

## **Interacting environmental stressors modulate reproductive output and larval performance in a tropical intertidal barnacle**

**Florian Freuchet, Réjean Tremblay, Augusto A. V. Flores\***

\*Corresponding author: guca@usp.br

*Marine Ecology Progress 532: 161–175 (2015)*

---

### **Supplement.**

**Lipid characterization of the experimental diet** - The quality of the food we used in these experiments was assessed by a detailed examination of its lipid contents. For dry weight analyses, we first removed any particles larger than 80 µm by sieving, and then filtered seawater through 25 mm GF/F filters (Whatman, Maidstone, UK, pre-combusted at 450°C during 2 hours). Filters were then dried at 70°C for 24h and weighed. In order to obtain lipid profiles, we first extracted lipids in dichloromethane–methanol, using the modified Folch procedure (Folch et al. 1957) described in Parrish (1987). For the characterization of food, fatty acids (FA) were extracted through direct transesterification (Lepage & Roy 1984). All fatty acid methyl esters (FAMES) were prepared as described in Lepage & Roy (1984) and analyzed in MSMS scan mode (ionic range: 60–650 m/z) on a Polaris Q ion trap coupled to a Trace GC (Thermo Finnigan, Mississauga, ON, CA) equipped with a Valcobond VB-5 capillary column (VICI Valco Instruments Co. Inc., Broakville, ON, CA); data were treated using Xcalibur v.1.3 software (Thermo Scientific, Mississauga, ON, CA). FAMES were identified by comparing retention times with known standards (Supelco 37 Component FAME Mix and menhaden oil; Supelco Inc., Belfonte, PA, USA). Methyl esters of fatty acids were prepared using toluene. Every sample was purified using a solution of hexane-acetate (C<sub>6</sub>H<sub>14</sub>/CH<sub>3</sub>COO<sup>-</sup>; 1:1), and analyzed by gas chromatography coupled with a mass spectrometer (GC-MS) (Toupoint et al. 2012). The sum of all identified fatty acids corresponded to the total lipids content in larvae and adults.

Table S1. Total fatty acid composition in the experimental diet (algal mix). Fatty acids accounting for less than 2%, both categories combined, were removed. SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; EFA: essential fatty acid; AA: arachidonic acid (20:4w6); EPA: eicosapentaenoic acid (20:5w3); DHA: docosahexaenoic acid (22:6w3).

<i>Fatty acid</i>	mean ( $\pm$ SE)
14:0	11.5 $\pm$ 0.3
16:0	29.6 $\pm$ 0.6
18:0	1.1 $\pm$ 0.1
$\Sigma$ SFA	43.0 $\pm$ 0.8
16:1w7	21.1 $\pm$ 0.2
16:1w5	0.0 $\pm$ 0.0
18:1w9	3.4 $\pm$ 0.0
18:1w7	1.9 $\pm$ 0.1
$\Sigma$ MUFA	26.6 $\pm$ 0.3
18:2w6	3.4 $\pm$ 0.1
18:3w3	0.4 $\pm$ 0.0
20:4w6 (AA)	3.1 $\pm$ 0.0
20:5w3 (EPA)	15.9 $\pm$ 0.3
22:6w3 (DHA)	7.7 $\pm$ 0.3
$\Sigma$ PUFA	30.5 $\pm$ 0.6
$\Sigma$ EFA	26.6 $\pm$ 0.6

#### LITERATURE CITED

- Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497–509
- Lepage G, Roy CC (1984) Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J Lipid Res* 25: 1391–1396
- Toupoint N, Gilmore-Solomon L, Bourque F, Myrand B, Pernet F, Olivier F, Tremblay R (2012) Match/mismatch between the *Mytilus edulis* larval supply and seston quality: effect on recruitment. *Ecology* 93: 1922–1934

Table S2. Fatty acid composition (mean percentage  $\pm$  SE) for neutral and polar lipids in adults collected in the field before beginning the experiment ( $T_0$ ), and both in adults and nauplii after acclimation ( $T_a$ ; food treatments pooled), and over exposure to chronic stress (TS1-TS3, food and stress treatments pooled). Fatty acids accounting for less than 2%, all categories combined, were removed. Abbreviations as in Table S1.

	Adults $T_0$		Adults $T_a$		Adults TS1-TS3		Nauplii $T_a$		Nauplii TS1-TS3	
	<i>Neutral</i>	<i>Polar</i>	<i>Neutral</i>	<i>Polar</i>	<i>Neutral</i>	<i>Polar</i>	<i>Neutral</i>	<i>Polar</i>	<i>Neutral</i>	<i>Polar</i>
<i>Fatty acid</i>										
14:0	2.2 $\pm$ 0.5	2.1 $\pm$ 0.5	2.4 $\pm$ 0.328	0.3 $\pm$ 0.1	2.1 $\pm$ 0.3	0.1 $\pm$ 0.0	0.5 $\pm$ 0.2	0.4 $\pm$ 0.2	0.9 $\pm$ 0.2	0.9 $\pm$ 0.2
16:0	21.0 $\pm$ 1.3	20.9 $\pm$ 1.2	24.4 $\pm$ 1.688	18.4 $\pm$ 1.9	22.0 $\pm$ 7.1	13.4 $\pm$ 0.9	13.9 $\pm$ 1.8	14.3 $\pm$ 1.6	23.6 $\pm$ 1.7	23.4 $\pm$ 1.7
18:0	11.2 $\pm$ 0.9	11.4 $\pm$ 0.9	11.4 $\pm$ 1.318	18.0 $\pm$ 1.7	11.0 $\pm$ 5.9	15.1 $\pm$ 0.9	23.9 $\pm$ 2.8	45.4 $\pm$ 4.5	38.3 $\pm$ 2.8	38.5 $\pm$ 2.8
20:0	0.8 $\pm$ 0.0	0.8 $\pm$ 0.0	1.3 $\pm$ 0.179	1.0 $\pm$ 0.1	1.2 $\pm$ 1.7	1.0 $\pm$ 0.1	3.0 $\pm$ 0.5	3.6 $\pm$ 0.5	2.3 $\pm$ 0.3	2.3 $\pm$ 0.3
22:0	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.2 $\pm$ 0.213	1.2 $\pm$ 0.2	1.3 $\pm$ 2.1	2.0 $\pm$ 0.3	4.1 $\pm$ 1.2	2.2 $\pm$ 0.5	1.9 $\pm$ 0.3	1.9 $\pm$ 0.3
ΣSFA	40.1 $\pm$ 1.3	40.1 $\pm$ 1.2	46.0 $\pm$ 3.46	42.7 $\pm$ 3.9	42.0 $\pm$ 6.8	35.2 $\pm$ 2.0	50.0 $\pm$ 3.0	73.3 $\pm$ 3.0	72.5 $\pm$ 2.6	72.5 $\pm$ 2.6
16:1w7	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2	5.7 $\pm$ 0.242	2.2 $\pm$ 0.1	4.5 $\pm$ 0.9	2.1 $\pm$ 0.1	3.2 $\pm$ 0.8	2.0 $\pm$ 0.3	1.3 $\pm$ 0.3	1.4 $\pm$ 0.3
16:1w5	5.4 $\pm$ 0.7	5.3 $\pm$ 0.7	0.4 $\pm$ 0.095	0.1 $\pm$ 0.0	0.2 $\pm$ 1.2	0.1 $\pm$ 0.0	1.8 $\pm$ 0.5	1.1 $\pm$ 0.2	1.7 $\pm$ 0.2	1.6 $\pm$ 0.2
17:1w7	0.6 $\pm$ 0.2	0.7 $\pm$ 0.2	0.3 $\pm$ 0.104	1.1 $\pm$ 0.2	0.2 $\pm$ 1.2	1.5 $\pm$ 0.1	4.2 $\pm$ 0.8	2.0 $\pm$ 0.5	0.9 $\pm$ 0.2	0.9 $\pm$ 0.2
18:1w9	7.6 $\pm$ 0.5	7.6 $\pm$ 0.5	2.5 $\pm$ 0.127	2.6 $\pm$ 0.1	2.2 $\pm$ 2.1	2.2 $\pm$ 0.1	3.8 $\pm$ 0.9	2.8 $\pm$ 0.3	9.0 $\pm$ 1.0	9.0 $\pm$ 1.0
18:1w7	2.7 $\pm$ 0.1	2.7 $\pm$ 0.1	5.9 $\pm$ 0.347	7.4 $\pm$ 0.3	5.6 $\pm$ 1.5	7.1 $\pm$ 0.3	13.2 $\pm$ 1.9	6.8 $\pm$ 1.0	2.0 $\pm$ 0.3	2.0 $\pm$ 0.3
20:1w11	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.7 $\pm$ 0.069	0.7 $\pm$ 0.1	0.5 $\pm$ 0.7	0.7 $\pm$ 0.1	1.3 $\pm$ 0.7	0.8 $\pm$ 0.5	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1
20:1w9	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2	0.7 $\pm$ 0.178	0.3 $\pm$ 0.1	0.3 $\pm$ 0.9	0.2 $\pm$ 0.1	1.6 $\pm$ 0.7	1.1 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2
ΣMUFA	18.8 $\pm$ 0.5	18.9 $\pm$ 0.5	18.7 $\pm$ 1.604	18.0 $\pm$ 2.6	15.2 $\pm$ 6.1	15.2 $\pm$ 0.6	17.5 $\pm$ 2.8	18.2 $\pm$ 4.1	17.4 $\pm$ 1.5	17.5 $\pm$ 1.5
18:2w6	1.2 $\pm$ 0.1	1.2 $\pm$ 0.0	1.4 $\pm$ 0.187	1.1 $\pm$ 0.1	1.3 $\pm$ 0.8	1.2 $\pm$ 0.1	3.9 $\pm$ 0.5	1.6 $\pm$ 0.5	3.0 $\pm$ 0.5	3.1 $\pm$ 0.5
18:3w3	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.2 $\pm$ 0.252	0.7 $\pm$ 0.2	2.1 $\pm$ 1.0	1.3 $\pm$ 0.3	1.4 $\pm$ 0.6	0.6 $\pm$ 0.3	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1
20:4w6 (AA)	2.1 $\pm$ 0.2	2.1 $\pm$ 0.2	1.7 $\pm$ 0.126	2.4 $\pm$ 0.2	1.9 $\pm$ 0.4	2.9 $\pm$ 0.2	1.0 $\pm$ 0.4	0.0 $\pm$ 0.0	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1
20:5w3 (EPA)	12.8 $\pm$ 0.7	12.9 $\pm$ 0.6	10.4 $\pm$ 1.04	14.0 $\pm$ 0.9	11.2 $\pm$ 0.4	15.6 $\pm$ 0.7	2.8 $\pm$ 0.5	0.9 $\pm$ 0.3	1.4 $\pm$ 0.2	1.4 $\pm$ 0.2
22:6w3 (DHA)	22.3 $\pm$ 1.0	22.2 $\pm$ 0.9	17.9 $\pm$ 2.798	20.1 $\pm$ 3.4	24.0 $\pm$ 0.6	27.5 $\pm$ 1.2	4.1 $\pm$ 0.8	2.2 $\pm$ 0.4	2.0 $\pm$ 0.3	2.0 $\pm$ 0.3
ΣPUFA	41.1 $\pm$ 1.4	41.0 $\pm$ 1.4	35.3 $\pm$ 3.57	39.3 $\pm$ 4.0	42.9 $\pm$ 3.1	49.6 $\pm$ 2.0	13.5 $\pm$ 1.8	14.7 $\pm$ 2.8	10.1 $\pm$ 1.5	10.0 $\pm$ 1.5
ΣEFA	37.2 $\pm$ 1.5	37.2 $\pm$ 1.5	30.0 $\pm$ 3.733	36.5 $\pm$ 3.9	37.1 $\pm$ 1.1	46.1 $\pm$ 2.0	5.7 $\pm$ 0.8	8.0 $\pm$ 1.6	4.0 $\pm$ 0.6	4.0 $\pm$ 0.6