Appendix

Dilute concentrations of maritime fuel can modify sediment reworking activity of high-latitude marine invertebrates

Thomas J. Williams[1], David Blockley[2], Andrew B. Cundy[1], Jasmin A. Godbold[1], Rebecca M. Howman[1,3], Martin Solan[1]

[1]University of Southampton, National Oceanography Centre Southampton, Waterfront Campus, European Way, Southampton, SO14 3ZH, UK
[2]Pinngortitaleriffik, Greenland Institute of Natural Resources, Kivioq 2, 3900 Nuuk, Greenland
[3]Québec Océan, Takuvik Joint International Laboratory CNRS, Université Laval, Quebec City, QC, Canada

Figure S1 | Cumulative sediment particle size distributions (n = 4) for sieved (1mm mesh size) sediment collected from the inner Kobbefjord, near Nuuk, Greenland (12m depth, 64°08.364 N, 51°23.621' W).

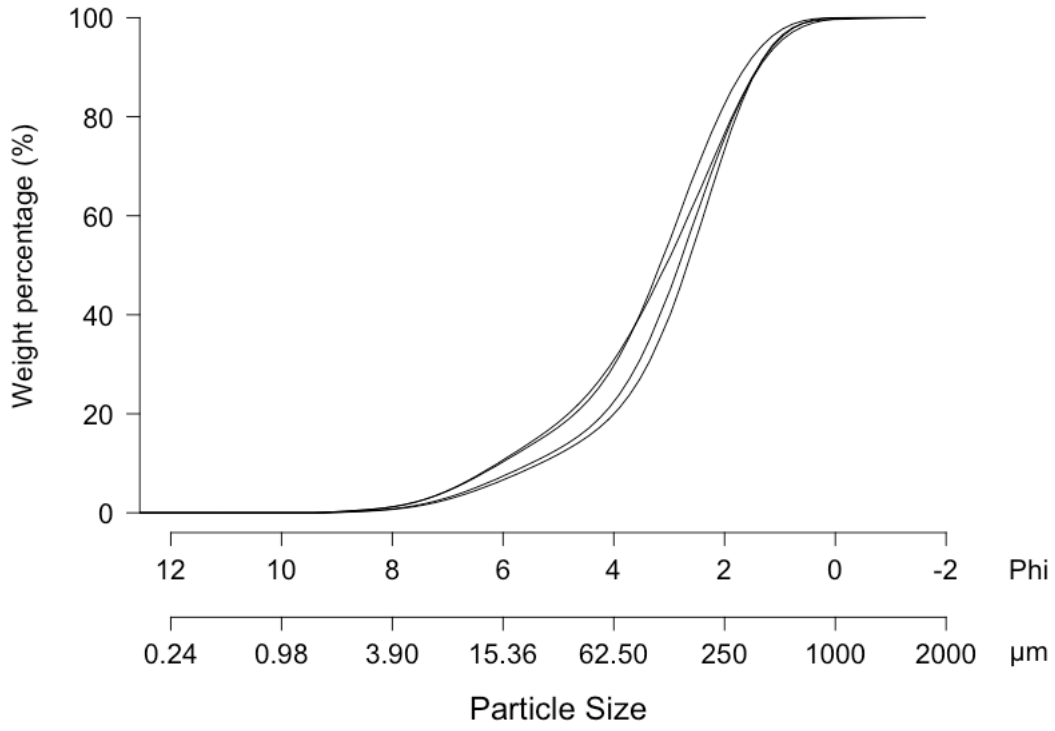


Figure S2 | The temperature-controlled insulated water bath housing randomly positioned square glass aquaria prior to faunal addition. Temperature (± 1℃) is maintained via continuous circulation of seawater through an external heater-chiller unit. Individual aquaria are supplied with continuous aeration via the rows of taps along the perimeter of the water bath.

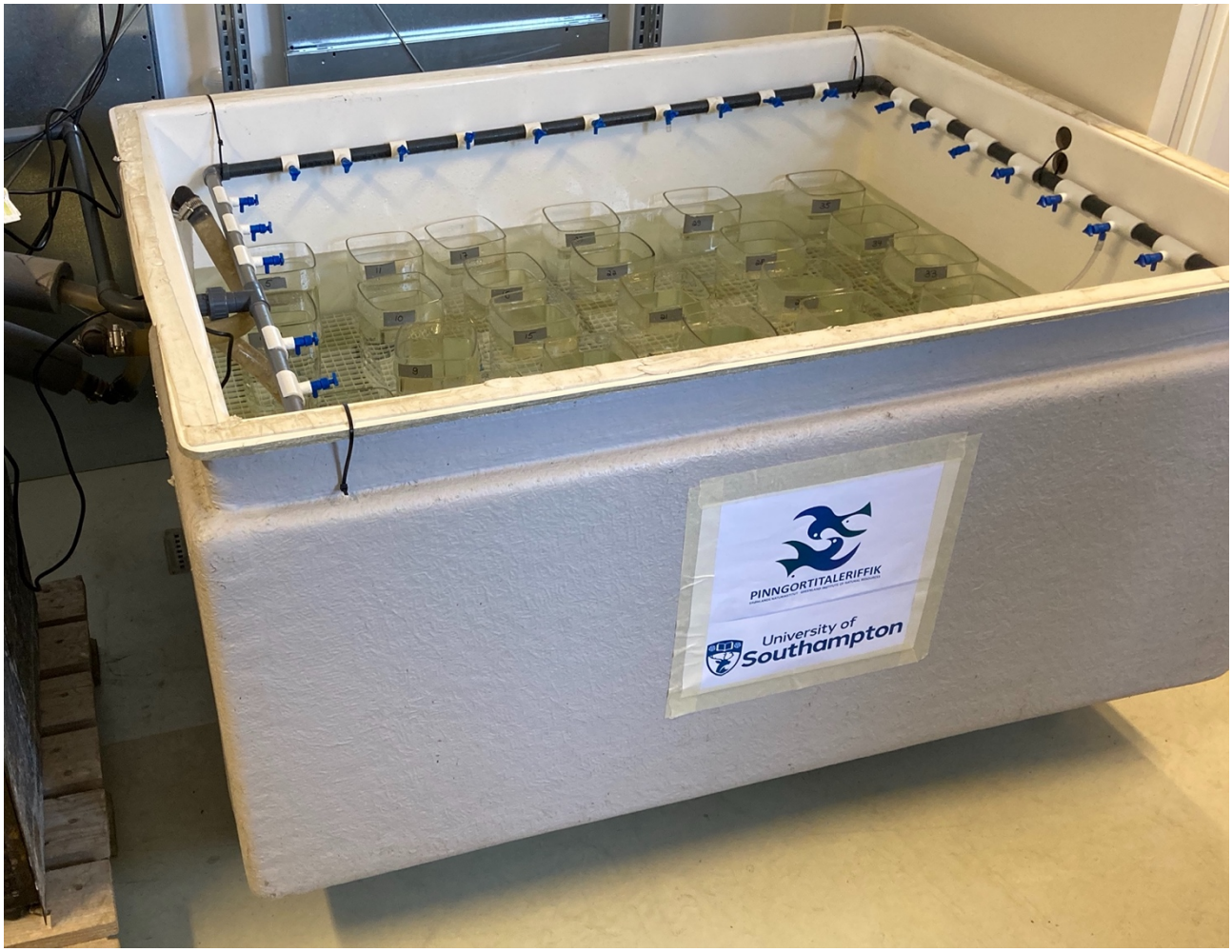


Figure S3 | Total biomass aquaria^{-1} for *Astarte crenata* (circle), *Cistenides hyperborea* (square) and *Macoma calcarea* (triangle) for each marine fuel oil (water accommodated fraction) dosing concentration (relative to above-sediment water volume).

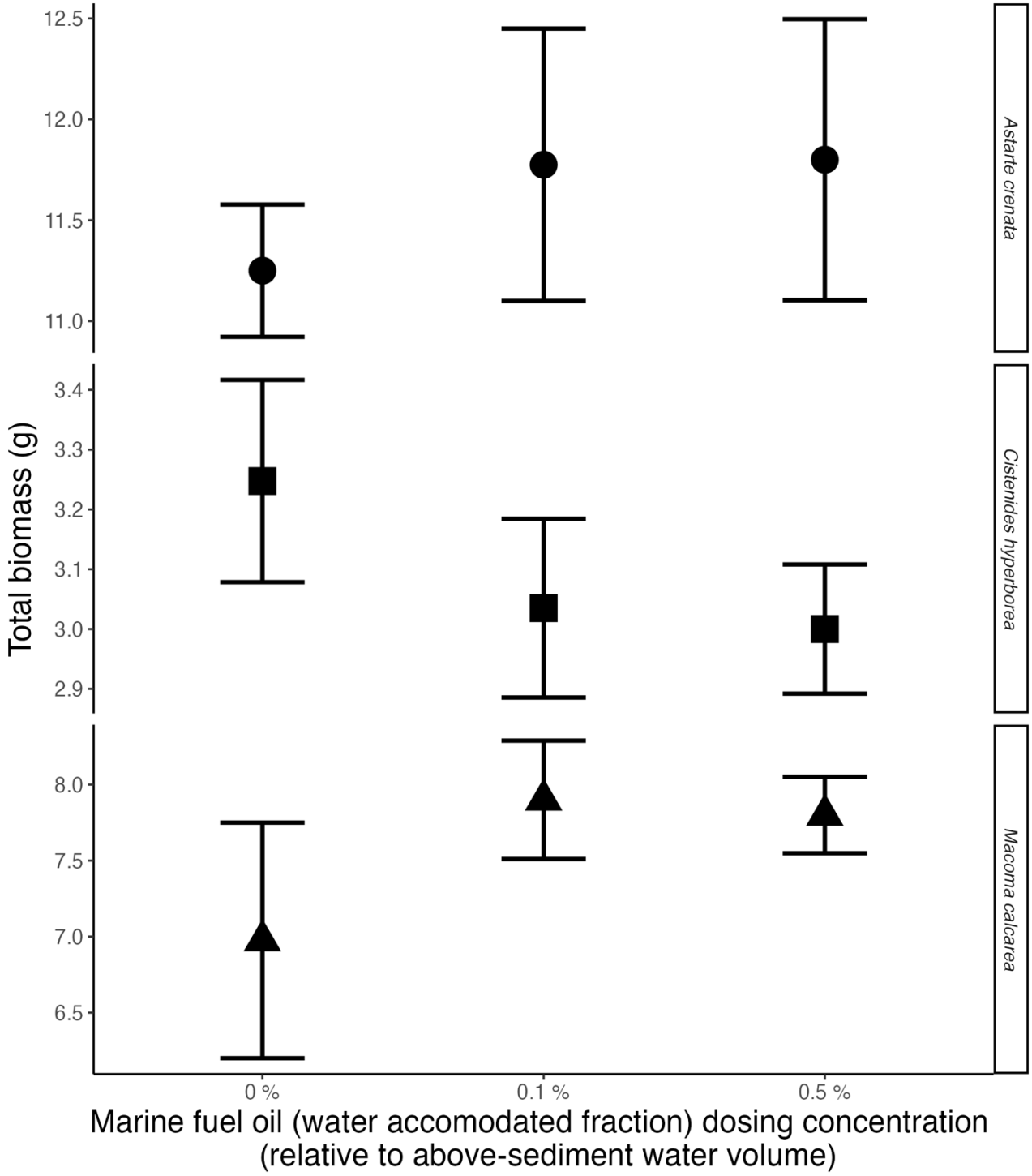
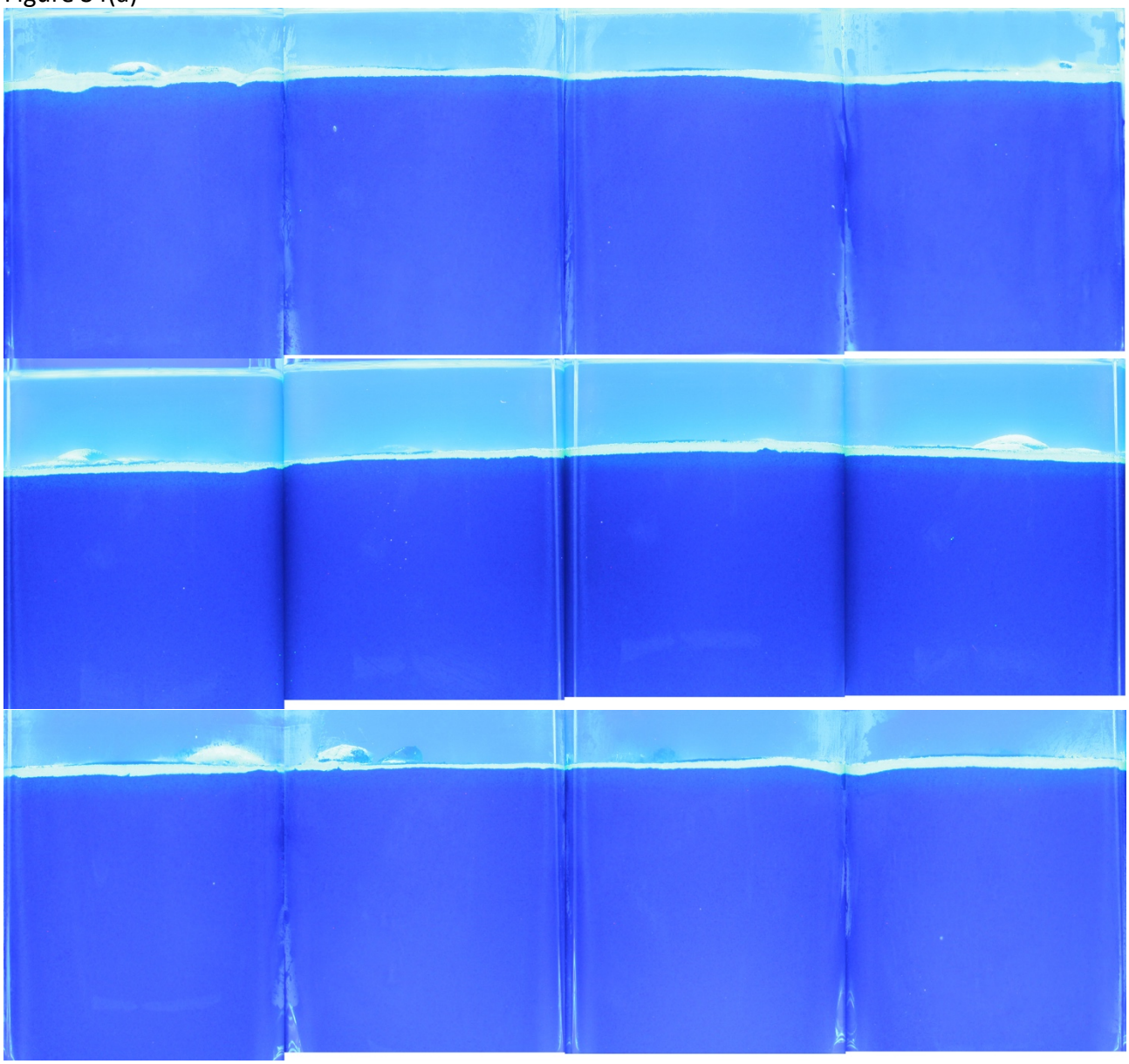


Figure S4 | Replicate (n = 4, with one exception of n = 3) f-SPI images for aquaria containing (a-c) *Astarte crenata* exposed to (a) 0%, (b) 0.1% and (c) 0.5% marine fuel oil (water accommodated fraction) dosing concentration (relative to above-sediment water volume). (d-f) *Cistenides hyperborea* exposed to (a) 0%, (b) 0.1% and (c) 0.5 % marine fuel oil concentrations, and (g-i) *Macoma calcarea* exposed to (g) 0%, (h) 0.1% and (i) 0.5% marine fuel oil concentrations. Each image shows the four aquarium sides, each ~10 cm width, stitched together after 7 days. The green coloration is the luminophore tracer.

Figure S4(a)



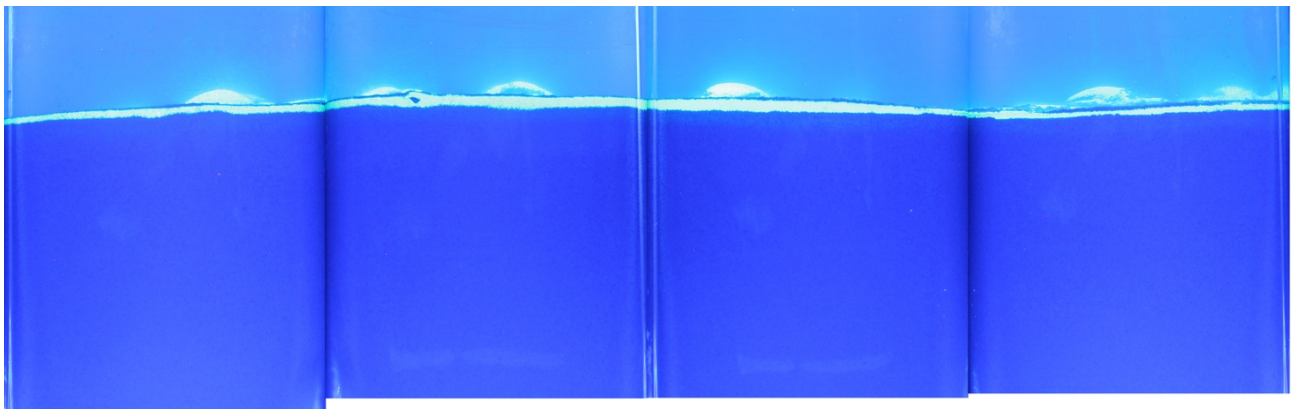


Figure S4(b)

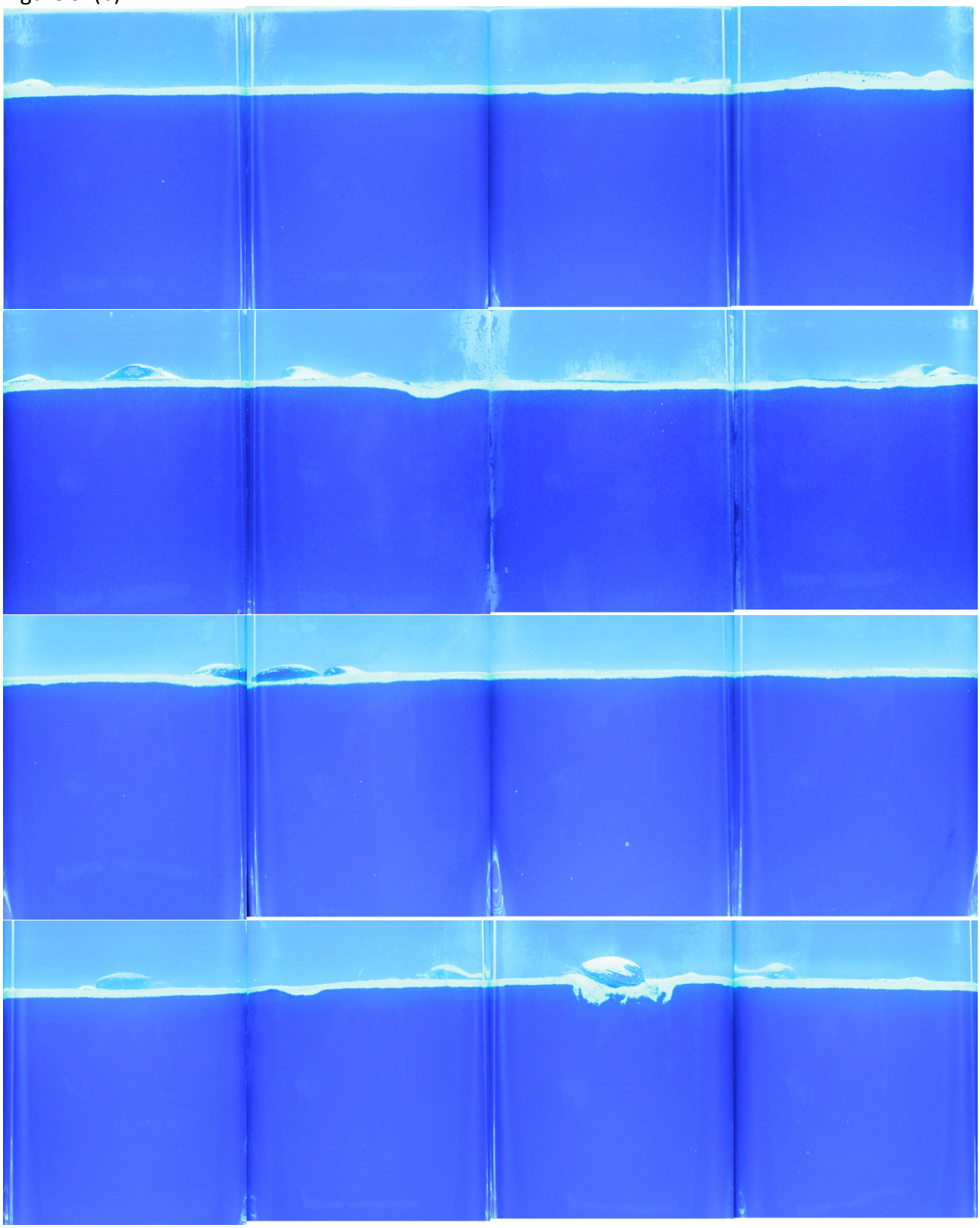


Figure S4(c)

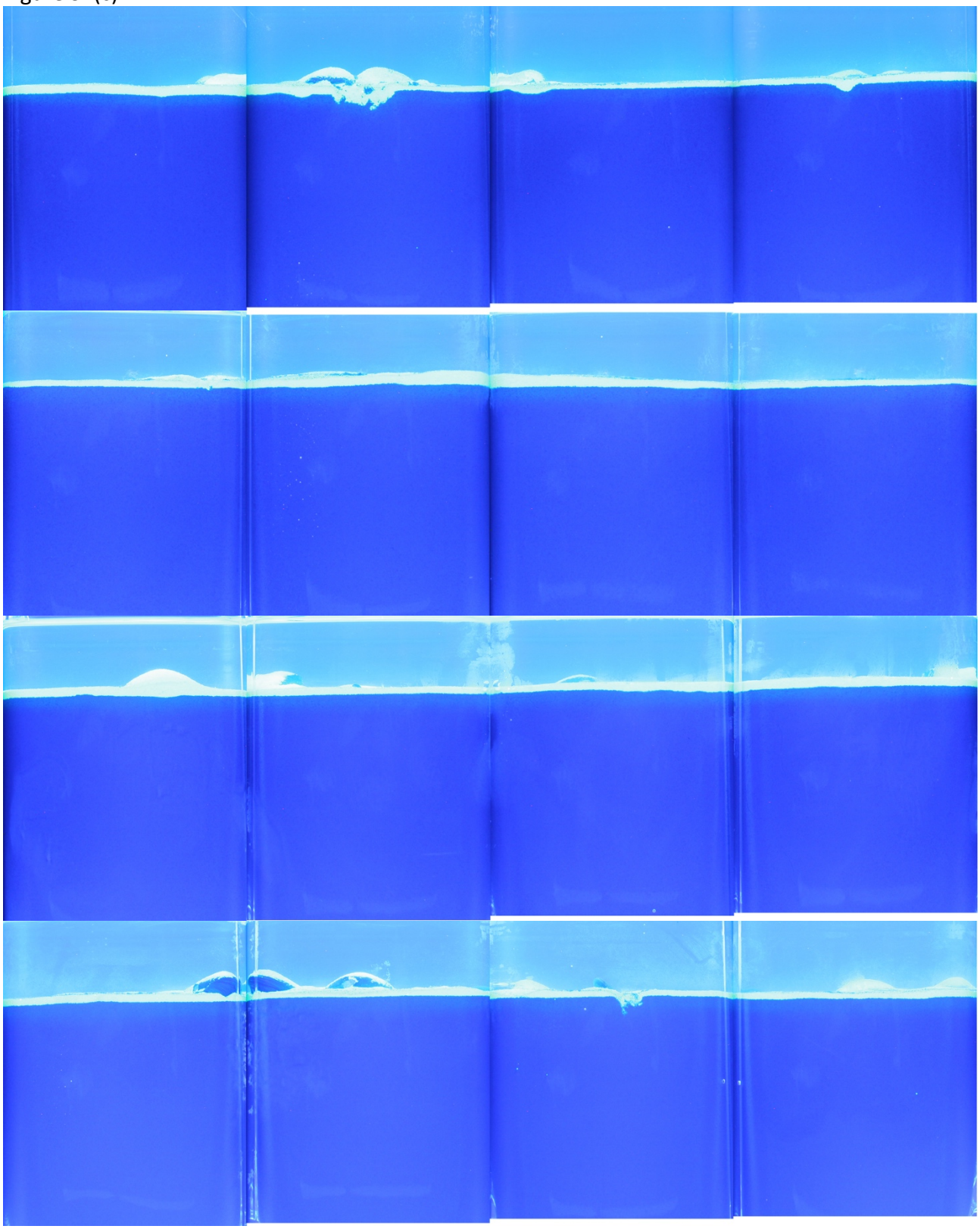


Figure S4(d)

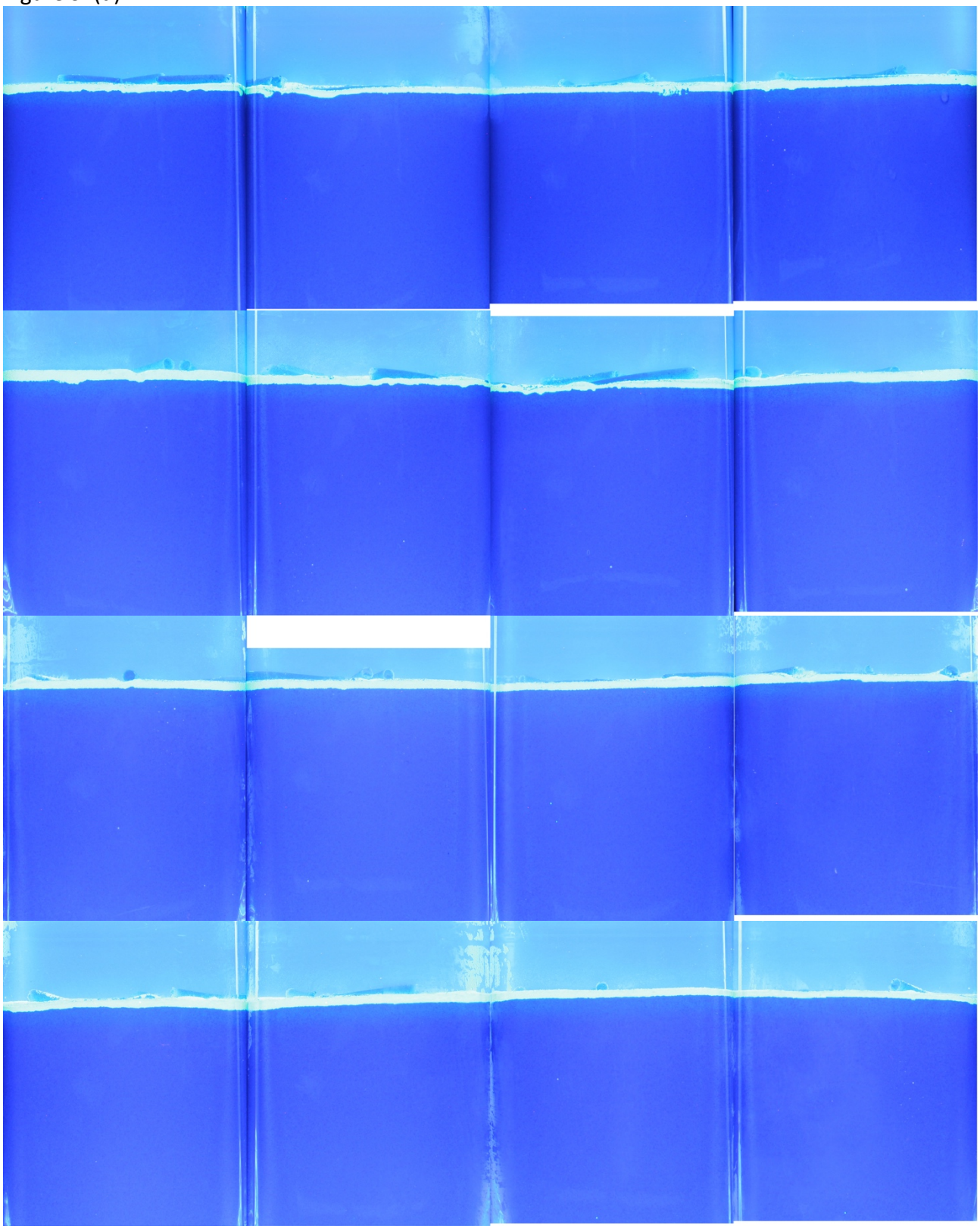


Figure S4(e)

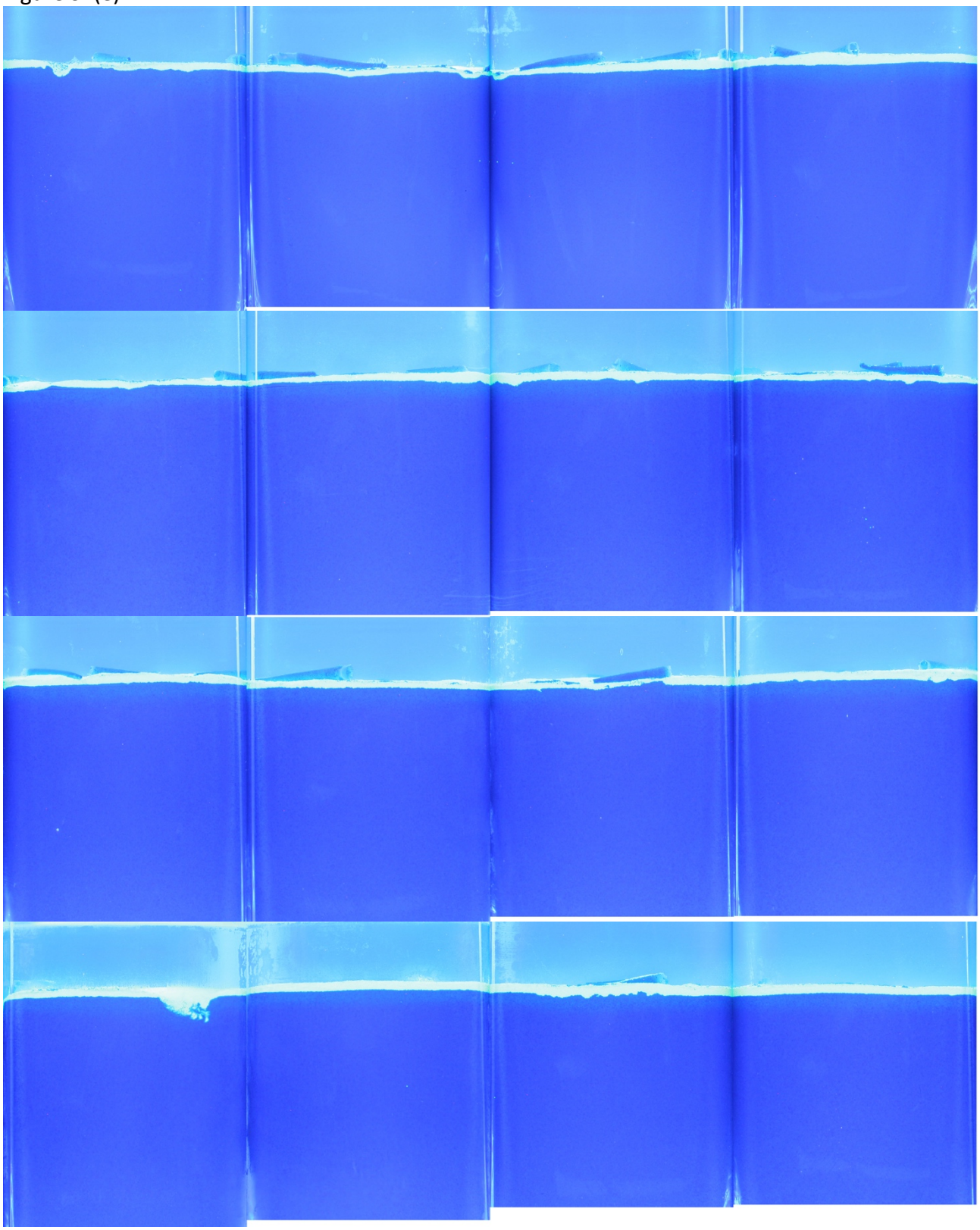


Figure S4(f)

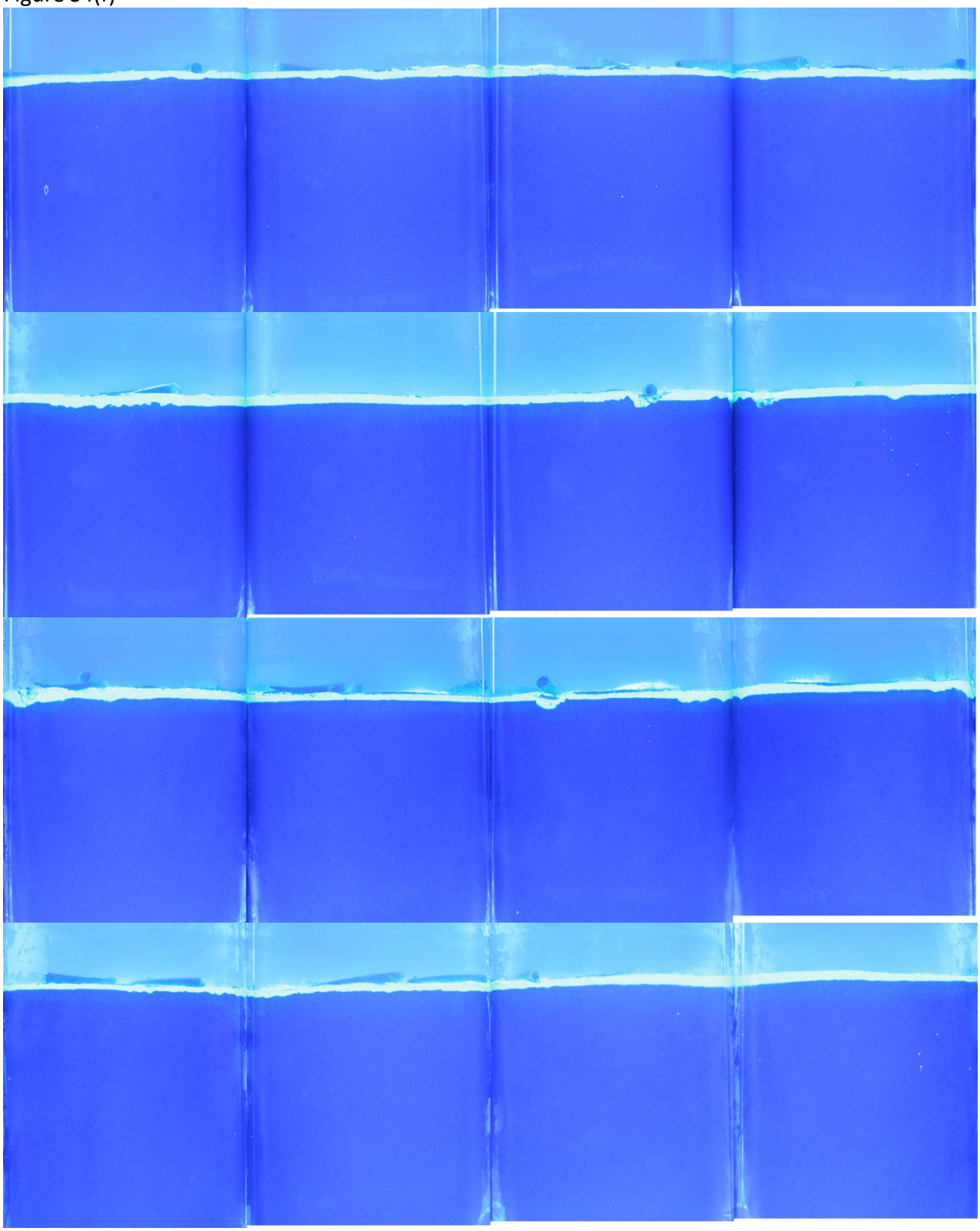


Figure S4(g)

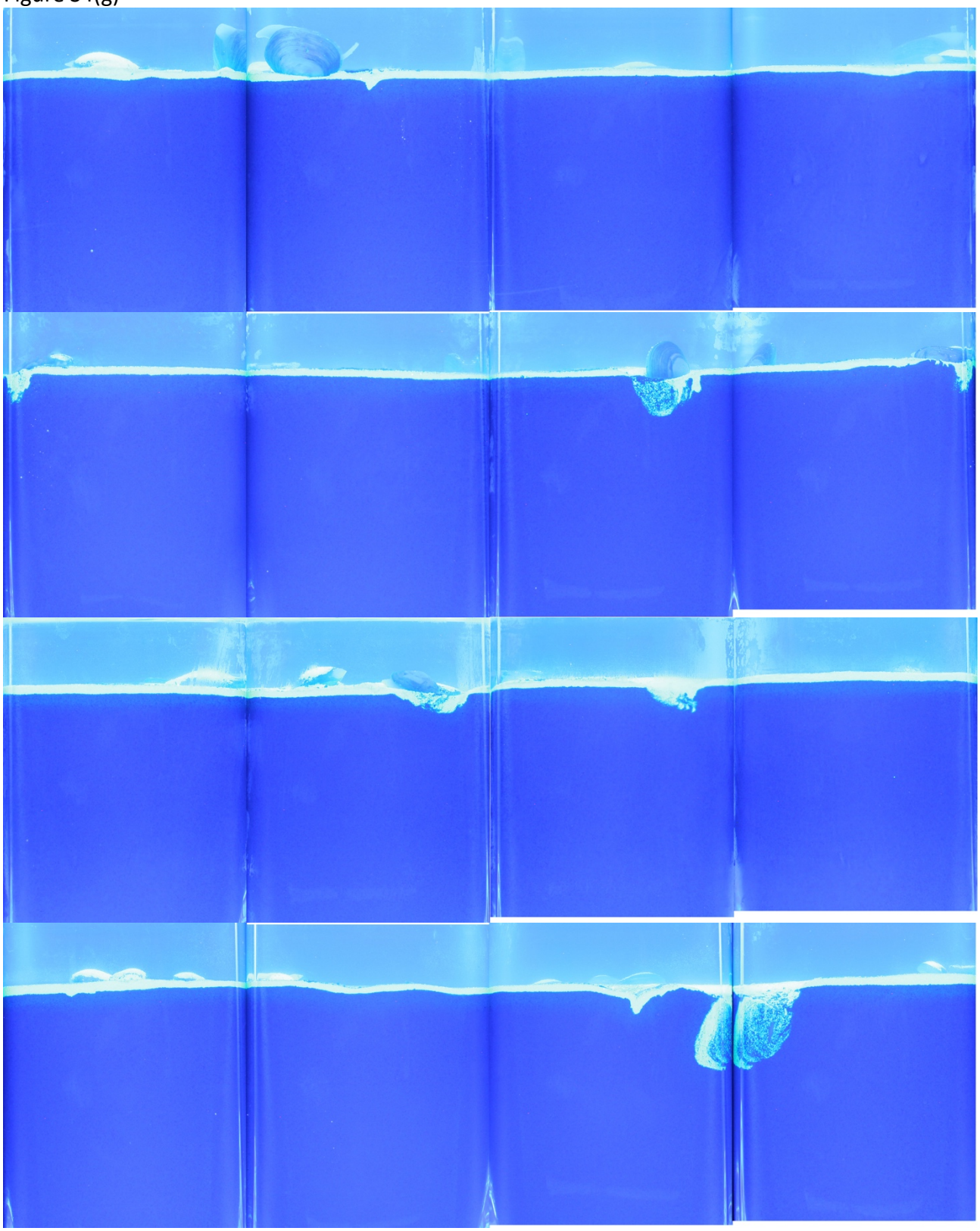


Figure S4(h)

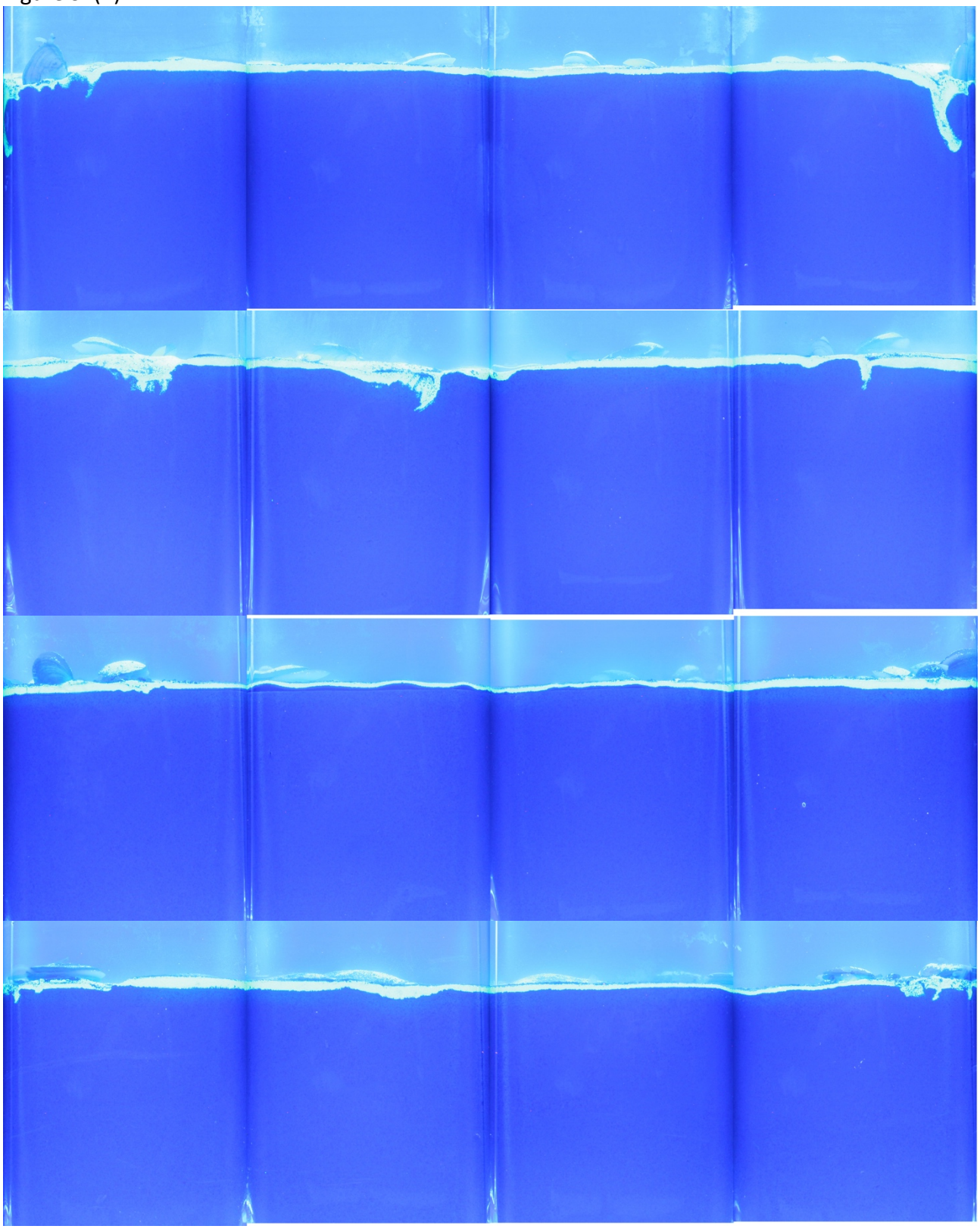


Figure S4(i)

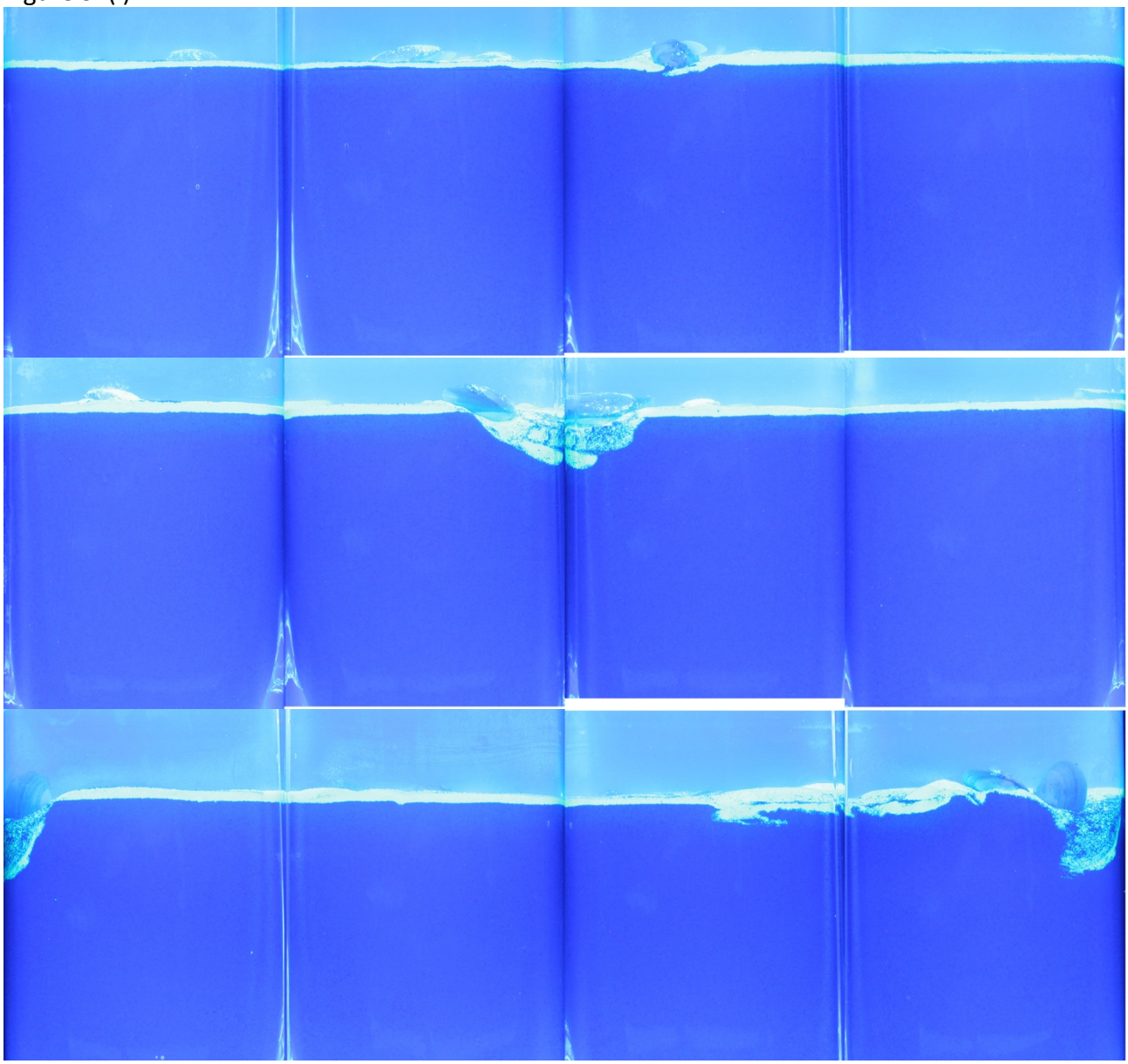
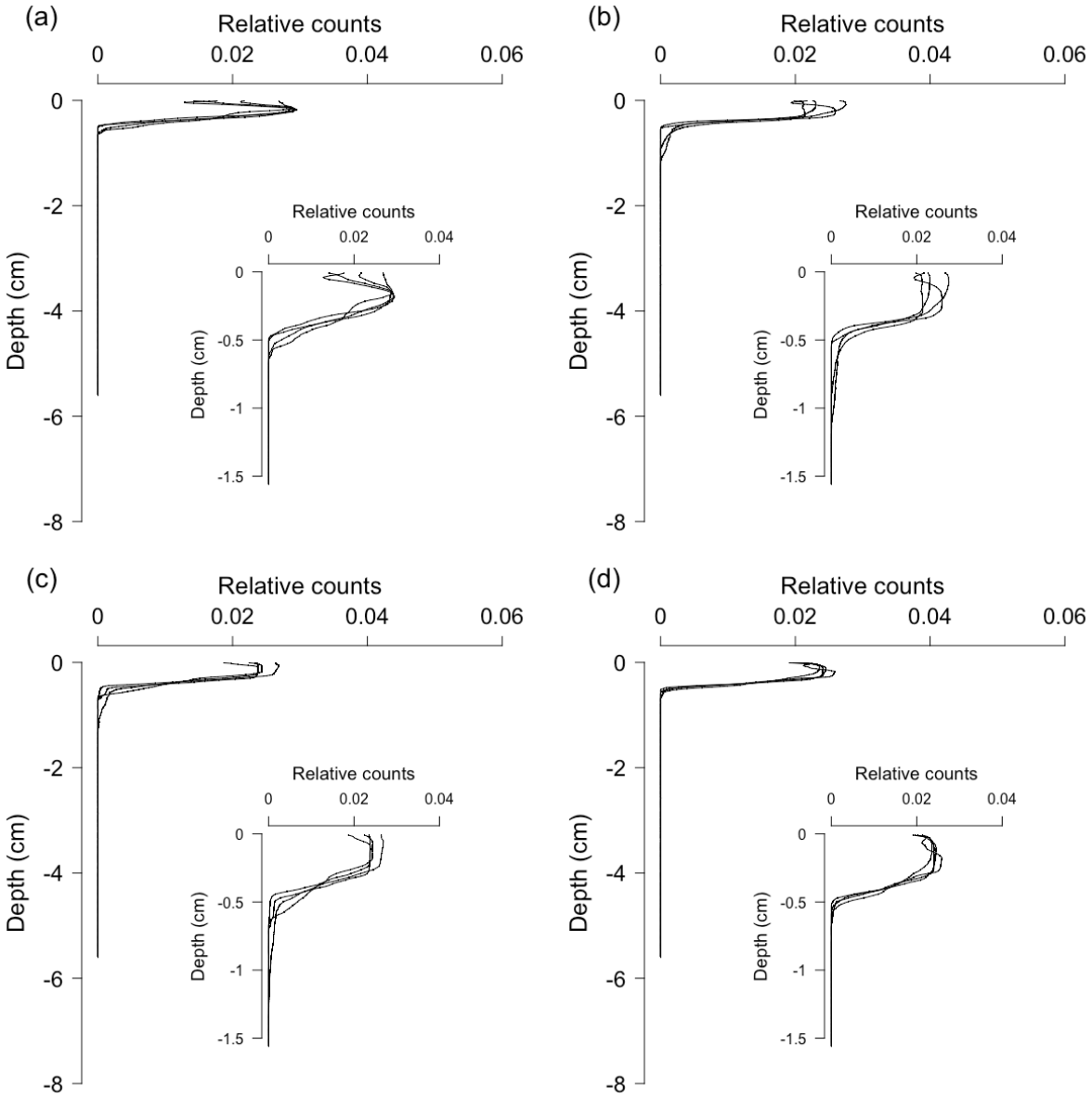


Figure S5 | Sediment profiles per aquaria containing (a-c) *Astarte crenata* (d-f) *Cistenides hyperborea* and (g-i) *Macoma calcarea* in the presence of (a,d,g) 0% (b,e,h) 0.1% and (c,f,i) 0.5% of the water accommodated fraction of marine fuel oil. Dosing concentrations are relative to above-sediment water volume.



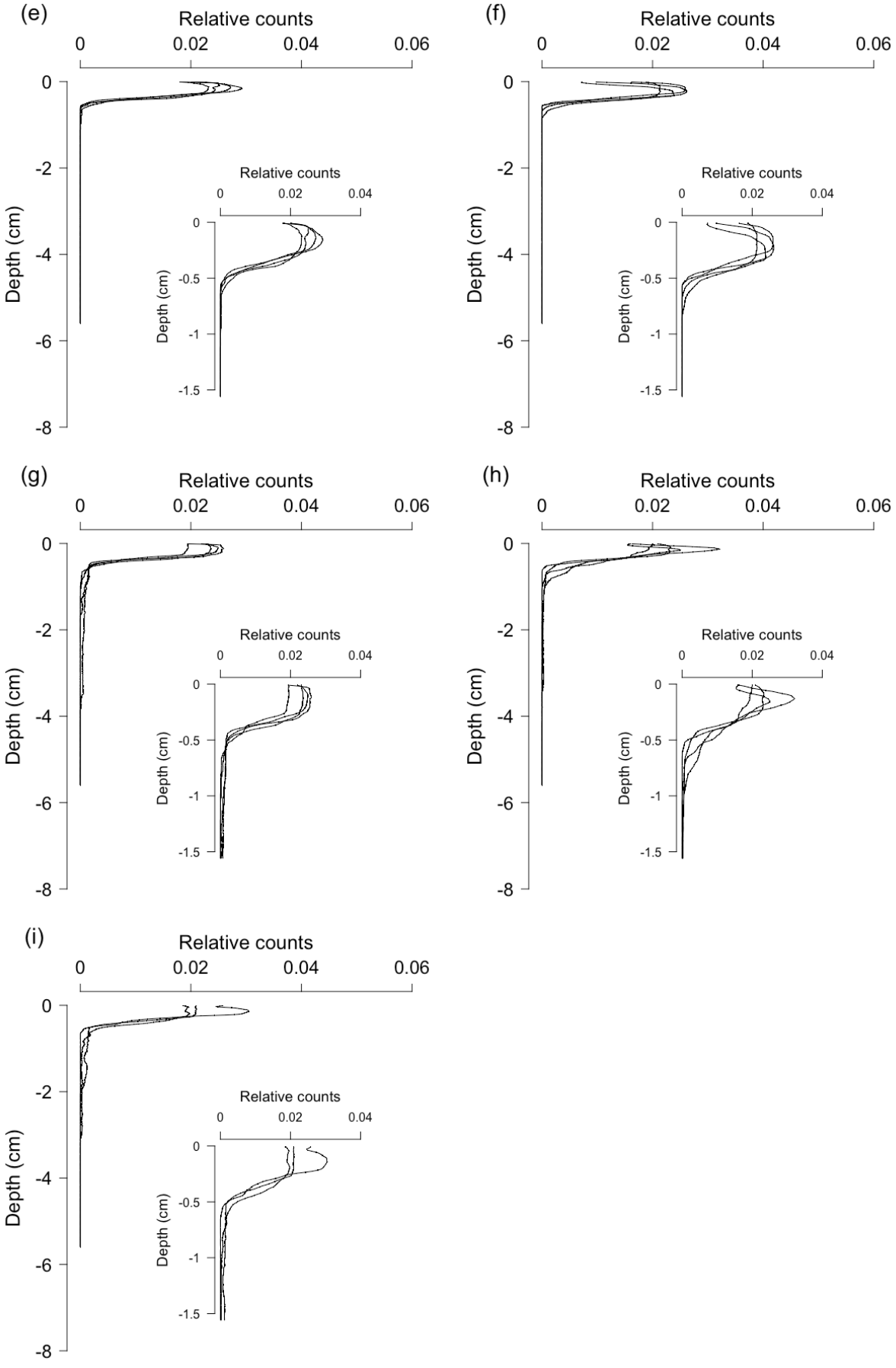

(e)
Relative counts
0
0.02
0.04
0.06
Depth (cm)
0
-2
-4
-6
-8
Relative counts
0
0.02
0.04
Depth (cm)
0
-0.5
-1
-1.5
(f)
Relative counts
0
0.02
0.04
0.06
Depth (cm)
0
-2
-4
-6
-8
Relative counts
0
0.02
0.04
Depth (cm)
0
-0.5
-1
-1.5
(g)
Relative counts
0
0.02
0.04
0.06
Depth (cm)
0
-2
-4
-6
-8
Relative counts
0
0.02
0.04
Depth (cm)
0
-0.5
-1
-1.5
(h)
Relative counts
0
0.02
0.04
0.06
Depth (cm)
0
-2
-4
-6
-8
Relative counts
0
0.02
0.04
Depth (cm)
0
-0.5
-1
-1.5
(i)
Relative counts
0
0.02
0.04
0.06
Depth (cm)
0
-2
-4
-6
-8
Relative counts
0
0.02
0.04
Depth (cm)
0
-0.5
-1
-1.5

Table S1 | Summary of sediment particle size statistics for four random mesocosms and sample of luminophores given by the default GRADISTAT output (Blott & Pye, 2001). Mean, sorting, skewness, kurtosis, the percentage of sample less than 63 µm and total organic material are presented for all sediment samples. Superscripts provide descriptive terminology as outlined by Blott & Pye (2001). **Mean, $\bar{x}$:** fs, fine sand; ms, medium sand; cs, coarse sand; vcs, very coarse sand. **Sorting, *σ*:** ps, poorly sorted; vps, very poorly sorted. **Skewness, *Sk*:** sy, symmetrical; vfsk, very fine skewed; fsk, fine skewed; csk, coarse skewed. **Kurtosis, *K*:** mk, mesokurtic; lk, leptokurtic; pk, platykurtic. Organic matter content was determined by loss on ignition.

Sample	Mean ($\bar{x}$, µm)	Sorting (σ, µm)	Skewness (Sk, µm)	Kurtosis (K, µm)	Sample <63 µm (%)	Organic material (%)	Sediment name
Sediment							
1	136^{fs}	228.51^{ps}	1.75^{sy}	6.56^{pk}	23.49	0.11	Poorly Sorted Very Fine Sand
2	160^{fs}	239.34^{ps}	2.07^{sy}	8.99^{pk}	16.62	0.44	Slightly Very Fine Gravelly Medium Sand
3	178^{fs}	248.04^{ps}	3.69^{fsk}	34.07^{pk}	15.15	0.30	Slightly Very Fine Gravelly Medium Sand
4	127^{fs}	196.00^{ps}	1.99^{sy}	8.11^{pk}	22.17	0.34	Poorly Sorted Very Fine Sand
Luminophores							
1	301^{ms}	124.75^{ws}	1.13^{sy}	4.85^{mk}	0	N/A	Well Sorted Medium Sand

Statistical model summary

Each species (*A. crenata*, *M. calcerea*, *C. hyperborea*) was analysed separately (Model S1 to S12). For each model we list the initial linear regression model and the minimal adequate model. When homogeneity of variance was violated, we used a linear regression with generalised least squares (GLS) estimation. We present a summary of the coefficient tables for single terms. The coefficients indicate the relative performance of each factor level in relation to the re-levelled baseline (as indicated). Coefficients ± SE, t-values and respective significance values are presented.

Abbreviations

(i) Explanatory variables

Oil, Marine fuel oil concentration (%, water accommodated fraction relative to above-sediment water volume).

(ii) Response variables

SBR, surface boundary roughness (cm)
$^{f\text{-}SPI}L_{mean}$, mean mixed depth of particle reworking (cm)
$^{f\text{-}SPI}L_{median}$, median mixed depth of particle reworking (cm)
$^{f\text{-}SPI}L_{max}$, maximum mixed depth of particle reworking (cm)

Data: All data used in the analyses are provided as Table S2.

Astarte crenata

Model S1 Surface boundary roughness (SBR, cm)

Initial linear regression model:

```
lm(SBR ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(SBR ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Model S2 Mean mixing depth ($^{f\text{-}SPI}L_{mean}$, cm)

Initial linear regression model:

```
lm(f-SPI Lmean ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(f-SPI Lmean ~ as.factor(Oil),
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Intercept ± SE (when baseline is for 0 % for marine fuel oil concentration): 0.35 ± 0.01, t = 32.24, **p < 0.0001**

Coefficient table for Oil

	0	0.1	0.5
0	/	0.07 ± 0.03 2.67 **0.026**	0.05 ± 0.01 4.40 **0.002**
0.1	0.07 ± 0.03 2.67 **0.026**	/	-0.01 ± 0.02 -0.06 0.597
0.5	0.05 ± 0.01 4.40 **0.002**	-0.01 ± 0.02 -0.06 0.597	/

Model S3 Median mixing depth ($^{f\text{-}SPI}L_{median}$, cm)

Initial linear regression model:

```
lm(f-SPI Lmedian ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(f-SPI Lmedian ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Model S4 Max mixing depth ($^{f\text{-}SPI}L_{max}$, cm)

Initial linear regression model:

```
lm(f-SPIL_max ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(f-SPIL_max ~ as.factor(Oil),
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Intercept ± SE (when baseline is for 0 % for marine fuel oil concentration): 0.58 ± 0.04, t = 13.18, **p < 0.0001**

Coefficient table for Oil

	0	0.1	0.5
0	/	0.22 ± 0.16 1.37 0.205	0.35 ± 0.16 2.19 **0.056**
0.1	0.22 ± 0.16 1.37 0.205	/	0.13 ± 0.22 0.58 0.574
0.5	0.35 ± 0.16 2.19 **0.056**	0.13 ± 0.22 0.58 0.574	/

Cistenides hyperborea

Model S5 Surface boundary roughness (SBR, cm)

Initial linear regression model:

```
lm(SBR ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(SBR ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Model S6 Mean mixing depth ($^{f\text{-}SPI}L_{mean}$, cm)

Initial linear regression model:

```
lm(f-SPIL_mean ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(f-SPIL_mean ~ as.factor(Oil),
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Intercept ± SE (when baseline is for 0 % for marine fuel oil concentration): 0.41 ± 0.01, t = 48.57, **p < 0.0001**

Coefficient table for Oil

	0	0.1	0.5
0	/	-0.03 ± 0.02 -1.52 0.162	0.04 ± 0.03 1.60 0.144
0.1	-0.03 ± 0.02	/	0.07 ± 0.03

	-1.52 0.162		2.36 **0.042**
0.5	0.04 ± 0.03 1.60 0.144	0.07 ± 0.03 2.36 **0.042**	/

Model S7 Median mixing depth ($^{f\text{-}SPI}L_{median}$, cm)

Initial linear regression model:

```
lm(f-SPI_Lmedian ~ as.factor(Oil))\
```

Minimal adequate model:

```
gls(f-SPI_Lmedian ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Model S8 Max mixing depth ($^{f\text{-}SPI}L_{max}$, cm)

Initial linear regression model:

```
lm(f-SPI_Lmax ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(f-SPI_Lmax ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Macoma calcerea

Model S9 Surface boundary roughness (SBR, cm)

Initial linear regression model:

```
lm(SBR ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(SBR ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Model S10 Mean mixing depth ($^{f\text{-}SPI}L_{mean}$, cm)

Initial linear regression model:

```
lm(f-SPI_Lmean ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(f-SPI_Lmean ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Model S11 Median mixing depth ($^{f\text{-}SPI}L_{median}$, cm)

Initial linear regression model:

```
lm(f-SPI_Lmedian ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(f-SPI_Lmedian ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Model S12 Max mixing depth ($^{f\text{-}SPI}L_{max}$, cm)

Initial linear regression model:

```
lm(f-SPI_Lmax ~ as.factor(Oil))
```

Minimal adequate model:

```
gls(f-SPI_Lmax ~ 1,
      weights = varIdent(form = ~ 1|as.factor(Oil), method = "REML")
```

Table S2 | Summary of bioturbation indicators used in our statistical analyses. Oil = marine fuel oil (%, water accommodated fraction relative to above-sediment water volume). * indicates loss of aquarium in error.

Species identity	Oil	Total biomass (g)	SBR (cm)	$^{f\text{-}SPI}L_{median}$ (cm)	$^{f\text{-}SPI}L_{mean}$ (cm)	$^{f\text{-}SPI}L_{max}$ (cm)
Astarte crenata	0	11.4	0.622	0.323	0.311	0.499
Astarte crenata	0	10.9	1.211	0.335	0.338	0.516
Astarte crenata	0	12.1	0.481	0.366	0.358	0.650
Astarte crenata	0	10.6	1.038	0.365	0.349	0.670
Astarte crenata	0.1	10.7	0.751	0.439	0.404	0.901
Astarte crenata	0.1	13.7	0.563	0.386	0.356	0.553
Astarte crenata	0.1	11.7	0.647	0.368	0.347	0.563
Astarte crenata	0.1	11	0.687	0.468	0.376	1.204
Astarte crenata	0.5	11.7	0.751	0.412	0.366	1.334
Astarte crenata	0.5	13.6	0.546	0.412	0.358	0.678
Astarte crenata	0.5	10.2	0.714	0.399	0.385	0.714
Astarte crenata	0.5	11.7	0.582	0.386	0.347	1.004
Cistenides hyperborea	0	2.8	0.667	0.403	0.376	0.695
Cistenides hyperborea	0	3.59	1.025	0.429	0.423	0.790
Cistenides hyperborea	0	3.2	0.461	0.390	0.386	0.593
Cistenides hyperborea	0	3.4	0.678	0.399	0.395	0.602
Cistenides hyperborea	0.1	3.41	0.989	0.370	0.339	0.640
Cistenides hyperborea	0.1	2.93	0.677	0.345	0.329	0.602
Cistenides hyperborea	0.1	3.1	0.490	0.381	0.377	0.640
Cistenides hyperborea	0.1	2.7	0.385	0.419	0.395	1.128
Cistenides hyperborea	0.5	3.3	0.464	0.415	0.388	0.577
Cistenides hyperborea	0.5	2.9	0.581	0.505	0.431	0.722
Cistenides hyperborea	0.5	3	0.715	0.461	0.432	0.921
Cistenides hyperborea	0.5	2.8	0.928	0.400	0.356	0.572
Macoma calcarea	0	9.2	0.481	0.386	0.340	0.991
Macoma calcarea	0	5.6	0.801	0.503	0.339	1.686
Macoma calcarea	0	6.6	1.108	0.450	0.376	1.221
Macoma calcarea	0	6.5	0.668	0.675	0.339	3.896
Macoma calcarea	0.1	7.3	1.148	0.471	0.348	3.453
Macoma calcarea	0.1	7.4	0.932	0.508	0.386	1.835
Macoma calcarea	0.1	9	0.562	0.350	0.290	0.655
Macoma calcarea	0.1	7.9	0.658	0.465	0.366	1.052
Macoma calcarea	0.5	7.5	0.591	0.365	0.282	0.657
Macoma calcarea	0.5	8.3	1.091	0.521	0.367	2.059
Macoma calcarea	0.5	7.2	*	*	*	*
Macoma calcarea	0.5	7.6	1.235	0.603	0.440	3.171

References

Blott, S. J., & Pye, K. (2001). GRADISTAT: a grain size distribution and statistics package for the analysis of unconsolidated sediments. *Earth Surface Processes and Landforms, 26*(11), 1237-1248. doi:10.1002/esp.261.

Ends.