Electronic Supplementary Material

S1. LABORATORY ANALYSES

OsHV-1 DNA

A sub-sample of ground oyster tissues (50-100 mg of fine powder) was diluted in artificial seawater (1:4 m/v). Total DNA was then extracted using a QIAgen QIAamp tissue mini kit according to the manufacturer's protocol. The extracted DNA was stored at -20°C prior to pathogen detection and quantification by qPCR.

The detection and quantification of OsHV-1 DNA was carried out using a previously published real-time PCR protocol [1]. Briefly, this protocol used SYBR® Green chemistry with specific DPFor/DPRev primers targeting the region of the OsHV-1 genome predicted to encode a DNA polymerase catalytic subunit [2]. Amplification reactions were performed using an Mx30005P real-time PCR thermocycler sequence detector (Stratagene, La Jolla, CA, USA) with 96-microwell plates [1]. Data are expressed as the number of experimental units (pearl nets containing the sampled oysters) in which DNA of OsHV-1 was detected.

Energetic reserves

For lipid analyses, powder aliquots (150 mg) were placed in amber glass vials filled with 3 ml chloroform-methanol (2:1 v/v) for further extraction following Folch [3]. Extracts were concentrated and applied to SIII Chromarods (Iatron laboratories, Tokyo, Japan). Lipid classes were separated in a stepwise procedure using developing solvents of increasing polarity [4] to separate aliphatic hydrocarbons, sterol and wax esters, ketones, triacylglycerols, free fatty acids, free fatty alcohol, free sterols, diacylglycerols, acetone mobile polar lipids and phospholipids. Between each development, the Chromarods were partially scanned by flame ionization detection (FID) on an Iatroscan MK-VI (Iatron

Laboratories, Tokyo, Japan). For the purpose of this study, only triacylglycerol in whole tissues of oysters is presented.

For carbohydrate analyses, powder aliquots (100 mg) were placed in Eppendorf tubes containing 1.5 mL nanopure water, homogenised for ~30 s with a T10 basic ultra Turrax (IKA, Germany). A subsample of the diluted powder was mixed with a phenol solution (5% m/v) and 2.5 mL H_2SO_4 and incubated for twenty minutes [5]. This subsample was then placed in a spectrophotometer and its absorbance measured at 490 and 600 nm. Total carbohydrate concentration was calculated using a standard calibration curve.

Triacylglycerol and carbohydrate are expressed in mg g^{-1} dry mass of tissues. The relative variation of these energetic reserves was calculated between 6 and 16 April according to the following formula:

$\Delta_{reserve} = \frac{(X_{06April} - X_{16April})}{X_{06April}}$, where *X* represents triacylglycerol or carbohydrate.

Fatty acids

An aliquot of the lipid extraction was evaporated to dryness and lipids recovered with three 500 mL washings of $CHCl_3$-MeOH (98:2 v/v). The lipids were placed at the top of a silica gel microcolumn (30 × 5 mm internal diameter; Kieselgel; 70–230 mesh [Merck, Lyon, France]; previously heated to 450°C and deactivated with 5% water). The neutral lipids were eluted with 10 mL $CHCl_3$-MeOH mixture (98:2 v/v). The polar lipids were recovered with 15 mL methanol. A known amount of 23:0 fatty acid was added as an internal standard. Lipids were transesterified with 10 wt% boron trifluoride-methanol [6] and analysed according to the method described by Marty et al. [7]. The fatty acid methyl esters (FAME) were analysed in a gas chromatograph with an on-column injector, a DB-Wax (30 m × 0.25 mm; 0.25 µm film thickness) capillary column and a flame ionization detector. Hydrogen was used as the carrier gas. Only the fatty acids in the neutral lipids of the animals are presented here, since neutral

lipids reflect the fatty acid profiles of food consumed and could reveal information about trophic sources, whereas fatty acids in the polar lipids are less sensitive to dietary changes [8].

References

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