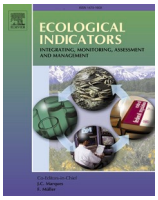
Ecological Indicators 129 (2021) 107879

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Effects of fish farm activities on the sponge *Weberella bursa*, and its associated microbiota

Olivier Laroche [a,b,*], Sonnich Meier [c], Svein A. Mjøs [d], Nigel Keeley [a]

[a] Institute of Marine Research, Tromsø, Norway
[b] Coastal and Freshwater Group, Cawthron Institute, Nelson, New Zealand
[c] Institute of Marine Research, Bergen, Norway
[d] Department of Chemistry, University of Bergen, P.O. Box 7803, N-5020 Bergen, Norway

ARTICLE INFO

Keywords:
(6 max): Transcriptomics
Gene expression
Functional profiling
Metabarcoding
16S rRNA
Fatty acids

ABSTRACT

Sustained growth of world-wide sea farming and the search of optimal growing conditions have driven several countries, including Norway, to establish new finfish sites in more exposed, high current locations. Characterized by a range of gravel, broken rock and/or bedrock, these complex environments and the associated diverse range of epifauna species are not easily monitored via traditional methodologies (e.g. morpho-taxonomic identification and enumeration, and compound analyses of sediment grabs). Consequently, little is known about many of the benthic inhabitants, or how they may respond to fish farming. In this study, we aimed to initiate addressing this knowledge gap by assessing the response of the sponge *Weberella bursa* (*Polymastidea*) to salmon aquaculture. Fourteen specimens were translocated along a distance gradient from a salmon farm located along the mid-west coast of Norway. Following 7 months of exposure, their epithelial tissue were analysed for gene expression analysis (mRNA), fatty acid (FA), stable isotope and taxonomic and functional microbiome characterization. Among all datasets, only fatty acid profiles showed significant changes associated with fish farm activities, with higher proportion of terrestrial FAs and long saturated and monounsaturated FAs near the farm. These results suggest that *W. bursa* sponges may be more resistant to organic enrichment than previously thought. Nonetheless, several putative indicators of non-lethal response could be identified. Specifically, *W. bursa* specimens located underneath the farm tended to have reduced ribosomal activity while having increased expression of genes controlling cell apoptosis (e.g. caspase-3, cytochrome *c* oxidase and death domain proteins). Based on predictive functional analysis, specimens near to the farm were also found to be particularly enriched in sulfur and nitrogen cycling bacteria, and in microbial taxa with anti-toxin and xenobiotic biodegradation capability, notably of benzyl benzoate compounds used in sea lice treatments. These results indicate that potentially harmful elements such as sulfite, nitrite and pesticides may be neutralized and degraded by a particularly enriched set of bacteria in *W. bursa* microbiome. While additional research is needed to validate these putative indicators, our study provides a first glimpse as to how sessile organisms may respond and adapt to environmental changes induced by fin fish farming, and pave the way to the development of novel monitoring tools adapted to mix and hard bottom habitats.

1. Introduction

In recent years, the sustained growth of world-wide sea farming (FAO 2018) and the pursuit of optimal growing conditions have led countries such as Norway to locate new production sites, traditionally placed in sheltered locations, in more exposed and high current areas (Holmer, 2010; Lader et al., 2017; Lekang et al., 2016). Contrary to protected sites that are typically characterized by soft bottom habitats, high current sites often encroach on headlands that are typified by a diverse of mix (e.g. gravel and broken rock) to hard bottom substrates (e.g. bedrock). These more complex environments usually support greater biodiversity than their soft bottom counterpart (Buhl-Mortensen et al., 2012; Dunlop et al., 2020; Sebens, 1991), yet little is known about the effect of fish farming induced organic enrichment on these types of benthic communities (Dunlop et al., 2020; Holmer, 2010; Salvo et al., 2017; Taranger et al., 2015). This information gap is not only due to the

* Corresponding author at: Cawthron institute, Private bag 2, Nelson 7042, New Zealand.
E-mail address: Olivier.Laroche@cawthron.org.nz (O. Laroche).

https://doi.org/10.1016/j.ecolind.2021.107879
Received 24 November 2020; Received in revised form 3 June 2021; Accepted 5 June 2021
Available online 9 June 2021

contemporary nature of the situation, but also the challenges associated with monitoring hard-bottom habitats with traditional methodologies (e.g. morpho-taxonomic identification and numeration, compound analyses of sediment grabs, Dunlop et al., 2020).

Alternative techniques, such as visual video-based surveys, have been proposed to characterize and monitor these habitats, but their effectiveness is limited by the irregular distribution of macro-benthic organisms over complex substrates (Hamoutene et al., 2018; 2015; 2016;; Wilding et al., 2012) and the limited information that can be gleaned from images in terms of organism health. In particular, visual methods primarily detect presence/absence information and require many observations and a lot of sampling effort to elucidate any trends. Another avenue currently investigated is the use of suction devices to collect particulate matter deposited at the bottom which could help characterize both physico-chemical and eDNA-based microbial community changes (e.g. Keeley et al., 2021). While preliminary results suggest tremendous potential for cost-efficient routine monitoring surveys (Keeley et al., 2021), this methodology does not provide information as to how macro-benthic organisms are being affected by the organic enrichment, smothering and additional side-effects of fish farm activities. Gaining some understanding of the internal response of these organisms is therefore essential in order to identify potential indicators of organism stress (based on tissue samples), and to provide an ecological rational for determining thresholds for other proxy indicators, such as microbial eDNA of the surrounding benthic biodeposits. Moreover, the ability to detect signs of stress in individuals provides a potential early warning mechanism of sub-lethal effects that may prevent the subsequent loss of potentially valuable ecological assemblages.

Suspension feeder and hard-substrate dependant benthic taxa such as sponges, bryozoans and corals are important biodiversity and ecosystem functioning contributors, notably due to their role in transferring energy from the pelagic to the benthic system, and in providing habitat for other taxa (Bell, 2008; De Goeij et al., 2013; Gili & Coma, 1998; Klitgaard, 1995; Maldonado et al., 2015). Sessile organisms are particularly vulnerable to environmental changes and while sponges may be differentially affected by environmental stressors, some have shown to be sensitive to anthropogenic stressors including sedimentation, notably due to the clogging of their aquiferous system (Sutherland et al., 2018). As such, they may represent fitting candidates for detecting and assessing the potential physiological impacts of salmon farming on the surrounding macro-benthos.

Recent developments in sequencing technologies and bioinformatics now enables broad gene expression analysis of non-model organisms (e.g. RNA-Seq) (Mutz et al., 2013) and host-microbiome characterization (DNA metabarcoding) (Yang et al., 2019). Such information can help assess the health and response of specimens towards anthropogenic activities and identify bio-indicators that can ultimately be used to monitor environmental conditions in a more targeted and efficient way (Clerissi et al., 2018; Dittami et al., 2020; Schirmer et al., 2010). Transcriptional and microbial community changes can lead to physiological alterations that can ultimately result in pathological conditions (Dean et al., 2017).

Fatty acids analysis has become a common tool for tracking effects of aquaculture waste into marine ecosystems (Fernandez-Jover et al., 2011; White et al., 2019; Woodcock et al., 2019). Marine aquaculture feeds are mainly constituted of lipids from terrestrial plants and consequently contain high levels of terrestrial fatty acids (FAs) such as oleic (OA, 18:1 (n-9)), linoleic (LA, 18:2 (n-6)) and a-linolenic (ALA, 18:3 (n-3)) acids. Marine animals eating aquaculture pellets or seston under aquaculture farms are therefore often found to have altered FA profiles characterized by higher levels of terrestrial versus marine FAs than animals feeding away from the farms (Ghanawi & McAdam, 2020; Gonzalez-Silvera et al., 2020; White et al., 2019). Similarly, stable carbon and nitrogen isotopes have been used as traces of carbon and nitrogen flux from organic waste from fish farms (Sarà et al., 2006; Woodcock et al., 2017; Yokoyama et al., 2006). Indeed, sedimentary organic matter in fish-farm areas are usually characterized by reduced δ13C and enriched in δ15N, due to the deposition of feces rich in terrestrial C3 plant (from feed pellets) and waste-feed-derived nitrogen (Yokoyama et al., 2006).

In this study, we aimed to begin to address the knowledge gap on how sessile benthic organisms living on mixed or hard-substrate habitats respond to the effects of salmon farming. To do so, *Weberella bursa* specimens, which are among the most common sponges in the Nordic Seas (Plotkin et al., 2018), were translocated along a distance gradient from a salmon farm located in the Sognefjord region along the mid-west coast of Norway. After being exposed for a period of seven months, these specimens were retrieved and their tissue analysed via 1) transcriptomics, for gene expression analysis, 2) 16S rRNA metabarcoding, for characterizing associated microbial communities and their predicted functions, 3) gas chromatography, for fatty acid profiling, and 4) ratio mass spectrometer for carbon and nitrogen stable isotope analysis. Specifically, our objectives were to find out 1) how these layers of information correlate to each other, 2) how sensitive they are to salmon farming, and 3) whether we could identify meaningful potential bio-indicators of fish farm activities that could be subsequently used in routine monitoring programs.

2. Materials and methods

2.1. Experimental set-up and sampling

A translocation experiment was set up to exploit the organic enrichment gradient created by elevated levels of organic waste being discharged from a fully functioning high capacity salmon (*Salmo salar*) farm in a fjord in northern Norway (Fig. 1). This study utilises samples from three stations arranged along a transect running parallel to the shoreline in the prevailing down-current (ENE) direction: 0 m (beside cage), 100 m (from cages) and at a reference station ~ 2,000 m distant. Mean sedimentation rates, measured from sediment traps deployed at the translocation sites during peak production following the methods of Keeley et al (2019), were of 44.4 (se = 11.1), 15.5 (se = 0.2) and 5.2 (se = 0.3) g/m2/d at 0 m, 100 m and Reference stations ,respectively.

Twelve *Weberella bursa* specimens were collected from a nearby reef approximately 2,800 m from the farm in comparable depths (~80 m) to the translocation sites (Fig. 1) using an ROV with a specially designed flexible (soft grab) surface-controlled pincer arm. 'Loose' or 'semi-loose' specimens (usually attached to small rocks on soft sediment) were selected (to avoid having to rip them form the substrate) and then placed in a reservoir in the ROV before being brought to the surface and transferred directly to an on-board holding tank (Fig. 2). The specimens were then individually weighed, photographed and tagged before being transferred to a grow-out frame and attached while immersed in water to avoid exposure to air (i.e. transferred in buckets). The grow-out frame constituted a mesh base that was open to the environment from all sides to ensure natural exposure to hydrodynamics and biodeposits, akin to what they would encounter on the seabed (Fig. 2). The grow-out frames were deployed close to the seabed (ca. 2 m above bottom) on mooring lines along a comparable depth band.

The sponges were cultured at the translocated positions for 7 months, from mid-March (15–18/03) until retrieval in mid-October 2018 (15–23/10). Upon retrieval, sponges were held in large transfer tanks before being transferred to small chambers for respirometry experiments (data not shown here), after which they were culled and dissected for tissue samples. Each specimen was divided radially under semi-sterile conditions (new gloves and clean cutting board and scalpel between individuals) to provide approximately 10 g of tissue from a cross-section (segment) of the animal, which was then placed in pre-labelled Eppendorf tubes and snap frozen in liquid nitrogen.

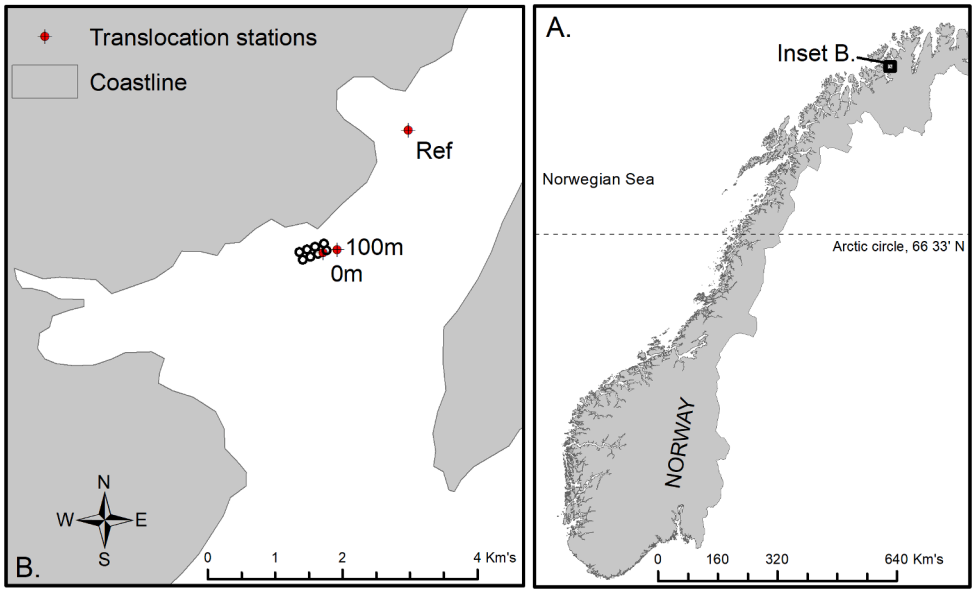


Fig. 1. Study site in Vargsundet, northern Norway, showing arrangement of sample stations in relation to the fish farm.

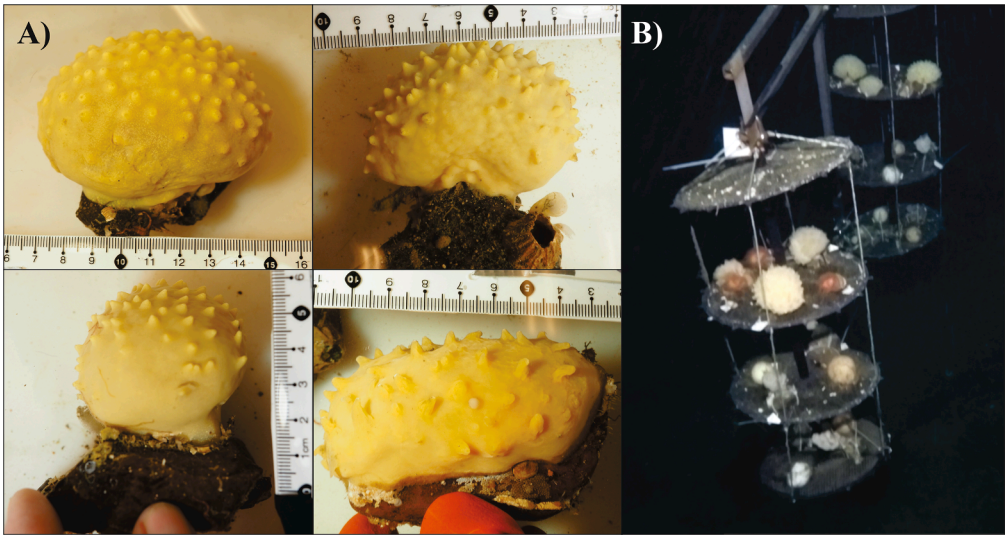


Fig. 2. Photos of four example *Weberella bursa* specimens before (A) and after (B) being transferred to the grow-out frames.

2.2. DNA and RNA extraction

Frozen tissue of *Weberella bursa* samples (−80 °C) were grinded and homogenized in liquid nitrogen with a sterilized mortar and pestle soaked in 50% commercial bleach solution (0.564 mol/L) for a minimum of 5 min and rinsed with double-distilled water [ddH_2O] between each sample. For each of the twelve samples (specimens), approximately 25 mg of powdered frozen tissue was lysed with the QIAshredder kit (QIAGEN, California, USA), and DNA/RNA co-extracted using the All-Prep DNA/RNA mini kit (QIAGEN, California, USA) following the manufacturer's instruction. RNA extracts were further processed to remove potential DNA contamination using the DNA-*free* kit (Life Technologies, California, USA) following the manufacturer's instruction. RNA integrity was assessed with the QIAxcel RNA QC kit v2.0 (QIAGEN, California, USA) and quantity measured with the Qubit™ RNA assay kit. Only samples with RNA interity number (RIN) above 7 or with a 28S rRNA / 18S rRNA ratio above 1.6 were used for transcriptome sequencing. To assess potential contamination, an extraction blank filled with ddH_2O was included.

2.3. Microbial metabarcoding library preparation

DNA extracts were quantified with the Qubit™ dsDNA BR (Life Technologies, California, USA), set at equimolar concentration (10 ng/μL) with 10 mM Tris for a total volume of 20 μL per sample and shipped on dry ice to the Norwegian Sequencing Center (NSC, Oslo, Norway) for 16S rRNA library preparation following the Fadrosh et al. (2014) protocol. Briefly, fusion primers that contained illumina overhang adaptors, a 12 base-pair (bp) sample index, a 0 to 7 bp heterogeneity spacer (to mitigate the issue of low diversity samples (Krueger et al., 2011), and the forward 319F: 5′-ACT CCT ACG GGA GGC AGC AG' or reverse 806R: 5′-GGA CTA CHV GGG TWT CTA AT-3′ 16S rRNA universal primers targeting the V3-V4 region (~469 bp; Fadrosh et al., 2014). Amplicons were purified and normalised using the SequalPrep™ kit (Life Technologies, California, USA), pooled together and paired-end sequenced (2x300 bp) with the Illumina MiSeq platform (California, USA). To assess potential contamination, a blank (ddH_2O) was added prior to sequencing. Raw sequence data is publicly available from the NCBI Sequence Read Archive (SRA) under project number PRJNA674901.

2.4. Transcriptomics library preparation

RNA extracts were set at equimolar concentration (25 ng/μL) and sent on dry-ice at the NSC for library preparation with the Strand-specific TruSeq™ mRNA-seq preparation kit following the manufacturer's instructions. A total of 12 libraries were prepared, pooled together and sequenced on an Illumina NovaSeq 6000 SP system (½ flow cell). Unprocessed sequence data is publicly available from the NCBI Sequence Read Archive (SRA) under project number PRJNA674901.

2.5. Bioinformatic analysis of 16S rRNA sequences

Fastq files were demultiplexed and primers trimmed using CUTADAPT (version 2.6; 2011) allowing no insertion or deletion and requiring a minimum overlap of 15. Sequences were then quality filtered and denoised with the default settings of the DADA2 R program (version 1.14; Callahan et al., 2016). The forward and reverse reads were merged using the default settings and a perfect minimum overlap of 10 bp. Chimera filtering was done using the consensus method where sequences found to be chimeric in the majority of samples (default value = 90%) are discarded. Remaining sequences were taxonomically assigned using the RDP Naïve Bayesian Classifier algorithm (Wang et al., 2007) trained on the SILVA 16S rRNA database (version 132 clustered at 99% similarity). Sequences found in the blanks, non-bacterial sequences, and those assigned to chloroplast were removed from the dataset. Additionally, only sequences with a minimum of 10 reads were kept for downstream analysis, so as to remove potentially spurious reads. Sampling depth per sample was visualized with the rarecurve function of the "vegan" R package (version 2.5.6; Oksanen et al., 2019) (Fig. S1). Sequences that remained post pre-processing were used with the PICRUST2 pipeline (Douglas et al., 2019) and default parameters used to predict the functional profile (KEGG orthologs) of *W. bursa* microbiomes.

2.6. Bioinformatics analysis of transcriptome sequences

The elvers automated RNA-seq workflow system (Johnson et al., 2019) was used to perform bioinformatics of the mRNA data as it allows de-novo assembly and facilitates the use of several tools in a repeatable manner. Briefly, Illumina adapters were trimmed from the sequences with TRIMMOMATIC (version 0.38; Bolger et al., 2014) and quality assessed with FASTQC (version 0.11.8; Andrews, 2010). Digital normalization, which aims to remove most artifacts, decrease sampling variation and reduce computational time and resources by eliminating redundant short reads (Brown et al., 2012) was performed with the khmer package (version 2.1.2; Crusoe et al., 2015). Filtered good quality reads were *de novo* assembled with TRINITY (version 2.8.5; Grabherr et al., 2011) and annotated with DAMMIT (version 1.2; 2016). Hash sketches (MinHash) were generated for each sequences with the SOURMASH program (version 3.1; Titus Brown & Irber, 2016) to enable quick similarity estimations. Read quantification was performed with the SALMON software (version 1.1; Patro et al., 2017) and differential abundance analyses with the "DeSeq2" R package (version ; 1.22.1; Love & Anders, 2014). Overall contig and read mapping metrics and estimation of the quality of the assembled contigs were computed by the TRANSRATE program (Smith-unna et al., 2016). Quantitative assessment of the transcriptome completeness was performed with the Benchmarking sets of Universal Single-Copy Orthologs (BUSCO, version 4.1.4; Seppey et al., 2019) using the metazoan_odb10 as reference. Details of the entire workflow and of the intermediary results are available in supplementary file 1.

2.7. Stable isotopes analysis

Homogenised samples of *W. bursa* specimens were dried at 60 °C for 24 h and were weighed out into tin capsules (1.28 ± 0.15 mg). All samples were sent to the Stable Isotope Facility of the University of California (Davis Campus, California, USA). Samples were analyzed for δ15N and δ13C using a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon). Stable isotope ratios were expressed in delta notation per the following formula:

$$\sigma X = \left[\frac{R\text{sample}}{R\text{standard}} - 1\right] x1000$$

where X is the heavy isotope, *R*sample is the ratio of heavy to light isotope in the sample, and *R*standard is the ratio of heavy to light isotope in the reference standard. The mean SD for reference materials replicates in this project is 0.06‰ for both δ15N and δ13C. In addition to giving the ratio of the two stable isotopes (δ15N and δ13C), the analysis also provides the total amount of carbon and nitrogen as percent of dry weight.

2.8. Fatty acids analysis

A sample of each *W. bursa* specimen (1–2 g) was homogenized by scalpel and a subsample (50–150 mg) weighed in a 16 ml glass tube for fatty acids analysis. Additional subsamples of 1.5–2 g were weighed on aluminium dishes for measuring water and ash content. To enable quantification of the lipids relative to ash free dry weight (total sample weight – water – ash), grounded *W. bursa* material was dried over night at 110 °C for determination of water content, thereafter the samples were burned at 450 °C for 6 h to determine the ash weight. The lipids are then quantified relative to ash free dry weight (total sample weight – water – ash).

All samples were methylated and the respective fatty acid methyl esters (FAME) were analysed on a HP-7890A gas chromatograph (Agilent, Santa Clara, CA, USA) with a flame ionization detector (GC-FID) according to a method described in (Meier et al., 2006) with the fatty acid 19:0 added as an internal standard. 2.5 M dry HCl in methanol was used as a methylation reagent. The FAMEs were extracted using 2 × 2 ml of hexane. The extracted hexane was diluted or concentrated to obtain a suitable chromatographic response. One ml was injected splitless (the split was opened after 2 min), with the injection temperature set to 270 °C. The column was a 25 m × 0.25 mm fused silica capillary, coated with polyethylene-glycol of 0.25 μm film thickness, CP-Wax 52 CB (Varian-Chrompack, Middelburg, Netherlands). Helium (99.9999%) was used as mobile phase at 1 ml/min and the temperature of the flame ionization detector was set at 300 °C. The oven temperature was programmed to hold at 90 °C for 2 min, then from 90 °C to 165 °C at 30 °C/min and then to 225 °C at 2.5 °C/min and held there for 20 min, for a total analysis time of 48.5 min. The chromatograms were integrated using the EZChrom Elite software (Agilent Technologies).

Identification of FAME and other lipids were conducted by comparing retention times with a FAME standard (GLC-463 from Nu-Chek Prep. Elysian, MN, USA) and retention index maps and mass spectral libraries (GC–MS; http://www.chrombox.org/data), performed under the same chromatographic conditions as the GC-FID (Wasta & Mjøs, 2013). Gas chromatography–mass spectrometry analysis of *W. bursa* samples was conducted using electron impact ionization (Agilent Technologies, 6890 N/5975B GC–MS system, Santa Clara, CA, USA).

2.9. Data analysis and statistics

Data analysis was performed on 5 layers of information (datasets), namely mRNA, fatty acids (FAs), stable isotopes, and the microbial ASVs and KOs profiles.

The taxonomic and functional profiles of *W. bursa* microbiomes were visualized with barplots and layered pie charts, respectively, using the "ggplot2" R package (version 3.3; Wickham, 2016). ASV alpha-diversity was assessed with the estimate_richness function from the "phyloseq" R package (version 1.30; McMurdie & Holmes, 2013) and visualized with

lineplots. Beta-diversity was assessed and visualized with a principal component analysis (PCA) using the Aitchison distance (Euclidean distance from centered-log ratio normalized data) as suggested in Gloor et al. (2017) for compositional data.

Correlation between datasets was tested with Protest analyses using the "vegan" R package (version 2.5.6; Oksanen et al., 2019) on centered-log ratio normalized datasets and 9,999 permutations. Briefly, Protest assess the correspondence between matching points (herein samples) of two datasets using least-squares orthogonal mapping and test for significant concordance using a permutational approach. The effect of taxonomic and functional richness of the microbiomes on *W. bursa* gene expression and fatty acid profile was tested with permutational analyses of variance (PERMANOVA) by using Aitchison distance with the "vegan" R package.

The response of each datasets towards the impacts of fish farming was tested with PERMONAVAs using Aitchison distance matrices against both the fourth root transformed distance from the pen and the estimated bacterial Metabarcoding Benthic index (bMBI) developed by (Keeley et al., 2018) and based on floc microbial data collected the same year along the same transect (see Keeley et al. (2021) for original data). Values for our specific stations (distances from pen) were obtained by using a linear regression of bMBI values on the square root of distance from the pen and by using the predict function of the "stats" R package (version 3.6.1; R Core Team, 2017) to obtain values for our specific stations.

Potential bioindicators of fish farm impacts were assessed using the "DeSeq2" R package (version 1.26; Love & Anders, 2014), with levels set to Reference, 100 m and Cage sites, using Reference as the control condition. For 16S ASVs, KOs, fatty acids and stable isotopes, the phyloseq_to_deseq2 function of the "phyloseq" R package (version 1.30; McMurdie & Holmes, 2013) was used to export the data into "DeSeq2" format. The geometric means were computed prior to estimating size factors with the estimateSizeFactors function (which control for difference in library/sample size), followed by the "DeSeq" and results functions. For fatty acids, the estimateSizeFactors function was followed by the estimateDispersionsGeneEst and the nbinomWaldTest functions to accommodate for the lack of overdispersion as recommended by (Love & Anders, 2014). The R script used to perform this analysis is publicly available at this github repository.

3. Results

3.1. 16S rRNA high-throughput sequencing output

Of the 12 samples, 2 failed to provide PCR products (Table S1). The remaining samples provided a total of 1,636,537 reads (mean of 125,887 per sample). Of those, 43.5% were discard after quality filtering, 0.2% after denoising, 15.5% after merging and 1.5% after removing chimeric sequences, resulting in 767,687 reads (mean of 59,053 per sample). None of the sequences found in blanks passed the pre-processing steps. Non-bacterial sequences and those either unidentified at kingdom level or assigned to chloroplast accounted for 32.6 and 10.6% of reads and ASV, respectively, and were removed from the dataset, leaving a total of 517,577 and 287 bacterial reads and ASVs, respectively. Removing rare ASVs (less than 10 reads) reduced the dataset to 371,707 reads and 49 ASVs. All samples had adequate read numbers (>10,000 reads) and reached saturation (Figure S1).

The microbial community was mainly composed of unclassified Proteobacteria families, followed by the Nitrospinaceae family and an unclassified Dadabacteriales family (Fig. 3A), with respective relative abundance varying substantially between specimens, irrespective of their location from the cage. Similarly, alpha-diversity metrics varied strongly between specimens, with a mean ASV richness of 23 (sd = 8.3), mean Shannon diversity of 0.96 (sd = 0.36) and mean Simpson diversity of 0.4 (sd = 0.17) (Fig. 3B). The 49 ASVs that passed the pre-processing step were associated with a total of 3,836 KOs, mostly falling in the transcription (24%), protein families, signalling and cellular processes (18%), unclassified KOs (18%), metabolism of cofactors and vitamins (6%) and amino acid (4%) (Fig. 3B). A PCA displaying beta-diversity of both ASV and inferred KO data is included in supplementary data

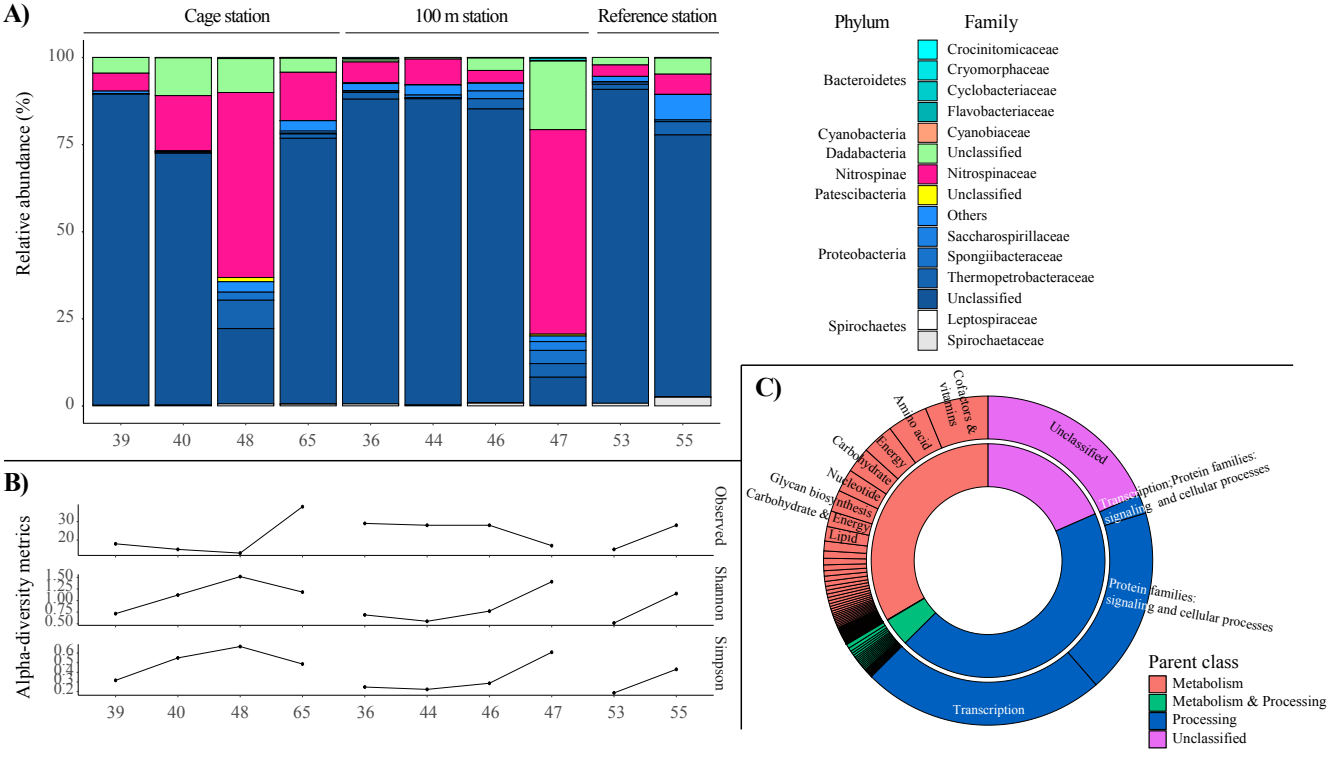


Fig. 3. Stacked barplot of microbial taxonomic community per sample (A), lineplot of alpha-diversity metrics per sample (B), and pie charts of the overall functional microbial community (C; parent classes (inner circle) and function groups (outer circle)) .

(Figure S4).

A total of 5 genera (17%) were found within all *W. bursa* specimens, composing the core microbiota (Figure S2). These included *Bdellovibrio*, *LS-NOB* (Nitrospinaceae), *Thermopetrobacter*, unclassified bacteria of the UBA10353 marine group, and unclassified Dadabacteriales. Most genera (62%) were found in at least half the specimens and none were solely found in a specific distance group from the pen (Figure S2). However, several genera were only found in proximity to the pen. These included *Alkalimarinus*, *Crocinitomix*, *Fulvivirga*, *Psychromonas*, *Sedimentitalea*, *Tenacibaculum* and an unclassified Rhodobacteraceae and Cryomorphaceae.

As for KOs, the great majority (64%) were found in all ASVs (Figure S3). While none were found to be specific to a distance group, 79 KOs (2%) were only present in the vicinity of the pen. These functions (those that were characterized) were mostly associated to carbohydrate metabolism and xenobiotics biodegradation (Table S2).

3.2. RNA-seq sequencing output

A total of 453,236,596 reads (mean of 37,769,716 per sample) were retrieved from the 12 samples sequenced on the NovaSeq 6000 SP (½ flow cell) (Table S1). Adapter trimming, quality filtering and paired-end read merging discarded ~ 15% of the reads. Merged reads were assembled into 376,266 contigs by Trinity, to which ~ 96% of reads could be mapped back (Table S3). The assembly score, percent of good contigs and overall contig and read mapping metrics, as estimated by the TransRate program, are available in Table S3, along with the BUSCO analysis results. Overall, the transcriptome completeness was estimated at 86.2%, with 7.1% and 6.7% of fragmented and missing BUSCOs, respectively. A PCA displaying dissimilarity between the transcriptome profile each specimen is included in supplementary data (Figure S4).

3.3. Fatty acids and stable isotopes

From 156 chromatographic peaks detected above the threshold of 0.05% of total peak area, 13 compounds were identified as fatty acids methyl esters (FAME) from the straight chain saturated FAs (SFA), 20 compounds were branched SFA, 3 compounds were isoprenic SFA, 23 compounds were straight chain monounsaturated FAs (MUFAs), 6 compounds were branched MUFAs, 6 compounds were polyunsaturated FAs (PUFAs), and 14 compounds were None-methyl-interrupted PUFAs. In addition, we found minor quantities of methoxy (2), cyclopropane (1) and hydroxylated (2) FAs. Several non-FAMEs compounds were also found, such as 8 dimethyl acetal compounds originating from ether lipids, 4 fatty alcohols, 19 sterols compounds and 17 compounds that could not be identified from the mass spectra. Retention time, retention index (ECL), name and percentages of the compounds found from GC–MS analysis can be found in the supporting information (Table S4).

Chromatogram and table of the compound identification are given in the supporting information, Figure S5-6 and Table S4.

Overall, >80% of the total FAs were represented by the 22 unique FAs (>1% of the total FAs) shown in Fig. 4. Lipids from *W. bursa* specimens were particularly dominated by in non-methyl-interrupted (NMI) PUFAs (32–40% of the total FAs), followed by MUFA (35–37% of total FAs) and SFA (19–20%), while the PUFAs that are normally found in high proportions in marine organism only contributed to 4–6% of the total FAs. The FA profiles per distance category are given in Table S5.

Only few apparent differences in FA profiles were related to proximity to the fish farm. In particular, sponges at the fish farm (0 m) had lower relative levels of the short chain SFAs (14:0 and 16:0) and higher levels of the long chain SFAs (22:0 and 24:0) compared with the reference area. Differences could also be found within the MUFAs, where the sponges at the fish farm (0 m) had lower levels of 16:1 (n-7) and 24:1 (n-7), and higher levels of 24:1 (n-9) and 26:1 (n-9) compared with the reference area (Fig. 4). Terrestrial fatty acid tropic markers (FATMs), which include 18:1 (n-9) + 18:2 (n-6) + 18:3 (n-3), accounted for a small fraction of total FAs and were found in higher relative abundance at the pen (Fig. 4).

Although non-significant, sponges close to the fish farm tended to have higher levels of total carbon relative to dry weight compared with

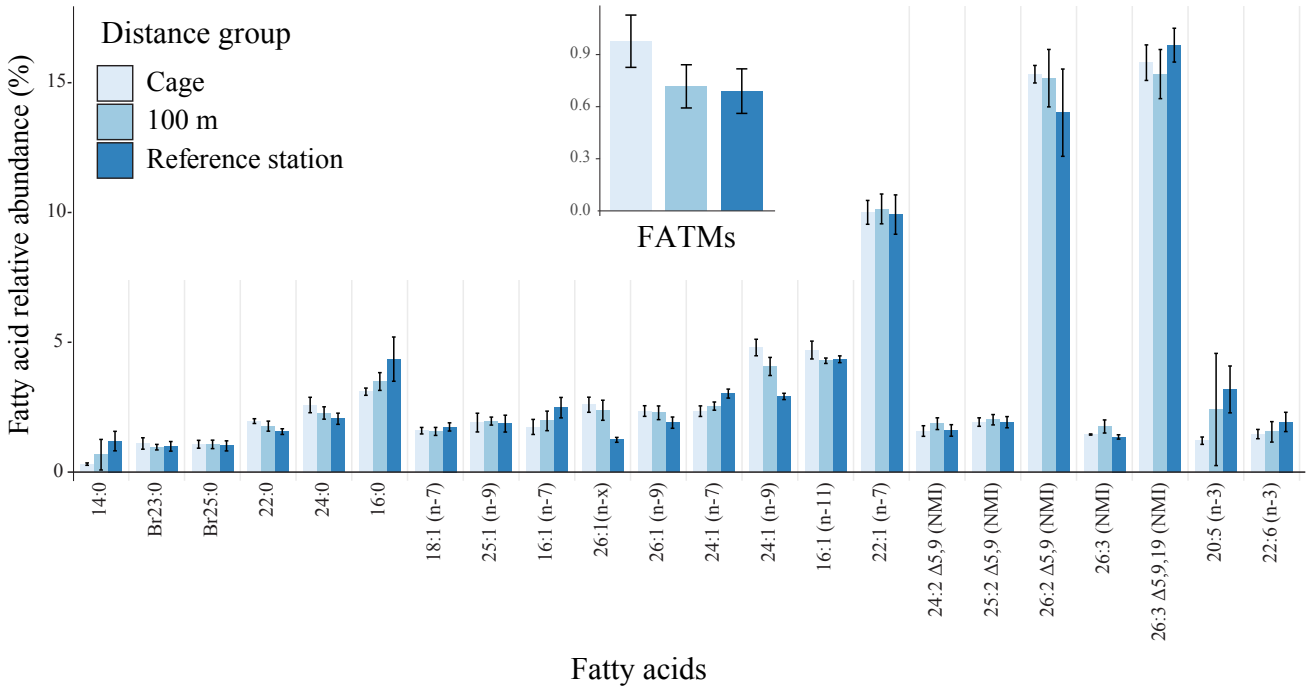


Fig. 4. Fatty acid (FAs) profiles of *W. bursa* specimens translocated at different distances from the fish farm (right under the cage [0 m], 100 m away, and at a references area 2 km away). Only FAs contributing to more that 1% of total FAs are shown in the figure. These FAs covered 81% of total FAs. Data are mean relative amounts (% of sum ± SD). The inlaid figure on the upper right corner corresponds to the sum of the terrestrial fatty acid tropic markers (FATMs; 18:1 (n-9) + 18:2 (n-6) + 18:3 (n-3)) per distance category.

the reference area (Fig. 5A), but there was no apparent difference for nitrogen content. Overall, the ratio of stable isotopes (δ15N (‰) / δ13C (‰)) tended to increase with distance from the farm (Fig. 5B), with lower variation in the ratio for sponges close to the fish farm.

3.4. Variance and relationships between datasets

Variance within layers of information was significantly different between all datasets (Table S6) and was by far the highest in mRNA data, followed by KOs, stable isotopes, ASVs and fatty acids (Figure S6). Overall, there was very little correspondence between datasets, with mRNA and stable isotopes (r = 0.49), fatty acids and stable isotopes (r = 0.43), mRNA and microbial ASVs (r = 0.408), microbial ASVs and KOs (r = 0.406) and microbial KOs and fatty acids (r = 0.406) being the most strongly associated data (Table 1). None of the relations were found to be significant. Additionally, permutational analyses of variance (PERMANOVA) showed no significant effect of microbial ASV and/or KO richness on *W. bursa* gene expression (Table S7), fatty acid (Table S8) or stable isotopes (Table S9) profiles.

3.5. Sensitivity of the datasets towards fish farm impacts

Using either the fourth root of distance from the pen or the bMBI values, the effect of fish farm activities on the taxonomic and functional richness of *W. bursa* microbiome was found to be non-significant (Table S10 and S11). The impact of fish farming on the beta-diversity of gene expression, microbial taxonomic and functional profiles, and stable isotopes was also found to be non-significant (Table 2 and S12). Only the fatty acid profile was found significantly impacted by fish farm activities (R^2 = 0.38, p.value = 0.02 and R^2 = 0.37, p.value = 0.02 for 4th root distance and bMBI, respectively).

3.6. Bioindicators of fish farm impacts

Indicators of fish farm activities were identified using the 'DeSeq2' methodology on each dataset, and by contrasting samples collected at the pen versus reference sites. On the mRNA data, 188 contigs were found significantly differentially expressed across the distance gradient. Among them, 33 could be annotated and are displayed in Fig. 6A. Notably, contigs associated with genes or proteins such as Caspase-3, Cytochrome *c* oxidase subunit 1 and death domain proteins were found to be primarily expressed at the pen while contigs associated with ribosomal proteins and androgen production were more expressed at reference sites (Fig. 6A).

Among the 49 microbial ASVs, 6 were found significantly differentially abundant between sites, all more present at the pen (Fig. 6B). These were affiliated to nitrite-oxidizing bacteria (LS-NOB), and the *Alkalimarinus*, *Colwellia*, *Crocinitomix* and *Sulfitobacter* genera. Of the 3,836 KOs identified, 19 were seemingly affected by fish farm activities, all significantly more abundant at the pen (Fig. 6E). Several of these KOs were associated to anti-toxin and xenobiotic biodegradation functions.

As for the fatty acids and stable isotopes, none were found to be significantly associated with a specific condition. Nonetheless, several

Table 1
Protest analysis testing for the non-randomness between the configurations of the four different datasets using 9,999 permutations. ASVs = amplicon sequence variants, KOs = KEGG orthologs.

Configurations	Sum of Squares	r	p.value
mRNA vs 16S ASVs	0.8339	0.408	0.421
mRNA vs 16S KOs	0.9281	0.268	0.860
mRNA vs fatty acids	0.8855	0.338	0.530
mRNA vs stable isotopes	0.7595	0.490	0.233
16S ASVs vs 16S KOs	0.8351	0.406	0.450
16S ASVs vs fatty acids	0.9502	0.223	0.896
16S ASVs vs stable isotopes	0.9662	0.184	0.961
16S KOs vs fatty acids	0.8355	0.406	0.477
16S KOs vs stable isotopes	0.8867	0.337	0.665
Fatty acids vs stable isotopes	0.8151	0.430	0.384

Table 2
Permutational analysis of variance per data source across the fourth root of distance from the pen. ASVs = amplicon sequence variants, KOs = KEGG orthologs.

Data type	Terms	Df	MeanSqs	F.Model	R^2	p.value
mRNA	Distance	1	78,917	1.029	0.093	0.264
	Residuals	10	76,698		0.907	
16S rRNA(ASVs)	Distance	1	31.831	0.1893	0.129	0.279
	Residuals	8	26.778		0.871	
16S rRNA(KOs)	Distance	1	3307.6	0.941	0.105	0.443
	Residuals	8	3514.8		0.895	
Fattyacids	Distance	1	26.056	5.02	0.386	0.02
	Residuals	8	41.528		0.614	
Stableisotopes	Distance	1	14.954	1.4913	0.157	0.28
	Residuals	8	10.027		0.843	

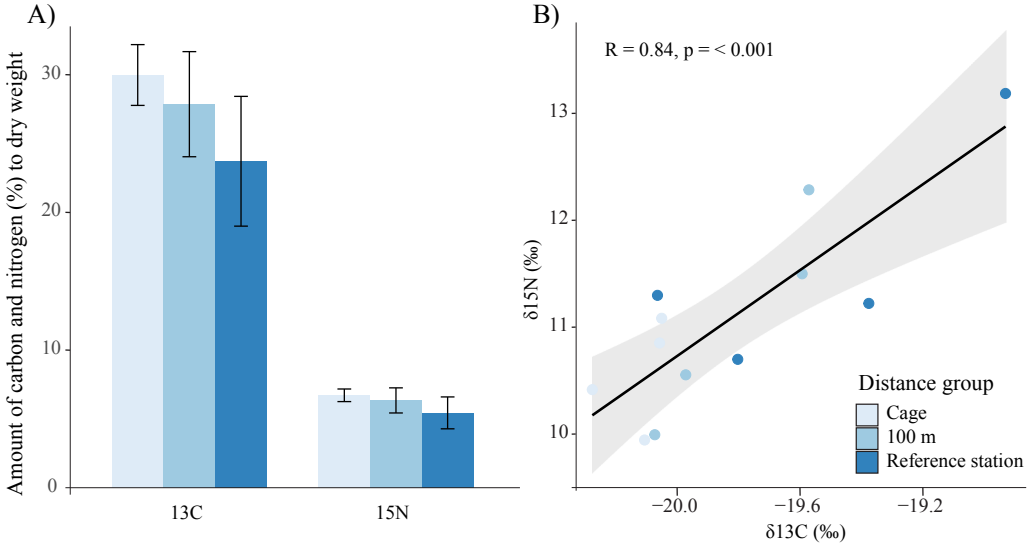


Fig. 5. Amount of carbon and nitrogen relative to dry weight (data giving in % as average ± SD) (A), and stable isotope ratios of δ15N (‰) over δ13C (‰) with regression line and Pearson correlation (B).

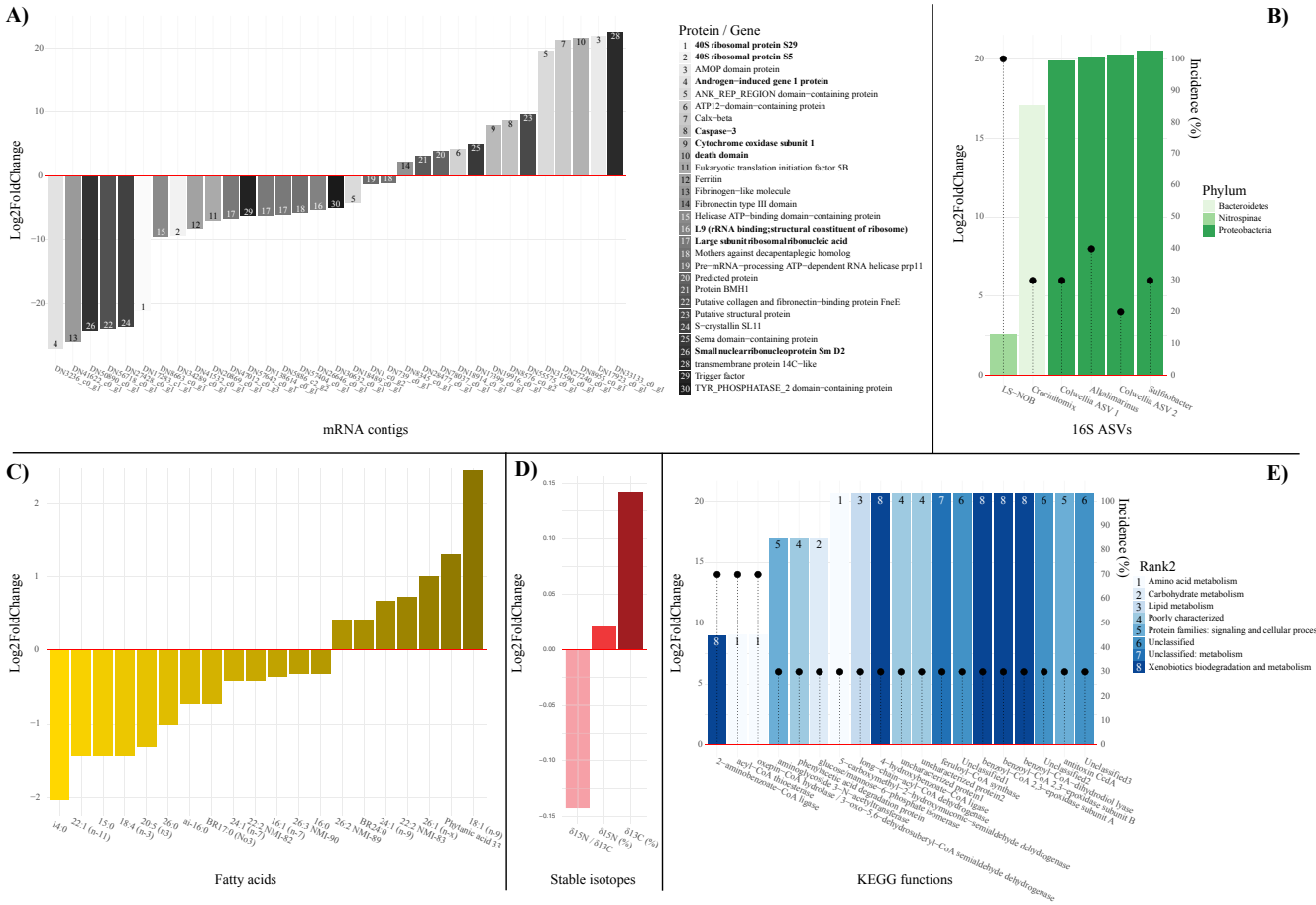


Fig. 6. Barplots of log2 fold change in abundance of mRNA contigs (A), 16S ASVs (B), fatty acids (C), stable isotopes (D) and 16S KEGG ortholog functions (E). A positive log2 fold change implies increased abundance near the pen and vice-versa. For A, B and E, displayed.

fatty acids such as 18:1 (n:9), Phytanic acid, 26:1 (n-x), 22:2 NMI, 24:1 (n-9) and BR24:0, and both stable isotope δ13C and δ15N showed increasing relative abundance near the pen while fatty acids 14:0, 22:1 (n-11), 15:0, 18:4 (n-3), 20:5 (n-3) and 26:0, and the δ13C / δ15N ratio were notably more abundant or higher away from the pen (6C-6D).

variables are limited to those with significant difference between the pen versus reference condition. For C, the 20 fatty acids with highest absolute log2 fold change are displayed. Incidence in B and E represents the percent of samples in which variables were found.

4. Discussion

Salmon farming generates and releases a substantial amount of organic and inorganic material in the surrounding environment including waste feed, faeces and by-products (e.g. medications and pesticides) (Carroll et al., 2003). This effluent not only has a smothering effect in the near-vicinity, but can also lead to eutrophication due to the high concentration of nutrients, and to toxic effects on the benthos (Bloodworth et al., 2019; Brown et al., 1987; Tett, 2008). By filtering large quantities of water, sponges remove dissolved organic matter and nutrients (e.g. nitrogen) from the water column (Bell, 2008) and as such they can alleviate the organic enrichment caused by fish farming. However, their slow growth and susceptibility to sedimentation makes them particularly vulnerable to these activities (Sutherland et al., 2018). In this study, we collected information on the transcriptome, fatty acid, stable isotopes and microbiome profiles of *W. bursa* specimens, with the main objectives of assessing their sensitivity towards salmon farming and finding potential bio-indicators of impact gradient.

4.1. *W. bursa microbiome and fatty acid characteristics*

Devoid of organs, sponges are known to harbour and rely on complex microbial assemblages to support various essential functions related to nutrition (e.g. dissolved organic matter [DOM] assimilation, nitrification, vitamin synthesis) and the defence system (e.g. synthesis of chemical compounds) (Pita et al., 2016). Consequently, they exert a firm control over the identity of their microbial occupants, resulting in species-specific consortia (Pita et al., 2016). Sponge microbial taxonomic diversity and density varies substantially between species, with sponges characterized by high microbial abundance (HMA) usually dominated by Chloroflexi, Acidobacteria and Actinobacteria (Moitinho-Silva et al., 2017; van Soest et al., 2012), while those with low microbial abundance (LMA) are mainly composed of Proteobacteria and Cyanobacteria (Bell, 2008; Giles et al., 2013; Gili & Coma, 1998). In this study, the recovered microbial taxonomic diversity of the *W. bursa* specimens was particularly low, with only 49 ASVs encompassing a total of 7 phyla. In comparison, the bacterial diversity in sediment samples (0.25 g) collected along the same transect and year by Keeley et al. (2021) averaged 565 ASVs. Low microbial diversity in sponges is typically characteristic of LMA sponges (Poppell et al., 2014; Rix et al., 2020). The overwhelming presence of bacteria from the UBA10353 marine group, followed by Nitrospinaceae and Dadabacteriales (Fig. 2A) is something that has also been observed in several other demosponges (see Moeller et al. (2019) and Fiore et al. (2020)). While we have no mean of estimating the microbial density of our specimens, the low ASV richness and clear dominance of Proteobacteria suggest that *W. bursa* belongs to the LMA group, characterized by both low microbial diversity and low microbial density. In regards to functional diversity, *W. bursa* microbiome

was predominantly composed of KOs responsible for transcription, signalling and cellular processes, and a relatively important proportion affiliated to the metabolism of cofactors and vitamins, energy and carbohydrates. Several other studies have found sponges to be enriched in microbial taxa capable of synthesising vitamins (Fan et al., 2012; Fiore et al., 2015; Lackner et al., 2017; Thomas et al., 2010) and carbon metabolisms (Kamke et al., 2013; Slaby et al., 2017), likely to satisfy their own needs (Pita et al., 2018). *W. bursa*'s potential reliance on its microbiome for nutritional and defence mechanisms may render it vulnerable to microbial turnover associated to a changing environment. It's important to note however that DOM assimilation from bacteria accounts for less than 5% of DOM uptake in LMA sponges, compared to 65–87% in HMA sponges (Rix et al., 2020). As such, LMA and HMA sponges may be differentially impacted by microbial dysbiosis.

Marine sponges are also known to have a very active lipid metabolism, resulting in very complex FA profiles, with many FAs not commonly found in marine lipids (Hahn et al., 1989; Rodkina et al., 2008). In this study, *W. bursa* specimens showed similar complexity, and like other demosponges, appear to synthetise large amount of NMI-PUFA such as 26:2 Δ5,9 (NMI) and 26:3 Δ5,9,19 (NMI), both predominant lipids usually found in sponges (Denis et al., 2009; Hahn et al., 1988).

4.2. Correspondence and sensitivity towards aquaculture activities

Sponges are known to rely on bacterial symbionts for supporting essential functions such as nutrition (e.g. carbon fixation, nitrogen metabolism), chemical defense and the synthesis of specific fatty acids (Li, 2019; Rod'kina, S.A, 2003), and to regulate their microbiome to match their needs. As such, we anticipated that the different components studied here (transcriptome, microbiome, fatty acid and stable isotope profiles) would be strongly interconnected. However, our results showed that none of them significantly correlated with each other, such as changes in microbiome beta-diversity for example was not followed by overall changes in gene expression, fatty acid or stable isotope composition. It is possible that the environmental conditions led to different effect and degree of impact on each data type, causing distinctive variance between them. For example, the lack of association between gene expression and the microbiome could be explained by the relatively high level of similarity in microbial beta-diversity between specimens, and the high variability naturally occurring in gene expression (Graw et al., 2021). While environmental factors may induce intraspecific variability in holobiont diversity (Steinert et al., 2016; Weigel & Erwin, 2016), sponge species-specific control over their microbiota is thought to be the principal driving factor of microbial diversity (Easson & Thacker, 2014; Erwin et al., 2012).

Another anticipated result was to find evidence of compromised health in *W. bursa* specimens exposed to salmon farming. Indeed, surveys conducted along the same transect within the translocation period showed a significant impact of fish farm activities on bacterial communities (Keeley et al., 2021) and on epifauna community assemblages (Dunlop et al., 2020), based on changes with respect to distance from the investigated farm. Additionally, several studies suggest that sponges are sensitive to anthropogenic activities (Edge et al., 2016; Scanes et al., 2018) and to sedimentation in particular (Sutherland et al., 2018). Under stress, sponges can respond by upregulating functions involved in maintaining cellular homeostasis and resistance for example, while downregulating housekeeping functions that are energetically expensive (Guzman & Conaco, 2016). This change can lead to a different selective pressure on the microbiota, disrupt the equilibrium found in healthy specimens, and render sponges more susceptible to diseases (Batista et al., 2018; Pita et al., 2016). Consequently, we expected the microbial consortia, and especially the variable fraction, to be influenced by the biological and environmental conditions associated with fish farming. As both gene expression and the microbiome can have a substantial effect on the host's fatty acid composition (Bennett et al., 2018; Rod'kina, S.A, 2005) we also expected to find a distinctive fatty acid signature of impact gradient. In addition, both fatty acid and stable isotope sedimentary profiles are known to be altered by the terrestrial components that make up fish feeds and that partly ends-up on the seafloor (e.g., Woodcock et al., 2017, 2019). To our surprise, our results suggest that fish farm activities had no significant effect on both alpha and beta-diversity of *W. bursa* microbiomes, nor on its gene expression and stable isotope profiles. Other studies have reported non-significant effect of environmental stress via sedimentation and nutrient enrichment on sponge microbiomes when under sub-lethal level (Baquiran & Conaco, 2018; Luter et al., 2014; Pineda et al., 2017; Simister et al., 2012; Strand et al., 2017) suggesting that the threshold to trigger such response may be relatively high. As for the transcriptome, variability introduced by temporal and spatial biological and environmental conditions in dynamic marine systems is relatively important (Goodwin et al., 2017), and is known to generate important background noise in transcriptomics due to the rapid response of gene expression to stimuli (Alvarez et al., 2015). While we could identify several genes that were differentially expressed along the exposure gradient (discussed in depth in section 4.3 below), our results show that the natural variability had a much greater effect on the transcriptome of *W. bursa*. Although our results indicate that sponges may be more resistant (herein the capacity of the holobiont to withstand perturbation; Pita et al. (2018)) than previously thought, they do suggest that sustained exposure to fish farming led to overall alterations in fatty acid composition of the exposed specimens. Indeed, individuals translocated near the farm were characterized by higher levels of terrestrial fatty acids, but also higher levels of long SFA (22:0 and 24:0) and MUFA 24:1 (n-9) and 26:1 (n-x) chains, and lower levels of de novo synthesis of 14:0 and 16:0, and desaturation of 16:0 to 16:1 (n-7). The increased amount of long-chain MUFA is likely to be due to the elongation of 18:1 (n-9) to 24:1 (n-9) and further to 26:1 (n-9). These differences seems to indicate that the lipid-rich diet of sponges in proximity to the farms was reducing the need of *de novo* synthesis of fatty acids and instead activates FA elongation enzymes that ultimately alter the fatty acid profiles (Bergé & Barnathan, 2005). In this regard, an interesting aspect for future studies would be to determine whether the effect of fish farms on sponge FA profiles is influenced by the host's dependence on its microbiome for nutrient assimilation (e.g. HMA versus LMA sponges).

4.3. Putative bio-indicators

In this study, we used four biological replicates per distance group to enable us to discriminate between natural variation and genuine transcriptomic signals induced by fish farming. We also measured gene expression 7 months after the specimens had being translocated to capture the sustained transcriptional change. Among the 33 annotated contigs found to be associated with exposure to fish farming, 20 were negatively impacted. These included several genes affiliated with ribosome activity (e.g. production of 40S ribosomal proteins, large ribosomal subunit, rRNA binding proteins) (Fig. 2). Ribosome are macromolecular systems responsible for the synthesis of protein and their activity provides a proxy of protein production, cell growth and metabolism activity (Calamita et al., 2018; Rudra & Warner, 2004). It is therefore not surprising to see their activity decrease under anthropogenic stress. Among the 13 genes significantly more expressed near the farm were caspase-3, genes associated to death domain proteins, and cytochrome *c* oxidase. The first two are directly involved in the regulation of cell apoptosis which, under stress, may be activated to eliminate damaged cells (Fulda et al., 2010). The mitochondrial cytochrome *c* gene is also known to control cell apoptosis by activating caspases (Cai et al., 1998). Together, the expression of these genes suggest that *W. bursa* specimens located near the pen did indeed experience some form of stress which could have affected their health in the mid/long term.

A few putative indicators of stress were also identified from *W. bursa*

microbiome. These included ASVs belonging to genera such as *Crocinitomix* and *Colwellia*, both previously identified as predominant taxa in coastal mariculture systems (Wang et al., 2018), *Sulfitobacter* and nitrite-oxidizing bacteria (LS-NOB). Sulfitobacter strains have been previously reported in aquaculture settings (Bourne et al., 2004; McIntosh et al., 2008) and are known to play a role in sulfur cycling from their ability to oxidize sulfite (Long et al., 2011). LS-NOB bacteria also represent an important bacterial group in aquaculture systems due to their ability to utilize nitrite and reduce eutrophication risk that may originate from the high nitrogen content of fish feed waste (McCaig et al., 1999). It has been suggested that sponges benefit from sulfur and nitrogen cycling bacteria for their ability to process and remove potentially toxic host-excreted compounds (Taylor et al., 2007). Interestingly, none of the indicators found associated to fish farm activities by Baquiran and Conaco (2018), which included members of the Bacilli and Fusobacteriia classes, were identified as such indicators in this study. This difference could be partly explained by the different primer set and reference database used to characterized microbial communities, and most likely by the different environmental conditions (shallow low-flow tropical site versus deep high-flow subarctic site) and sponge species (*Gelliodes obtusa* versus *Weberella bursa*) under study.

In addition to microbial taxa, 19 molecular pathways (KOs) were found positively associated with fish farm activities. Several of these are responsible for anti-toxin and xenobiotic biodegradation functions such as 2-aminobenzoate-CoA ligase, 4-hydroxybenzoate-CoA ligase and benzoyl-CoA 2,3 epoxidase subunit A and B. To treat and eliminate parasitic sea lice, a common blight in salmon farming that causes physical damage and stress to the fish, various medicinal compounds are administrated via feed additives or by bath treatment (Urbina et al., 2019). These include emamectin benzoate and benzoyl ureas, both used at the investigated site and known to negatively affect non-target benthic taxa at environmentally relevant concentration (Bloodworth et al., 2019; Macken et al., 2015). Our results suggest that these potentially harmful compounds, including sulfite and nitrite, may be neutralized and degraded by a particularly enriched set of bacteria in *W. bursa* microbiome specimens located near the pen, demonstrating the important role of these microorganisms for their host.

Because marine aquaculture pellets are partly made of terrestrial plants, they contain high concentration of terrestrial lipids such as oleic (OA, 18:1 (n-9), linoleic (LA, 18:2 (n-6)) and a-linolenic (ALA, 18:3 (n-3)) acids, and of stable isotopes such as δ15N and δ13C. As such, these elements are often used as indicators of mariculture impact gradient (Fernandez-Jover et al., 2011; Sarà et al., 2006; White et al., 2019; Woodcock et al., 2017, 2019; Yokoyama et al., 2006). In this study, *W. bursa* specimens close to the salmon farm tended to have elevated level of terrestrial fatty acids, and especially of 18:1 (n-9). Nonetheless, these contributed minimally (<1%) to the overall FA profile and as such, may not be effective biogeochemical tracers for aquaculture effect in marine sponges. Other more abundant FAs such Phytanic acid and 26:1 (n-9) were positively correlated to the impact gradient while 14:0, 22:1 (n-11), 15:0 and 18:4 (n-3) were negatively associated. Phytanic acid are derived from enzymatic degradation of chlorophyl and may indicate a higher consumption of algae material in sponges close to the fish farm (Roca-Saavedra et al., 2017). The FAs 14:0, 22:1 (n-11) and 18:4 (n-3) are FATMs found in calanoid copepods, and the presence of small amounts of fatty alcohols; Alc-16:0, Alc-16:1 (n-7), Alc-20:1 (n-9) and Alc-22:1 (n-11) in *W. bursa* specimens also suggest that *Calanus* copepods are part of their diet (Dalsgaard, 2003). However, further research is needed to confirm their response to salmon farming, and to determine whether it is a side-effect of differential gene expression or changes in microbial communities, or a direct effect of specific environmental variables. As for stable isotopes, both δ15N and δ13C tended to be elevated closer to the pen, with a slightly increasing of δ15N / δ13C ratio moving away from fish farm activities. Interestingly, lower variation in the stable isotopes was observed in specimens translocated near the pen, indicating these individuals had a more uniform food source (i.e. fish farm waste) versus those located at the reference site. While it is the first time stable isotopes have been assessed in *W. bursa* specimens to trace organic effluent from fish farms, our results are congruent with reported results within sediment (McGhie et al., 2000; Woodcock et al., 2017; Yokoyama et al., 2006). Additional investigations will be necessary to confirm the trend of these observations and validate the putative indicators highlighted here.

5. Conclusions

Overall, our results indicate that sponges (herein *W. bursa*) are more resistant to exposure (~7 months) to organic enrichment occurring at high-flow aquaculture sites than anticipated. Indeed, only fatty acid profiles changed significantly across the impact gradient from the salmon farm. The high flow conditions may have significantly reduced the smothering effect of farming activities on the sponges. It is also possible that the duration of exposure of sponges to sedimentation and smothering, and of ingesting potentially toxic waste, was insufficient to significantly affect the health of individuals. Future studies should consider investigating the effects over longer exposures (ca. 1–3 years). Nevertheless, our study identified potentially important indicators of non-lethal responses to exposure to fish farm activities from *W. bursa* tissue. These included genes associated to ribosome activity and to the control of cell apoptosis, microbial taxa with predicted capability for anti-toxin and xenobiotic biodegradation, and fatty acids such as phytanic acid and terrestrial fatty acids. Additional research will be necessary to validate these putative indicators for use in benthic monitoring surveys.

Author contributions

NK conceptualized the study and provided funding and equipment support. NK conducted field work and sampling. OL, SM and SAM generated the data and performed analyses, and OL wrote the manuscript with intellectual contributions from all co-authors.

Data accessibility

Unprocessed sequences are accessible from the NCBI Sequence Read Archive (SRA) under project number PRJNA674901. Metadata for the samples are available in the Supplementary Tables.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Support and funding for this project was provided by the Norwegian Research Council under the project 267829 SUSTAINable AQUAculture in the North: identifying thresholds, indicators and tools for future growth. We thank Raymond Bannister and Astrid Harenza for valuable assistance setting up the experiment and the captain and crew of Fangst for assistance at sea. We thank the Norwegian Academic HPC Services for access and support to their infrastructure to perform bioinformatics, and the Stable Isotope Facility of the University of California (Davis Campus, California, USA) for analysing the stable isotopes.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107879.

References

Alvarez, M., Schrey, A.W., Richards, C.L., 2015. Ten years of transcriptomics in wild populations: What have we learned about their ecology and evolution? Mol. Ecol. 24, 710–725. https://doi.org/10.1111/mec.13055.

Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data.

Baquiran, J.I.P., Conaco, C., 2018. Sponge-microbe partnerships are stable under eutrophication pressure from mariculture. Mar. Pollut. Bull. 136, 125–134. https://doi.org/10.1016/j.marpolbul.2018.09.011.

Batista, D., Costa, R., Carvalho, A.P., Batista, W.R., Rua, C.P.J., de Oliveira, L., Leomil, L., Fróes, A.M., Thompson, F.L., Coutinho, R., Dobretsov, S., 2018. Environmental conditions affect activity and associated microorganisms of marine sponges. Mar. Environ. Res. 142, 59–68. https://doi.org/10.1016/j.marenvres.2018.09.020.

Bell, J.J., 2008. The functional roles of marine sponges. Estuar. Coast. Shelf Sci. 79, 341–353. https://doi.org/10.1016/j.ecss.2008.05.002.

Bennett, H., Bell, J.J., Davy, S.K., Webster, N.S., Francis, D.S., 2018. Elucidating the sponge stress response; lipids and fatty acids can facilitate survival under future climate scenarios. Glob. Chang. Biol. 24, 3130–3144. https://doi.org/10.1111/gcb.14116.

Bergé, J.-P., Barnathan, G., 2005. Fatty Acids from Lipids of Marine Organisms: Molecular Biodiversity, Roles as Biomarkers, Biologically Active Compounds, and Economical Aspects. Adv. Biochem. Eng. Biotechnol. 49–125. https://doi.org/10.1007/b135782.

Bloodworth, J.W., Baptie, M.C., Preedy, K.F., Best, J., 2019. Negative effects of the sea lice therapeutant emamectin benzoate at low concentrations on benthic communities around Scottish fish farms. Sci. Total Environ. 669, 91–102. https://doi.org/10.1016/j.scitotenv.2019.02.430.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bourne, D.G., Young, N., Webster, N., Payne, M., Salmon, M., Demel, S., Hall, M., 2004. Microbial community dynamics in a larval aquaculture system of the tropical rock lobster, Panulirus ornatus. Aquaculture 242, 31–51. https://doi.org/10.1016/j.aquaculture.2004.08.047.

Brown, C.T., Howe, A., Zhang, Q., Pyrkosz, A.B., Brom, T.H., 2012. A Reference-Free Algorithm for Computational Normalization of Shotgun Sequencing Data 1–18.

Brown, J.R., Gowen, R.J., McLusky, D.S., 1987. The effect of salmon farming on the benthos of a Scottish sea loch. J. Exp. Mar. Bio. Ecol. 109, 39–51. https://doi.org/10.1016/0022-0981(87)90184-5.

Buhl-Mortensen, L., Buhl-Mortensen, P., Dolan, M.F.J., Dannheim, J., Bellec, V., Holte, B., 2012. Habitat complexity and bottom fauna composition at different scales on the continental shelf and slope of northern Norway. Hydrobiologia 685, 191–219. https://doi.org/10.1007/s10750-011-0988-6.

Cai, J., Yang, J., Jones, D.P., 1998. Mitochondrial control of apoptosis: The role of cytochrome c. Biochim. Biophys. Acta - Bioenerg. 1366, 139–149. https://doi.org/10.1016/S0005-2728(98)00109-1.

Calamita, P., Gatti, G., Miluzio, A., Scagliola, A., Biffo, S., 2018. Translating the game: Ribosomes as active players. Front. Genet. 9, 1–8. https://doi.org/10.3389/fgene.2018.00533.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Carroll, M.L., Cochrane, S., Fieler, R., Velvin, R., White, P., 2003. Organic enrichment of sediments from salmon farming in Norway: Environmental factors, management practices, and monitoring techniques. Aquaculture 226, 165–180. https://doi.org/10.1016/S0044-8486(03)00475-7.

Clerissi, C., de Lorgeril, J., Petton, B., Lucasson, A., Escoubas, J.-M., Gueguen, Y., Mitta, G., Toulza, E., 2018. Diversity and stability of microbiota are key factors associated to healthy and diseased Crassostrea gigas oysters. bioRxiv 378125. https://doi.org/10.1101/378125.

Crusoe, M.R., Alameldin, H.F., Awad, S., Boucher, E., Caldwell, A., Cartwright, R., Charbonneau, A., Constantinides, B., Edvenson, G., Fay, S., Fenton, J., Fenzl, T., Fish, J., Garcia-Gutierrez, L., Garland, P., Gluck, J., González, I., Guermond, S., Guo, J., Gupta, A., Herr, J.R., Howe, A., Hyer, A., Härpfer, A., Irber, L., Kidd, R., Lin, D., Lippi, J., Mansour, T., McA'Nulty, P., McDonald, E., Mizzi, J., Murray, K.D., Nahum, J.R., Nanlohy, K., Nederbragt, A.J., Ortiz-Zuazaga, H., Ory, J., Pell, J., Pepe-Ranney, C., Russ, Z.N., Schwarz, E., Scott, C., Seaman, J., Sievert, S., Simpson, J., Skennerton, C.T., Spencer, J., Srinivasan, R., Standage, D., Stapleton, J.A., Steinman, S.R., Stein, J., Taylor, B., Trimble, W., Wiencko, H.L., Wright, M., Wyss, B., Zhang, Q., zyme, en, Brown, C.T., 2015. The khmer software package: enabling efficient nucleotide sequence analysis. F1000Research 4, 900. https://doi.org/10.12688/f1000research.6924.1.

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. pp. 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7.

De Goeij, J.M., Van Oevelen, D., Vermeij, M.J.A., Osinga, R., Middelburg, J.J., De Goeij, A.F.P.M., Admiraal, W., 2013. Surviving in a marine desert: The sponge loop retains resources within coral reefs. Science (80-.). 342, 108–110. https://doi.org/10.1126/science.1241981.

Dean, J.L., Jay Zhao, Q., Lambert, J.C., Hawkins, B.S., Thomas, R.S., Wesselkamper, S.C., 2017. Application of Gene Set Enrichment Analysis for Identification of Chemically-Induced, Biologically Relevant Transcriptomic Networks and Potential Utilization in Human Health Risk Assessment. Toxicol. Sci. 157, kfx021. https://doi.org/10.1093/toxsci/kfx021.

Denis, C., Wielgosz-Collin, G., Bretéché, A., Ruiz, N., Rabesaotra, V., Boury-Esnault, N., Kornprobst, J.-M., Barnathan, G., 2009. New 17-Methyl-13-Octadecenoic and 3,16-Docosadienoic Acids from the Sponge Polymastia penicillus. Lipids 44, 655–663. https://doi.org/10.1007/s11745-009-3291-9.

Dittami, S.M., Arboleda, E., Auguet, J.C., et al., 2020. A community perspective on the concept of marine holobionts: current status, challenges, and future directions. PeerJ Prepr. https://doi.org/10.5281/zenodo.3696771.

Douglas, G.M., Maffei, V.J., Zaneveld, J., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2019. PICRUSt2: An improved and extensible approach for metagenome inference. bioRxiv 672295. https://doi.org/10.1101/672295.

Dunlop, K., Harendza, A., Plassen, L., Keeley, N., 2020. Epifaunal Habitat Associations on Mixed and Hard Bottom Substrates in Coastal Waters of Northern Norway. https://doi.org/10.3389/fmars.2020.568802.

Easson, C.G., Thacker, R.W., 2014. Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges. Front. Microbiol. 5, 1–11. https://doi.org/10.3389/fmicb.2014.00532.

Edge, K.J., Johnston, E.L., Dafforn, K.A., Simpson, S.L., Kutti, T., Bannister, R.J., 2016. Sub-lethal effects of water-based drilling muds on the deep-water sponge Geodia barretti. Environ. Pollut. 212, 525–534. https://doi.org/10.1016/j.envpol.2016.02.047.

Erwin, P.M., Pita, L., López-Legentil, S., Turon, X., 2012. Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. Appl. Environ. Microbiol. 78, 7358–7368. https://doi.org/10.1128/AEM.02035-12.

Fadrosh, D.W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R.M., Ravel, J., 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome 2, 6. https://doi.org/10.1186/2049-2618-2-6.

Fan, L., Reynolds, D., Liu, M., Stark, M., Kjelleberg, S., Webster, N.S., Thomas, T., 2012. Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. Proc. Natl. Acad. Sci. U. S. A. 109, E1878–E1887. https://doi.org/10.1073/pnas.1203287109.

FAO, 2018. The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. Rome.

Fernandez-Jover, D., Arechavala-Lopez, P., Martinez-Rubio, L., Tocher, D., Bayle-Sempere, J., Lopez-Jimenez, J., Martinez-Lopez, F., Sanchez-Jerez, P., 2011. Monitoring the influence of marine aquaculture on wild fish communities: benefits and limitations of fatty acid profiles. Aquac. Environ. Interact. 2, 39–47. https://doi.org/10.3354/aei00029.

Fiore, C.L., Jarett, J.K., Steinert, G., Lesser, M.P., 2020. Trait-Based Comparison of Coral and Sponge Microbiomes. Sci. Rep. 10, 2340. https://doi.org/10.1038/s41598-020-59320-9.

Fiore, C.L., Labrie, M., Jarett, J.K., Lesser, M.P., 2015. Transcriptional activity of the giant barrel sponge, Xestospongia muta Holobiont: Molecular evidence for metabolic interchange. Front. Microbiol. 6 https://doi.org/10.3389/fmicb.2015.00364.

Fulda, S., Gorman, A.M., Hori, O., Samali, A., 2010. Cellular stress responses: Cell survival and cell death. Int. J. Cell Biol. 2010, 1–23. https://doi.org/10.1155/2010/214074.

Ghanawi, J., McAdam, B.J., 2020. Using fatty acid markers to distinguish between effects of salmon (Salmo salar) and halibut (Hippoglossus hippoglossus) farming on mackerel (Scomber scombrus) and whiting (Merlangius merlangus). Aquac. Res. 51, 2229–2242. https://doi.org/10.1111/are.14568.

Giles, E.C., Kamke, J., Moitinho-Silva, L., Taylor, M.W., Hentschel, U., Ravasi, T., Schmitt, S., 2013. Bacterial community profiles in low microbial abundance sponges. FEMS Microbiol. Ecol. 83, 232–241. https://doi.org/10.1111/j.1574-6941.2012.01467.x.

Gili, J.-M.M., Coma, R., 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. Trends Ecol. Evol. 13, 316–321. https://doi.org/10.1016/S0169-5347(98)01365-2.

Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., Egozcue, J.J., 2017. Microbiome datasets are compositional: And this is not optional. Front. Microbiol. 8, 1–6. https://doi.org/10.3389/fmicb.2017.02224.

Gonzalez-Silvera, D., Izquierdo-Gomez, D., Sanchez-Jerez, P., Elbal, M.T., López-Jiménez, J.A., Martínez-López, F.J., 2020. Influence of aquaculture waste on fatty acid profiles and gonad maturation of wild fish aggregations at fish farms. Mar. Environ. Res. 156 https://doi.org/10.1016/j.marenvres.2020.104902.

Goodwin, K.D., Thompson, L.R., Duarte, B., Kahlke, T., Thompson, A.R., Marques, J.C., Caçador, I., Andrew, R., Cluster, C.C., Oceanic, N., Chemistry, O., Division, E., Oceanographic, A., Oceanic, N., Thompson, A.R., Marques, J.C., Caçador, I., Andrew, R., Cluster, C.C., Oceanic, N., Chemistry, O., Division, E., Oceanographic, A., Oceanic, N., 2017. DNA Sequencing as a Tool to Monitor Marine Ecological Status. Front. Mar. Sci. 4, 1–14. https://doi.org/10.3389/fmars.2017.00107.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–652. https://doi.org/10.1038/nbt.1883.

Graw, S., Chappell, K., Washam, C.L., Gies, A., Bird, J., Robeson, M.S., Byrum, S.D., 2021. Multi-omics data integration considerations and study design for biological systems and disease. Mol. Omi. https://doi.org/10.1039/d0mo00041h.

Guzman, C., Conaco, C., 2016. Comparative transcriptome analysis reveals insights into the streamlined genomes of haplosclerid demosponges. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep18774.

Hahn, S., Lam, W., Wu, I., Silva, C.J., Djerassi, C., 1989. Unusual pattern of fatty acid biosynthesis. Evidence for C-19 desaturase activity in freshwater sponges. J. Biol. Chem. 264, 21043–21046.

Hahn, S., Stoilov, I.L., Ha, T.B.T., Raederstorff, D., Doss, G.A., Li, H.T., Djerassi, C., 1988. Biosynthetic studies of marine lipids. 17. The course of chain elongation and desaturation in long-chain fatty acids of marine sponges. J. Am. Chem. Soc. 110, 8117–8124. https://doi.org/10.1021/ja00232a025.

Hamoutene, D., Salvo, F., Bungay, T., Mabrouk, G., Couturier, C., Ratsimandresey, A., Dufour, S.C., 2015. Assessment of Finfish Aquaculture Effect on Newfoundland Epibenthic Communities through Video Monitoring. N. Am. J. Aquac. 77, 117–127. https://doi.org/10.1080/15222055.2014.976681.

Hamoutene, D., Salvo, F., Cross, S., Dufour, S.C., Donnet, S., 2018. Linking the presence of visual indicators of aquaculture deposition to changes in epibenthic richness at finfish sites installed over hard bottom substrates. Environ. Monit. Assess. 190, 750. https://doi.org/10.1007/s10661-018-7108-2.

Hamoutene, D., Salvo, F., Donnet, S., Dufour, S.C., 2016. The usage of visual indicators in regulatory monitoring at hard-bottom finfish aquaculture sites in Newfoundland (Canada). Mar. Pollut. Bull. 108, 232–241. https://doi.org/10.1016/j.marpolbul.2016.04.028.

Holmer, M., 2010. Environmental issues of fish farming in offshore waters: Perspectives, concerns and research needs. Aquac. Environ. Interact. 1, 57–70. https://doi.org/10.3354/aei00007.

Johnson, K.L., Brown, C.T., Reid, C., Leinweber, K., 2019. dib-lab/elvers: v0.1. https://doi.org/10.5281/zenodo.3345045.

Kamke, J., Sczyrba, A., Ivanova, N., Schwientek, P., Rinke, C., Mavromatis, K., Woyke, T., Hentschel, U., 2013. Single-cell genomics reveals complex carbohydrate degradation patterns in poribacterial symbionts of marine sponges. ISME J. 7, 2287–2300. https://doi.org/10.1038/ismej.2013.111.

Keeley, N., Laroche, O., Birch, M., Pochon, X., 2021. A Substrate Independent Benthic Sampler (SIBS) for hard and mixed- bottom marine habitats: a proof of concept study. Front. Mar. Sci. https://doi.org/10.3389/fmars.2021.627687.

Keeley, N., Wood, S.A., Pochon, X., 2018. Development and preliminary validation of a multi-trophic metabarcoding biotic index for monitoring benthic organic enrichment. Ecol. Indic. 85, 1044–1057. https://doi.org/10.1016/j.ecolind.2017.11.014.

Klitgaard, A.B., 1995. The fauna associated with outer shelf and upper slope sponges (porifera, demospongiae) at the faroe islands, northeastern Atlantic. Sarsia 80, 1–22. https://doi.org/10.1080/00364827.1995.10413574.

Krueger, F., Andrews, S.R., Osborne, C.S., 2011. Large scale loss of data in low-diversity illumina sequencing libraries can be recovered by deferred cluster calling. PLoS ONE 6, 4–10. https://doi.org/10.1371/journal.pone.0016607.

Lackner, G., Peters, E.E., Helfrich, E.J.N.N., Piel, J., 2017. Insights into the lifestyle of uncultured bacterial natural product factories associated with marine sponges. Proc. Natl. Acad. Sci. 114, E347–E356. https://doi.org/10.1073/pnas.1616234114.

Lader, P., Kristiansen, D., Alver, M., Bjelland, H. V., Myrhaug, D., 2017. Classification of Aquaculture Locations in Norway With Respect to Wind Wave Exposure, in: Volume 6: Ocean Space Utilization. American Society of Mechanical Engineers, pp. 1–10. https://doi.org/10.1115/OMAE2017-61659.

Lekang, O.I., Salas-Bringas, C., Bostock, J.C., 2016. Challenges and emerging technical solutions in on-growing salmon farming. Aquac. Int. 24, 757–766. https://doi.org/10.1007/s10499-016-9994-z.

Li, Z., 2019. Sponge and Coral Microbiomes, in: Symbiotic Microbiomes of Coral Reefs Sponges and Corals. Springer Netherlands, Dordrecht, pp. 17–28. https://doi.org/10.1007/978-94-024-1612-1_2.

Long, C., Lu, X.-L., Gao, Y., Jiao, B.-H., Liu, X.-Y., 2011. Description of a Sulfitobacter Strain and Its Extracellular Cyclodipeptides. Evidence-Based Complement. Altern. Med. 2011, 1–6. https://doi.org/10.1155/2011/393752.

Love, M.I., Anders, S., Huber, W., 2014. Differential analysis of count data - the DESeq2 package. Genome Biol. 15, 550. https://doi.org/110.1186/s13059-014-0550-8.

Luter, H.M., Gibb, K., Webster, N.S., 2014. Eutrophication has no short-term effect on the Cymbastela stipitata holobiont. Front. Microbiol. 5, 1–10. https://doi.org/10.3389/fmicb.2014.00216.

Macken, A., Lillicrap, A., Langford, K., 2015. Benzoylurea pesticides used as veterinary medicines in aquaculture: Risks and developmental effects on nontarget crustaceans. Environ. Toxicol. Chem. 34, 1533–1542. https://doi.org/10.1002/etc.2920.

Maldonado, M., Aguilar, R., Bannister, R.J., Bell, J.J., Conway, K.W., Dayton, P.K., Díaz, C., Gutt, J., Kelly, M., Kenchington, E.L.R., Leys, S.P., Pomponi, S.A., Rapp, H.T., Rützler, K., Tendal, O.S., Vacelet, J., Young, C.M., 2015. Sponge Grounds as Key Marine Habitats: A Synthetic Review of Types, Structure, Functional Roles, and Conservation Concerns, in: Marine Animal Forests. Springer International Publishing, Cham, pp. 1–39. https://doi.org/10.1007/978-3-319-17001-5_24-1.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10. https://doi.org/10.14806/ej.17.1.200.

McCaig, A.E., Phillips, C.J., Stephen, J.R., Kowalchuk, G.A., Martyn Harvey, S., Herbert, R.A., Martin Embley, T., Prosser, J.I., 1999. Nitrogen cycling and community structure of proteobacterial β-subgroup ammonia-oxidizing bacteria within polluted marine fish farm sediments. Appl. Environ. Microbiol. 65, 213–220. https://doi.org/10.1128/aem.65.1.213-220.1999.

McGhie, T.K., Crawford, C.M., Mitchell, I.M., O'Brien, D., 2000. The degradation of fish-cage waste in sediments during fallowing. Aquaculture 187, 351–366. https://doi.org/10.1016/S0044-8486(00)00317-3.

McIntosh, D., Ji, B., Forward, B.S., Puvanendran, V., Boyce, D., Ritchie, R., 2008. Culture-independent characterization of the bacterial populations associated with cod (Gadus morhua L.) and live feed at an experimental hatchery facility using denaturing gradient gel electrophoresis. Aquaculture 275, 42–50. https://doi.org/10.1016/j.aquaculture.2007.12.021.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. https://doi.org/10.1371/journal.pone.0061217.

Meier, S., Mjøs, S.A., Joensen, H., Grahl-Nielsen, O., 2006. Validation of a one-step extraction/methylation method for determination of fatty acids and cholesterol in marine tissues. J. Chromatogr. A 1104, 291–298. https://doi.org/10.1016/j.chroma.2005.11.045.

Moeller, F.U., Webster, N.S., Herbold, C.W., Behnam, F., Domman, D., Albertsen, M., Mooshammer, M., Markert, S., Turaev, D., Becher, D., Rattei, T., Schweder, T., Richter, A., Watzka, M., Nielsen, P.H., Wagner, M., 2019. Characterization of a thaumarchaeal symbiont that drives incomplete nitrification in the tropical sponge Ianthella basta. Environ. Microbiol. 21, 3831–3854. https://doi.org/10.1111/1462-2920.14732.

Moitinho-Silva, L., Nielsen, S., Amir, A., Gonzalez, A., Ackermann, G.L., Cerrano, C., Astudillo-Garcia, C., Easson, C., Sipkema, D., Liu, F., Steinert, G., Kotoulas, G., McCormack, G.P., Feng, G., Bell, J.J., Vicente, J., Björk, J.R., Montoya, J.M., Olson, J.B., Reveillaud, J., Steindler, L., Pineda, M.-C.C., Marra, M.V., Ilan, M., Taylor, M.W., Polymenakou, P., Erwin, P.M., Schupp, P.J., Simister, R.L., Knight, R., Thacker, R.W., Costa, R., Hill, R.T., Lopez-Legentil, S., Dailianis, T., Ravasi, T., Hentschel, U., Li, Z., Webster, N.S., Thomas, T., 2017. The sponge microbiome project. Gigascience 6, 1–7. https://doi.org/10.1093/gigascience/gix077.

Mutz, K.-O.O., Heilkenbrinker, A., Lönne, M., Walter, J.-G.G., Stahl, F., 2013. Transcriptome analysis using next-generation sequencing. Curr. Opin. Biotechnol. 24, 22–30. https://doi.org/10.1016/j.copbio.2012.09.004.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package.

Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., Kingsford, C., 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods 14, 417–419. https://doi.org/10.1038/nmeth.4197.

Pineda, M.-C.C., Strehlow, B., Sternel, M., Duckworth, A., Jones, R., Webster, N.S., 2017. Effects of suspended sediments on the sponge holobiont with implications for dredging management. Sci. Rep. 7, 1–15. https://doi.org/10.1038/s41598-017-05241-z.

Pita, L., Fraune, S., Hentschel, U., 2016. Emerging sponge models of animal-microbe symbioses. Front. Microbiol. 7, 1–8. https://doi.org/10.3389/fmicb.2016.02102.

Pita, L., Rix, L., Slaby, B.M., Franke, A., Hentschel, U., 2018. The sponge holobiont in a changing ocean: from microbes to ecosystems. Microbiome 6, 46. https://doi.org/10.1186/s40168-018-0428-1.

Plotkin, A., Gerasimova, E., Rapp, H.T., 2018. Polymastiidae (Porifera: Demospongiae) of the Nordic and Siberian Seas. J. Mar. Biol. Assoc. United Kingdom 98, 1273–1335. https://doi.org/10.1017/S0025315417000285.

Poppell, E., Weisz, J., Spicer, L., Massaro, A., Hill, A., Hill, M., 2014. Sponge heterotrophic capacity and bacterial community structure in high- and low-microbial abundance sponges. Mar. Ecol. 35, 414–424. https://doi.org/10.1111/maec.12098.

R Core Team, 2017. R: A Language and Environment for Statistical Computing.

Rix, L., Ribes, M., Coma, R., Jahn, M.T., de Goeij, J.M., van Oevelen, D., Escrig, S., Meibom, A., Hentschel, U., 2020. Heterotrophy in the earliest gut: a single-cell view of heterotrophic carbon and nitrogen assimilation in sponge-microbe symbioses. ISME J. 14, 2554–2567. https://doi.org/10.1038/s41396-020-0706-3.

Roca-Saavedra, P., Mariño-Lorenzo, P., Miranda, J.M., Porto-Arias, J.J., Lamas, A., Vazquez, B.I., Franco, C.M., Cepeda, A., 2017. Phytanic acid consumption and human health, risks, benefits and future trends: A review. Food Chem. 221, 237–247. https://doi.org/10.1016/j.foodchem.2016.10.074.

Rod'kina, S.A., 2005. Fatty acids and other lipids of marine sponges. Russ. J. Mar. Biol. 31, S49–S60. https://doi.org/10.1007/s11179-006-0015-3.

Rod'kina, S.A., 2003. Comparative Characterization of the Microbial Community in Two Species of Sponges from Sea of Japan Using Fatty Acid Markers. Russ. J. Mar. Biol. 29, 255–258. https://doi.org/10.1023/A:1025441111489.

Rodkina, S.A., Imbs, A.B., Krasokhin, V.B., 2008. Fatty acids of sponges from the Sea of Okhotsk. Russ. J. Mar. Biol. 34, 384–390. https://doi.org/10.1134/S1063074008060060.

Rudra, D., Warner, J.R., 2004. What better measure than ribosome synthesis? Genes Dev. 18, 2431–2436. https://doi.org/10.1101/gad.1256704.

Salvo, F., Mersereau, J., Hamoutene, D., Belley, R., Dufour, S.C., 2017. Spatial and temporal changes in epibenthic communities at deep, hard bottom aquaculture sites in Newfoundland. Ecol. Indic. 76, 207–218. https://doi.org/10.1016/j.ecolind.2017.01.019.

Sarà, G., Scilipoti, D., Milazzo, M., Modica, A., 2006. Use of stable isotopes to investigate dispersal of waste from fish farms as a function of hydrodynamics. Mar. Ecol. Prog. Ser. 313, 261–270. https://doi.org/10.3354/meps313261.

Scanes, E., Kutti, T., Fang, J.K.H.H., Johnston, E.L., Ross, P.M., Bannister, R.J., 2018. Mine waste and acute warming induce energetic stress in the deep-sea sponge Geodia atlantica and coral Primnoa resedeaformis; results from a mesocosm study. Front. Mar. Sci. 5, 1–14. https://doi.org/10.3389/fmars.2018.00129.

Schirmer, K., Fischer, B.B., Madureira, D.J., Pillai, S., 2010. Transcriptomics in ecotoxicology. Anal. Bioanal. Chem. 397, 917–923. https://doi.org/10.1007/s00216-010-3662-3.

Scott, C., 2016. dammit: an open and accessible de novo transcriptome annotator.

Sebens, K.P., 1991. Habitat structure and community dynamics in marine benthic systems. In: Bell, S.S., McCoy, E.D., Mushinsky, H.R. (Eds.), Habitat Structure: The Physical Arrangement of Objects in Space. Springer, Netherlands, Dordrecht, pp. 211–234. https://doi.org/10.1007/978-94-011-3076-9_11.

Seppey, M., Manni, M., Zdobnov, E.M., 2019. BUSCO: Assessing Genome Assembly and Annotation Completeness. Methods Mol. Biol. 227–245. https://doi.org/10.1007/978-1-4939-9173-0_14.

Simister, R., Taylor, M.W., Tsai, P., Webster, N., 2012. Sponge-Microbe Associations Survive High Nutrients and Temperatures. PLoS ONE 7, 21–23. https://doi.org/10.1371/journal.pone.0052220.

Slaby, B.M., Hackl, T., Horn, H., Bayer, K., Hentschel, U., 2017. Metagenomic binning of a marine sponge microbiome reveals unity in defense but metabolic specialization. ISME J. 11, 2465–2478. https://doi.org/10.1038/ismej.2017.101.

Smith-unna, R., Boursnell, C., Patro, R., Hibberd, J.M., Kelly, S., 2016. TransRate : reference-free quality assessment of de novo transcriptome assemblies 1134–1144. https://doi.org/10.1101/gr.196469.115.Freely.

Steinert, G., Taylor, M.W., Deines, P., Simister, R.L., de Voogd, N.J., Hoggard, M., Schupp, P.J., 2016. In four shallow and mesophotic tropical reef sponges from Guam the microbial community largely depends on host identity. PeerJ 4, e1936. https://doi.org/10.7717/peerj.1936.

Strand, R., Whalan, S., Webster, N.S., Kutti, T., Fang, J.K.H.H., Luter, H.M., Bannister, R. J., 2017. The response of a boreal deep-sea sponge holobiont to acute thermal stress. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-01091-x.

Sutherland, T.F., Sterling, A.M., Ou, M., 2018. Influence of salmonid aquaculture activities on a rock-cliff epifaunal community in Jervis Inlet, British Columbia. Mar. Pollut. Bull. 127, 297–309. https://doi.org/10.1016/j.marpolbul.2017.12.005.

Taranger, G.L., Karlsen, Ø., Bannister, R.J., Glover, K.A., Husa, V., Karlsbakk, E., Kvamme, B.O., Boxaspen, K.K., Bjørn, P.A., Finstad, B., Madhun, A.S., Morton, H.C., Svåsand, T., 2015. Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. ICES J. Mar. Sci. 72, 997–1021. https://doi.org/10.1093/icesjms/fsu132.

Taylor, M.W., Radax, R., Steger, D., Wagner, M., 2007. Sponge-Associated Microorganisms: Evolution, Ecology, and Biotechnological Potential. Microbiol. Mol. Biol. Rev. 71, 295–347. https://doi.org/10.1128/mmbr.00040-06.

Tett, P., 2008. Fish Farm Wastes in the Ecosystem, in: Aquaculture in the Ecosystem. Springer Netherlands, Dordrecht, pp. 1–46. https://doi.org/10.1007/978-1-4020-6810-2_1.

Thomas, T., Rusch, D., DeMaere, M.Z., Yung, P.Y., Lewis, M., Halpern, A., Heidelberg, K. B., Egan, S., Steinberg, P.D., Kjelleberg, S., 2010. Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. ISME J. 4, 1557–1567. https://doi.org/10.1038/ismej.2010.74.

Titus Brown, C., Irber, L., 2016. sourmash: a library for MinHash sketching of DNA. J. Open Source Softw. 1, 27. https://doi.org/10.21105/joss.00027.

Urbina, M.A.A., Cumillaf, J.P.P., Paschke, K., Gebauer, P., 2019. Effects of pharmaceuticals used to treat salmon lice on non-target species: Evidence from a systematic review. Sci. Total Environ. 649, 1124–1136. https://doi.org/10.1016/j.scitotenv.2018.08.334.

van Soest, R.W.M.M., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., de Voogd, N.J., Santodomingo, N., Vanhoorne, B., Kelly, M., Hooper, J.N.A.A., 2012. Global diversity of sponges (Porifera). PLoS ONE 7, e35105. https://doi.org/10.1371/journal.pone.0035105.

Wang, J.-H., Lu, J., Zhang, Y.-X., Wu, J., Zhang, C., Yu, X., Zhang, Z., Liu, H., Wang, W.-H., 2018. High-throughput sequencing analysis of the microbial community in coastal intensive mariculture systems. Aquac. Eng. 83, 93–102. https://doi.org/10.1016/j.aquaeng.2018.10.001.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07.

Wasta, Z., Mjøs, S.A., 2013. A database of chromatographic properties and mass spectra of fatty acid methyl esters from omega-3 products. J. Chromatogr. A 1299, 94–102. https://doi.org/10.1016/j.chroma.2013.05.056.

Weigel, B.L., Erwin, P.M., 2016. Intraspecific variation in microbial symbiont communities of the sun sponge, Hymeniacidon heliophila, from intertidal and subtidal habitats. Appl. Environ. Microbiol. 82, 650–658. https://doi.org/10.1128/AEM.02980-15.

White, C.A., Woodcock, S.H., Bannister, R.J., Nichols, P.D., 2019. Terrestrial fatty acids as tracers of finfish aquaculture waste in the marine environment. Rev. Aquac. 11, 133–148. https://doi.org/10.1111/raq.12230.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Wilding, T.A., Cromey, C.J., Nickell, T.D., Hughes, D.J., 2012. Salmon farm impacts on muddy-sediment megabenthic assemblages on the west coast of Scotland. Aquac. Environ. Interact. 2, 145–156. https://doi.org/10.3354/aei00038.

Woodcock, S., Troedsson, C., Strohmeier, T., Balseiro, P., Skaar, K., Strand, Ø., 2017. Combining biochemical methods to trace organic effluent from fish farms. Aquac. Environ. Interact. 9, 429–443. https://doi.org/10.3354/aei00242.

Woodcock, S.H., Meier, S., Keeley, N.B., Bannister, R.J., 2019. Fate and longevity of terrestrial fatty acids from caged fin-fish aquaculture in dynamic coastal marine systems. Ecol. Indic. 103, 43–54. https://doi.org/10.1016/j.ecolind.2019.03.057.

Yang, Q., Franco, C.M.M.M., Lin, H.-W.W., Zhang, W., 2019. Untapped sponge microbiomes: Structure specificity at host order and family levels. FEMS Microbiol. Ecol. 95, 1–16. https://doi.org/10.1093/femsec/fiz136.

Yokoyama, H., Abo, K., Ishihi, Y., 2006. Quantifying aquaculture-derived organic matter in the sediment in and around a coastal fish farm using stable carbon and nitrogen isotope ratios. Aquaculture 254, 411–425. https://doi.org/10.1016/j.aquaculture.2005.10.024.