**Food availability, but not tidal emersion, influences the combined effects of ocean acidification and warming on oyster physiological performance**

Coline Caillona,1, Elodie Fleurya, Carole Di Poib, Frédéric Gazeauc, Fabrice Perneta,\*

a Ifremer, Univ Brest, CNRS, IRD, Laboratoire des Sciences de l’Environnement Marin (LEMAR), 29280 Plouzané, France

b Ifremer, Univ Brest, CNRS, IRD, Laboratoire des Sciences de l’Environnement Marin (LEMAR), 29840 Argenton-en-Landunvez, France

c Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche (LOV), 06230 Villefranche-sur-Mer, France

1 Present address: Department of Biological Sciences, University of Rhode Island, Kingston, Rhode Island 02881, USA

Corresponding author.

E-mail address: fabrice.pernet@ifremer.fr (F. Pernet).

**Supplementary data**

 

**Fig. S1.** Ingestion rate of *Crassostrea gigas* oysters under different temperature and pH conditions, tidal treatment and food level during the 81-day acclimation period, prior to viral exposure. Ingestion rates were averaged over the whole acclimation period (n = 72) and reported over a day considering the number of hours of immersion. ‘Subtidal’ and ‘Intertidal’ indicate the tidal treatment in which the oysters were placed (subtidal: constant immersion; intertidal: 8.5 h immersion and 3.5 h emersion). Data correspond to means ± s.e.m. (n = 3 tanks). Different letters represent significant differences (P < 0.05) between conditions.

**Table S1.** Summary of results from generalized linear models to assess the effect of ’Condition’ (6 levels) on growth-related parameters, ingestion and oxygen consumption rates, energy reserves and membrane fatty acids of *Crassostrea gigas* oysters during the 81-day acclimation period. Significant P-values (P < 0.05) are indicated in bold.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Effect | SS | df | F | P | Effect | SS | df | F | P |
| **Shell length** |  |  |  |  | **TAG:ST ratio** |  |  |  |  |
| Condition | 361.2 | 5 | 76.7 | **<0.001** | Condition | 25.2 | 5 | 7.3 | **0.002** |
| Residuals | 11.3 | 12 |  |  | Residuals | 8.2 | 12 |  |  |
| **Total body mass** |  |  |  |  | **Total carbohydrate content** |  |  |  |  |
| Condition | 35.0 | 5 | 124.6 | **<0.001** | Condition | 1259.6 | 5 | 19.2 | **<0.001** |
| Residuals | 0.7 | 12 |  |  | Residuals | 157.6 | 12 |  |  |
| **Shell mass** |  |  |  |  | **Total protein content** |  |  |  |  |
| Condition | 12.0 | 5 | 122.0 | **<0.001** | Condition | 488.8 | 5 | 4.5 | **0.015** |
| Residuals | 0.2 | 12 |  |  | Residuals | 261.5 | 12 |  |  |
| **Dry flesh mass** |  |  |  |  | **Unsaturation index** |  |  |  |  |
| Condition | 0.2 | 5 | 86.9 | **<0.001** | Condition | 3776.8 | 5 | 11.4 | **<0.001** |
| Residuals | 0.0 | 12 |  |  | Residuals | 796.6 | 12 |  |  |
| **Growth rate (length)** |  |  |  |  | **Arachidonic acid (ARA)** |  |  |  |  |
| Condition | 0.1 | 5 | 77.4 | **<0.001** | Condition | 0.0 | 5 | 1.3 | 0.323 |
| Residuals | 0.0 | 12 |  |  | Residuals | 0.0 | 12 |  |  |
| **Growth rate (mass)** |  |  |  |  | **Docosahexaenoic acid (DHA)** |  |  |  |  |
| Condition | 5334.6 | 5 | 124.6 | **<0.001** | Condition | 9.5 | 5 | 4.8 | **0.012** |
| Residuals | 102.7 | 12 |  |  | Residuals | 4.7 | 12 |  |  |
| **Ingestion rate** |  |  |  |  | **Eicosapentaenoic acid (EPA)** |  |  |  |  |
| Condition | 0.6 | 5 | 82.5 | **<0.001** | Condition | 94.9 | 5 | 15.7 | **<0.001** |
| Residuals | 0.0 | 12 |  |  | Residuals | 14.6 | 12 |  |  |
| **Oxygen consumption rate** |  |  |  |  | **Non-methylene-interrupted FA** |  |  |  |  |
| Condition | 1.9 | 5 | 8.4 | **0.001** | Condition | 10.4 | 5 | 7.8 | **0.002** |
| Residuals | 0.5 | 12 |  |  | Residuals | 3.2 | 12 |  |  |

TAG:ST, triacylglycerol:sterol; FA, fatty acid.

**Table S2.** Composition of polar fatty acids (mass %) in *Crassostrea gigas* oysters under different temperature and pH conditions, tidal treatment and food level at the end of the 81-day acclimation period, prior to viral exposure. Only fatty acids (FA) accounting for >1 % of total polar FA in at least one condition are shown. Data correspond to means ± s.e.m. (n = 3 tanks).

|  |  |  |  |
| --- | --- | --- | --- |
|  | Current temperature and pH conditions |  | Future temperature and pH conditions |
|  | Subtidal, HF | Intertidal, HF | Intertidal, LF |  | Subtidal, HF | Intertidal, HF | Intertidal, LF |
| 14:0 | 3.1±0.0 | 2.7±0.2 | 3.4±0.2 |  | 3.0±0.0 | 3.2±0.2 | 3.4±0.2 |
| 16:0 | 15.3±0.4 | 13.5±0.7 | 17.0±0.7 |  | 16.4±0.8 | 15.1±0.9 | 18.5±0.8 |
| 17:0 | 0.7±0.0 | 0.6±0.1 | 0.9±0.0 |  | 0.8±0.1 | 0.7±0.1 | 1.0±0.1 |
| 18:0 | 6.7±0.1 | 6.0±0.7 | 8.3±0.2 |  | 7.2±0.5 | 6.4±0.1 | 8.6±0.3 |
| **Σ SFA** | **26.8±0.4** | **23.6±1.4** | **30.4±0.9** |  | **28.4±1.4** | **26.3±1.4** | **32.5±1.0** |
| 16:1n-7 | 1.9±0.0 | 2.0±0.0 | 1.9±0.1 |  | 2.0±0.1 | 2.1±0.1 | 1.9±0.1 |
| 18:1n-7 | 7.2±0.1 | 7.0±0.1 | 6.6±0.1 |  | 6.7±0.1 | 7.1±0.0 | 6.0±0.1 |
| 20:1n-11 | 2.5±0.1 | 2.5±0.1 | 2.2±0.1 |  | 2.8±0.1 | 2.9±0.1 | 2.8±0.1 |
| 20:1n-9 | 0.3±0.0 | 0.3±0.0 | 0.3±0.0 |  | 0.3±0.0 | 0.3±0.0 | 0.3±0.0 |
| 20:1n-7 | 5.3±0.1 | 5.3±0.1 | 4.9±0.2 |  | 4.7±0.1 | 4.9±0.1 | 4.5±0.2 |
| **Σ MUFA** | **18.5±0.0** | **18.6±0.1** | **16.7±0.3** |  | **18.3±0.2** | **18.9±0.5** | **16.5±0.4** |
| 18:2n-6 | 1.3±0.1 | 1.4±0.1 | 1.1±0.0 |  | 1.3±0.1 | 1.6±0.1 | 1.1±0.0 |
| 18:3n-3 | 0.8±0.0 | 0.6±0.0 | 0.8±0.1 |  | 0.7±0.0 | 0.8±0.1 | 0.9±0.1 |
| 20:4n-6 (ARA) | 1.0±0.0 | 0.9±0.0 | 0.9±0.0 |  | 1.0±0.0 | 0.9±0.0 | 0.9±0.0 |
| 20:5n-3 (EPA) | 18.2±0.1 | 20.3±1.1 | 16.0±0.7 |  | 16.0±0.6 | 17.6±0.4 | 12.8±0.4 |
| 22:2n-6 | 0.0±0.0 | 0.3±0.1 | 0.3±0.3 |  | 0.2±0.2 | 0.0±0.0 | 0.8±0.4 |
| 22:5n-6 | 2.1±0.0 | 1.0±0.5 | 3.3±0.4 |  | 2.3±0.4 | 1.7±0.3 | 4.0±0.4 |
| 22:5n-3 | 1.0±0.0 | 1.1±0.0 | 1.1±0.0 |  | 1.1±0.0 | 1.1±0.0 | 1.1±0.0 |
| 22:6n-3 (DHA) | 7.4±0.1 | 8.1±0.6 | 6.5±0.3 |  | 7.0±0.3 | 7.7±0.4 | 6.0±0.3 |
| **Σ PUFA** | **62.5±0.1** | **65.7±1.7** | **56.2±1.7** |  | **58.8±1.7** | **62.0±1.4** | **53.0±0.7** |
| 18:0DMA | 6.6±0.6 | 7.6±0.6 | 8.8±0.5 |  | 8.8±0.6 | 7.8±0.1 | 9.8±0.9 |
| 20:1DMA | 1.4±0.1 | 1.7±0.1 | 1.3±0.0 |  | 1.3±0.1 | 1.2±0.1 | 1.1±0.2 |
| **Σ DMA** | **8.8±0.6** | **10.1±0.5** | **10.9±0.6** |  | **10.8±0.8** | **9.7±0.3** | **11.6±0.9** |
| 22:2i.j | 6.0±0.1 | 6.6±0.3 | 4.7±0.5 |  | 5.4±0.2 | 5.5±0.3 | 4.6±0.3 |
| **Σ NMI** | **7.0±0.1** | **7.6±0.3** | **5.7±0.4** |  | **6.5±0.3** | **6.6±0.3** | **5.4±0.3** |

HF, high food; LF, low food; SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; DMA, dimethyl acetals; NMI, non-methylene-interrupted FA.

**Table S3.** Levels of OsHV-1 DNA in *Crassostrea gigas* oysters acclimated to different temperature and pH conditions, tidal treatment and food level, and further exposed to viral pathogens (5 dpi). Summary of generalized linear model was performed on log-transformed (x+1) data. Data correspond to means ± s.e.m. (n = 3 tanks). Different letters represent significant differences (P < 0.05) between conditions.

|  |
| --- |
| (Condition, F=9.4, p<0.001) |
| Condition | OsHV-1 DNA (cp mg-1) | Post-hoc |
| Current, Subtidal, HF | 4.7 101 ± 4.7 101 | ab |
| Future, Subtidal, HF | 2.7 10-1 ± 1.4 10-1 | a |
| Current, Intertidal, HF | 1.2 104 ± 1.2 104 | bc |
| Future, Intertidal, HF | 6.4 100 ± 4.7 100 | ab |
| Current, Intertidal, LF | 9.0 104 ± 1.9 104 | c |
| Future, Intertidal, LF | 6.9 104 ± 3.7 104 | c |

HF, high food; LF, low food.