Ecological Indicators
March 2019, Volume 98 Pages 49-60
https://doi.org/10.1016/j.ecolind.2018.10.044
https://archimer.ifremer.fr/doc/00464/57608/

Connecting organic to mineral: How the physiological state of an ecosystem-engineer is linked to its habitat structure

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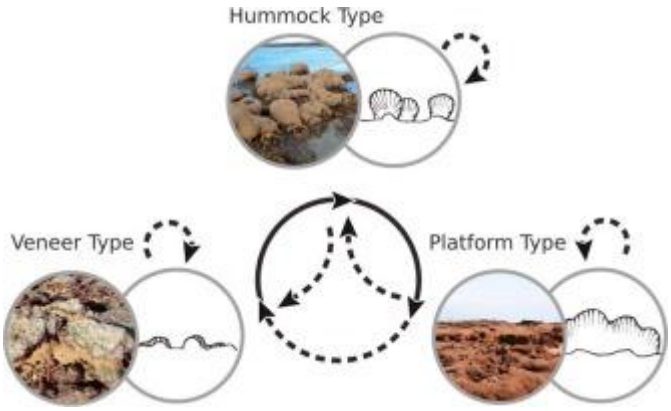
Abstract :

The honeycomb worm Sabellaria alveolata is capable of building extensive bioconstructions, including what are currently considered Europe's largest biogenic reefs. The size and volume of these bioconstructions, however, vary greatly, such that not all habitats engineered by S. alveolata may be easily identified as reefs. Given that European environmental legislation protects marine habitats that are classified as "reefs", it is important to identity a clear set of definition criteria. Furthermore, quantifiable and unequivocal criteria are also needed to evaluate the ecological (health) state of these reefs, in order to best monitor and protect them. Here we propose new terminology to describe the physical appearance of these bioconstructions and attempt to link these physical criteria to the physiological state of the tube-building polychaete. We tested whether a bioconstruction displaying outward signs of growth is built by "healthy" worms devoid of physiological stress by analysing three macromolecules (carbohydrates, proteins, lipids), four polar lipid fatty acids, six neutral lipid fatty acid markers and three metabolic enzymes (citrate synthase, catalase and superoxide dismutase). The worms were sampled in bioconstructions of different "Type" (veneer vs. hummock), "Phase" (progradation vs. retrogradation), and "Shore Level" (high shore vs. low shore) at Champeaux in Mont-Saint-Michel Bay, France. Our results show that worms sampled in retrograding reefs (i.e. displaying signs of erosion and colonisation by epibionts such as oysters or mussels), were less physiologically stressed than worms sampled in prograding bioconstructions, possibly due to lower intraspecific competition and hence greater food availability. We therefore suggest management measures should encompass the whole mosaic of biogenic construction Types and Phases. We propose the inclusion of the polar lipid fatty acid arachidonic acid, in combination with the activity of two metabolic enzymes, citrate synthase and superoxide dismutase, as the three key biochemical markers to consider for quantitative information on the physiological state of this particular ecosystem engineer. Our results also revealed the influence of both sex and size on fatty acid and enzyme levels, highlighting the importance of taking into account both these variables when sampling and subsequently pooling individuals by sex and size category for laboratory analyses. Once seasonal and site variation have been addressed, these biochemical indicators could be examined in parallel with S. alveolata bioconstruction physical criteria as part of a European-wide protocol for monitoring ecological status in this potential reef habitat.

Graphical abstract



Highlights

► Biochemical markers can be informative for monitoring bioengineered habitats. ► Management measures should cover multiple bioconstruction types and phases. ► Physiological distress is not correlated with retrograding bioconstructions. ► Worms in retrograding bioconstructions have a higher quantity of available food. ► Three biochemical markers stand out as relevant physiological state descriptors.

Keywords : Biochemical indicators, Health, Ecological status, *Sabellaria alveolata*, Biogenic reef, Engineer species

1. Introduction

Biogenic reefs are among the most biologically diverse and functionally important habitats on Earth (Goldberg, 2013). Their physical structure is known to provide numerous provisioning and regulating services, such as unique habitat provision and coastal protection (Hattam et al., 2015). Tropical coral reefs are often the first image that springs to mind upon hearing the term "reef", and are the subject of enormous literature. Scleractinian corals, however, are only one of many marine taxa capable of building biogenic reefs, which can be defined as follows: "*solid, massive structures which are created by accumulations of organisms*" and "*clearly forming a substantial, discrete community or habitat which is very different from the surrounding seabed*" (Holt et al., 1998). In temperate waters, shellfish and tube-dwelling polychaetes also fall under this definition (Bartol et al., 1999; Dubois et al., 2006).

67 The term “healthy reefs” is ubiquitous throughout the literature on marine conservation;
68 healthy reefs are often the focus of protection efforts (Abelson et al., 2017). Yet when it
69 comes to assessing reef health status, the metrics most commonly described assess either
70 ecological (*i.e*. species richness and presence of species of interest), or landscape
71 characteristics (*i.e*. elevation, extent, coverage). It is unknown whether these assessments are
72 indicative of the health of the engineering organism itself.

73 Reef-building sabellariid worms are ubiquitous globally in both intertidal and shallow subtidal
74 zones. The species responsible for building what are currently considered as Europe’s largest
75 biogenic reefs (Gruet, 1986) is the honeycomb worm *Sabellaria alveolata* (Linnaeus, 1767).
76 This sedentary colonial polychaete creates tubes of coarse sand grains and shell fragments
77 cemented together; dense aggregations of which may be regarded as reefs (Holt et al., 1998).
78 This Lusitanian species is widely distributed from southwest Scotland to Morocco where it
79 inhabits the low- to mid-shore (Gruet, 1986; Dubois et al., 2002). As an ecosystem engineer,
80 *S*. *alveolata* generates small to large scale topographic complexity, creating numerous spatial
81 and trophic niches for other species to colonise (Dubois et al., 2002; 2006; Dubois and
82 Colombo 2014; Jones et al., 2018). Their bioconstructions buffer physical and chemical
83 stresses, protect from predators and competitors, and alter resource availability (Porras et al.,
84 1996). Consequently, these bioconstructions host highly diverse and unique communities,
85 composed of species originating from hard, muddy and sandy substrates, in addition to both
86 subtidal and intertidal habitats (Dubois et al., 2002; 2006; Schimmenti et al., 2015), and are
87 broadly considered as local hotspots of biodiversity (Jones et al., 2018).

88 Honeycomb worm bioconstructions constitute a highly dynamic habitat subject to numerous
89 natural (*e.g*. cold winters or storms) and anthropogenic disturbances (*e.g*. trampling,
90 harvesting, shellfish farming, coastal development) (Dubois et al., 2002; Firth et al. 2015;

91 Plicanti et al., 2016). These bioconstructions may take on three main structural types (see *e.g.*
92 Cunningham, 1984 and Holt et al., 1998, Fig. 1). Gruet (1982) described a cycle whereby a
93 bioconstruction evolves from (1) "veneers" in which the tubes overlap and lie at an acute
94 angle to the substratum, to (2) "hummocks" in which the tubes radiate out from the initial
95 settlement point before reaching (3) "platforms" formed of extensive areas of hummocks
96 fused together. In the majority of locations throughout Europe, however, neither the
97 "hummock" nor "platform" type is ever reached. Any one of these bioconstruction types can
98 display outward signs of being in a "progradation" or "retrogradation" phase (Figure 1a).
99 Gruet (1982) was the first to refer to *S. alveolata* reef phases, which he referred to as
100 "growth" and "destruction". Here we propose new terminology for *S. alveolata*
101 bioconstruction development. The engineered structure is constantly in a delicate balance
102 between these two phases, and can therefore display some or all of the characteristics listed in
103 Figure 1 B. Patches can undergo cyclic or erratic changes whereby they prograde or
104 retrograde either partially or totally through resettlement, as *S. alveolata* larvae, as with all
105 sabellariid species, preferentially settle on conspecific adult tubes (Pawlik, 1986).

106

107 Currently there is no single definition or guidance of what constitutes a *S. alveolata "*reef".
108 Far from being a semantic dispute, this has far-reaching implications in terms of management
109 and conservation, as throughout the majority of its distribution (*i.e.* Europe) the sole
110 legislative instrument affording any protection is the Habitats Directive (Council Directive
111 92/43/EEC), which only protects the "reef" form of biogenic constructions. A "reefiness"
112 scoring system in the context of the Habitats Directive was developed by Hendrick and
113 Foster-Smith (2006) for subtidal *Sabellaria spinulosa* reefs, based on a series of physical,
114 biological and temporal characteristics weighted according to data quality of and perceived

115 importance of each feature. Desroy et al. (2011) developed a Health Status Index for *S*.
116 *alveolata* bioconstructions at the Bay of Mont-Saint-Michel, based on fragmentation,
117 proportion of different bioconstruction morphological types and coverage by three key
118 epibionts (*i.e*. oysters, *Magallana* (formerly *Crassostrea*) *gigas*, mussels, *Mytilus* spp., and
119 green macroalgae, *Ulva* spp.). Existing rapid assessments of intertidal *S*. *alveolata* "quality"
120 (*i.e*. Cunningham, 1984; Firth et al., 2015) are based on visual evaluations of the
121 bioconstructions, and do not consider environmental characteristics of the site. Whilst all of
122 these scoring indices are very useful, they may not easily translate beyond the species or
123 biogeographic regions that they were developed for. Due to differences in morphology
124 between the two species, the "reefiness" score for *S*. *spinulosa* developed by Hendrick and
125 Foster-Smith (2006) is more helpful as a means of comparing the relative values of two
126 different areas of *S*. *spinulosa* reef. The Health Status Index developed by Desroy et al. (2011)
127 is only applicable in areas where *S*. *alveolata* bioconstructions reach the platform type and
128 become colonised by *M*. *gigas* or *Mytilus* spp., thus restricting its application. Furthermore,
129 not only do both of these indices require a considerable amount of field and laboratory work
130 with numerous field experts, they also rely entirely on the physical looking-aspect of the
131 bioconstruction. For example, muddy and fragmented bioconstruction patches have a low
132 score on Desroy et al. (2011) health scale, disregarding the actual physiological state of the
133 worm.. The main objective of the present study was to develop a generic tool that would be
134 applicable across the entire geographic range of *S*. *alveolata*, able to help linking the physical
135 state of the bioconstructions with the physiological state of the individuals.
136
137 Biochemical proxies may provide generic health indicators well suited for *S*. *alveolata*. Cell
138 health and survival rely on a series of biochemical fluxes and reactions that are highly
139 conserved among species and biogeographic regions (Hochachka and Somero, 2002).

Therefore, biochemical indicators can be used as a snapshot of the physiological condition of the individual at the time it was sampled (Fraser, 1989; Dahlhoff, 2004), and could potentially have similar applicability across a variety of taxa. In order to understand how the physiological condition of *S*. *alveolata* ties in with their bioconstruction structure (*i*.*e*. type, phase and shore level), we focused on a suite of metabolic parameters that reflect several key physiological processes.

Information on the organic macromolecules of a species is fundamental to understanding its biochemical characteristics. Carbohydrates and neutral lipids constitute major energy reserves for fuelling growth, reproduction, and defence against stressors in many marine invertebrates (*e*.*g*. Gallager et al., 1986; Berthelin et al., 2000; Rivest et al., 2017). Glycogen is the primary polysaccharide (polymeric carbohydrate) in annelids (Scheer, 1969) and has long been recognised as the principal energy reserve in juvenile and adult bivalves (Lucas and Beninger, 1985). It serves both as an energy reserve under unfavourable environmental conditions, and also for the formation of gametes (Gabbott, 1975). Quantifying glycogen levels may therefore provide an indication of the level of energy reserves in *S*. *alveolata*.

Fatty acids, which are key constituents of the lipid compartment, vary with environmental factors such as temperature (*e*.*g*. Pernet et al., 2007) or salinity (Fuhrmann et al., 2018), trophic sources (Winder et al., 2017) and life history stage (*e*.*g*. Soudant et al., 1999; Lourenço et al., 2017). On one hand, the fatty acid composition of neutral lipids, which generally consists of triglycerides, reflects the fatty acid profile of the food consumed, thus revealing useful information about quality and assimilation of trophic sources (Dalsgaard, 2003). On the other hand, the fatty acid composition of polar lipids, which mainly originate from cell membranes (phospholipids), is altered to enable physiological adaptation of

165 organisms to their physical environment (Hazel and Williams, 1990; Hochachka and Somero,
166 2002). The best example of this is the remodelling of membrane lipids by ectothermic
167 animals, including *S. alveolata*, to counteract the effect of temperature on membrane fluidity
168 (Hazel, 1995; Muir et al., 2016). Some fatty acids are particularly informative. The fatty acid
169 20:4n-6 (arachidonic acid) is a precursor of hormones involved in stressful or energetically
170 expensive situations, namely gametogenesis and spawning (Osada, Nishikawa and Nomura,
171 1989), stimulation of immune functions in marine invertebrates (Delaporte, 2003) and
172 acclimation to increasing seawater temperatures in *S. alveolata* (Muir et al., 2016). Therefore,
173 fatty acid composition acts as a good stress bioindicator in marine organisms.
174
175 Metabolism is the biological processing of energy and materials through a series of
176 biochemical reactions catalysed by enzymes (Brown et al., 2004). Metabolic enzymes are
177 proteins which help maintain physiological homeostasis. Citrate synthase is a central enzyme
178 in the process of sugar oxidation involved in adenosine triphosphate (ATP) generation. It is
179 the first step of the citric acid or Krebs cycle, consisting of a series of chemical reactions
180 which generate energy through the oxidation of acetyl-Coenzyme A derived from
181 carbohydrates, lipids and proteins. Citrate synthase activity (CS) is correlated with respiration
182 rate in marine invertebrates and can be used as indicator of oxidative stress (Dahlhoff et al.,
183 2002). Reactive oxygen species (ROS) are generated through cell respiration and their
184 production is exacerbated by environmental stressors such as contaminants, pathogens and
185 dietary restrictions (*e.g.* Abele and Puntarulo, 2004 for review). Accumulation of ROS in
186 aerobic cells can result in oxidative stress in the host (Lesser, 2006) which is normally
187 prevented by an enzymatic antioxidant system. Studies have highlighted the importance of the
188 enzymes superoxide dismutase (SOD) and catalase (CAT) in the prevention of tissue damage
189 from oxidative stress in marine invertebrates (Abele and Puntarulo, 2004 for review).

The overall objective of this study was to investigate the relationship between the outward structural appearance of the bioconstruction and the physiology of the resident individuals. From a practical standpoint, we tested the effect of the physical appearance of the bioconstructions in terms of their structural Type (veneer *vs*. hummock, but not platform due to this type being absent from the majority of locations), Phase (progradation *vs*. retrogradation) and shore level (high *vs*. low-shore) on several well-known biochemical indicators of health. Key macromolecules, enzyme activity and fatty acid composition of neutral and polar lipids may change between different bioconstruction phases of growth and deterioration and are likely to modulate the individual's response to environmental stressors.

2. Materials and Methods

2.1 Study location: Champeaux Reef

Our study took place in the Bay of Mont-Saint-Michel (north-western France), a megatidal (>14m) ecosystem with one of the highest maximum spring tidal range values in the world (Levoy et al., 2017). It is home to the largest and most extensive biogenic constructions in Europe, namely the Saint-Anne reef (2.25 km²) and the Champeaux reef (0.29 km²) (Desroy et al., 2011). The current study focused on the Champeaux reef affixed to rocky substrate in the upper intertidal zone, situated in the southeastern part of the Bay (48.7318, 01.5520; Figure 2). The tidal amplitude in a megatidal regime implies that the water column is very well mixed. The surrounding substrate is known as "*tangue*", a heterolithic sediment displaying an alternate structure of sandy and silty-muddy beds which represent the deposit of each semi-diurnal tidal cycle (Tessier, 1993).

2.2 Sampling design

Twelve patches of *S*. *alveolata* were sampled on the bioconstruction during low water on the 22nd February 2016. Five individual worms were extracted from each patch of either veneer or hummock type (Table 1). For hummocks, the distinction was made between formations presenting outward signs of either progradation or retrogradation. Most treatments were well interspersed over the study area, but within ~50 m from each other. Although we would have ideally wished to sample all shore heights and phases for all types, our study location only allowed for prograding hummocks to be sampled both on the high and low shore, whilst retrograding hummocks were only present in the eastern part of the study site (Figure 2).

Small clumps of tubes were broken off, and five females were gently extracted from their tubes for each sampling point. In addition, five males were sampled from one low-shore hummock patch located in the centre of the reef. Worms were randomly sampled within mature individuals. Maturation stage was assessed by visual examination of the extracted worms. Unspent (*i*.*e*. those that had not shed their gametes) worms are creamy white for males and purplish/rose-violet for females, while spent worms are thin-bodied brownish (see Wilson, 1971, Gruet and Lassus, 1983, Wilson, 1968 cited in Culloty et al., 2010). All sampled worms were placed in cryotubes and immediately flash frozen in liquid nitrogen *in situ* before they could start releasing their gametes. Samples were then long-term stored at -80°C until laboratory analyses.

Table 1. Summary of the sampling design. Abbreviations: nc, not considered for analysis; F, Female; M, Male.

Bioconstruction	Shore level	Sex	Patch	Number of	Code

Type	Phase				individuals	
Hummock	Retrogradation	nc	F	1	5	HR1
Hummock	Retrogradation	nc	F	2	5	HR2
Hummock	Retrogradation	nc	F	3	5	HR3
Hummock	Progradation	High	F	1	5	HPHs1
Hummock	Progradation	High	F	2	5	HPHs2
Hummock	Progradation	High	F	3	5	HPHs3
Hummock	Progradation	Low	F	1	5	HPLs1
Hummock	Progradation	Low	F & M	2	5 & 3	HPLs2
Hummock	Progradation	Low	F	3	5	HPLs3
Veneer	nc	nc	F	1	5	V1
Veneer	nc	nc	F	2	5	V2
Veneer	nc	nc	F	3	4	V3

2.3 Laboratory analyses

Individual worms were weighed and ice-cold milliQ water was added at ¼ mass volume ratio. Then, samples were grinded and homogenised 2×20 seconds at 4.5 m sec^{-1} using the FastPrep-24™ 5G Instrument (MP Biomedicals SARL, Illkirch, France). Tissue samples were aliquoted in one tube containing 100-200 µL for lipid analyses and in six tubes containing 180 µL for carbohydrate and protein extraction.

2.3.1 Carbohydrates

An aliquot of 180 µl of tissue sample was used for determination of total carbohydrate concentrations. Briefly, samples were diluted 10 times by addition of 820 µL MilliQ water. Carbohydrate concentrations were determined by colorimetric method according to DuBois (1956). Then, samples (250 µL) were mixed with phenol (0.5 ml, 5% m/v) and sulfuric acid (2.5 ml, 98%), and incubated for 40 min. Absorbance was read at 490 nm with a UV 941 spectrophotometer (Kontron instruments, San Diego, California, USA). Carbohydrate

concentrations were determined using a standard calibration curve and expressed as mg of carbohydrates per g of wet weight.

2.3.2 Lipids

2.3.2.1 Extraction

One aliquot tube of 180µl was used for determination of fatty acid profiles of neutral and polar lipids. The sample was transferred into a 6 mL glass vial. Lipids were extracted according to Bligh and Dyer (1959). A mixture of $CHCl_3$:MeOH 1:2 (v/v, 750 µL) was added to the sample. Then, the sample was vortexed before adding pure $CHCl_3$ (250 µL), vortexed again, diluted with MilliQ water (250 µL), vortexed again, and centrifuged at 3000 rpm for 5 minutes. The lower organic phase was transferred into another clean 2-mL glass vial. The remaining aqueous phase was washed with 500 µL $CHCl_3$, vortexed, and centrifuged again. The lower phase was recovered, pooled with the first one and evaporated under a nitrogen flow. The sample was stored at -20°C in 1 mL of $CHCl_3$:MeOH 98:2 (v/v).

2.3.2.2 Neutral and Polar Lipid Separation

Subsamples (250 µL) were then placed on the top of a silica gel micro column (30×5 mm internal diameter; Kieselgel; 70–230 mesh (Merck, Lyon, France); previously heated to 450°C and deactivated with 5% water) (Marty et al., 1992). Neutral lipids were eluted with 10 ml of $CHCl_3$:MeOH (98:2, v/v) and the polar lipids were recovered with 15 ml of MeOH. A known amount of tricosanoic acid (23:0) was added to both fractions as an internal standard. The lipid fractions were evaporated to dryness under nitrogen, re-suspended in $CHCl_3$:MeOH 2:1 (v/v) before transesterification.

2.3.2.3 Transesterification

Neutral and polar lipids were transesterified at 100°C for 10 min with 1 mL of boron trifluoride-methanol (12% MeOH) (Metcalfe and Schmitz, 1961). This transesterification produces fatty acid methyl esters (FAME) from the fatty acid esterified at the sn-1 and sn-2 position of diacylphospholipids, and the sn-2 position of plasmalogen phospholipids. It also produces dimethyl acetals (DMA) from the alkenyl chains at the sn-1 position of plasmalogens (Morrison and Smith, 1964). FAME and DMA were analysed in a HP6890 gas-chromatography system (Hewlett-Packard) equipped with a DB-Wax capillary column (30 m × 0.25 mm; 0.25 μm film thickness; Agilent technologies). Peaks were analysed by comparison with those of a standard 37 component FAME mix (Supelco® 37, Merck) together with other known FAME mixes from marine invertebrates. Each fatty acid was expressed as the peak area percentage of the total fatty acid content. Total DMA was used as an indicator of the plasmalogen level.

The fatty acid trophic markers investigated in this study were the ratio of 16:1n-7/16:0 and 16:4n-1 both of which indicate the contribution of diatoms to the diet; the ratio of 22:6n-3/20:5n-3 which is used as an indicator of dinoflagellate contribution relative to diatoms, all of the sum of 18:2n-6 and 18:3n-3, which is generally considered as a marker of terrestrial inputs; the ratio of 18:1n-9/18:1n-7, which is generally used as an indicator of carnivory; the ratio of polyunsaturated/saturated fatty acid (PUFA/SFA), which is an indicator of food freshness; and the sum of iso- and anteiso-branched chain fatty acids and unbranched 15:0 and 17:0, which reflects the contribution of bacteria to the organic matter. These fatty acid food web markers are routinely used in trophic ecology (Dalsgaard, 2003). The ratio of neutral to polar lipid content was also calculated, as this proxy reflects the relative

293 contribution of reserve to structural lipids and is used as a nutritional condition index that is
294 scaled to body size (Hentschel, 1998).

295 **2.3.3. Enzymes**

296 **2.3.3.1. Protein extraction and quantification**

297 Tissue samples of 150µl were diluted by 2/3 in ice-cold lysis buffer solution and
298 homogenized with a Polytron® PT 2500 E (Kinemetica, Luzernerstrasse, Switzerland). The
299 buffer solution was made of 150 mM NaCl, 10 mM Tris, 1 mM EDTA, 1 mM EGTA, 1%
300 Triton X-100, 0.5% Igepal, 1 tablet of complete EDTA free protease inhibitor cocktail (Roche
301 Diagnostics, Risch-Rotkreuz, Switzerland) in 25 ml of buffer, phosphatase inhibitor cocktail
302 III (Merck KGaA, Darmstadt, Germany) all at pH 7.4 (Le Foll et al., 2006). Homogenates
303 were incubated for 1 hour before being centrifuged twice at 4000 rpm for 1 hour at 4°C and at
304 11700 rpm for 45 min at 4°C to eliminate the lipid fraction of the samples, using GR412 and
305 MR22 Jouan centrifuges (Thermo Scientific, Waltham MA, USA), respectively. The resulting
306 supernatants were aliquoted and stored at -80°C until protein quantification and enzyme
307 assays.

308

309 To determine total protein concentration, an aliquot of protein extract was diluted by $1/10^{th}$
310 and quantified according to Lowry et al. (1951) using the *DC*tm protein assay kit (Bio-Rad,
311 Hercules, California, USA). Absorbance was read at 750 nm and protein concentrations were
312 determined by comparison with a calibration curve of Bovine Serum Albumin provided with
313 the kit. Results were expressed as mg of proteins per g of dry tissue weight.

2.3.3.1 Citrate Synthase

Enzyme assays were performed in triplicate at room temperature and enzyme activities were expressed and related to the total protein concentration for each sample.

Citrate synthase activity (CS; EC 4.1.3.7) was assayed at room temperature according to Childress and Somero (1979). Protein extract (20 µL) was added in wells containing an assay buffer solution (160 µL) which consisted of 100 mM Tris-HCl, 0.2 mM acetyl-coenzyme A, 0.1 mM 5, 5' -dithio-bis-[2-nitrobenzoic] acid (DTNB). The reaction was initiated by adding oxaloacetate (0.5 mM; 20 µL). Absorbance was recorded for 10 min at 412 nm using a Synergy HT microplate reader (BioTek, Winooski VT, USA). Results were expressed in mU mg^{-1} protein, where 1 U is the amount of enzyme to catalyze 1 µmole of TNB per minute (using $\varepsilon_{TNB,412} = 13.6\ mM^{-1}$ cm-1).

2.3.3.2 Superoxide Dismutase

Total superoxide dismutase activity (SOD; EC 1.15.1.1) was determined using an assay kit (Merck KgaA, Darmstadt, Germany), following the manufacturer's instruction. Superoxide dismutase activity (SOD) was measured by adding 200µL of Water-Soluble Tetrazolium salt (WST-1) to 15µL of total protein lysates 10 times diluted, and the reaction was initiated by adding 20µL of xanthine oxidase (XO) and xanthine mix. After a 20 min incubation at 37°C, absorbance was read at 450nm using a Synergy HT microplate reader (BioTek, Winooski VT, USA). SOD is quantified by comparing the decrease in the colour development at 450nm to a standard inhibition curve, performed using SOD from bovine erythrocytes. Results were expressed in units per mg of protein (U/mg), where 1 U of SOD is the amount of enzyme necessary to inhibit by 50% the xanthine/XO complex formation.

2.3.3.3 Catalase

Catalase activity (CAT; EC 1.11.1.6) was assessed at room temperature following Aebi (1984). Briefly, 5 µL of 10 times diluted protein supernatant were added to 195 µL of hydrogen peroxide solution (10 mM) to initiate the reaction. Absorbance was immediately recorded for every 15 s for 4 min using a Synergy HT microplate reader (BioTek, Winooski VT, USA). CAT is expressed in U/mg protein where 1 U is the amount of enzyme necessary for catalysing 1 µmole of H_2O_2 per min (using εH_2O_2, 39.4 mM^{-1} cm^{-1}).

2.4. Statistical analyses

Mixed design analyses of covariance (ANCOVA) were used to compare lipid, carbohydrate, and protein contents, enzyme activities and relative fatty acid concentration of the *S. alveolata* samples among the different bioconstruction types, phases and shore level. In the model, the random factor was the patch where the samples were taken and the covariate was individual worm mass (*i.e.* wet weight measures for 59 individual females). The individual worm mass did not vary with bioconstruction characteristics (ANOVA F-test=2.35, p=0.148) and can therefore be used as an independent factor. The categorical fixed variables were the four combinations of bioconstruction types and phases: veneer (V), retrograding hummock (HR) and prograding hummock located either on the high-shore (HPHs) or low-shore (HPLs). Interactions between bioconstruction characteristics and worm mass were systematically not significant and therefore omitted from the model (Table 2). When F-tests were significant the following *a priori* null hypotheses ($H0$) were tested using contrasts:

$$H0_1: \mu_{V=} \frac{\mu_{HR} + \mu_{HPHs} + \mu_{HPLs}}{3}$$

$$H0_2: \mu_{HR=} \frac{\mu_{HPHs} + \mu_{HPLs}}{2}$$

$H0_3: \mu_{HPHs} = \mu_{HPLs}$

To compare males and females, the unequal variance *T*-test was used. The normality of residuals and homogeneity of variances were investigated using Box-Cox transformations (Box and Cox, 1964). When necessary data were log-transformed. All statistical tests were performed with SAS software (SAS 9.4, Carry NC, USA).

Table 2. Summary of the mixed analyses of covariance (ANCOVA) model used for comparing females. df = degrees of freedom

Sources of variation	**df**
Main-plot analysis	
Bioconstruction characteristics	3
$H0_1$: Veneer (V) = Hummock (H)	1
$H0_2$: Retrograding H (HR) = Prograding H (HP)	1
$H0_3$: HP High-shore (HPHs) = HP Low-shore (HPLs)	1
Error a: Patch (Bioconstruction characteristics)	8
Subplot analysis	
Individual worm mass	1
Error b: Individual worm mass × Patch (Bioconstruction characteristics)	47
Total	59

3. Results

We investigated the effect of bioconstruction characteristics, worm mass and sex on 16 different parameters grouped into four categories (Figures 3 to 6). We report that

366 bioconstruction characteristics were associated with variations in three out of four indicator
367 categories, whereas both individual mass and sex drove differences in all four categories.

368 **3.1 Organic macromolecules**

369 There was a significant relationship between the neutral:polar lipid ratio and bioconstruction
370 characteristics (Fig. 3c), where the two bioconstruction phases contrasted significantly
371 ($F_{1,8}$=20.1, p=0.002), with a higher neutral:polar lipid ratio found in the retrogradation phase.
372 Individual wet weight was negatively correlated with both carbohydrate and protein
373 concentration, and positively correlated with neutral:polar lipid ratio (Fig.3d-f). Females had a
374 neutral:polar lipid ratio that was 5.9 times higher than males (Fig.3i), however sex had no
375 effect on either proteins or carbohydrates (Fig.3g and h).

376 **3.2 Fatty acids**

377 **3.2.1 Polar lipids**

378 There was a significant effect of bioconstruction characteristics on both gadoleic (20:1n-11)
379 and arachidonic (20:4n-6) acid after controlling for individual worm mass. Gadoleic acid
380 varied with bioconstruction type and was significantly higher in worms sampled in hummocks
381 than in worms from veneers ($F_{1,8}$=12.0, p=0.009) (Fig.4a). Arachidonic acid levels varied
382 with bioconstruction phase and were significantly lower in retrograding hummocks ($F_{1,8}$=7.8,
383 p=0.024) (Fig.4b). 20:4n-6 levels were near-significantly lower on the low shore ($F_{1,8}$=4.8,
384 p=0.059) at 1.9%, compared to 2.2% on the high shore. Both eicosapentaenoic acid (20:5n-3)
385 and docosahexaenoic acid (22:6n-3) were not influenced by bioconstruction characteristics
386 (Fig. 4c and d). 22:6n-3 was the only polar lipid fatty acid to be significantly negatively
387 correlated with individual wet weight (Fig.4h). Sex clearly influenced levels of 20:1n-11 and
388 22:6n-3 content but had no effect on the other markers (Fig.4i and l); the former is present in

389 greater quantity in females than in males whereas the latter is present in greater quantity in
390 males than in females.

391 **3.2.2 Neutral lipids**

392 Fatty acid composition in neutral lipids was not influenced by bioconstruction characteristics
393 (Fig. 5a-f). With the exception of bacterial and carnivory markers, all neutral lipids were
394 positively influenced by individual wet weight (Fig. 5gh and kl). A strong difference between
395 male and female was observed for all trophic markers, with female worms exhibiting higher
396 values for all markers except for carnivory, where males have 18:1n-9/18:1n-7 levels twice as
397 high as those found in females (Fig. 5o). The 16:1n-7/16:0 ratio (Fig. 5a), which is used as a
398 diatom tracer, was corroborated by a second diatom tracer, 16:4n-1 (File S1), whose
399 concentration was significantly influenced by both individual wet weight and sex.

400 **3.2 Enzyme assays**

401 Citrate synthase activity (CS) was significantly affected by bioconstruction characteristics
402 (Fig. 6a); average CS was near significantly higher in worms sampled in hummocks than in
403 veneers (p=0.054), and twice as high in worms sampled in the retrogradation phase compared
404 to the progradation phase ($F_{1,8}$=10.1, p=0.013). Although superoxide dismutase activity
405 (SOD) was not significantly influenced by bioconstruction characteristics (Fig. 6), SOD
406 positively correlated with CS (File S1, CS=0.95+3.17×SOD, r^2=0.400 p<0.001). Individual
407 wet weight was negatively correlated with CS, but not with catalase activity (CAT) or SOD
408 (Fig.6). Sex clearly influenced CS activity but had no effect on either CAT or SOD (Fig.3cfi).
409 Mean CS was 3.8 times higher in males than in females (Fig.6c). Relationships among all
410 variables measured in our study were represented in a principal component analysis biplot
411 (File S1).

412 **4. Discussion**

413 Our study revealed that a retrograding *Sabellaria alveolata* bioconstruction does not
414 necessarily equate with reserve-depleted worms. Indeed, carbohydrate levels did not vary
415 between retrograding and prograding bioconstructions, and the ratio of neutral:polar lipids
416 was in fact higher in the retrogradation phase. Neutral lipids are essential energy reserves for
417 sustaining early life stages and play a key role in settlement, habitat selectivity and
418 recruitment in marine invertebrates (*e.g*. Tremblay et al., 2007). Also, higher levels of
419 energetic reserves are associated with a better ability to cope with stressful situations such as
420 pathogen resistance (Lochmiller and Deerenberg 2000, Pernet et al., 2014, Ellis et al., 2011).
421 It is therefore likely that worms living in retrograding reefs are better equipped to face
422 stressful situations.
423
424 We also found that levels of 20:4n-6 in the polar lipids of worms sampled in the
425 retrogradation phase were lower than in the progradation phase, unexpectedly compounding
426 the fact that worms are more stressed in progradation phase bioconstructions. Arachidonic
427 acid is a precursor of hormones involved in stress response and levels increase for example
428 with increasing temperature in *S. alveolata* (Muir et al., 2016). These authors found that
429 across six sampling stations spanning Scotland to Morocco, the level of 20:4n-6 was lowest in
430 worms collected in the Bay of Mont-Saint-Michel where environmental conditions were
431 perceived to be the most favourable (*e.g*. hydrodynamics and sediment supply). Indeed, this is
432 a central population within the geographic distribution of *S. alveolata* and supports the largest
433 living biogenic reefs in Europe. Differences in 20:4n-6 observed between the two
434 bioconstruction phases in our study were however weak (0.25%) compared to existing natural
435 variability across the range (1-5%, Muir et al., 2016). Membrane 20:4n-6 is therefore a good

436 indicator of stress, as it can detect differences locally as well as across a species distribution
437 range and is therefore applicable at several scales.

438 Citrate synthase activity (CS) was twice as high in worms sampled in the retrogradation phase
439 compared to the progradation phase. The CS involved in ATP generation reflects the oxygen
440 consumption rate of animals and has been used in community ecology to evaluate the
441 physiological status of rocky intertidal invertebrates (Dahlhoff et al., 2002). CS varies in
442 response to multiple variables. For example, CS in marine invertebrates increases with food
443 availability (Dowd et al., 2013), growth rate (Garcia-Esquivel et al., 2002), maintenance costs
444 and mitochondrial density (Moyes, 2003). Therefore, the elevated levels of CS presented here
445 require interpretation in conjunction with other markers. In our study, the observed increase in
446 CS in retrograding bioconstructions coincided with higher levels of stored lipid reserves, as
447 reflected by the higher neutral:polar lipid ratio. Therefore, it may reflect a feeding-induced
448 increase in aerobic metabolism as reported in fish larvae (Ferron and Leggett, 1994) and
449 intertidal invertebrates (Dahlhoff, 2002). Although superoxide dismutase activity (SOD) was
450 not significantly influenced by overall bioconstruction characteristics, SOD correlated with
451 CS and was higher in the retrogradation phase. This also points towards worms sampled in
452 retrograding bioconstructions having a faster metabolism, as SOD processes the ROS
453 metabolic by-products produced through aerobic respiration (Abele and Puntarulo, 2004).

454 To sum up, high neutral:polar lipids, low 20:4n-6, high CS, high SOD and undepleted
455 carbohydrates all indicate that worms sampled in retrograding bioconstructions were not
456 stressed, but rather had either a higher quantity or quality of food source. As all trophic
457 markers were remarkably similar between the two bioconstruction phases, worms sampled in
458 the retrogradation phase reef probably had access to food of the same quality but in greater
459 quantity. Intraspecific competition in a densely-populated progradation-phase bioconstruction

460 is a source of physiological stress due to limited food availability (Connell, 1983).
461 Alternatively, retrograding bioconstructions harbour more small cracks and crevices filled
462 with deposited sediment on top of which microphytobenthos can grow and that can be made
463 available through resuspension processes (Dubois et al., 2007; Lefebvre et al., 2009; Jones et
464 al., 2018). Microphytobenthos is mostly composed of benthic diatoms, which are of great
465 dietary value, compared to phytoplankton (Miller et al., 1996).

466 Existing assessments of the health of biogenic habitats depend primarily on visual criteria.
467 Certain aspects of the health of tropical coral reef polyps have made use of biochemical
468 markers, by assessing the size of energy stores (as lipid content) as a proxy for physiological
469 condition (Anthony, 2006) or by using CS and energy-storage lipids to see how scleractinian
470 coral larvae respond to ocean acidification and warming (Rivest and Hoffmann 2014; Rivest
471 et al., 2017). However, none of these studies made a link between the coral polyps and their
472 calcareous skeleton. Attempting to join the physical appearance of a bioconstruction with the
473 physiological status of the engineering species is surprisingly new.

474 Our study confirms that designating a given *S. alveolata* bioconstruction as retrograding or
475 prograding leaves room for interpretation, as shown for coral reefs (Abelson et al., 2017).
476 Health criteria based on the physical appearance of the bioconstruction did not yield any
477 information on the processes regulating individuals. Yet our results show that worms present
478 in retrogradation phase bioconstructions are in a better physiological state compared to
479 progradation phase worms. In a recent study, Jones et al. (2018) demonstrated the ecological
480 value of retrograding (referred to as "degraded reef") *S. alveolata* bioconstructions which, due
481 to their increased micro-habitat availability, act as biodiversity and recruitment promoters and
482 harbour more diversified food sources. *S. alveolata* colonies, in their role as habitats, may be
483 of considerable value to certain species of very small animals (Wilson, 1971), with

484 retrograding bioconstructions showing high numbers of associated species (Porras et al.,
485 1996; Dubois et al., 2002; Jones et al., 2018).
486
487 Although further work is needed to address large-scale variation over time, our results suggest
488 that *S. alveolata* sampled in retrograding bioconstructions were in fact healthier. The only
489 signs of ecosystem distress in the retrogradation phase are value judgments about the
490 bioconstruction appearance. Prograding bioconstructions, with their rounded shapes and crisp
491 porches, are often considered more aesthetically appealing than silty, pitted retrograding
492 bioconstructions. Although visual beauty correlates with ecosystem health for tropical coral
493 reefs (Haas et al., 2015), this does not appear to be the case for other *S. alveolata*
494 bioconstructions.
495
496 The majority of shorelines where *S. alveolata* are present host a mosaic of bioconstruction
497 types of varying heights and surface areas. With the exception of 20:1n-11, all of the
498 examined parameters did not vary between bioconstruction types. Hummock and veneer
499 sampling points were well interspersed and, due to the Mont-Saint-Michel's bay megatidal
500 regime, were subjected to a homogeneous water mass. None of the biochemical parameters
501 varied significantly between the high and low shore. Although the intertidal zone is a stressful
502 environment (Helmuth et al., 2002; Bertness et al., 2009; Firth and Williams 2009; Firth et al.,
503 2011), the buffering role that *S. alveolata* bioconstructions play diminishes fluctuations in
504 temperature and humidity to the point where they are able to host a number of subtidal species
505 (Dubois et al., 2002; Jones et al., 2018 and unpublished temperature data). It is worth
506 mentioning that the bathymetric differences between the high and low shore sampling points
507 were minor (*ca*. 1.5 m) compared to the tidal amplitude in the Mont-Saint-Michel (>14 m).
508 This could explain why shore level had no effect in our sampling design. Hummocks found

lower down the shore reach a greater height relative to sea level. However, this feature is common for *S. alveolata* bioconstructions which develop around the mid-tide level, with the bioconstructions located highest on the shore being submerged at least one hour less than the bioconstructions in the low intertidal zone. In an area experiencing a semidiurnal tide cycle, this translates as four less hours to filter-feed per lunar day, hence explaining why the largest individuals are typically found in the bioconstructions lowest down the shore.

There was a five-fold difference in individual wet weight across sampled individuals, with specimens ranging from ~70 to ~350 mg. We found that energetic reserves, CS, 22:6n-3 in phospholipids and four out of six trophic markers were correlated with size. It is therefore vital to take *S. alveolata* individual size into consideration when analyzing biochemical variables, something that is not systematically done in marine invertebrate biochemical studies. Carbohydrate and protein concentrations diminished with individual mass whereas neutral lipids increased, reflecting a size-specific biochemical composition. All of the sampled individuals were mature and collected at the adult stage. Therefore, size may be confounded with age, and differences in biochemical composition could reflect either. Both CS and 22:6n-3 decreased with increasing individual mass. Body mass and basal metabolic rates are allometrically related (Hochachka and Somero , 2002). Our results suggest that differences in respiration rate in *S. alveolata* relate to cell membrane fatty acid composition. This is consistent with Hulbert's theory of membranes as metabolic pacemakers (Hulbert et al., 2005).

We found that the neutral:polar lipid ratio and five out of six neutral lipid fatty acid trophic markers were higher in female worms, whereas 22:6n-3, the carnivory trophic marker and CS were all significantly higher in male worms. Docosahexaenoic acid (DHA – 22:6n-3) is

534 abundant in spermatozoa (Masuda, 2003), and hence is the only polar lipid fatty acid
535 significantly higher in males. Invertebrate eggs typically contain few carbohydrates and are
536 mainly composed of energy dense proteins and lipids, hence explaining higher neutral lipid
537 contents in females (Pernet and Jaeckle, 2004). Female worms stored four times more neutral
538 lipids than males. The neutral lipid fatty acid composition is transferred more conservatively
539 than the polar lipid fraction and therefore mirrors more closely that of the diet. Females
540 containing high neutral lipid levels are more susceptible to dietary change than males which
541 contain mostly polar lipids. This renders neutral lipid-rich female *S. alveolata* more suitable
542 than males for representing trophic-related changes in the environment, especially when
543 investigating diets or changes in food source composition. In order to use biochemical
544 indicators for studying trophic interactions, it is therefore important to be able to distinguish
545 the two sexes and to sample and analyse female *S. alveolata*.

546 Our study examined a one-time sampling event at a single study location. Increased spatio-
547 temporal sampling may further clarify differences between the long-term physical changes of
548 the bioconstruction and the short-term physiological state of the worm. In the progradation
549 phase, *S. alveolata* individuals displayed biochemical stress markers. However, this may be
550 considered as temporary stress due to, for example, momentarily lacking sufficient food. A
551 bioconstruction can move rapidly between progradation and retrogradation phases and is
552 therefore an inherent or "natural" fluctuation. An alternative scenario is that the worm
553 population is physiologically stressed beyond recovery. When resilience is exceeded, the
554 future of the bioconstruction is entirely dependent on primary or secondary settlement, thus
555 reflecting some kind of fundamental change in underlying dynamics (Johnson, 2009).
556 Although retrograding bioconstructions support worms in a better physiological condition, the
557 high diversity this phase supports may have a negative effect both on adult worms through

558 interspecific competition, and on *S. alveolata* larval recruitment, as epiflora can lower
559 successful recruit density due to the mechanical action of algal fronds sweeping the reef
560 surface (Dubois et al., 2006). Bioconstructions will no doubt naturally undergo a cycle of
561 progradation and retrogradation. However, when resilience is exceeded the balance between
562 these two phases is upset and may cause the system to "flip" to an alternative state (Fung et
563 al., 2011). An ecosystem's health can be defined in terms of system vigour, organisation and
564 resilience (Costanza, 1992). Whilst existing ecological studies attest to retrograding *S.*
565 *alveolata* bioconstruction vigour and organisation (Dubois et al., 2002; Jones et al., 2018),
566 further work is needed to quantify their resilience.
567
568 The European Habitats Directive, and the overarching Marine Strategy Framework Directive
569 (MSFD, 2008/56/EC) give statutory significance to the definition of a "reef". Crucially, the
570 distinction between what is considered as "reef" and what is not, is imprecise. Which *S.*
571 *alveolata* bioconstruction type, and which progradation/retrogradation phase criteria are of
572 conservation value, are yet to be defined. This has far-reaching implications in terms of
573 environmental management and conservation. The offset between the physical state of the
574 bioconstruction and the physiological state of the worm makes it vital to analyse the two
575 together, and to understand the natural history and physiology of one's study organisms when
576 using biochemical indicators for ecological studies (Dahlhoff, 2004). Seasonal, broad-scale
577 studies are therefore warranted to further our understanding of the link between the engineer
578 species and the engineered habitat it creates.

579 **5. Conclusion**

580 The metabolic enzymes citrate synthase and superoxide dismutase, when analysed in
581 conjunction with the polar lipid fatty acid 20:4n-6, serve as stress markers for the

physiological state of *S. alveolata*. Once seasonal and inter-site variation have been addressed, these three biochemical indicators could be looked at in priority, in concurrence with *S. alveolata* bioconstruction physical criteria, as part of a European-wide monitoring protocol. Member states will be updating their MSFD monitoring programme and measures in the coming years and are being urged by the European Commission for a coherent and coordinated approach within and between marine regions. Although exploratory biochemical analyses time and money costs are high, they greatly diminish with subsequent routine screening of a few key indicators. Furthermore, few worm specimens are needed and their collection is substantially less destructive than the sediment core sampling typically used for obtaining biodiversity metrics. Whereas physical bioconstruction parameters are site-specific, biochemical indicators are applicable across a species range and therefore serve as large-scale, quantitative metrics, provided that sex and size are controlled for.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors thank C. Quéré and V. Le Roy for technical assistance with enzyme assays and fatty acid analysis. C. Cordier, S. Androuin and M. Marzloff are gratefully acknowledged for help with the figures. AC was funded by a PhD grant from Ifremer. The authors would like to thank the two anonymous reviewers for their valuable comments and suggestions. This work was supported by the Total Foundation [Grant No. 1512 215 588/F, 2015] and the French National Program EC2CO-Biohefect/Dril REEHAB [Grant No. AO2016-99 3962, 2016].

References

Aebi, H., 1984. [13] Catalase in vitro, in: Methods in Enzymology. Elsevier, pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Abele, D., Puntarulo, S., 2004. Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 138, 405–415. https://doi.org/10.1016/j.cbpb.2004.05.013

Abelson, A., Nelson, P.A., Edgar, G.J., Shashar, N., Reed, D.C., Belmaker, J., Krause, G., Beck, M.W., Brokovich, E., France, R. and Gaines, S.D., 2016. Expanding marine protected areas to include degraded coral reefs. Conservation Biology, 30: 1182–1191. doi:10.1111/cobi.12722

Anthony, K., 2006. Enhanced energy status of corals on coastal, high-turbidity reefs. Marine Ecology Progress Series 319, 111–116. doi:10.3354/meps319111

Bartol, I.K., Mann, R., Luckenbach, M., 1999. Growth and mortality of oysters (Crassostrea virginica) on constructed intertidal reefs: effects of tidal height and substrate level. Journal of Experimental Marine Biology and Ecology 237, 157–184. https://doi.org/10.1016/S0022-0981(98)00175-0Berthelin, C., Kellner, K., Mathieu, M., 2000. Storage metabolism in the Pacific oyster (*Crassostrea gigas*) in relation to summer mortalities and reproductive cycle (West Coast of France). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 125, 359–369. https://doi.org/10.1016/S0305-0491(99)00187-X

Bertness, M.D., Leonard, G.H., Levine, J.M., Schmidt, P.R., Ingraham, A.O., 1999. Testing the relative contribution of positive and negative interactions in rocky intertidal communities. Ecology 80, 2711–2726. https://doi.org/10.1890/0012-9658(1999)080[2711:TTRCOP]2.0.CO;2

Bligh, E.G., Dyer, W.J., 1959. A Rapid Method of Total Lipid Extraction and Purification. Canadian Journal of Biochemistry and Physiology 37, 911–917. https://doi.org/10.1139/o59-099

Box, G.E.P., Cox, D.R., 1964. An Analysis of Transformations. Journal of the Royal Statistical Society. Series B (Methodological) 26, 211–252.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a Metabolic Theory of Ecology. Ecology 85, 1771–1789. https://doi.org/10.1890/03-9000

Childress, J.J., Somero, G.N., 1979. Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. Marine Biology 52, 273–283. https://doi.org/10.1007/BF00398141

Connell, J.H., 1983. On the Prevalence and Relative Importance of Interspecific Competition: Evidence from Field Experiments. The American Naturalist 122, 661–696. https://doi.org/10.1086/284165

Costanza, R., 1992. Towards an operational definition of ecosystem health, in: Ecosystem Health: New Goals for Environmental Management. Island Press, pp. 239–256.

Culloty, S.C., Favier, E., Ní Riada, M., Ramsay, N.F., O'Riordan, R.M., 2010. Reproduction of the biogenic reef-forming honeycomb worm *Sabellaria alveolata* in Ireland. Journal of the Marine Biological Association of the United Kingdom 90, 503. https://doi.org/10.1017/S0025315409990932

Cunningham, P.N., 1984. The geographical distribution of *Sabellaria alveolata* (L.) in England, Wales and Scotland, with investigations into the community structure of, and the effects of trampling on *Sabellaria alveolata* colonies. (N.C.C. Contract No. HF3/11/22.).

Dalsgaard J., St. John, M., Kattner, G., Muller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46:225-340. doi: 10.1016/S0065-2881(03)46005-7

Dahlhoff, E.P., Stillman, J.H., Menge, B.A., 2002. Physiological community ecology: variation in metabolic activity of ecologically important rocky intertidal invertebrates along environmental gradients. Integrative and comparative biology 42, 862–871.

Dahlhoff, E.P., 2004. Biochemical Indicators of Stress and Metabolism: Applications for Marine Ecological Studies. Annual Review of Physiology 66, 183–207. https://doi.org/10.1146/annurev.physiol.66.032102.114509

Delaporte, M., 2003. Effect of a mono-specific algal diet on immune functions in two bivalve species - *Crassostrea gigas* and *Ruditapes philippinarum*. Journal of Experimental Biology 206, 3053–3064. https://doi.org/10.1242/jeb.00518

Desroy, N., Dubois, S.F., Fournier, J., Ricquiers, L., Le Mao, P., Guerin, L., Gerla, D., Rougerie, M., Legendre, A., 2011. The conservation status of *Sabellaria alveolata* (L.) (Polychaeta: Sabellariidae) reefs in the Bay of Mont-Saint-Michel. Aquatic Conservation: Marine and Freshwater Ecosystems 21, 462–471. doi:10.1002/aqc.1206

Dowd, W.W., Felton, C.A., Heymann, H.M., Kost, L.E., Somero, G.N., 2013. Food availability, more than body temperature, drives correlated shifts in ATP-generating and antioxidant enzyme capacities in a population of intertidal mussels (*Mytilus californianus*). Journal of Experimental Marine Biology and Ecology 449, 171-185.

Dubois, S., Retière, C., Olivier, F., 2002. Biodiversity associated with *Sabellaria alveolata* (Polychaeta: Sabellariidae) reefs: effects of human disturbances. Journal of the Marine Biological Association of the UK 82, 817–826. doi:10.1017/S0025315402006185

Dubois, S., Commito, J.A., Olivier, F., Retière, C., 2006. Effects of epibionts on *Sabellaria alveolata* (L.) biogenic reefs and their associated fauna in the Bay of Mont Saint-Michel. Estuarine, Coastal and Shelf Science 68, 635–646. https://doi.org/10.1016/j.ecss.2006.03.010

Dubois, S., Orvain, F., Marin-Léal, J., Ropert, M., Lefebvre, S., 2007. Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. Marine Ecology Progress Series 336, 151–160. https://doi.org/10.3354/meps336151

Dubois, S.F., Colombo, F., 2014. How picky can you be? Temporal variations in trophic niches of co-occurring suspension-feeding species. Food Webs 1, 1–9. https://doi.org/10.1016/j.fooweb.2014.07.001

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28, 350–356. https://doi.org/10.1021/ac60111a017

688 Ellis, R.P., Parry, H., Spicer, J.I., Hutchinson, T.H., Pipe, R.K., Widdicombe, S., 2011.
689 Immunological function in marine invertebrates: Responses to environmental
690 perturbation. Fish & Shellfish Immunology 30, 1209–1222.
691 https://doi.org/10.1016/j.fsi.2011.03.017

692 Ferron, A., Leggett, W.C., 1994. An Appraisal of Condition Measures for Marine Fish
693 Larvae, in: Advances in Marine Biology. Elsevier, pp. 217–303.
694 https://doi.org/10.1016/S0065-2881(08)60064-4

695 Firth, L.B., Williams, G.A., 2009. The influence of multiple environmental stressors on the
696 limpet *Cellana toreuma* during the summer monsoon season in Hong Kong. Journal of
697 Experimental Marine Biology and Ecology 375, 70–75.
698 https://doi.org/10.1016/j.jembe.2009.05.011

699 Firth, L.B., Knights, A.M., Bell, S.S., 2011. Air temperature and winter mortality:
700 Implications for the persistence of the invasive mussel, Perna viridis in the intertidal
701 zone of the south-eastern United States. Journal of Experimental Marine Biology and
702 Ecology 400, 250–256. https://doi.org/10.1016/j.jembe.2011.02.007

703 Firth, L.B., Mieszkowska, N., Grant, L.M., Bush, L.E., Davies, A.J., Frost, M.T., Moschella,
704 P.S., Burrows, M.T., Cunningham, P.N., Dye, S.R., Hawkins, S.J., 2015. Historical
705 comparisons reveal multiple drivers of decadal change of an ecosystem engineer at the
706 range edge. Ecology and Evolution 5, 3210–3222.
707 https://doi.org/10.1002/ece3.1556Fraser, A.J., 1989. Triacylglycerol Content as a
708 Condition Index for Fish, Bivalve, and Crustacean Larvae. Canadian Journal of
709 Fisheries and Aquatic Sciences 46, 1868–1873. https://doi.org/10.1139/f89-235

710 Fuhrmann, M., Delisle, L., Petton, B., Corporeau, C., Pernet, F., 2018. Metabolism of the
711 Pacific oyster, *Crassostrea gigas* , is influenced by salinity and modulates survival to
712 the Ostreid herpesvirus OsHV-1. Biology Open 7, bio028134.
713 https://doi.org/10.1242/bio.028134

714 Fung, T., Seymour, R.M., Johnson, C.R., 2011. Alternative stable states and phase shifts in
715 coral reefs under anthropogenic stress. Ecology 92, 967–982.
716 https://doi.org/10.1890/10-0378.1

717 Gabbott, P.A., 1975. Storage cycles in marine bivalve molluscs: a hypothesis concerning the
718 relationship between glycogen metabolism and gametogenesis. Presented at the Ninth
719 European Marine Biology Symposium, Aberdeen University Press, Oban, pp. 191–211.

720 Gallager, S.M., Mann, R., Sasaki, G.C., 1986. Lipid as an index of growth and viability in
721 three species of bivalve larvae. Aquaculture 56, 81–103. https://doi.org/10.1016/0044-
722 8486(86)90020-7

723 Garcia-Esquivel, Z., Bricelj, V.M., Felbeck, H., 2002. Metabolic depression and whole-body
724 response to enforced starvation by *Crassostrea gigas* postlarvae. Comparative
725 Biochemistry and Physiology Part A: Molecular & Integrative Physiology 133, 63-77.
726
727 Goldberg, W.M., 2013. The biology of reefs and reef organisms. University of Chicago Press,
728 Chicago.

729 Gruet, Y., 1982. Recherches sur l'écologie des "récifs" d'hermelles édifiés par l'Annélide
730 Polychète *Sabellaria alveolata* (Linné). (Thèse Doctorat d'Etat). Nantes.

731 Gruet, Y., 1986. Spatio-temporal Changes of Sabellarian Reefs Built by the Sedentary
732 Polychaete *Sabellaria alveolata* (Linné). Marine Ecology 7, 303–319.
733 doi:10.1111/j.1439-0485.1986.tb00166.x
734 Haas, A.F., Guibert, M., Foerschner, A., Co, T., Calhoun, S., George, E., Hatay, M., Dinsdale,
735 E., Sandin, S.A., Smith, J.E., Vermeij, M.J.A., Felts, B., Dustan, P., Salamon, P.,
736 Rohwer, F., 2015. Can we measure beauty? Computational evaluation of coral reef
737 aesthetics. PeerJ 3, e1390. https://doi.org/10.7717/peerj.1390

738 Hattam, C., Atkins, J.P., Beaumont, N., Börger, T., Böhnke-Henrichs, A., Burdon, D., Groot,
739 R. de, Hoefnagel, E., Nunes, P.A.L.D., Piwowarczyk, J., Sastre, S., Austen, M.C., 2015.
740 Marine ecosystem services: Linking indicators to their classification. Ecological
741 Indicators 49, 61–75. https://doi.org/10.1016/j.ecolind.2014.09.026
742 Hazel, J.R., 1995. Thermal Adaptation in Biological Membranes: Is Homeoviscous
743 Adaptation the Explanation? Annual Review of Physiology 57, 19–42.
744 https://doi.org/10.1146/annurev.ph.57.030195.000315

745 Hazel, J.R, Williams, E.E., 1990. The role of alterations in membrane lipid composition in
746 enabling physiological adaptation of organisms to their physical environment. Progress
747 in Lipid Research 29, 167–227. https://doi.org/10.1016/0163-7827(90)90002-3

748 Helmuth, B., Harley, C.D.G., Halpin, P.M., O'Donnell, M., Hofmann, G.E., Blanchette, C.A.,
749 2002. Climate Change and Latitudinal Patterns of Intertidal Thermal Stress. Science
750 298, 1015–1017. https://doi.org/10.1126/science.1076814

751 Hendrick, V.J., Foster-Smith, R.L., 2006. *Sabellaria spinulosa* reef: a scoring system for
752 evaluating 'reefiness' in the context of the Habitats Directive. Journal of the Marine
753 Biological Association of the UK 86, 665. doi:10.1017/S0025315406013555
754 Hentschel, B.T., 1998. Spectrofluorometric quantification of neutral and polar lipids suggests
755 a food-related recruitment bottleneck for juveniles of a deposit-feeding polychaete
756 population. Limnology and Oceanography 43, 543–549.
757 https://doi.org/10.4319/lo.1998.43.3.0543

758 Hochachka, P.W., Somero, G.N., 2002. Biochemical adaptation: mechanism and process in
759 physiological evolution. Oxford University Press, New York.
760 Holt, T.J., Rees, E.I., Hawkins, S.J., Seed, R., 1998. Biogenic Reefs. An overview of dynamic
761 and sensitivity characteristics for conservation management of marine SACs. (No.
762 volume IX). Scottish Association for Marine Science (UK Marine SACs Project).
763 Hulbert, A.J., Turner, N., Storlien, L.H., Else, P.L., 2005. Dietary fats and membrane
764 function: implications for metabolism and disease. Biological Reviews 80, 155–169.
765 https://doi.org/10.1017/S1464793104006578

766 Johnson, C.R., 2009. Natural Length Scales of Ecological Systems: Applications at
767 Community and Ecosystem Levels. Ecology and Society 14. doi:10.5751/ES-02749-
768 140107
769 Jones, A.G., Dubois, S.F., Desroy, N., Fournier, J., 2018. Interplay between abiotic factors
770 and species assemblages mediated by the ecosystem engineer *Sabellaria alveolata*
771 (Annelida: Polychaeta). Estuarine, Coastal and Shelf Science 200, 1–18.
772 doi:10.1016/j.ecss.2017.10.001

773 Le Foll, C., Corporeau, C., Le Guen, V., Gouygou, J.-P., Berge, J.-P., Delarue, J., 2006.
774 Long-chain n-3 polyunsaturated fatty acids dissociate phosphorylation of Akt from
775 phosphatidylinositol 3'-kinase activity in rats. AJP: Endocrinology and Metabolism
776 292, E1223–E1230. https://doi.org/10.1152/ajpendo.00446.2006

777 Lefebvre, S., Marín Leal, J.C., Dubois, S., Orvain, F., Blin, J.-L., Bataillé, M.-P., Ourry, A.,
778 Galois, R., 2009. Seasonal dynamics of trophic relationships among co-occurring
779 suspension-feeders in two shellfish culture dominated ecosystems. Estuarine, Coastal
780 and Shelf Science 82, 415–425. https://doi.org/10.1016/j.ecss.2009.02.002

781 Lesser, M.P., 2006. Oxidative Stress in Marine Environments: Biochemistry and
782 Physiological Ecology. Annual Review of Physiology 68, 253–278.
783 https://doi.org/10.1146/annurev.physiol.68.040104.110001

784 Levoy, F., Anthony, E.J., Monfort, O., Larsonneur, C., 2000. The morphodynamics of
785 megatidal beaches in Normandy, France. Marine Geology 171, 39–59.
786 https://doi.org/10.1016/S0025-3227(00)00110-9

787 Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: just what is
788 the cost of immunity? Oikos 88, 87–98. https://doi.org/10.1034/j.1600-
789 0706.2000.880110.x

790 Lourenço, S., Roura, Á., Fernández-Reiriz, M.-J., Narciso, L., González, Á.F., 2017. Feeding
791 Relationship between *Octopus vulgaris* (Cuvier, 1797) Early Life-Cycle Stages and
792 Their Prey in the Western Iberian Upwelling System: Correlation of Reciprocal Lipid
793 and Fatty Acid Contents. Frontiers in Physiology 8.
794 https://doi.org/10.3389/fphys.2017.00467

795 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., 1951. Protein measurement
796 with the Folin phenol reagent. The Journal of Biological Chemistry 193, 265-75.
797 Lucas, A., Beninger, P.G., 1985. The use of physiological condition indices in marine bivalve
798 aquaculture. Aquaculture 44, 187–200. https://doi.org/10.1016/0044-8486(85)90243-1

799 Marty, Y., Delaunay, F., Moal, J., Samain, J.-F., 1992. Changes in the fatty acid composition
800 of *Pecten maximus* (L.) during larval development. Journal of Experimental Marine
801 Biology and Ecology 163, 221–234. https://doi.org/10.1016/0022-0981(92)90051-B
802 Masuda, R., 2003. The critical role of docosahexaenoic acid in marine and terrestrial
803 ecosystems: from bacteria to human behavior, in: The Big Fish Bang. Proceedings of
804 the 26th Annual Larval Fish Conference. Howard I. Browman & Anne Berit Skiftesvik,
805 Os, Norway, pp. 249–256.
806 Metcalfe, L.D., Schmitz, A.A., 1961. The Rapid Preparation of Fatty Acid Esters for Gas
807 Chromatographic Analysis. Analytical Chemistry 33, 363–364.
808 https://doi.org/10.1021/ac60171a016
809 Miller, D.C., Geider, R.J. and MacIntyre, H.L., 1996. Microphytobenthos: the ecological role
810 of the "secret garden" of unvegetated, shallow-water marine habitats. II. Role in
811 sediment stability and shallow-water food webs. Estuaries, 19(2), 202-212.
812 Morrison, W. R. and Smith, L. M., 1964. Preparation of fatty acid methyl esters and
813 dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5,
814 600-8.
815 Moyes, C.D., 2003. Controlling muscle mitochondrial content. Journal of Experimental
816 Biology 206, 4385–4391. https://doi.org/10.1242/jeb.00699

817 Muir, A.P., Nunes, F.L.D., Dubois, S.F., Pernet, F., 2016. Lipid remodelling in the reef-
818 building honeycomb worm, *Sabellaria alveolata*, reflects acclimation and local
819 adaptation to temperature. Scientific Reports 6, 35669. doi:10.1038/srep35669

820 Osada, M., Nishikawa, M., Nomura, T., 1989. Involvement of prostaglandins in the spawning
821 of the scallop, *Patinopecten yessoensis*. Comparative Biochemistry and Physiology. C:
822 Comparative Pharmacology 94, 595–601.
823
824 Pawlik, J.R., 1986. Chemical induction of larval settlement and metamorphosis in the reef-
825 building tube worm *Phragmatopoma californica* (Sabellariidae: Polychaeta). Marine
826 Biology 91, 59–68. https://doi.org/10.1007/BF00397571

827 Pernet, F., Lagarde, F., Jeannée, N., Daigle, G., Barret, J., Le Gall, P., Quere, C., D'orbcastel,
828 E.R., 2014. Spatial and Temporal Dynamics of Mass Mortalities in Oysters Is
829 Influenced by Energetic Reserves and Food Quality. PLoS ONE 9, e88469.
830 https://doi.org/10.1371/journal.pone.0088469
831 Pernet, F., Tremblay, R., Comeau, L., Guderley, H., 2007. Temperature adaptation in two
832 bivalve species from different thermal habitats: energetics and remodelling of
833 membrane lipids. Journal of Experimental Biology 210, 2999–3014.
834 https://doi.org/10.1242/jeb.006007

835 Pernet, B., Jaeckle, W.B., 2004. Size and Organic Content of Eggs of Marine Annelids, and
836 the Underestimation of Egg Energy Content by Dichromate Oxidation. The Biological
837 Bulletin 207, 67–71. https://doi.org/10.2307/1543629

838 Plicanti, A., Domínguez, R., Dubois, S.F., Bertocci, I., 2016. Human impacts on biogenic
839 habitats: Effects of experimental trampling on *Sabellaria alveolata* (Linnaeus, 1767)
840 reefs. Journal of Experimental Marine Biology and Ecology 478, 34–44.
841 https://doi.org/10.1016/j.jembe.2016.02.001

842 Porras, R., Bataller, J.V., Murgui, E., Torregrosa, M.T., 1996. Trophic Structure and
843 Community Composition of Polychaetes Inhabiting Some *Sabellaria alveolata* (L.)
844 Reefs Along the Valencia Gulf Coast, Western Mediterranean. Marine Ecology 17,
845 583–602. https://doi.org/10.1111/j.1439-0485.1996.tb00419.x

846 Rivest, E.B., Chen, C.-S., Fan, T.-Y., Li, H.-H., Hofmann, G.E., 2017. Lipid consumption in
847 coral larvae differs among sites: a consideration of environmental history in a global
848 ocean change scenario. Proceedings of the Royal Society B: Biological Sciences 284,
849 20162825. https://doi.org/10.1098/rspb.2016.2825

850 Rivest, E.B., Hofmann, G.E., 2014. Responses of the Metabolism of the Larvae of
851 *Pocillopora damicornis* to Ocean Acidification and Warming. PLoS ONE 9, e96172.
852 https://doi.org/10.1371/journal.pone.0096172

853 Scheer, B.T., 1969. Carbohydrates and carbohydrate metabolism: Annelida, Sipuncula,
854 Echiura. *Chemical zoology*, *4*.

855 Schimmenti, E., Musco, L., Lo Brutto S., Mikac, B., Nygren, A., Badalamenti, F., 2015 « A
856 Mediterranean Record of *Eulalia Ornata* (Annelida: Phyllodocidae) Corroborating Its
857 Fidelity Link with the *Sabellaria Alveolata*-Reef Habitat ». Mediterranean Marine
858 Science 17(2): 359-70.
859

Soudant, P., Van Ryckeghem, K., Marty, Y., Moal, J., Samain, J.F., Sorgeloos, P., 1999. Comparison of the lipid class and fatty acid composition between a reproductive cycle in nature and a standard hatchery conditioning of the Pacific Oyster *Crassostrea gigas*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 123, 209–222. https://doi.org/10.1016/S0305-0491(99)00063-2

Tessier, B., 1993. Upper intertidal rhythmites in the Mont-Saint-Michel Bay (NW France): perspectives for paleoreconstruction. Marine Geology 110, 355–367.

Tremblay, R., Olivier, F., Bourget, E., Rittschof, D., 2007. Physiological condition of *Balanus amphitrite* cyprid larvae determines habitat selection success. Marine Ecology Progress Series 340, 1–8.

Wilson, D. 1968. The settlement behaviour of the larvae of *Sabellaria alveolata* (L.). Journal of the Marine Biological Association of the United Kingdom, 50, 1-45.

Wilson, D.P., 1971. *Sabellaria* Colonies At Duckpool, North Cornwall, 1961–1970. Journal of the Marine Biological Association of the United Kingdom 51, 509. https://doi.org/10.1017/S002531540001496X

Winder, M., Carstensen, J., Galloway, A.W.E., Jakobsen, H.H., Cloern, J.E., 2017. The land-sea interface: A source of high-quality phytoplankton to support secondary production: Nutritious phytoplankton support coastal systems. Limnology and Oceanography 62, S258–S271. https://doi.org/10.1002/lno.10650

Figure legends

Figure 1 Conceptual diagram of a) the different bioconstruction Types of *S. alveolata* (adapted from Gruet, 1986). Progradation and Retrogradation, as defined in b), are represented by full versus hatched arrows respectively. Note that a bioconstruction can cycle between the two Phases – as represented by the circular arrows – within the same bioconstruction Type. Images © Ifremer.

Figure 2 Map of the study site with the twelve sampling points. V=Veneer, HR= Retrograding Hummock, HPHs=Prograding Hummock High Shore, HPLs=Prograding Hummock Low Shore.

892 **Figure 3** Organic macromolecules in the honeycomb worm *Sabellaria alveolata* as a function
893 of bioconstruction type, bioconstruction phase and shore level (a-c, n=59), worm mass (d-f,
894 n=59) and sex (g-i n=8 (5 female, 3 male)). All y axis units are $mg.g^{-1}$ tissue. V=Veneer,
895 H=Hummock, HR=Retrograding Hummock, HP=Prograding Hummock, HPHs=Prograding
896 Hummock High shore, HPLs=Prograding Hummock Low shore. Carbohydrate statistical test
897 results are log-transformed. The letters "a" and "b" represent a significant difference in the
898 contrast test ($p=0.002$). * = $p \leq 0.05$.

899

900 **Figure 4** Polar lipids in the honeycomb worm *Sabellaria alveolata* as a function of
901 bioconstruction type, bioconstruction phase and shore level (a-d, n=59), worm mass (e-h,
902 n=59) and sex (i-l, n=8 (5 female, 3 male)). All y axis units are a percentage of total
903 phospholipids. Note that the y axis scale varies between fatty acids. V=Veneer, H=Hummock,
904 HR=Retrograding Hummock, HP=Prograding Hummock, HPHs=Prograding Hummock High
905 shore, HPLs=Prograding Hummock Low shore. 22:6n-3 statistical test results are log-
906 transformed. The letters "a" and "b" represent a significant difference in the bioconstruction
907 type contrast test ($p=0.009$), and the letters "c" and "d" in the bioconstruction type contrast
908 test ($p=0.024$). * = $p \leq 0.05$.

909

910 **Figure 5** Neutral lipids in the honeycomb worm *Sabellaria alveolata* as a function of
911 bioconstruction type, bioconstruction phase and shore level (a-f, n=59), worm mass (g-k,
912 n=59) and sex (m-r, n=8 (5 female, 3 male)). All y axis units are a percentage of total neutral
913 lipids. Note that the y axis scale varies between fatty acids. Diatoms=16:1n-7/16:0;
914 Dinoflagellates=22:6n-3/20:5n-3; Carnivory=18:1n-9/18:1n-7; Bacterial = 15:0 + 17:0 +

915 branched fatty acids; Terrestrial= 18:2n-6 + 18:3n-3; Freshness= PUFA/SFA. V=Veneer,
916 H=Hummock, HR=Retrograding Hummock, HP=Prograding Hummock, HPHs=Prograding
917 Hummock High shore, HPLs=Prograding Hummock Low shore. * = p≤0.05

918 **Figure 6** Enzyme levels in the honeycomb worm *Sabellaria alveolata* as a function of
919 bioconstruction type, bioconstruction phase and shore level (a-c, n=59), worm mass (d-f,
920 n=59) and sex (g-I, n=8 (5 female, 3 male)). CS y axis units are micro Units $mU.mg^{-1}$) of
921 protein, whereas CAT and SOD units are $U.mg^{-1}$ of protein. CS= Citrate synthase,
922 CAT=Catalase, SOD=Superoxide dismutase. V=Veneer, H=Hummock, HR=Retrograding
923 Hummock, HP=Prograding Hummock, HPHs=Prograding Hummock High shore,
924 HPLs=Prograding Hummock Low shore. All statistical test results are log-transformed. The
925 letters "a" and "b" represent a significant difference in the contrast test (p=0.013). * = p≤0.05.

*Manuscript (revision changes marked)

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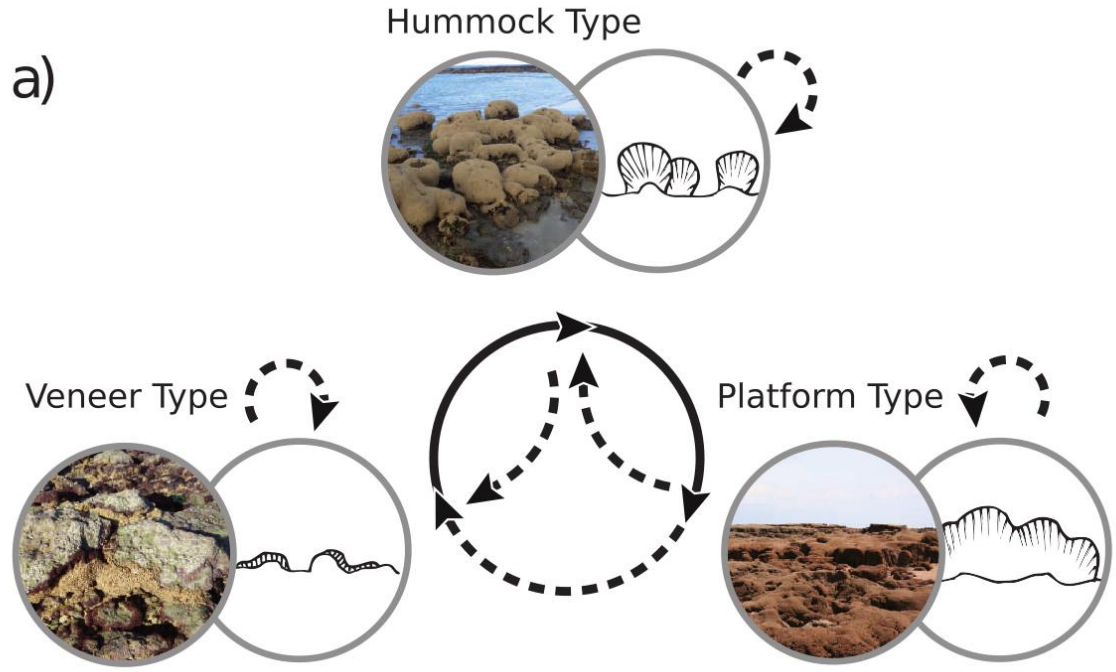
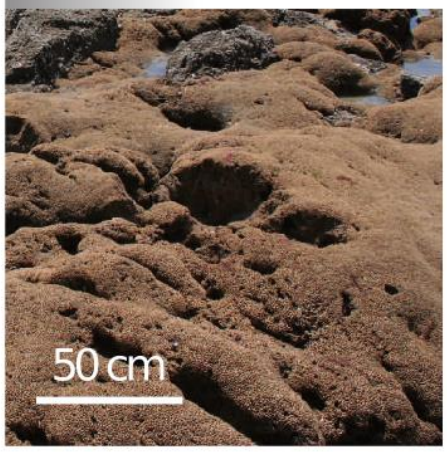
Figure 1



b)

Progradation Phase

- Dominated by occupied tubes identified by porches
- Limited/No epibionts
- Uniform aspect
- Presence of small tube openings (aperture <2.5 mm) indicating recruitment
- Surface of reef of similar colour to surrounding sediment



Retrogradation Phase

- Mosaic of occupied and unoccupied tubes (identified by lack of porches)
- Often featuring epibionts/biofilms
- Signs of reef erosion
- No visible small tube openings (aperture <2.5 mm)
- Surface of reef highly fragmented and dark brown to grey in colouration

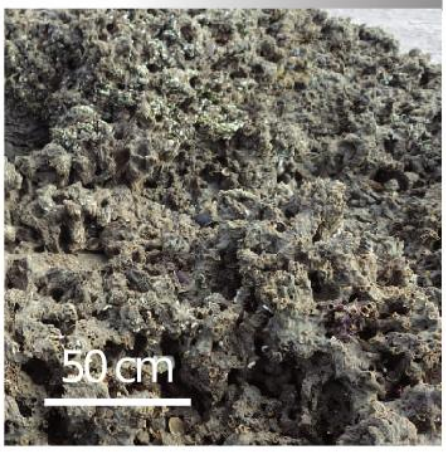


Figure 2

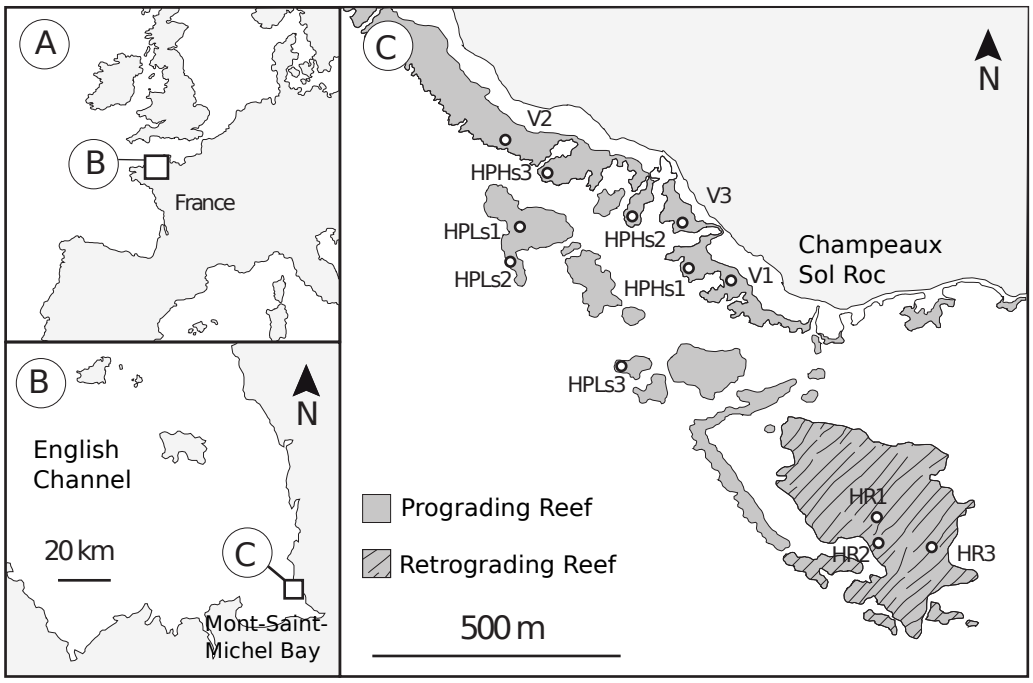

A
B
France
B
N
English
Channel
20 km
C
Mont-Saint-
Michel Bay
C
N
V2
HPHs3
V3
HPLs1
HPHs2
Champeaux
Sol Roc
HPLs2
HPHs1
V1
HPLs3
Prograding Reef
Retrograding Reef
500 m
HR1
HR2
HR3

Figure 3

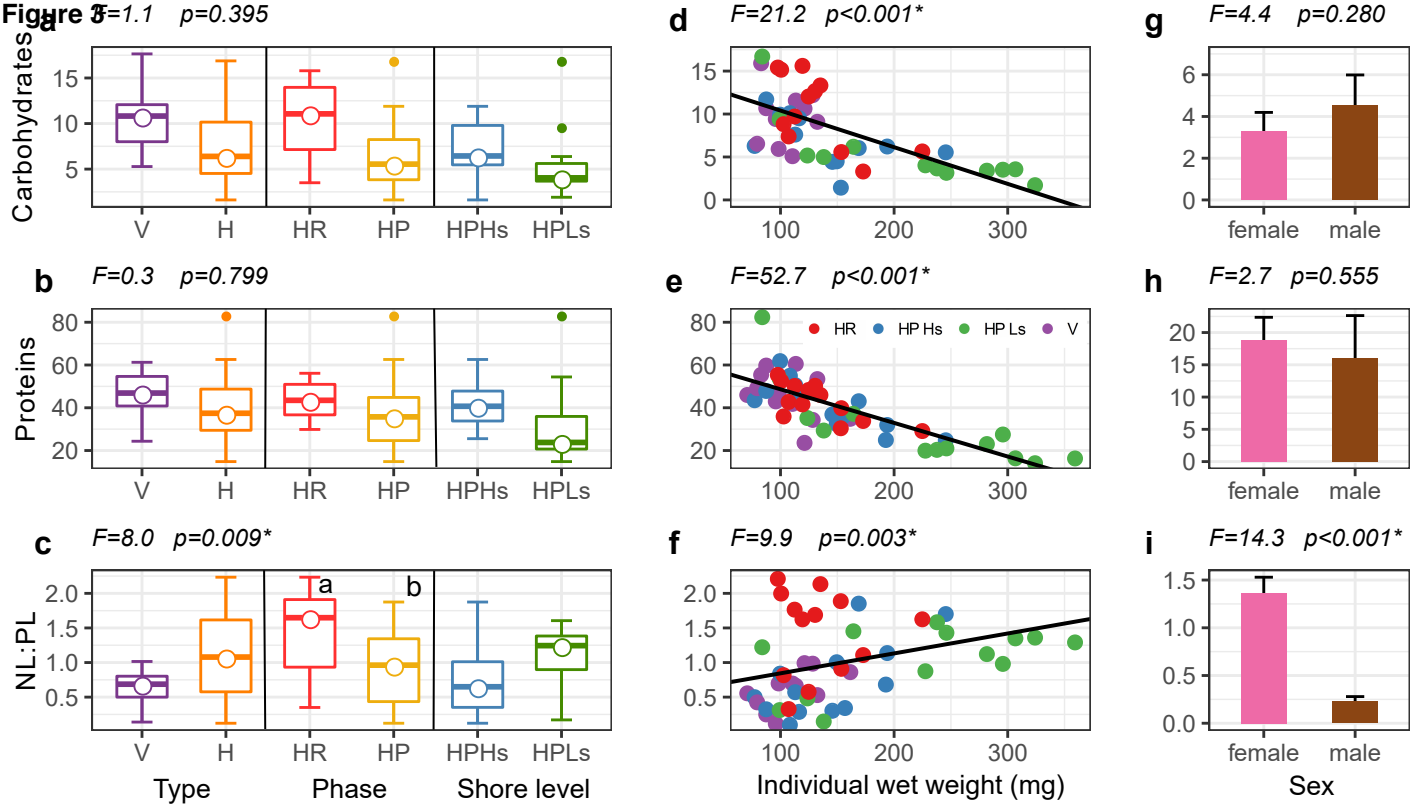
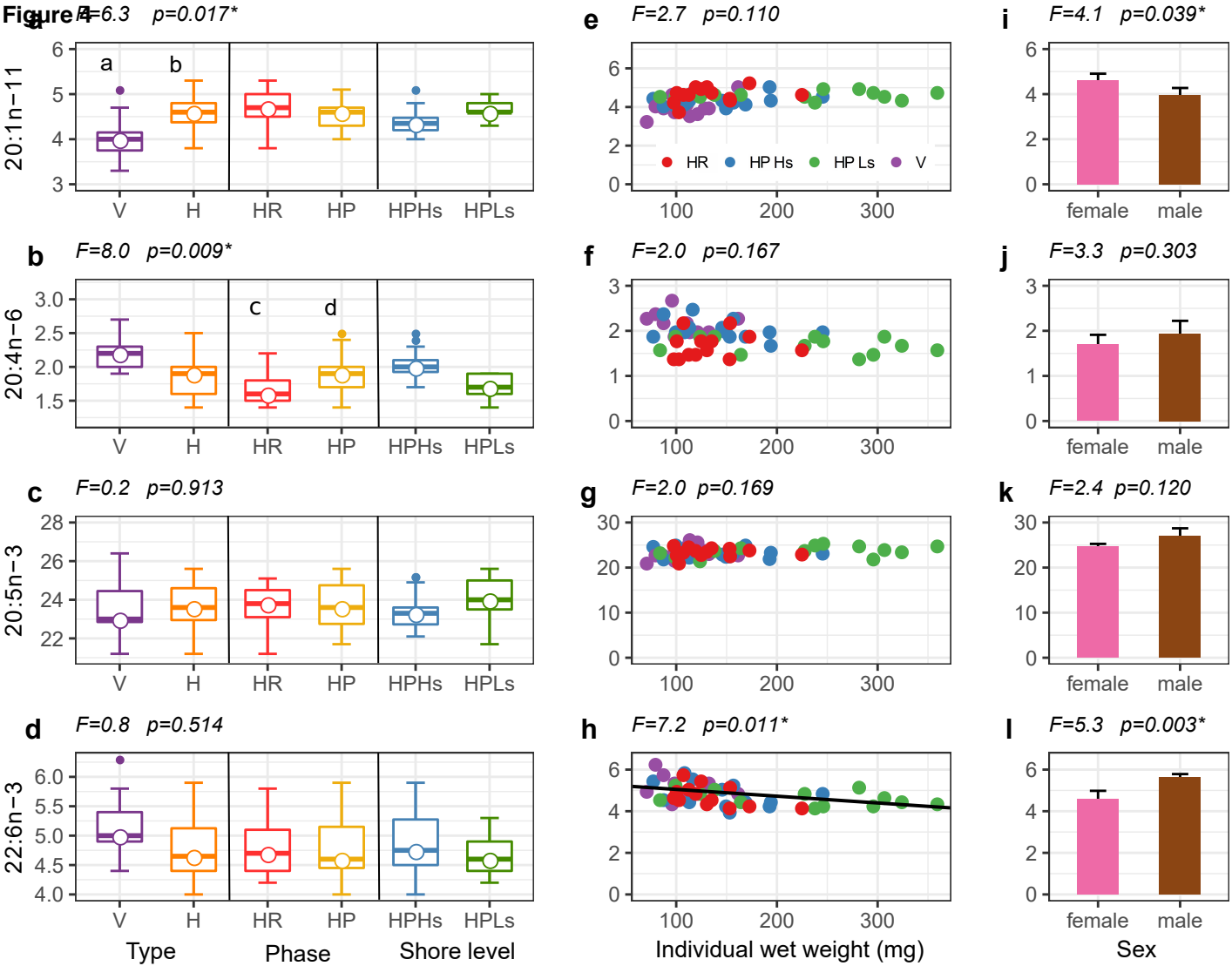

a
F=1.1 p=0.395
Carbohydrates
V
H
HR
HP
HPHs
HPLs
b
F=0.3 p=0.799
Proteins
c
F=8.0 p=0.009*
NL:PL
a
b
Type
Phase
Shore level
d
F=21.2 p<0.001*
e
F=52.7 p<0.001*
HR
HP Hs
HP Ls
V
f
F=9.9 p=0.003*
Individual wet weight (mg)
g
F=4.4 p=0.280
female
male
h
F=2.7 p=0.555
i
F=14.3 p<0.001*
Sex

Figure 4


a
F=6.3 p=0.017*
20:1n−11
a
b
V
H
HR
HP
HPHs
HPLs
b
F=8.0 p=0.009*
20:4n−6
c
d
c
F=0.2 p=0.913
20:5n−3
d
F=0.8 p=0.514
22:6n−3
Type
Phase
Shore level
e
F=2.7 p=0.110
HR
HP Hs
HP Ls
V
f
F=2.0 p=0.167
g
F=2.0 p=0.169
h
F=7.2 p=0.011*
Individual wet weight (mg)
i
F=4.1 p=0.039*
female
male
j
F=3.3 p=0.303
k
F=2.4 p=0.120
l
F=5.3 p=0.003*
Sex

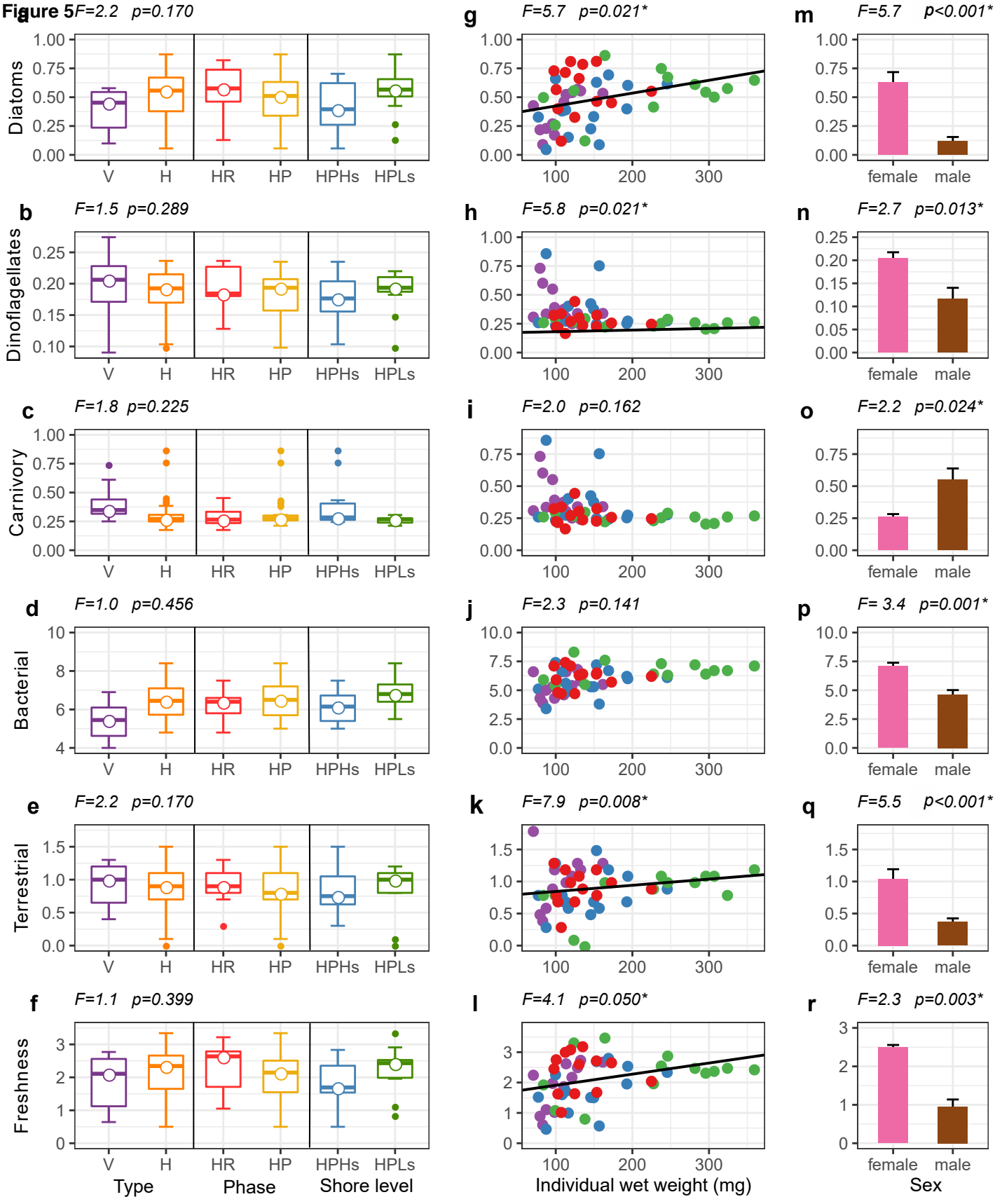
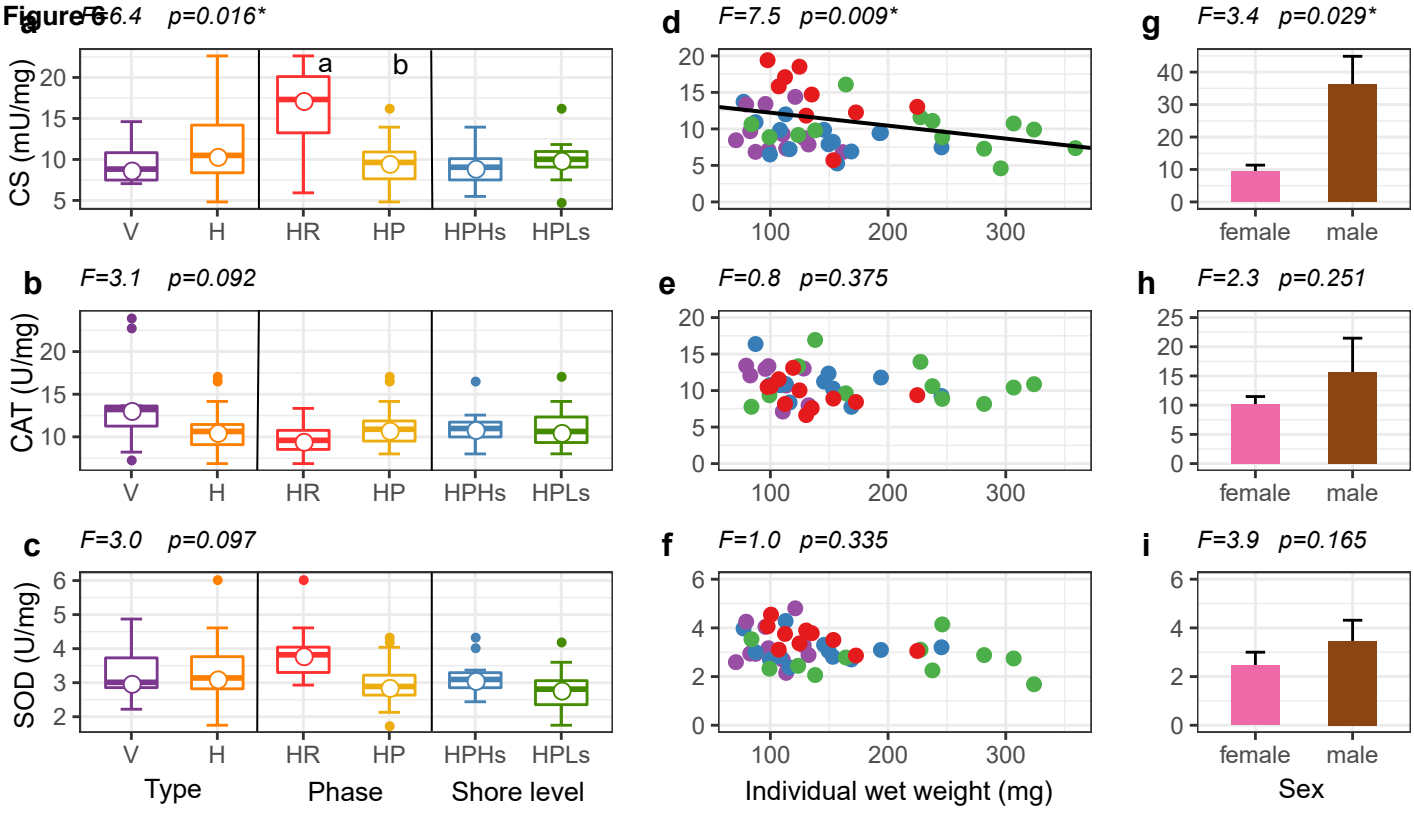


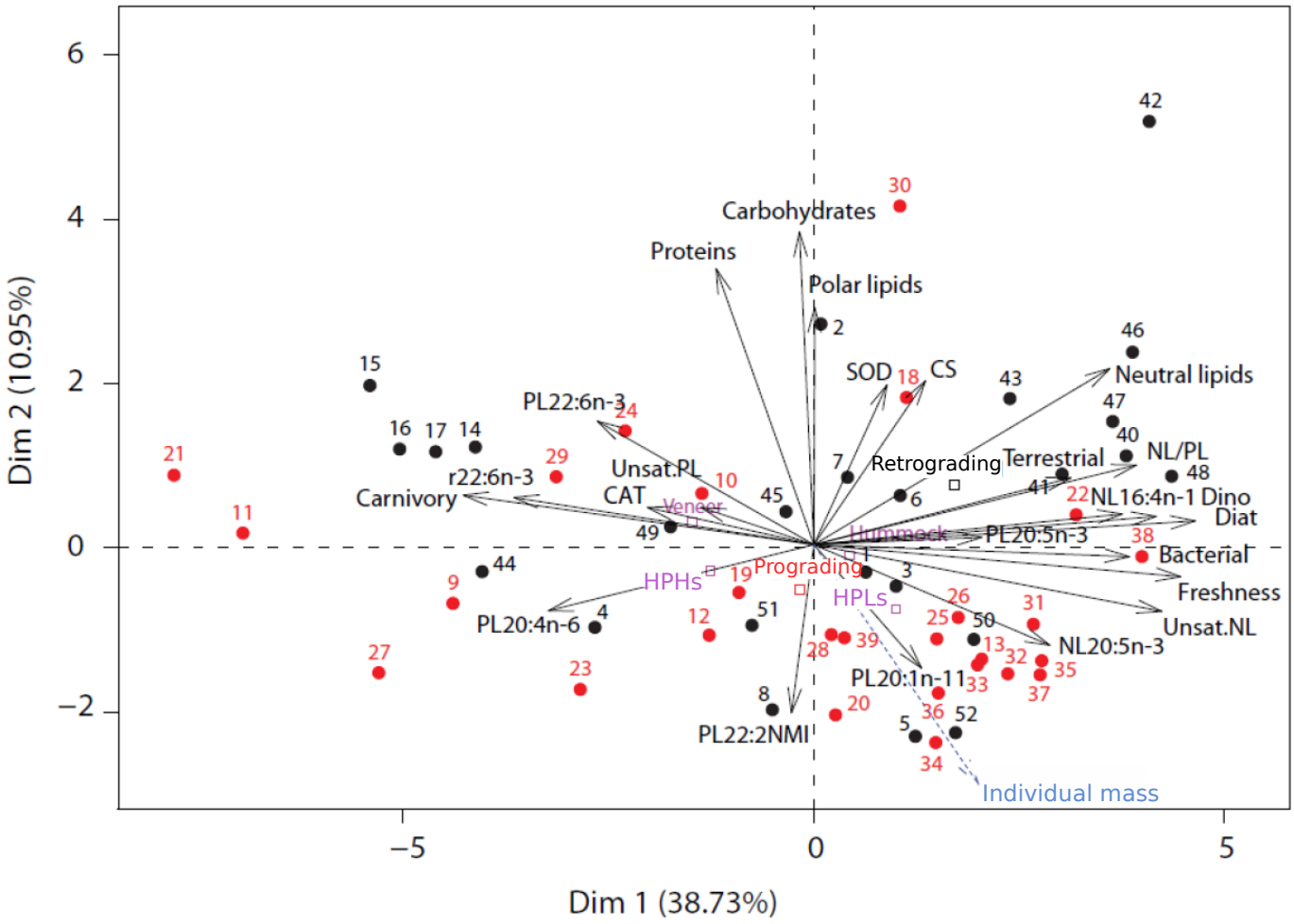
Figure 5
a
F=2.2 p=0.170
Diatoms
b
F=1.5 p=0.289
Dinoflagellates
c
F=1.8 p=0.225
Carnivory
d
F=1.0 p=0.456
Bacterial
e
F=2.2 p=0.170
Terrestrial
f
F=1.1 p=0.399
Freshness
V
H
HR
HP
HPHs
HPLs
Type
Phase
Shore level
g
F=5.7 p=0.021*
h
F=5.8 p=0.021*
i
F=2.0 p=0.162
j
F=2.3 p=0.141
k
F=7.9 p=0.008*
l
F=4.1 p=0.050*
Individual wet weight (mg)
m
F=5.7 p<0.001*
n
F=2.7 p=0.013*
o
F=2.2 p=0.024*
p
F= 3.4 p=0.001*
q
F=5.5 p<0.001*
r
F=2.3 p=0.003*
female
male
Sex

Figure 6


a
F=6.4 p=0.016*
CS (mU/mg)
a
b
V
H
HR
HP
HPHs
HPLs
d
F=7.5 p=0.009*
g
F=3.4 p=0.029*
female
male
b
F=3.1 p=0.092
CAT (U/mg)
e
F=0.8 p=0.375
h
F=2.3 p=0.251
c
F=3.0 p=0.097
SOD (U/mg)
f
F=1.0 p=0.335
i
F=3.9 p=0.165
Type
Phase
Shore level
Individual wet weight (mg)
Sex

S1_PCA

Click here to download Supplementary Material: File_S1_PCA.pdf



Principal Component Analysis (PCA) biplot of 52 female *Sabellaria alveolata* individuals and 26 biochemical variables. Individual mass is considered as an illustrative quantitative variable, and reef type and phase are considered as illustrative qualitative variables. The first two dimensions of the PCA express 49.68% of the total dataset inertia. Individuals are coloured after their category for the variable "Reef Phase": progradation phase individuals are in red, retrogradation phase individuals are in black. PCA calculated using the PCA function of the package FactoMineR (Lê et al., 2008).

Lê, S., Josse, J. & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*. **25(1)**. pp. 1-18