

Crude oil contamination interrupts settlement of coral larvae after direct exposure ends

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Supplement 1

Total mineral oil in seawater

Crude oil hydrocarbons were quantified as the total concentration of mineral oil in seawater. Seawater samples, which were used for larval exposure experiments with *Agaricia humilis* and *Orbicella faveolata*, were collected from the field on Days 18 and 21 after the spill, respectively. Total mineral oil was also quantified in the undiluted laboratory-contaminated seawater prior to its use in larval exposure experiments.

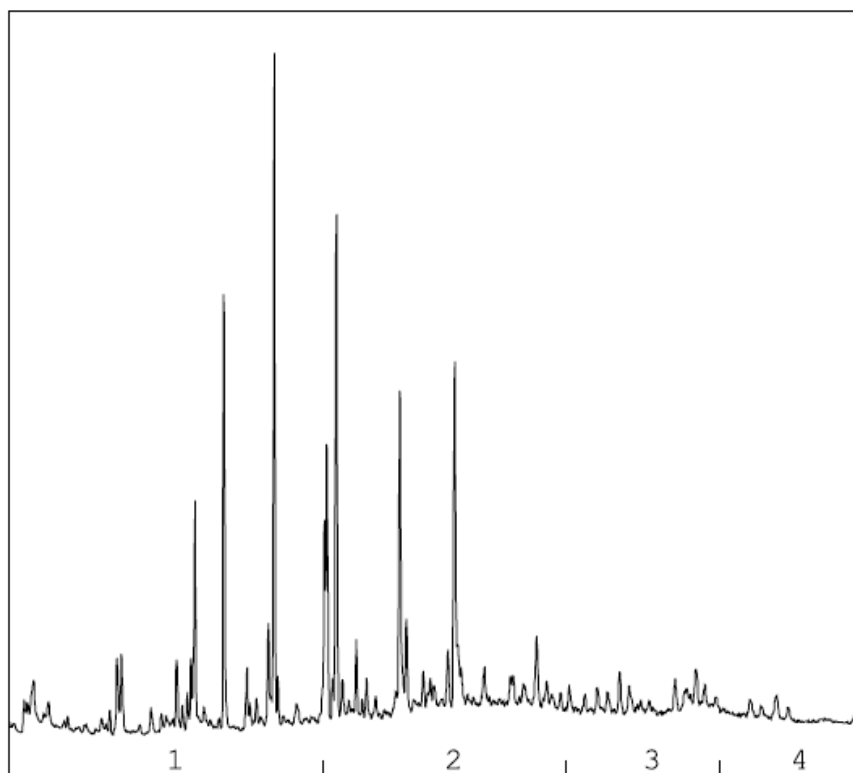
Water samples for measuring total mineral oil concentrations in seawater were collected from the field in acid-washed (0.4 M HCl) 100 ml syringes from the spill site ('Oil West site'). Water was also sampled from the undiluted laboratory-prepared, oil-contaminated seawater mixture. Water samples were divided over two 40 ml pre-combusted (4 h, 450°C) amber-glass EPA vials (Fisherbrand, USA) with autoclaved and acid-washed (0.4 M HCl) PTFE screw caps. Water samples were acidified with 0.4 ml of 12 M HCl. Samples were kept refrigerated (4°C) in the dark until further processing in The Netherlands, within 25 days after the samples were taken.

The sample preparation procedure for the analysis of mineral oil in seawater was based on the ISO 9377-2:2000 (www.iso.org), under accreditation-certificate L086 of Omegam Laboratoria BV (Amsterdam, The Netherlands). This procedure follows a hexane extraction, florisil clean-up and analysis by gas chromatography. Detection limit of this procedure is 0.1 mg l⁻¹ mineral oil in seawater.

Detected quantities were as follows:

Seawater Source	Total Mineral Oil
Oil West site 18 days after oil spill	145 µg/L
Oil West site 21 days after oil spill	135 µg/L
Laboratory-contaminated seawater (undiluted)	550 µg/L

The chromatogram below was generated from the undiluted laboratory-contaminated seawater sample. The table that follows describes the distribution of hydrocarbon chains detected based on carbon chain length. Note that the height of the peaks are not a direct measure of the concentration of mineral oil in seawater, but indicates carbon fraction distribution of the crude oil sample.



Label	Carbon chain range	Percentage of sample
1	C10—C19	30%
2	C19—C29	51%
3	C29—C35	17%
4	C35—C40	2%

Supplement 2

Table S1 – The single and multi-parameter models used to determine the effect of site and oil contamination on larval survival and settlement. *A priori* model structures were compared as follows: a single-parameter (null) model for which survival/settlement was equal among sites was contrasted against a two-parameter model of oil spill sites vs. non-oil spill sites using a likelihood ratio test (LRT). Following the results of the LRT, the two-parameter model was determined to be a statistically significant improvement in maximum likelihood relative to the one-parameter, null model if the likelihood value was different by 1.96 or greater (corresponding to one half of the X^2 critical value with one degree of freedom and a level of significance of 0.05). The *a priori* model with the better statistical fit is presented in italics.

Following this hypothesis-driven model comparison, a *post hoc*, directed search of all other permutations was used to identify the most parsimonious model fit (when graphed, these results are used to identify the *post hoc* groupings). An “NA” indicates that no model was a better fit than one or both of the *a priori* models. The most parsimonious model is bolded if it was a statistically better fit than all models tested (the groupings associated with the model are indicated by letters on Figs. 3 & 4). If the most parsimonious model could not be statistically differentiated from other models but was a better fit than both *a priori* models, the best-fit model is reported but not bolded (and groupings are not shown on Figs. 3 & 4).

Model	Params.	a_{SB}	a_{BS}	a_{VB}	a_{OE}	a_{OM}	a_{OW}	ML
All sites are equal	1	a_o	a_o	a_o	a_o	a_o	a_o	
Oiled vs. Non-oiled sites	2	a_1	a_1	a_1	a_2	a_2	a_2	
Most parsimonious	?	$a_?$	$a_?$	$a_?$	$a_?$	$a_?$	$a_?$	

Table S2 – Parameter estimation of the probability that *A. humilis* larvae survived the six-day exposure period. The model parameters (e.g., a_{sb}) and maximum likelihood (ML) of the two *a priori* models and the most parsimonious model (if applicable) are reported below and graphical representations of the data are presented in Figure 3C.

Model	Params.	a_{SB}	a_{BS}	a_{VB}	a_{OE}	a_{OM}	a_{OW}	ML
All sites are equal	1	0.93	0.93	0.93	0.93	0.93	0.93	80.4
Oiled vs. Non-oiled sites	2	0.92	0.92	0.92	0.94	0.94	0.94	79.8
Most parsimonious	NA							

Table S3 – Parameter estimation of the probability that *A. humilis* larvae survived the ten-day post-exposure period. Graphical representations of the data are presented in Figure 3D.

Model	Params.	a_{SB}	a_{BS}	a_{VB}	a_{OE}	a_{OM}	a_{OW}	ML
All sites are equal	1	0.91	0.91	0.91	0.91	0.91	0.91	59.2
Oiled vs. Non-oiled sites	2	0.88	0.88	0.88	0.93	0.93	0.93	57.6
Most parsimonious	2	0.93	0.82	0.82	0.82	0.82	0.82	55.6

Table S4 – Parameter estimation of the probability that *A. humilis* larvae settled by the end of the ten-day post-exposure period. Graphical representations of the data are presented in Figure 4B.

Model	Params.	a_{SB}	a_{BS}	a_{VB}	a_{OE}	a_{OM}	a_{OW}	ML
All sites are equal	1	0.23	0.23	0.23	0.23	0.23	0.23	131.5
Oiled vs. Non-oiled sites	2	0.29	0.29	0.29	0.18	0.18	0.18	127.1
Most parsimonious	2	0.38	0.17	0.38	0.17	0.17	0.17	118.3

Table S5 – Parameter estimation of the probability that *A. humilis* larvae settled of those that survived the ten-day post-exposure period. No graphical representation is shown.

Model	Params.	a_{SB}	a_{BS}	a_{VB}	a_{OE}	a_{OM}	a_{OW}	ML
All sites are equal	1	0.23	0.23	0.23	0.23	0.23	0.23	119.2
Oiled vs. Non-oiled sites	2	0.28	0.28	0.28	0.19	0.19	0.19	116.9
Most parsimonious	4	0.52	0.01	0.33	0.19	0.19	0.19	91.3

Table S6 – Parameter estimation of the probability that *O. faveolata* larvae survived the six-day exposure period. Graphical representations of the data are presented in Figure 3A.

Model	Params.	a _{SB}	a _{BS}	a _{VB}	a _{OE}	a _{OM}	a _{OW}	ML
All sites are equal	1	0.89	0.89	0.89	0.89	0.89	0.89	96.2
Oiled vs. Non-oiled sites	2	0.93	0.93	0.93	0.84	0.84	0.84	87.2
Most parsimonious	3	0.96	0.88	0.96	0.88	0.88	0.78	72.7

Table S7 – Parameter estimation of the probability that *O. faveolata* larvae survived the ten-day post-exposure period. Graphical representations of the data are presented in Figure 3B.

Model	Params.	a _{SB}	a _{BS}	a _{VB}	a _{OE}	a _{OM}	a _{OW}	ML
All sites are equal	1	0.83	0.83	0.83	0.83	0.83	0.83	81.2
Oiled vs. Non-oiled sites	2	0.95	0.95	0.95	0.71	0.71	0.71	56.3
Most parsimonious	3	0.97	0.88	0.97	0.63	0.63	0.88	45.9

Table S8 – Parameter estimation of the probability that *O. faveolata* larvae settled by the end of the ten-day post-exposure period. Graphical representations of the data are presented in Figure 4A.

Model	Params.	a _{SB}	a _{BS}	a _{VB}	a _{OE}	a _{OM}	a _{OW}	ML
All sites are equal	1	0.22	0.22	0.22	0.22	0.22	0.22	103.2
Oiled vs. Non-oiled sites	2	0.39	0.39	0.39	0.06	0.06	0.06	64.5
Most parsimonious	3	0.55	0.30	0.30	0.06	0.06	0.06	58.4

Table S9 – Parameter estimation of the probability that *O. faveolata* settled of those that survived the ten-day post-exposure period. No graphical representation is shown.

Model	Params.	a _{SB}	a _{BS}	a _{VB}	a _{OE}	a _{OM}	a _{OW}	ML
All sites are equal	1	0.27	0.27	0.27	0.27	0.27	0.27	91.2
Oiled vs. Non-oiled sites	2	0.41	0.41	0.41	0.08	0.08	0.08	63.7
Most parsimonious	3	0.55	0.33	0.33	0.08	0.08	0.08	59.0

Table S10 – A comparison of the relative fit of a single mean survival/settlement across all oil contamination concentrations (null) versus a logistic fit of survival/settlement in response to the degree of contamination. Reported are the model parameters (a_0 and a_1), maximum likelihood values, and pseudo- R^2 values (logistic fit). Statistically distinct and better fitting models are italicized ($\alpha = 0.05$). “6-day exposure” refers to the end of the 6-day period during which larvae were exposed to laboratory-generated, oil-contaminated seawater (Fig. 3E & G). “10-day post-exposure” refers to the end of the 10-day period during which larvae were exposed to non-contaminated seawater, which immediately followed the 6-day exposure to contaminated seawater (Fig. 3F & H, Fig. 4C & D).

Experiment	Species	Model	a_0	a_1	ML	R^2
6-day exposure: Survival	<i>A. humilis</i>	Null	2.86	--	28.5	--
		<i>Logistic</i>	2.86	0.003	28.5	0.00
	<i>O. faveolata</i>	Null	3.27	--	29.2	--
		<i>Logistic</i>	3.03	-0.15	28.9	0.01
10-day post- exposure: Survival	<i>A. humilis</i>	Null	1.80	--	46.1	--
		<i>Logistic</i>	<i>0.91</i>	<i>-0.69</i>	<i>36.6</i>	<i>0.21</i>
	<i>O. faveolata</i>	Null	1.88	--	50.1	--
		<i>Logistic</i>	<i>0.82</i>	<i>-0.91</i>	<i>30.9</i>	<i>0.38</i>
10-day post- exposure: Settlement	<i>A. humilis</i>	Null	-3.67	--	15.1	--
		<i>Logistic</i>	<i>-4.71</i>	<i>-0.48</i>	<i>13.8</i>	<i>0.09</i>
	<i>O. faveolata</i>	Null	-1.67	--	53.9	--
		<i>Logistic</i>	<i>-3.28</i>	<i>-0.72</i>	<i>32.0</i>	<i>0.41</i>
10-day post- exposure: Settlement of survivors	<i>A. humilis</i>	Null	-3.51	--	15.0	--
		<i>Logistic</i>	<i>-4.46</i>	<i>-0.42</i>	<i>14.1</i>	<i>0.06</i>
	<i>O. faveolata</i>	Null	-1.50	--	48.0	--
		<i>Logistic</i>	<i>-3.03</i>	<i>-0.66</i>	<i>30.8</i>	<i>0.36</i>