The following supplement accompanies the article

The cost of emersion for the barnacle *Balanus glandula*

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Supplementary Methods

Section 1 – Set-up of Respirometry Trials

All trials took place in October - November 2016. The barnacles started each trial immersed in seawater for 30 minutes at 10°C. Three respirometry chambers containing barnacles, plus one control, were filled with seawater and placed into a 30-L water bath connected to a recirculating chiller (AC200-A25, ThermoFisher Scientific, Waltham, MA, USA) maintained at 10°C (Supplementary Fig. 1A). After 30 minutes, the water was siphoned out of the chambers, initiating the emersion period. After the oxygen sensors stabilized, we heated the barnacles at a rate of 10°C/hour until the target temperature was reached. Barnacles were held at the target temperature for the reminder of the emersion period. After five hours, the barnacles were transferred to an aquatic respirometry chamber, placed in a second water bath maintained at 10°C (SC100-A10 ThermoFisher) and monitored for 6 more hours.

Aerial respiration chambers consisted of 18 mL glass vials, filled with a layer of hot melt adhesive to reduce the volume to approximately 10 mL. We measured oxygen consumption with a fluorometric oxygen sensor (Fibox 4, PreSens Precision Sensing GmbH, Regensburg, Germany), recording % air saturation (%a.s.) every 10 seconds. The oxygen sensor spot (SP-PSt3-NAU, PreSens Precision Sensing GmbH, Regensburg, Germany) was attached to the exterior bottom of a glass shell vial (8mm OD), which was fit into one hole of a rubber stopper, capping the chamber. A P100 Temperature sensor (PreSens Precision Sensing GmbH, Regensburg, Germany) was fit into the second hole of the rubber stopper and connected to the Fibox 4 unit.

Aquatic respirometry was conducted with 0.22 μM filtered (GSWP02500 filter, MilliporeSigma, St. Louis, MO, USA) natural seawater, aerated at 10°C for 18 hrs before use. Aquatic respirometry chambers consisted of 55mm tall x 24mm ID cylindrical glass chambers fitted with two-holed glass stoppers (Loligo vertical chambers #CH20400, Loligo Systems, Viborg, Denmark). Aquatic chambers were filled with approximately 20mL of filtered seawater.

The barnacle rested on a polyethelene mesh platform, above an 8-mm glass-coated magnetic stir bar to keep the water well mixed throughout the trial (Supplementary Fig. 1B). We affixed an oxygen sensor spot (as described above) on the interior of each chamber near the base. The water bath contained four mini-stir plates (Cimarec 50088118, Thermofisher), and 4 miniature DC pumps (#PU10700 Loligo Systems, Viborg, Denmark), for periodic flushing with fresh seawater. We flushed the water in all four chambers for five minutes whenever the O_2 levels dropped below 80% of the initial reading in any chamber.

Section 2 – Calculations of Oxygen Consumption

All calculations were completed using the Presens Oxygen Calculator (v 2.2.6) and R Software (v 3.4.2). For all aerial data, we estimated the initial chamber pressure as the pressure that led to an initial oxygen reading of 100 %a.s. in the chamber. To control for pressure changes during the heating phase, we rescaled the %a.s. values by dividing each 10-second reading in the barnacle chambers by the control chamber. We converted %a.s. to μmol L^{-1} using the ideal gas law and the pressure and temperature of the room at the start of the emersion period, and multiplied by the volume of fluid in the chamber to convert to μmol O_2. We calculated the fluid volume from the mass of water that filled the chamber when the barnacle was present.

We excluded a six to ten minute period from the aquatic respiration data sets whenever the chambers were flushed with fresh seawater. We also removed any bad data identified by visual inspection of the time series and replaced them with linear interpolation. Finally, we calculated a rate of oxygen use for each five-minute period and then used each slope to calculate the total oxygen consumed during the five-minute period. For the aquatic trials, slopes were corrected by subtracting off the slope of the control vials.

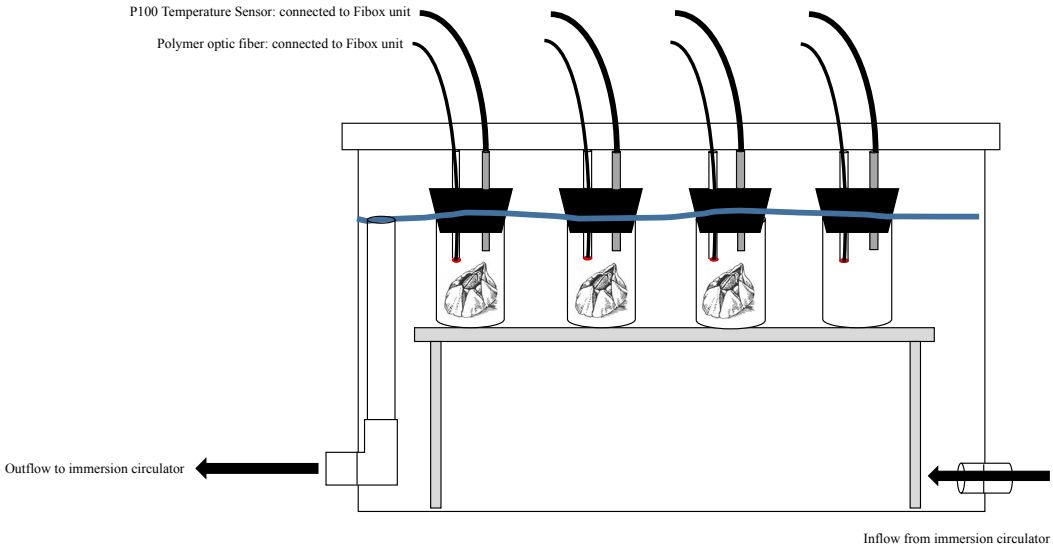
Section 3 – Ash weight protocol and determining size covariate

We calculated the dry tissue mass of the barnacles after all experimental trials were complete by drying them at 60°C for 48 hrs and then incinerating at 400°C for four hours. Dry mass and ash mass were recorded to the nearest 0.1mg, and ash free dry weights for individual barnacles were calculated from their difference.
For all statistical models, we initially compared models using operculum length and ash free dry mass as different metrics of size. In all cases, operculum length resulted in the strongest models, based on AIC scores.

Supplementary Figure

Fig. S1. Experimental set-up for aerial respiration trials (A) and aquatic respiration trials (B).

A.



B.