

Supplementary material of the manuscript “*Effect of carbonate chemistry manipulations on calcification, respiration, and excretion of a Mediterranean pteropod*”

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Supplementary Materials and Methods

Carbonate chemistry measurements

Seawater temperature and pH were measured daily in the experimental beakers at IAEA and before and after incubation at LOV. pH was measured using a Metrohm pH meter (826 pH mobile) with a glass electrode (Metrohm, electrode plus) calibrated on the total scale using Tris/HCl and 2-aminopyridine/HCl buffer solutions with a salinity of 38.0 (Dickson et al. 2007). A 100 mL seawater sample was taken for determination of A_T before and after the 20 h incubations at LOV and before addition of ^{45}Ca at the IAEA. Samples were filtered on GF/F, poisoned with HgCl_2 and stored in the dark pending measurement (within 2 months). A_T was determined potentiometrically using a Metrohm titrator (Titrand 80) and a glass electrode (Metrohm, electrode plus) calibrated as described above. Triplicate measurements were carried out on 25 mL sub-samples at 25°C and A_T was calculated as described by Dickson et al. (2007). Titrations of standard seawater provided by A. G. Dickson (batch 90) yielded A_T values within $0.9 \mu\text{mol kg}^{-1}$ of the nominal value (standard deviation = $1.3 \mu\text{mol kg}^{-1}$; $n = 5$). All parameters of the carbonate chemistry were determined from pH_T , A_T , temperature and salinity using the R package seacarb (Lavigne and Gattuso 2010). A Monte-Carlo procedure, by randomly using values of the measured parameters (temperature, pH and A_T) between the mean \pm SD over 1000 iterations, was used to estimate the propagation of errors to the computed parameters (C_T , pCO_2 , Ω_a and Ω_c).

Rates normalization, error propagation and statistics

At the end of the incubation period during LOV experiment, animals ($n = 20$) were collected, dried at 60°C during 24 h and the dry weight measured on a Cahn microbalance (± 0.1

μg). For the IAEA experiment, wet weights were measured on a Mettler Toledo balance (± 0.01 mg). Wet weights were converted to dry weights based on the linear relationship obtained from the wet and dry weights measurements of 63 pteropods (dry weight = 0.152 x wet weight) collected prior to the experimental period (data not shown). All physiological rates have been normalized by the dry weight of the incubated organisms.

As all estimated processes were based on average values of triplicate measurements (or slope ± SE of the linear fitted model in the case of respiration rates) that were corrected from “blank” variations (also in triplicates), the propagated standard deviations (SD) from the means were calculated as:

$$SD_{t+b} = \sqrt{SD_t^2 + SD_b^2}$$

Where SD_{t+b} is the propagated standard deviations and SD_t and SD_b are, respectively, the standard deviation obtained in the treatment and blank.

In order to relate the physiological rates to the carbonate chemistry parameters at which the organisms were exposed during the incubations, linear and non-linear regressions were performed and the significance of the slopes was tested using student's *t*-tests.

Supplementary References

Dickson, A. G., Sabine, C. L., and Christian, J. R. (Eds), Guide to best practices for CO₂ measurements. PICES Special Publication 3, 2007.

Lavigne, H., and Gattuso, J.-P., seacarb: seawater carbonate chemistry with R. R package version 2.3.3. <http://CRAN.Rproject.org/package=seacarb>, 2010.