

## Supplementary Methods

### *High-Pressure Respirometry System (HPRS)*

The HPRS was housed in a temperature-controlled intermodal shipping container maintained at 15–17°C, approximating the median temperature experienced by *Alviniconcha in situ* [1, 2]. Surface seawater from the ship's metal-free seawater system was filtered through 0.2- $\mu\text{m}$  inline cartridge filters (Millipore Inc.), UV-treated, and then pumped into a 200 L polypropylene storage and cooling tank. Seawater was subsequently pumped from the storage tank into an acrylic gas equilibration column, where it was supplied with carbon dioxide, oxygen, and nitrogen gas using mass flow controllers (Aalborg Instruments and Controls, Inc.) to achieve concentrations of 4 mM, >250  $\mu\text{M}$ , and 400  $\mu\text{M}$ , respectively. Depending on the experiment, hydrogen or hydrogen sulfide gas was also supplied via mass flow controllers (Aalborg Instruments and Controls, Inc.) to achieve concentrations of approximately  $\sim 25$   $\mu\text{M}$  and  $\sim 120$   $\mu\text{M}$ , respectively. In addition, sodium nitrate ( $\text{NaNO}_3$ ) was added to achieve a final concentration of 40  $\mu\text{M}$ , which is comparable to deep-ocean water. The pH of the resulting input water was 6–7. Seawater from the equilibration column was then supplied to three high-pressure metering pumps (Lewa GmbH) equipped with titanium wetted parts. The pumps delivered fluid into three titanium high-pressure aquaria at a rate of 20 ml  $\text{min}^{-1}$ . Pressure was maintained via 316 stainless steel backpressure valves (StraVal Inc). Input water and effluent samples were taken every four hours for monitoring of pH using a handheld pH meter.

### *Reductant and oxygen concentrations*

Every 4 hours, 2 ml of input or effluent water was preserved with a 2 mM zinc acetate solution and frozen at  $-80^\circ\text{C}$  until analysis as in [3]. At the same timepoints, dissolved  $\text{H}_2$  was analyzed at sea by gas chromatography using a 5 $\text{\AA}$  molecular sieve packed column and thermal conductivity detection after headspace extraction in a gas-tight glass syringe. Oxygen concentrations were continuously monitored with the Oxy-4 SMA multi-channel optical oxygen meter and flow-through cell sensors FTC-SU-Pst3 (PreSens Precision Sensing, GmbH).

### *Empty and estimated controls*

Some loss of both hydrogen and sulfide was observed in the control aquaria devoid of snails, which equaled  $\sim 32$  to 37% of the reductant used by the snails. These loss rates in our controls are likely due to unexpected reactivity with metals within the control vessels or diffusional loss from the

system, and not due to depletion by free-living microbes, because dissolved oxygen concentrations did not change proportionally. Nevertheless, this loss is insufficient to account for the observed differences in holobiont uptake and, therefore, does not likely explain the observed differences in reductant utilization between each holobiont.

#### *Mitochondrial COI gene and transcriptomic sequencing*

Gill tissue pieces were excised from each animal and homogenized in TRIzol™ reagent (Thermo Fisher Scientific, Inc.) using a Tissue-Tearor (Biospec Products, Inc.). Additionally, gill tissue pieces from all individuals were preserved in RNALater™. All samples were stored at –80°C until RNA and DNA extraction with the Direct-zol RNA and Quick-DNA 96 Plus extraction kits (Zymo Research, Inc.), respectively. Species identities were confirmed by their mitochondrial *COI* gene signatures as in [4]. Transcriptomic sequencing libraries were created with the RNAtag-Seq approach [5] after rRNA depletion, and then sequenced with a 2 x 33–75 bp paired-end protocol on Next- and NovaSeq (Illumina, Inc.) instruments at the Broad Institute Microbial ‘Omics Core (Cambridge, MA, USA).

#### *Bioinformatic analyses*

Raw sequence reads were quality checked with FASTQC [6] and then trimmed with TRIMMOMATIC [7] using all built-in adaptor sequences and the following clipping settings: ILLUMINACLIP:Illumina.fa:2:30:10 SLIDINGWINDOW:4:20 LEADING:5 TRAILING:5 MINLEN:25. To remove potential sequence contaminants the trimmed reads were aligned against the human and PhiX genomes with BOWTIE2 [8]. All unmapped paired-end reads were extracted with SEQTK (<https://github.com/lh3/seqtk>). Ribosomal sequence removal in SORTMERA [9] was done with default settings except that we excluded paired-end reads where only one mate aligned to the rRNA databases (i.e., --paired\_in option). BBSPLIT (<https://sourceforge.net/projects/bbmap/>) was applied to sort host and symbiont sequences by mapping the reads against draft genomes of the three *campylobacterial* and *gammaproteobacterial Alviniconcha* symbiont phylotypes [10]. Reads with ambiguous alignments were discarded. Sequencing of *A. boucheti* #79 from Experiment 3 failed and this sample was therefore excluded from further analyses. Read quantification was done in SALMON [11] with the following parameters: -l ISR --meta --rangeFactorizationBins 4 --numBootstraps 1000 --seqBias --gcBias -s -u. Differential gene expression was analyzed with *DESeq2* in R [12, 13]. To account for batch effects and pseudo-replication we grouped all snail

specimens according to tank and included this factor into the model design. We further corrected for variability in animal weight and time to dissection after depressurization. P-values were adjusted for type I error with the Benjamini-Hochberg method [14] at a significance threshold of 0.05. Heatmaps, PCA plots and Likert graphs were generated in R using the *DESeq2*, *gplots* and *HH* packages [12, 15, 16].

## References

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