Supporting Information

Leray and Knowlton 10.1073/pnas.1424997112

SI Text

I. ARMS Specifications

ARMS consist of ten 22.5 × 22.5-cm PVC plates separated by 1.27-cm spacers, anchored to a baseplate (Fig. S1). In alternate layers, water flow through the spaces was obstructed by bars running from the corners to the center of the plate. The total surface area sampled was 0.869 m² per ARMS, and the total volume between plates was 0.005 m³ per ARMS.

II. Field Sampling Protocols

A. Deployment and Recovery. ARMS were deployed subtidally adjacent to natural oyster reefs on September 19, 2013 in VA and on November 6, 2013, in FL. We used stainless-steel stakes to anchor the structures to the reef at a level guaranteeing that they remained submerged at low tide. ARMS were collected on May 3–4, 2014, and May 26–29, 2014, in VA and FL, respectively, for a soak time of ~6 mo. To prevent loss of community members, a 100-μm Nitex-lined crate was placed over the ARMS structure and fastened with two or three hooked elastic cords (bungies) before removal of the ARMS from the bottom. Lined crates are designed to cover the central structure made of 10 PVC plates only, which means that mobile specimens occurring on the base plate are able to escape during ARMS handling in the field. Stakes used for anchoring ARMS to the substrate were then removed, and a small cable tie was placed at the northern corner of the baseplate to keep track of orientation. ARMS were then placed in a large plastic container with seawater and at least two aeration stones and transported to the wet laboratory.

B. Disassembly. The lined crate was removed, rinsed over the plastic container in which the structure was transported with seawater from the plastic container, and examined for any hiding organisms. The ARMS were positioned upside-down to unscrew nuts and bolts at each corner (long bolts are left in place to allow the removal of each plate one by one). The baseplate was removed first, brushed minimally inside the plastic container to remove any mobile animals, and placed aside (not analyzed). Each of the 10 plates was then removed one by one and lightly brushed, a small cable tie was placed at the northern corner of the plate, and the plate was photographed on both sides and finally placed in labeled (with ARMS and plate number) 5-gallon buckets containing 45-μm filtered seawater and an air stone.

C. Processing Sieved Fractions. Water from the large plastic container was filtered through three sets of sieves [2 mm (no. 10), 500 μm (no. 32), and 106 μm (no. 140)], and each fraction was placed in an individual tray with an air stone. Mobile specimens retained by the 2-mm sieve were sorted to morphospecies; photographed alive to document color patterns; and anesthetized using clove oil, magnesium chloride, or chilling before preservation in 95% EtOH. Pieces of algae and other sessile organisms retained by the 2-mm sieve were not processed. The two smaller sieved fractions (2 mm to 500 μm and 500 to 106 μm) were washed with seawater into a 45-μm Nitex net and preserved in falcon tubes (or larger jars depending on the volume of the sample) containing 95% EtOH. Both individual mobile animals larger than 2 mm and smaller sieved fractions were kept at −20 °C until DNA extraction.

D. Processing the Sessile Fraction. The most common and conspicuous sessile taxa found on the plates were photographed, and a small tissue sample was preserved in salt-saturated 25% (vol/vol) DMSO buffer [0.25 M EDTA (pH 7.5), DMSO, NaCl-saturated] for DNA barcoding. The surface and sides of all PVC plates were then scraped into a tray of seawater or EtOH, and the total content was poured into a kitchen blender with 45-μm filtered seawater (roughly 1:1 in volume) for homogenization for 30 s at maximum speed. Blended material was then immediately poured into a collection net (45-μm Nitex mesh) and rinsed with seawater or 95% EtOH, squeezing out the liquid through the mesh at least twice. On-site homogenization followed by the washing step was found to give high-molecular-weight DNA as detailed below. After the last wash and squeezing out of liquid, ~15 g of material was placed inside each of three falcon tubes that were then filled with DMSO buffer. Falcon tubes were placed at −20 °C, along with any remaining tissue that was frozen, in plastic bags.

E. Avoiding Contamination. Because the PCR-based approach to characterize communities is very sensitive to contamination, each piece of equipment was soaked in 10% bleach (sodium hypochlorite) for a minimum of 5 min before first use and between samples for sterilization. Nitrile gloves were used to manipulate equipment at all times.

III. Tests of DNA Preservation of Sessile Fraction

Preliminary tests were conducted to determine the best approach to obtain high-molecular-weight DNA from the sessile fraction. An initial protocol was designed in which plates were first submerged in 95% EtOH for several hours to reduce the amount of water in animal tissues. Then, tissues were scraped into a large container filled with EtOH (ratio of ~1:10) and preserved at −20 °C for several days or weeks. Finally, tissues were homogenized (using a blender) in a small amount of EtOH, and ~10 g of material was immediately collected for DNA extraction in the laboratory. That approach provided very low DNA quality [100% of DNA fragments shorter than 300 bp as measured by a TapeStation (Agilent Technologies)] for 90% of samples tested. After ruling out the potential effect of mechanical shearing during tissue homogenization, the protocol was modified to minimize storage time by conducting tissue homogenization and DNA extraction in the field. Nevertheless, DNA was still degraded, which suggested that chemical denaturation potentially caused by substances released by sessile animals occurred quickly following tissue homogenization. We were able to obtain very high-quality DNA (75% of DNA fragments longer than 10 kb as measured by the Agilent TapeStation) across all samples by homogenizing samples shortly after scraping (plates are kept in aerated seawater) and immediately rinsing the homogenate in a 45-μm mesh collection net using seawater or EtOH. We used DMSO buffer for tissue preservation because it was shown to be more effective than EtOH for preserving DNA of several sessile taxonomic groups (1).

IV. Decantation of Sieved Fractions

Small sieved fractions (2 mm to 500 μm and 500 to 106 μm) contain sediments that should be separated from the organic fraction before DNA extraction. Each sample was therefore transferred into a 2-L cylinder and filled up to the 1.5-L level with deionized water. The cylinder was sealed with parafilm and shaken vigorously to resuspend animals and other organic matter, and the water was poured quickly into a 45-μm sieve. Sample resuspension was repeated five times (or until no organic particulates could be observed after shaking). The material retained by the sieve was weighed and homogenized with a spatula. Half

of the sample was then crushed using a mortar and pestle for 2 min and preserved in a falcon tube with 95% EtOH for DNA extraction. The other half was archived (in 95% EtOH) or used for morphological analysis, as in the present study. Sediments collected in the bottom of the cylinder were also archived. All equipment used for decantation was bleached and UV-sterilized between samples.

We examined the 2-mm to 500-μm sediments from VA and FL to quantify the abundance and diversity of mollusks and other organisms. There was a mean (±SD) of 4.1 (±4.3) and 5 (±4.7) specimens in the 2-mm to 500-μm sediments from VA and FL, respectively, with a majority being mollusks [2 (±2.3) and 3.7 (±3.7) specimens per ARMS, respectively]. We also found a few amphipods and isopods. We obtained COI sequences from 18 specimens found in sediments from VA. They belonged to nine OTUS, all of them (including two mollusk OTUs) matching reference OTUs in the metabarcoding dataset, which shows the effectiveness of the decantation process in retaining organisms for metabarcoding.

V. Laboratory Protocols

A. DNA Barcoding. A small piece of tissue was collected from each specimen retained by the 2-mm sieve and placed individually in 96-well Costar plates (Corning) for phenol DNA extraction performed on an AutoGeneprep 965 (Autogen). DNA from sessile taxa and whole specimens from the 500-μm to 2-mm fractions were also extracted using the same procedure. Eluted DNA was used for PCR amplification of a ~658-bp fragment of the mitochondrial COI gene using the following PCR mixture: 19-μL reaction with 10 μL of Promega GoTaq G2 Hot Start Master Mix, 0.6 μL of 10 μM each forward or reverse primer [jgLCO/jgHCO (2)] and 0.2 μL of 20 mg/mL BSA. PCR thermal cycling conditions were as follows: 5 min at 95 °C; four cycles of 30 s at 94 °C, 45 s at 50 °C, and 60 s at 72 °C; 34 cycles at 45 °C annealing temperature; and a final extension of 8 min at 72 °C. PCR product was purified using ExoSAP-IT (Affymetrix), and sequences were generated in both directions with the Sanger sequencing platform. We then repeated the PCR assay with the mlCOIintF/jgHCO primer combination (3) whenever the initial reaction was not successful (~8% of samples) to increase our success rate.

B. DNA Metabarcoding.

DNA extractions. DNA was extracted from 10 g of homogenized sessile tissue, and the crushed half of the 2-mm to 500-μm and 500- to 106-μm samples using the MO-BIO Powermax Soil DNA Isolation Kit. The initial bead-beating step of the kit was found to shear DNA. Therefore, we added proteinase K (0.4 mg/mL) to the powerbead solution (+ C1 solution) instead and incubated samples in a shaking incubator overnight at 56 °C, which ensured effective tissue lysis. We followed the manufacturer's instructions for the rest of the protocol. However, for sessile samples, we only used one-third of the homogenized tissue lysate for extraction to prevent clogging of the silica membrane of the spin column. Extracted DNA was purified using the MO-BIO Powerclean DNA Clean-Up Kit and quantified with a Qubit fluorometer (dsDNA HS Assay kit; Invitrogen) before PCR amplification.

PCR amplification, tagging, and sequencing. We used a hierarchical tagging approach (as in ref. 3) combining seven tailed PCR primers (Table S4) and eight Ion Xpress barcode adapters (Life Technologies) for sample multiplexing. Three replicate PCR assays were performed to amplify an ~313-bp COI fragment for each of the 54 bulk samples using the following PCR mixture: 20-μL reaction with 1 μL of 10 μM each forward or reverse primer (tailed-mlCOIintF/tailed-jgHCO; Table S4), 1.4 μL of 10 mM dNTP, 0.4 μL of Clontech Advantage 2 Polymerase Mix, 2 μL of Clontech Advantage 2 PCR buffer, and 1 μL (10 ng) of purified DNA. We used the touchdown PCR profile with 16 initial cycles:

denaturation for 10 s at 95 °C, annealing for 30 s at 62 °C (−1 °C per cycle), and extension for 60 s at 72 °C, followed by 20 cycles at an annealing temperature of 46 °C. Triplicate PCR products were pooled and purified using Agencourt AMPure XP beads, and equimolar amounts of each sample were pooled, with each pool containing amplicons generated with each of the seven tailed-primer pairs (total of eight pools). End-repair (Ion Plus Fragment Library kit) and ligation of Ion Xpress barcode adapters were conducted following the manufacturer's instructions (Life Technologies). Library templates were clonally amplified using the OT2 400-bp kit on the Ion One Touch 2, and enriched template ISPs were sequenced on the Ion Torrent platform using the Ion PGM 400-bp version 2 protocol (all from Life Technologies).

VI. Data Analysis

A. DNA Barcoding. Forward and reverse sequences were assembled, checked for stop codons or frame shifts, and edited in Geneious (Biomatters). Our dataset comprised a diversity of taxonomic groups so that using a fixed sequence dissimilarity cutoff (i.e., 5%) for clustering OTUs would not result in accurate species delineations. Therefore, we used the Bayesian clustering algorithm implemented in CROP (4) to delineate OTUs based on the natural distribution of sequence dissimilarity in the dataset. Lower and upper bound variance was set to 3 and 4, respectively, because these settings were shown to provide the best results for marine invertebrates (3). CROP outputs a representative sequence per OTU that was used for taxonomic identification.

B. DNA Metabarcoding. Higher quality reads prefiltered by Torrent Suite Software version 4.0.2 (Life Technologies) were assigned to samples based on the combination primer tail-Ion Xpress barcode. Additional sequences were removed from the prefiltered dataset if they (*i*) were shorter than 250 bp, (*ii*) had more than two mismatches in the primer sequence, (*iii*) had any ambiguous base call, or (*iv*) had at least one homopolymer region longer than 8 bp. We then used the option "enrichAlignment" in Multiple Alignment of Coding Sequences (MACSE) (5) to align our reads to the high-quality library of COI barcodes of the Moorea Biocode project (7,675 sequences from 30 animal phyla represented), an all-taxa biodiversity inventory of the Moorea Island ecosystem (6), retaining sequences that had zero stop codons (using invertebrate mitochondrial translation table), zero frame shifts, zero insertions, and no more than three deletions. This latter step maximizes the reliability of the sequence dataset.

C. Taxonomic Assignments. We performed BLASTn searches (7) of OTU representative sequences of the barcoding and metabarcoding datasets in GenBank and BOLD. Previous papers that used similar DNA sequencing approaches to look at terrestrial (8) and marine diversity (3, 9) used 98% assignments. However, the distribution of sequence similarity in our dataset showed a significant number of matches between 97% and 98% before rapidly dropping below 90%, a pattern that may be driven by matches to specimens collected across the Atlantic Ocean. Therefore, we accepted species level matches when similarity to the reference barcode was higher than 97%.

In the absence of a direct match we used a phylogenetic approach implemented in the Statistical Assignment Package (10) to assign OTUs to higher taxonomic levels. The program was set to download up to 40 homologs from GenBank with ≥70% sequence identity. We accepted taxonomic assignments at an 80% posterior probability cutoff, but we did not consider assignment lower than order level to minimize misidentifications due to the lack of data for some taxonomic groups in GenBank. OTUs that matched or were assigned to bacteria were removed.

VII. Assessment of Reliability of Metabarcoding Approach

A. Fraction Sized 2 mm to 500 μm. One ARMS from each of the three sites at each location was randomly chosen for analysis. Archived bulk samples (see *SI Text*, section IV), which correspond to half of the sample measured by weight, were resuspended in a graduated beaker containing 100 mL of 95% EtOH and homogenized with a spatula, and 20 mL was immediately collected using a Hensel–Stempel pipette. All specimens were isolated and identified to the lowest taxonomic level using morphology, the entire specimen was used for phenol DNA extraction according to the protocol described in *SI Text* (section V.A), and the mitochondrial COI gene was sequenced for OTU delineation.

The amount of DNA in each individual extract was measured with a Qubit fluorometer. The total amount of DNA represented by each OTU was calculated by summing the amount of DNA of each specimen belonging to that same OTU according to COI barcodes. To identify OTUs shared between datasets, we ran local BLAST searches [using Geneious (Biomatters)] of one representative sequence per OTU obtained via barcoding against the full database of OTU representative sequences obtained via metabarcoding.

To evaluate the efficacy of metabarcoding at detecting diversity, we first calculated the overall proportion of OTUs shared by the barcoding and metabarcoding datasets at each location. We then conducted similar calculations but between datasets of corresponding ARMS. For example, we compared the proportion of shared OTUs between the barcoding and metabarcoding datasets of ARMS 1 from FL. To evaluate the efficacy of metabarcoding at estimating OTU relative abundance, we first tested the relationship between the amount of DNA per OTU and

number of reads per OTU in the metabarcoding dataset. We pooled amounts of DNA and read number for OTUs belonging to the same phylum to test for the same relationship at the level of functional groups.
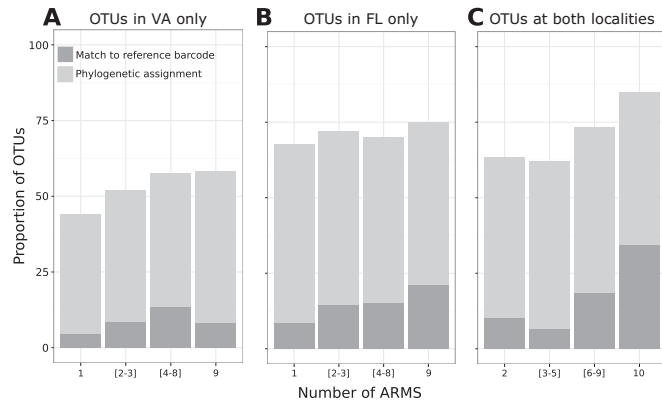
We sorted and photographed a total of 251 and 954 animals in three 2-mm to 500-μm fractions from VA and FL, respectively (representing one ARMS from each of the three sites at the two locations). A total of 671 specimens were individually barcoded, which includes all specimens except Tanaidacea and Ostracoda from FL, for which only ~25% of specimens were individually barcoded because of their high abundance. Based on the subset of specimens analyzed, abundant Ostracoda and Tanaidacea belonged to one and three OTUs, respectively. We extrapolated the amount of DNA for each of these four OTUs for subsequent analysis.

B. Sessile Fraction. We individually subsampled and barcoded morphologically distinctive sessile taxa to identify matching OTUs in the metabarcoding dataset using local BLASTn searches (as discussed above). The number of reads per OTU was then compared with the estimated cover of each OTU on each ARMS as measured by a point count approach implemented in Coral Point Count with Excel extensions (CPCe) (11). A 15 × 15 grid was positioned over each plate photograph, and the taxon located under each intersection of the grid was recorded. All 10 plates (19 sides) were scored for each ARMS.

The efficacy of metabarcoding at detecting diversity and relative abundance of sessile taxa was evaluated using similar calculations as presented in the previous section (*SI Text*, section VII.A).

1. Gaither MR, Szabó Z, Crepeau MW, Bird CE, Toonen RJ (2010) Preservation of corals in salt-saturated DMSO buffer is superior to ethanol for PCR experiments. *Coral Reefs* 30(2):329–333.
2. Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Mol Ecol Resour* 13(5):851–861.
3. Leray M, et al. (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Front Zool* 10:34.
4. Hao X, Jiang R, Chen T (2011) Clustering 16S rRNA for OTU prediction: A method of unsupervised Bayesian clustering. *Bioinformatics* 27(5):611–618.
5. Ranwez V, Harispe S, Delsuc F, Douzery EJP (2011) MACSE: Multiple Alignment of Coding SEquences accounting for frameshifts and stop codons. *PLoS ONE* 6(9): e22594.

6. Leray M, Boehm JT, Mills SC, Meyer CP (2012) Moorea BIOCODE barcode library as a tool for understanding predator–prey interactions: Insights into the diet of common predatory coral reef fishes. *Coral Reefs* 31(2):383–388.
7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215(3):403–410.
8. Yu DW, et al. (2012) Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods Ecol Evol* 3(4):613–623.
9. Machida RJ, Hashiguchi Y, Nishida M, Nishida S (2009) Zooplankton diversity analysis through single-gene sequencing of a community sample. *BMC Genomics* 10:438.
10. Munch K, Boomsma W, Huelsenbeck JP, Willerslev E, Nielsen R (2008) Statistical assignment of DNA sequences using Bayesian phylogenetics. *Syst Biol* 57(5):750–757.
11. Kohler KE, Gill SM (2006) Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. *Comput Geosci* 32(9):1259–1269.

Fig. S1. Illustration of study design and diversity encountered. (*A*) Map of experimental design. (*B*) Photographs of Virginia location, ARMS, and ARMS recovery. (*C*) Photographs of representative ARMS plates and organisms in the 2-mm to 500-μm fraction. (*D*) Sample processing workflow. In *C*, scale bars are provided for individual organisms, and the square plates are 22.5 cm on each side.

Fig. S2. Proportion of identified OTUs in the metabarcoding dataset according to the number of ARMS where they were detected. (*A*) Virginia only. (*B*) Florida only. (*C*) Both localities. OTUs were considered to match a reference barcode if they had >97% similarity to a COI sequence in the BOLD or GenBank or in a reference barcode generated in this study.
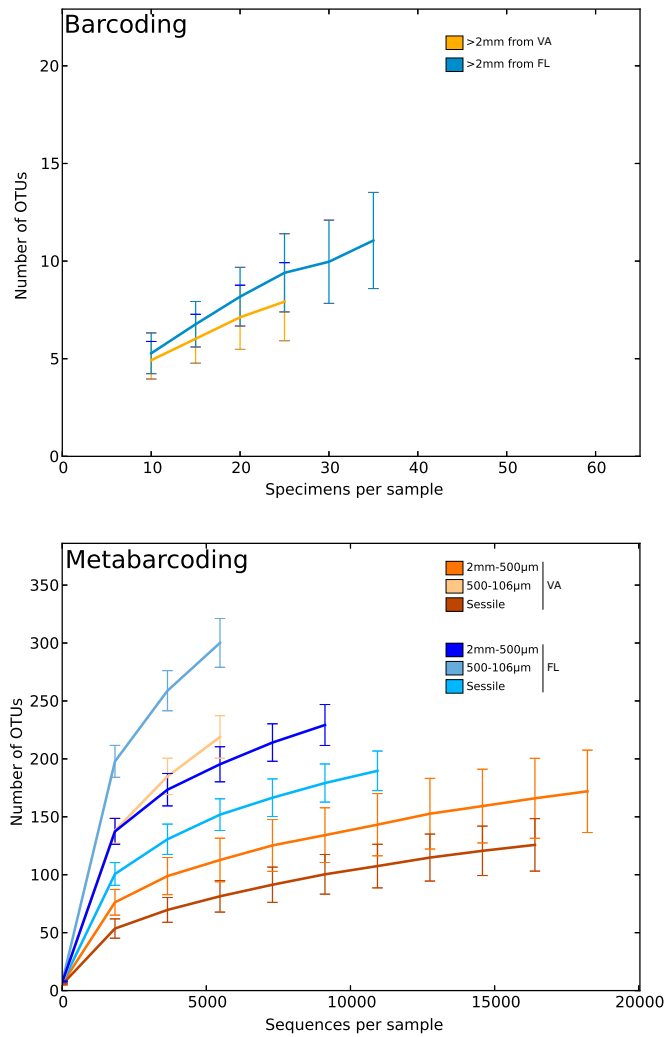
Fig. S3. Individual-based rarefaction curves.
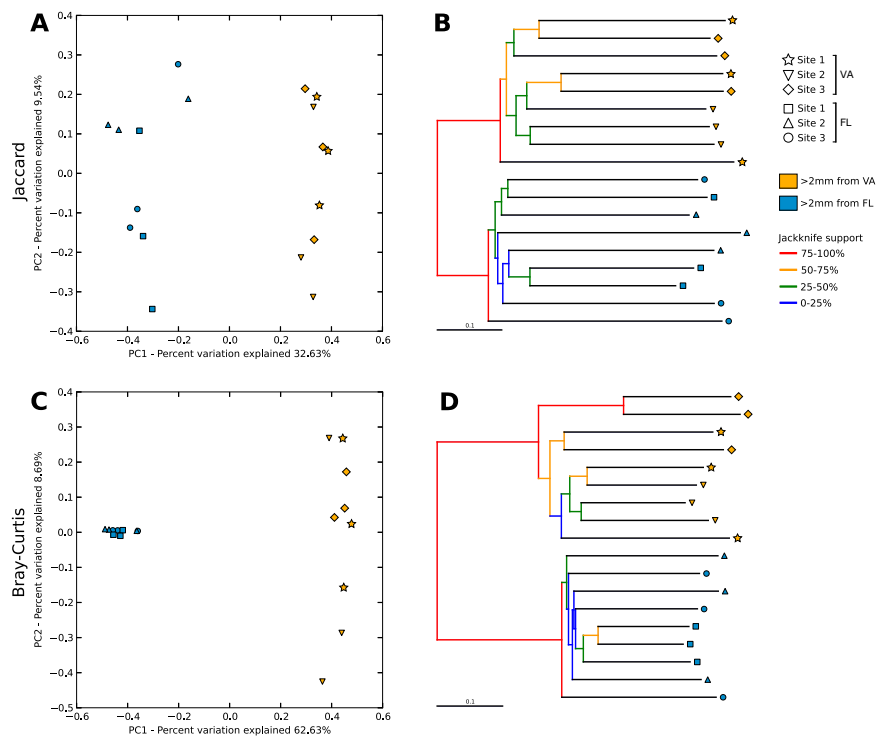
Fig. S4. Clustering analyses [PCoA (*A* and *C*) and UPGMA trees (*B* and *D*)] depicting similarity in community composition among >2-mm samples based on OTU incidence (Jaccard; *A* and *B*) and relative abundance (Bray–Curtis; *C* and *D*). PC, principal component.

Fig. S5. PCoA with coordinates of the 10 most abundant phyla. The size of the sphere is proportional to the mean relative abundance of the taxon across samples.

Table S1. OTU diversity and abundance as revealed by DNA barcoding and DNA metabarcoding in ARMS from VA and FL

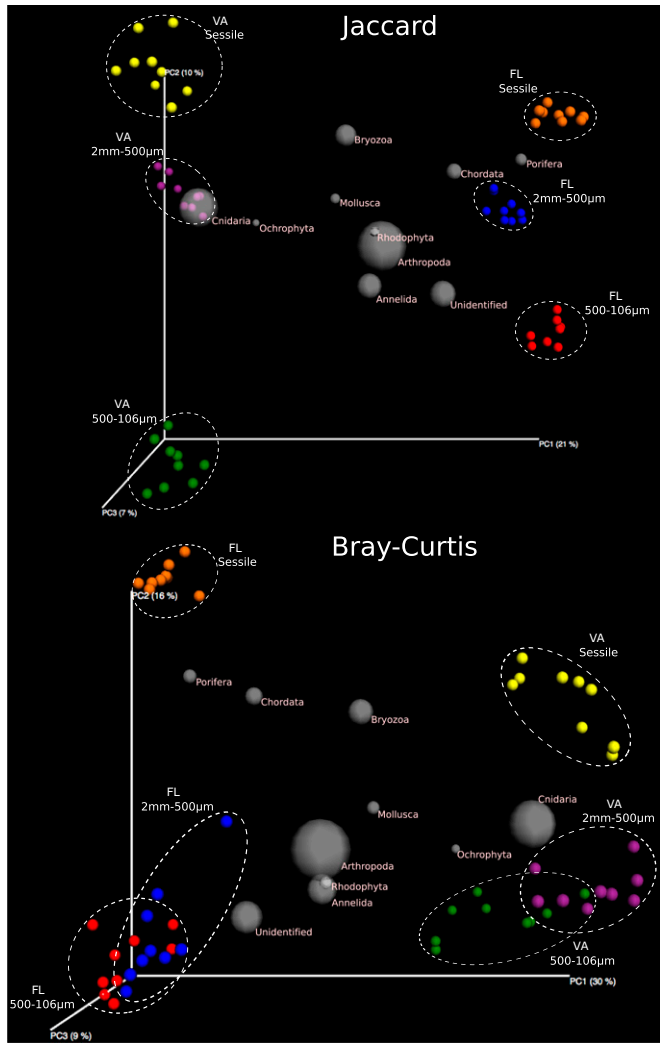| Diversity descriptors	VA					FL					
	Barcoding	Metabarcoding				Barcoding		Metabarcoding			
	>2 mm	2 mm to 500 μm	500 to 106 μm	Sessile	Total	>2 mm	2 mm to 500 μm	500 to 106 μm	Sessile	Total
No. of sequences	498	256,147	97,439	218,704	572,290	655	155,232	86,350	168,031	409,613
Total no. of OTUs	38	651	828	436	1,204	64	821	976	591	1,391
Mean (±SD) no. of OTUs	11.2 ± 4.1	203.3 ± 52.3	290.3 ± 32.1	146.6 ± 28.9	434.2 ± 55.7	15.8 ± 4.9	277.2 ± 37.6	360.1 ± 28.5	222.9 ± 24.3	536.7 ± 30.8
Mean (±SD) rarefied no. of OTUs	8.2 ± 2.1	117.1 ± 21.4	229.7 ± 20.5	85.7 ± 15.1	333.5 ± 34.9	9.6 ± 2.2	202.6 ± 16.4	312.1 ± 23.2	157.4 ± 15.5	484.4 ± 30.7
Chao1 [95% CI]	46.0 [40.1, 67.8]	1,075.6 [953.3, 1,247.2]	1,204.9 [1,106.4, 1,338.2]	638.7 [568.1, 746.9]	1,711.4 [1,596.5, 1,859.9]	104.8 [80.2, 166.9]	1,183.0 [1,082.1, 1,322.8]	1,486.0 [1,356.2, 1,660.2]	858.0 [769.8, 989.7]	1,945.7 [1,821.2, 2,106.2]
Chao1 [95% CI] (rarefied)	49.2 [39.4, 80.8]	552.7 [469.3, 690.4]	917.8 [843.7, 1,021.6]	451.5 [380.0, 569.8]	1,174.0 [1,082.3, 1,298.7]	144.7 [70.7, 403.5]	866.5 [772.2, 1,007.4]	1,213.9 [1,108.9, 1,358.7]	562.1 [505.6, 653.5]	1,483.7 [1,384.1, 1,617.3]
Chao2 [95% CI]	50.5 [41.7, 80.3]	1,126.2 [1,001.7, 1,295.0]	1,309.5 [1,191.1, 1,466.5]	706.7 [621.0, 832.0]	1,891.0 [1,746.4, 2,074.1]	140.06 [94.3, 254.8]	1,213.3 [1,117.7, 1,339.8]	1,536.0 [1,406.2, 1,705.1]	880.2 [796.2, 998.5]	2,056.3 [1,921.3, 2,225.7]
Chao2 [95% CI] (rarefied)	49.4 [39.7, 79.4]	535.6 [468.3, 638.5]	1,007.3 [913.2, 1,136.2]	557.3 [449.4, 730.4]	1,256.3 [1,149.8, 1,398.0]	116.1 [64.6, 280]	892.4 [804.3, 1,015.4]	1,280.5 [1,167.8, 1,431.1]	596.6 [533.6, 692.6]	1,562.7 [1,455.1, 1,702.1]
ACE	47.6	1,062.8	1,223.0	628.3	1,743.0	108.6	1,197.8	1,483.1	819.38	1,982.0
ACE (rarefied)	57.6	515.4	975.7	459.1	1,217.8	91.6	837.7	1,213.2	556.02	1,521.3
ICE	56.5	1,205.5	1,345.6	741.2	1,928	139.9	1,326.1	1,537.2	892.0	2,078.5
ICE (rarefied)	70.6	580.0	1,066.3	551.3	1,300.7	105.6	942.8	1,269.0	588.6	1,583.0
OTUs with match to BOLD/GenBank, %	60.5	14.1	10.6	16.2	10.2	57.8	15.7	11.8	16.9	11.9
Unidentified OTUs, %	NA	35.6	38.8	31.2	40.9	NA	26.8	27.1	23.8	28.3
Singletons, %	31.6	39.8	36.5	34.9	34.8	46.9	32.3	34.6	30.3	31.1

Each diversity estimate was calculated using both raw and rarefied OTU tables. ACE, abundance-based coverage estimator; CI, confidence interval; ICE, incidence-based coverage estimator; NA, not applicable.

Table S2. Percent contribution of individual OTUs to differences between localities and fractions (based on similarity of percentage analyses)

OTU no.	Kingdom	Phylum	Class	Subclass/order	Genus/species	VA-FL	VA			FL		
							500 to 106 µm	500-µm sessile	106-µm sessile	500 to 106 µm	500-µm sessile	106-µm sessile
x17	Animalia	Annelida	Clitellata	Haplotaxida	*Tubificoides parapectinatus*		1.9	2.2				
x19	Animalia	Annelida	Polychaeta	Eunicida	*Marphysa sanguinea*	2.9	5.5	1.6	4.6	3.0	3.0	1.6
x350	Animalia	Annelida	Polychaeta	Sabellida	*Branchiomma cf. bairdi*						1.6	1.8
x1039	Animalia	Annelida	Polychaeta	Spionida	*Polydora cornuta*		1.7		1.7			
x283	Animalia	Annelida	Polychaeta	Spionida	*Streblospio benedicti*	1.2	4.2		4.2			
x432	Animalia	Annelida	Polychaeta	Terebellida						1.0		
x1066	Animalia	Annelida	Polychaeta	Terebellida	*Polycirrus*		1.1		1.0			
x26	Animalia	Annelida	Polychaeta							1.3		1.4
x282	Animalia	Annelida	Polychaeta							1.9	1.7	
x38	Animalia	Annelida	Polychaeta							1.0	1.0	
x791	Animalia	Arthropoda	Malacostraca	Amphipoda	*Caprella penantis*		1.0	1.1				
x320	Animalia	Arthropoda	Malacostraca	Amphipoda	*Monocorophium acherusicum*			1.0				
x136	Animalia	Arthropoda	Malacostraca	Amphipoda	*Gammarus mucronatus*	1.4	4.1	5.1				
x165	Animalia	Arthropoda	Malacostraca	Amphipoda		1.1	4.1	4.4				
x372	Animalia	Arthropoda	Malacostraca	Amphipoda			1.5	1.3				
x83	Animalia	Arthropoda	Malacostraca	Amphipoda		2.3	8.3	9.0				
x102	Animalia	Arthropoda	Malacostraca	Decapoda								
x352	Animalia	Arthropoda	Malacostraca	Decapoda	*Dyspanopeus sayi*		1.2	1.3		2.8	2.5	
x998	Animalia	Arthropoda	Malacostraca	Decapoda	*Panopeus occidentalis*			2.5	2.3			
x418	Animalia	Arthropoda	Malacostraca	Isopoda	*Cilicaea*					2.1	1.8	
x37	Animalia	Arthropoda	Malacostraca	Stomatopoda	*Neogonodactylus bredini*					2.3	2.2	
x597	Animalia	Arthropoda	Maxillopoda	Calanoida	*Centropages hamatus*		1.2		1.5			
x574	Animalia	Arthropoda	Maxillopoda	Calanoida							1.9	1.3
x983	Animalia	Arthropoda	Maxillopoda	Calanoida			1.3		1.1			
x62	Animalia	Arthropoda	Maxillopoda	Harpacticoida						1.8		1.7
x607	Animalia	Arthropoda	Maxillopoda	Siphonostomatoida			1.5		1.4			
x39	Animalia	Arthropoda	Maxillopoda			2.0				6.1		5.4
x397	Animalia	Arthropoda	Maxillopoda							1.3		1.2
x492	Animalia	Arthropoda	Maxillopoda							1.4		1.3
x871	Animalia	Arthropoda	Maxillopoda							1.9		1.4
x84	Animalia	Arthropoda	Ostracoda			4.1				6.2	7.1	5.8

Table S2. Cont.

OTU no.	Kingdom	Phylum	Class	Subclass/order	Genus/species	VA-FL	VA			FL		
							500 to 106 µm	500-µm sessile	106-µm sessile	500 to 106 µm	500-µm sessile	106-µm sessile
x688	Animalia	Arthropoda	Pycnogonida	Pantopoda						1.0		1.0
x1054	Animalia	Arthropoda						2.7	2.7			
x2	Animalia	Arthropoda						2.7	2.4	1.0		
x651	Animalia	Arthropoda						1.2	1.0			
x799	Animalia	Arthropoda						4.1	3.9			
x5	Animalia	Bryozoa	Gymnolaemata	Cheilostomatida	*Bugula neritina*	1.2						
x3	Animalia	Bryozoa	Gymnolaemata	Cheilostomatida	*Biflustra arborescens*	1.0		2.5	2.9			
x182	Animalia	Bryozoa	Gymnolaemata	Cheilostomatida	*Schizoporella errata*	4.0		11.8	10.6		7.7	8.0
x245	Animalia	Bryozoa	Gymnolaemata	Cheilostomatida		1.6				2.4	1.6	2.3
x198	Animalia	Chordata	Ascidiacea	Phlebobranchia	*Ascidia virginea*	2.7					8.5	8.6
x13	Animalia	Chordata	Ascidiacea	Stolidobranchia	*Symplegma rubra*	3.7				1.4	9.4	8.9
x1	Animalia	Chordata					1.9		1.9			
x437	Animalia	Chordata				1.1		3.9	3.5			
x841	Animalia	Cnidaria	Hydrozoa	Leptothecata	*Obelia bidentata*	11.5	12.4	12.2	11.0			
x1114	Animalia	Cnidaria	Hydrozoa			7.3	7.7	9.5	9.0			
x139	Animalia	Echinodermata	Ophiuroidea	Ophiurida	*Amphipholis cf. squamata*					1.8	1.6	1.6
x132	Animalia	Mollusca	Bivalvia	Ostreoida	*Ostrea equestris*						1.6	1.6
x544	Animalia	Mollusca	Gastropoda	Littorinimorpha	*Crepidula plana*			1.1	1.1			
x575	Animalia	Porifera	Demospongiae	Halichondrida							2.2	2.3
x1061	Animalia	Porifera	Demospongiae	Homosclerophorida	*Oscarella*	3.1	1.3	1.4	1.1	3.0	6.5	6.2
x8	Chromista	Ochrophyta	Phaeophyceae	Ectocarpales	*Ectocarpus siliculosus*	1.0						
x423	Plantae	Rhodophyta	Florideophyceae	Ceramiales	*Polysiphonia*		1.2	1.3				
x366	Plantae	Rhodophyta	Florideophyceae	Gracilariales	*Gracilaria vermiculophylla*		2.2	2.5				
x733	Plantae	Rhodophyta	Florideophyceae							1.1	1.0	
x112	Unidentified						1.5		1.4	1.3		1.2
x131	Unidentified						1.8		1.7			
x147	Unidentified									2.1		
x158	Unidentified					1.0	3.7		3.7	1.5		1.9
x21	Unidentified									1.0	1.4	
x36	Unidentified											
x581	Unidentified						1.1		1.1			

Only OTUs with a contribution superior to 1% are presented.

Table S3. Biomass and sediment in samples from VA and FL

Location: fraction	Site	Mean (±SD) biomass, g	Mean (±SD) sediment, g
VA: 2 mm to 500 μm	1	19 (±13)	59 (±87)
	2	19 (±7)	82 (±29)
	3	17 (±13)	9 (±5)
VA: 500 to 100 μm	1	19 (±2)	791 (±386)
	2	18 (±3)	497 (±400)
	3	14 (±4)	835 (±216)
VA: Sessile	1	148 (±54)	NA
	2	249 (±28)	NA
	3	85 (±9)	NA
FL: 2 mm to 500 μm	1	6 (±1)	4 (±2)
	2	8 (±1)	25 (±19)
	3	11 (±2)	8 (±4)
FL: 500 to 100 μm	1	11 (±2)	110 (±109)
	2	12 (±2)	34 (±36)
	3	21 (±10)	175 (±139)
FL: Sessile	1	118 (±25)	NA
	2	127 (±18)	NA
	3	225 (±8)	NA

NA, not applicable, because there was no sediment in the sessile fraction.

Table S4. Tailed PCR primers

Primer label	Primer sequence (5′–3′)
mlCOIint_Tag1	AGACGCGGWACWGGWTGAACWGTWTAYCCYCC
mlCOIint_Tag2	AGTGTAGGWACWGGWTGAACWGTWTAYCCYCC
mlCOIint_Tag3	ACTAGCGGWACWGGWTGAACWGTWTAYCCYCC
mlCOIint_Tag4	ACAGTCGGWACWGGWTGAACWGTWTAYCCYCC
mlCOIint_Tag5	ATCGACGGWACWGGWTGAACWGTWTAYCCYCC
mlCOIint_Tag6	ATGTCGGGWACWGGWTGAACWGTWTAYCCYCC
mlCOIint_Tag7	ATAGCAGGWACWGGWTGAACWGTWTAYCCYCC
jgHCO_Tag1	AGACGCTAIACYTCIGGRTGICCRAARAAYCA
jgHCO_Tag2	AGTGTATAIACYTCIGGRTGICCRAARAAYCA
jgHCO_Tag3	ACTAGCTAIACYTCIGGRTGICCRAARAAYCA
jgHCO_Tag4	ACAGTCTAIACYTCIGGRTGICCRAARAAYCA
jgHCO_Tag5	ATCGACTAIACYTCIGGRTGICCRAARAAYCA
jgHCO_Tag6	ATGTCGTAIACYTCIGGRTGICCRAARAAYCA
jgHCO_Tag7	ATAGCATAIACYTCIGGRTGICCRAARAAYCA