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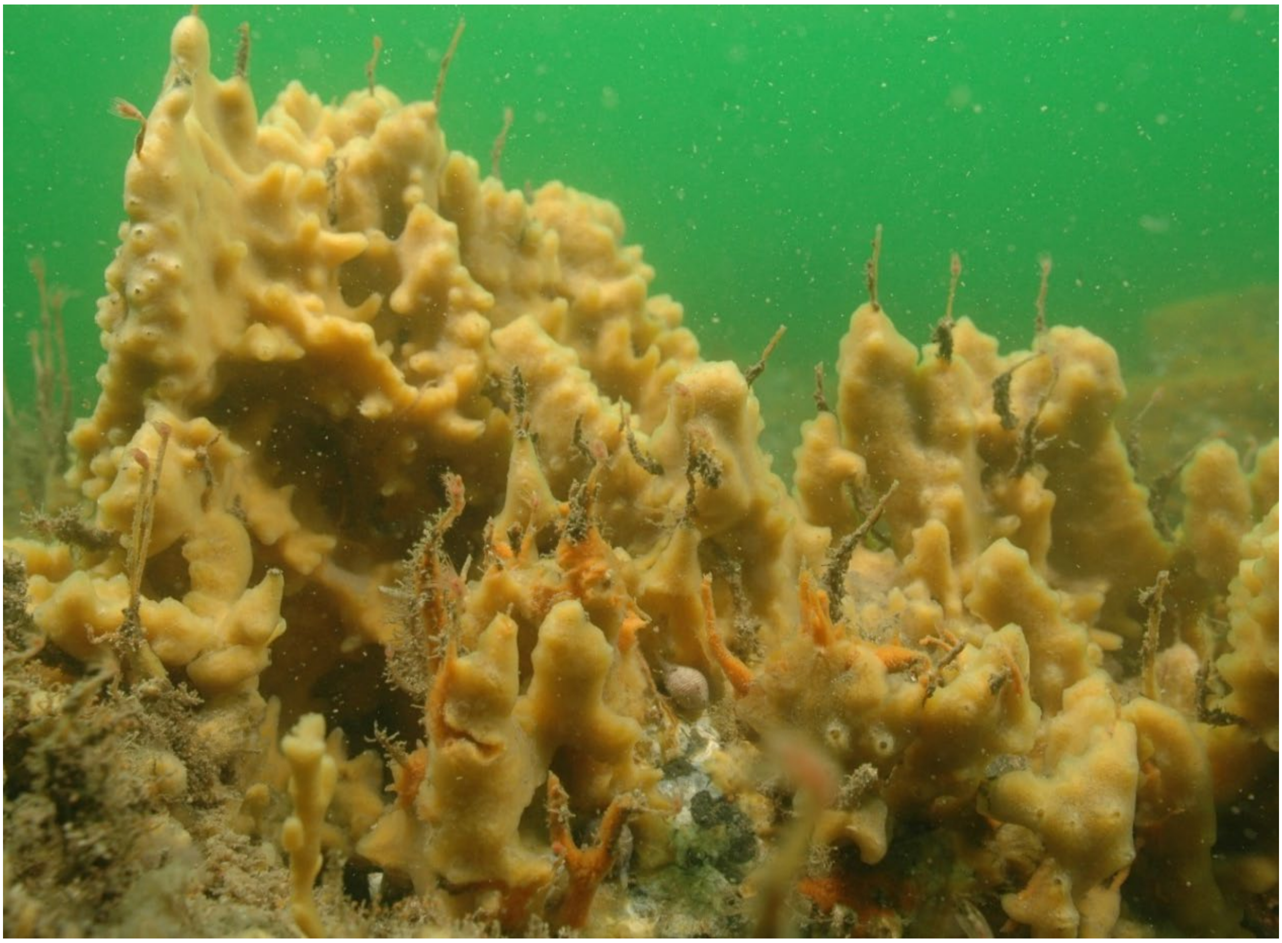
Investigation into the decline of the sponge community in the Menai Strait & Conwy Bay Special Area of Conservation



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Contents

About Natural Resources Wales 2

Evidence at Natural Resources Wales 2

Recommended citation for this volume: 3

Contents 4

List of figures 7

List of tables 10

Crynodeb gweithredol 11

Executive summary 12

General introduction 14

- Background to sponges (*Porifera*) 14
- Menai Strait and Conwy Bay (MS&CB) SAC sponge community 16
- Objectives of this investigation 17
- Monitoring and current status 17
  - Coleg Normal 18
  - Nelson's Column 22
  - Warrior 25
  - NN Marine Nature Conservation Review (MNCR) surveys 27
- Pressures assessment methodology 30

Organic enrichment 32

- Sensitivity of species 32
- Evidence of organic enrichment at Coleg Normal 33
- Pressure assessment scores 40

Aquaculture 40

- Sensitivity of species 41
- Evidence of aquaculture impacts at Coleg Normal 42
- Pressure assessment scores 45

Ocean warming 45

- Sensitivity of species 46

- Evidence of global warming at Coleg Normal 48
- Pressure assessment scores 50

Nutrient enrichment (i.e. nitrogen, phosphorus & silicon) 51

- Sensitivity of species 51
- Evidence of nutrient enrichment at Coleg Normal 52
- Pressure assessment scores 55

Changes in suspended solids (water clarity) 56

- Sensitivity of species 56
- Evidence of changes in suspended solids at Coleg Normal 57
- Pressure assessment scores 60

Smothering and siltation rate changes (Light) 60

- Sensitivity of species 61
- Evidence of smothering and siltation rate changes at Coleg Normal 62
- Pressure assessment scores 62

Introduction of microbial pathogens 63

- Sensitivity of species 63
- Evidence of introduction of microbial pathogens at Coleg Normal 64
- Pressure assessment scores 67

Abrasion/disturbance of the substrate on the surface of the seabed 68

- Sensitivity of species 68
- Evidence of Abrasion/disturbance of the substrate on the surface of the seabed at Coleg Normal 69
- Pressure assessment scores 70

Synthetic compound contamination (incl. pesticides, antifoulants, pharmaceuticals). 70

- Sensitivity of species 70
- Evidence of synthetic compound contamination (incl. pesticides, antifoulants, pharmaceuticals) at Coleg Normal 71
- Pressure assessment scores 72

Wave exposure changes 73

- Sensitivity of species 73
- Evidence of wave exposure changes at Coleg Normal 74
- Pressure assessment scores 76

Introduction or spread of invasive non-native species (INNS) 77
  Sensitivity of species 77
  Evidence of introduction or spread of invasive non-native species at Coleg Normal 78
  Pressure assessment scores 78
Pressures summary 79
Discussion 80
  Importance and decline of the Menai Strait and Conwy Bay (MS&CB) SAC sponge community 80
  Potential drivers of the decline in the sponge community at Coleg Normal 80
  Evaluating drivers of sponge decline at Coleg Normal 81
  Management Considerations 83
  Ongoing monitoring 84
  Knowledge gaps and research needs 84
References 86
Appendices 102
  A. Sponge microbiome report 102
  Data Archive Appendix 135

List of figures

Figure 1: The sponge holobiont 15

Figure 2: Map of the designated features of the Menai Strait and Conwy Bay SAC (taken from Wynter et al. 2025) 16

Figure 3: Location of reef monitoring sites and the feature monitored within the MS&CB SAC (Irving & Smith, 2013) 18

Figure 4: Divers using a wire probe to measure the thickness of encrusting sponges at the Coleg Normal monitoring site 18

Figure 5: *Haliclona oculata,* key erect sponge recorded at Coleg Normal monitoring site . 19

Figure 6: Percentage occurrence (%) (left axis) and mean size (cm) (right axis) of the sponge *Haliclona oculata* along Coleg Normal’s 30m southwest transect in the Menai Strait and Conwy Bay (MS&CB) SAC 20

Figure 7: Key encrusting sponge species recorded at Coleg Normal monitoring site 20

Figure 8: Percentage occurrence (%) (left axis) and mean thickness (cm) (right axis) of encrusting (crustose and cushion) sponges along Coleg Normal’s 30m southwest transect in the Menai Strait and Conwy Bay (MS&CB) SAC 21

Figure 9: Mean thickness of key encrusting (crustose and cushion) sponges (*Halichondria panicea*, *Amphilectus fucorum* and “crusts” including *Microciona spp.*) along Coleg’s Normal 30 southwest transect in the Menai Strait and Conwy Bay (MS&CB) SAC during the monitoring period 2004 – 2023. 21

Figure 10: Nelson’s column diving site in the Menai Strait 22

Figure 11: Decrease in sponge morphotypes at Nelson’s column in the Menai Strait and Conwy Bay (MS&CB) SAC during the monitoring period 2005 – 2023. 23

Figure 12: Percentage cover of *Amphilectus fucorum, Halichondria panicea* and *Haliclona oculata* at Nelson’s column in the Menai Strait and Conwy Bay (MS&CB) SAC during the monitoring period 2005 – 2023. 24

Figure 13: Location of the Pembrokeshire Marine SAC subtidal reef monitoring sites, including Warrior 25

Figure 14: Percentage occurrence and mean thickness of encrusting (cm) (crustose and cushion) sponges at Warrior in the Pembrokeshire Marine SAC during the monitoring period 2005 – 2023. 26

Figure 15: Percentage occurrence and mean thickness of encrusting sponges at Warrior in the Pembrokeshire Marine SAC during the monitoring period 2005 – 2023 26

Figure 16: Marine Nature Conservation Review (MNCR) dive survey sites in the central area of the Menai Strait 28

Figure 17: Major sewage discharges into the Menai Strait in 1991, prior to infrastructure upgrades, and proposed improvements planned for implementation by 2000 35

Figure 18: Left*:* Trends in *E.coli* concentrations in England and Wales from 1999 to 200836

Figure 19: Average faecal coliform levels in Menai Strait East shellfish flesh 37

Figure 20: Map of the Menai Strait showing locations of phased sewage infrastructure improvements under the UK's Asset Management Plans 38

Figure 21: Plots show trends in the occurrence and size of erect sponges (*Haliclona oculata*), and the occurrence and thickness of encrusting (crustose and cushion) sponges along the Coleg Normal transect between 2004 and 2023 39

Figure 22: The Menai Strait with shellfish waters protected area...................................... 43

Figure 23: Annual mussel harvest from the Menai Strait (East) fishery 43

Figure 24: Conductivity, temperature and depth (CTD) profile in the Menai Strait............ 44

Figure 25: Summary of effects of ocean warming on sponges 46

Figure 26: Changes in in-situ and modelled annual mean seawater temperature in the Menai Strait.. 48

Figure 27: The duration of marine heatwave events recorded in the western Irish Sea between 1997 and 2022 49

Figure 28: Sea surface temperature (SST) logger data 50

Figure 29: Trends in winter silicate, nitrate and phosphate measurements in the Menai (MS) and wider Irish Sea (Cypris station (CS)) between 1960 and 2002.......................... 53

Figure 30: Water Framework Directive Cycle 1 (2009) River catchment classification map, focussed on the Menai Strait 54

Figure 31: a) Time series of Secchi disk depth measurements in the Menai Strait from 1960-1999.. 58

Figure 32: Long term water quality monitoring in the Menai Strait 59

Figure 33: The size and abundance of *Haliclona oculata* at Warrior monitoring site in Pembrokeshire Marine (PM) SAC 64

Figure 34: *Haliclona oculata* at Nelson’s Column monitoring site in the Menai Strait and Conwy Bay (MS&CB) SAC 65

Figure 35: Sponge sampling locations 65

Figure 36: Changes in bacterial community between tissue type and sampling locations 66

Figure 37: The average annual concentration of pesticides (µg/l) recorded at “Gazelle - Anglesey Shore” long-term monitoring location in the Menai Strait between 1983 and 2010. 72

Figure 38: The decadal wind distribution at the Liverpool WaveNet Buoy for the periods 1990-2000 and 2000-2010 75

Figure 39: Decreasing trend (-) in the monthly-mean wave height in September (-x-) in Liverpool Bay from 1960 to 2100 76

List of tables

Table 1: Abundance data for the key sponge species (*H. panicea, A. fucorum, and H. oculata*) at the six surveyed sites in the Menai Strait in 1982, 2002, and 2023, along with relative changes between survey years 29

Table 2: Pressure impact scoring definitions 30

Table 3: Assessment for **organic enrichment** pressure 40

Table 4: Assessment for **aquaculture** pressure 45

Table 5: Assessment for **ocean warming** pressure 50

Table 6: Assessment for **nutrient enrichment** pressure 55

Table 7: Assessment for **changes in suspended solids** pressure 60

Table 8: Assessment for **smothering and siltation rate changes** pressure 63

Table 9: Assessment for **introduction of microbial pathogens** pressure 68

Table 10: Assessment for **abrasion/disturbance of the substrate on the surface of the seabed** pressure 70

Table 11: Assessment for **synthetic compound contamination** pressure 73

Table 12: Assessment for **wave exposure** pressure 76

Table 13: Assessment for **introduction or spread of invasive non-native species** pressure 78

Table 14: Pressures spreadsheet summary table 79

Crynodeb gweithredol

Mae'r rhaglen Rhwydweithiau Natur, a ariennir gan Lywodraeth Cymru, yn ceisio mynd i'r afael â'r argyfwng natur drwy wella bioamrywiaeth a chynyddu gwydnwch ar safleoedd gwarchodedig. O fewn Ardal Cadwraeth Arbennig (ACA) Y Fenai a Bae Conwy, gwelwyd dirywiad amlwg yn helaethrwydd y gymuned sbwng a ysgubir gan y llanw rhwng 2004 a 2009. Canfuwyd hyn mewn gwaith monitro hirdymor ar safle Coleg Normal. Mae hyn yn ddangosydd ar gyfer cyflwr nodwedd y riff creigiog, ac arweiniodd at statws ACA “anffafriol”. Adlewyrchir y dirywiad hwn yn y ffaith bod y rhywogaethau crawennu (ar ffurf clustogau a chramennau) wedi teneuo. Fe’i gwelir hefyd yn y gostyngiad yn uchder a chylchedd y rhywogaethau sy’n tyfu at i fyny (e.e. Haliclona oculata), ac yng nghanran is eu hamlder ar hyd y trawslun. Sbardunodd y newidiadau hyn yr angen am yr ymchwiliad hwn, er mwyn deall yr achosion a llywio strategaethau rheoli yn y dyfodol.

Cynhaliwyd yr ymchwiliad mewn dau gam. I ddechrau, adolygwyd y data monitro presennol, a defnyddio arolygon newydd i’w hategu er mwyn llunio asesiad cyfredol o gyflwr y gymuned sbwng a ysgubir gan y llanw yng Ngholeg Normal ac ar draws ACA ehangach y Fenai a Bae Conwy. Roedd gwaith diweddar Rhwydweithiau Natur yn cynnwys dadansoddiad o ddata monitro (2005 - 2023) o golofn Nelson (safle ychwanegol a sefydlwyd er mwyn helpu i ddeall newidiadau a welwyd yng Ngholeg Normal), ac arolygon plymio Adolygiad Cadwraeth Natur Morol (MNCR) (2023) lle ailymwelwyd â safleoedd a arolygwyd yn gyntaf yn 1982 a 2002. Roedd y canlyniadau’n cadarnhau’r dirywiad yng Ngholeg Normal, gyda sbyngau crawennu (ar ffurf clustogau a chramennau) yn sefydlogi ar lefelau isel a rhywogaethau sy’n tyfu at i fynnu fel H. oculata yn absennol o’r trawslun ers 2019. Datgelodd arolygon MNCR hefyd ostyngiadau eang yn y sbwng crawennu dominyddol H. panicea o gymharu â data hanesyddol, tra bod A. fucorum yn dangos cynnydd mewn mannau penodol. Cafwyd achosion tebyg o ddirywiad yn Warrior yn ACA Morol Sir Benfro (2005-2009), gyda phatrymau cylchol yn H. oculata yng Ngholofn Warrior a Nelson yn awgrymu cylch twf naturiol posibl. Ar y cyfan, mae patrymau yn nodi dirywiadau rhywogaeth-benodol, digonedd cylchol yn H. oculata, a newidiadau amgylcheddol ar raddfa eang posibl sy'n effeithio ar gymunedau sbwng ar draws sawl ACA.

Roedd yr ail gam yn cynnwys asesiad pwysau strwythuredig i nodi’r ffactorau sy’n ysgogi’r dirywiad, gan ddechrau gydag Asesiad Sensitifrwydd yn seiliedig ar Dystiolaeth Forol (MARESA) a'i fireinio ar gyfer amodau safle-benodol drwy adolygiad llenyddol, sgorio yn erbyn pum maen prawf, a'i adolygu gydag arbenigwyr mewnol. Cafodd un ffactor pwysau ar ddeg eu nodi a’u grwpio yn ôl y rhai a ddigwyddodd cyn neu yn ystod y cyfnod o ddirywiad, pwysau cefndirol, a bygythiadau posibl yn y dyfodol. Nodwyd mai gostyngiad mewn cyfoeth organig oedd sbardun mwyaf tebygol i dirywiad a welwyd mewn sbwng, yn dilyn gostyngiad sylweddol cyn y cyfnod monitro. Rhoddwyd ystyriaeth i ddyframaethu a chyfoethogi maetholion, ond diystyriwyd hynny oherwydd sefydlogrwydd y gweithgarwch a’r lefel isel o hyder ynghylch a fyddai hynny’n effeithio’n benodol ar sbwng. Yn gyffredinol, mae tystiolaeth yn awgrymu bod newidiadau i fewnbwn organig, ynghyd ag amodau mewn mannau penodol, wedi cyfrannu at y dirywiad.

Mae statws cadwraeth “anffafriol” nodwedd y riffiau yn ACA y Fenai a Bae Conwy yn deillio’n bennaf o’r ffaith bod y sbwng yn llai helaeth yng Ngholeg Normal. Gall y dirywiad hwn adlewyrchu adferiad o gyfoethogi organig hanesyddol, gan amlygu'r angen am linellau sylfaen cadarn a gwaith monitro aml-baramedr i ddehongli newid ecolegol. Os yw'r newidiadau hyn yn gysylltiedig â gwell ansawdd dŵr, maent yn atgyfnerthu gwerth sbyngau nid yn unig fel dangosyddion o gyflwr y riff ond hefyd i fonitro’r amgylchedd yn ehangach, ac ystyried eu gallu hidlo a'u gallu i gronni llygryddion. Er bod H. oculata yn parhau i fod yn absennol yng Ngholeg Normal, mae arolygon ehangach yn awgrymu mai ymateb safle-benodol yw hwn yn hytrach na thuedd ehangach.

Mae'r asesiad pwysau yn tynnu sylw at gynhesu cefnfor a chlefydau fel bygythiadau sy'n dod i'r amlwg, gan danlinellu'r angen am waith monitro parhaus wedi’i dargedu, gyda blaenoriaethau yn canolbwyntio ar gynnal asesiadau o helaethrwydd sbwng ac ar ymchwilio i ail-dwf H. oculata, er mwyn gwahaniaethu rhwng cylchoedd naturiol ac ymatebion straen, ynghyd â gosod a chynnal cofnodwyr tymheredd i olrhain tymheredd y môr a thonnau gwres morol posibl. Mae angen ymchwilio ymhellach i sensitifrwydd sbwng, ymatebion straen, a rôl yr holobiont sbwng (y sbwng cynnal a'i gymuned ficrobaidd gysylltiedig), sy'n cael ei gydnabod fwyfwy fel ffactor allweddol mewn swyddogaeth ecolegol a goddefgarwch straen. Mae camau gweithredu a argymhellir yn cynnwys arbrofion tanc dan reolaeth, astudiaethau maes ar draws graddiannau maetholion, ac ymchwiliadau microbiom i gryfhau rôl sbyngau fel bioddangosyddion.

Executive summary

The Nature Networks (NN) programme, funded by Welsh Government, seeks to address the nature emergency by improving biodiversity and resilience in protected sites. Within the Menai Strait & Conwy Bay (MS&CB) Special Area of Conservation (SAC), long-term monitoring at the Coleg Normal site has identified a marked decline in the luxuriance (an estimate of both abundance and volume) of the tide-swept sponge community between 2004 and 2009. This is an indicator of the condition of the rocky reef feature and led to an “unfavourable” SAC status. This decline is reflected in the reduced thickness of the encrusting (cushion and crust) species and the reduced height and circumference of the erect species (e.g. *Haliclona oculata)*, and a lower percentage occurrence along the transect. These changes prompted the need for this investigation to understand the causes and guide future management strategies.

The investigation was carried out in two stages. First, existing monitoring data were reviewed and supplemented with new surveys to provide an up-to-date assessment of condition of the tide-swept sponge community at Coleg Normal and across the wider MS&CB SAC. Recent NN work included analysis of monitoring data (2005 – 2023) from Nelson’s column (an additional site established to help understand changes observed at Coleg Normal), and Marine Nature Conservation Review (MNCR) dive surveys (2023) revisiting sites first surveyed in 1982 and 2002. Results confirmed the decline at Coleg Normal, with encrusting (cushion and crust) sponges stabilising at low levels and erect species such as *H. oculata* absent from the transect since 2019. MNCR surveys also revealed widespread reductions in the dominant encrusting sponge *H. panicea* compared to historic data, while *A. fucorum* showed localised increases. Similar declines occurred at

Warrior in the Pembrokeshire Marine (PM) SAC (2005–2009), with cyclical patterns in *H. oculata* at both Warrior and Nelson's Column suggesting a possible natural growth cycle. Overall, patterns indicate species-specific declines, cyclical abundance in *H. oculata*, and potential widescale environmental changes affecting sponge communities across multiple SACs.

The second stage consisted of a structured pressures assessment to identify drivers of the decline, starting with Marine Evidence based Sensitivity Assessment (MarESA) and refined for site-specific conditions through literature review, scoring against five criteria, and reviewed with internal experts. Eleven pressures were identified and grouped by those occurring before or during the decline period, background pressures, and potential future threats. A reduction in organic enrichment was identified as the most likely driver of the observed sponge decline, following a significant reduction prior to the monitoring period. Aquaculture and nutrient enrichment were considered but discounted due to stable activity and low confidence in sponge-specific impacts. Overall, evidence suggests that changes in organic input, combined with localised site conditions, contributed to the decline.

The "unfavourable" conservation status of the reefs feature in the MS&CB SAC is primarily due to reduced sponge luxuriance at Coleg Normal. This decline may reflect recovery from historic organic enrichment, highlighting the need for robust baselines and multi-parameter monitoring to interpret ecological change. If these changes are linked to improved water quality, it reinforces the value of sponges not only as indicators of reef condition but also for broader environmental monitoring, given their filtration capacity and ability to accumulate pollutants. While *H. oculata* remains absent at Coleg Normal, wider surveys suggest this is a site-specific response rather than a broader trend.

The pressures assessment highlights ocean warming and disease as emerging threats, underscoring the need for continued, targeted monitoring, with priorities focused on maintaining assessments of sponge luxuriance and investigating *H. oculata* regrowth to distinguish natural cycles from stress responses, alongside installing and maintaining temperature loggers to track rising sea temperatures and potential marine heatwaves. Further research is needed on sponge sensitivity, stress responses, and the role of the sponge holobiont (the host sponge and its associated microbial community), which is increasingly recognised as a key factor in ecological function and stress tolerance. Recommended actions include controlled tank experiments, field studies across nutrient gradients, and microbiome investigations to strengthen sponges' role as bioindicators.

General introduction

Nature Networks (NN) is a multi-year programme funded by Welsh Government aimed at addressing the nature emergency in Wales through increasing biodiversity, improving the condition of protected sites and enhancing the resilience and connectivity of our habitats and species. Within our Marine Protected Areas (MPAs), some habitats and species are in decline. As part of the NN program, the "Investigations into the decline of benthic habitats and species" project seeks to identify the drivers of these declines, with the goal of supporting the recovery of these vulnerable habitats and species. This report investigates the decline of the sponge community in the Menai Strait, within the Menai Strait & Conwy Bay (MS&CB) Special Area of Conservation (SAC).

Background to sponges (*Porifera*)

Sponges (*Porifera)* are an ancient and diverse group (>8500 species) of sessile benthic invertebrates, with recent evidence confirming them as the sister group to all other animals (Soest et al. 2012; Redmond and McLysaght 2021).They are widely distributed being found in both marine and freshwater systems (predominantly marine), in temperate, tropical, polar and abyssal regions and can be found from shallow coastal areas to up to depths of 2000 m in a broad range of turbidity and flow conditions (Gili and Coma 1998).

They are one of the most abundant suspension feeders in benthic communities and fulfil several important functional roles such as bentho-pelagic coupling (the two-way exchange of energy, nutrients and organic matter between the seafloor and the water column) (Bell 2008), due to their ability to efficiently filter large volumes of water (up to 24,000 litres of seawater day per 1 kg sponge) (Vogel 1974).They have a simple body structure with no nervous, digestive or circulatory system and feed by pumping water into their internal canal system. Water reaches flagellated choanocyte chambers, which create flow and capture cells <5 µm, while larger cells are engulfed by pinacocytes lining the incurrent canals (Duckworth and Pomponi 2005). This pumping system allows them to retain dissolved nutrients and food such as bacteria and phytoplankton. They are one of the few filter feeders able to efficiently retain particulate matter in the size range of bacterial cells (0.2 – 1.0 µm), which are usually only able to be utilised once adhered to detrital particles (Reiswig 1975; Stuart and Klumpp 1984). This is an important part of the sponge diet in high organic coastal marine waters.

In addition to feeding on microorganisms, sponges can host a large diversity of them within their mesohyl tissues (the space between the canals and chambers (Soest et al. 2012). This can make up to 40% of the sponge volume with impacts to host function such as enabling photosynthesis and for defence (Figure 1) (Webster and Taylor 2012). Importantly, these microbes facilitate the assimilation of large quantities of dissolved organic matter (DOM), which serves as a nutritional source for the sponge. This process also contributes significantly to DOM cycling in marine ecosystems. Within the sponge DOM that has been released by primary producers is converted into particulate organic matter (POM), which can then be utilised by other benthic organisms, a mechanism known as the "sponge loop (Pita et al. 2018)

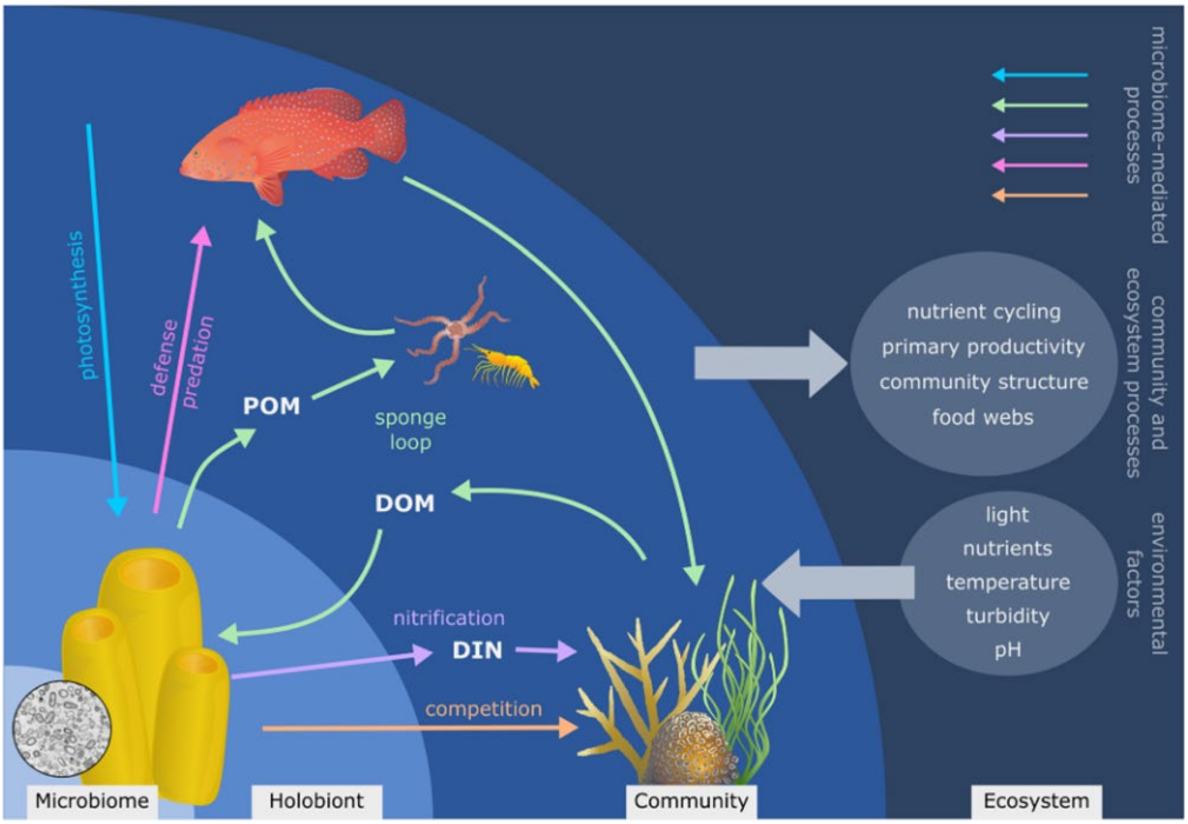


Figure 1: The sponge holobiont. Key functions carried out by the microbiome (coloured arrows) influence holobiont functioning and, through cascading effects, subsequently influence community structure and ecosystem functioning. Environmental factors act at multiple scales to alter microbiome, holobiont, community, and ecosystem scale processes. Thus, factors that alter microbiome functioning can lead to changes at the holobiont, community, or even ecosystem level and vice versa. DOM, dissolved organic matter; POM, particulate organic matter; DIN, dissolved inorganic nitrogen (Adapted from (Pita et al. 2018)

Sponge life-histories vary depending on species and location. They can reproduce sexually (usually hermaphrodites) or asexually (e.g. through budding), releasing short-lived ciliated larvae that settle and then metamorphosise (Naylor 2011). Reproduction is also possible via rejuvenation of fragmented parts. Growth and reproduction are usually seasonal, with dieback often observed over winter in temperate regions (Hayward and Ryland 1990). Life span varies depending on species and environmental conditions with quick growth and short lifespan (i.e. approx. 1-10 yrs) in temperate regions, but with many tropical and deep sea sponges living for thousands of years (McMurray et al. 2008). Erect sponges tend to be longer-lived and grow slower than smaller encrusting or cushion species (Lancaster et al. 2014).

All these life history traits make sponges highly adaptable and resilient to a diverse range of conditions. For example, they are able to restructure their body shape to adapt to changes in environmental conditions and increase feeding efficiency (Schönberg 2021). They are also able to slough off tissue in response to pressures such as heavy siltation/sedimentation that would otherwise block their inhalant pores (Goldstein and Funch 2022). Despite this resilience, recent evidence suggests sponges are increasingly threatened by anthropogenic pressures, including climate change (Bell et al. 2023) and disease (Webster 2007).

Menai Strait and Conwy Bay (MS&CB) SAC sponge community

The MS&CB SAC is situated in north-west Wales and includes the whole of the Menai Strait, from its south-western entrance at Abermenai Point through to Red Wharf Bay and the Little Orme in the north (Figure 2).

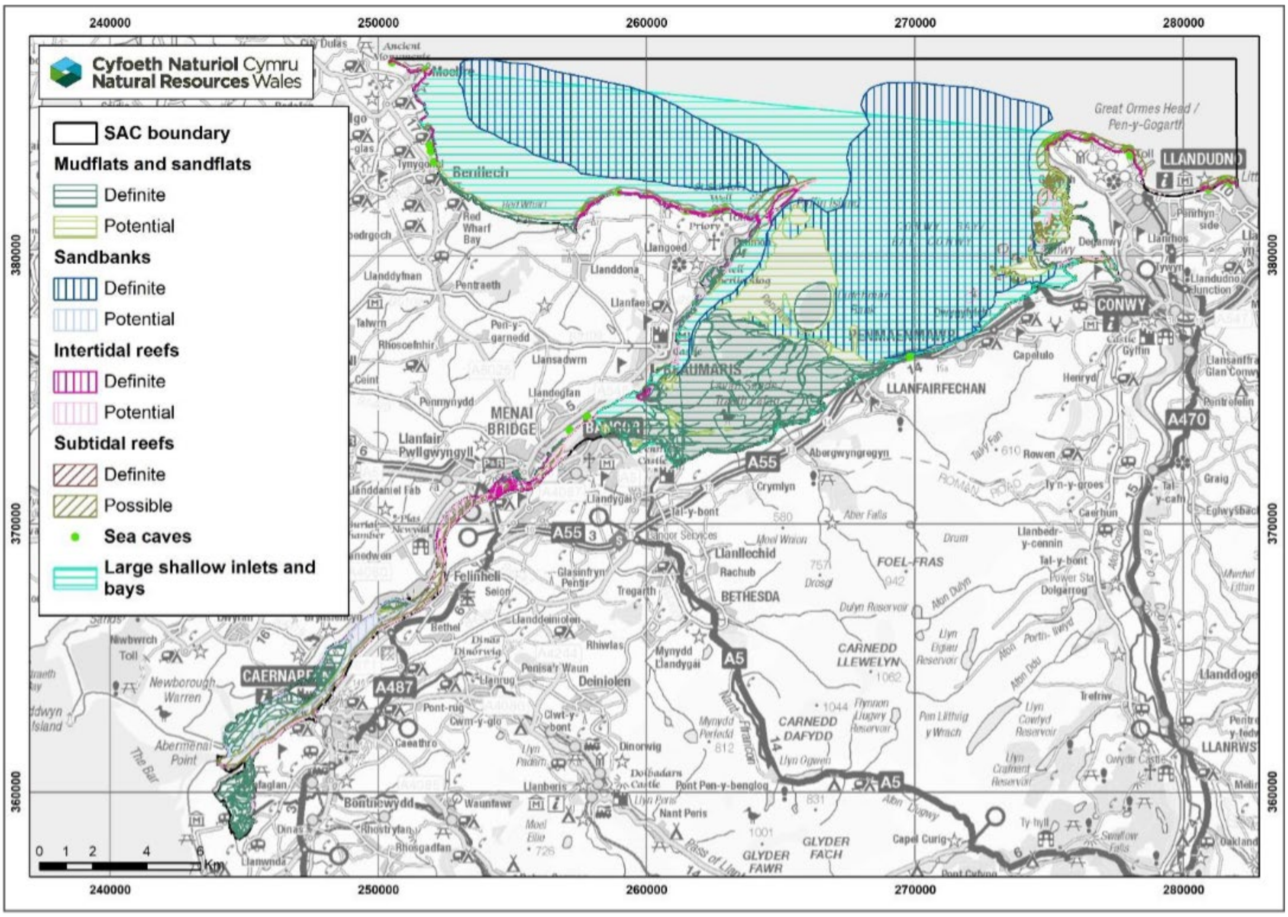


Figure 2: Map of the designated features of the Menai Strait and Conwy Bay SAC (taken from Wynter et al. 2025)

The Menai Strait contains some of the best examples of strongly tide-swept reef in the UK. It is a wave-sheltered but extremely tidal and turbid channel with tidal flows reaching up to 4 m/s at the “Swellies”, in the central area of the Strait between the two bridges (the Britannia and the Menai/Telford Suspension Bridge). These strong tidal streams result in characteristic communities, dominated by filter feeders attached in or onto the substrate, typically including species such as hydroids, bryozoans, anemones and sponges. Historically, this area has been dominated by exceptionally high abundances of several sponge species including *Halichondria panicea* (breadcrumb sponge), *Amphilectus fucorum* (shredded carrot sponge), *Haliclona oculata* (Mermaid's glove sponge) and various encrusting sponges (e.g. *Cliona spp)*. The fast-flowing and turbid waters bring a good supply of food and nutrients, supporting their growth, with unusually large sizes observed in many areas. High levels of suspended material reduce light penetration and limit seaweed growth, thereby reducing competition for space from photosynthetic species. In areas with extremely strong tidal currents, only encrusting or low-profile species can persist, as larger organisms are swept away. These conditions favour opportunistic, fast-growing sponges that can thrive under high turbidity and strong hydrodynamic stress.

Objectives of this investigation

This report brings together historical and newly collected data to:

- Evaluate recent changes in the condition of the MS&CB tide-swept sponge community, describing its current state and ongoing decline.
- Identify and assess potential pressures contributing to the decline observed at Coleg Normal monitoring site.
- Inform future conservation and management strategies to support the tide-swept reef and sponge community.

The following sections will address these objectives.

Monitoring and current status

In 2004, subtidal monitoring sites were established for the reef features within three of the marine SACs. Within the MS&CB SAC the features being monitored included the limestone reef communities at Ynys Moelfre and Bottle Rock (situated off the Southern end of Puffin Island) and the tide-swept sponge communities at the Coleg Normal and Nelson's Column monitoring sites in the Menai Strait (Figure 3).

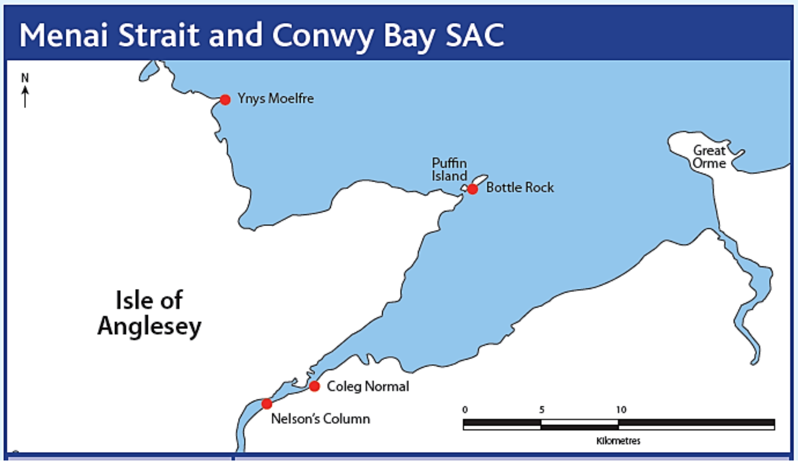


Figure 3: Location of reef monitoring sites at Ynys Moelfre, Bottle Rock, Coleg Normal and Nelson 's Column within the MS&CB SAC (Irving & Smith, 2013)

At all of these sites the sponge fauna is monitored as an indicator of the condition of the benthic community. For example, the diversity of sponge morphologies has been used to assess the richness of the subtidal communities associated with the limestone bedrock at Ynys Moelfre (2005-2023) and Bottle Rock (2011-2023). The following sections will describe in detail the monitoring at Coleg Normal and Nelson's Column in the Menai Strait as well as at Warrior, another tide-swept sponge community monitored in Pembrokeshire Marine (PM) SAC.

Coleg Normal

The Coleg Normal site is located on the southern shore of the Menai Strait, about 0.5 km east of the Menai Suspension Bridge, and named after the nearby Coleg Normal building (Figure 4). The site was selected for monitoring as it was representative of the tide-swept circalittoral communities of the Menai Strait and previous evidence indicated that the sponge communities were well-developed here. It was considered that the vigorous sponge growth was a result of the combined effects of the hydrodynamic regime at the site together with the high nutrient and turbidity levels affecting the community.

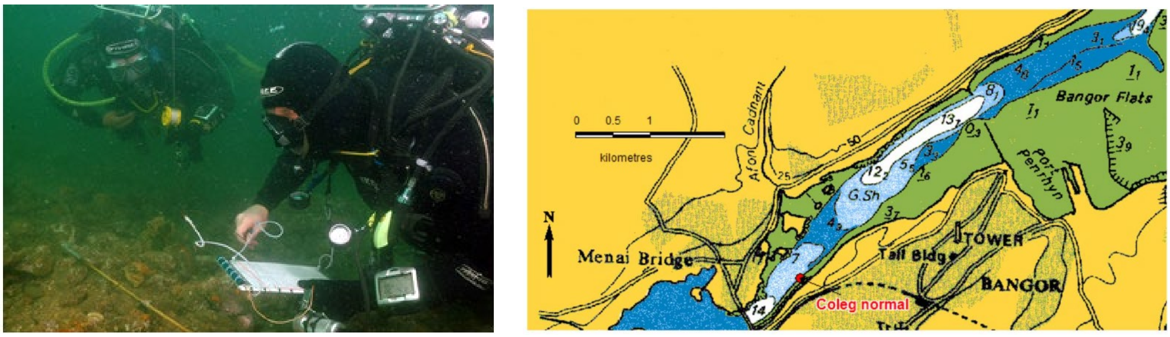


Figure 4: Divers using a wire probe to measure the thickness of encrusting sponges at the Coleg Normal monitoring site (left). Location of the Coleg Normal monitoring site opposite Menai Bridge in the Menai Strait (right).

The site comprises a steep boulder and cobble slope dominated by the sponges *A. fucorum*, *H. panicea*, *H. oculata* and *Microciona spp as* well as other encrusting species. The initial objective was to create a baseline of data to assess the condition of the rocky reef feature, based on the overall condition of the sponge community. Sponge "luxuriance", an estimate of both abundance and volume, is used as an indicator for assessing the health of subtidal reef habitat with the aim of meeting favourable condition target.

Monitoring is intended to be carried out annually or as frequently as possible, and initially comprised two subtidal transects running parallel to the shore in a south-westerly direction. From 2008, two transects were added to the north east. The transects follow two depth contours, the 'shallow' at approximately 5 m depth below chart datum (BCD) and the 'deep' at approximately 8 m depth BCD. There is no bedrock to fasten markers for permanent quadrats at the site and tidal flows reach up to 4 m/s, so leaving any form of marker or buoy is not practical. Instead, measurements from a known feature on the shore are used to orientate the transects parallel to the shore. The transects extend 30 m either side of the measuring tape. Every 0.5 m a data point is taken measuring the thickness of encrusting (term used here to cover crustose and cushion sponges combined) sponges and the height and circumference of erect sponges (Irving et al. 2012).

Since monitoring began, there has been a dramatic decline in the luxuriance of the sponges at this site, seen as both a reduction in the thickness of the encrusting species and the height and circumference of the erect species, alongside a reduction in percentage occurrence along the transect. There is some anecdotal evidence from regular divers of the Menai Strait to suggest this may have been occurring since the 1990s. This has resulted in “unfavourable” status in the SAC condition assessments (NRW 2018; Wynter et al. 2025).

Erect sponges

Between 2004 and 2009 the occurrence of *H. oculata* along the main SW transects (shallow and deep) decreased from approximately 30% to around 5% alongside an approximately 95% reduction in mean size (a measure of circumference and height) (Figure 5 & Figure 6). After this dramatic initial decrease the decline has continued at a slower rate with no *H. oculata* recorded at these transects since 2019. At the additional NE transect *H. oculata* have also declined. Though sampling here only began in 2008 and has not been carried out regularly, the data suggests larger individuals occurred in this area.

Figure 5: *Haliclona oculata,* key erect sponge recorded at Coleg Normal monitoring site (Image taken by R. Holt in 2002 at Coleg Normal, Countryside Council for Wales, CCW)

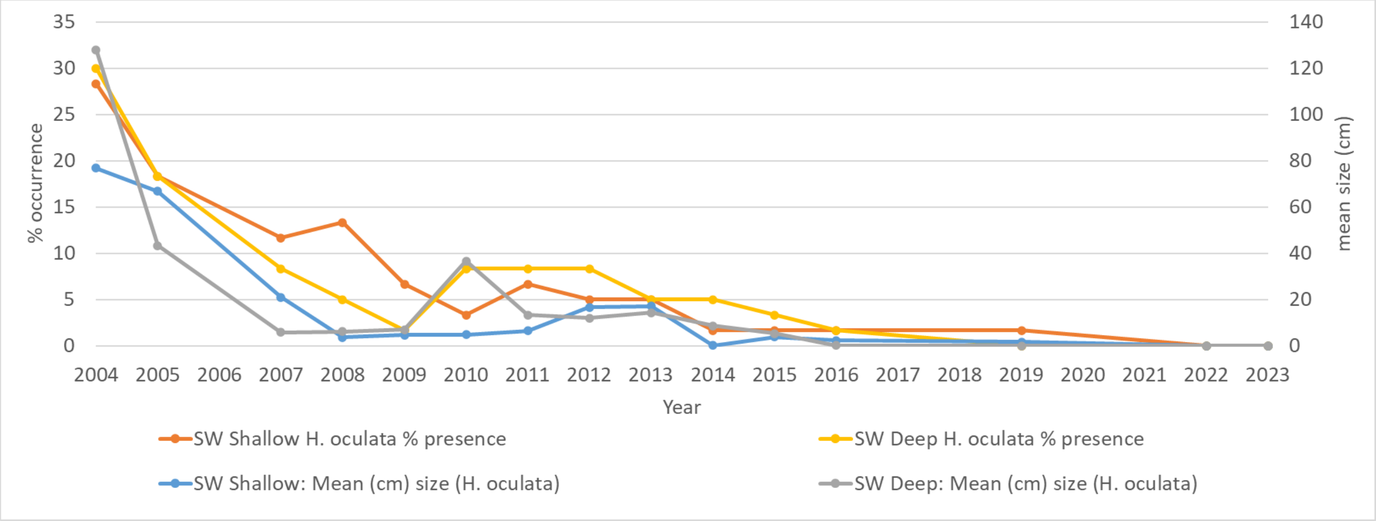


Figure 6: Percentage occurrence (%) (left axis) and mean size (cm) (right axis) of the sponge *Haliclona oculata* along Coleg Normal's 30m southwest transect in the Menai Strait and Conwy Bay (MS&CB) SAC during the monitoring period 2004 – 2023.

Encrusting sponges

Between 2004 and 2008 there was an overall decline in both the thickness and occurrence of encrusting (crustose and cushion) sponge species along the main SW transects (shallow and deep) (Figure 7 & 8). A 94% reduction in thickness in sponge was recorded at the shallow transect with percentage occurrence dropping from 73% to 8%. The deep transect showed an approximately 32% reduction in thickness over the same time period but with a different pattern of decline. After an initial decline between 2004 and 2005 there was an increase in 2007 before the decline continued. The occurrence also followed this pattern. From 2009 both the thickness and occurrence of encrusting sponges have fluctuated but remained at this reduced level.

Figure 7: Key encrusting sponge species recorded at Coleg Normal monitoring site: (A) *Halichondria panicea*, (B) *Amphilectus fucorum* and (C) *Microciona spp*. (Images taken by NRW divers during annual monitoring activities)

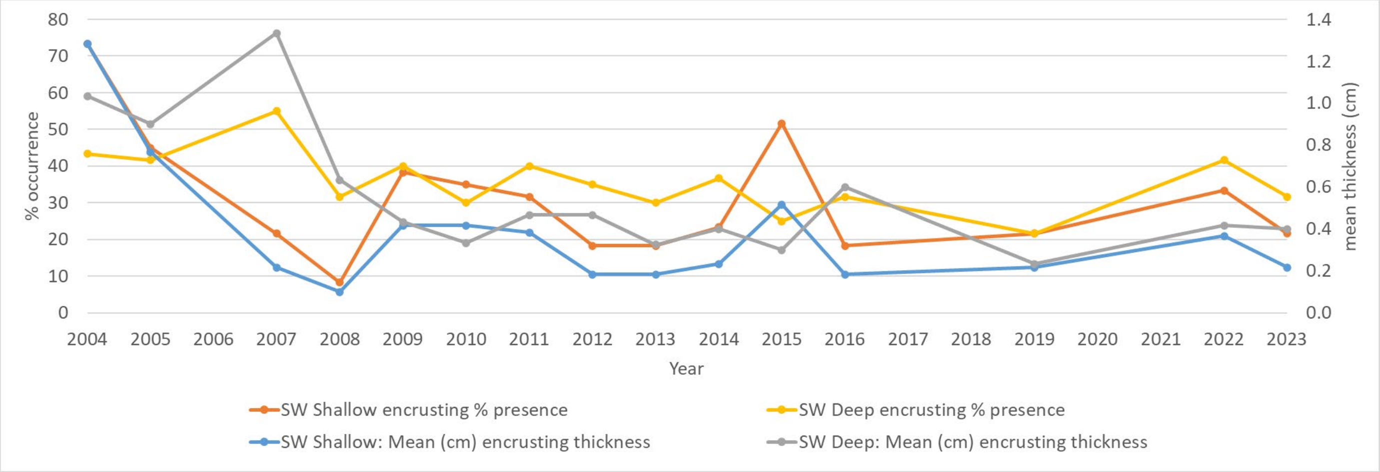


Figure 8: Percentage occurrence (%) (left axis) and mean thickness (cm) (right axis) of encrusting (crustose and cushion) sponges along Coleg Normal's 30m southwest transect in the Menai Strait and Conwy Bay (MS&CB) SAC during the monitoring period 2004 – 2023.

The key encrusting species recorded at the site were *H. panicea* and *A. fucorum*, with the remaining species (e.g. *Microciona spp.*) being grouped as "crusts" (Figure 9). When looking more closely at the data, the overall decline in luxuriance observed was driven by the reduction in the dominant, large growth of *H. panicea* and *A. fucorum* with the crusts showing general fluctuations with no clear decline.

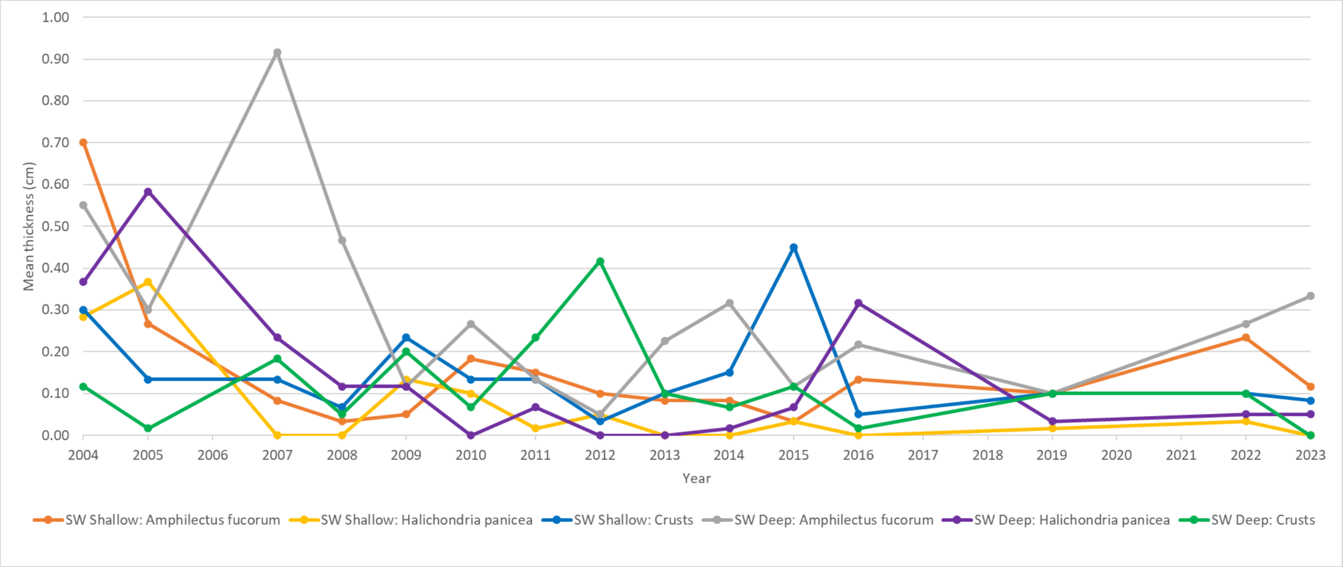


Figure 9: Mean thickness of key encrusting (crustose and cushion) sponges (*Halichondria panicea*, *Amphilectus fucorum* and "crusts" including *Microciona spp.*) along Coleg's Normal 30 southwest transect in the Menai Strait and Conwy Bay (MS&CB) SAC during the monitoring period 2004 – 2023.

At the additional NE transect the luxuriance of the sponges since recording began in 2008 has shown the same reduced level that has been recorded on the main SW transect

(Figure 9). There was no data collected here during the dramatic decline period between 2004 and 2008.

In addition to the dive monitoring data there is also anecdotal evidence of the wider site recorded in the site visit logs. This highlights:

*2014: "All divers **reporting low density of sponges"***

2021: "No erect sponges seen…Explored area - saw only 2 Halichondria in whole site"

In 2022 a wider search was carried out further to the SW and NE of the transects to search for H.oculata. In total 4 were noted to the SW but higher abundances recorded to the NE. With "good sponge cover" also noted from 60 m along the shallow and deep transect.

Nelson's Column

In addition to the Coleg Normal monitoring site, described above, a second site in the Menai Strait was established, Nelson's Column (Figure 10). The data obtained from this site provides an overview of the tide-swept community at the site to help understand changes observed at Coleg Normal. The site is located on the northern shore of the Strait, about 0.5 km west of the Britannia Bridge, and named after the Nelson's Column monument located on the shore.

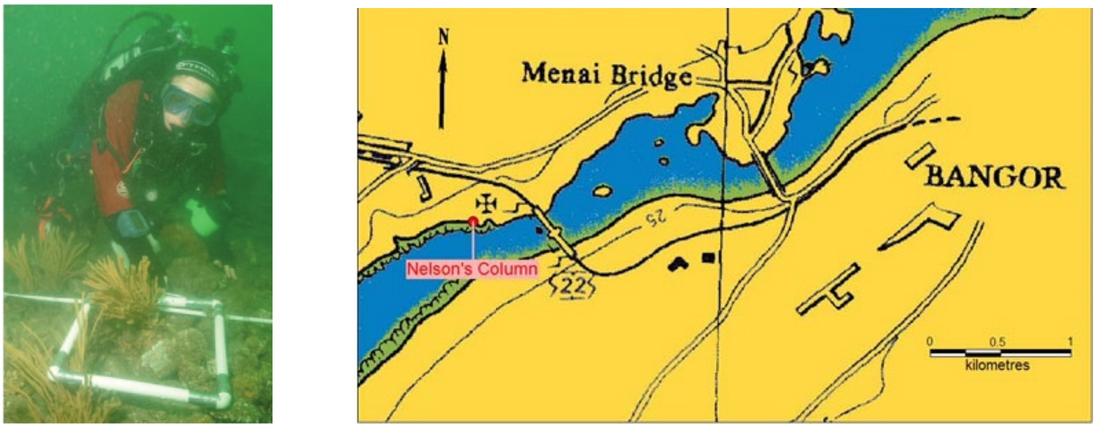


Figure 10: Nelson's column diving site in the Menai Strait: Diver positioning the 0.3 m x 0.3 m quadrat along the transect tape during monitoring at Nelson's column (left). Location of the Nelson's column monitoring site beside the Britannia Bridge in the Menai Strait (right).

The base of the column acts as mark for a transect which runs perpendicular to the shore in a SW-NE direction down to approximately mid-channel. The subtidal transect is 100 m long with photos taken of quadrats (0.3 m x 0.3 m) at 2 m intervals from 1 to 14 m depth (x 30 quadrats). The substrate here is mixed pebbles, cobbles and small boulders. A video record of the whole transect is also taken to show the main seabed habitats and the associated conspicuous species/communities, providing an overall impression of the site.

As part of this NN investigation, Marine Ecological Solutions Ltd (Marine EcoSol) were contracted to undertake the analysis of the available images from the monitoring surveys. The percentage cover was estimated to species level where possible and sponge morphology and substrate were recorded. In addition, the size and health of *H. oculata* were recorded due to concerns over the health of this species as it had been observed with necrotic tissue (Jones 1988, NRW divers, *pers.comm*, 2023). This will be discussed further under “Introduction of microbial pathogens”.

Multivariate analyses, using Primer v7 software (Clarke and Gorley 2015), were conducted by NRW to assess temporal changes in the abundance and composition of the benthic community along the transect between 2005 and 2023. Over this period there was a decline in both species richness and abundance, with a 20% reduction in the number of species and a 50% reduction in total abundance. Diversity metrics (Shannon, Simpson and Pielou’s evenness) peaked around 2011–2014, indicating a period of higher diversity and more balanced community structure, but subsequently declined by 2019, with only partial recovery by 2023. The abundance and diversity of sponges also decreased, with a 50% reduction in the number of species and a 60% reduction in total abundance. Diversity indices for sponges mirrored the overall community trend, peaking mid-period (2014) before declining sharply, although evenness improved overall, reflecting a more balanced distribution among the remaining species. This contraction was further evidenced by a decline in sponge morphotypes recorded along the transect, reducing from eight morphotypes in 2005 to just two by 2023 (Figure 11). These results indicate that sponges have been more severely affected than the wider benthic assemblage, potentially reducing habitat complexity and associated biodiversity.

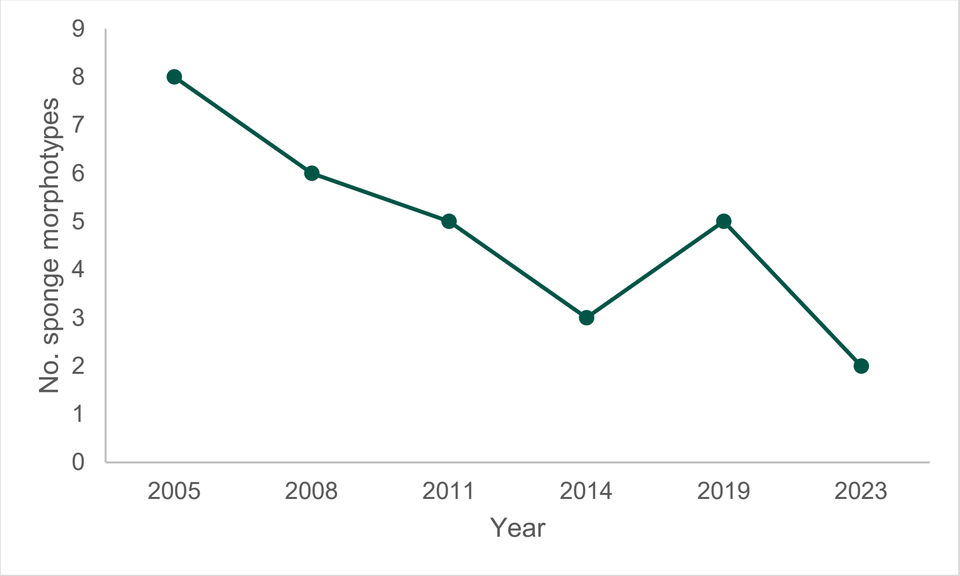


Figure 11: Decrease in sponge morphotypes at Nelson’s column in the Menai Strait and Conwy Bay (MS&CB) SAC during the monitoring period 2005 – 2023.

The key sponge species did not reflect the same pattern of decline observed at Coleg Normal. The percentage cover of *Halichondria sp.* along the transect did show a slight decline between 2004 and 2014, remaining at this low level, but not like the dramatic reduction in occurrence seen at Coleg Normal. The percentage cover of *A. fucorum* declined considerably, though this reduction did not occur until the period between 2011 and 2014, after which it has remained consistently low. The percentage cover of *H. oculata* has fluctuated over time on an approximately 6 year cycle. Size of *H. oculata* was also estimated and showed a reduction in size between 2005 and 2011, with no large or medium specimens being recorded, only small. This may be related to a cycle of growth as by 2019 small, medium and large specimens were recorded at the same time as percentage cover had increased. Other encrusting sponge species have fluctuated with no trend from 2004 to 2023. It is important to note the different survey methods employed at both sites when comparing this data. For example, percentage cover does not account for changes that may be occurring in the thickness of the sponges present.

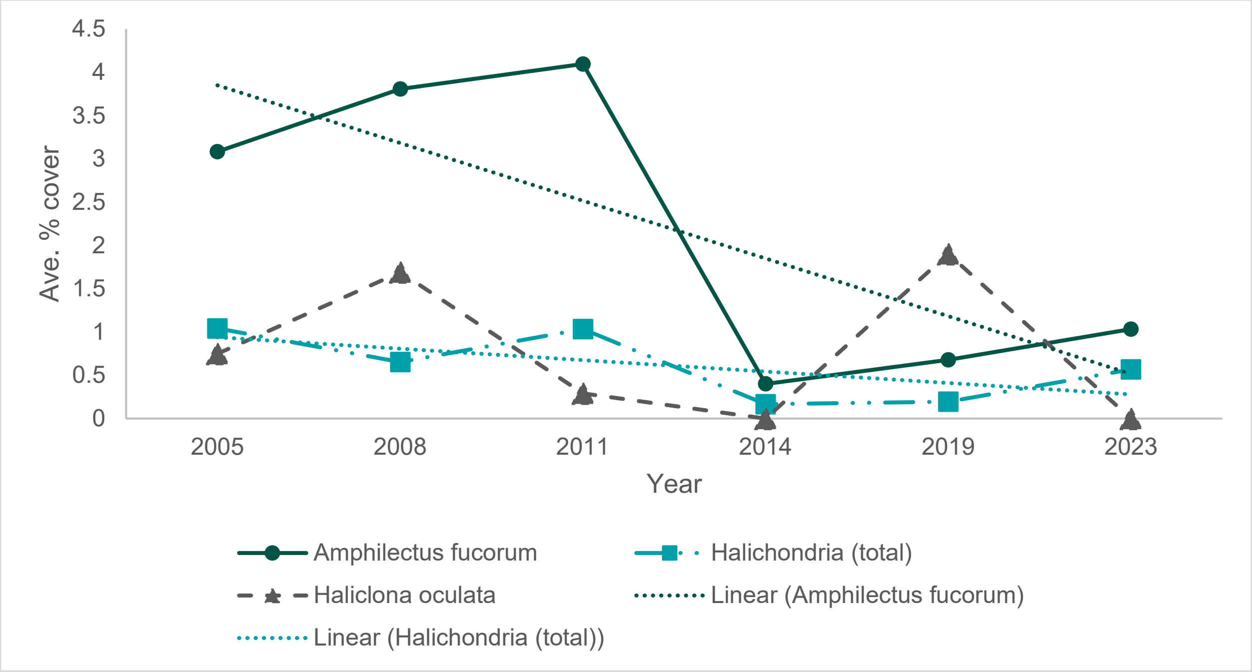


Figure 12: Percentage cover of *Amphilectus fucorum, Halichondria panicea* and *Haliclona oculata* at Nelson's column in the Menai Strait and Conwy Bay (MS&CB) SAC during the monitoring period 2005 – 2023.

Declines were also observed in other filter-feeding species, including *Alcyonium digitatum* (Dead man's fingers) and *Aplidium* sp. The reduction in *A. digitatum* is particularly notable given its large size and distinctive morphology, which lends confidence to the observed trend. Substrate composition data did not indicate any significant changes over time, such as shifts in fine sediment cover, suggesting that habitat structure remained relatively stable. However, interpretation of species trends must be approached with caution due to the variability in image quality across survey years and the non-fixed nature of the transect, which introduces spatial variation in habitat. Image quality was highest in 2005, likely contributing to the greater species diversity recorded in that year. In contrast, no high-quality images were available for the later years (2019 and 2023), which likely resulted in fewer species being detected due to limitations in resolution and clarity.

Warrior

In the Pembrokeshire Marine (PM) SAC, the Warrior monitoring site is another strongly tide-swept sponge-dominated community (Figure 13). This is the only other monitoring site where a sponge luxuriance transect is undertaken with the same methodology as described for Coleg Normal. The Warrior sponge transect is carried out at only one depth (10.7 m BCD).

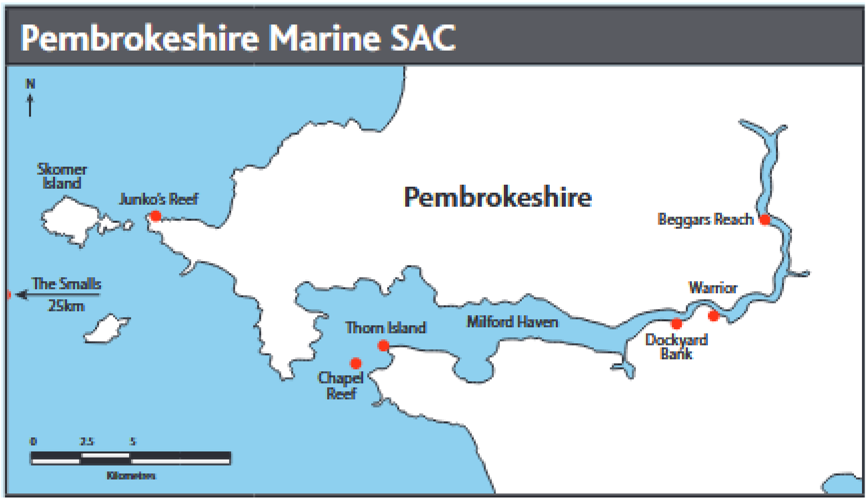


Figure 13: Location of the Pembrokeshire Marine SAC subtidal reef monitoring sites, including Warrior here a sponge luxuriance transect is conducted to monitor the tide-swept sponge community (taken from Irving and Stanwell-Smith 2013).

Encrusting sponges

Data from Warrior showed a 74% decrease in the thickness of encrusting sponge species between 2005 and 2009 (

Figure 14). There are no data available for 2004. Between 2009 and 2023 the thickness of encrusting sponges has fluctuated but remained at this reduced level. During this time period the occurrence has fluctuated with no trend.

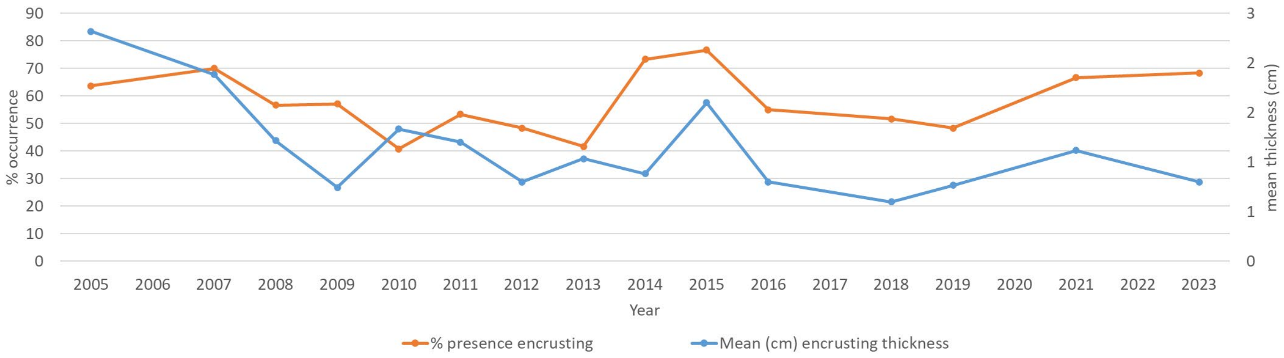


Figure 14: Percentage occurrence and mean thickness of encrusting (cm) (crustose and cushion) sponges at Warrior in the Pembrokeshire Marine SAC during the monitoring period 2005 – 2023.

Erect sponges

Between 2005 and 2023 the occurrence of *H. oculata* has fluctuated without trend over the time period on an approximately 5 or 6 year cycle as also observed at Nelson’s Column in the Menai Strait. The average size (height x circumference) of *H. oculata* has seen a slight reduction over the same time period (Figure 15).

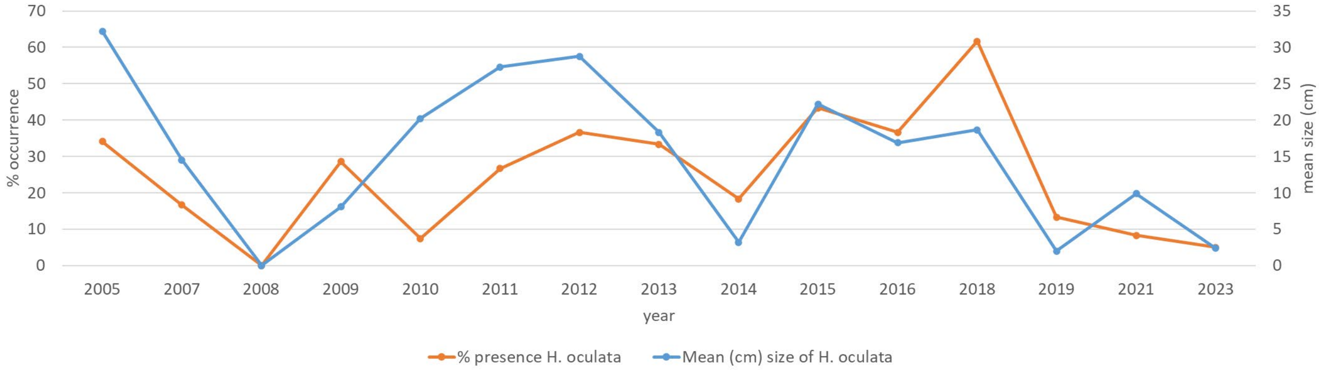


Figure 15: Percentage occurrence and mean thickness of encrusting sponges at Warrior in the Pembrokeshire Marine SAC during the monitoring period 2005 – 2023.

In addition to the dive monitoring data the following notes were also recorded in the site visit logs:
2013: “lots of dead and decaying Haliclona oculata”
2018: “collect die-back of H. oculata on next visit”
2019: “Few large Haliclona, numerous small/young ones”

In addition, analyses carried out for the 2025 condition assessments identified changes over time at two in the community composition of the five subtidal reef monitoring sites within the PM SAC (Warrior Reef and Beggars Reach) suggesting possible disturbance (Jackson-Bué et al. 2025). At Beggars Reach, located in the inner Milford Haven waterway, preliminary data suggest a possible decline in the abundance of *Halichondria* spp. between 2009 and 2023, alongside indications that other filter feeders such as *Ascidia* sp. may have increased.

Overall, the indicator for abundance, distribution and species composition of subtidal reef communities in the SAC failed to meet its conservation target with high confidence. This reflects concerns raised across multiple sites within the Milford Haven Waterway and the Skomer MCZ, particularly regarding the condition and resilience of sponge-dominated reef habitats.

NN Marine Nature Conservation Review (MNCR) surveys

In 2023, as part of this NN investigation, a series of Marine Nature Conservation Review (MNCR) dive surveys were conducted in the Menai Strait by NRW, Aquatic Survey and Monitoring Ltd (ASML) and Marine EcoSol. This aimed to collect comparative data to understand the scale of the decline observed at Coleg Normal, by revisiting sites previously surveyed in 1982 (Lumb 1983) and 2002 (Moore 2002).

Methods

Seven sites were identified in the central area of the Menai Strait from Nelson’s Column to Coleg Normal (Figure 16). These locations were selected due to the availability of previous survey data that classified them as having sponge dominated biotopes (Cushion sponges, hydroids and ascidians on tide-swept, turbid, sheltered circalittoral rock, CR.MCR.CFAVS.CuSpH.As) (Lumb 1983; Moore 2002). Survey priority was given to sites that had data available from both 1982 and 2002 surveys. Abundance data were recorded by divers using the semi-quantitative Superabundant, Abundant, Common, Frequent, Occasional, Rare and Present (SACFORP) scale (Hiscock 1996) along with video and images to characterise the sites.

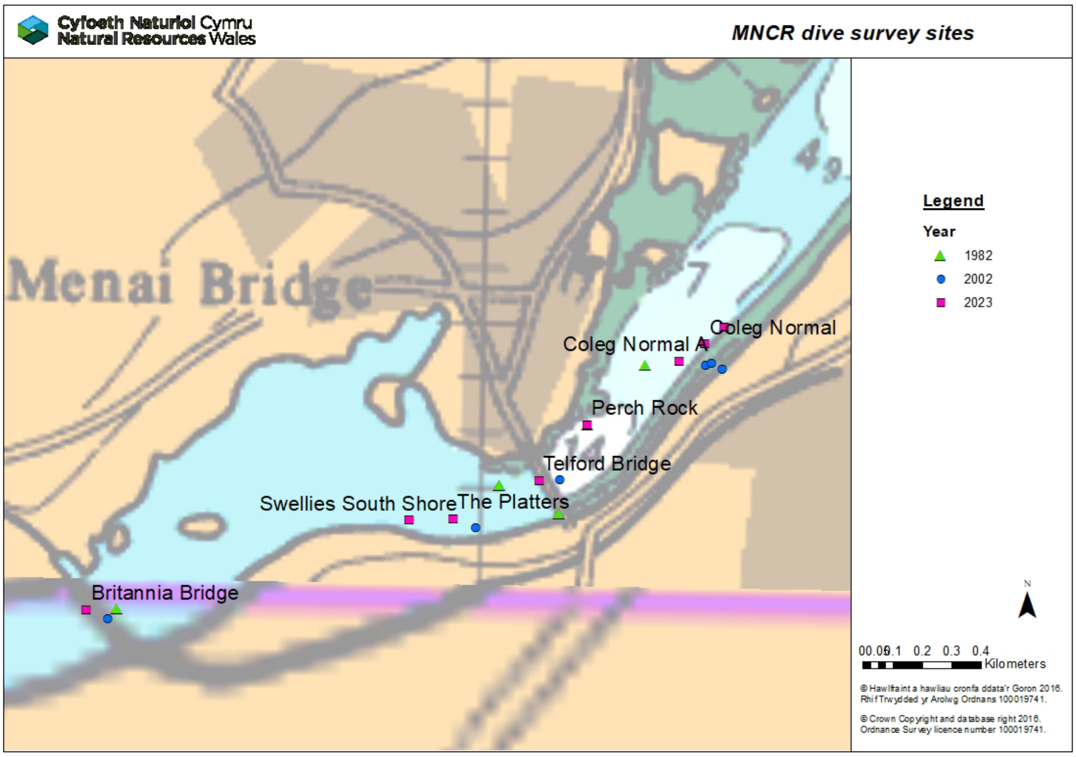


Figure 16: Marine Nature Conservation Review (MNCR) dive survey sites in the central area of the Menai Strait. Pink squares indicate the 2023 survey locations, blue circles the 2002 sites and green triangles the 1982 sites.

Abundance data was recorded using the ACFOR scale in the 1982 Menai Sublittoral Survey. To enable comparison with data from this survey for detection of broadscale change this data was downgraded to the truncated ACFOR scale by reclassifying Super Abundant records to Abundant (Burrows et al. 2009). Values were also converted to a numerical scale (1-6) for analysis of the data. Multivariate analyses, using Primer v7 software (Clarke and Gorley 2015), were carried out by NRW to visualise changes in the abundance and composition of the benthic community at each site over time (e.g. non-metric multidimensional scaling plots). There was no site replication within each survey year, so it was inappropriate to analyse the data statistically and instead the data was visually interpreted for patterns. A difference of a least 2 SACFOR categories is generally considered ecologically significant (Baker et al. 1981; Simkanin et al. 2005; Firth et al. 2015) and due to the subjective nature of the SACFOR abundance scale a jump of one category accounts for natural fluctuations and inter-surveyor differences.

Results

The abundance of the key sponge species (*H. panicea, A. fucorum* and *H. oculata*) were compared for each site to identify notable change over time, based on a two-step category change (Table 1).

The results show an overall decline in abundance of *H. panicea* at multiple sites (Britannia Bridge, Telford Bridge and Coleg Normal), especially between 2002 and 2023. No change was recorded at Swellies south shore, the Platters and Perch Rock. Swellies south shore was not surveyed in 1982, and Perch Rock was not surveyed in 2002. No sites recorded an increase in abundance between 1982 and 2023.

A. fucorum abundance showed a general increase at several sites (Britannia Bridge, the Platters, Perch Rock and Coleg Normal). Swellies south shore and Telford Bridge showed no change. At Coleg Normal, MNCR habitat survey data showed no change in abundance between 2002 and 2023 in contrast to monitoring data for the same period which recorded a reduction in thickness and abundance. This difference likely reflects the contrasting methodologies: habitat surveys rely on visual estimations of presence and abundance, whereas monitoring uses quantitative transects and measurements. Spatial variation between transects (e.g., deep vs. shallow) may also contribute to these differing patterns.

H. oculata abundance showed a general trend of low and declining abundance. It was not recorded at Britannia Bridge or Telford Bridge. At the Platters it was only recorded in 2002 (Common) and at Swellies south shore only in 2023 (Rare). At Perch Rock a 2 step decline was observed between 1982 and 2023 with no data available for 2002. At Coleg Normal no change was recorded between 1982 and 2002 but a significant decline recorded between 2002 and 2023 in line with the monitoring data.

As no data were available for 2002 at Perch Rock, future assessments could consider incorporating Seasearch data to help fill this gap and improve temporal resolution. Additionally, anecdotal evidence from regular divers in the Menai Strait suggests that sponge abundance has declined over time, supporting the observed trends and highlighting the value of local ecological knowledge in long-term monitoring.

Table 1: Abundance data for the key sponge species (*H. panicea, A. fucorum, and H. oculata*) at the six surveyed sites in the Menai Strait in 1982, 2002, and 2023, along with relative changes between survey years. Abundance is recorded categorically: A (Abundant), C (Common), F (Frequent), O (Occasional), R (Rare), N (Not recorded), and / (Not surveyed). Change columns (1982–2002, 1982–2023, 2002–2023) use arrows to indicate direction: ↑ for increase, ↓ for decrease, with the number showing the number of category steps changed. A dash (–) indicates no change; N/A denotes insufficient data for comparison. Cells are colour-coded to indicate trend direction and magnitude: blue shades represent increasing trends and orange shades represent decreasing trends, with darker colours indicating greater change than lighter colours.

Site ***Halichondria panicea***	**Abundance 1982**	**Abundance 2002**	**Abundance 2023**	**Short-term comparison (~20yr) 1982/2002**	**Short-term comparison (~20yr) 2002/2023**	**Long-term comparison (~40yr) 1982/2023**
Britannia Bridge	C	C	O	-	N/A	↓ 2
Swellies South Shore	/	C	F	N/A	-	N/A
The Platters	C	A	C	-	-	-
Telford Bridge	A	A	O	-	↓ 3	↓ 3
Perch Rock	F	/	F	N/A	N/A	-
Coleg Normal	C	A	O	-	↓ 3	↓ 2
Site ***Amphilectus fucorum***	**1982**	**2002**	**2023**	**1982/2002**	**2002/2023**	**1982/2023**
Britannia Bridge	N	A	C	↑ 5	N/A	↑ 4
Swellies South Shore	/	C	C	N/A	-	N/A
The Platters	F	O	A	-	↑ 3	↑ 2
Telford Bridge	F	C	F	-	-	-
Perch Rock	O	/	C	N/A	N/A	↑ 2
Coleg Normal	N	C	F	↑ 4	-	↑ 4
Site ***Haliclona oculata***	**1982**	**2002**	**2023**	**1982/2002**	**2002/2023**	**1982/2023**
Britannia Bridge	N	N	N	-	N/A	-
Swellies South Shore	/	N	R	N/A	-	N/A
The Platters	N	C	N	↑ 5	↓ 5	-
Telford Bridge	N	N	N	-	-	-
Perch Rock	F	/	R	N/A	N/A	↓ 2
Coleg Normal	F	O	N	-	↓ 2	↓ 3

Pressures assessment methodology

Initially, potential pressures were identified using the Marine Evidence based Sensitivity Assessment (MarESA) carried out for the tide-swept sponge community biotope classified for the MS&CB SAC *(*Cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock, CR.MCR.CFaVS) (Readman et al. 2023). Since the MarESA assessments are generic and not site-specific, this list of pressures was then reviewed to identify pressures relevant to the area of concern, in this case the Menai Strait, and pressures that were not relevant to the investigation were removed from the list. A spreadsheet of these pressures was generated and an extensive literature review was then conducted building on the MarESA evidence base, covering both the sensitivity of the species and the history of the pressure in the area. The impact of the identified pressures was scored and ranked to assess the relative risk of pressures to tide-swept sponge community resilience (Table 2). Impact scoring was based on five criteria: timing, intensity, frequency, spatial scale, and species sensitivity, each evaluated on a four-point scale (Table 2). These scores were then multiplied to generate an overall risk score. A confidence rating (low, medium, high) was also assigned to reflect the strength and availability of supporting evidence. These assessments, including the evidence base and pressure evaluations, were presented during an internal workshop, where both the pressure scores and confidence ratings were reviewed and validated by a panel of experts, including site officers, benthic specialists, and water quality specialists.

Table 2: Pressure impact scoring definitions. The decline period for this assessment was defined as 2004 to 2009 as this is the timeframe in which a marked, well quantified reduction in sponge luxuriance was recorded at Coleg Normal. These data led to the feature being assessed as ‘unfavourable’ in the SAC condition assessments and prompted this investigation.

Impact score criteria	Definition
Timing	Likelihood of a pressure occurring during the observed decline period (2004-2009) **1** = Only outside the time decline period **2** = Mostly outside the time decline period (including continuous pressures that occurred before and after the decline period as well as throughout) **3** = Mostly within the time decline period (including pressures present before and/or after for a limited time duration) **4** = Only within the time decline period

Impact score criteria	Definition
Intensity	The relative magnitude of the pressure. **1** = Very low intensity **2** = Low intensity **3** = High intensity **4** = Very high intensity
Frequency	How often the pressure occurs **1** = One-off or short-term event **2** = Frequent for a short duration, or infrequent over a longer period **3** = Frequent (may be intermittent but ongoing) **4** = Continuous
Spatial scale	Proportion of the feature affected by the pressure **1** = <10% of the feature **2** = >10% – <50% of the feature **3** = >50% – 80% of the feature **4** = >80% – 100% of the feature

Impact score criteria	Definition
Sensitivity of species	The likelihood of change when a pressure is applied to a feature. This reflects the feature’s ability to tolerate or resist change, and its capacity to recover from impact: **1** = Not sensitive **2** = Low sensitivity **3** = Medium sensitivity **4** = High sensitivity
Risk	Timing x Intensity x Frequency x Spatial scale x Sensitivity of species

Organic enrichment

Definition of pressure: The accumulation of carbon-rich matter, such as plant debris, decaying organisms, faecal material, and organic waste often introduced through sewage discharges, aquaculture, or terrestrial runoff, which can alter the chemical and biological conditions of the ecosystem.

Sensitivity of species

The sensitivity assessment (Readman et al. 2023) classified “cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock” (the biotope at Coleg Normal) resistance to organic enrichment as ″high" with resilience rated as "high" and overall sensitivity as "not sensitive" at a benchmark of a deposit of 100 $gC/m^2/yr$.

Organic enrichment increases the availability of particulate and dissolved organic matter, enhancing productivity for suspension and deposit feeders, including sponges. However, when organic input exceeds the environment's assimilative capacity, it leads to oxygen depletion, altered sediment chemistry, and a decline in species richness and biomass (Pearson and Rosenberg 1978). As suspension feeders, sponges often show increased biomass in organically enriched waters due to elevated food supply, particularly bacteria (Rose and Risk 1985; Gökalp et al. 2021; Amato et al. 2024). Several studies show that sponges can effectively uptake and utilise bacteria as a food source. *Hymeniacidon perlevis* efficiently removed bacteria from sewage-contaminated water, indicating that bacteria alone can constitute a major source for sponges in coastal waters of high organic content (Longo et al. 2022); *Haliclona cinerea* and *Suberites ficus* sustained growth solely on bacteria, with excess intake supporting rapid growth (Reiswig, 1975); and *Microcionia prolifera* fed effectively on *E. coli,* the more bacteria present, the more efficiently the sponge utilised them for growth (Claus et al. 1967). However, bacterial nutrition alone may be insufficient for some species. For example, *Halichondria melanadocia* required unnaturally high bacterial concentrations just to maintain weight and showed better growth on mixed diets (Duckworth and Pomponi 2005). Interestingly, individuals exposed to the highest bacterial concentrations did show the best growth but also the lowest survival, with mortality associated with a white biofilm. Sponge responses are therefore likely to vary depending on the species and the environmental conditions.

Broader studies also show increased sponge biomass near sources of organic input, such as coastal runoff or sewage outfalls (Wilkinson and Cheshire 1990; Fonnegra Chaves et al. 2007). For example, *Cliona delitrix* increased in abundance near sewage sources, likely due to elevated suspended matter rather than *E. coli* alone. In wider benthic communities, historical organic enrichment often supported deposit and suspension feeders, but wastewater treatment improvements have reduced nutrient inputs, altering food availability and community composition (Smith and Shackley 2006; Zubikarai et al. 2014; Oliveira et al. 2014; Callaway 2022). The extent of impact depends on local hydrodynamics: in well-flushed systems, high organic input can support high sponge biomass due to dispersed suspended food, whereas in sheltered environments, organic matter accumulates, reducing oxygen availability and leading to declines in diversity and biomass (Pearson and Rosenberg 1978; Puente and Diaz 2015).

In addition, organic enrichment can have impacts on the sponge microbiome, with increases in antimicrobial activities for example (Batista et al. 2018) . A recent study on *H. panicea* indicated the microbiome is highly affected by its surrounding environment responding to changes in water quality (Al-Haddad et al. 2025). However more research is needed to understand potential impacts to the sponge itself, such as growth.

There is limited direct evidence on the impacts of organic enrichment on the key species found in the Menai Strait (*H. panicea, H. oculata* and *A. fucorum*). However, their historic success in this turbid coastal location indicates tolerance to organic enrichment. *H. panicea* has been shown to exhibit seasonal plasticity in its organic and inorganic content, correlating with local environmental conditions, showing rapid growth during spring and summer when food availability increases (Broadribb et al. 2021). In addition, a recent study highlights *H. panicea* can maintain growth even under elevated organic input, and under optimal conditions exhibits exponential growth (Kumala and Riisgård 2024). Studies on *H. oculata* showed a positive correlation between its growth rate and the concentration of carbon and nitrogen in suspended particulate matter, such as algae and bacteria, suggesting that increased particulate organic matter availability can enhance its growth (Koopmans and Wijffels 2008).

Evidence of organic enrichment at Coleg Normal

The Menai Strait has historically experienced high levels of organic enrichment. In 1993, the National Rivers Authority (NRA) expressed concern over the significant volume of untreated sewage being discharged at the Menai Strait’s northeastern entrance (NRA

1993). Around the same time, the Countryside Council for Wales (CCW) was proposing to designate the Menai Strait as a Marine Nature Reserve (MNR), with the ecologically rich Swellies region at its core. These concerns and proposals coincided with the introduction of the 1991 EC Urban Waste Water Treatment Directive (UWWTD), which drove widespread improvements in sewage infrastructure across Europe. In response, Dŵr Cymru committed over £20 million to upgrading sewage discharges in the area. The Menai Straits' status as a Designated Shellfish Water was also an important factor influencing these upgrades, particularly measures aimed at improving water quality and meeting microbiological standards for shellfish harvesting. As part of this effort, studies were undertaken to model sewage discharges into the Menai Strait and guide the design of new treatment systems (Robinson 1983).

A key environmental objective of the scheme was to address the Bangor outfall, then the largest source of crude sewage into the Menai Strait. At the time, untreated domestic effluent entered the Menai Strait from more than 25 locations. Two options were considered: upgrading and extending the Bangor outfall further into the Menai Strait, or diverting all Bangor flows 4 km southwest to the existing Treborth treatment works for processing and discharge there. The Treborth option was chosen despite a CCW-commissioned modelling study (Jones 1991) showing that while the Treborth option would improve water quality at the Bangor Shellfish Beds, it could worsen water quality in the Swellies, raising concerns about its potential ecological impact.

Following this, phased improvements to the sewage infrastructure were carried out as part of the UK's Asset Management Plans (AMPs). Initial assessments took place under AMP1 (1990–1995), followed by major investments under AMP2 (1995–2000) and AMP3 (2000–2005). The primary goal was to eliminate crude sewage discharges into the Menai Strait (including private discharges) and upgrade treatment facilities to comply with the requirements of the UWWTD.

Prior to these upgrades, the Coleg Normal monitoring site, located at the northeastern end of the Menai Strait, was likely affected by at least 17 major crude sewage outfalls, along with several secondary discharges. As part of AMP2, discharges from Menai Bridge, Llandegfan, Port Dinorwic, and Bangor were redirected to the Treborth treatment works, and many crude outfalls were upgraded to primary or secondary treatment (Figure 17). These interventions, completed by 2000, likely resulted in significant improvements in water quality, particularly through reductions in organic enrichment and suspended solids. Additionally, by 1998, there was a ban on all dumping of sewage sludge at sea (Wright 1992). However, there is limited published data available to clearly quantify these changes.

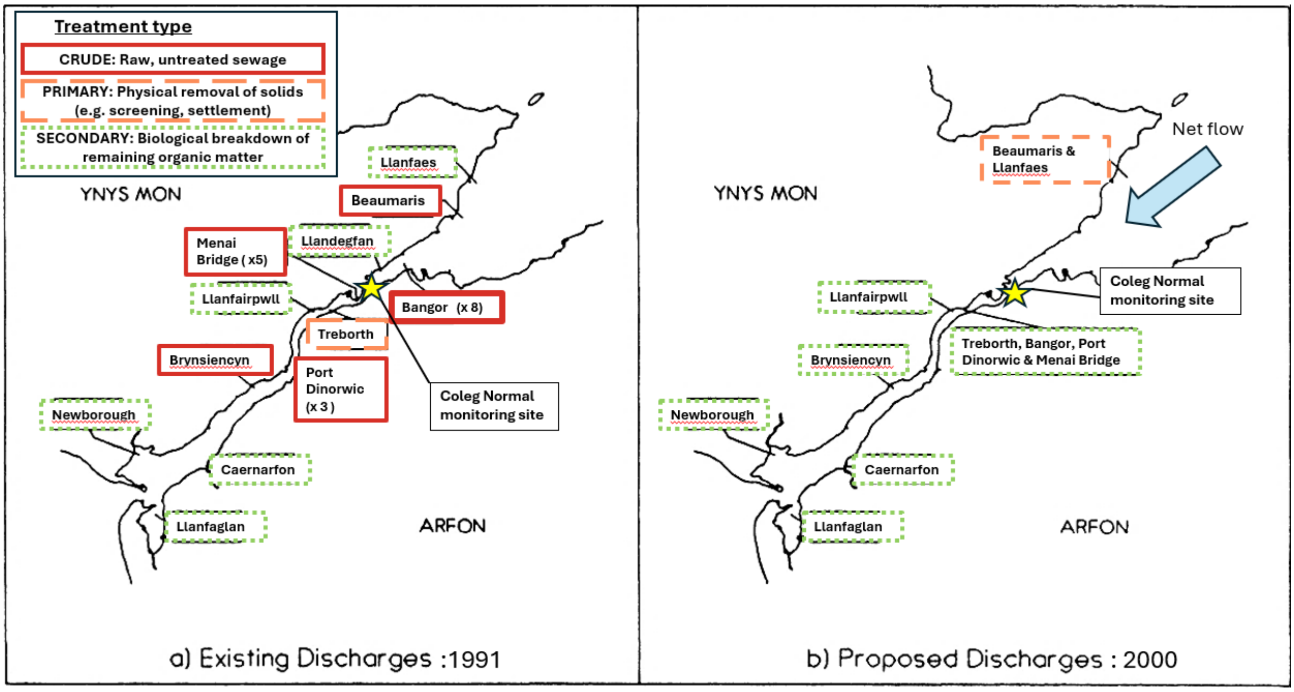


Figure 17: Major sewage discharges into the Menai Strait in 1991, prior to infrastructure upgrades, and proposed improvements planned for implementation by 2000 (adapted from Robinson, 1993). Crude sewage is raw, untreated wastewater. Primary treatment uses physical processes (e.g. screening, sedimentation) to remove some suspended solids and organic matter. Secondary treatment uses biological processes to further break down and remove remaining solids and organic matter. Note: This figure includes only major direct discharges into the Menai Strait and excludes private discharges and riverine inputs.

Microbiological monitoring by Dr. John Latchford at Bangor University began in 1994 at four sites along the Menai Strait, including Menai Bridge and Britannia Bridge (near the new outfall location), sampled during both high and low tides. Although this dataset remains unanalysed and unpublished, Latchford's personal observations indicate a substantial improvement in water quality following the upgrades in the 1990s (Latchford, pers. comm., 2006, Morris and Goudge 2006). In 2005, under AMP3, the Treborth works were further upgraded to include tertiary ultraviolet (UV) treatment, reflecting the conservation importance of the receiving waters. A few years after this upgrade, monitoring was discontinued, as coliform concentrations had dropped so low that continued sampling was no longer considered necessary (Latchford, pers. comm.,2006). This valuable dataset was unfortunately not available for inclusion in this report. However, these observations suggest that concerns about the potential negative impact of relocating the outfall on the core conservation area may not have been realised, possibly due to the secondary and tertiary upgrades at Treborth, and that overall water quality in the Menai Strait appears to have improved.

E.coli monitoring in shellfish flesh has been shown to reflect broad-scale improvements in coastal water quality following sewage infrastructure upgrades (Acornley et al. 2010). Between 1999 and 2008, *E. coli* levels declined significantly in 12% of the 57 shellfish areas assessed across England and Wales, mainly in regions where major sewerage upgrades eliminated crude sewage discharges and introduced secondary or tertiary treatments such as UV disinfection (Figure 18). This included the Menai Strait and areas in the wider Liverpool Bay, which directly influence the water quality in the Menai Strait. As a result of these improvements, the proportion of shellfish areas achieving Class B hygiene status increased from 69% to 86%. This included the Menai East shellfish beds, which were previously classified as Class B and C during the 1990s (NRA 1995). These beds subsequently improved to Class A and B, although they have reverted to Class B status from 2023 onwards (Carcinus Ltd 2025). The improvements in water quality were reflected not only in reduced *E. coli* levels but also in lower average faecal coliform counts in shellfish flesh from 2003 onwards (Figure 18).

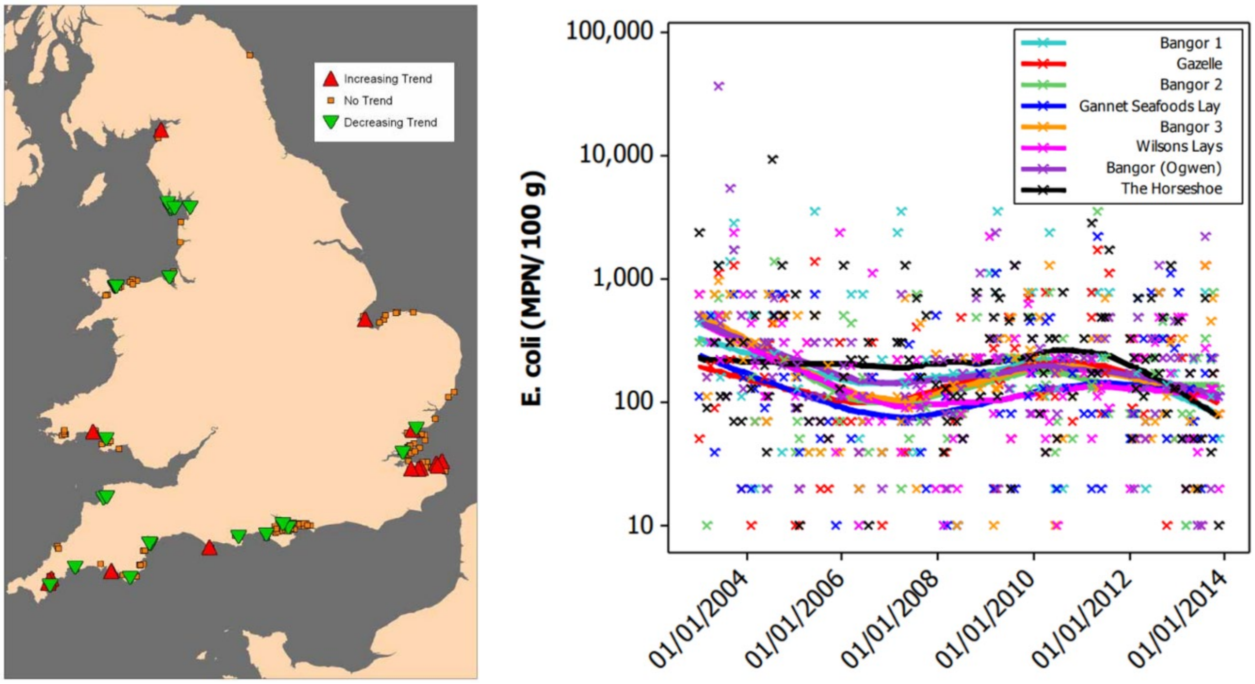


Figure 18: Left*:* Trends in *E.coli* concentrations in England and Wales from 1999 to 2008 (taken from Acornley et al. 2010) and Right*:* scatterplot of *E.coli* results from mussels at Menai East shellfish beds, showing decreasing trend in *E.coli* in samples of shellfish flesh pre-2004 to 2007 (Cefas 2013).

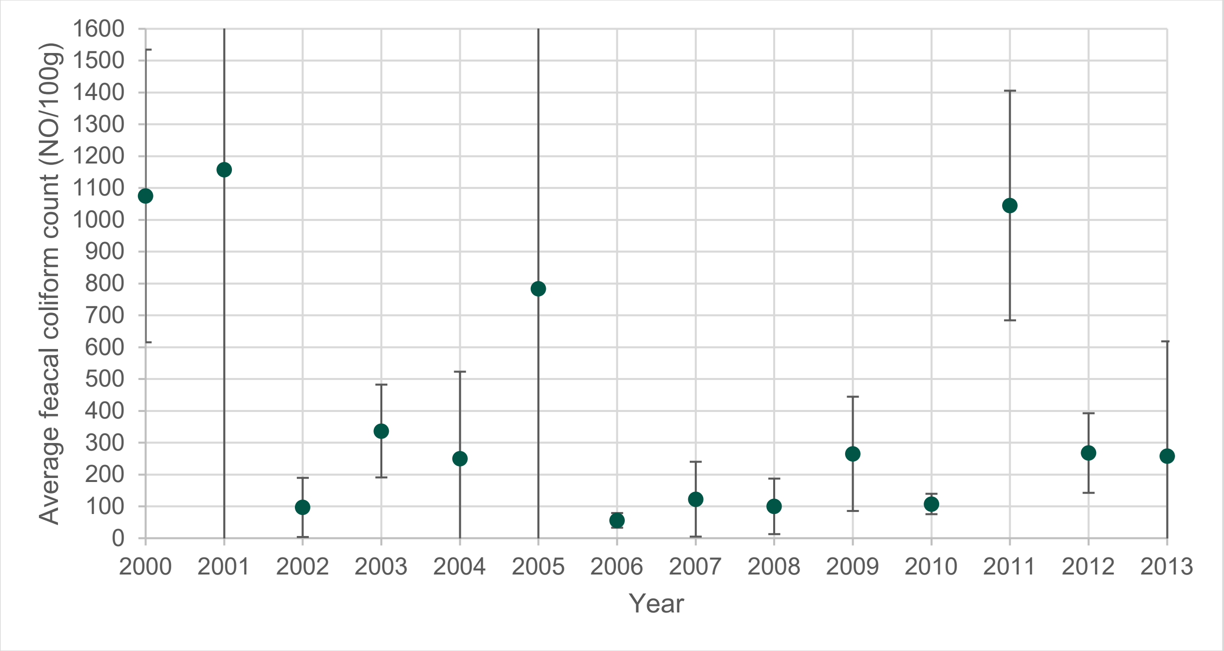


Figure 19: Average faecal coliform levels in Menai Strait East shellfish flesh. Data show a general decline in levels from 2000 onwards, though some variability remains. Lack of data before 2000 and possible differences in sampling frequency introduce some uncertainty in interpreting long-term trends. Data: Natural Resources Wales.

Further improvements under AMP3 (2000–2005) included upgrades to smaller local discharges, including the crude outfall at Coleg Normal, located directly adjacent to the Coleg Normal monitoring site (Figure 20). This was upgraded to a combined storm overflow (CSO), with the consent signed on 31 March 2004. Monitoring at this site began just over three months later, on 14 July 2004. At the same time, crude discharges near the Menai Suspension Bridge were also upgraded to CSOs. These upgrades coincide with the sharp decline in sponge luxuriance at the Coleg Normal site, suggesting a potential link between improved sewage treatment and reduced organic inputs, that may have supported suspension feeders such as sponges.

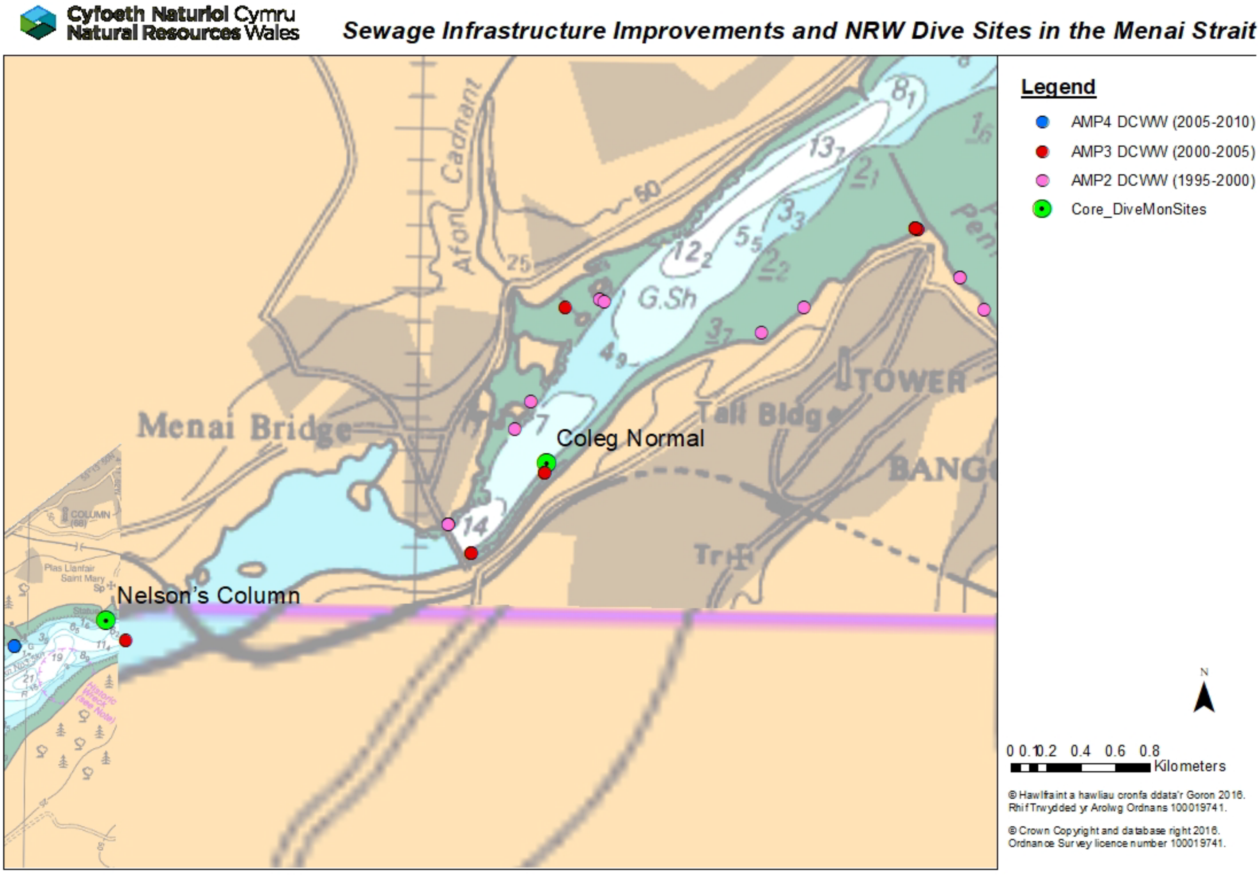


Figure 20: Map of the Menai Strait showing locations of phased sewage infrastructure improvements under the UK’s Asset Management Plans (AMPs). Coloured dots indicate the time period of each phase: pink (AMP2, 1995–2000), red (AMP3, 2000–2005), blue (AMP4, 2005–2010), and green marks the location of the NRW dive monitoring sites. Initial assessments began under AMP1 (1990–1995), with major investments in subsequent phases to eliminate crude sewage discharges and upgrade treatment facilities in line with UWWTD requirements.

Figure 21: Plots show trends in the occurrence and size of erect sponges (*Haliclona oculata*), and the occurrence and thickness of encrusting (crustose and cushion) sponges along the Coleg Normal transect between 2004 and 2023. Overlaid are the timings of the crude outfall upgrades at Coleg Normal and Menai Bridge to combined sewer overflows (CSOs) (box with dashed outline), the upgrade of Treborth to UV treatment (box with dotted outline), and the period of reduction in *E. coli* levels in Menai East mussel samples (pale green box).

These improvements to the sewage treatment in the Menai Strait and the resulting reductions in organic enrichment potentially had significant impacts on the sponge community. Prior to the upgrades, elevated organic inputs would have enhanced food availability for suspension feeders like sponges, potentially supporting high biomass. The available evidence for *H. panicea* and *H. oculata* suggests they may benefit from organically enriched environments. However, following the widespread removal of crude sewage discharges, and localised inputs at Coleg Normal and Menai Bridge, declines in suspended and particulate organic matter may have reduced their food supply, leading to the observed declines in sponge luxuriance.

However, it is important to note that high sponge biomass under organically enriched conditions does not necessarily indicate a healthy community. Duckworth & Pomponi

(2005) observed that while sponges exposed to the highest bacterial concentrations exhibited the greatest growth, they also experienced the lowest survival rates, with mortality associated with the development of a white biofilm. Notably, during 1988–89 in North Wales/Menai Strait, a potential disease in *H. panicea* was noted, characterised by a white biofilm, attributed to a possible fungal pathogen (Webster 2007). This occurred during the period of particularly poor water quality in the Menai Strait, suggesting that although sponges may have achieved high biomass in response to abundant organic inputs, their overall health and resilience may have been compromised under such degraded conditions.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of a *decline* in organic enrichment on the sponge community at the Coleg Normal monitoring site (Table 3). A timing score of 3 was assigned because there was a localised decrease in organic input during the decline period, but there were wide scale decreases in organic enrichment ongoing before and after this time period. Intensity was assigned 4 reflecting the level of change that occurred (crude to CSO). Frequency was assigned 4 as this was a permanent (continuous) change, and spatial scale was scored 4 since it would have affected the whole site. A medium/high sensitivity score (3) was given to reflect the moderate vulnerability to a decrease in organic enrichment, though with some recovery potential. The confidence was high for this assessment due to the availability of supporting literature and data.

Table 3: Assessment for **organic enrichment** pressure. Scores reflect the impact of a decrease in organic enrichment during the decline period (2004–2009), rather than an increase. Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature's susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
3	4	4	4	3	576	High

Aquaculture

Definition of pressure: The farming of aquatic organisms such as fish, shellfish and seaweed in controlled or semi-controlled environments

Sensitivity of species

This pressure was not included in the MarESA sensitivity assessment but has been considered here due to the presence of commercial mussel and oyster aquaculture sites within the MS&CB SAC, located in both the eastern and western Menai Strait. While sites in the eastern Menai Strait may pose potential risks to sponge communities, those in the western Menai Strait are unlikely to have a significant impact due to the tidal regime, which limits exchange between the two areas and reduces the likelihood of interaction with key sponge habitats.

Typical impacts from various forms of aquaculture on the marine environment are well documented and include organic and nutrient enrichment, sedimentation, spread of disease, introduction of invasive species and genetic impacts on wild stocks (Tičina et al. 2020).

The presence of mussel cultures (both suspended and bottom) can change the sedimentary environment with elevated levels of organically rich suspended particles, altering the infaunal benthic community (Smith and Shackley 2004; Ysebaert et al. 2009). This effect is localised, generally limited to the mussel beds and direct surroundings, though dependent on the biomass of the mussels present and the hydrodynamics of the site (Chamberlain et al. 2001; Beadman et al. 2003). Additionally, mussels filter large volumes of water and studies have shown a reduction in phytoplankton biomass in the water column, ranging from 10-74%, as a result of mussel cultures (Beadman et al. 2003).

Both the increase in sedimentation and reduction in phytoplankton could have negative impacts on sponge species as a result of smothering and siltation, and competition for food. However, there is some evidence that the size of particles retained by bivalves and sponges has little overlap suggesting effective food-resource partitioning (Stuart and Klumpp 1984). Many studies have also shown positive associations of sponge with aquaculture sites. The sponges benefit from the increased availability of organic particles and bacteria in the vicinity of the culture sites showing increased growth and survival in some cases (Gökalp et al. 2019). This has led to recent research focussed on their potential use in Integrated Multi-Trophic Aquaculture (IMTA) (Gökalp et al. 2021; Amato et al. 2024; Longo et al. 2025). These studies have primarily focussed on fish farming though the use of sponges to reduce the bacterial load in shellfish culture areas has also been demonstrated (Longo et al. 2016).

Sponges feed on microscopic particles suspended in the water, primarily phytoplankton, bacteria, and dissolved organic matter (DOM). The growth of both *H. panicea* and *H. oculata,* key sponges in the Menai Strait, is dependent on temperature and food concentration, with growth rates following seasonal trends (Koopmans and Wijffels 2008). In temperate waters, filter-feeding sponges face seasonal fluctuations in food availability. During winter, reduced light limits primary production, leading to low phytoplankton and bacterial biomass, resulting in seasonal tissue regression in many species. However, the relative importance of bacteria and phytoplankton in the diet of sponges remains unclear, with several studies focusing on *H. panicea*. One study suggests that phytoplankton alone is insufficient to meet the sponge's carbon requirements (Thomassen and Riisgård 1995), while another suggests that *H. panicea* may be able to sustain itself on a phytoplankton-only diet under natural conditions (Riisgård et al. 2016). Further research has shown that the sponge can efficiently retain both food sources, with bacteria consistently making up a smaller portion approximately 20% of its diet (Lüskow et al. 2019).

Overall, aquaculture activities such as shellfish farming can alter the marine environment which may negatively affect sponges by causing smothering and intensifying competition for food. While some studies suggest that sponges benefit from elevated organic particles and bacteria near culture sites, the overlap in phytoplankton use between bivalves and sponges raises concerns about resource competition, particularly during winter when food is limited. Although evidence of food partitioning exists, the extent to which this mitigates competition remains unclear.

Evidence of aquaculture impacts at Coleg Normal

The whole Menai Strait is classified as a shellfish harvesting area (including cockles, mussels and oysters), but the main aquaculture sites are the Eastern and Western Fishery Order areas. The Menai Strait (East) Mussel and Oyster Fishery Order area is the largest and located at the Northeastern entrance to the Menai Strait around 1km from the Coleg Normal sponge monitoring site (Figure 22). This is therefore the most likely to have an impact and will be discussed here. Notably, the Western Fishery does not currently operate under a Several Order, which limits the extent of regulated aquaculture activity in that area.

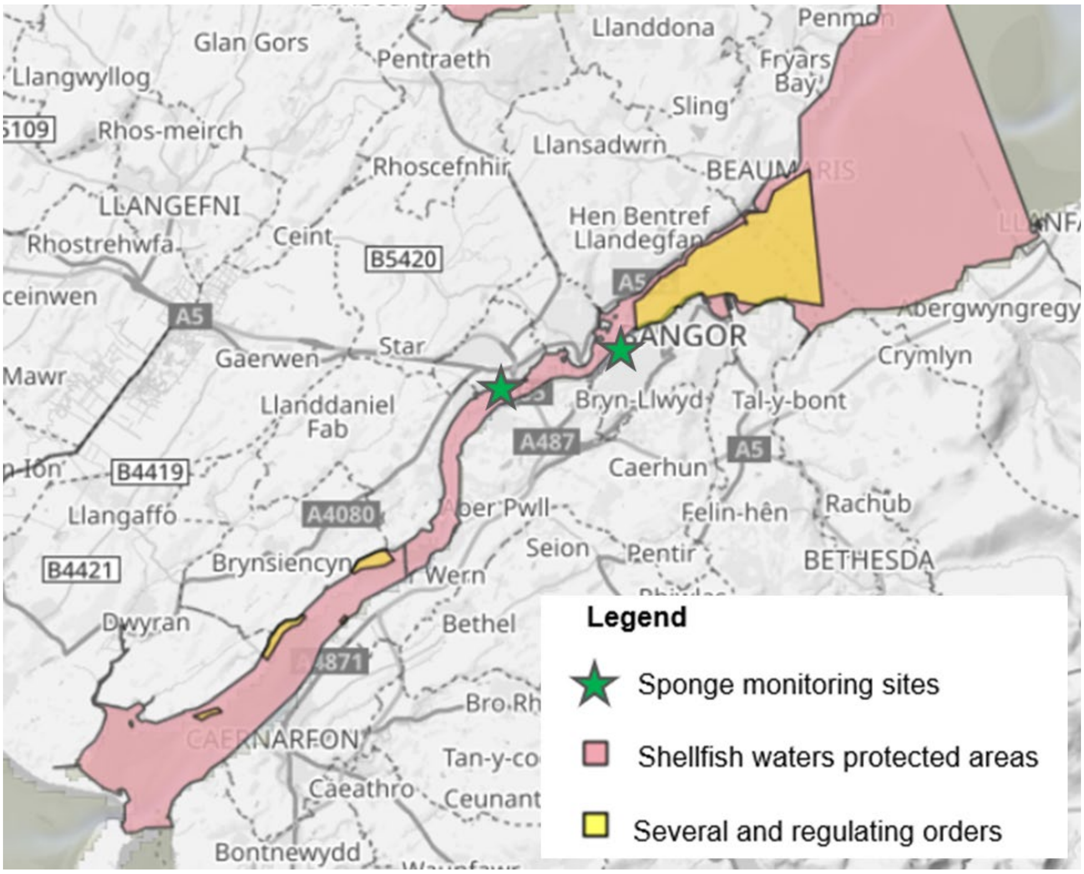


Figure 22: The Menai Strait with shellfish waters protected area shown in pink, the several and regulating orders for shellfish aquaculture shown in yellow, and the sponge monitoring sites within the Menai Strait indicated with green stars (adapted from Wales Marine Planning Portal, Welsh Government 2020).

The area is approximately 700ha and has been under a fishery order since 1962, though the history of shellfish cultivation in the eastern Menai Strait extends back to the late 19th century (Beadman et al. 2003). The fishery operates by collecting seed mussels from several areas and relaying them on ‘lays’ in the northern Menai Strait. After two years of cultivation, they are sold as adult mussels.

Mussel farming increased at the site following the signing of the fishery order (1962), remaining generally stable, though there has been some variability over time depending on seed availability and environmental conditions (Figure 23). In recent years there has been a decline in the fishery (reduction of mussels on the ground) related to a lack of seed mussel and a change to export rules following Brexit.

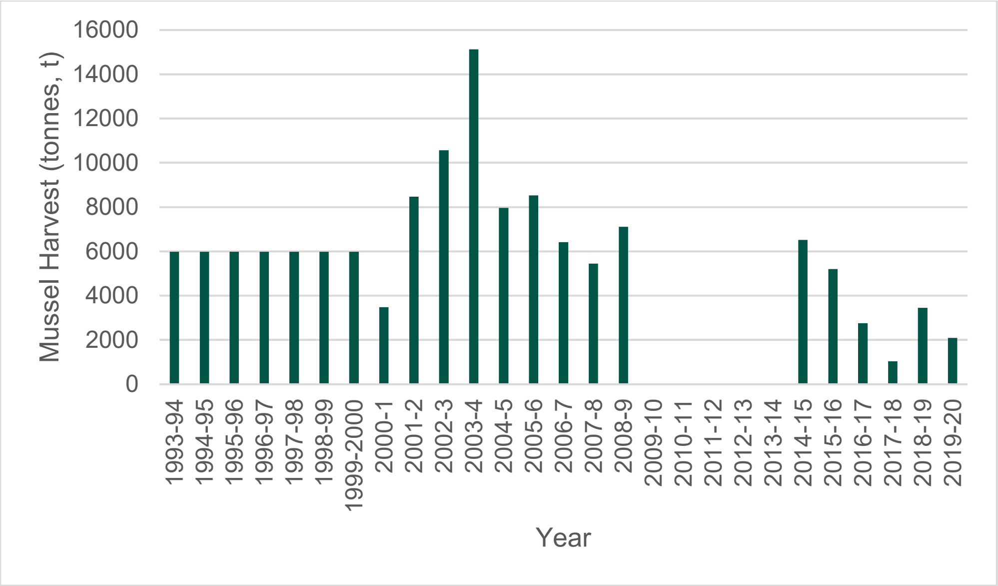


Figure 23: Annual mussel harvest from the Menai Strait (East) fishery, in tonnes, compiled from reports (1993-2000, Beadman 2003; 2000-2009, MacAlister Elliott and Partners Ltd 2010; 2014 – 2017, Welsh Government 2021. No data could be found for 2009-2014.

Several studies exist assessing the potential impacts of the fishery to the marine environment. Significant differences have been identified in the infaunal community (reduced abundance of individuals and species present) between mussel and mussel-free areas (between 0-10 m from the bed) becoming undetectable at a distance of between 20 and 80 m from the bed. These were positively correlated to mussel density (i.e. with increasing density, a reduction in abundance of individuals and species) (Beadman et al. 2004). Since the impact is localised this would be unlikely to affect the sponge community at Coleg Normal.

Further studies have investigated the carrying capacity of the Menai Strait by examining the phytoplankton consumption of the mussels, using chlorophyll a (Chl *a*) as an indicator. A gradient of Chl *a* concentration was identified, decreasing by half between the northeast entrance and Menai Bridge, attributed to mussel feeding (Figure 24) (Tweddle et al. 2005). Despite this reduction, Chl *a* remained available to the mussels, with potential food depletion only occurring above the mussel bed and for short-lived periods at slack water, when concentrations dropped just above the minimum threshold (Saurel et al. 2007). Additionally, the seawater in the Menai Strait has a short residence time of 2 to 3 days, while the clearance time by mussels was calculated at approximately 15 days in the subtidal area. It has been estimated that the total supply of phytoplankton imported into the Strait is 9 tonnes of carbon per day, and the amount consumed by filter feeders in the area of the mussel bed is 4.5 tonnes of carbon per day (Tweddle et al. 2005).

Although mussel beds clearly reduce phytoplankton concentrations, it remains uncertain how this affects competition for food among other components of the ecosystem that rely on similar resources. In particular, it is not known whether this reduction limits food availability for the sponge community at Coleg Normal, which may be sensitive to changes in phytoplankton abundance.

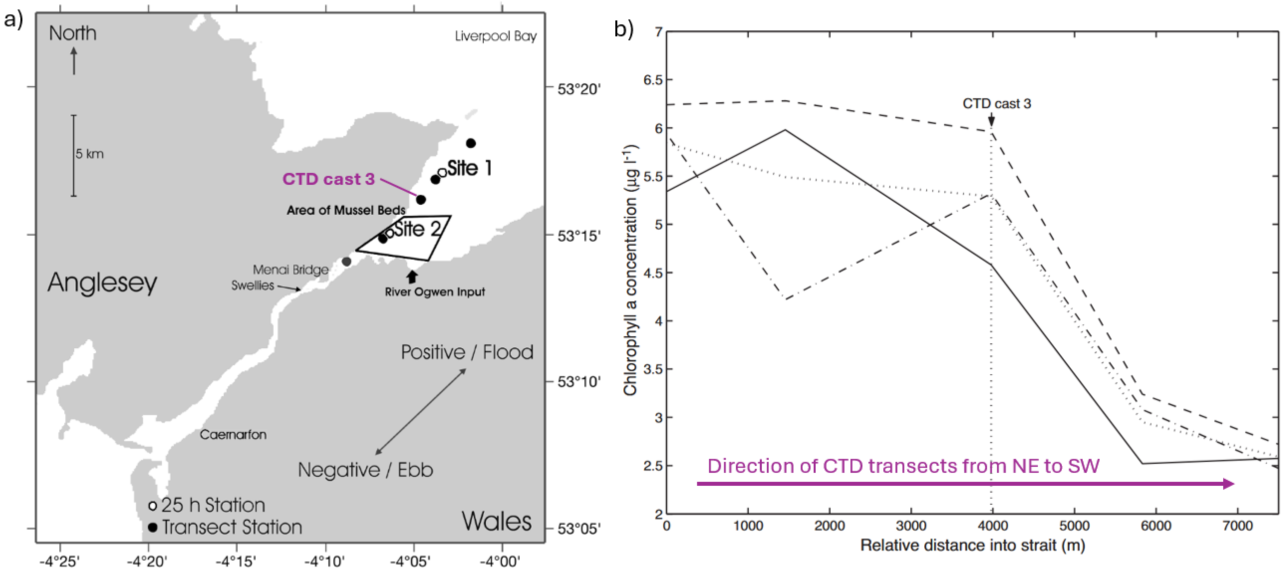


Figure 24: Conductivity, temperature and depth (CTD) profile in the Menai Strait: a) Location of sampling positions within the Menai Strait - ○: Site 1 and Site 2; •: transect stations; black outline: approximate mussel area. Purple text indicates the location of the CTD profile cast 3 b) CTD profiles collected along the transect through the Menai Strait (see location map a)) during ebb flow (towards the southwest), crossing over the mussel beds to estimate the along-channel gradient of chlorophyll. Measurements taken up to 4000 m into the strait (CTD cast 3) reflect upstream conditions relative to the mussel beds, while those beyond 4000 m represent downstream conditions. Chlorophyll-*a* concentrations were recorded at multiple depths: 1 m below the surface (———), 2.5 m above the seabed (– – – –), and 1 m above the seabed (– · – · –), with vertically averaged values shown as ·········· (taken from Tweddle et al. 2005).

Phytoplankton constitutes only part of the sponge diet, as sponges can efficiently retain extremely small particles, down to 0.1 µm, enabling them to compete effectively with other filter feeders (Thomassen & Riisgård, 1995; Stuart & Klump, 1984). Mussel beds may also have beneficial effects by enhancing nutrient remineralisation through the release of faeces and pseudofaeces, which can increase nutrient availability and stimulate primary production, potentially helping to offset phytoplankton depletion (e.g. Asmus & Asmus, 1991). Further research is needed to assess the extent of this nutrient-driven primary production and to better understand the nutrient requirements of the sponge community.

Monitoring began at Coleg Normal in 2004 and the immediate decline observed has continued despite a decrease in the mussel cultivation over this period. The fishery order under went an HRA and based on the available evidence this determined there would be “No adverse effect” on the integrity of the SAC. This suggests the mussel fishery is unlikely to have driven the decline, though more evidence is needed.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of a aquaculture on the sponge community at the Coleg Normal monitoring site (Table 4). A timing score of 2 was assigned as the occurrence of this activity is mostly outside the time period (a continuous pressures that occurred before and after the decline period as well as throughout). Intensity was given a 2 to reflect low intensity changes during the time period. Frequency was assigned 4 as this was a permanent (continuous) change, and spatial scale was scored 4 since it would have affected the whole site. A medium/low sensitivity score (2) was given to reflect the likely resilience of the sponge community to this activity. Though a confidence of medium was given for this assessment due to the limited availability of supporting literature and data related to food availability for the sponge community.

Table 4: Assessment for **aquaculture** pressure. Scores reflect the impact during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature’s susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
2	2	4	4	2	128	Medium

Ocean warming

Definition of pressure: The long-term increase in the average temperature or the world’s oceans caused by the retention of thermal energy due to the buildup of ‘greenhouse’ gases, such as CO_2 and CH_4

Sensitivity of species

The sensitivity assessment classified “cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock” (the biotope at Coleg Normal) resistance to temperature increase (local) as "high" with resilience rated as "high" and overall sensitivity as "not sensitive" (Readman et al. 2023) . This evaluation was based on a 5°C increase in temperature for one month, or 2°C for one year related to events or activities increasing local water temperature e.g. thermal discharges. Temperature increase related to global warming was not assessed (Readman et al. 2023) .

Increased sea temperatures as a result of global warming have generally been associated with negative impacts on sponges, though studies suggest they may be more tolerant to temperature increases than other benthic organisms (Kelmo et al. 2013; Bell et al. 2018). Temperature can affect sponges in multiple ways, ultimately leading to tissue necrosis, bleaching and disease (Figure 25). Mass sponge mortalities have been linked to elevated sea temperatures (e.g. Cebrian et al. 2011; Bell et al. 2023), and are thought to be driven by a combination of increased pathogen prevalence and virulence under warmer conditions, destabilization of sponge symbiotic microbial communities, and exceeding physiological thermal thresholds (Webster 2007). Tolerance of sponges varies between species and the temperature they are exposed to, with different responses also seen depending on life stage (Bell et al. 2018). For example, sponge settlers from heat-wave exposed sponges had greater growth rates under heatwave conditions than those from sponges under ambient conditions (Strano et al. 2023).

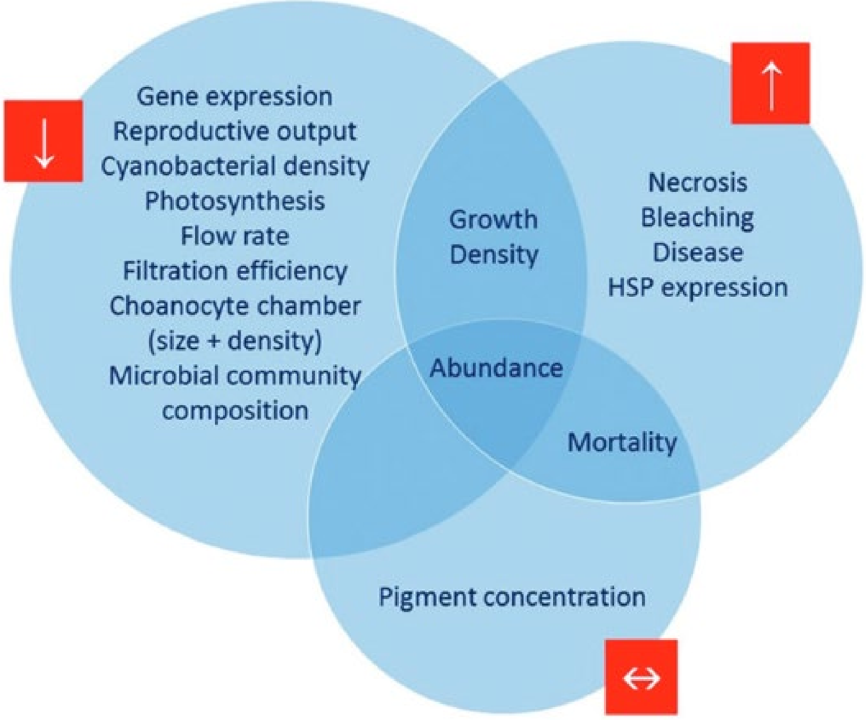


Figure 25: Summary of effects of ocean warming on sponges based on 44 studies from between 2000-2017. The effects of ocean warming include decreased reproductive output,

photosynthetic rates, pumping rates, filtration efficiency, choanocyte chamber size and density, and changes to microbial community composition and microbial function. OW also causes increases in necrosis, bleaching, disease, and heat shock protein production, whereas mortality, abundance, and growth are more variable responses (adapted from Bell et al. 2018)

Temperature effects are particularly pronounced during marine heatwaves (MHWs), which have impacted both tropical and temperate species. For example, a series of prolonged, high-intensity (>100 days duration, >2°C increase) MHWs in New Zealand caused widespread bleaching and necrosis with only limited impact from shorter events (Bell et al. 2023). In the Mediterranean, mass mortality was correlated with temperature anomalies of just 1–2 °C above normal, with a significant positive relationship between mortality rate and the proportion of time sponges were exposed to temperatures above a critical threshold (Cebrián et al. 2011). Species with microbial symbionts were especially vulnerable, likely due to heat-induced dysbiosis and increased susceptibility to opportunistic pathogens. In another example the temperate sponge, *Crella incrustans*, showed a significant increase in respiration, tissue necrosis, mortality, and a decrease in survivability of sponge settlers only under simulated MHW conditions (16 days at 22°C) (Strano et al. 2022). Additionally, heat stress altered the sponge microbiomes, with carryover effects on the larval microbial community, impacting development (Strano et al. 2023). An increase in respiration rate is a common response to elevated temperatures reported in several studies (Taylor et al. 2021; Strano et al. 2022; Maggioni et al. 2023; Bosch-Belmar et al. 2023).

Although prolonged and extreme MHWs can have severe impacts, responses are variable with adaptation and recovery seen in some species, particularly under gradual warming rather than acute heatwave conditions (Kelmo et al. 2013; Strano et al. 2022, 2023; Bosch-Belmar et al. 2023). Recent research highlights the importance of considering the combined effects of elevated temperature and other anthropogenic stressors, such as nutrient enrichment and deoxygenation. Some studies indicate negative interactions with elevated seawater temperatures, leading to metabolic shifts and a reduced capacity for nutrient cycling, potentially disrupting the sponge loop pathway (Botté et al. 2023; Maggioni et al. 2023). However, evidence is mixed, as other studies suggest that short-term thermal exposure has limited effects (Simister et al. 2012).

There is limited direct evidence on the thermal tolerance of key sponge species found in the Menai Strait. Increased pumping rates have been observed in *H. panicea* with rising temperature, though this only looked at a temperature change from 6 to 12°C, within the species' normal environmental range (Riisgård et al. 1993). The Menai Strait is the centre of the range for *H. panicea*, *H. oculata*, and *A. fucorum* (Readman et al. 2023), suggesting that short-term increases in temperature are unlikely to affect them significantly, as they are not living near their upper thermal limits. Additionally, *H. panicea* is also found intertidally, where it experiences naturally higher temperatures, further indicating a degree of thermal resilience. There is some indication of temperature-related impacts on *H. oculata,* though thermal tolerances have never been directly tested. For example, a mass mortality was recorded off the Dutch coast following a warm summer, where seawater temperatures rose to 23°C, approximately 3°C warmer than usual (Koopmans and Wijffels 2008) . Additionally, thermal stress was suggested as the primary cause of the complete loss of *H. oculata* at Stratford Shoal in the northwest Atlantic where temperatures rose as high as 26°C (Stefaniak et al. 2014). No evidence could be found for *A. fucorum*.

Evidence of global warming at Coleg Normal

Available data for sea surface temperature (SST) in the Menai Strait show a long-term warming trajectory, with the annual mean nearly 2°C higher in 2021 than in the 1950s, with most of this increase occurring since the late 1980s to early 1990s (Figure 26). A period of lasting high sea temperatures began in the mid-1990s **Error! Reference source not found.**. During the period of steep decline in sponge luxuriance (2004-2008) modelled data show a sustained rise in mean annual SST, approximately 0.5°C over three years (Figure 26). However, this was followed by a period of lower-than-average temperatures that did not correspond with a recovery in sponge luxuriance, suggesting that warming may not be the sole driver of the decline.

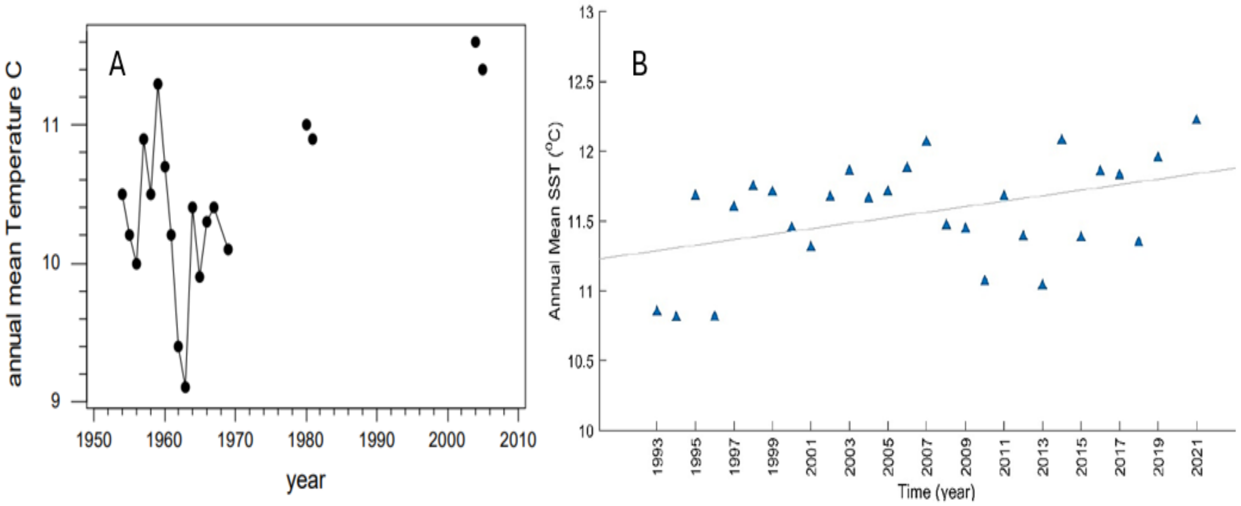


Figure 26: Changes in in-situ and modelled annual mean seawater temperature in the Menai Strait: (A) Time series of annual mean water temperature in the Menai Strait between 1950-1981 and additional data 2004 and 2005 (taken from Bowers 2006), (B) Modelled annual mean sea surface temperature (SST) between 1993-2021 (taken from Smyth et al. 2022).

A marine heatwave (MHW) is a period of anomalously warm SST. Between 2000 and 2016, the frequency of MHWs around the UK increased by an average of four events per year. A 25-year continuous monitoring station in the Western Irish Sea recorded 45 MHW events between 1997 and 2022, though with no evidence of a change in duration (Cornes et al. 2023) (Figure 27). The general duration of MHW events is between 10 and 20 days around the UK. Long-term observational records from the Menai Strait show typical seasonal temperatures fluctuated between approximately 4 and 17°C from 1961 to 1999, and between 4 and 18°C from 2011 – 2019 (Evans and Mitchelson-Jacob 2001; Smyth et al. 2022). A peak temperature of 20.2°C was recorded in August 1995, and similar high temperatures (19°C and above) were recorded during summer 2014 and 2018, with a duration of more than 20 days (in 2014 and 2018) (Figure 28). In addition, spring tides have been observed to raise temperatures by up to 1°C, especially during winter, potentially contributing to localised short term microclimatic conditions (Bowers 2006).

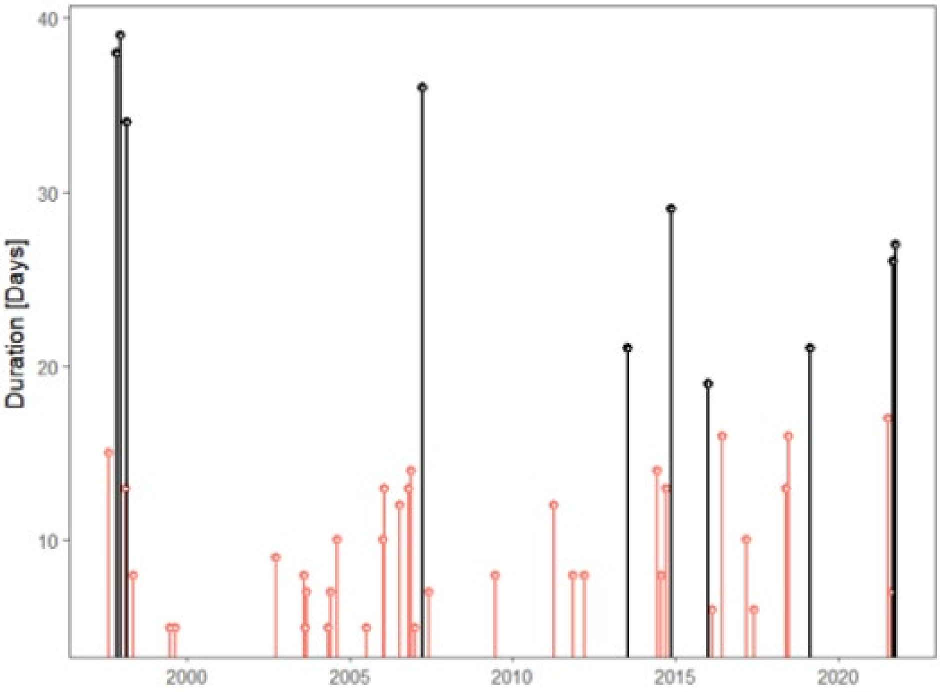


Figure 27: The duration of marine heatwave events recorded in the western Irish Sea between 1997 and 2022, with values above 20 days highlighted in black (taken from Cornes et al. 2023)

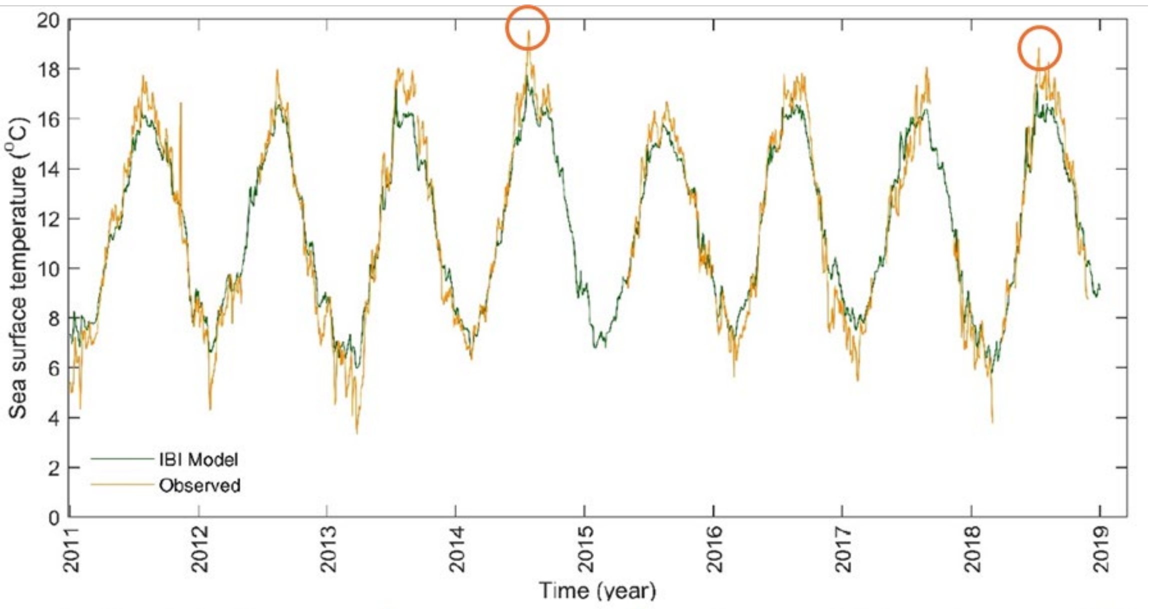


Figure 28: Sea surface temperature (SST) logger data obtained from a temperature logger attached to Menai Bridge Pier, at 1m depth (in orange) and modelled data (in green), from 2011-2019. Orange circles indicate temperatures 19°C and above experienced in 2014 and 2019 (taken from Smyth et al. 2022)

The Menai Strait is the centre of the range for *H. panicea, H. oculata* and *A. fucorum* (Readman et al. 2023), suggesting that short-term increases in temperature are unlikely to affect them significantly as they are not living near their upper thermal limits. Additionally, *H. panicea* is also found intertidally where it will experience higher temperatures. Cyclical changes in *H. oculata* abundance and cover, observed at the monitoring sites Nelson's Column (Menai Strait) and Warrior (Milford Haven), do not coincide temporally, indicating these patterns are unlikely driven by widespread temperature changes. However, exposure to MHW conditions may still cause adverse effects, including tissue regression, disease, and mortality, as reported in other temperate sponge species even after short-term exposure (Taylor et al. 2021; Strano et al. 2022; Bosch-Belmar et al. 2023). Available data suggest that MHWs in the region typically involve temperature increases of 1–2 °C. Further research is needed to determine whether such increases, particularly when combined with other stressors, are sufficient to cause harm to these species.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of ocean warming on the sponge community at the Coleg Normal monitoring site (Table 5). A timing score of 2 was assigned because this is an ongoing pressure that occurred throughout the time period but mostly outside (a continuous pressure that occurred before and after the decline period as well as throughout). Intensity was assigned 2 to reflect the low intensity changes over the short 2004 – 2009 assessment window, though it should be noted this is part of a long-term and significant warming trajectory. Frequency was assigned 4 as this was a permanent (continuous) change, and spatial scale was scored 4 since it would have affected the whole site. A medium/low sensitivity score (2) was given to reflect the likely resilience of the sponge community to ocean warming at this level, though MHWs are likely to have greater impact due to short-term thermal extremes; further evidence is needed to assess this risk. The confidence was medium for this assessment due to the limited availability of supporting literature and data specific to the sensitivities of the Menai Strait sponges.

Table 5: Assessment for **ocean warming** pressure. Scores reflect the impact during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature's susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
2	2	4	4	2	128	Medium

Nutrient enrichment (i.e. nitrogen, phosphorus & silicon)

Definition of pressure: increased levels of inorganic nutrients, especially nitrogen, phosphorus and silicon in the marine environment.

Sensitivity of species

The sensitivity assessment (Readman et al. 2023) classified “cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock” (the biotope at Coleg Normal) resistance to nutrient enrichment as ″not relevant" with resilience rated as "not relevant" and overall sensitivity as "not sensitive", at a benchmark set at compliance with Water Framework Directive (WFD) criteria for good status, based on nitrogen concentration.

Nutrient enrichment relates to the input of nutrients, particularly inorganic, into the marine environment, through natural processes (e.g. decomposition of detritus) or anthropogenic inputs (e.g. agricultural runoff/sewage discharges). Key nutrients are dissolved inorganic nitrogen (DIN - which comprises nitrate, nitrite, or ammonium), phosphorous (as phosphate) and silicon (as silicate). This can lead to eutrophication, particularly in sheltered water bodies.

Sponges are highly efficient filter feeders, and many studies increasingly recognise their importance in benthic-pelagic coupling and, in particular, nutrient cycling (e.g. nitrogen, phosphate and silicate). While much of the research is focused on coral reef systems (Holmes 2000; Ward-Paige et al. 2005; Simister et al. 2012), this has also been demonstrated in temperate environments (Jiménez and Ribes 2007; Perea-Blázquez et al. 2012). These nutrient cycling processes are primarily driven by sponge-associated microbial communities capable of processing both organic and inorganic nutrients through mechanisms such as nitrification, denitrification and ammonium oxidation (Maldonado et al. 2008; Pita et al. 2018; Maggioni et al. 2023).

However, the direct impact of nutrient enrichment on sponge growth and health is variable. Unlike organic matter, inorganic nutrients do not act as a direct food source for sponges. Instead, any positive effects are likely to be indirect, resulting from increased microbial and phytoplankton productivity, which can elevate levels of particulate organic carbon (POC) and dissolved organic carbon (DOC) that sponges utilise (Pita et al. 2018). Some studies have reported increases in sponge size in nutrient-enriched environments (Holmes 2000; Ward-Paige et al. 2005), but this is not consistently observed. In many cases, nutrient enrichment shows no significant effect on sponge growth, physiology, or microbial symbiont health (Gochfeld et al. 2012; Simister et al. 2012; Luter et al. 2014; Ramsby et al. 2020). For example, Ramsby et al. (2020) found that DIN enrichment had no measurable effect on growth or condition in five common Great Barrier Reef sponge species and Simister et al. (2012) found no adverse effect on sponges exposed to a range of nutrient concentrations. Although limited, there are some available studies that indicate potential negative impacts on sponges under nutrient enrichment. For example, *H. oculata*, showed negative growth rates in response to elevated concentrations of nitrate, ammonium, phosphate, and salinity (Koopmans and Wijffels 2008), while eutrophication was linked to sponge cover loss and necrosis in the enclosed system of Lough Hyne (Micaroni et al. 2021).

Overall, sponges responses to moderate nutrient enrichment appear relatively stable and species-dependent. Although they can utilise the enrichment for nutrient cycling and helping to buffer eutrophication (Bell 2008), it does not consistently enhance growth. In addition, when nutrient inputs are combined with stressors such as warming or hypoxia, they may exceed physiological thresholds, leading to microbial dysbiosis (imbalance of microorganisms in the microbiome), disease, and sponge decline (Pita et al. 2018; Campana et al. 2021).

Evidence of nutrient enrichment at Coleg Normal

A long term data set compiled by Evans et al. (2003) looks at nutrient trends in the Menai Strait from 1963 to 2002. The data includes chemical (nitrate, nitrite, total particulate nitrogen, phosphate, total dissolved phosphorus, ammonia, silicate), physical (salinity, temperature and light attenuation) and biological observations. This shows a significant increase in nutrient concentrations from the 1960s, reaching a peak in the 1980s, followed by a decline in the 1990s (Figure 29). Comparable patterns were observed in the wider Irish Sea, identified by data recorded at the Cypris station, Isle of Man, though the changes through the decades are smaller. Nutrient concentrations were higher in the Menai Strait, attributed to its inshore location and proximity to Liverpool Bay, a major source of nutrient input. The observed variations were largely linked to anthropogenic influences, particularly the phosphate loading. The reduction in phosphate concentrations from the late 1980s into the early 1990s aligns with similar trends across Europe, which have been associated with the implementation of phosphate reduction measures and UWWTD upgrades (see Civan et al. 2018). However, for other nutrients, anthropogenic sources alone do not account for the observed changes. Climatic factors, including variability in the North Atlantic Oscillation (NAO) and wind strength, are also implicated as significant drivers (Evans et al. 2003).

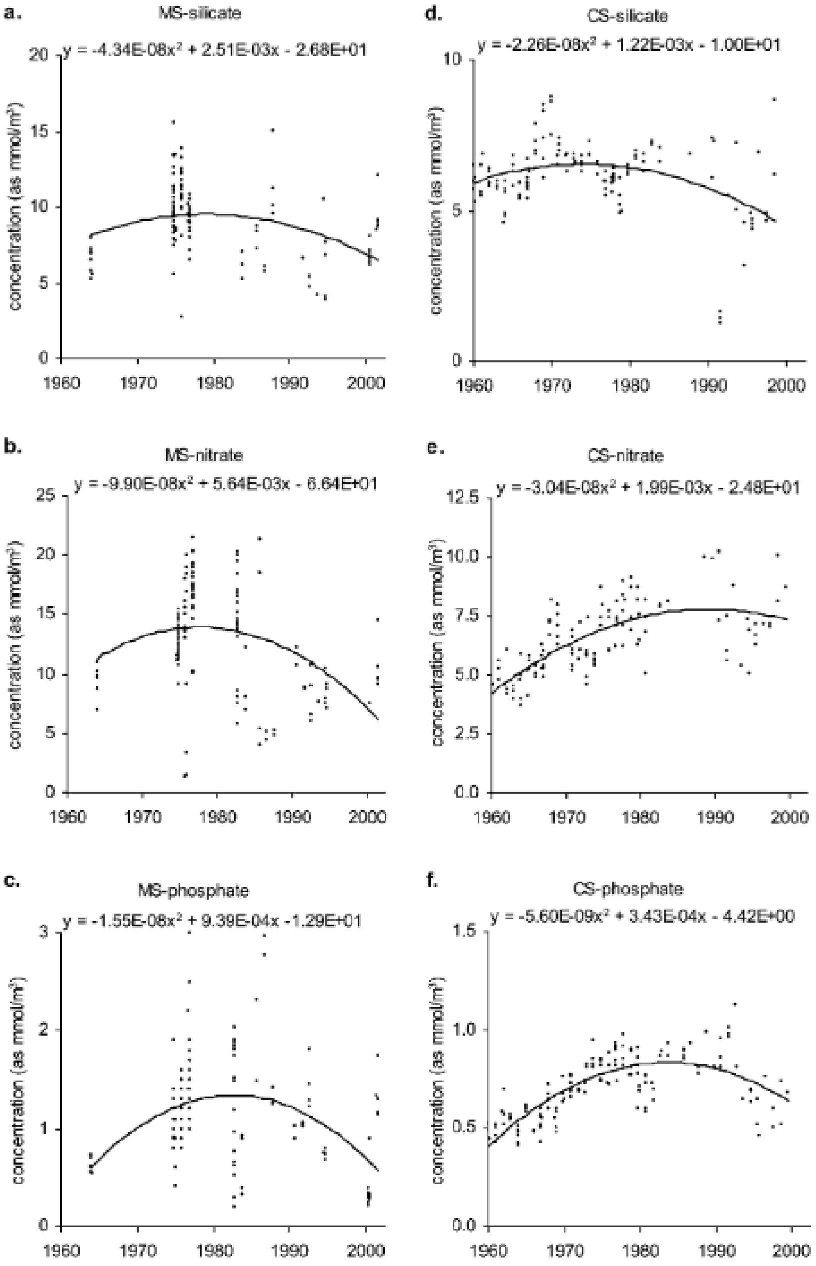


Figure 29: Trends in winter silicate, nitrate and phosphate measurements in the Menai (MS) and wider Irish Sea (Cypris station (CS)) between 1960 and 2002 (taken from Evans et al. 2003).

Although this data stops in 2002, a continued decline in nutrient levels, particularly phosphorus and nitrogen, is supported by other sources, including the OSPAR assessment of nutrient inputs across the North-East Atlantic (Axe et al. 2022). According to the 2023 Quality Status Report, total nitrogen inputs to the Celtic Seas region decreased by approximately one-third between 1990 and 2018. This reduction has been largely attributed to declining atmospheric emissions.

Sponges may respond to low nutrient conditions by developing elongated (1–2 cm) tissue outgrowths that increase surface area for feeding (Duckworth & Pomponi 2005). These thin, filamentous structures can enhance particle capture without significantly increasing biomass or luxuriance. The apparent rise in *A. fucorum* abundance in MNCR data from the Menai Strait may reflect this morphological shift, or simply improved detection as a result of the more visible outgrowths being recorded as increased abundance.

Locally, the Water Framework Directive (WFD) Cycle 1 assessment, reported in 2009 and based on monitoring from 2004–2008, classified the Ogwen lower catchment as “poor” for ecological, biological and overall status (Figure 30). Several other rivers feeding into the northeastern Menai Strait were rated “moderate” indicating regional impacts to water quality at the time. The Menai Strait waterbody itself was classified as “moderate” for ecological and overall status, and “good” for DIN. This marine classification likely reflects the influence of riverine inputs overall, though the poor rating for the Ogwen catchment was not directly mirrored in the marine assessment. More recent WFD assessments in Cycles 2 and 3 (2015 and 2021) show improvement, with the Menai Strait achieving “good” status, indicating a general enhancement in water quality.

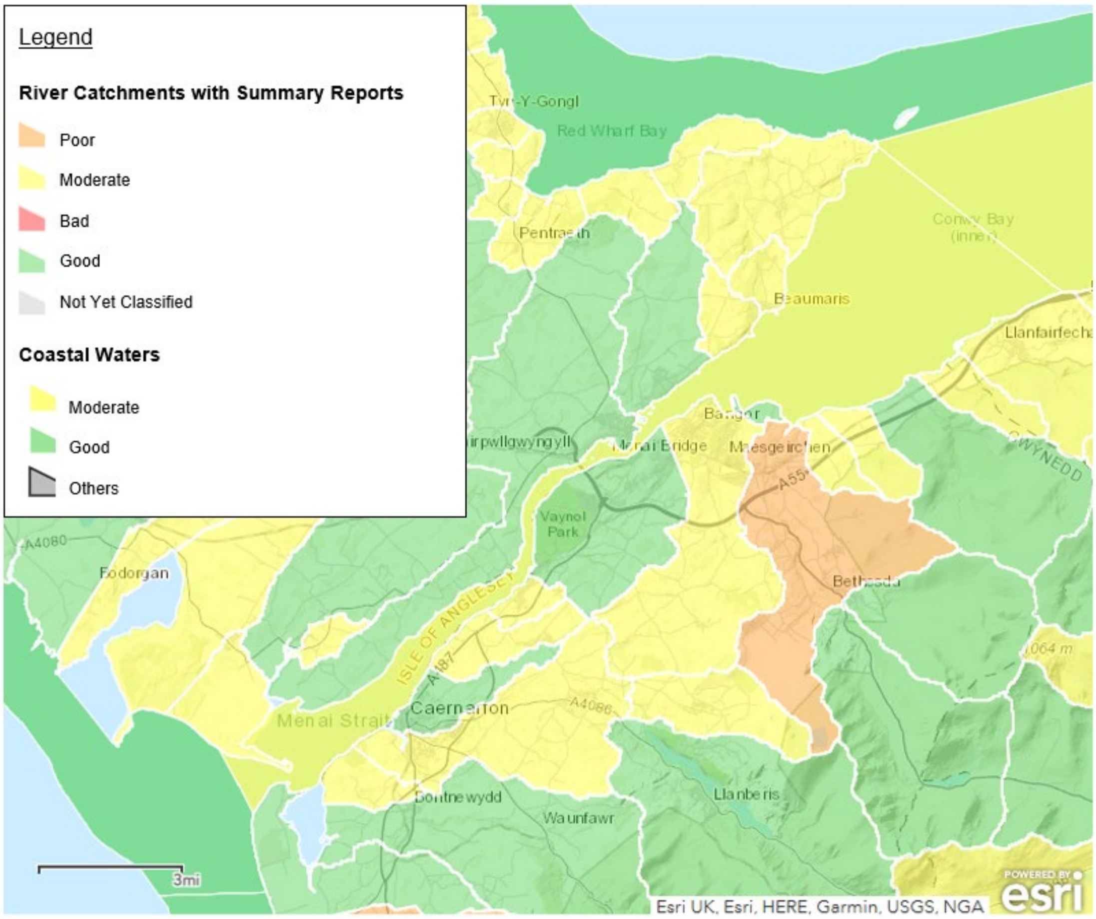


Figure 30: Water Framework Directive Cycle 1 (2009) River catchment classification map, focussed on the Menai Strait. The Ogwen – lower is classified as Poor, indicated by orange fill (NRW 2025).

Agricultural pressures, particularly intensified upland grazing in North Wales, likely continue to influence nutrient levels in the Menai Strait through runoff. Since the 1950s, sheep numbers in England and Wales have more than doubled, with a steady increase beginning in the mid-1970s. By around 2000, grazing pressure on the Carneddau uplands had risen from 1.2 sheep per hectare to 5–6 sheep per hectare (Britton et al. 2005) with associated land disturbance from livestock, vehicles, and drainage increasing the risk of nutrient-rich runoff entering the Menai Strait via the Ogwen river. In addition to sheep, changes in cattle farming systems from the 1980s, shifting from straw-based winter housing and farmyard manure to slurry systems, likely increased the risk of organic pollution and runoff incidents (SSAFO Regulations 1991; Ramos et al. 2006; Lloyd et al. 2015). However, the "good" WFD status achieved for the Menai Strait after 2009 suggests either that this input was not significant at the marine scale or that effective land management practices were in place to mitigate their impact.

Available evidence suggests that nutrient enrichment is unlikely to be the primary cause of a sponge decline at Coleg Normal. Nutrient levels in the Menai Strait peaked in the 1980s and declined from the 1990s onward, with continued improvements supported by recent assessments. The relevant biotope is considered "not sensitive" to nutrient enrichment at levels compliant with WFD "good" status. While sponges may benefit indirectly from increased organic matter associated with nutrient inputs, most studies report variable or neutral responses to moderate enrichment. The observed decline in sponge abundance between 2004 and 2009 occurred after nutrient levels had already decreased, making a direct link unlikely. However, it is possible this decline is part of a longer-term trend coinciding with nutrient reductions, though current site-specific data are insufficient to confirm this. Further research is needed to clarify species-specific responses and the role of interacting stressors.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of a *decrease* in nutrient enrichment on the sponge community at the Coleg Normal monitoring site (Table 6). A timing score of 2 was assigned as a decrease in nutrient (part of a Irish sea wide decrease) was largely outside the time period, although there were some potential localised changes during the time period. Intensity was given a 2 to reflect low intensity changes during the time period, though it should be noted there were higher intensity changes in the past. Frequency was assigned 3 reflecting an intermittent but ongoing pressure, and spatial scale was scored 4 since it would have affected the whole site. A medium/low sensitivity score (2) was given to reflect the likely resilience of the sponge community to changes in nutrients. However, a low confidence score was given for this assessment due to the limited availability of supporting literature and data related to nutrient levels and the impacts on sponge growth in the Menai Strait.

Table 6: Assessment for **nutrient enrichment** pressure. Scores reflect the impact of a decrease in nutrient enrichment during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the

pressure; sensitivity = a feature's susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
2	2	3	4	2	96	Low

Changes in suspended solids (water clarity)

Definition of pressure: Changes in water clarity from sediment & organic particulate matter concentrations in the water column.

Sensitivity of species

The sensitivity assessment classified "cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock" (the biotope at Coleg Normal) resistance to changes in suspended solids (water clarity) as ″high" with resilience rated as "high" and overall sensitivity as "not sensitive" (Readman et al. 2023).

As filter feeders, sponges are susceptible to changes in suspended solids. This can have both negative and positive impacts, depending on the species, habitat, environmental conditions and duration of exposure. The impacts of both settled and suspended sediment on different aspects of sponge ecology and physiology are summarised by Bell et al. (2015). Potential negative impacts of increased levels of suspended solids include 1) clogging of inhalant pores and canals, reducing pumping rates and thereby impacting feeding efficiency, respiration and growth (Gerrodette and Flechsig 1979; Bannister et al. 2012; Pineda et al. 2017); 2) scouring of external surfaces (Nava and Carballo 2013); and 3) increasing turbidity, limiting light penetration, impacting phototrophic species (Lemloh et al. 2009).

However, despite these negative impacts, many sponge species are commonly found in environments with high levels of suspended solids. For example at Lough Hyne and Skomer Island, rich sponge assemblages are found in highly sedimented environments (Bell and Barnes 2000; Bell et al. 2006; Bell 2007). Studies suggest that some species that regularly experience high levels of suspended solids may have adaptive mechanisms to survive in these conditions such as changing their morphology type, incorporating sediments into their tissues or employing mechanisms to remove sediment from their tissue, though this may all come at a metabolic cost (Schönberg 2016, 2021; McGrath et al. 2017; Goldstein et al. 2024). Because sponges show a wide variety of responses to sediment, the sensitivity of each species to suspended sediments should be evaluated individually where possible. For the key species found in the Menai Strait (*H. panicea, H. oculata* and *A. fucorum*), the limited available information, combined with their persistent presence in this highly turbid environment, a defining feature of the biotope's physical description, suggests a degree of resilience to suspended sediment.

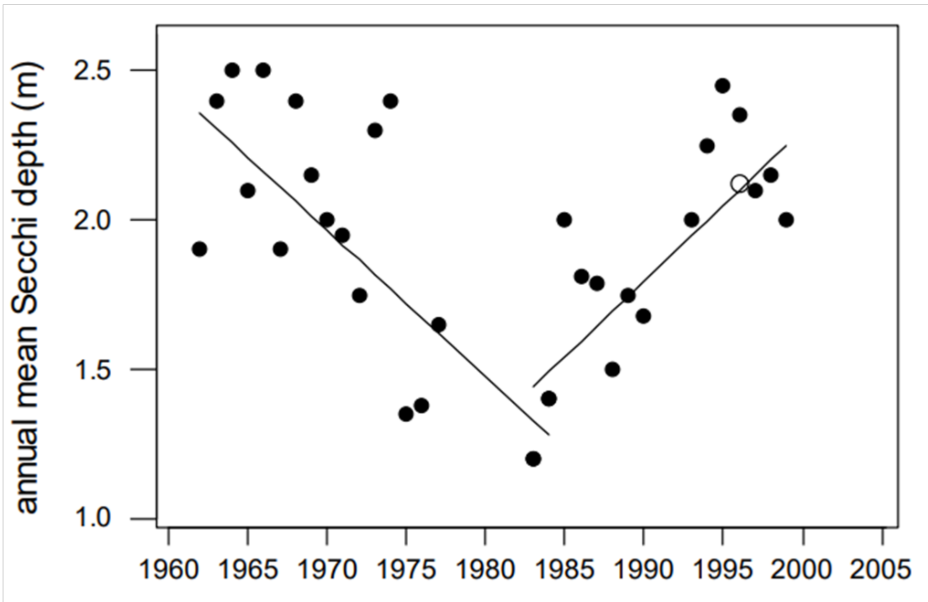
For example, *H. panicea* is commonly found in low-light environments, indicating that reductions in water clarity due to suspended solids are unlikely to be problematic under typical conditions. However, there is some evidence that very high concentrations of suspended solids (greater than 50 mg/l) can be detrimental (Goldstein et al. 2024). *H. oculata* is often associated with silted waters, typically occurring on rocky or sandy substrates and frequently attached to stones and rocks. This species is known to tolerate both low salinity and turbid water with suspended silt (Mayhew 2006). Notably, one study found that the growth rate of *H. oculata* correlated with the availability of carbon from suspended particulate matter, suggesting that a sudden increase in suspended organic matter may even stimulate growth (Koopmans and Wijffels 2008). Specific evidence for *A. fucorum* is limited, but its co-occurrence with these other species in turbid habitats implies similar tolerance.

Evidence of changes in suspended solids at Coleg Normal

Between 1960 and 2000, Secchi disk depth recordings were taken in the Menai Strait near Menai Bridge which provide one of the only time series data sets available to look at changes in suspended solids. This data indicates that there has been a trend of increasing water clarity since 1985, when peak turbidity was recorded, returning to conditions seen in the 1960s (Figure 31) (Bowers 2006). Analysis of the sediment type identified that a considerable proportion was organic (i.e. phytoplankton, bacteria and detritus) but that the majority of the suspended sediments were inorganic (e.g. clay and mud). Several reasons have been discussed for the changes in suspended solids but a single cause has not been attributed (Birkett and Maggs 2001; Kratzer et al. 2003; Bowers 2006). For example, dredge spoil was dumped north of Puffin Island during the 1960s and 70s, with a large inorganic component, and the cessation of this activity could have contributed to the improvement in water clarity. Changes in land management practices and canalisation and reclamation of land in the Conwy estuary during this period could also have contributed by increasing run-off from land. Alternatively, the turbidity changes may be linked to wind speed, though the relationship is not perfect (White et al. 2003; Bowers 2006).

Two further important activities affecting suspended solids in the Menai Strait, aquaculture and wastewater inputs, are discussed in detail elsewhere in this report. However, it is relevant to note here that mussel cultivation removes chlorophyll a (Chl *a*) but may also influence suspended solids, either reducing or producing them. The introduction of EU wastewater regulations in 1991 led to ongoing improvements in water quality, likely reducing suspended solid inputs, as has been observed in other regions through Secchi disk records (Borkman 1998). Additional local improvements included the 1998 ban on sewage sludge dumping in Liverpool Bay. Agricultural inputs have generally increased over this period, potentially contributing to suspended solids, while aquaculture activity expanded around 2000 and wastewater treatment improvements were most pronounced in the 1990s. Coastal erosion, rainfall and associated runoff may also play a role, but further analysis would be required to disentangle these effects.

a)



b)

Secchi depth (m)	0.5	1	1.5	2	3	4
Total suspended sediment load (mg/l)	38.8	18.0	11.1	7.6	4.1	2.4

Figure 31: a) Time series of Secchi disk depth measurements in the Menai Strait from 1960-1999. Black points represent annual mean Secchi depth measured manually with a Secchi disk. Up to 1988 data has been taken from Lumb (1989, 1990), and from 1989-99 from Birkett and Maggs (2001). The slightly larger open circle shows an estimate of Secchi depth for 1996 based on optical data from a recording sensor, taken from Kratzer et al., 2003 b) Secchi depths and corresponding suspended sediment load (Taken from Bowers, 2006)

The decline observed at the Coleg Normal monitoring site occurred after this data set ends. However, additional water quality data collected by NRW from 1983 to 2013 includes two long-term monitoring sites located northeast and southwest of Coleg Normal. Both sites show annual fluctuations in suspended solids, but overall concentrations have remained consistently low and are comparable to the levels recorded in 2000 (Figure 32).

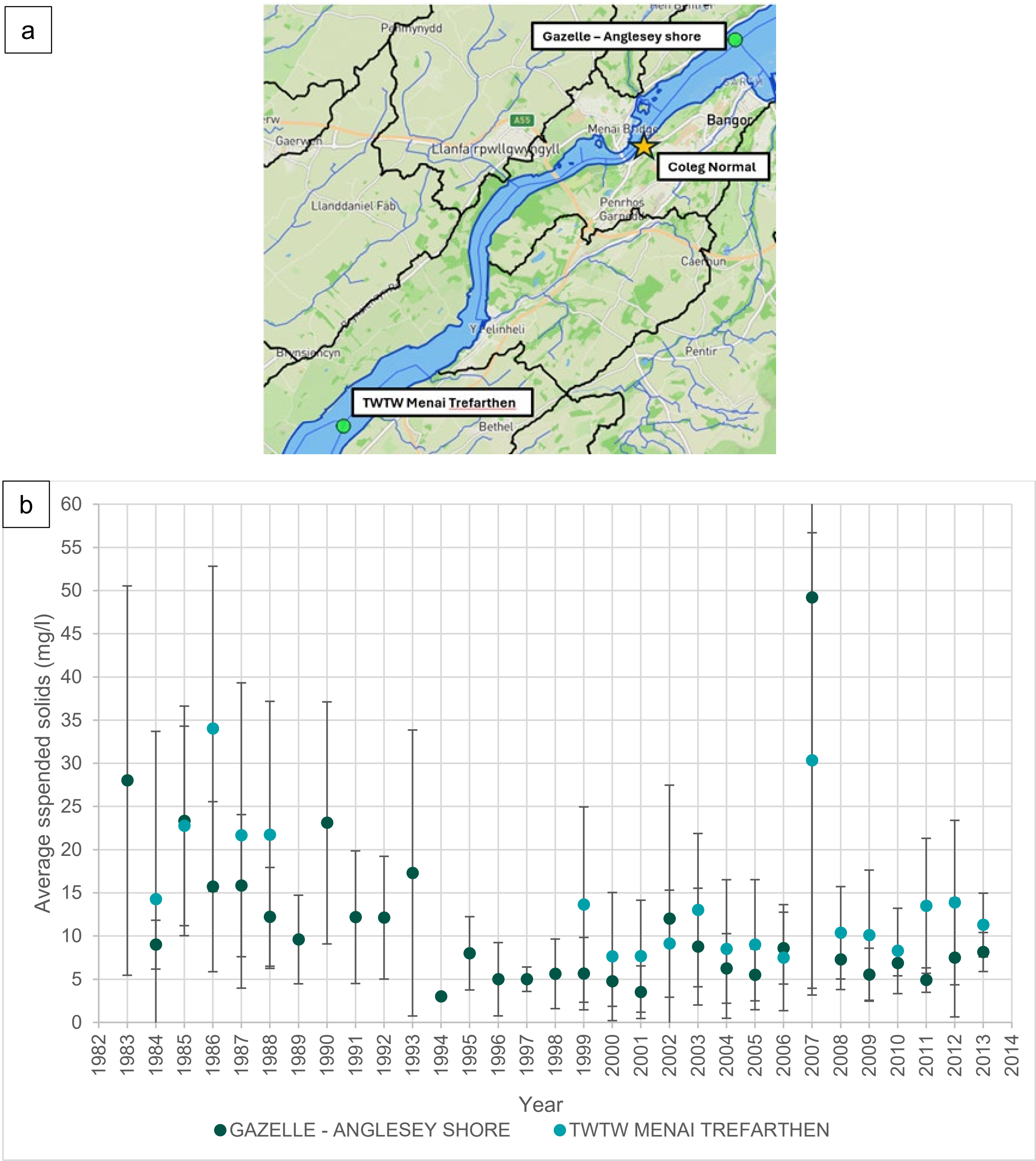


Figure 32: Long term water quality monitoring in the Menai Strait. a) Two long-term water quality monitoring sites (TWTW Menai Trefarthen and Gazelle - Anglesey shore) in the Menai Strait (green circles), and the Coleg Normal monitoring site (yellow star), b) Average annual suspended solid concentrations measured in mg/l at two long-term water quality monitoring sites in the Menai Strait between 1983 and 2013.

Early surveys in the 1970s and 1980s describe the large and abundant sponge community in the Menai Strait (Hoare and Peattie 1979; Peattie and Hoare 1981; Lumb 1983). These would have occurred during the period when the highest levels of suspended solids were present. The sponge monitoring at Coleg Normal only began in 2004 so it is not known if the steep decline observed in the years following this was part of an ongoing decline that could have been related to changes in suspended solids. However, the key sponges recorded are known to be fast growing and therefore likely to respond rapidly to environmental changes. The levels of suspended solids had remained relatively constant the decade before and after monitoring began (based on the available data), they therefore do not correlate with the observed changes in sponge luxuriance.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of a *decrease* in suspended solids on the sponge community at the Coleg Normal monitoring site (Table 7). A timing score of 1 was assigned as the data indicates no change in suspended solid concentrations during the decline period, though there have been long-term changes outside this time period. Intensity was given a 2 to reflect low intensity changes during the time period, though it should be noted there were higher intensity changes in the past. Frequency was assigned 4 as this was a permanent (continuous) change, and spatial scale was scored 4 since it would have affected the whole site. A medium/low sensitivity score (2) was given to reflect the likely resilience of the sponge community to the turbid environment where they are found. The confidence was high for this assessment due to the availability of supporting literature and data.

Table 7: Assessment for **changes in suspended solids** pressure. Scores reflect the impact of a decrease in suspended solids during the decline period (2004–2009), rather than an increase. Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature’s susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
1	2	4	4	2	64	High

Smothering and siltation rate changes (Light)

Definition of pressure: ‘Light’ (deposition of up to 5 cm) of fine material added to the seabed in a single, discrete event.

Sensitivity of species

The sensitivity assessment classified “cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock” (the biotope at Coleg Normal) resistance to smothering and siltation rate changes (Light) as "medium" with resilience rated as "high" and overall sensitivity as "low" (Readman et al. 2023).

As benthic organisms, sponges are susceptible to smothering and siltation rate changes. However, as discussed previously (within the section on sponge sensitivity to “changes in suspended solids”), increased sediments in the marine environment can have both negative and positive impacts on sponges. These are dependent on the species, habitat, environmental conditions and duration of exposure. The impacts of both settled and suspended sediment on different aspects of sponge ecology and physiology are summarised by Bell et al (2015). Potential negative impacts of increased levels of settled sediments include: 1) clogging of inhalant pores and canals, reducing pumping rates and consequently impacting feeding efficiency, and growth (Gerrodette and Flechsig 1979; Bannister et al. 2012; Pineda et al. 2017b); 2) scouring of external surfaces (Nava and Carballo 2013); 3) limiting light availability, impacting photosymbionts (e.g. cyanobacteria) in phototrophic species (Lemloh et al. 2009); and 4) preventing larvae from settling due to sediment laden surfaces or smothering of sponge recruits (Maldonado et al. 2008).

Despite these negative impacts, many sponge species are found in highly sedimented environments, for example at Lough Hyne and Skomer Island (Bell and Barnes 2000, 2002; Bell and Smith 2004). Studies show that some species have adaptions to deal with these conditions and even utilise sediments to reinforce body structures and gain shelter from potentially harmful environmental conditions and predation (Schönberg 2016). Other species may have adaptive mechanisms to survive in these conditions such as changing their morphology type or the production of mucus to remove sediment from their tissue, though this may all come at a metabolic cost (Schönberg 2016, 2021; Biggerstaff et al. 2017; Pineda et al. 2017a; Goldstein et al. 2024). Since sponges exhibit a range of responses with varying adaptations to sedimentation, the sensitivity of each relevant species should be assessed for an impact where information is available. Studies show *H. panicea* has mechanisms to cope with sedimentation including contractile responses to prevent clogging of the filtering system and tissue sloughing to clear debris from its surface (Barthel and Wolfrath 1989; Goldstein et al. 2024). However, *H. panicea* was only found in silt-free areas in the Oosterschelde and assumed intolerant to sedimentation (Vethaak et al. 1982). This is in line with other research suggesting sedimentation primarily affects massive, encrusting and wide cup sponge morphologies (Pineda et al. 2016). Although it can grow quickly and alter its morphology, there is an energetic cost of this and other sediment clearing mechanisms, and it is therefore unlikely to survive smothering for a significant length of time (Readman et al. 2023).

No direct evidence was found on the effects of sediment on *A. fucorum.* At Lough Hyne it was predominantly recorded on shallow, vertical surfaces at sites with fast flow and low sedimentation (Bell and Barnes 2000). Although typically encrusting in form, the species is fast-growing and capable of altering its shape within weeks developing lobes and tassels (Readman et al. 2023). This suggests the potential for short-term adaptation to high-sediment environments, though its long-term tolerance remains uncertain.

H. oculata can be associated with silted waters. For example, its abundance increased following the construction of a storm barrier in the Oosterschelde Estuary, which reduced tidal flow and increased sedimentation (de Kluijver and Leewis 1994). This pattern aligns with the general observation that branching and erect morphologies are better adapted to sedimented environments as their elevated structures keeps them above sediment layers and results in lower sediment accumulation compared to encrusting forms (Bell et al. 2015; Schönberg 2016).

Evidence of smothering and siltation rate changes at Coleg Normal

No available data sets could be identified to look at smothering and siltation rate changes in the Menai Strait. There is anecdotal evidence suggesting increased siltation along the shores of the Menai Strait during the 1970s and 1980s (Lumb 1983). Potential causes have been discussed such as the dumping of dredge spoil north of Puffin Island in the 1960s and 1970s, increased storminess between 1970s and the 1990s and changes in the water quality (Bowers 2006). The eastern Menai Strait mussel fishery may also influence sediment dynamics (Beadman et al. 2003).

The strong tidal currents at Coleg Normal and in the Menai Strait as a whole limit the potential for long-term sediment accumulation. Overall, while past human activities and environmental changes may have caused localised and temporary increases in siltation, there is not enough data to identify any significant long-term trends, and monitoring is needed to clarify current conditions. Anecdotal evidence from NRW divers suggest the sponge habitats in the Menai Strait may be experiencing less sediment coverage, though data from Nelson’s column monitoring site does not indicate any changes since monitoring began here in 2005.

Activities such as harbour dredging (e.g. Port Penrhyn in Bangor and Victoria Dock in Caernarfon) may contribute to sediment movement in the Menai Strait. These operations are managed to minimise impacts. For example, dredging at the southwestern end is timed with the ebb tide, allowing sediments to disperse away from central areas and reducing the likelihood of localised effects.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of smothering and siltation rate changes on the sponge community at the Coleg Normal monitoring site (Table 8). A timing score of 2 was assigned because the evidence indicates this pressure occurred largely outside the time period, although there were some potential localised changes during the time period. Intensity was assigned 1 reflecting the low level of change that occurred. Frequency was assigned 3 as this is a likely a frequent (may be intermittent but ongoing) pressure, and spatial scale was scored 4 since it would have affected the whole site. A medium/low sensitivity score (2) was given to reflect the low vulnerability to smothering and siltation rate changes. The confidence was low for this assessment due to the lack of supporting literature and data particularly on siltation changes in then Menai Strait.

Table 8: Assessment for **smothering and siltation rate changes** pressure. Scores reflect the impact during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature's susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
2	1	3	4	2	48	Low

Introduction of microbial pathogens

Definition of pressure: The introduction of relevant microbial pathogens or metazoan disease vectors to an area where they are currently not present (e.g. *Martelia refringens* and Bonamia, Avian influenza virus, viral Haemorrhagic Septicaemia virus) (Readman et al. 2023).

Sensitivity of species

The sensitivity assessment classified "cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock" (the biotope at Coleg Normal) resistance, resilience and overall sensitivity to introduction of microbial pathogens as "No evidence" (Readman et al. 2023).

Reports of sponge disease have increased globally in recent years, but with failure to identify the cause and whether this is in part due to increased monitoring and awareness rather than factors such as disease prevalence or reduced host resilience (Webster 2007; Luter and Webster 2017). There is some evidence for correlations between sponge disease and environmental factors such as climate change and water quality (Luter and Webster 2017; Pita et al. 2018; Taylor et al. 2021). One of the first investigations into disease in a temperate sponge species was in black and fouled *Cliona celata* at Skomer Marine Nature Reserve (MNR) in Wales in 2013 (Preston 2015). This described a shift in the sponge microbial community as it changed from a healthy sponge to a black necrotic sponge due to a pathogen or environmental stressor. This dysbiosis and a correlated decline in sponge health is a commonly described stress response as a result of altered environmental conditions, but identifying a specific pathogen is difficult and rarely achieved due to the diverse host-specific microbes which include bacteria, archaea, algae and fungi (Webster and Taylor 2012). Additionally, whether the dysbiosis is a cause or a consequence of the change in sponge health remains unclear (Pita et al. 2018).

Most reports describe visual signs of sponge stress and disease as the appearance of white spots or a white bacterial overgrowth often followed by tissue regression, lesions, and necrosis (Webster 2007). For example, a white bacterial film has been observed in *H.*

panicea under low water flow conditions, and on *Scopalina* sp. under elevated temperature stress in lab studies (Taylor et al. 2021). There is some suggestion that branching sponges are better able to recover from disease than encrusting, due to their ability to exclude diseased branches from the main body, and that disease is easier to detect than in encrusting species where it can go undetected inside the tissue (Webster et al. 2007).

Evidence of introduction of microbial pathogens at Coleg Normal

A record of a potential disease or environmental stressor affecting sponges in the Menai Strait was noted in 1988–89, when a white film was observed covering a specimen of *H. panicea* (Luter and Webster 2017). During the same period, a decline in both the abundance and health of *H. oculata* was also noted in the Menai Strait, with dark spots and decaying branches (Jones 1988). As previously discussed in this report, this period coincided with high turbidity levels and concerns over water quality, with high levels of untreated sewage being discharged into the Menai Strait, conditions that may have caused environmental stress to marine organisms. In one study sponges exposed to high bacterial concentrations exhibited good growth but low survival, often accompanied by the formation of a white, unidentified biofilm on dead tissue, suggesting that organic enrichment can contribute to mortality and potentially disease (Duckworth and Pomponi 2005). There was no evidence or investigation of a pathogen related to the white biofilm covering the specimen of *H. panicea* observed in the late 1980s.

The die-back of *H. oculata* was investigated at the time with the conclusion that this may be part of a previously unobserved natural life cycle phase (C. Jones, 1988). Similar patterns have been observed in recent years in Milford Haven and in the Menai Strait at the Nelson's Column monitoring site, where populations periodically undergo mass necrosis, followed by recovery within one or two years (Figure 33 & Figure 34). This evidence suggests the observed die back may reflect natural population dynamics rather than a disease outbreak.

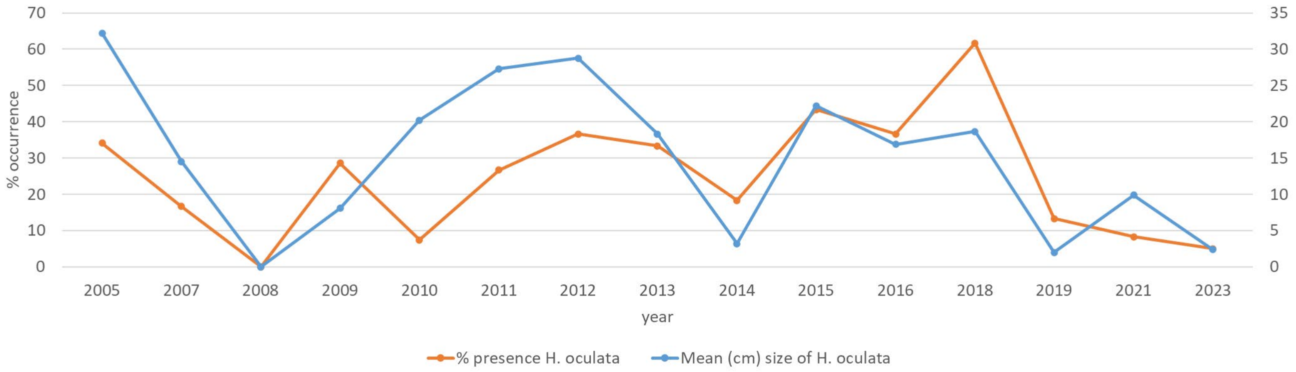


Figure 33: The size and abundance of *Haliclona oculata* at Warrior monitoring site in Pembrokeshire Marine (PM) SAC, showing a cyclical pattern of growth (NRW monitoring data).

Figure 34: *Haliclona oculata* at Nelson’s Column monitoring site in the Menai Strait and Conwy Bay (MS&CB) SAC, 2019, showing healthy and unhealthy branches with decay and algal overgrowth (image: NRW).

To further investigate this dieback, Bangor University were contracted to carry out an investigation of the microbiome of *H. oculata* in the Menai Strait. Samples were collected in June 2023 by NRW divers during the routine monitoring programme at four locations, three within the central Menai Strait and one at a far site just outside the northern entrance (Figure 35). High-throughput metabarcoding was used to compare bacterial, fungal and algal microbiota in *H. oculata* and *A. fucorum* (a common encrusting sponge species) to investigate: 1) how their microbiomes differ, 2) whether there were differences in the microbiomes of healthy and necrotic *H. oculata*; and 3) whether bacterial, algal, and fungal microbiota are similarly linked to *H. oculata* health status.

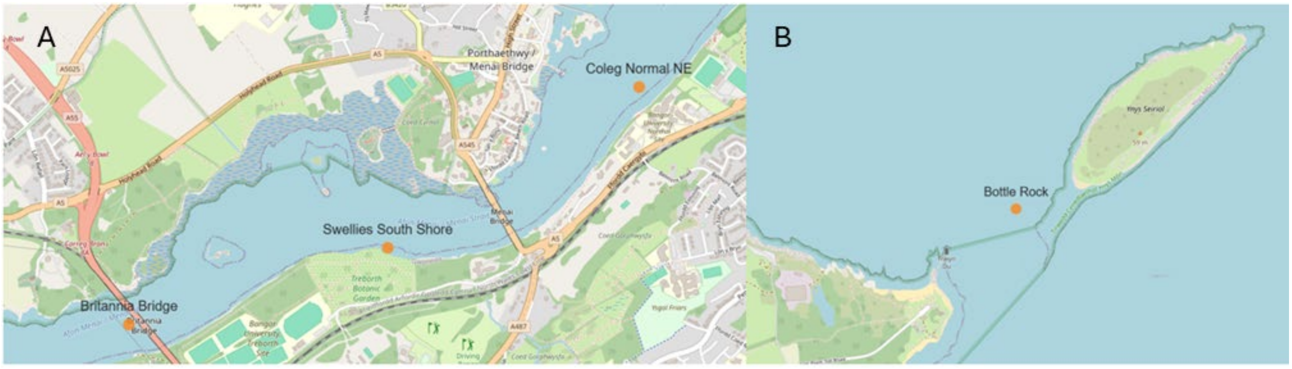


Figure 35: Sponge sampling locations A) within the central Menai Strait at Britannia Bridge, Swellies South Shore and Coleg Normal NE & B) at Bottle Rock, outside the northern entrance

Samples of *H. oculata* were taken from specimens with no visual signs of necrosis (HealthyAllHO), and tissue from specimens showing necrosis, including both the necrotic tissue (NecroticHalfHO) and apparently healthy tissue (HealthyHalfHO). Samples of *A. fucorum* were referred to as HealthyAllAF since there were no unhealthy parts. In addition, water samples were taken at every site. For full details of the methodology see the final report (A).

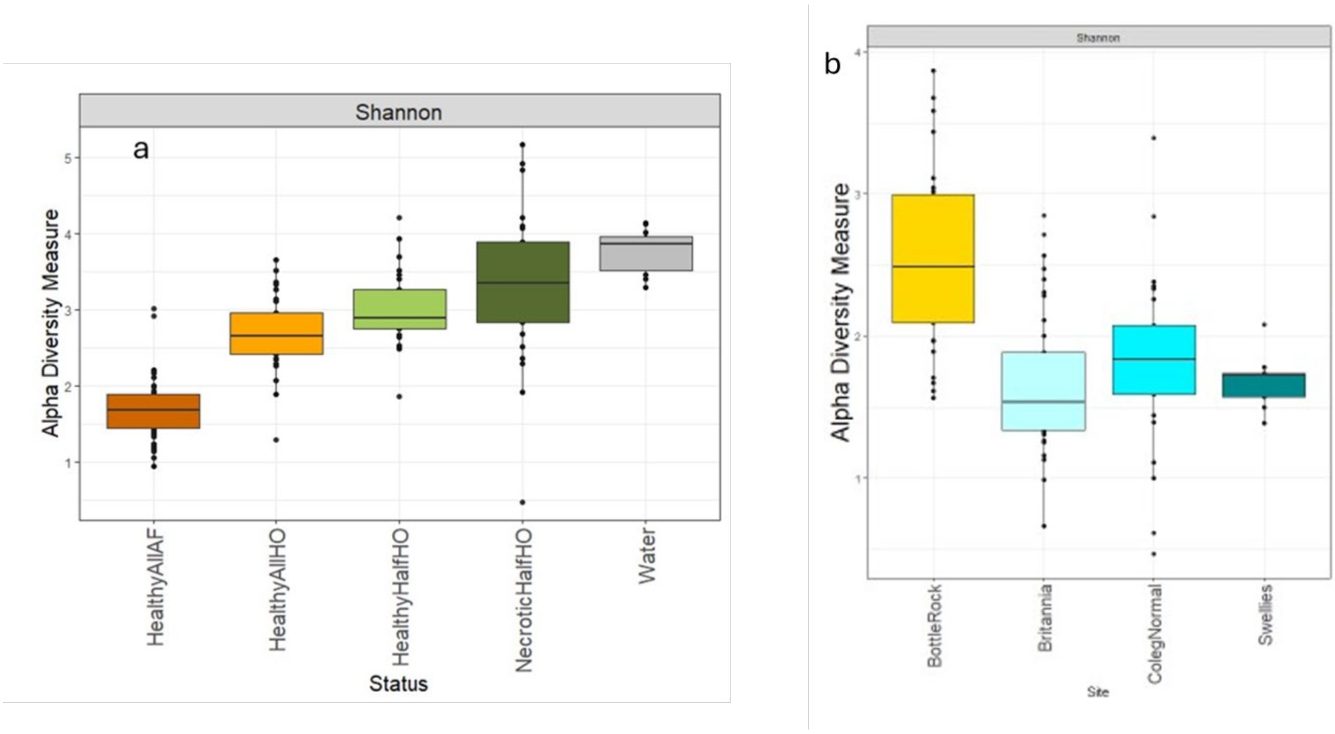


Figure 36: Changes in bacterial community between tissue type and sampling locations. a) Boxplot of bacterial alpha diversity (Shannon index) comparing species and health states, b) Boxplot of algal alpha diversity (Shannon index) comparing sampling locations. Health state codes: HealthyAllAF - tissue from *Amphilectus fucorum* with no visible signs of disease, HealthyAllHO - tissue from *Haliclona oculata* showing no visible signs of disease, HealthyHalfHO - apparently healthy tissue from *H. oculata*, NecroticHalfHO - necrotic tissue from *H. oculata*.

Key findings showed no significant difference in bacterial communities by location, but significant differences between sponge species and health status (Figure 36, (a)). Both *H. oculata* and *A. fucorum,* displayed typical low microbial abundance (LMA) profiles dominated by Proteobacteria. *A. fucorum* had the lowest microbial diversity, followed by fully healthy *H. oculata*. Necrotic tissue had the highest diversity though largely different to the bacteria found in the healthy tissue and the surrounding sea water. However, no sediment samples were taken so it is unknown if the bacterial community within the sponge was becoming more similar to the sediment as it decayed, as seen in the black sponge disease at Skomer (Preston 2015). Stress induced dysbiosis is often characterised by an increase in alpha diversity (i.e., a higher number and variety of microbial taxa within a sample), where the hosts immunity is compromised and there is a loss of control over the microbiome (Pita et al. 2018).

Recent research on the impact of water quality on the microbiome of *H. panicea* found reduced bacterial diversity related to the presence of faecal bacteria, suggesting these groups may compete with the main sponge microbiome (Al-Haddad et al. 2024). No location effect on the bacterial community was observed in the current investigation, perhaps due to strong tidal mixing exposing all sites to similar water. The data presented here is from a single time point and potentially lacks comparison with sites of suitably varied water quality, so it is not possible to determine whether changes in water quality affect the microbiome of these species. This remains an important area for future investigation.

In contrast, location did have a significant influence on the algal microbiome community, and little difference was found between species and health status (Figure 36(b)). Bottle Rock, located outside the northern entrance of the Menai Strait had significantly higher alpha diversity than all sites within the Menai Strait. Low diatom alpha diversity has been linked to sewage outflows in France (Chonova et al. 2019), so it is possible that the algal microbiome community in the sponges from sites nearer to sewage inputs in the Menai Strait could be showing an effect, however further research is needed to investigate this. Further to this although the alpha diversity was lower in samples from within the Menai Strait, they had both distinct and shared taxa, indicating they did not share the same community composition.

Healthy *H. oculata* tissues contained algal symbionts from the *Mamiellaceae* family, typically linked to primary production, carbon fixation, and nutrient cycling. These were largely absent in necrotic tissues, which instead showed dominance of *Derbesiaceae*, rare in healthy tissue, suggesting it may be an opportunistic invader or disease indicator. Bacterial taxa more abundant in healthy tissues also included metabolically active groups, such as the cyanobacterium *Synechococcus*, known for carbon fixation. In contrast, necrotic samples showed a more erratic microbial profile, including *Lewinella* (common in wastewater), *Aranicella* (linked to harmful *Dinophysis* blooms), and the Sva0996 clade (associated with high nitrate), pointing to an environment influenced by human activity.

The fungal community analysis methodology was novel and needed improvement so was not included in the report. Overall, this study provides the first comprehensive overview of the microbiome differences between healthy and diseased *H. oculata* and a novel resource for investigating the ecology and health of temperate sponges. Further work is needed to explore temporal changes in microbiome and with a greater range of water quality parameters.

Environmental stressors such as temperature, turbidity, and nutrient or organic enrichment may drive shifts in the sponge microbiome, leading to microbial dysbiosis and potentially enabling opportunistic pathogen infections as a secondary impact. While microbial pathogens cannot be ruled out as contributing to the Menai Strait sponge decline, there is currently no direct evidence linking a specific pathogen to the observed necrosis in *H. oculata* or indicating that disease was the primary cause of the decline in luxuriance observed at Coleg Normal monitoring site.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of introduction of microbial pathogens on the sponge community at the Coleg Normal monitoring site (Table 9). A timing score of 2 was assigned because the evidence indicates this pressure occurred largely outside the time period, although there were some potential localised changes during the time period. Intensity was assigned 2 reflecting the low level of change that occurred. Frequency was assigned 2 reflecting a potentially infrequent pressure over a long time period, and spatial scale was scored 2 since it may affect only part of the site (e.g. not all species). A medium/high sensitivity score (3) was given to reflect the moderate vulnerability to introduction of microbial pathogens. The confidence was low for this assessment due to the lack of supporting literature and data.

Table 9: Assessment for **introduction of microbial pathogens** pressure. Scores reflect the impact during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature's susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
2	2	2	2	3	48	Low

Abrasion/disturbance of the substrate on the surface of the seabed

Definition of pressure: Damage to seabed surface features (species and habitats).

Sensitivity of species

The sensitivity assessment classified "cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock" (the biotope at Coleg Normal) resistance to an abrasion or disturbance to the surface of the substratum or seabed as ″low" with resilience rated as "medium" and overall sensitivity as "medium" (Readman et al. 2023)

Key anthropogenic activities that cause abrasion/disturbance to the surface of the seabed include anchoring, infrastructure development and fishing. Fishing has the most widespread impact on benthic habitats. Impact studies have predominantly focussed on mobile gears (e.g. bottom-towed gear) with static fishing gears (e.g. pots) assumed to have low or no impact (Fennell et al. 2021). However, a series of long-term (>4 year) studies in the Lyme Bay MPA concluded that potting was more destructive than previously thought with negative effects on the benthos, significantly on reef builders (typically long-lived and slow growing species) (Gall et al. 2020; Rees et al. 2021). In contrast, other short (2 month) and long (4 year) term studies found no impact from commercial crustacean potting (Coleman et al. 2013; Stephenson et al. 2017).

There is limited direct evidence on the impacts of abrasion or disturbance of the seabed on sponges. Studies looking at fishing impacts show varying sensitivity. Erect and branching sponges are especially vulnerable to abrasion and dislodgement due to their morphology and life history traits (Jennings and Kaiser 1998). Studies have shown 32-50% of sponges were damaged from trawl events, with possible recovery within a year (Tilmant 1979; Van Dolah et al. 1987). Gall *et al.* (2020) reported significant potting-related damage to erect sponges, including abrasion and removal, with damage reaching up to 50%. Rocky reef habitats which can support these communities, are considered to have medium-high sensitivity to potting and moderate resilience (Eno 2001). However not all studies report significant impacts (Eno 2001; Coleman et al. 2013). Direct physical damage may also leave sponges more vulnerable to disease (Readman et al. 2023).

No direct evidence could be found on impacts to *H. panicea* and *A. fucorum*, though as generally encrusting and fast-growing species they are assumed less vulnerable with quick recovery. One study observed no significant impact to *H. oculata* from a single trawl event (Van Dolah et al. 1987). However as an erect species it is expected to be more vulnerable with potentially longer recovery.

Evidence of abrasion/disturbance of the substrate on the surface of the seabed at Coleg Normal

There is some evidence of pot fishing occurring at Coleg Normal. Potting was directly observed during NRW dive monitoring surveys in 2023, indicating recent and possibly ongoing activity. A 2007 NRW site visit log recorded the transect tape becoming entangled in a pot line, suggesting potting has been occurring here for over 15 years at least. Anecdotal evidence suggests pot fishing to have been ongoing since the 1950s and 60s, with a decrease in recent years (R. Sharp, pers. comm, 2024). However, there are no historic data available on fishing effort in the Menai Strait to confirm these observations and to relate this activity with the observed decline in sponge luxuriance.

Gall *et al.* (2020) found potting caused significant abrasion and partial removal of erect sponges as well as other key species such as *A. digitatum, Alcyonidium diaphanum,* and *Cliona celata*. Analysis of Coleg Normal MNCR survey data does not indicate a decline in these species at the same time as the observed decline in *H. oculata*. This suggests that this decline may not be solely due to potting but driven by other environmental or biological factors. Although *H. oculata* has been recorded in nearby areas to the northeast of the Coleg Normal transect, the level of potting activity in these areas is also currently unknown. Without confirmed effort data, it is not possible to determine whether potting pressure varies spatially across the Menai Strait or whether any relationship exists between fishing activity and the patterns of *H. oculata* decline observed at the monitored site.

Further potential sources of seabed disturbance could come from anchoring, but this is thought to be limited due to the strong tides in the Menai Strait and observations of anglers drift fishing rather than anchoring (R. Sharp, Pers.comm, 2024).

Overall, while there is evidence of potting at the site and the potential for abrasion-related impacts, there is not enough data to determine if potting could have caused the observed decline. Further data is needed to understand historic potting effort.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of abrasion/disturbance of the substrate on the surface of the seabed, on the sponge community, at the Coleg Normal monitoring site (Table 10). A timing score of 2 was assigned because this was likely an ongoing pressure that occurred throughout the time period but mostly outside (a continuous pressures that occurred before and after the decline period as well as throughout). Intensity was assigned 1 reflecting the likely low level intensity activity. Frequency was assigned 3 as this pressure may be intermittent but ongoing, and spatial scale was scored 2 since it could affect only part of the site. A medium/high sensitivity score (3) was given to reflect the moderate vulnerability to introduction of abrasion/disturbance of the substrate on the surface of the seabed to some sponge species. The confidence was low for this assessment due to the lack of supporting literature and data particularly on fishing activity.

Table 10: Assessment for **abrasion/disturbance of the substrate on the surface of the seabed** pressure. Scores reflect the impact during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature’s susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
2	1	3	2	3	36	Low

Synthetic compound contamination (incl. pesticides, antifoulants, pharmaceuticals).

Definition of pressure: The introduction of relevant contaminant into the local environment e.g. via spills, approved and incidental discharges.

Sensitivity of species

This pressure was recorded as “Not assessed” under the sensitivity assessment for “cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock” (the biotope at Coleg Normal) (Readman et al. 2023)

Sponges are abundant, widely distributed and highly efficient filter feeders, capable of processing large volumes of seawater. They can accumulate a wide range of contaminants and as result recent research has focussed on their use as bio-monitors for assessing pollution levels and the overall health of marine ecosystems. Contaminants include metals, trace elements and organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (Hansen et al. 1995; Batista et al. 2013; Gentric et al. 2016; Orani et al. 2018), microplastics (Girard et al. 2021; Soares et al. 2022; Corti et al. 2023) and emerging contaminants including pharmaceuticals and pesticides (Rizzi et al. 2023; Saliu et al. 2024). In addition, sponges and their associated microbial communities are also being studied for their potential in bioremediation, due to their ability to remove or degrade certain pollutants from the marine environment (Loredana et al. 2017; Amato et al. 2024).

However, research on the impacts of these pollutants on sponges and their associated microbial communities (the holobiont) is limited. Existing evidence from *H. panicea* indicates that exposure to heavy metals, hydrocarbons, and dispersants can impair pumping activity causing a reduction in filtration efficiency, lowering internal oxygen levels, disrupting microbial communities, leading to mortality (Hansen et al. 1995; Vad et al. 2022). Emerging contaminants, such as pharmaceuticals, have been shown to trigger cellular stress responses, indicating potential toxicity (Rizzi et al. 2023), while toxins associated with microparticles may interfere with sponge development and filtration processes (Girard et al. 2021). Overall, there is not enough available evidence to determine the long-term impacts of contaminants on sponge health and further research is needed to assess their sensitivity.

Evidence of synthetic compound contamination (incl. pesticides, antifoulants, pharmaceuticals) at Coleg Normal

Available water quality monitoring data from a long-term monitoring site, Gazelle - Anglesey Shore, northeast of the Coleg Normal sponge site indicates an overall decline in pesticide concentrations present in the Menai Strait (Figure 37). The levels of legacy pesticides such as Lindane, Isodrin and Dichlorodiphenyltrichloroethane (DDT) have all been recorded at very low concentrations (<0.005 µg/l) and would therefore be unlikely to have an impact on the sponge community. For example, Loredana *et al.* (2017) recorded low mortality when sponges were exposed to a Lindane concentration of 1 µg/L. While these substances persist in the environment, pesticides more likely to have been detected in recent years include MCPA (2-methyl-4-chlorophenoxyacetic acid) (still permitted for professional use in the UK; HSE 2024), Metaldehyde (banned for outdoor use in the UK from April 2022; HSE 2022), and Diazinon (withdrawn from EU pesticide approval by 2011; European Commission 2011). Monitoring data indicate that even these more contemporary pesticides, where detected, occur at low concentrations and show a declining trend, suggesting limited risk to the sponge community. In addition, the Menai Strait waterbody received a pass for chemicals in the 2024 cycle 3 interim classification and a pass in the 2009 cycle 1 classification (Wynter et al. 2025).

Figure 37: The average annual concentration of pesticides (µg/l) recorded at “Gazelle - Anglesey Shore” long-term monitoring location in the Menai Strait between 1983 and 2010.

Heavy metal concentrations in the seawater of the Menai Strait have not been considered a significant concern, and the area has been regarded as relatively uncontaminated by several researchers since the 1990s (Morris and Goudge 2006). In accordance with the EC Shellfish Hygiene Directive, heavy metal levels have been regularly monitored at two sites in the central Menai Strait, with data indicating a steady decline from the higher concentrations observed in the 1980s (Morris and Goudge 2006).

Potential localised sources of contamination that have been suggested include the re-painting of the Menai Bridge in 2005, which may have introduced lead-based paints and other chemicals into the water, and an oil spill near Felinheli in 2006. However, there is no available data to assess these potential impacts, and as isolated events, they are unlikely to have caused the ongoing decline, with recovery likely to have occurred. The development of nearby land into a football stadium may have increased surface run-off and introduced local sources of pesticides; however, no data are available to assess this, and construction did not begin until after 2009, later than main period of observed decline at Coleg Normal. Similarly, a nearby caravan park could represent a potential source of contamination, although there has been no expansion in recent years. More significantly the site is situated close to a storm overflow, which could introduce localised contaminants.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of synthetic compound contamination on the sponge community at the Coleg Normal monitoring site (Table 11). A timing score of 2 was assigned because this is an ongoing pressure occurring mostly before the decline period. Intensity was assigned 1 reflecting the low/no change that occurred. Frequency was assigned 2 as this was a permanent (continuous) change, and spatial scale was scored 4 since it would have affected the whole site. A low sensitivity score (2) was given to reflect possible sensitivity to synthetic compound contamination. However, the confidence was low for this assessment due to the lack of supporting literature and data on contaminants and the sensitivities of sponges in the Menai Strait.

Table 11: Assessment for **synthetic compound contamination** pressure. Scores reflect the impact during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature's susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
2	1	2	4	2	32	Low

Wave exposure changes

Definition of pressure: Local changes in wavelength, height and frequency.

Sensitivity of species

The sensitivity assessment classified “cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock” (the biotope at Coleg Normal) resistance to wave exposure changes as ″high" with resilience rated as "high" and overall sensitivity as "not sensitive" (Readman et al. 2023). This was assessed with a benchmark of a change in near shore significant wave height of >3% but <5% for more than one year.

The impact of wave exposure on sponges varies with relation to their morphology. Erect, branching and cushion sponges are predominantly found in sheltered water as they are more vulnerable to dislodgment and fragmentation in high energy environments. In contrast, encrusting sponges are highly resilient to wave-exposed environments due to their low profile and firm attachment to the substrate (Ávila et al. 2011; Schönberg 2021). Massive sponges while generally more robust, can fragment under storm surge or strong current conditions (Bell and Barnes 2000; Schönberg 2021).

Sponge resistance to wave exposure is also related to their internal skeletal composition. Species with a high proportion of spicules in their structure are stiffer but more brittle making them more susceptible to fragmentation. Species with a high proportion of spongin have a more flexible structure, but they may lack structural strength so may still fragment.

However, sponges that have a combination of spicules and spongin are generally both flexible and robust, and therefore more resistant to dislodgment or fragmentation in high energy environments (Ávila et al. 2011; Schönberg 2021). In addition, sponges exhibit significant phenotypic plasticity in response to hydrodynamic conditions allowing them to adapt to conditions by altering their morphology and composition of their tissues (Kaandorp and De Kluijver 1992; Schönberg 2021; Broadribb et al. 2021).

Studies indicate sponge recovery from increased wave exposure related to storm events. This has been recorded as early as 10 weeks post storm, with sponge fragments able to adhere to the substrate, re-growth of damaged individuals, and with some potential positive impacts associated with increased suspended sediments and nutrient inputs (Ávila et al. 2011; Mary George et al. 2018; Gochfeld et al. 2020).

The Menai Strait sponge community is dominated by the encrusting and simple-massive formed sponge species *H. panicea* and *A. fucorum*, and the erect *H. oculata* adapted to the strong turbulent flows. *H. panicea* is found in habitats experiencing a range of exposures and has been shown to acclimate over short time frames to changes in environmental conditions (Broadribb et al. 2021). Though some large, poorly attached colonies may be displaced by wave action (Readman et al. 2023). Similarly, *H. oculata* has been shown to rapidly alter its growth form in response to changes in wave exposure (Kaandorp and De Kluijver 1992). It is therefore expected that these species will have low sensitivity to changes in wave exposure, with impacts, such as a reduction in biomass likely to reverse quickly once normal conditions resume.

Evidence of wave exposure changes at Coleg Normal

The Coleg Normal monitoring site is situated in an extremely tidal and turbid channel, but it is very sheltered in relation to wave exposure due to its position at the northeastern end of the Menai Strait facing away from the prevailing south-westerly winds (see MNCR wave exposure scale in Tyler-Walters et al. 2023). In addition, its position over 5 km from the narrow entrance results in very low fetch and high dissipation of potential wave energy. Increased wave exposure is likely to occur only during periods of strong north-easterly winds.

Studies indicate a general decline in wind speeds between 1990 and 2010 after a peak in the early 1990s, related to a shift from a positive to a negative phase of the North Atlantic Oscillation (NAO) (Earl et al. 2013). This can result in an increase in northeasterlies. Modelled past and future conditions for Liverpool Bay (validated using tide gauges and wave stations) are available which provide insights into regional wind and wave conditions (Brown et al. 2010, 2012). Looking at the decades approximately either side of the decline, 1990 to 2010, these follow the same pattern of a slight decrease in average wind speeds. Looking at extreme events, there is a reduction in frequency but increase in intensity of extreme wind events, which is reflected in a decrease in average wave heights, but an increase in intensity of the most severe waves. However little change in wind-distribution was observed with the dominant direction of wind and waves remaining from the southwest so overall wave exposure shows little change (Figure 38).

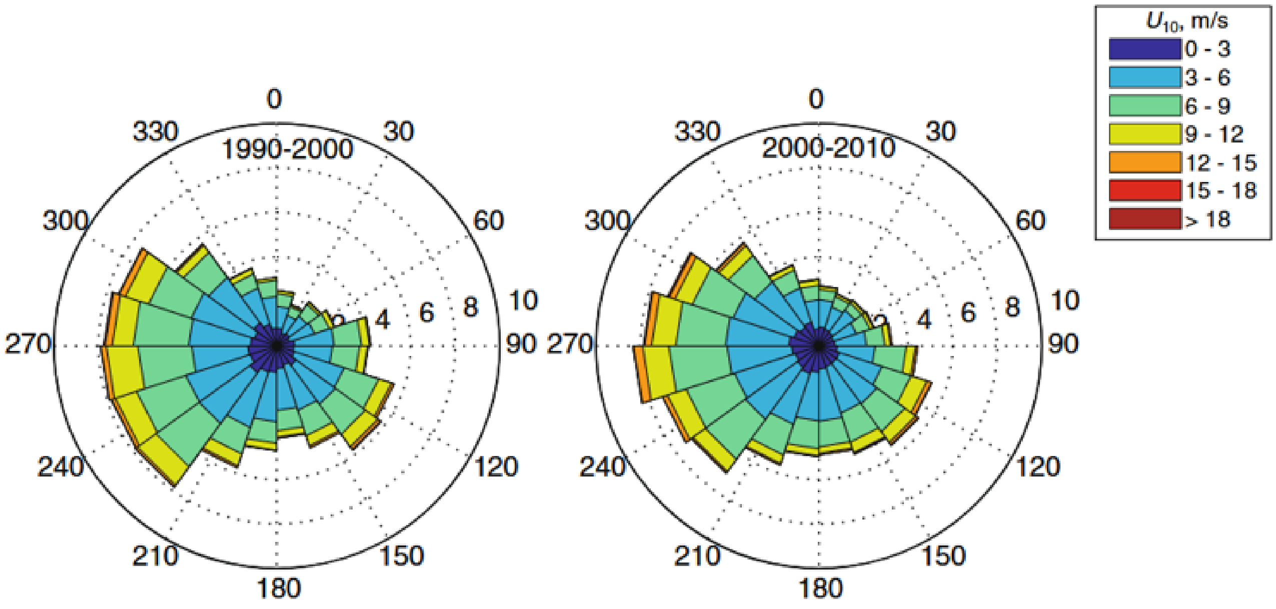


Figure 38: The decadal wind distribution at the Liverpool WaveNet Buoy for the periods 1990-2000 and 2000-2010 (adapted from Brown et al. 2012)

The latest Marine Climate Change Impacts Partnership (MCCIP) report on storms and waves (2025) states there has been a decrease in mean wave height over the last 30 years in northern UK waters and increase in the south, with a poleward shift in the storm track since the 1990s (Bricheno et al. 2025). It has been suggested that Liverpool Bay is positioned at the boundary between these areas and as a result is relatively less effected by changing storm effects, with predicted decreasing wave height (Figure 39) (Brown et al. 2012).

There are anecdotal reports suggesting an increase in wind and wave exposure in the Menai Strait. In 2018, the “Beast from the East” brought strong easterly winds, causing damage to moored boats that would typically be sheltered from such conditions. Additionally, observations from Treborth Botanic Gardens indicate increased erosion along the shoreline, negatively impacting coastal trees (K. Davies, Bangor University, pers. comm.). However, no studies have been conducted to establish the timing or causes of these changes. Other potential causes of localized increases in wave exposure include the rise in high-speed tourist boat operations and a growing number of jet skis in the area.

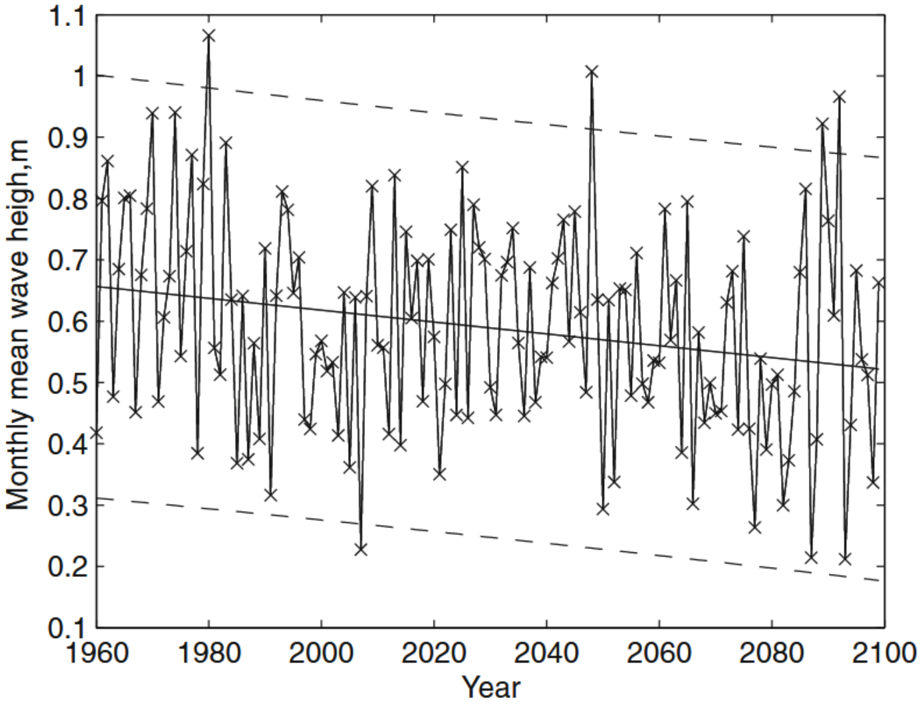


Figure 39: Decreasing trend (-) in the monthly-mean wave height in September (-x-) in Liverpool Bay from 1960 to 2100, with 95% confidence interval (C.I.) (- -) (Taken from Brown et al. 2012).

Anecdotal evidence of sponge fragments washed up on local beaches correlates with the period when recorded wind and wave levels were higher in the early 1990s and sponge luxuriance was greater (C. Lindenbaum, *Pers. comm, 2024)*. However, any damage would likely have been occasional, and since sponges grow rapidly and show good recovery from storm damage there would likely not have been long term effects. In addition, wave energy and storms are considered natural physical disturbance in temperate subtidal reefs (Roberts et al. 2006). Overall, from the available evidence it is unlikely that changes in wave exposure caused the current decline.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of wave exposure on the sponge community at the Coleg Normal monitoring site (Table 12). A timing score of 1 was assigned because there was no change during the decline period. Intensity was assigned 2 reflecting the sheltered location. Frequency was assigned 2 as increased wave exposure would occur infrequently over a longer period, and spatial scale was scored 2 since it would likely affect only the shallow half of the site. A medium/low sensitivity score (2) was given to reflect adaptability and recovery to wave exposure. The confidence was low for this assessment due to the limited supporting literature and data.

Table 12: Assessment for **wave exposure** pressure. Scores reflect the impact during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative

magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the pressure; sensitivity = a feature’s susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
1	2	2	2	2	16	Low

Introduction or spread of invasive non-native species (INNS)

Definition of pressure: The direct or indirect introduction of non-native species, e.g. Chinese mitten crabs, slipper limpets, Pacific oyster and their subsequent spreading and out-competing of native species (Readman et al. 2023).

Sensitivity of species

The sensitivity assessment classified “cushion sponges and hydroids on turbid tide-swept sheltered circalittoral rock” (the biotope at Coleg Normal) resistance, resilience and overall sensitivity to or spread of invasive non-native species as "Insufficient/No evidence" (Readman et al. 2023).

There are three high risk INNS present in Wales that could potentially impact the sponge community in the Menai Strait. The carpet sea squirt, *Didemnum vexillum,* the American slipper limpet, *Crepidula fornicata* and the Japanese weed (*Sargassum muticum*).

D.vexillum is an invasive colonial ascidian that is present in Holyhead marina and Milford Haven in Wales. It is considered an "ecosystem engineer" due to its ability to alter habitats and biodiversity (McKenzie et al. 2017). It can form extensive mats smothering the benthic community below. Observations in Holyhead Marina show it overgrowing native tunicates like *Ciona intestinalis* (Griffith et al. 2009). Although there is no direct evidence available on impact of this species on sponges, they would likely be negatively affected due to competition for space, and smothering.

C. fornicata is an invasive mollusc present in the UK since the 1870s. It can smother species and habitats due to being highly gregarious, and producing large amounts of pseudofaeces (Blanchard 1997). There is no direct evidence available of *C. fornicata* impacts on sponge communities, with a lack of evidence of significant colonisation on infralittoral or circalittoral bedrock. It occurs at low densities or is absent in boulder-dominated areas and shows a preference for shell over stone. *C. fornicata* is rarely found in faunal turf habitats, likely due to high larval predation and competition from fast-growing suspension feeders, which limit its ability to establish (Readman et al. 2023). It is therefore expected to be a low-risk pressure.

S. muticum is an invasive brown algae from the Pacific present in the UK since the 1970s (Pizolla 2008). It grows rapidly on hard substrata in both estuarine and full-salinity waters, enabling it to outcompete local species and colonise a broad range of habitats. There is no direct evidence of *S. muticum* impacting UK sponge communities. However, canopy studies in Scotland show changes in underlying sessile epifaunal communities, indicating potential effects on other sessile taxa through shading and competition for space (Harries et al. 2007). Broader assessments note reduced light and increased sedimentation beneath mats, conditions likely to affect sponges even though this has not been documented directly (Invasive Species Scotland 2026).

Evidence of introduction or spread of invasive non-native species at Coleg Normal

There is currently no evidence of *D. vexillum* in the Menai Strait. *C. fornicata* was first introduced in 2006 with mussel seed laid in the eastern mussel fishery. Eradication operations were undertaken and it was thought to have been successfully removed. However, since 2019 it has become established at the southwestern end of the Menai Strait, with numbers increasing substantially. Other INNS known to be present include the Chilean Oyster (*Ostrea chilensis*), the Pacific oyster (*Magallana gigas*) and the wireweed (*Sargassum muticum*). *O.chilensis* is thought to have been present for around 30 years.

The cover of *S. muticum* is considered extensive in parts of the SAC, although this species is not consistently recorded (Wynter et al. 2025). In some areas of the Menai Strait, *S. muticum* has replaced the zone formerly dominated by sugar kelp. However, its distribution does not appear to have expanded significantly in recent years. Despite its presence elsewhere in the Strait, there are currently no records of *C. fornicata*, *O. chilensis*, *M. gigas*, or *S. muticum* at the Coleg Normal monitoring site, and there is no indication that these species have occurred there historically or contributed to the observed decline.

Pressure assessment scores

The sensitivities and evidence described above were used to assess the impact of introduction or spread of invasive non-native species on the sponge community at the Coleg Normal monitoring site (Table 13). A timing score of 2 was assigned because this pressure occurred mostly outside the time period. Intensity was assigned 1 reflecting the low level of change that occurred. Frequency was assigned 1 as this was thought a one-off or short-term event, and spatial scale was scored 2 since it would have likely affect only part of the site. A low sensitivity score (2) was given to reflect the potential resilience to introduction or spread of invasive non-native species. The confidence was high for this assessment due to the availability of supporting literature and data on INNS in the Menai Strait.

Table 13: Assessment for **introduction or spread of invasive non-native species** pressure. Scores reflect the impact during the decline period (2004–2009). Scores range from 1 (low) to 4 (high). Timing = the likelihood that the pressure occurred during the decline period (2004–2009); intensity = the relative magnitude of the pressure; frequency = how often the pressure occurs; spatial scale = the proportion of the feature affected by the

pressure; sensitivity = a feature's susceptibility to change and ability to recover under pressure. For detailed definitions, see Table 2.

Timing	Intensity	Frequency	Spatial scale	Sensitivity	Risk	Confidence
2	1	1	2	2	8	High

Pressures summary

Table 14: Pressures spreadsheet summary table. Pressures assessed in this report ranked from highest to lowest risk of causing the decline in the sponge community at the Coleg Normal monitoring site in the Menai Strait & Conwy Bay SAC. **Note:** The risk score for organic enrichment and nutrient enrichment reflects ecological impacts associated with a **decline** in inputs, not an increase.

Pressure	Risk	Confidence
Organic enrichment	576	High
Aquaculture	128	Medium
Ocean warming	128	Medium
Nutrient enrichment (i.e. nitrogen, phosphorus & silicon)	96	Low
Changes in suspended solids (water clarity)	64	High
Smothering and siltation rate changes (Light)	48	Low
Introduction of microbial pathogens	48	Low
Abrasion/disturbance of the substrate on the surface of the seabed	36	Low
Synthetic compound contamination (incl. pesticides, antifoulants, pharmaceuticals).	32	Low
Wave exposure changes	16	Low
Introduction or spread of invasive non-native species (INNS)	8	High

Discussion

Importance and decline of the Menai Strait and Conwy Bay (MS&CB) SAC sponge community

The MS&CB SAC contains one of the best examples of strongly tide-swept reef in the UK, supporting historically dominant sponge communities. Existing monitoring data were reviewed and supplemented with new surveys to provide an up-to-date assessment of the sponge community condition. At Coleg Normal, a significant decline between 2004 and 2009 resulted in "unfavourable" SAC status (NRW 2018; Wynter et al. 2025), with encrusting species (*H. panicea* and *A. fucorum*) stabilising but remaining low and erect species (*H. oculata*) absent since 2019. MNCR dive surveys in 2023 confirmed widespread reductions in *H. panicea* and *H. oculata* compared to historic data, while *A. fucorum* showed localised increases. Declines in sponge also occurred at Warrior in the PM SAC between 2005 and 2009 (Jackson-Bué et al. 2025), with similar trends in encrusting species but with *H. oculata* showing a cyclical pattern of abundance at both Warrior and Nelson's Column (in the MS&CB SAC) suggesting a potential natural growth cycle. Overall, patterns across sites suggest species-specific declines, cyclical abundance in *H. oculata*, and possible widescale environmental changes affecting sponge communities in multiple SACs.

Potential drivers of the decline in the sponge community at Coleg Normal

To investigate potential causes of the observed decline, eleven pressures relevant to the sponge community at Coleg Normal were identified and assessed. These were broadly grouped into four categories, with the pressure receiving the highest risk score highlighted in bold, and the next most relevant indicated by asterix (*):

1. Occurring before or during the decline period

These pressures were active before or during the observed decline and may have contributed to long-term changes:

- **Organic enrichment**
- Nutrient enrichment *
- Changes in suspended solids

2. Background Pressures

Ongoing pressures that have persisted before, during, and after the decline period:

- Aquaculture *
- Abrasion/disturbance of the substrate on the surface of the seabed

3. Potential Future Threats

Emerging or increasing pressures that may impact sponge communities in the future:

- Invasive non-native species (INNS)
- Introduction of microbial pathogens
- Ocean warming *
- Synthetic compound contamination

4. Low Risk / Data-Limited Pressures

Pressures considered low risk or lacking sufficient data to assess their impact:

- Wave exposure changes
- Smothering and siltation rate changes

Evaluating drivers of sponge decline at Coleg Normal

Though populations of *H. panicea* and *A. fucorum* have remained stable since the decline observed at Coleg Normal between 2004 and 2009, the continued lack of recovery in abundance and luxuriance to pre-2004 levels suggest a persistent shift in environmental conditions during or just prior to that period.

Of the pressures assessed, organic enrichment, aquaculture and ocean warming received the highest risk scores. However, aquaculture and ocean warming are unlikely to have caused the decline, as they are ongoing pressures with no significant change during the specific time period. Nutrient enrichment, while ranked third in risk, lacked direct evidence of sponge-specific impacts and was assessed with low confidence. Therefore, of the pressures assessed, the most likely driver of the observed decline was the significant, widespread reduction in organic enrichment leading up to the decline, combined with localised changes at the start of the monitoring period at Coleg Normal.

Organic enrichment has been shown to enhance sponge biomass (Gökalp et al. 2021; Longo et al. 2022; Amato et al. 2024). Prior to sewage infrastructure upgrades, implemented under the 1991 EC Urban Waste Water Treatment Directive (UWWTD), the Menai Strait experienced high levels of organic input, with the Bangor outfall being the largest source of crude sewage and Coleg Normal the nearest monitoring site. The stronger response observed at Coleg Normal compared to Nelson’s Column likely reflects its proximity to both the main inputs at the North Eastern end of the Menai Strait and a local discharge point (Puente and Diaz 2015; Nasi et al. 2023).

Improvements to wastewater infrastructure likely reduced not only organic enrichment but also suspended solids and nutrient inputs, pressures that were present prior to the observed decline and showed downward trends over time. The Urban Waste Water

Treatment Directive (UWWTD) was one of several legislative measures, alongside the Nitrates Directive (1991) and the Environmental Protection Act (1990), that would have contributed to enhanced water quality in the region.

Overall, sponge responses to moderate nutrient enrichment appear relatively stable and species-specific, though further research is needed to understand the responses of key species in the Menai Strait. Suspended solids received a low-risk score, as available data suggest levels remained relatively constant in the decade before and after monitoring began. Consequently, no clear correlation was found between suspended solids and the observed changes in sponge luxuriance. However, if a sponge decline had already begun before monitoring commenced, as suggested by anecdotal evidence, it is possible it may have contributed. It is important to note that no site-specific data are available for suspended solids at Coleg Normal the existing data reflect broader trends across the Menai Strait.

Comparative sponge abundance data from the Oosterschelde estuary show similar patterns. *H. panicea* declined gradually between 1997 and 2001, followed by a sharp drop between 2001 and 2004 (Koopmans and Wijffels 2008). This may reflect impacts of the UWWTD, although a major dam project could also have influenced local conditions through increased fine sediment and reduced flow. Although the dam may have influenced local conditions, its completion ten years earlier suggests the benthic community might have stabilised in that time, and the changes could be linked to another pressure. Interestingly, *H. oculata* in the Oosterschelde, alongside a gradual decrease in abundance, showed a similar growth cycle to that observed at Nelson's Column and Warrior. This again indicates that this is likely a lifecycle/growth pattern.

Another decline in temperate sponges was observed in Lough Hyne, Ireland, where most three-dimensional sponges declined between 1990 and 2019, likely due to one or more mass mortality events between 2010 and 2015 (Micaroni et al. 2021). Temperature and eutrophication were suggested as possible causes, with nutrient levels rising in the lough and surrounding coast. Recovery since 2017 suggests the stressors are no longer present at levels that prevent recolonisation (Micaroni et al. 2025). The authors hypothesised that areas with higher currents or generally favourable conditions for sponge growth may show greater resilience. Given the well-flushed nature of the Menai Strait, a lack of recovery at Coleg Normal suggests either an ongoing pressure or a regime shift, such as the major wastewater improvements.

Notably, an increase in ascidians was observed during the sponge decline in Lough Hyne, and preliminary data suggest a similar pattern may occur at Beggars Reach in Milford Haven Inner waterbody (2009–2023), where *Halichondria* spp. appear to have decreased. This potential trend should be verified through the latest analysis and confirmed with future monitoring surveys. This area was classified with a poor status for DIN and moderate for opportunistic macroalgae in the latest WFD assessments, and have been designated a sensitive area (eutrophic) under the Urban Waste Water Treatment Regulations (Jackson-Bué et al. 2025). This suggests a nutrient enrichment pressure could be having an impact here. Ascidians are also known to increase with rising silt levels (Roberts et al. 1998), which have also been reported in parts of the Milford Haven Waterway. Alternatively, ascidians may be opportunistic colonisers following sponge loss, potentially indicating early recovery (Gili and Coma 1998). These pressures are more pronounced in the Haven, a more enclosed system with greater freshwater input and industrial activity, compared to the well-flushed Menai Strait.

The sustained decline of *H. oculata* at Coleg Normal, with no recent records, contrasts with other species and sites in the MS&CB SAC and PM SAC, where the species remains present despite declines. This suggests a site-specific and species-specific pressure may be influencing its continued absence. Its proximity to a sewage outfall may have previously supported the population via crude discharges. Evidence indicates *H. oculata* growth is strongly correlated with suspended particulate matter (Koopmans & Wijffels, 2008). However, it remains unclear whether historical inputs supported persistence, or whether other pressures, such as contaminants, seabed abrasion, disease, or temperature, are contributing to the ongoing decline.

Management considerations

The “unfavourable” conservation status of the reefs feature within the MS&CB SAC has largely been attributed to a reduction in sponge luxuriance and abundance at the Coleg Normal monitoring site. However, the observed decline may reflect a shift away from historically elevated sponge biomass associated with past organic enrichment, rather than a deterioration in reef condition. It is possible that the previously high levels of sponge cover were an artefact of anthropogenic enrichment and that the community now present is closer to its natural state. In this context, the baseline used to assess sponge condition may not have reflected natural levels, and it is recommended that work is undertaken to establish what an appropriate baseline should be. In addition, these findings suggest that the condition assessment for the site may need to be revisited to ensure that natural recovery is not misinterpreted as unfavourable change. More broadly this highlights the importance of establishing robust environmental baselines and monitoring multiple parameters to accurately detect and interpret ecological change, even though such comprehensive data collection is often unfeasible for long-term marine monitoring programmes with limited resources.

If the observed changes in the sponge community are linked to improvements in water quality, this highlights the value of using sponges as indicators of environmental condition. Sponges are increasingly recognised for their potential role in environmental monitoring due to their ability to filter large volumes of water and accumulate pollutants in their tissues. Their use has been demonstrated in biomonitoring for emerging contaminants and pharmaceuticals (Rizzi et al. 2023; Saliu et al. 2024) as well as heavy metals (Hansen et al. 1995; Aljahdali and Alhassan 2023).

Although there is uncertainty as to the cause of the absence of *H. oculata* at Coleg Normal, wider survey beyond the transects at this site and at Nelson’s column suggest this has not occurred elsewhere and is a site-specific reaction.

Ongoing monitoring

The pressures assessment identified several pressures that may become increasingly relevant in the future, particularly global warming and disease. Monitoring should be continued and targeted to ensure relevant data is collected.

Key Priorities:

- Sponge Luxuriance and Regrowth:

Ongoing assessments of sponge abundance and luxuriance should be maintained. For *H. oculata*, further investigation into regrowth at previously occupied sites could help confirm whether observed patterns reflect natural life cycles or persistent environmental stress.

- Temperature Monitoring:

Ongoing temperature monitoring is recommended, especially in light of increasing sea temperatures and the potential for longer marine heatwaves (MHWs). These conditions may exacerbate disease prevalence and stress in sponge communities. A temperature logger at the Menai Bridge Pier is recommended, if not already maintained by Bangor University or other local initiatives.

Knowledge gaps and research needs

The pressures assessment identified several areas where data on sponge sensitivity and ecological responses are limited, restricting our ability to fully understand the impacts of environmental stressors and the underlying drivers of change. To effectively use sponges as bioindicators of ecosystem health, a deeper understanding is needed, not only of their responses to external pressures but also of their internal biological dynamics. This includes the complex interactions within the sponge holobiont (the host sponge and its associated microbial community), which play a critical role in resilience and stress response (Webster and Taylor 2012).

This could be addressed through:

- Field and Tank-Based Studies

To better understand sponge responses to environmental stressors, a combination of controlled tank experiments and field-based research is recommended. Tank studies can isolate specific factors such as temperature fluctuations, nutrient loading (organic and inorganic), and contaminants, providing insights into species-specific growth, recovery, and stress thresholds. Complementary field studies across sites with varying nutrient regimes, particularly those influenced by agricultural runoff and sewage inputs, could be conducted to assess impacts on sponge health and community composition in natural settings.

- Microbiome Investigations

The sponge microbiome is increasingly recognised as a key factor in ecological function and stress tolerance. Establishing baseline microbial profiles for commonly monitored species in the Menai Strait would support their use as bioindicators and improve interpretation of environmental change. This could also help distinguish between natural life cycle patterns and stress-induced declines.

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Appendix

A. Sponge microbiome report

Associations between health status, locality, and microbiota of the Menai Strait sponge *Haliclona oculata*

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Abstract

Sponges perform vital ecological roles within their benthic ecosystem. In recent decades, die-offs and declines in certain species have been linked to environmental pressures and/or pathogens, either via directly affecting the sponge itself or the microbiomes of the sponge holobiont. The microbiome of sponges often contains members unique to Porifera, and characterising their community compositions can reveal a great deal about their function. Sponges separate into two groups based on whether they have a low microbial abundance (LMA), or high (HMA). *Haliclona oculata* is an LMA sponge living in the Menai Strait, North Wales with recent reported declines in health. To investigate this, samples were taken from healthy and necrotic tissue, a comparison species *A. fucorum*, living sympatrically with *H. oculata*, and seawater samples, in multiple locations. Multi-marker DNA metabarcoding was utilised to profile and compare the bacterial (16S), algal (23S) and fungal (ITS) communities. In *H. oculata*, health status was more strongly associated with bacteria alpha and beta diversity than site. In contrast, site had a significant influence on algal microbiome community differences. Differential abundance of specific taxa and/or taxonomic groups between healthy and necrotic sponges may provide health indicators or suggest that sponges might be experiencing adverse conditions. Overall, this study provides a comprehensive overview of the microbiome differences between healthy and diseased *H. oculata* and a novel resource for investigating the ecology and health of temperate sponges.

Introduction

Microbiomes are the diverse communities of microorganisms, including bacteria, archaea and fungi, that inhabit various environments, including in and on multicellular animals. Animal-associated microbiomes play crucial roles in the health and functioning of their hosts. These microbial communities are integral to numerous biological processes, including nutrient provision, nutrient cycling, immune system modulation, and protection against pathogens (Pita et al., 2018; Nicholsen, 2012). While much of the focus has traditionally been on the bacterial microbiome of the host, which undoubtedly contributes the majority of species (Getzke et al., 2019), there is increasing interest in the roles of other kingdoms, such as fungi and algae, and their interactions (Getzke et al., 2019; Deveau et al., 2018). Collectively, the various microbiomes and the host to which they belong are referred to as a holobiont (Dheilly & Rall, 2014; Carthey et al., 2019). Within the holobiont, residents coexist in a balanced state, but perturbations can lead to dysbiosis (Hacquard et al., 2015). Dysbiosis can have cascading effects on other partners in the holobiont and the host itself, potentially resulting in ill-health (Simon et al., 2019). With the rapidly growing recognition of the importance of microbiomes for host-health, there is increasing interest in how perturbations of “healthy” microbiota may contribute to population health and fitness in a wide range of animals including marine sponges.

Sponges (phylum Porifera) are sessile metazoan animals that feed by filtering water through specialized tissues in freshwater or marine environments (Taylor et al., 2007). Sponges are classified into four classes: Calcarea, Hexactinellida, Demospongiae, and Sclerospongiae, with Demospongiae being the dominant class, encompassing 83% of known species, which total upwards of 8,500 (van Soest, 2012). They appear in fossil records from the early Cambrian, with examples of all current groups present at that time (Bergquist, 1978). As one of the oldest animal lineages, having evolved in the pre-Cambrian, their cellular structure is comparatively simple. Besides organized epithelial tissue, sponges lack other organized tissue systems and organs (Simpson, 1984). However, they use their tissues effectively to filter food and particulate organic carbon (POC) from the water column. Water enters through inhalant pores and exits via oscules. The surface of a sponge consists of pinacocyte cells, while the inside contains chambers lined with choanocyte cells. These specialized flagellated cells create the water flow necessary for filtration (Van Soest, 2012). The remaining tissue is mesohyl tissue, which houses the skeleton, collagenous fibres, and the microbial communities that contribute to the sponge holobiont (Van Soest, 2012).

Sponges perform several important functions within their ecosystems. Firstly, they are reef-building organisms. Many species stabilize and bind the substrate, allowing other organisms such as corals to utilize the space (Bell, 2008). Others, such as the *Cliona* genus, act as bioeroders, influencing sediment creation and subsequent mineral availability. These two opposing mechanisms can balance each other, maintaining reef size and structure. Secondly, sponges provide shelter for various creatures, such as crustaceans (Butler et al., 1995), and serve as a food source for certain fish, starfish, turtles, and nudibranchs (Wulff, 2013; Wulff, 2006). Sponges also contribute to carbon fixation through photosynthesizing symbionts including single-celled algae and cyanobacteria (primary production), and through the filtering and digestion of plankton (Bell, 2008). Additionally, sponge microbiomes process nitrogen into more usable forms for other organisms and processes (Fiore et al., 2010). Changes in the abundance of sponges can lead to over-erosion, excess sedimentation, over-accretion, habitat/food loss, or effects on the balance of the carbon and nitrogen cycles in which sponges and their symbionts play an important role. Lastly, sponges have been cultivated by humans for commercial purposes and are studied for the potential usefulness of unique compounds in biotechnology (Sipkema et al., 2005).

There is increasing concern for global sponge populations with a growing number of reports of mass population declines. Sponge die-offs have been linked to various factors such as cyanobacteria blooms, temperature changes, and other abiotic stressors. For example, in Florida in 1991, a cyanobacteria bloom resulted in a significant loss of 23% to 80% of sponge populations across a large area, which also implied a shelter loss for spiny lobsters that use them for refuge (Butler et al., 1995). The exact mechanism of sponge death due to cyanobacteria blooms was not fully understood, but possible explanations included clogging of sponge canals or necrosis caused by commensal bacteria under altered conditions (Butler et al., 1995). Similarly, in the Mediterranean in 2008-9, a mass die-off of the sponge *Ircinia fasciculata* was linked to a marine heat wave that affected its photosynthesizing cyanobacteria, resulting in an 80-95% decline. In contrast, the sympatric sponge *Sarcotragus spinosulum*, which lacks phototrophic bacteria, was largely unaffected (Cebrian et al., 2011). This indicates that temperature-induced stress on non-sponge members of the holobiont can lead to significant sponge mortality due to the essential functions performed by sponge-associated microbiota.

Field and lab-based studies have confirmed elevated temperatures to be an important stressor to sponges, with tipping points for certain species occurring where damage becomes irreversible (Bell et al., 2023; Garrabou et al., 2009; Pantile & Webster., 2011; Posedas et al., 2022). However, not all sponges respond negatively to elevated temperatures and in some climate change scenarios sponges are predicted to do well (Bell et al., 2018). It seems that, while some sponge species will suffer, others may thrive at the expense of other organisms such as corals, leading to increased sponge density but lower ecosystem diversity. Since sponge species perform different ecological roles, any change in population densities will alter the balance within that ecosystem. Other known stressors include pH (Posedas et al, 2022), increased sedimentation (Pineda et al., 2017) and possibly increased nutrients such as nitrogen and phosphorus from farming and sewage (Webster, 2008). The only pathogen identified as directly responsible for sponge mortality is a strain of alpha-proteobacteria, which was confirmed as causing disease in *Rhopaloeides odorabile (Webster et al., 2002). Ultimately, pathogens and environmental changes may affect the sponge directly or cause dysbiosis in their microbiota, indirectly impacting their* health and susceptibility to stressors (Simon et al., 2019). The symbiotic relationship between sponges and their microbiomes, and its effect on sponge health under stressors, is complex, enigmatic, and has been the subject of much research over the last half century.

Research on sponge microbiomes began as early as the 1970s with the pioneering work of researchers such as Wilkinson, Vacelet, and Reiswig. Histological studies revealed differences in choanocyte cell size and aquiferous system complexity among sponge species, correlating with the uptake of bacteria from the environment (Wilkinson, 1978a' Wilkinson, 1978b). This uptake did not correlate with bacteria visible in mesohyl tissue, suggesting that filtered bacteria were largely consumed or destroyed by the immune system rather than integrating into the microbiome. In these early studies, it was clear from using microscopy that there were sponges whose mesohyl contained a lot of bacteria ("bacteriosponges") and those that did not. Today, work continues on these two types, now referred to as low microbial-abundance (LMA) and high microbial abundance (HMA) sponges (Taylor et al., 2007). LMA sponges tend to be dominated by a single clade of Proteobacteria or Cyanobacteria while HMA sponges contain representatives from a wider range of phyla including Poribacteria, Acidobacteria, Chloroflexi and Gemmatimonadetes (Giles et al., 2012).

Today, over 40 phyla have been detected in sponges, including Proteobacteria, Chloroflexi and Cyanobacteria (Thomas et al., 2016). In an analysis of sponges from 20 countries, approximately 40,000 operational taxonomic units (OTUs) in sponges were identified with up to 12,000 in any one species. Sponges host unique microbes, such as Poribacteria, named after their hosts (Porifera), which are almost exclusively found in sponges (Fieseler et al., 2004). "Sponge-specific clusters" (SSCs) of bacterial symbionts, identified by Hentschel et al. (2002), are defined as bacterial 16S sequences from different geographical locations and species that are more closely related to each other than to non-sponge sequences. This concept has been revisited with larger datasets, showing that 27% of bacterial, 41% of archaeal, and 14% of fungal sequences are classified as SCCs (Simister et al., 2012).

Sponge-associated microbes play essential roles in nutrient cycling, primary production, and host protection. Bacteria can secrete antimicrobial peptides, photosynthetic cyanobacteria contribute to carbon fixation, and sponge symbionts process nitrogen into more usable forms (Cuvelier et al., 2014). In addition, dissolved organic matter is converted by sponges to particulate organic matter, making carbon available to different organisms (Pita et al., 2018). Fungal and algal components of sponge microbiomes are less studied but crucial. Fungi contribute to nutrient cycling and may play roles in pathogen defence (Pérez-Llano et al., 2023). Algae, particularly cyanobacteria, are vital for primary production (Worden et al., 2019). The interactions between these non-bacterial microorganisms and their sponge hosts are complex and not fully understood, representing an under-studied area in sponge microbiome research. However, it is clear that the defences, production and needs of all the members of the holobiont create a balance whereby if one species is affected, there can be knock-on effects across the whole holobiont.

Haliclona oculata is an LMA sponge (Naim et al., 2014) showing declines and necrosis in the Menai Strait, North Wales (Natural Resources Wales). In this study, high-throughput metabarcoding was used to compare bacterial, fungal and algal microbiota in *H. oculata* and another LMA sponge *Amphilectus fucorum* that has shown no similar health issues to address the following questions: What are the differences and similarities between healthy *H. oculata* and *A. fucorum* as representatives of temperate LMA sponges?; Are there differences in the microbiomes of healthy and necrotic *H. oculata*? And are bacterial, algal and fungal members of the microbiota similarly associated with *H. oculata* health status?

Methods

Sample Collection

Samples of *Haliclona oculata* and *Amphilectus fucorum* were collected from four distinct locations (Figure 1) by scuba divers from Natural Resources Wales between June 22nd and July 1st, 2023. The divers aimed to collect 10 samples of each sample type per site: *Amphilectus fucorum* (referred to as HealthyAllAF), tissue from *Haliclona oculata* showing no visual signs of disease (HealthyAllHO), and tissue from specimens showing disease, including both necrotic tissue (NecroticHalfHO) and apparently healthy tissue (HealthyHalfHO) (Figure 2). Photographs were taken of all individuals prior to tissue collection. Upon collection, all samples were immediately placed into labelled falcon tubes. Upon reaching shore, seawater was decanted from tubes and tissue samples were submerged in RNAlater (ThermoFisher), transported to the laboratory, and stored at -80°C until processing. Additionally, three replicates of seawater samples (1 litre each) were obtained during each dive. Seawater samples were kept chilled, stored at 4 °C and filtered within 24 hours using 0.22 μm Sterivex filters. Blank controls were included for each filtration batch using UV-sterilized laboratory water. All filters were subsequently filled with Qiagen ATL buffer and stored at -80 °C.

Figure 1: Map showing the sampling locations used in this study. Coleg Normal, Swellies and Britannia are all in the Menai Strait. Britannia is close to a highly treated sewage outlet and Coleg Normal is close to a storm overflow. Bottle Rock is just outside the Menai Strait next to Puffin Island.

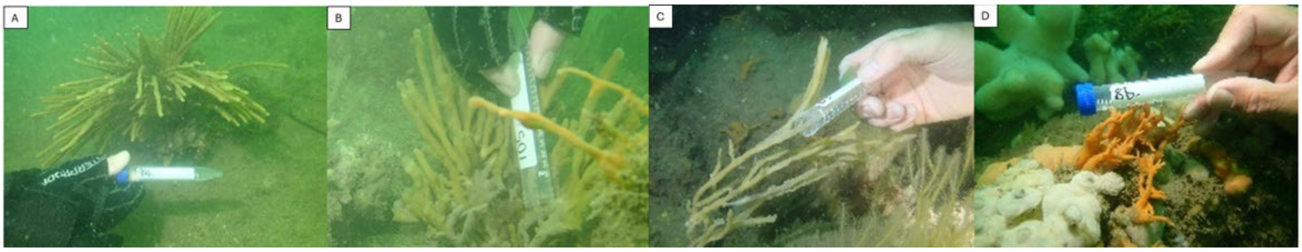


Figure 2: Photographic examples of specimens that exemplify the different kinds of tissue (Statuses) that were collected and compared in this study: A – *Haliclona oculata,* tissue from a fully healthy-looking individual (HealthyAllHO); B – *H. oculata*, tissue from an individual that is partly healthy and diseased (HealthyHalfHO and NecroticHalfHO). In this example, the different tissue types are easy to distinguish; C – HealthyHalfHO and NecroticHalfHO. On this site (Bottle Rock), clear examples were hard to locate and healthy and necrotic tissue is harder to differentiate; D – *Amphilectus fucorum*, the comparison species, which to our current knowledge is experiencing no health issues in the Menai Strait.

DNA Extraction

Tissue samples were thawed on ice and a cross-section of tissue was excised using a sterile scalpel on a sterile surface, taken from midway along a branch. The tissue was cut into small pieces and DNA extraction was performed using Qiagen Blood and Tissue Kits (Qiagen) following the manufacturer's protocol, with the following modifications: After tissue digestion, samples were centrifuged for 5 minutes at 14,000 rpm. The liquid was transferred to new Eppendorf tubes before adding ethanol. Additionally, samples were eluted with 100 µl Buffer AE instead of the standard 200 µl. Extracted DNA was stored at -20°C until further use. Filters used for seawater sampling were thawed and DNA was extracted using Qiagen Blood and Tissue Kits, following the same protocol as tissue samples.

PCR Library Preparation

To amplify bacterial DNA, the V4 region of the 16S rRNA gene was targeted using Earth Microbiome primers (515F: 5'- GTGBCAGCMGCCGCGGTAA -3'; 806R: 5'- GGACTACHVGGGTWTCTAAT -3') (Caporaso et al., 2012). Algal DNA was amplified using 23S primers (p23SrV_f1: 5'- GGACAGAAAGACCCTATGAA -3'; p23SrV_r1: 5'- TCAGCCTGTTATCCCTAGA -3'; Sherwood & Presting, 2007), and fungal DNA with Earth Microbiome Project ITS primers (ITSf1: 5'- CTTGGTCATTTAGAGGAAGTAA -3'; ITS2: 5'- GCTGCGTTCTTCATCGATGC -3'). All primers were uniquely indexed with a forward and reverse 12 bp barcode. All samples, negative controls, and extraction blanks were amplified using 12.5 µl of Qiagen Multiplex PCR Mastermix, 8.5 µl ultrapure water, 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), and 2 µl DNA template. Amplification was carried out using a Biorad T100 Thermal Cycler under the following conditions:

- **16S**: Initial denaturation at 95°C for 15 minutes, followed by 35 cycles of 94°C for 45 seconds, 50°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 5 minutes.
- **23S**: Initial denaturation at 95°C for 15 minutes, followed by 35 cycles of 94°C for 45 seconds, 55°C for 45 seconds, 72°C for 1 minute, and a final extension at 72°C for 5 minutes.
- **ITS**: Initial denaturation at 95°C for 15 minutes, followed by 35 cycles of 94°C for 30 seconds, 50°C for 45 seconds, 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

Agarose gel electrophoresis (1.5% TBE) was performed on a subset of samples for PCR visualization. DNA quantification was carried out using a Qubit Fluorometer (ThermoFisher Scientific). PCR products were pooled at equimolar concentrations. Prior to sequencing, all pools were bead-cleaned (0.9x volume) using AMPure beads following the manufacturers protocol (Beckman Coulter). Subsequently, DNA libraries were submitted to Novogene for Illumina Novaseq 2x150 bp sequencing.

Sequence Processing

The DADA2 pipeline integrated with the cutadapt package (Callahan et al., 2016) was utilized for sequence processing, including demultiplexing, read merging, error identification, and removal of primer sequences, low quality reads, and chimeras, and (Amplicon Sequence Variant) ASV for identification using default/recommended parameters. Taxonomy assignment using the DADA2 naive Bayesian classifier was performed using the SILVA reference database version 138 (https://zenodo.org/records/4587955) for 16S amplicon sequence variants (ASVs) and the UNITE database version 10 (https://unite.ut.ee/repository.php) was employed for ITS ASVs. In the absence of an equivalent curated 23S algae database, 23S ASVs were taxonomically assigned by BLAST searches against the NCBI nr database. The top 20 hits (e-value = 1E-4) were evaluated by Assign-Taxonomy-with-BLAST python script (Assign_Taxonomy_with_BLAST, 2024) to provide final taxonomic assignments.

Rarefaction curves were used to determine required minimum sequencing depth per sample for each marker**,** retaining only those with a minimum of 2000 reads for 16S and 23S libraries and 1000 reads for ITS libraries. This threshold ensured adequate sequencing depth for capturing microbial diversity comprehensively. The decontam package (Davis et al., 2017) was used to remove potential laboratory contaminants using the prevalence-based method and all extraction and PCR negative controls. ASV count tables, sequences and taxonomic assignments were imported in phyloseq (McMurdie & Holmes, 2013) for further analyses. For the 16S marker, ASVs labelled as "mitochondria” and “chloroplast” were excluded. Additionally, ASVs all makers that could not be taxonomically classified at least to the phylum level were filtered out.

Data Analysis

Rarefied datasets were generated based on the smallest library size and used for. Alpha diversity metrics including Observed richness, Shannon diversity index, and Simpson diversity index were computed on rarefied data. Significant differences in alpha diversities between groups (sponge tissue types and water), controlling for sampling site, was determined using Wilcoxon rank-sum tests. Beta diversity was assessed using the Bray-Curtis dissimilarity metric on rarefied data. Statistical significance was determined using permutational analysis of variance (PERMANOVA) through the vegan package (Oksanen et al., 2024) and adonis function. Post-hoc pairwise comparisons were conducted for both Site and Status factors. Non-metric multidimensional scaling (NMDS) plots were generated using phyloseq to visualize community structure based on beta diversity metrics. Differential abundance analysis was performed using DESeq2 (Love et al, 2014) on unrarefied data to identify taxa that significantly differed in relative abundance between groups. Various visualization techniques such as stacked bar plots, nested bar plots, and bubble plots were constructed using Phyloseq and fantaxtic to explore microbial community composition and dynamics across different experimental conditions.

Results and Discussion

Overall, after quality control and removal of mitochondria and chloroplasts sequences, 5666 ASVs were recovered from the bacteria (16S) samples with an average number of reads per sample of 12,665. For the algae (23S) dataset, 744 ASVs were recovered with an average number of reads per sample of 5503 and for the fungi (ITS) dataset 431 ASVs were recovered with an average reads per sample of 20,234.

Species and health status drives differences in bacterial diversity more than site

Overall, there was a significant difference in alpha diversity (Shannon index: F (4) = 44.8287, $p < 0.001$) of bacteria communities between sample types (hereafter “status”; species and health category). However, geographic location (“site”) had no significant impact (F (3) = 0.5185), p = 0.6703). Significant differences in alpha diversity were found between all status groups except for healthy and necrotic tissues sampled from *H. oculata* showing signs of necrosis (Figure 3b). However, dive notes show that there was sometimes difficulty finding both healthy and unhealthy tissue in the same individual sponge. Therefore, there was some uncertainly on allocating samples to these two statuses, especially at the Bottle Rock site. Photos were taken of each sponge sampled and indeed, some sponges are more visually healthy than others. It was decided not to re-label samples to avoid the risk of bias. In future, it may be beneficial to devise a more robust fine-scale necrosis score/index to categorise the health status of individual sponges.

Sites were chosen for potential differences in environmental conditions. Britannia is near a sewage outlet that is highly treated before expulsion, Coleg Normal is near to storm overflow point, and Bottle Rock is outside the Menai Strait near Puffin Island. Despite this, there appeared to be no differences in alpha diversity between the sites in both water and sponge-associated microbiomes. This could be because it is a highly tidal zone with twice-daily high and low tides of up to 10 metres with accompanying change in water direction, so water is frequently moved and mixed.

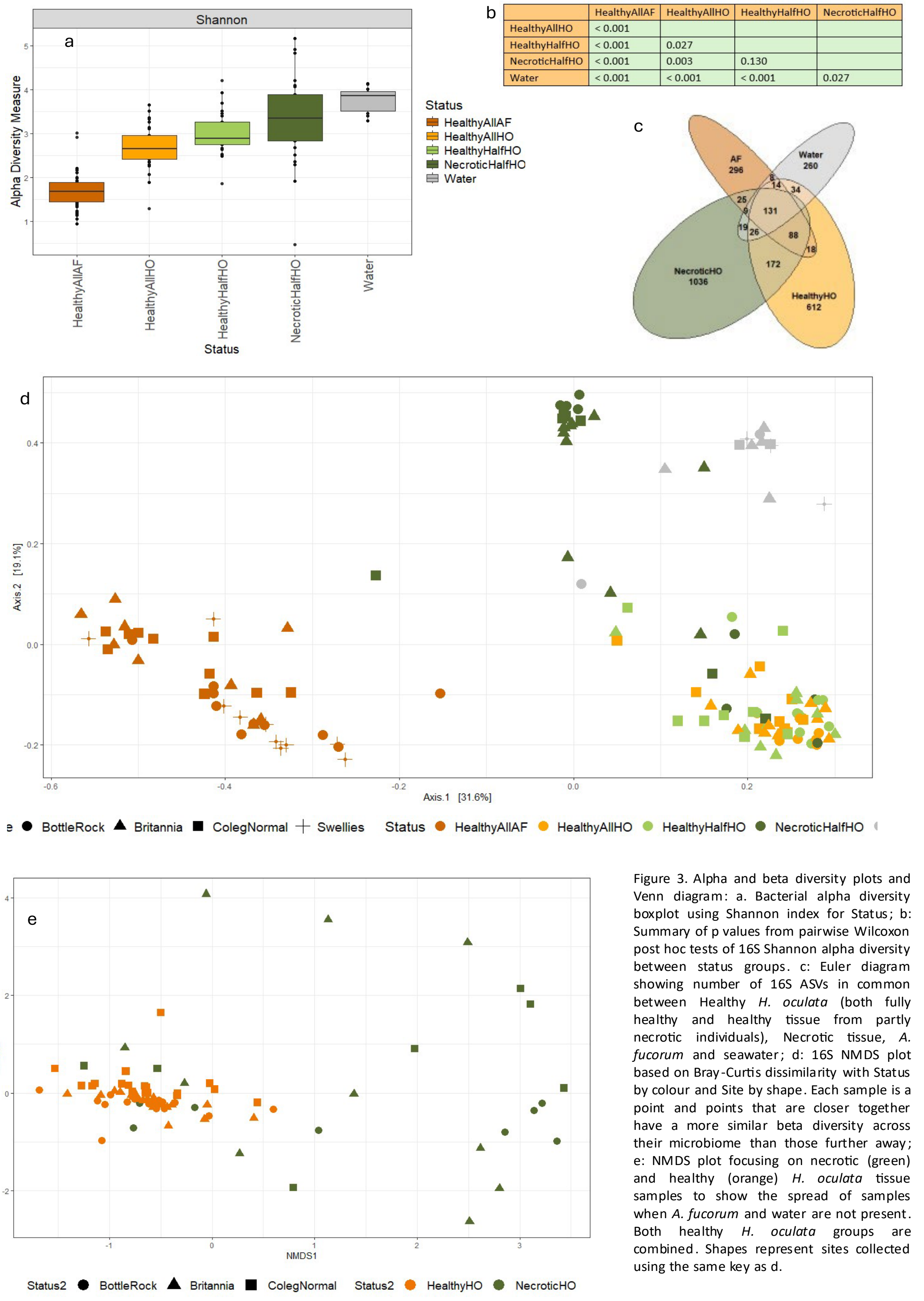
Seawater had the highest alpha diversity (mean Shannon index value = 3.77) and A. *fucorum*, which is not known to have any health issues in the Menai Strait, had the lowest alpha diversity (1.68). For *H. oculata,* alpha diversity seemed to decrease with health; fully healthy individuals had the lowest diversity and necrotic *H. oculata* tissues had the highest diversity (Figure 3a). The similarity of necrotic *H. oculata* tissues and water samples alpha diversities provoke questions about how far those similarities extend. If sick individuals lose control over their own microbiome, they could potentially become more similar to the environment as they die. Therefore, a large overlap in ASVs would be expected between necrotic tissue and water. However, this did not appear to be the case; only 185 bacteria ASVs were shared between necrotic tissue and seawater (Figure 3c), which is substantially lower than those shared with healthy *H. oculata* tissue (417 ASVs). Thus, despite sharing a similar alpha diversity, the bacteria found in necrotic tissue were largely different from those found in the environment and healthy sponge tissue. These differences may be due to factors such as differences in nutrient/resource availability (Pita et al, 2018), or adaptation of seawater bacteria to a planktonic lifestyle compared to within a (albeit dysbiotic) animal microbiome where an immune response and other microbial interactions are at work.

Differences in bacteria community composition between sample groups and sites were further examined via beta diversity assessment. Overall, both sample type and location had a significant impact on bacteria community composition (Status: F (4) = 36.144, R^2 = 0.508, $p < 0.001$; Site: F (3) = 5.364, R^2 = 0.057, $p < 0.001$). However, status had the strongest impact (contributing 51% to the differences), while site has a smaller influence at 6%.

Ordination of bacteria community composition distances (Non-metric Multi-Dimensional Scaling, NMDS, plots) showed distinctive groupings according to status (Figure 3d). *A. fucorum* samples clustered together regardless of site, as do the water samples, demonstrating a distinct community residing within each of these groups. Samples from fully healthy *H. oculata* and healthy tissue sections from individuals showing necrosis were largely intermingled. This shows that, in terms of beta diversity, the bacterial microbiomes of healthy tissue samples are very similar regardless of whether there is necrotic tissue elsewhere on the same sponge. While PERMANOVA pairwise tests showed a difference between these two groups, it was only marginally significant ($p = 0.043$) while all other pairwise comparisons were extremely significantly different ($p < 0.001$).

A large proportion of necrotic *H. oculata* samples were clustered together near the water samples, indicating that the bacterial microbiome composition of necrotic tissue is more similar to water than either *A. fucorum* or healthy *H. oculata*. However, there was also a spread of necrotic samples in the direction of and intermingled with the healthy tissue samples of *H. oculata*. Examining the photographs and diver notes for these samples, there was uncertainly at times about how to label the samples due to difficulty finding good examples of *H. oculata* showing both healthy and sick tissue, especially at the Bottle Rock site. This uncertainty could ultimately explain why both healthy *H. oculata* tissue types were still marginally significantly different.

When samples from fully healthy individuals and healthy sections from necrotic individuals were combined into one group and their beta diversity compared to necrotic tissue (Figure 3e), the healthy tissues clumped together quite closely while the necrotic tissue sample points were widely dispersed. This supports the Anna Karenina principle (Zaneveld, McMinds & Thurber, 2017) which is a concept applied to animal microbiomes from the opening to the novel which states that “all happy families look alike; each unhappy family is unhappy in its own way.” In other words, unhealthy animals’ microbiota will often show different forms of dysbiosis resulting in the erratic pattern shown here. This trend of increased alpha and beta diversity in necrotic tissue samples mirrored the findings in a freshwater sponge Lubomirskia baicalensis, (Belikov et al., 2019) and marine sponges Ircinia fasciculata (Blanquer et al., 2016) and Aplysina aerophoba (Webster et al., 2008). An environmental stressor, such as elevated temperature, may be contributing to this form of dysbiosis. For example, *Xestospongia mutasponge* produce proteins such as heat shock proteins under temperature stress that will divert resources away from microbiota regulation allowing pathogenic bacteria to enter (Blanquer et al., 2016) and influence the immune response, further promoting pathogen entry (Rosenberg & Zilber-Rosenberg, 2016).



	HealthyAllAF	HealthyAllHO	HealthyHalfHO	NecroticHalfHO
HealthyAllHO	< 0.001			
HealthyHalfHO	< 0.001	0.027		
NecroticHalfHO	< 0.001	0.003	0.130	
Water	< 0.001	< 0.001	< 0.001	0.027

Figure 3. Alpha and beta diversity plots and Venn diagram: a. Bacterial alpha diversity boxplot using Shannon index for Status; b: Summary of p values from pairwise Wilcoxon post hoc tests of 16S Shannon alpha diversity between status groups. c: Euler diagram showing number of 16S ASVs in common between Healthy *H. oculata* (both fully healthy and healthy tissue from partly necrotic individuals), Necrotic tissue, *A. fucorum* and seawater; d: 16S NMDS plot based on Bray-Curtis dissimilarity with Status by colour and Site by shape. Each sample is a point and points that are closer together have a more similar beta diversity across their microbiome than those further away; e: NMDS plot focusing on necrotic (green) and healthy (orange) *H. oculata* tissue samples to show the spread of samples when *A. fucorum* and water are not present. Both healthy *H. oculata* groups are combined. Shapes represent sites collected using the same key as d.

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Depending on which bacteria capitalise on the new opportunity first, the colonisation pattern will be unique in different animals of the same species undergoing difficulties causing microbial beta diversity that is quite different intraspecifically, leaving the pattern of raised alpha diversity uniformly higher overall. To qualitatively examine the taxonomic composition and differences in bacteria communities, the relative abundances of bacteria phyla and genera were visualised using stacked bar plots (Figure 4a & 4b). Overall, 42 phyla were detected across all samples, with Proteobacteria and Planctomycetota dominating healthy tissue samples in both sponge species (Figure 4a). A study of another LMA sponge *Cinachyrella kuekenthalli* identified 21 phyla (Cuvelier et al., 2014) and one of *Haliclona simulans* detected 8 in similar proportions to our top phyla (Kennedy et al., 2008) but since then techniques have become more sensitive. Phyla that modern metabarcoding techniques are now detecting include, as an example, Bdellovibrio, a known intracellular parasite of cyanobacteria (Wilkinson, 1979; Duncan et al., 2019). Similar to these studies, no Poribacteria were found, which are thought to be largely unique to sponges but mainly appear in HMA sponges (Hochmuth et al., 2010). Our results also compare to more than 60 phyla found across a wide range of LMA and HMA sponge types analysed for the Sponge Microbiome Project (Moitinho-Silva et al., 2017). The dominant phyla in all samples were Proteobacteria (49 – 75%), Planctomycetota (2 – 33%), Bacteroidota (1 – 15%), Verrucomicrobiota (4 – 8%) and Cyanobacteria (0 – 5%).

In contrast to healthy sponge samples, necrotic *H. oculata* samples contained a much more erratic mixture of phyla, which mirrors their erratic spread in beta diversity ordinations. In addition, at phylum level, water samples possessed similar taxa but with a distinct distribution. For example, water contains a larger proportion of Bacteroidota and a smaller proportion of Planctomycetota (figure 4a).

Examining the most abundant bacterial genera, a similar picture emerges, with consistencies in healthy *H. oculata* samples and an erratic distribution in necrotic tissues (Figure 4b). However, profiles of bacterial genera were highly distinct between sponge species and water. For example, only *A. fucorum* was dominated by an unknown proteobacteria of the Family “EC94”. Interestingly, the genus Endozoicomonas appears in all groups from all sites in the Menai strait, but not in any Bottle Rock samples. This genus is an indicator of health in other sponges and corals (Alex & Antunes, 2019; Bayer et al., 2013). The genus Synechococcus (phylum Cyanobacteria) appears to be more prevalent in healthy *H. oculata* than *A. fucorum*. There is evidence that cyanobacteria often contribute to the healthy microbiome of *Haliclona spp.* (Thacker & Freeman, 2012; Steindler et al., 2005) and may play a role in carbon fixation via photosynthesis (Webster & Taylor, 2012).

Abundance analysis of bacteria found in healthy and necrotic *H. oculata* samples found 35 ASVs that could be resolved to genera level that were significantly different in their relative abundance. 23 genera were higher in abundance in healthy samples, while 12 were more abundant in necrotic tissue (Figure 4c). Of the bacteria taxa that were more abundant in healthy samples, many are thought to contribute to animal health through their metabolic processes. *Synechococcus* is a cyanobacterium and as such contributes substantially to carbon fixation through photosynthesis (Palenik et al., 2003). Similarly, members of the NOR5/OM60 clade are photosynthetic bacteria known in marine environments that also contribute to the carbon cycle and primary production (Yan et al., 2009). Members of Candidatus Puniceispirillum genus and its clade SAR116 are known to carry genes for various metabolic processes that contribute to nutrient cycling (Oh et al., 2010). The chemoautotrophs of SUP05 possess genes that assimilate carbon, metabolise carbon, sulfur, amino acids and coenzymes, fix CO_2 and produce N_2O (Walsh et al., 2009). All of these bacteria may contribute to overall holobiont health by processing certain compounds to make others available to either the sponge itself or other symbionts of the sponge. Their lowered abundance in necrotic tissue could be direct (i.e. a stressor is affecting the bacteria first and the sponge as a result) or secondarily after impacting the sponge first.

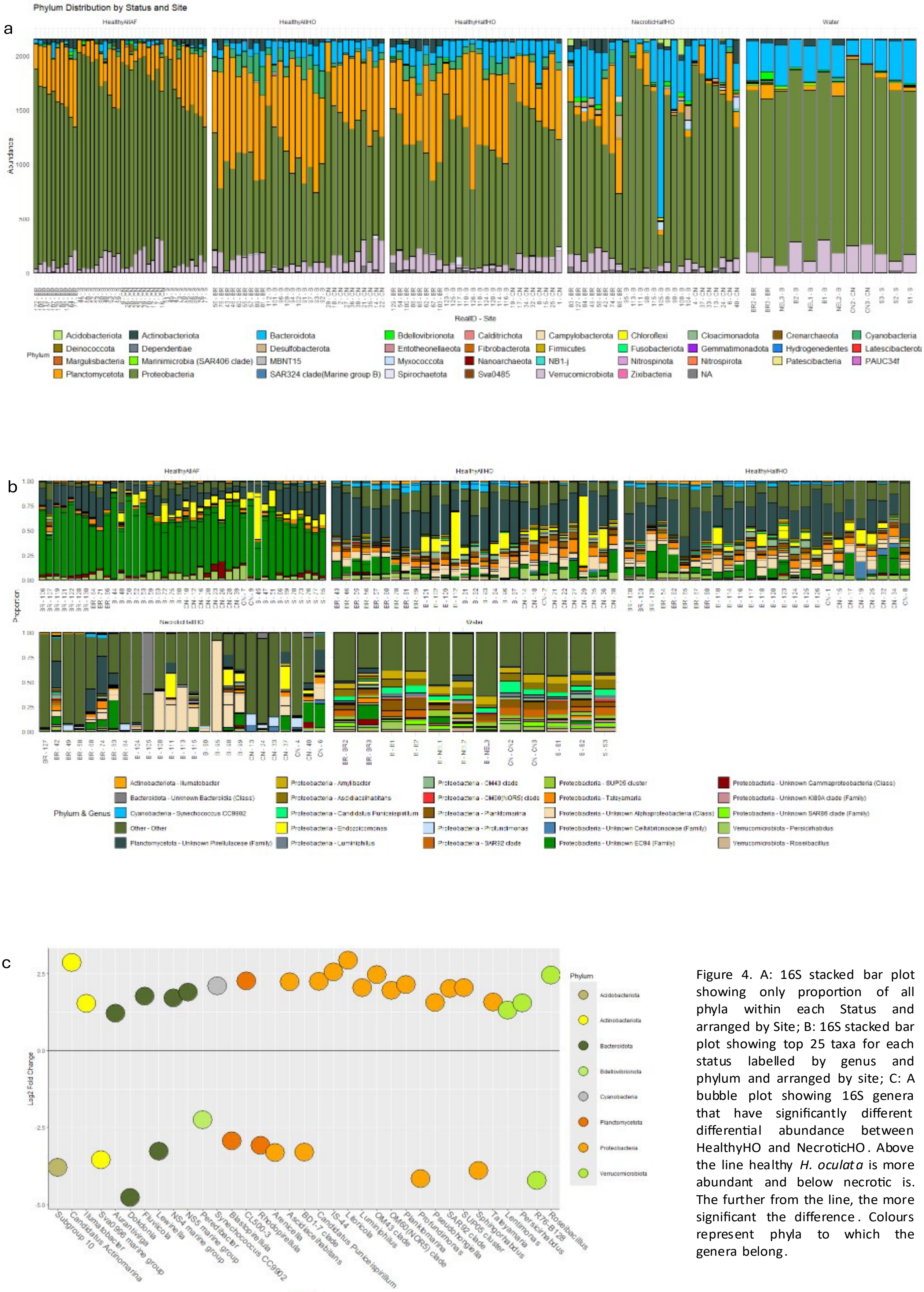


Figure 4. A: 16S stacked bar plot showing only proportion of all phyla within each Status and arranged by Site; B: 16S stacked bar plot showing top 25 taxa for each status labelled by genus and phylum and arranged by site; C: A bubble plot showing 16S genera that have significantly different differential abundance between HealthyHO and NecroticHO. Above the line healthy *H. oculata* is more abundant and below necrotic is. The further from the line, the more significant the difference. Colours represent phyla to which the genera belong.

Figure 4: A: 16S stacked bar plot showing only proportions of all phyla within each Status and arranged by site. B: 16S stacked bar plot showing top 25 taxa for each status labelled by genus and phylum and arranged by site. C: A bubble plot showing 16S genera that have significantly different differential abundance between HealthyHO and NecroticHO. Above line healthy H. oculata is more abundant and below necrotic is. The further from the line, the more significant the difference. Colours represent phyla to which the genera belong.

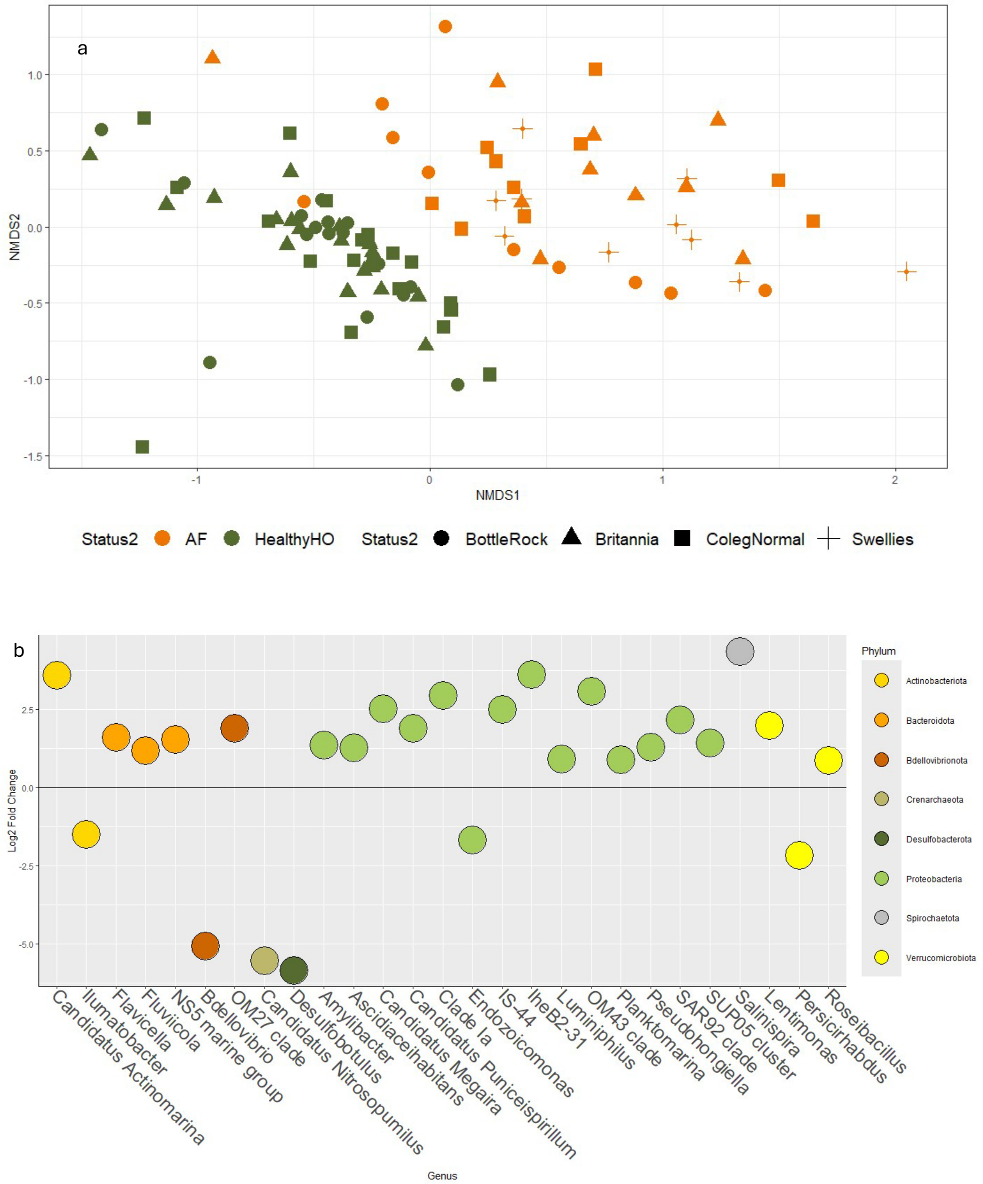


Figure 5: a: NMDS plot showing 16S beta diversity of bacterial community between the two species with site shown by shape and species by colour. c: Bubble plot showing 16 S genera with significant differences in differential abundance between the two species. Points above

the line are more abundant in *H. oculata* and points below the line more abundant in *A. fucorum*. The further the point from the line, the more significant the difference is. The Phylum that the Genera belong to is shown by colour.

Of the bacteria that were more abundant in necrotic *H. oculata* tissue, certain bacteria are worth comment. The genus *Lewinella* is common in wastewater and breaks down complex organic compounds (Kang et al., 2017). The genus *Aranicella* is strongly associated with a harmful blooming algae *Dinophysis*, which also appears in the list of taxa from this study. These algae can alter the planktonic communities and thereby affect other organisms such as shellfish (Hattenrath-Lehmann & Gobler, 2017). Both the bacterial and algal presence here could indicate a water quality issue. However, when the algal abundance was compared between sites, *Dinophysis* was not significantly different in abundance between Britannia/Coleg Normal (sites near sewage treatment areas) and Bottle Rock/Swellies. Interestingly, another toxic blooming algae – *Alexandrium catanella* – is also known to have strong associations with members of the BD1-7 clade (Yarimizu et al., 2024), which also appears in our list of differentially abundant bacteria. The BD1-7 clade was also not significantly associated with a particular site. This suggests that, although putatively harmful taxa were more abundant in necrotic sponge tissues, they were no obvious associations with proximity to human inputs.

Three genera of bacteria more abundant in necrotic *H. oculata*, Synechococcus (discussed above), Blastopirellula and Rhodopirellula have been linked with polyphosphate (PolyP) accumulation. PolyP is used by sponges in adverse conditions as a reserve of energy, participating in biochemical pathways involved with sugars, fatty acids, proteins and nucleosides (Ou et al., 2019). The increased abundance of these PolyP-associated taxa in necrotic tissue indicates that these bacteria may thrive in deteriorating tissue conditions due to their ability to utilise stored PolyP and could be useful as bioindicators. Lastly, the Sva0996 clade is of interest due to research that indicates it is an opportunistic scavenger, able to utilise degrading material, possibly from lysing cells in necrotic tissues (Brunet et al., 2021; Prins et al., 2024). This clade is associated with high nitrate concentrations (Indraningrat et al., 2022) which could again originate from degrading sponge tissue or elevated nitrates in the environment. We suggest this taxon could be further investigated as a bioindicator of sponge health.

Geographic location influences algal microbiomes

In contrast to bacteria communities, algal alpha diversity was not significantly different between status groups (F (4) = 0.774, p = 0.545) whereas site had a significant influence (F (3) = 14.97, p < 0.001). All pairwise comparisons between sites in the Menai Strait were not significant, yet Bottle Rock had significantly higher alpha diversity than all sites in the Menai Strait (p < 0.001, Figure 6a). It is not clear what could be causing higher alpha diversity outside the Menai Strait. It is worth remarking that samples near to storm overflows and sewage outlets in the Menai Strait have the lowest alpha diversity. Diatom alpha diversity has been linked with sewage outflows in France, where lower diversity existed near urban and hospital outflows than in natural river systems (Chonova et al., 2019). Generally, microalgae in marine environments are known for both their ability to detoxify pollutants and for their sensitivity to them, whereby photosynthesis and growth can be inhibited (Torres et al., 2009). The microalgae we detected in sponge tissues in this study are potentially symbiotic with sponges and so their response to stress may also depend on other effects within the holobiont. However, the impact of different nutrients and pollutants resulting from sewage outputs on algae associated with sponges warrants further investigation.

Despite no significant differences in algal alpha diversity between status groups, variability in the necrotic *H. oculata* was much larger than in any other sample type (i.e. sometimes it is lower than healthy tissue and sometimes higher, Figure 6b). This is possibly due to environmental algae that are not usually a permanent symbiotic partner opportunistically invading diseased tissue in some of the samples, while algae typical of healthy microbiomes in this species have left in others. Healthy and necrotic *H. oculata* samples had distinct algae as well as sharing number of algal taxa (Figure 6c). Necrotic tissue both gained many taxa (194 ASVs) and lost others that appear in healthy samples (56 ASVs). Interestingly, examining the overlap of algae ASVs between sites showed that the Menai Strait sites also have both distinct and shared taxa (Figure 6d). Thus, although their alpha diversity is similar, this does not mean that they share the same community composition.

Sampling location and sample status had a significant influence on algae beta diversity (Status: F (4) = 21.744, $p < 0.001$, $R^2 = 0.415$; Site: F (3) = 9.111, $p < 0.001$, $R^2 = 0.142$). The only pairwise comparison that was not significant was between the two healthy *H. oculata* groups ($p = 0.134$). Algae community composition of healthy *H. oculata* tissue was very similar regardless of whether sampled from a fully healthy sponge or one showing necrosis. This is evidenced by their largely overlapping beta diversity ordination (Figure 6e). The other sample types are largely clustered with some apparent site segregation, especially in *A. fucorum* samples (Figure 6e). Similar to bacteria community composition, healthy tissue sections from necrotic *H. oculata* individuals shows a spread towards the necrotic group perhaps also indicating a transition phase from algal microbiome of healthy to that of dysbiosis. In contrast to bacteria, however, the water algae community is quite different from the necrotic *H. oculata* and occupies a space distinctly separate from all sponge tissues (Figure 6e).

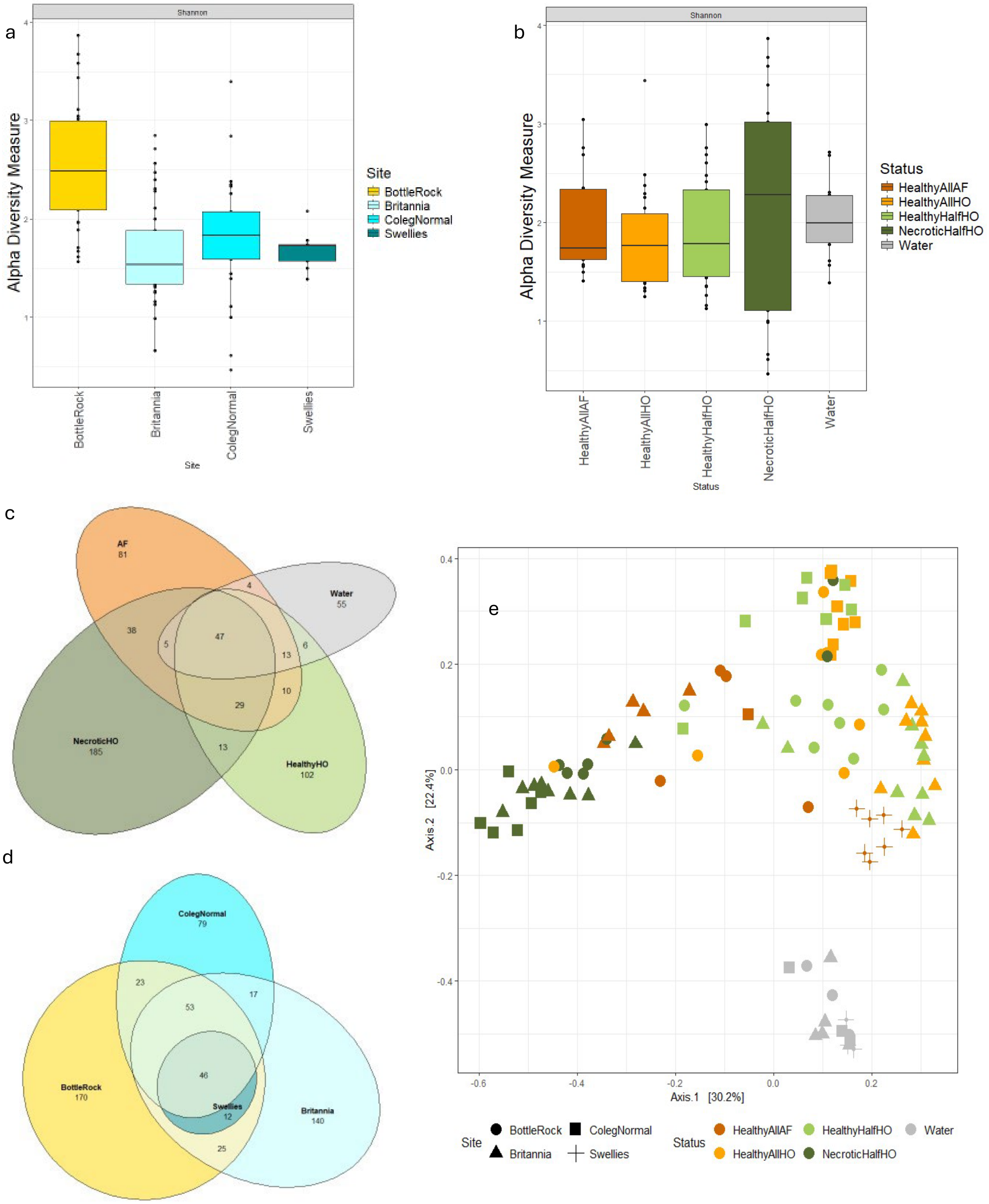


Figure 6: Alpha and beta diversity plots and Euler diagrams (proportional Venn diagrams). A: 23S alpha diversity plot using the Shannon index for Site. B: 23S alpha diversity using the Shannon index for Status. C: Euler diagram showing the overlaps between algal statuses

for taxa. The central number is common to all groups, while the outer numbers are unique to individual statuses with varying levels of overlapping in-between. The sizes of the ellipses reflect the overall number of taxa belonging to a status. D: 23S Euler diagrams for sites; E: An NMDS plot based on Bray-Curtis dissimilarity for both status (colour) and site (shape) for the algal dataset.

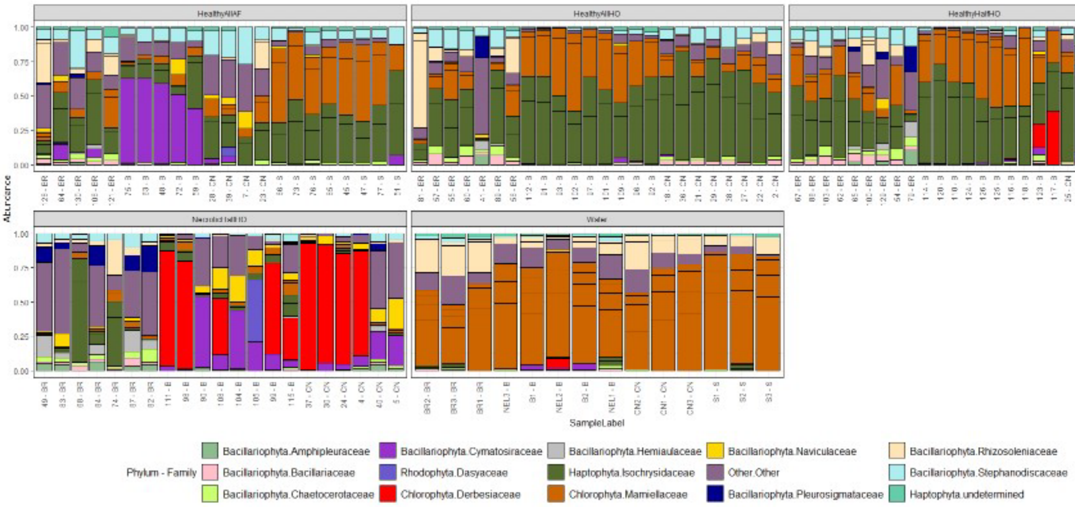
Six algae phyla were detected across all samples (Figure 7a) with Bacillariophyta (diatoms), Chlorophyta (green algae) and Haptophyta dominating healthy sponge tissue. Similar to the bacterial dataset, the pattern across necrotic samples is more erratic than other groups and water has a distinct profile largely dominated by Chlorophyta. As with alpha diversity, there are some site patterns noticeable especially in among *Amphilectus* samples. The erratic pattern in diseased tissue is repeated when focusing on the most abundant families (Figure 7b) and the similarity between the two Healthy *H. oculata* groups is also evident at the family level. The Family Cymatosiraceae (diatom) represents a much larger proportion of the community in the healthy Britannia *A. fucorum* samples but also appears in many of the diseased tissues and water samples at this site. As diatoms are known to be sensitive to pollutants and nutrient changes (Desrosiers et al., 2019), this may indicate highly localised distinct water quality and/or nutrient inputs in this location. The Family Mamiellaceae, which makes up most of the seawater algal community, also changes with site within tissue samples but is largely absent from necrotic tissue. The only genus within this family identified in our data – Micromonas – are microalgae known to be a key primary producer in ecosystems (Worden et al., 2019) and as such is likely to be an indicator of healthy microbiomes. This alga makes up a smaller proportion in the microbiome of *A. fucorum* samples, which are instead dominated by Cymatosiraceae. Lastly, Derbesiaceae from the phylum Chlorophyta appears in large proportions in necrotic tissue, but in heathy tissue samples from necrotised individuals it is less dominant, and only a minor component of completely healthy tissue communities. This suggests that it is an opportunistic invader and indicative of diseased or partially diseased tissue even if disease is not visually apparent.

Abundance analysis indicated that there are 15 different taxa that are differentially abundant between healthy and necrotic sponge tissues including taxa belonging to Mamiellaceae and Derbesiaceae (Figure 7c). The algae more abundant in healthy tissue are typically associated with primary production and thus carbon fixation or nutrient cycling. For example, Bathycoccaceae, which is closely related to Mamiellaceae, is a photosynthetic primary producer (Tragin & Vaulot, 2019), while Isochrysidaceae are known producers of essential fatty acids (Patil et al., 2007). These algae as a whole contribute to the holobiont and are indicators of a healthy microbiome. Stephanodiscaceae are reported to decrease with anthropogenic influence (Wolin & Stoermer, 2005) and therefore might act as bioindicators of anthropogenic disturbance.

a)


HealthyAllAF
HealthyAllHO
HealthyHalfHO
NecroticHalfHO
Water
Abundance
1.00
0.75
0.50
0.25
0.00
BR2 - BR
BR3 - BR
BR1 - BR
NEL3 - B
B1 - B
NEL2 - B
B2 - B
NEL1 - B
CN2 - CN
CN1 - CN
CN3 - CN
S1 - S
S2 - S
S3 - S
SampleLabel
phylum
Bacillariophyta
Chlorophyta
Haptophyta
Prasinodermophyta
Rhodophyta
Streptophyta

b)


HealthyAllAF
HealthyAllHO
HealthyHalfHO
NecroticHalfHO
Water
Abundance
1.00
0.75
0.50
0.25
0.00
SampleLabel
Phylum - Family
Bacillariophyta.Amphipleuraceae
Bacillariophyta.Bacillariaceae
Bacillariophyta.Chaetocerotaceae
Bacillariophyta.Cymatosiraceae
Rhodophyta.Dasyaceae
Chlorophyta.Derbesiaceae
Bacillariophyta.Hemiaulaceae
Haptophyta.Isochrysidaceae
Chlorophyta.Mamiellaceae
Bacillariophyta.Naviculaceae
Other.Other
Bacillariophyta.Pleurosigmataceae
Bacillariophyta.Rhizosoleniaceae
Bacillariophyta.Stephanodiscaceae
Haptophyta.undetermined

c)

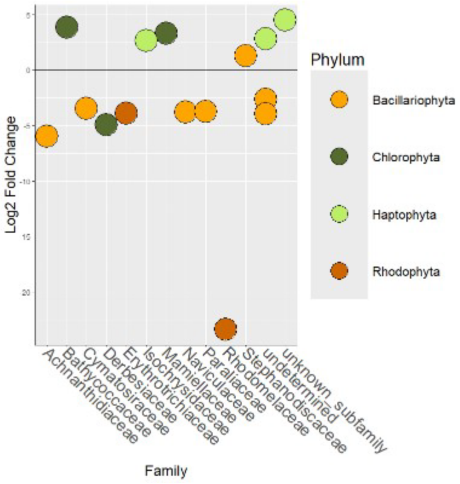

Log2 Fold Change
5
0
-5
-10
-15
-20
Achnanthidiaceae
Bathycoccaceae
Cymatosiraceae
Derbesiaceae
Erythrotrichiaceae
Isochrysidaceae
Mamiellaceae
Naviculaceae
Paraliaceae
Rhodomelaceae
Stephanodiscaceae
undetermined
unknown_subfamily
Family
Phylum
Bacillariophyta
Chlorophyta
Haptophyta
Rhodophyta

Figure 7: Differential and proportional abundance plots. a: bar plot showing all algal phyla proportionately represented in the different Status groups ordered by Site; b: Top 14 algal genera with phylum shown in the legend for the 5 Status groups and ordered by Site; c: 23S bubble plot showing the differential abundances between *H. oculata* (healthy) at family level. More abundant families in healthy is above and more abundant family in necrotic is below with the distance from the line indicating strength of difference; d: Bubble plot comparing algae from and *H. oculata* and *A. fucorum* with genera from genera more abundant in *H. oculata* above the line and *A. fucorum* below the line.

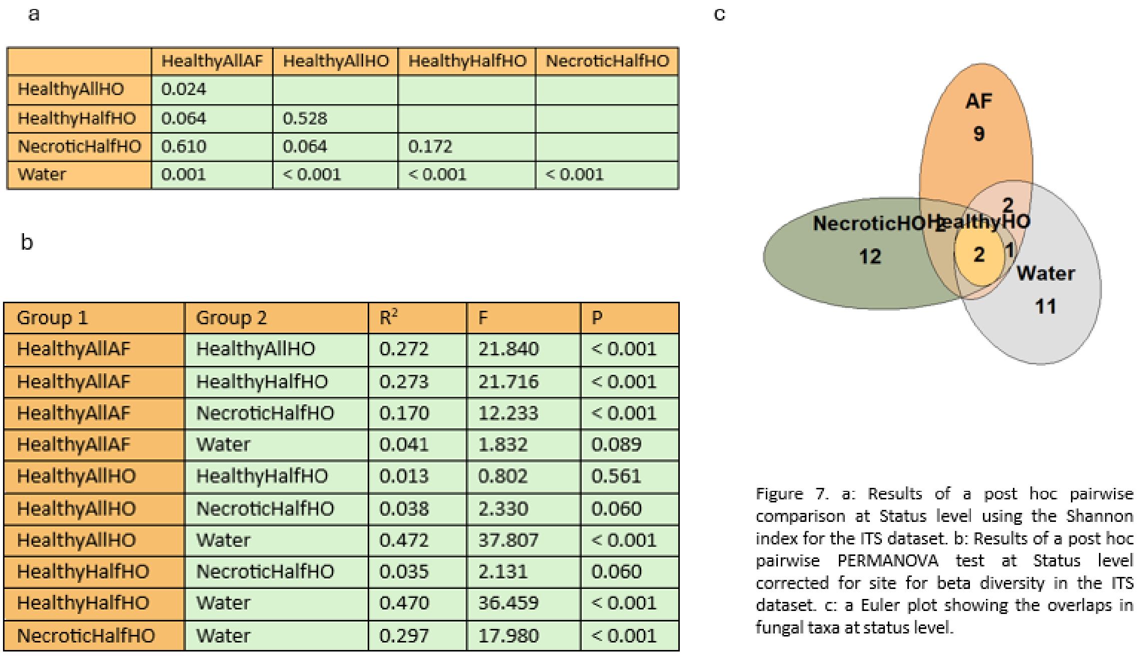
Naviculaceae is more abundant in the necrotic group. A freshwater study found different species of Naviculaceae were more abundant in either polluted or unpolluted water, so was is hard to conclude much from our own results without more taxonomic resolution (Benhassane et al., 2020). *Paralia* appears to be a tolerant alga that grows well under nutrient enrichment, which can happen as a result of vertical mixing of sediment or external inputs. It also tolerates low salinity (brackish) water and high salinity water from vertical mixing (McQuoid & Nordberg, 2003; Zong, 1997; Gebühr et al., 2009). This indicates that it grows well under disturbed or stressful conditions. If we had higher taxonomic resolution and data on nutrient levels, it might be possible to glean more from the differential abundance analysis of algae associated with these sponges.

Limited fungal diversity in Menai Strait sponges

Analyses of alpha diversity of fungi showed no overall significant influence of site (F (3) = 1.593, $p = 0.194$) but sample type was significant (F (4) = 27.252, $p < 0.001$). Pairwise comparisons revealed that this significance stems only from differences between seawater and tissue while all pairwise comparisons between tissue types regardless of species or health is not significant (Figure 8a). Overlap between detected fungal taxa in water and sponge samples is low (Figure 8c), suggesting that taxa present in these sponge species are not due to filtering from the water column. In addition, necrotic *H. oculata* tissue had more taxa than healthy tissue. It would be interesting to examine sediment samples to determine if these taxa may be opportunistically colonising necrotic tissue from the seabed. Otherwise, all sample types were quite distinct with only a small number of shared taxa between groups (Figure 8c).

Fungal community composition (beta diversity) was not influenced by site (F (3) = 1.467, $p = 0.139$, $R^2 = 0.023$) but sample status was significant (F (4) = 15.185, $p < 0.001$, $R^2 = 0.318$). All *H. oculata* tissue pairwise comparisons were not significant nor was the contrast between *A. fucorum* and water. All other pairwise comparison were significant (Figure 7b). Fungal beta diversity ordination showed clustering of samples into small groups, but this clustering does not seem to be as a result of either their status or site, which combined only contribute to approximately 34% of differences. These data suggest that something else that has not been measured in this experiment is affecting the beta diversity resulting in the clustering pattern shown. This could be true for the bacteria and algae microbiota too since site and sample status contributed to beta diversity differences by only 55% and 57% respectively.

Much of the data collected using the ITS marker was poor quality and did not resolve beyond "Fungi", suggesting further optimisation of primers and/or PCR conditions may be needed for their use in sponges. Of the ASVs that did resolve further, between 90% and 98% of the tissue samples belonged to Basidiomycota while only 32% belonged to this division in seawater. The remainder of all groups belonged to Ascomycota. The poor results may not be linked to marker choice despite other marine fungi research opting for the ITS2 region (Retter et al., 2019; Ogaki et al., 2021; da Silva et al., 2021) since there was a good quantity of ASVs obtained and reads per sample. The databases for marine fungi at this stage in time are not comprehensive and many of the entries in the UNITE database are from terrestrial studies. It is likely that as more research focuses on marine fungi and more sequences added, this kind of analysis will become more powerful.



a

	HealthyAllAF	HealthyAllHO	HealthyHalfHO	NecroticHalfHO
HealthyAllHO	0.024			
HealthyHalfHO	0.064	0.528		
NecroticHalfHO	0.610	0.064	0.172	
Water	0.001	< 0.001	< 0.001	< 0.001

b

Group 1	Group 2	R^2	F	P
HealthyAllAF	HealthyAllHO	0.272	21.840	< 0.001
HealthyAllAF	HealthyHalfHO	0.273	21.716	< 0.001
HealthyAllAF	NecroticHalfHO	0.170	12.233	< 0.001
HealthyAllAF	Water	0.041	1.832	0.089
HealthyAllHO	HealthyHalfHO	0.013	0.802	0.561
HealthyAllHO	NecroticHalfHO	0.038	2.330	0.060
HealthyAllHO	Water	0.472	37.807	< 0.001
HealthyHalfHO	NecroticHalfHO	0.035	2.131	0.060
HealthyHalfHO	Water	0.470	36.459	< 0.001
NecroticHalfHO	Water	0.297	17.980	< 0.001

Figure 7. a: Results of a post hoc pairwise comparison at Status level using the Shannon index for the ITS dataset. b: Results of a post hoc pairwise PERMANOVA test at Status level corrected for site for beta diversity in the ITS dataset. c: a Euler plot showing the overlaps in fungal taxa at status level.

Figure 8: a: Results of a post hoc pairwise comparison between sample types using the Shannon index for the ITS dataset. b: Results of a post hoc pairwise PERMANOVA test between sample types corrected for site for beta diversity in the ITS dataset. c: a Euler plot showing the overlaps in fungal taxa in sample types.

Conclusion

This study provides a comprehensive analysis of microbial diversity associated with healthy and diseased *H. oculata* and a comparison species *A. fucorum* in the Menai Strait, North Wales. The results reveal significant insights into how microbiomes differ at alpha and beta diversity levels depending on sponge health, species and site. Additionally, an analysis of specific bacteria and algae that differ significantly in their abundance across groups offers suggestions for what might be contributing to the difference or more detail on deeper impact to the holobiont.

The 16S dataset results demonstrate that health status exerts a more pronounced effect on bacterial diversity than geographic location. Significant differences in bacterial alpha diversity were observed across different health statuses, with necrotic tissue exhibiting higher diversity than healthy tissue. In terms of community composition (beta diversity), all tissue samples largely clustered according to group. However, healthy and necrotic tissue from the same sponges often intermingled, which suggests a range between very healthy and diseased with many samples falling somewhere between the two states. Despite the pronounced impact of health status on bacterial communities, geographic site variations contributed little towards the differences seen. In addition, the two sponge species, *H. oculata* and *A. fucorum*, conform to typical LMA profiles with a dominant Proteobacteria group. Overall, the abundance analysis indicated that *H. oculata* necrotic tissue samples had an erratic pattern of phylum and top genera composition. The genera found to be differentially abundant between healthy and necrotic tissues reveal bacteria taxa likely indicative of healthy microbiomes in this species while also drawing attention to other bacteria in necrotic samples that could hint at sponges living in somewhat adverse conditions.

In contrast to bacteria, algal alpha diversity was significantly influenced by geographic location, with higher diversity observed at the Bottle Rock site compared to the Menai Strait locations. This geographic effect highlights potential environmental or nutrient-based factors influencing algal communities associated with sponges. Algae beta diversity was impacted by both health status and site, and abundance analyses highlighted algae that may be indicative of health and/or issues either within the sponge holobiont itself or within the environment as a whole.

The study found no significant site effects on fungal alpha diversity, with significant differences emerging primarily between sponge tissues and seawater. Notably, necrotic tissues showed higher fungal diversity compared to healthy tissues, suggesting potential opportunistic colonization. However, fungal community composition did not exhibit strong clustering based on site or health status, indicating that other unmeasured factors may be influencing fungal diversity. The low taxonomic resolution of the dataset as a whole impacted the ability to interrogate diversity within the mycobiome comprehensively highlighting the need to improve the methods to fully capture this component of sponge microbiomes. Aside from this improvement, it would have been interesting to have had sediment sample data to compare samples to in addition to seawater.

Few sponge studies have extensively resolved down to the species level for members of the microbiome, so long-read sequencing could play a pivotal role in the future to enable us to investigate key species that might be playing a role in the holobiont-environment-health dynamic. Additionally, there is scope for future research to focus more on gene expression through the use of techniques such as RNA-seq to investigate how function is being affected by changes in conditions. At the moment, it is not always easy to unravel whether environmental changes are affecting the sponge directly and the microbiome secondarily, or whether the microbiome is affected first, causing the sponge to lose health as an effect. Both long-read sequencing and RNA-seq might shed more light on cause and effect in this intricate and complex system.

Overall, this study provides the first comprehensive overview of the microbiome differences between healthy and diseased *H. oculata* and a novel resource for investigating the ecology and health of temperate sponges.

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Data Archive Appendix

Metadata for this project is publicly accessible through Natural Resources Wales' Data Discovery Service https://metadata.naturalresources.wales/geonetwork/srv (English version) and https://metadata.cyfoethnaturiol.cymru/geonetwork/cym/ (Welsh Version).

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