling between corals and endosymbiotic algae during heat stress can be a primary driver of symbiotic breakdown [35]. Given the GVC's role in nutrient acquisition and cycling, characterising nutrient dynamics in this compartment (e.g., via low-volume metabolomics and isotopic tracers; Box 1) could resolve important regulatory mechanisms for coral stress responses.

A “bioreactor” analogue?

The semi-enclosed GVC exhibits characteristics of a **bioreactor**: a maximised surface area within a compact space, periodic mixing and exchange of fluid (i.e., seawater), and the existence of chemical gradients that potentially favour specific biological processes. Tentative evidence for bioreactor-like functioning of the GVC is the presence of elevated concentrations of vitamin B12 that is produced only by a small subset of bacteria and archaea living in anoxic or low oxygen environments [36]. Vitamin B12-producing microorganisms are commonly associated with the gut of herbivores, yet the anoxic zones present in the GVC may also create an ideal niche for these taxa in corals. To date only one study has characterised bacterial assemblages from GVC fluid [4], identifying bacterial ribotypes commonly found in the gut/abdomen microbiomes of other animal taxa, including honey bees and humans. This limited data indicates that the coral GVC is not occupied by opportunistic bacteria ingested with food items, rather that this microenvironment selects for specific microbial communities [4]. These distinct bacterial communities seem to populate the GVC fluid, with cell densities approximately two orders of magnitude higher than surrounding seawater [4]. Given the tiny volume of fluid likely present within many scleractinians (e.g., ~20 µL per polyp in *Galaxea fascicularis* [4]), application of novel low-input omics techniques will be needed to elucidate the full nature and functional capacity of the GVC microbiome (Box 1). Determining whether anaerobic microbial processes such as denitrification and fermentation occur inside the GVC and influence overall coral fitness and nutrient cycling represents a logical avenue for future research.

Competition for space

Competition for substrate is fierce on coral reefs [37], and the GVC mediates physical interactions between scleractinians and neighbouring competitors (e.g., other corals, macroalgae), via extrusion of mesenterial filaments discharging nematocysts into the epidermis of nearby competitors, which can

dissolve tissues of other corals [38] and cause bleaching of macroalgae [39] (Fig 1). Mesenterial filaments also sweep and remove detritus from surrounding surfaces, facilitating tissue expansion for skeletal deposition and colony growth [40]. The mechanical or chemical cues triggering mesenterial filament behaviour are largely unknown.

A gateway to the wider reef environment

Chemicals originating from the GVC can influence processes at the ecosystem scale. For example, coral mucus partially originating from specialised cells in the GVC [12] can be released in large quantities into the surrounding seawater, where it stimulates growth of planktonic bacteria and algae, and permeates into nearby reef sands fueling benthic food webs [41].

Coral GVC activity also mediates episodic, large-scale release of organic matter to the reef during sexual reproduction (Fig 1) by providing a temporary storage site for sperm and egg bundles, which are synchronously released by multiple coral species during annual **mass spawning events** [42]. Such coordinated release of organic material rich in C, N and P into otherwise oligotrophic reef waters profoundly affects microbial activity, primary productivity and biogeochemical cycling in the surrounding water column and sediment [43,44]. In rare instances, decay of spawning material can increase benthic oxygen demand and trigger environmental hypoxia [45], leading to widespread mortality of reef-associated organisms [46].

Analogous compartments in other sessile invertebrates

Although compartments in sessile invertebrates are morphologically and functionally diverse, they are always delineated by specific and often fluctuating physico-chemical conditions, providing many analogies with the coral GVC. Even organisms with simple body plans and lacking true tissue, e.g., sponges (phylum Porifera), exhibit internal O_2 gradients conducive to microenvironment formation. For

example, steep O_2 gradients are regulated by their water pumping activities [5,47] and pauses in pumping can lead to tissue anoxia within minutes [48]. Low-oxygen microenvironments can persist even after pumping resumes and certain sponge species can survive under very low O_2 levels [49], where their inner body can remain constantly suboxic with intermittent anoxic conditions [50] creating niches for anaerobic microorganisms [50,51].

Other sessile filter feeders (e.g., the phyla Bryozoa, Phoronida, Entoprocta, and Brachiopoda) are far less-studied than corals or sponges. Although the anatomy of their digestive systems is well-described, the different physico-chemical environments they likely harbour have not been characterised. Ascidians (phylum Chordata) have received more attention, where microsensor measurements (e.g., in the colonial species *Cystodytes dellechiajei*) revealed that nitrification, presumably carried out by Crenarchaeota, occurs deep into the tunic tissue (i.e., the outer covering that acts as an exoskeleton), where pH is three units lower than surrounding seawater [52]. Tropical didemnid ascidians harbour a diversity of microorganisms, including the obligate cyanobacterial symbiont, *Prochloron* in their tissues and cloacal cavity. The internal microenvironment of the ascidians becomes anoxic and acidic (~pH 7) after several minutes of darkness, while illumination results in O_2-supersaturation and alkaline conditions (~pH 10) over a similar timeframe [6]. This highly-dynamic physico-chemical environment is central to our understanding of ascidian-*Prochloron* symbioses [53] and might hold the key to successful cultivation of these important cyanobacteria.

More structurally-complex sessile invertebrates such as Bivalves (phylum: Mollusca) and Echinoderms harbour organs, including developed digestive systems, and their spatial heterogeneity has routinely been considered when studying their biology and microbiology. For example, anoxic aggregates, covered with sulphate-reducing bacteria belonging to the genus *Desulfonema* have been identified in the intestine of the sea urchin *Echinocardium cordatum* [7]. More recently, investigation of the gills of the

deep-sea mussel *Bathymodiolus puteoserpentis* revealed strong spatial partitioning in the distribution of bacterial symbionts and metabolites [54]. Such examples show how studying compartments within sessile invertebrates can reveal their internal functional partitioning and ultimately illuminate their biology. Insight into compartment functions could be fast-tracked through use of model organisms, which has been key to our understanding of human physiology and biochemistry and has led to many important achievements in medicine [64]. Cnidarians, such as *Nematostella vectensis* (Anthozoa), *Hydra vulgaris* (Hydrozoa) or *Exaiptasia diaphana* (Anthozoa), have been used extensively as model organisms in developmental biology [65] or as surrogate models to study coral biology [66-68]. These species could also serve as a proxy for other sessile invertebrates and accelerate our understanding of specific compartments since they are amenable to genetic manipulation, and a vast array of omics resources are already available for them (e.g., genomes, transcriptomes and proteomes). Given that some processes occurring in the GVC are likely conserved across many benthic invertebrate taxa, the advantages derived from using model organisms will enable us to more easily control or isolate specific variables to disentangle the roles of compartments in sessile invertebrates.

Concluding remarks

Insights from the coral GVC reveal a compartment harbouring distinct microorganisms and displaying dynamic gradients in pH, O_2, and nutrients. Although clearly central to many aspects of coral biology, only a handful of studies have focussed on the GVC, several of which are now >40 years old. Yet, the coral GVC has arguably received far more attention than that of many other sessile invertebrates, where targeted experimental work is needed to answer many unsolved questions (see Outstanding Questions). Although in-depth studies are still lacking for corals, the gastric cavity can serve as a template to study such compartments in a wider range of benthic taxa. Studies focusing on sessile invertebrates should no longer begin by homogenising large amounts of tissue, but instead specifically target compartments at

appropriate scales. Multiple techniques now permit deconvolution of the physical, chemical, and ecological interactions occurring at the microscale (Box 1). For example, specific cell types can be captured using laser microdissection, before being analysed using low-input sequencing techniques (Box 1). Additionally, use of model organisms (e.g., the anemone, *Exaiptasia diaphana*) may fast-track our understanding of compartments. Sessile invertebrates are facing mounting anthropogenic pressures. Studying the functions played by their inner compartments and associated microbiomes could transform understanding of their health, as these internal structures may determine their capacity to absorb environmental stress while maintaining ecosystem functioning.

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Glossary

Bioreactor

A manufactured vessel designed to effectively support a biologically-active environment, and in which biological or biochemical reactions occur.

Broadcast spawner

Describes coral species that release eggs and sperm into the water column for external fertilisation - usually during annual "mass spawning" events.

Coenosarc

Thin layer of living tissue that connects individual polyps within a coral colony. Secretes the coenosteum (underlying skeletal matrix).

Coral bleaching

Loss of photosynthetic endosymbionts and/or photosynthetic pigments resulting from environmental stress (notably from elevated seawater temperature).

Coral polyp

A soft-bodied Cnidarian animal (related to anemones and jellyfish) that lives individually, or forms extensive coral colonies of inter-connected polyps.

Gastrodermis

Inner cell layer lining the gastrovascular cavity of cnidarians that harbours endosymbiotic algae (Symbiodiniaceae) in corals and anemones.

Gastrovascular cavity (GVC)

(Synonyms: gastric cavity, coelenteron). The central body cavity of cnidarians such as corals, anemones and jellyfish.

Gastrovascular canal

A network of tubes lined with gastrodermis connecting the gastrovascular cavities of polyps within a coral colony. Canals extend through the coenosarc of all colonial scleractinians; while species with a perforate skeletal architecture benefit from an

additional subsurface gastrovascular canal system that permeates the coenosteum between polyps.

Hypoxia

A term describing a condition where oxygen levels are insufficient to sustain normal biological functioning (at either the cellular/individual or ecosystem level).

Imperforate

A type of gastrovascular architecture describing a solid skeletal structure with an absence of subsurface gastrovascular channels.

Mass spawning events

Synchronous reproductive events where multiple corals release eggs and sperm into the water column, often on only a single night of the year.

Nematocysts

(Synonym: Cnidocyst). Stinging capsules embedded in the ectodermis of Cnidarians and used in predatory capture of prey such as phytoplankton and zooplankton.

Perforate

A type of gastrovascular architecture, where skeletal voids allow connectivity between all polyps within a colony via subsurface gastrovascular canals.

Phagocytosis

Process by which a cell utilizes its plasma membrane to ingest other cells or particles, during which a vesicle (termed the phagosome) is formed around the cell or particle.

Phagolysosome

A digestive vesicle formed within a cell by the fusion of a lysosome to a phagosome, creating a microbicidal environment where destruction of microorganisms occurs.

Symbiodiniaceae

Family of photosynthetic microalgae (Class: Dinophyceae) that form symbioses with several reef invertebrates including corals, where they reside within gastrodermal cells.

Symbiosome

Specialised membrane compartment within a coral host cell, where Symbiodiniaceae reside.

Figure Legends

Figure 1: Anatomy of the coral gastrovascular cavity (GVC), its identified functions and emergent properties. The anatomy of the GVC is depicted centrally with the following annotated features: 1) polyp mouth, 2) actinopharynx, 3) mesenteries, 4) gonads, 5) mesenterial filaments and 6) coenosarc. Specific GVC functions are depicted in the circular side panels, including: export of particulate organic carbon (POC) through gamete and mucus release into the water column, competition for space via mesenterial filament extrusion, prey digestion, fluid circulation within and between polyps and recruitment of symbiont cells (Symbiodiniaceae). The presence of steep chemical gradients reflects an emergent property of the GVC and generates microenvironments conducive to the production of specific secondary metabolites by microbial associates (e.g., vitamin B12). Artwork: Philippe Plateaux.

Figure 2: Current tools enabling the exploration of compartments in sessile invertebrates. A wide range of techniques now allow studying organisms at the compartment, cellular or even subcellular levels. These tools include: microsensing to characterise microscale gradients in specific physico-chemical variables, chemical tracers and chemical imaging enabling to follow the connectivity within colonial organisms, microscale inspection using microendoscopy, imaging mass-spectrometry techniques that can be coupled with stable isotope tracers to investigate the assimilation of specific substrates, particle image velocimetry commonly used to characterise fluid flow dynamics at the microscale, and low-input omics techniques that are currently emerging. For example, recent advances in DNA extraction enable us to use input volumes that are six-orders of magnitude lower (small droplet) than traditional approaches (larger drop). A more extensive description of these techniques can be found in Box 1. Artwork: Philippe Plateaux.

Box 1. A toolbox to explore compartments in sessile invertebrates.

Many techniques enable the dissection of biological, chemical and physical processes at microscales. For example:

Imaging mass spectrometry: This umbrella term encompasses many instruments enabling the spatial mapping of atoms or molecules [55]. Although the strong beam of nanoscale secondary ion mass spectrometry (NanoSIMS) allows for an unparalleled spatial resolution (50-100 nm), it fragments molecules, thus preventing their identification. However, recent advances in matrix-assisted laser desorption/ionization (MALDI) allow the identification of molecules within a 3 μm resolution [54]. These techniques can be combined with electron microscopy (identifying specific microscale structures) or fluorescent *in situ* hybridization (identifying specific microorganisms).

Microsensors: Minimally-invasive measurement devices with tip diameters of ~5-100 μm that convert the physical property (e.g., light, temperature) or chemical quantity (e.g., O_2, pH, CO_2, H_2, H_2S, NO, N_2O) of an analyte, into an electrochemical or optical signal [56,57]. Microsensors can quantify these parameters at unmatched spatiotemporal resolution, but the profiles generated are unidimensional.

Low-input omics techniques: Most omics techniques still require large inputs of starting material (e.g., litres of seawater, or centimetre-sized fragments of the target organism) necessitating the filtration of several litres of seawater or the collection of centimeter-size fragments of the target organism(s). However, approaches requiring much lower input are rapidly emerging and we can now obtain metagenomes from 1 μL of seawater [58]. In addition, laser-capture microdissection can be coupled with low-input sequencing approaches to selectively isolate specific cell types [59]. Input requirements are also dropping quickly for proteomics [60] and metabolomics [61], enabling further characterization of microenvironments, such as the GVC fluid.

Chemical tracers and imaging: Fluorescent dyes and other tracers have been used to study the uptake, connectivity, or residence time of fluids within organisms. The low detection limit of these tracers means that small concentrations are needed during aquaria or field experiments. For example, spatial O_2 and pH distributions can be realized by coating samples with planar sensing foils [6] or optical sensor particles [62] that are read-out with luminescence intensity or lifetime imaging systems

Particle image velocimetry (PIV): A common approach to visualise and quantify flow using small particles. PIV enables the calculation of speed and direction of the flow in a two-dimensional space, and has often been used at the microscale [11].

Microendoscopy: Widely-employed in the medical field, microendoscopes use imaging fibre bundles with an outer diameter as small as 500 μm that can be coupled with multiple imaging approaches, such as fluorescence [63]. Although their sizes now permit easy insertion into most sessile invertebrates, thin endoscopic fibres currently have low pixel resolution.