Cowart DA, Matabos M, Brandt MI, Marticorena J and Sarrazin J (2019) *Exploring Environmental DNA (eDNA) to Assess Biodiversity of Hard Substratum Faunal Communities on the Lucky Strike Vent Field (Mid-Atlantic Ridge) and Investigate Recolonization Dynamics After an Induced Disturbance.* Front. Mar. Sci. 6:783. doi: 10.3389/fmars.2019.00783

**Supplementary Material S1:** PCR protocols for DNA barcoding of animal tissue.

*Eukaryotic 18SV1V2 small subunit rRNA gene amplicon (300 – 400 bp)*
SSUF04 5'-GCTTGTCTCAAAGATTAAGCC -3'
R22mod 5'-CCTGCTGCCTTCCTTRGA -3' (Sinninger et al. 2016)
PCR mixtures (30 µl final volume) contained 1 µL of DNA, 1.20 µL of each primer (10 µM), 2.40 µL of dNTP mix (10 mM each), 3 µL of 5X buffer, 4 µL of MgCl2 (25mM), 0.60 µL of GoTaq® G2 Hot Start Taq Polymerase and 16.60 µL of ddH2O. Cycling conditions were as follows: 2 min at 95 °C for one cycle, followed by 30 cycles of 1 min at 95 °C, 45 s at 57 °C and 3 min at 72 °C, finalizing with an extension of 10 min at 72 °C.

*Eukaryotic 18SV7V8 small subunit rRNA gene amplicon (350 - 450-bp)*
Forward#3 5'-GYGGTGCATGGCCGTTSKTRGTT-5'
Reverse#5RC 5'-GTGTGYACAAAGGBCAGGGAC-3' (Machida and Knowlton 2012)
PCR mixtures (30 µl final volume) contained 2 µL of DNA, 1.20 µL of each primer (10 µM), 2.40 µL of dNTP mix (10 mM each), 3 µL of 5X buffer, 4 µL of MgCl2 (25mM), 0.60 µL of GoTaq® G2 Hot Start Taq Polymerase and 15.60 µL of ddH2O. PCR cycling conditions were as follows: 3 min at 94 °C for one cycle, followed by 30 cycles of 30 s at 94 °C, 30 s at 58 °C, 45 s at 72 °, followed a final extension 10 min at 72 °C.

*Eukaryotic 18SV9 small subunit rRNA gene amplicon (130 – 150 bp)*
1389F 5′-GTACACACCGCCCGTC-3′
EukB 5'-TGATCCTTCTGCAGGTTCACCTAC-3' (Stoeck et al. 2010)
PCR mixtures (30 µl final volume) contained 2 µL of DNA, 1.20 µL of each primer (10 µM), 2.4 µL of dNTP mix (10 mM each), 3 µL of 5X buffer, 4 µL of MgCl2 (25mM), 0.60 µL of GoTaq® G2 Hot Start Taq Polymerase and 15.60 µL of ddH2O. Cycling conditions were as follows: 5 min at 95 °C for one cycle, followed by 30 cycles of 30 s at 94 °C, 45 s at 57 °C and 1 min at 72 °C, finalizing with an extension of 2 min at 72 °C.

Eukaryotic *COI gene amplicon (313 bp)*
m1COIintF 5'-GGWACWGGWTGAACWGTWTAYCCYCC -3'
HCO2198 5'-TAAACTTCAGGGTGACCAAAAATCA -3' (Lear et al. 2018)
PCR mixtures (25 µl final volume) contained 1 µL of DNA, 0.60 µL of each primer (10 µM), 1 µL of dNTP mix (10 mM each), 5 µL of 5X buffer, 1.60 µL of MgCl2 (25mM), 0.20 µL of GoTaq® G2 Hot Start Taq Polymerase and 15 µL of ddH2O. Touchdown PCR cycling conditions were as follows: 3 min at 95 °C for one cycle, followed by 16 cycles of 30 s at 95 °C, 1 min at 62 °C, 1

min at 72 °C while decreasing annealing temperature by 1 °C each cycle. Next, 25 cycles of 30 s at 95 °C, 30 s at 46 °C and 1 min at 72 °C, followed a final extension 10 min at 72 °C.

Eukaryotic *COI gene amplicon (500 - 660-bp)*

General (used for *Mirocaris*, 658 bp)

HCO: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

LCO: 5'-GGTCAACAAATCATAAAGATATTGG-3' (Folmer et al. 1994)

PCR mixtures (25 µl final volume) contained 2 µL of DNA, 0.50 µL of each primer (10 µM), 1.20 µL of dNTP mix (10 mM each), 5 µL of 5X buffer, 2 µL of MgCl2 (25mM), 0.25 µL of BSA (20mg/mL, ThermoScientific), 0.10 µL of GoTaq® G2 Hot Start Taq Polymerase and 14.45 µL of ddH2O. PCR cycling conditions were as follows: 2 min at 94 °C for one cycle, followed by 5 cycles of 35 s at 94 °C, 35 s at 48 °C, 1 min 10 s at 72 °C. Next, 35 cycles of 35 s at 94 °C, 35 s at 52 °C and 1 min 10 s at 72 °C, followed a final extension 10 min at 72 °C.

Polynoids (659 bp)

HCO poly: 5'-TAMACTTCWGGGTGACCAAARAATCA-3'

LCO poly: 5'-GAYTATWTTCAACAAATCATAAAGATATTG-3' (Carr et al. 2011)

PCR mixtures (25 µl final volume) contained 2 µL of DNA, 2.50 µL of each primer (10 µM), 0.50 µL of dNTP mix (10 mM each), 5 µL of 5X buffer, 2.50 µL of MgCl2 (25mM), 0.13 µL of GoTaq® G2 Hot Start Taq Polymerase and 9.88 µL of ddH2O. PCR cycling conditions were as follows: 4 min at 95 °C for one cycle, followed by 35 cycles of 30 s at 94 °C, 1 min at 52 °C, 1 min 15 s at 72 °C, followed a final extension 8 min at 72 °C.

*Bathymodiolus* (544 bp)

BathCOI-F: 5'-TGTGGTCTGGAATAATTGGAAC-3'

BathHCO-R:5'-ATAAAAAGATGTATTRAARTGACG-3' (Olu-Le Roy et al. 2007)

PCR mixtures (25 µl final volume) contained 1 µL of DNA, 0.50 µL of each primer (10 µM), 1.20 µL of dNTP mix (10 mM each), 5 µL of 5X buffer, 2 µL of MgCl2 (25mM), 0.25 µL of BSA (20mg/mL, ThermoScientific), 0.10 µL of GoTaq® G2 Hot Start Taq Polymerase and 14.45 µL of ddH2O. PCR cycling conditions were as follows: 2 min at 94 °C for one cycle, followed by 5 cycles of 35 s at 94 °C, 35 s at 48 °C, 1 min 10 s at 72 °C. Next, 35 cycles of 35 s at 94 °C, 35 s at 52 °C and 1 min 10 s at 72 °C, followed a final extension 10 min at 72 °C.

Eukaryotic *28S D9D10 large subunit rDNA gene amplicon (400 – 550bp)*

Forward #8 5'-GGGAAAGAAGACCCTGTTGAG-3'

Reverse#11 5'-GCTTGGCBGCCACAAGCCAGTTA-3' (Machida and Knowlton 2012)

PCR mixtures (15 µl final volume) contained 1 µL of DNA, 0.60 µL of each primer (10 µM), 1.20 µL of dNTP mix (10 mM each), 1.5 µL of 5X buffer, 2 µL of MgCl2 (25mM), 0.30 µL of GoTaq®

G2 Hot Start Taq Polymerase and 7.80 µL of ddH2O. PCR cycling conditions were as follows: 10 min at 95 °C for one cycle, followed by 30 cycles of 10 s at 95 °C, 30 s at 53 °C, 1 min at 72 °C, followed a final extension 10 min at 72 °C.

Eukaryotic *28S D2D3 large subunit rDNA gene amplicon (600 – 1000 bp)*

D2A 5´-ACA AGT ACC GTG AGG GAA AGT TG-3´
D3B 5´-TCG GAA GGA ACC AGC TAC TA-3´ (De Ley et al. 1999)
PCR mixtures (25 µl final volume) contained 2 µL of DNA, 2 µL of each primer (10 µM), 0.5 µL of dNTP mix (10 mM each), 5 µL of 5X buffer, 2 µL of MgCl2 (25mM), 0.125 µL of GoTaq® G2 Hot Start Taq Polymerase and 11.375 µL of ddH2O. PCR cycling conditions were as follows: 2 min at 94 °C for one cycle, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C, followed a final extension 10 min at 72 °C.

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