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RESEARCH ARTICLE

Physiological responses to self-induced burrowing and metabolic rate depression in the ocean quahog *Arctica islandica*

Julia Strahl[1,2], Thomas Brey[1], Eva E. R. Philipp[3], Gudrun Thorarinsdóttir[4], Natalie Fischer[5], Wiebke Wessels[6] and Doris Abele[1,*]

[1]Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany, [2]Center for Biomolecular Interactions Bremen, University of Bremen, Leobener Str., 28359 Bremen, Germany, [3]Institute of Clinical Molecular Biology, Christian-Albrechts University Kiel, Schittenhelmstrasse 12, 24105 Kiel, Germany, [4]Marine Research Institute, Skulagata 4, P.O. Box 1390, 121 Reykjavik, Iceland, [5]Hamburg University of Applied Sciences, Faculty of Life Sciences, FTZ-ALS, Lohbruegger Kirchstrasse 65, 21033 Hamburg, Germany and [6]University of Bremen, Leobener Str., 28359 Bremen, Germany

*Author for correspondence (Doris.Abele@awi.de)

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SUMMARY

Arctica islandica **is the longest-lived non-colonial animal found so far, and reaches individual ages of 150 years in the German Bight (GB) and more than 350 years around Iceland (IC). Frequent burrowing and physiological adjustments to low tissue oxygenation in the burrowed state are proposed to lower mitochondrial reactive oxygen species (ROS) formation. We investigated burrowing patterns and shell water partial pressure of oxygen (P_{O_2}) in experiments with live *A. islandica*. Furthermore, succinate accumulation and antioxidant defences were recorded in tissues of bivalves in the normoxic or metabolically downregulated state, as well as ROS formation in isolated gills exposed to normoxia, hypoxia and hypoxia/reoxygenation. IC bivalves burrowed more frequently and deeper in winter than in summer under *in situ* conditions, and both IC and GB bivalves remained burrowed for between 1 and 6 days in laboratory experiments. Shell water P_{O_2} was <5 kPa when bivalves were maintained in fully oxygenated seawater, and ventilation increased before animals entered the state of metabolic depression. Succinate did not accumulate upon spontaneous shell closure, although shell water P_{O_2} was 0 kPa for over 24 h. A ROS burst was absent in isolated gills during hypoxia/reoxygenation, and antioxidant enzyme activities were not enhanced in metabolically depressed clams compared with normally respiring clams. Postponing the onset of anaerobiosis in the burrowed state and under hypoxic exposure presumably limits the need for elevated recovery respiration upon surfacing and oxidative stress during reoxygenation.**

Key words: *Arctica islandica*, metabolic rate depression, burrowing, mantle cavity water P_{O_2}, adenylates, antioxidative enzymes, reactive oxygen species formation, succinate.

INTRODUCTION

Marine ectotherms with a wide latitudinal and geographical distribution often feature distinct population-specific maximum lifespan potentials (MLSPs). Longer MLSPs are frequently associated with cold adaptation when comparing with temperate populations of the same species or species of similar lifestyle (Brey, 1991; Brey et al., 1995; Ziuganov et al., 2000; Cailliet et al., 2001; La Mesa and Vacchi, 2001; Philipp et al., 2006). Extension of life expectancy in the cold presumes a delay in physiological ageing, which relates to lower metabolic rates and correspondingly reduced formation of mitochondrial reactive oxygen species (ROS) in cold environments (Philipp et al., 2005; Abele et al., 2009). The allometric hypothesis of ageing suggests that slower growth of water-breathing ectotherms in cold climates may delay ageing by increasing the time needed to reach a crucial maximum size (reviewed in Pauly, 2010). Generally body mass in these organisms tends to increase faster than the size of the organs involved in oxygen uptake (e.g. gills and mantle in bivalves). Thus, in marine invertebrates, oxygen uptake per body mass steadily diminishes as mass increases. The decreasing oxygen uptake with age is anticipated to accelerate ageing as marine invertebrates reach their near-maximum size and become energy limited (Pauly, 2010).

The ocean quahog, *Arctica islandica* (Linnaeus 1767), is a prominent example for marine invertebrate longevity (Ridgway and Richardson, 2010). Recorded MLSPs ($\mathrm{MLSP_{rec}}$) of *A. islandica* differ regionally between populations from sub-Arctic environments around Iceland (IC) with individual ages of more than 350 years (Schöne et al., 2005; Wanamaker et al., 2008), and from the German Bight (GB) with recorded individual MLSPs of 150 years (Witbaard and Klein, 1994; Witbaard et al., 1999; Epplé et al., 2006). Low salinity populations with conspicuously shorter life expectancies have established in brackish waters of the sub-Arctic White Sea with a $\mathrm{MLSP_{rec}}$ of 53 years and the temperate Baltic Sea with $\mathrm{MLSP_{rec}}$ of 40 years (Begum et al., 2010).

Arctica islandica are characterised by extremely low standard metabolic rates, slow but stable cell turnover and high antioxidant protection in cells, which are maintained constant with age (Abele et al., 2008; Begum et al., 2009; Strahl and Abele, 2010; Basova et al., in press). A characteristic behaviour, which might link lifestyle to mitochondrial ROS production, is the self-induced hypoxia and metabolic rate depression (MRD) described for *A. islandica* from an Irish Sea population (Taylor, 1976). Usually, the bivalves burrow directly beneath the sediment surface and mantle cavity partial pressure of oxygen (P_{O_2}) fluctuates between anoxia (0 kPa) and normoxia (21 kPa) from ventilation through short siphons, which are in contact with the overlying seawater (Abele et al., 2010). At irregular intervals ocean quahog burrow into deeper sediment horizons for periods of 1–7 days, close the shell and reduce the heart rate 10-fold compared with animals under normoxic conditions (Taylor, 1976). After several days of experimental anoxia in Baltic Sea *A. islandica*, caloric energy release is even lowered to 1% of fully aerobic rates (Oeschger, 1990). Thus, intermittent metabolic reduction could reduce lifetime ROS production in tissues of the ocean quahog and limit levels of oxidative damage compared with other bivalves [see also protein carbonyl comparison in Abele et al. (Abele et al., 2008)].

A problem with burrowing and self-induced hypoxia or anoxia is that this behaviour could cause oxidative stress when the bivalves come to the surface again, similar to hypoxia/reoxygenation injury in humans (Li and Jackson, 2002). However, in several hypoxia- and anoxia-tolerant invertebrates and ectothermic vertebrates that undergo aestivation, hibernation or freezing, MRD appears to trigger antioxidant functions, presumably to reduce hypoxia/reoxygenation injury during emergence from MRD (Hermes-Lima et al., 1998; Boutilier and St-Pierre, 2000; Hermes-Lima and Zenteno-Savín, 2002; Lushchak et al., 2005; Lushchak and Bagnyukova, 2007; Larade and Storey, 2009). The question is whether similar mechanisms control oxidative stress in surfacing *A. islandica*. Both MRD duration (in days) and MRD frequency, as well as the regulation of antioxidant capacities in *A. islandica* might depend on environmental temperature, salinity and/or food availability and thus differ between geographically distinct populations.

If metabolic depression is self-induced in *A. islandica*, it should be accompanied by energy-saving mechanisms in order to avoid considerable accumulation of acidic anaerobic metabolites (i.e. succinate, lactate, opines and short-chain organic acids). The ocean quahog should have evolved strategies to balance ATP supply and demand during MRD. Earlier works confirm that *A. islandica* features high capacities of anaerobic ATP production and that mitochondrial anaerobic pathways are used that maximise the yield of ATP formed per mol of H^+ during periods of oxygen deficiency (Livingstone et al., 1983; Oeschger, 1990; Oeschger and Storey, 1993; Strahl et al., 2011). This enables the bivalves to survive extended periods of hypoxia and anoxia caused by eutrophication in the North and Baltic Seas (Rosenberg et al., 1992; Oeschger and Storey, 1993; Diaz and Rosenberg, 1995).

In the present study, we studied the burrowing behaviour of IC and GB *A. islandica* of similar shell size and age in the laboratory at 10°C water temperature, well within the temperature tolerance window of both populations. The seasonality of burrowing was investigated *in situ* in an IC population. P_{O_2} measurements in the mantle cavity water of GB specimens were made with implanted needle optodes to investigate whether the animals expose their tissues to hypoxia/anoxia voluntarily in a normoxic environment. ROS production rates were determined in isolated gill tissue at normoxia, hypoxia and after hypoxia/reoxygenation. Tissue samples of GB and IC *A. islandica* from the burrowing experiments and the P_{O_2} measurements were analysed for antioxidant enzyme activities, total adenylates and citrate synthase (CS) activity, as well as for the accumulation of the anaerobic metabolite succinate.

MATERIALS AND METHODS

An overview of the experimental design of the study, comprising two biogeographically separate populations (IC and GB), a field experiment at IC, as well as different laboratory studies and physiological parameter comparisons from the two different populations, is given in Fig. 1.

Field study

In June 2003 and February 2004, the burrowing activity of *A. islandica* was determined by divers *in situ* in Eyjafjördur, North Iceland (65°47.86′N, 18°3.76′W; Fig. 2) at 10 m depth. The seafloor at the site of investigation consists of medium-grained sand of 0.25–0.49 mm grain size. Seawater temperatures were not recorded during the field study, but mean monthly surface water temperatures in Eyjafjördur between 1987 and 2000 ranged between 7.5°C in June and 1.5°C in February (Jónasson et al., 2004). Mean phytoplankton concentrations, measured in the years 1992 and 1993 (Kaasa and Gudmundsson, 1994) at a nearby locality (2.5 km away), were $0.03\pm0.01\,\mathrm{mg\,chl\,m^{-3}}$ in February and $0.9\pm0.4\,\mathrm{mg\,chl\,m^{-3}}$ in June. On each sampling date, divers positioned 1 m × 1 m frames on the seabed and individually collected all clams of >10 mm shell length using an underwater suction sampler. *Arctica islandica* has two short siphons, and its burrow openings appear as paired cylindrical holes. When the siphon openings were visible at the sediment surface, the burrowing depth was considered to be 0 cm. In deeper burrowed clams, where siphons were invisible, the depth of burial was measured with a ruler to the nearest 0.5 cm and was recorded as the distance from the seafloor surface to the uppermost part of the clam. Ocean quahog were encountered at depths as deep as 12 cm. Shell height of investigated bivalves ranged from 19 mm to 91 mm in June 2003 and from 7 mm to 92 mm in February 2004.

Bivalve collection and maintenance

Arctica islandica were collected in May 2008 at Helgoland ‘Tiefe Rinne’ in the GB (54°09.05′N, 07°52.06′E; Fig. 2) at 40–45 m water depth, using a trawl net. Surface water temperature was 12°C. In August 2008 *A. islandica* were collected northeast of IC (66°01.44′N, 14°50.91′W; Fig. 2) at 8–15 m water depth at a surface water temperature of 9°C. Bivalves from the GB and IC were transported in cooled containers to the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. *Arctica islandica* were acclimated for two weeks at 10°C and 33 PSU (practical salinity unit) in 60-litre aquaria with re-circulating seawater containing 10 cm of pea gravel sediment of 2–3 mm grain size and fed once a week with a mixture of *Nannochloropsis oculata*, *Phaeodactylum tricornutum* and *Chlorella* sp. (DT’s Plankton Farm, Sycamore, IL, USA; $3\,\mathrm{ml\,bivalve^{-1}\,week^{-1}}$).

Laboratory burrowing experiment

Wire straps of 18 cm length and 0.3 cm diameter were numbered and attached to the shells of 30 IC and 30 GB *A. islandica* next to the siphon openings with epoxy adhesive glue (Reef construct, Aqua medic, Bissendorf, Germany), which solidified completely underwater within 24 h. Clams were kept at 10°C in 60-litre aquaria containing 20 cm of pea gravel sediment. Daily burrowing depth of each clam was determined to the nearest 0.5 cm by measuring the length of the wire, which was visible above the sand. GB and IC *A. islandica*, which had burrowed for 3.5 days in more than 3 cm sediment depth were sampled as MRD animals ($\mathrm{IC_{bur}}$, $\mathrm{GB_{bur}}$). Bivalves with their siphons open for 3.5 days at the sediment surface were sampled as normoxic, non-burrowed control animals ($\mathrm{IC_{non\text{-}bur}}$, $\mathrm{GB_{non\text{-}bur}}$). Ten individuals of each population and state were dissected, and the mantle, gill and adductor muscle were snap frozen in liquid nitrogen for the analysis of physiological parameters. The shells of all IC clams (shell height: 62–90 mm) and GB clams (shell height: 68–84 mm) were cleaned and numbered for individual age determination.

Additionally, the siphon status of another 10 GB and 10 IC *A. islandica* was documented individually for two weeks using a video

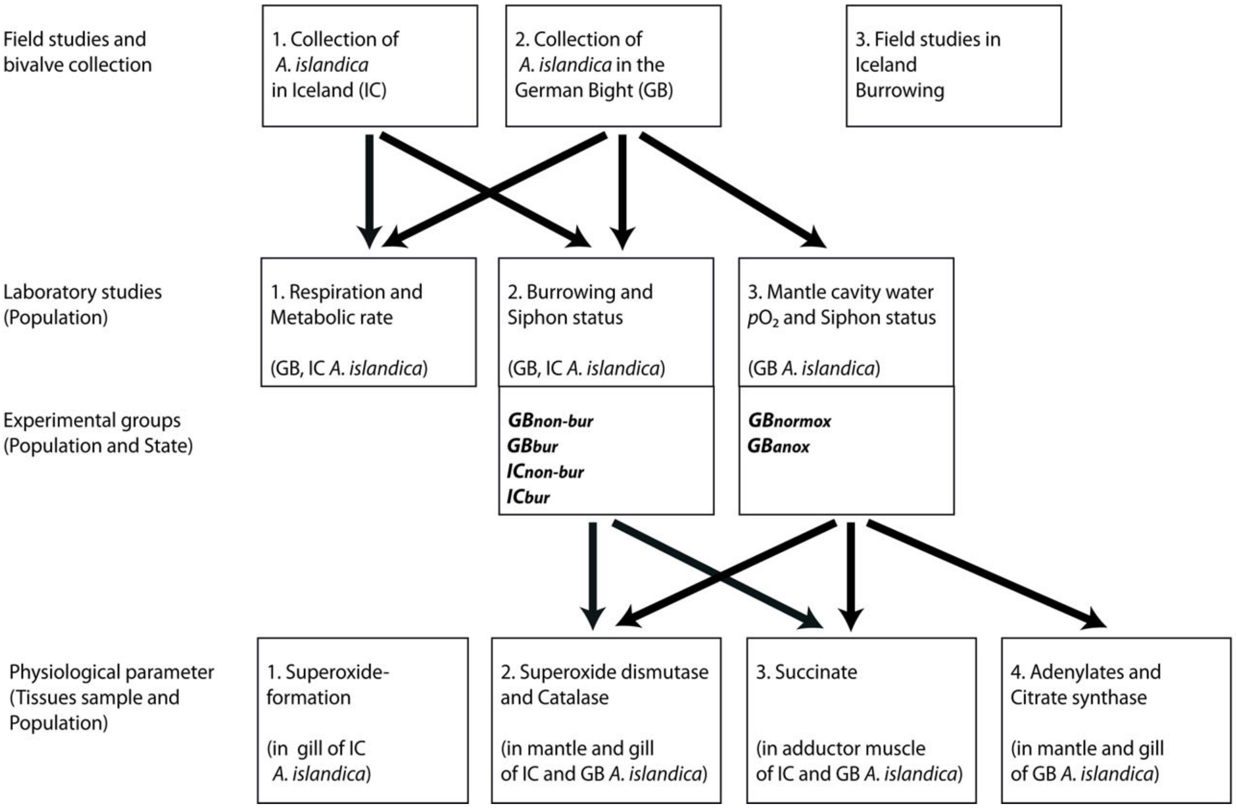

Fig. 1. Flow diagram of fieldwork, laboratory experiments and measurements of the physiological parameters in *Arctica islandica* from Iceland (IC) and the German Bight (GB). Experimental bivalves were either non-burrowed ($GB_{non\text{-}bur}$, $IC_{non\text{-}bur}$) or burrowed for 3.5 days (GB_{bur}, IC_{bur}), and in a second experimental approach of changing the partial pressure of oxygen (P_{O_2}) in the mantle cavity water (GB_{normox}) or for ≥24 h of 0% O_2 in the mantle cavity water (GB_{anox}), *N*=9–11.

camera (Panasonic NV-GS 180, Hamburg, Germany) and VisionGS Software (Business edition-V1.51 test release, Vision GS, Berlin, Germany). Every 10 min a snapshot of the siphon opening of one clam was taken and stored for further analysis.

Oxygenation in the mantle cavity water

Measurements of mantle cavity water P_{O_2} were carried out using single channel Microx TX-3 oxygen metres equipped with PreSens oxygen needle optodes (12×0.04 mm, PSt1-L5-TF, Precision Sensing GmbH, Regensburg, Germany) that had fluorescent coated flat tips of 140 μm diameter. Prior to taking the measurements, the optodes were calibrated to 100% air saturation with aerated seawater and to 0% using water saturated with nitrogen at 10°C. *Arctica islandica* were kept in fully aerated seawater of 10°C and 33 PSU in 20 l aquaria, which were laminated with black foil and covered during the experiments to minimise disturbances to the clams resulting from movements in the laboratory. In order to avoid damage to the optodes, bivalve mobility was restricted. At least 24 h before an experiment, a Teflon nut was glued to the bivalve's lower shell to fix the animals with a screw to an experimental platform in the aquarium (see also Abele et al., 2010). A 1 mm hole was drilled into the shell approximately 1 cm from the edge, which was covered with thin elastic latex foil (Rubber Dam, Heraeus Kulzer, Werhem im Taunus, Germany) and isolation material (Armaflex, Armacell, Münster, Germany) to avoid exchange between mantle cavity water and aquarium water. One hour before starting the oxygen measurements, a hole was pinched through the isolation material and the optode was gently introduced into the mantle cavity. Air saturation was recorded at 30 s intervals using the TX3_v520 software of PreSens (Precision Sensing GmbH). Additionally, the siphon status of each experimental clam was documented during oxygen measurements by taking 10-minute snapshots of the siphon opening with a video camera placed outside the aquaria, where part of the black foil was removed. Bivalves with a recorded permanent P_{O_2}=0 for ≥24 h were identified as MRD animals (GB_{anox}). P_{O_2} measurements were terminated and the clams were dissected, and the mantle, gill and adductor muscle tissue was snap frozen in liquid nitrogen. Bivalves that did not exhibit prolonged periods of zero P_{O_2} in mantle cavity water but frequently fluctuating P_{O_2} between 100% and 0% were identified as GB_{normox} clams, and were also dissected and tissues snap frozen in liquid nitrogen. Oxygen saturation data (%) were converted to P_{O_2} (kPa) and to frequencies corresponding to 1 kPa classes ranging from class 0 kPa to class 21 kPa, according to Abele et al. (Abele et al., 2010). Nine GB_{anox} and nine GB_{normox} animals were analysed and processed, and all bivalve shells with a shell height ranging between 73 mm and 87 mm were cleaned and numbered for individual age determination.

ROS formation in isolated gill tissue

ROS formation was measured with the redox-sensitive, cell-permeable fluorophore Dihydroethidium (DHE), which directly detects cellular superoxide production *in vivo*, using a method modified after Kalivendi et al. (Kalivendi et al., 2003). DHE is oxidised by cellular (including cytosolic) superoxide with the

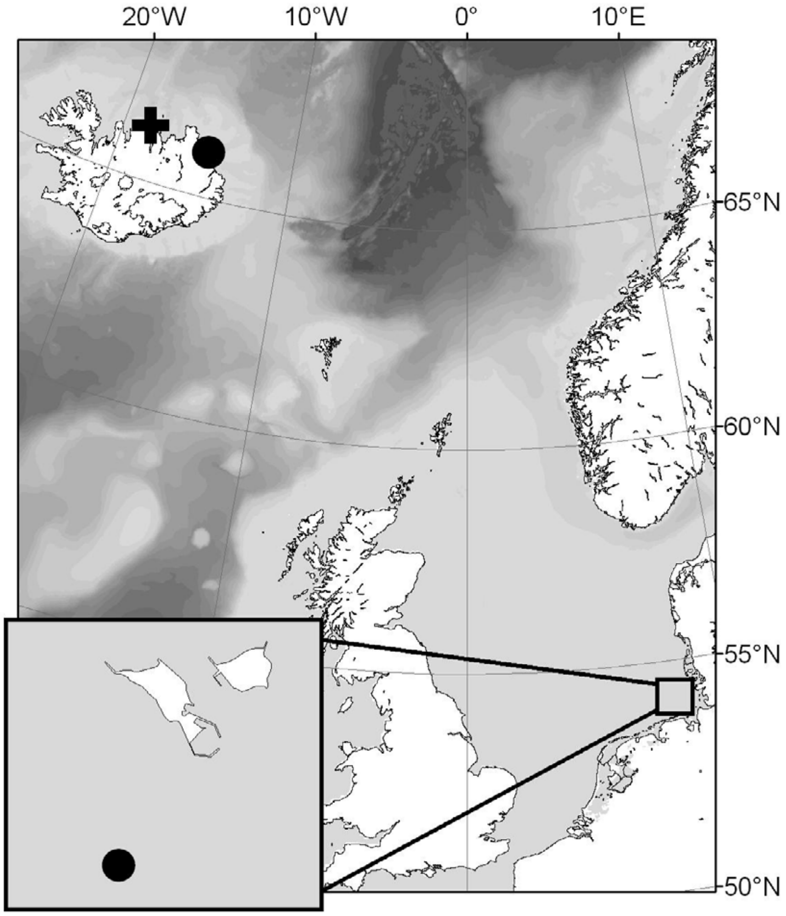

Fig. 2. Location of the field study (black cross) north of Iceland and sampling locations (black circles) of experimental *Arctica islandica* near Iceland [mean sea surface temperature (SST): 5°C, annual range: 2–10°C, 34 PSU, http://www.hafro.is/Sjora] and the German Bight near Helgoland (see frame insert, SST: 9°C, annual range: 4–19°C, 34 psu, http://www.bsh.de/de/Meeresdaten/Beobachtungen/MURSYS-Umweltreportsystem/index.jsp). Continental shelves appear light grey, deeper areas are shaded darker; source of bathymetry: Smith and Sandwell (Smith and Sandwell, 1997).

substrate entering the nucleus and binding to DNA, thus enhancing fluorescence only in the nucleus (Zhao et al., 2003). Small IC *A. islandica* (shell height 35–55 mm) were kept without food for three days before starting the experiments. Bivalve gills were dissected and small gill pieces of 2 or 3 filaments were excised and transferred to incubation vials filled with incubation buffer (450 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl, 20 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} Hepes, 1 mmol l^{-1} EGTA, 0.5 mmol l^{-1} dithiothreitol, 0.055 mmol l^{-1} glucose, pH 7.4) cooled to 8°C and of varying P_{O_2}. One part of the filamentous tissue was incubated immediately after dissection for 20 min in 6 ml of incubation buffer with 10 µmol l^{-1} DHE at 21 kPa to measure ROS formation in the freshly excised (FE) tissue. Two pieces of similar mass were incubated for 2 h in 6 ml of incubation buffer at 21 kPa or 5 kPa. The hypoxic P_{O_2} was adjusted to 5 kPa by using a Wösthoff gas mixing pump and mixing oxygen and nitrogen gas (Wösthoff GmbH, Bochum, Germany). After 2 h of incubation, 10 µmol l^{-1} DHE was added directly to the incubation medium at 21 kPa or 5 kPa. After 20 min the gill filaments were washed in clean buffer, transferred to a microscope slide and bathed in clean buffer to determine the superoxide production during the 20 min incubation. For measurements under hypoxia, filaments were washed and bathed in incubation buffer at 5 kPa. Hypoxic conditions were maintained during the measurements by closing the chamber with a coverslip. In order to determine superoxide production under hypoxia/reoxygenation, a fourth part of the filamentous tissue was incubated for 2 h at 5 kPa and reoxygenated for 20 min after adding 10 µmol l^{-1} DHE. Chambers were cooled to 4°C using a refrigerated microscope stage connected to a thermostat (Ecoline RE 106, Lauda, Lauda-Königshofen, Germany), to avoid radical formation in the gill filaments due to artifactual warming. Fluorescence images were obtained with a fluorescence microscope (Leica TCS, Solms, Germany) at an excitation of 488 nm and emission at 560/60 nm. To evaluate ROS production, the ratio of the stained area of the total area in defined image sections was calculated using the software ImageJ (Version 1.43U, National Institutes of Health, Bethesda, MD, USA).

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined after Livingstone et al. (Livingstone et al., 1992). Briefly, 100–180 mg of frozen mantle and gill tissues were ground in liquid nitrogen and homogenised with a small pestle that fits into a microcentrifuge tube in Tris-HCl buffer (20 mmol l^{-1} Tris, 1 mmol l^{-1} EDTA, 20 mmol l^{-1} HCl, pH 7.6) 1:10 (wt/vol.). Samples were centrifuged for 3 min at 18,000 ***g*** and 4°C. SOD activity was measured as the degree of inhibition of the reduction of cytochrome *c* by superoxides generated by a xanthine oxidase/xanthine system at 550 nm in 43 mmol l^{-1} potassium buffer with 0.1 mmol l^{-1} EDTA, pH 7.8, 20°C. One unit of SOD causes 50% inhibition under assay conditions. Mitochondrial and cytosolic SOD isoforms were not distinguished.

Catalase activity

Catalase (CAT) activity was determined after Aebi (Aebi, 1984). Briefly, 20–50 mg of frozen mantle and gill tissues were ground in liquid nitrogen and homogenised with a small pestle in 50 mmol l^{-1}

phosphate buffer (50 mmol l^{-1} KH_2PO_4, 50 mmol l^{-1} Na_2HPO_4, pH 7.0) with 0.1% Triton X-100 at 1:30 (wt/vol.). Samples were centrifuged for 15 min at 13,000 ***g*** and 4°C, and CAT activity was determined by recording the period required for H_2O_2 decomposition at 20°C, resulting in a decrease of absorption from 0.45 to 0.4 at 240 nm (1 U).

Adenylate concentrations

Concentrations of ATP, ADP and AMP were determined after Lazzarino et al. (Lazzarino et al., 2003), using high-performance liquid chromatography (HPLC). Frozen samples of mantle and gill tissues were ground in liquid nitrogen and homogenised with a micropistil in nitrogen-saturated precipitation solution [CH_3CN (Acetonitril) + 10 mmol l^{-1} KH_2PO_4 at a 3:1 ratio, pH 7.4] at 1:11 (wt/vol.). Samples were centrifuged for 10 min at 20,690 ***g*** and 4°C and clear supernatants were stored on ice. Pellets were resuspended in 1 ml of precipitation solution using an Ultra Turrax (IKA-Werke, Staufen, Germany) for 5 s and centrifuged for 10 min at 20,690 ***g*** and 4°C. Secondary supernatants were combined with primary, washed with a double volume of chloroform (CH_3Cl HPLC grade) and centrifuged for 10 min at 20,690 ***g*** and 4°C. The upper aqueous phase contains the water-soluble, low molecular weight compounds and was washed twice with chloroform, centrifuged for 10 min at 20,690 ***g*** and 4°C and stored at –80°C. Samples were defrosted, centrifuged for 20 min at 20,690 ***g*** and 4°C and measured by HPLC using a Kromasil 250×4.6 mm, 5 μm particle size column (Eka Chemicals, AB, Sweden) and a guard column. 50 μl of samples was injected, and HPLC conditions such as solvents, gradients, flow rate and detection were applied according to Lazzarino et al. (Lazzarino et al., 2003). Adenylate standards were purchased from Sigma-Aldrich (Steinheim, Germany), adenylate concentrations were calculated using Karat Software 7.0 (Beckmann Coulter, Krefeld, Germany), and the total amount of adenylates (=ATP+ADP+AMP) was added.

CS activity

The activity of the mitochondrial key enzyme CS was determined after Sidell et al. (Sidell et al., 1987). Frozen samples of mantle and gill tissues were ground in liquid nitrogen and homogenised with a glass homogeniser (Nalgene, Rochester, NY, USA) in Tris-HCl buffer (20 mmol l^{-1} Tris-HCl, 1 mmol l^{-1} EDTA, 0.1% Tween-20, pH 7.4) at 1:10 (wt/vol.). Samples were sonicated for 15 min at 2°C in a Branson Sonifier 450 (duty cycle 50%, output control 8; G. Heinemann Ultraschall- und Labortechnik, Schwäbisch Gmünd, Germany) and centrifuged for 5 min at 7400 ***g*** at 4°C. CS activity was measured at 20°C by recording the absorbance increase of 5 mmol l^{-1} DTNB [5,5′dithiobis(2-nitrobenzoic acid)] in 75 mmol l^{-1} Tris-HCl (pH 8.0), 0.4 mmol l^{-1} acetyl-CoA and 0.4 mmol l^{-1} oxalacetate at 412 nm. CS activity was calculated using the mmolar extinction coefficient ε_{412} of 13.61 mmol l^{-1} cm^{-1}.

Succinate concentration

Frozen samples of the adductor muscle were homogenised on ice using an Ultra Turrax and ultrasound (Branson Sonifier 450, G. Heinemann Ultraschall- und Labortechnik) in 0.5 mol l^{-1} perchloric acid (PCA) at 1:6 (wt/vol.) and centrifuged for 15 min at 12,000 ***g*** and 4°C. The supernatant of each sample was neutralised with a pre-defined amount of 2 mol l^{-1} KOH and centrifuged for 5 min at 12,000 ***g*** and 4°C. The succinate content was determined after Michal et al. (Michal et al., 1976) using the succinic acid assay kit (Cat. No. 10 176 281 035, Boehringer Mannheim/R-Biopharm, Mannheim, Germany). The absorbance of NADH was recorded in UV-DU 800 spectrophotometer (Beckmann Coulter) at 340 nm and 37°C. The incubation time was prolonged to 30 min for complete enzymatic reaction of succinate in the sample.

Age determination

Age determination for *A. islandica* was carried out as described by Begum et al. (Begum et al., 2009). Briefly, the right shell was embedded in epoxy resin and sectioned along the axis of maximum shell growth (=shell height) with a table diamond saw (FK/E PROXXON-28070, PROXXON, Föhren, Germany). Cross sections were ground using grits of P80, P600, P1200, P2400 and P4000. Annual shell growth bands were counted using a stereomicroscope (Olympus SZX12, Hamburg, Germany) at 10- to 80-fold magnification.

Statistical analysis

All data sets were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett's test) before statistical analysis. A *t*-test (or a Mann–Whitney *U*-test for non-Gaussian distributed data) was used to evaluate the effect of season (independent, categorical) on the burrowing behaviour in the field (dependent variable) and of 'siphon status' (open *vs* closed; independent, categorical) on the mantle cavity P_{O_2} (dependent variable, numerical). The effect of the independent variables, i.e. population (categorical), experimental P_{O_2} (numerical), tissue type (categorical) and metabolic status (normoxic *vs* metabolic reduction/anoxic; categorical) on the dependent variables, i.e. siphon status, superoxide formation, enzyme activity (SOD, CAT, CS), as well as on adenylate and succinate concentration were tested using one-way ANOVA (model I type) and Tukey's *post hoc* test (for adenylates, CS, succinate, siphon status) or, for non-Gaussian distributed data, using Kruskal–Wallis test and Dunn's *post hoc* test (for SOD, CAT).

Effects of status and exposure time on mantle cavity water P_{O_2}

We reduced temporal resolution of each individual P_{O_2} time series from the original 30 s to 5 min by averaging the measurements over subsequent 5 min intervals (i.e. 12 values per interval). All individual time series were reversed in order to start the comparison from a common endpoint and cut to the period 0–2350 min to obtain equal time series length for all GB_{normox} and GB_{anox} clams. The pooled P_{O_2} data were Box-Cox transformed (Sokal and Rohlf, 1981) to meet the pre-conditions of ANCOVA and were tested for effects of status (GB_{normox} and GB_{anox} clams), time and body mass (shell free wet mass) by a fully factorial analysis of covariance model (ANCOVA, i.e. $P_{O_2,\text{Box-Cox}}$ *vs* status and covariates time and body mass). As all parameters and the status interaction terms were found to affect P_{O_2} significantly (see Results), we used the residuals of the ANCOVA model to check for status-specific temporal patterns in P_{O_2}. Visual inspection of the residuals *vs* time plots indicated distinct differences in the temporal development of mantle cavity P_{O_2}. Accordingly, we compared the time intervals 100–2350 min in GB_{normox} clams, and 800–2350 min as well as 100–800 min in the GB_{anox} clams by means of one-way ANOVA with subsequent Tukey's *post hoc* test on differences between means.

Endogeneous rhythms in mantle cavity water P_{O_2}

From the 18 GB_{normox} and GB_{anox} individual time series produced, we selected those 13 series with at least 450 consecutive 5 min data points and not more than one gap (malfunction of optode) in the time series. These gaps (three series) were closed by linear interpolation. We removed the long-term trend (across >100 data points) from each time series by means of cubic spline interpolation (settings: shape parameter λ=10,000). The time series of the residuals of the cubic

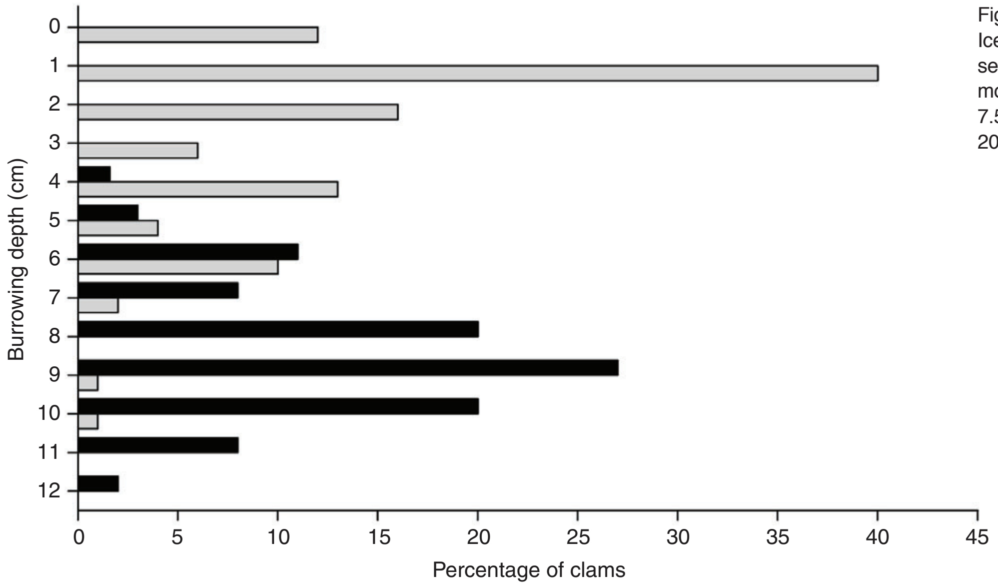

Fig. 3. Percentage of *Arctica islandica* in Iceland seabed studies burrowed at different sediment depths in June 2003 at a mean monthly sea surface temperature (SST) of 7.5°C (grey bars, *N*=111) and in February 2004 at a SST of 1.5°C (black bars, *N*=79).

spline interpolation was taken as representative for short-term variability in mantle cavity P_{O_2}. Correlation analysis of the residual time series failed to detect any significant positive correlation between the specimen numbers 2, 3 and 4 that had been measured simultaneously, i.e. there was no external forcing of the oscillations in P_{O_2} detectable. We analysed each P_{O_2} time series by a two-step procedure using the software package kSpectra (SpectraWorks Inc., Milwaukee, WI, USA). In the first step we applied singular spectrum analysis [SSA; settings: window length 60, covariance estimation using the approach of Vautard and Ghil (Vautard and Ghil, 1989), Monte Carlo significance test] to identify the strongest oscillatory components. Ranked by variance explained, the first 10 SSA components (singular values) captured close to 50% of total variance in each time series. These 10 components were used to reconstruct a 'filtered' P_{O_2} time series. In the second step, the reconstructed P_{O_2} time series was subjected to the non-parametric multi-taper method (MTM; settings: significance='red noise', three tapers, adaptive procedure, robust background noise) of spectral analysis, which is a common tool in geophysics, oceanography, climatology and geochemistry (Mann and Lees, 1996). Within the frequency range 0–0.5, spectral density is computed for 512 equally spaced frequencies [see Ghil et al. (Ghil et al., 2000) for more detailed information on SSA and MTM]. The resulting 512 frequencies × 13 individuals matrix was subjected to principal component analysis (on the covariance matrix) and the first principal component was taken to represent the common spectral pattern of all 13 time series.

RESULTS

Burrowing behaviour and mantle cavity P_{O_2} in GB and IC *A. islandica*

In situ burrowing depth of IC *A. islandica* differed significantly between winter and summer (Fig. 3). In February 2004, *A. islandica* were found in 4–12 cm sediment depth [mean=8.5, standard deviation (s.d.)=1.7] whereas in June 2003, clams were found in 0–10 cm sediment depth and were, on average, located significantly closer to the surface (mean=2.4, s.d.=2.2, Mann–Whitney *U*-test $P<0.0001$; Fig. 3). The 'burrowing and siphon status' experiments indicated no significant differences in siphon activity (open/closed) or burrowing status between GB and IC animals. GB individuals had siphons open 27±19% of the investigated time, closed 43±27%

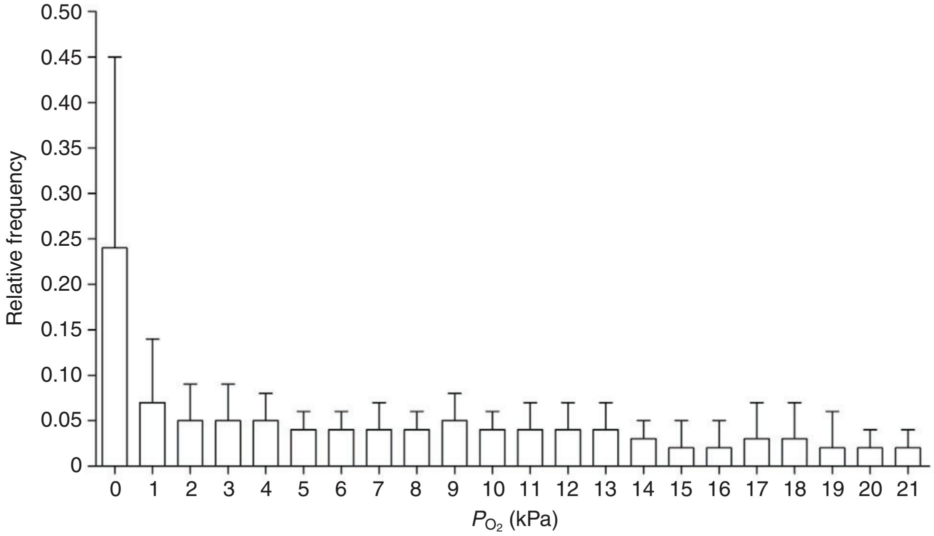

Fig. 4. Relative frequencies of partial pressure of oxygen (P_{O_2}) in the mantle cavity water of *Arctica islandica* (means ± s.d.) from the German Bight registered with a resolution of 1 kPa P_{O_2} classes between anoxia at 0 kPa and 100% saturation at 21 kPa. Values include all P_{O_2} measurements of normoxic-exposed clams (GB_{normox}) and of GB_{anox} clams prior to initiating the metabolic rate depression (MRD) status (defined as P_{O_2}=0 kPa for ≥24 h in the mantle cavity water). *N*=18 different test animals experimentally maintained in fully oxygen saturated water (21 kPa) at 10°C.

of the time and individuals were burrowed 30±32% of the time. IC clams had open siphons 39±26% of the time, closed 26±24% of the time and were burrowed 35±34% of the investigated time. The exact burrowing depth of the clams was not determined in the experiment, but periods during which clams remained constantly burrowed lasted equally long, between 1 day and 6 days, in both populations.

P_{O_2} measurements in the mantle cavity water of GB *A. islandica* showed that the clams actively regulated oxygen concentrations when deprived of their sedimentary retreat (Fig. 4). Ventilation behaviour differed between individuals, and mantle cavity P_{O_2} fluctuations were strong and fast in some clams and less pronounced in others (individual recordings are not shown). The mean P_{O_2} during the intervals where siphon closure was confirmed by photographic recording in nine GB_{normox} and nine GB_{anox} clams, prior to entering the MRD status, was 4.03±5.27 kPa and was significantly lower than during siphon opening, i.e. 4.96±6.29 kPa (*t*-test $P<0.0001$, $N=3214$ data points of a total of 18 clams). The frequency distribution of recorded P_{O_2} values (5 min means) over kPa classes from 0 kPa to 21 kPa (=100%) indicated that the P_{O_2} in the mantle cavity water was zero approximately 25% of the total observation time, whereas P_{O_2} values ≥14 kPa were recorded for 16% of the total time (Fig. 4).

Mantle cavity water P_{O_2} was significantly affected by status, time, body mass and the interaction terms status × time and status × body mass (ANCOVA $P<0.0001$ for all terms). The residuals of this model revealed a distinctly different behaviour of P_{O_2} in GB_{anox} clams, particularly during the last 700 min prior to entering MRD. In GB_{anox} clams, P_{O_2} was significantly lower than in GB_{normox} clams (100–2350 min, mean residuals=–0.2115±0.072) when animals were more than 700 min away from the MRD status (800–2350 min, mean residuals=–0.541±0.089, Tukey's test $P<0.001$), but significantly higher in the 100–800 min interval directly before entering the MRD status (mean residuals=1.918±0.133, Tukey's test $P<0.001$, Fig. 5A,B).

Spectral analysis revealed a prominent signal at a frequency of $0.02\ h^{-1}$, i.e. the 13 clams analysed exhibited a common oscillatory pattern in mantle cavity water P_{O_2} for a period of approximately 50 min.

Antioxidant enzyme activities and ROS formation in tissues of GB and IC *A. islandica*

In mantle and gill tissues no significant differences were found in CAT and SOD activity between GB_{normox} and GB_{anox} clams and between clams that had burrowed for 3.5 days and non-burrowed clams of both GB and IC *A. islandica* (Table 1). Nevertheless, enzyme activities in the tissues of GB_{bur} and IC_{bur} clams were mildly but consistently higher than in $GB_{non\text{-}bur}$ and $IC_{non\text{-}bur}$ clams (Table 1). SOD and CAT activities were similar in mantle and gill tissues of GB and IC *A. islandica* (Table 1).

Superoxide formation in the isolated gill tissue was highest immediately after animal dissection and gill extraction (Fig. 6). ROS signal intensities were significantly lower after 2 h at 5 kPa (hypoxia) compared with values in FE tissues or control tissue pieces from the same animal maintained for 2 h at 21 kPa. After 2 h at 5 kPa with subsequent 20 min of reoxygenation (21 kPa), the signals of superoxide formation were slightly higher than in tissues incubated under hypoxia, but still significantly lower than in FE tissues and 4-fold lower than after 2 h at 21 kPa (Fig. 6).

Content of adenylates, CS activity and succinate accumulation in tissues of GB and IC *A. islandica*

The overall adenylate concentration (ATP+ADP+AMP) was significantly higher in the gills of GB_{normox} clams compared with the mantle, which was due to higher ATP and ADP concentrations (Table 2). AMP concentrations and AMP/ATP ratios were similar in both tissues of *A. islandica*. Gills and mantle differed in their response to MRD condition. In the mantle total adenylate concentrations were significantly higher in GB_{anox} clams compared with GB_{normox} clams, mainly due to higher ADP and AMP concentrations after 24 h of 0% O_2 in mantle cavity water. In contrast, the gill had a similar content of total adenylates, especially of ADP and AMP, but significantly lower ATP concentrations under GB_{anox} compared with GB_{normox} conditions. The AMP/ATP ratio was higher in mantle and gill in GB_{anox} compared with GB_{normox} clams, but these differences were not statistically significant (Table 2).

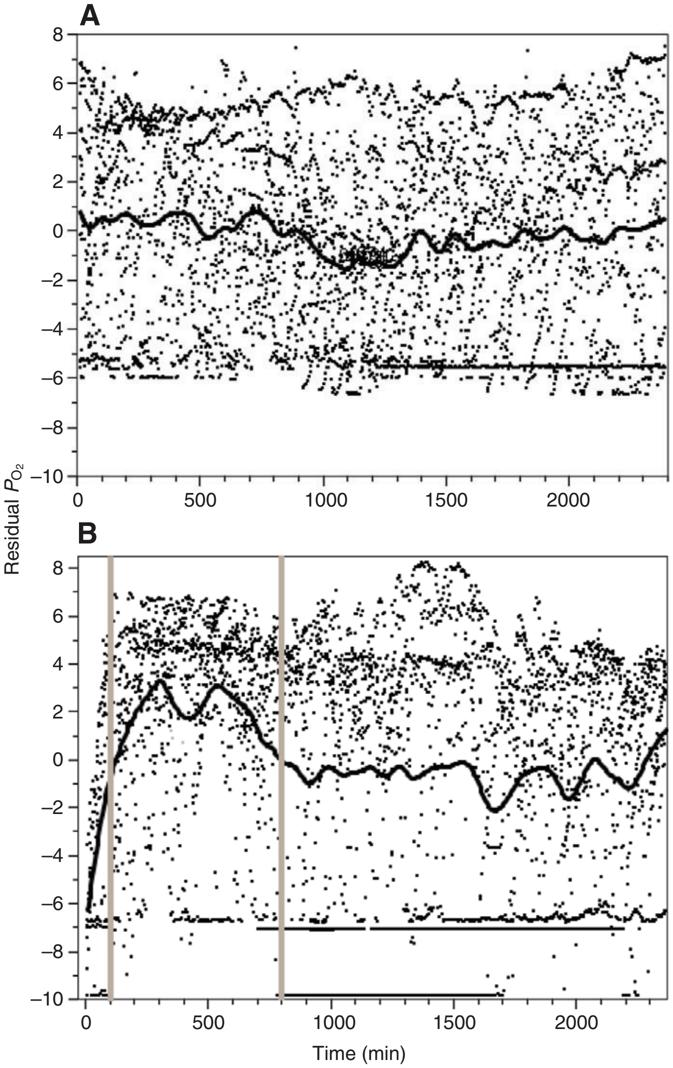

Fig. 5. Residuals of the ANCOVA model plotted over time for (A) normoxic (GB_{normox}) *Arctica islandica* (N=8) and (B) anoxic (GB_{anox}) *A. islandica* prior to the metabolic rate depression (MRD) status [defined as partial pressure of oxygen (P_{O_2})=0 kPa for ≥24 h in the mantle cavity water, N=8] from the German Bight, experimental temperature=10°C. The black line represents the cubic spline interpolation ($\lambda=10^6$). Time axis is reversed; zero represents the end of the oxygen measurements in A and the onset of MRD in B. Grey bars in B indicate the 100–800 min time window with specific pre-MRD P_{O_2} development.

CS activity was similar in different tissues and different states of mantle cavity P_{O_2} (Table 2).

Table 1. Activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in gill and mantle tissues of non-burrowed ($GB_{non\text{-}bur}$, $IC_{non\text{-}bur}$) and 3.5 days-burrowed (GB_{bur}, IC_{bur}) German Bight (GB) and Iceland (IC) *Arctica islandica*, and of GB clams with changing partial pressure of oxygen (P_{O_2}) in the mantle cavity water (GB_{normox}) or with ≥24 h of 0% O_2 in the mantle cavity water (GB_{anox})

	Age (years)	Duration of MRD	Gill SOD ($U\,g^{-1}$ wet mass)	Mantle SOD ($U\,g^{-1}$ wet mass)	Gill CAT ($U\,g^{-1}$ wet mass)	Mantle CAT ($U\,g^{-1}$ wet mass)
$GB_{non\text{-}bur}$	33–98	–	705±324	545±265	2944±1016	3362±1114
GB_{bur}	33–99	3.5 days	729±226	790±431	4036±1472	2998±1098
$IC_{non\text{-}bur}$	29–141	–	870±447	657±389	3888±524	2435±540
IC_{bur}	29–142	3.5 days	940±328	839±408	4161±880	2832±1138
GB_{normox}	28–62	–	732±83	767±277	2465±818	2752±693
GB_{anox}	43–83	24 h	745±185	674±204	2769±769	2804±431

Data are means ± s.d., each group N=6–10, experimental temperature=10°C, assay temperature=20°C. MRD, metabolic rate depression.

The succinate concentration in the adductor muscle of both GB and IC *A. islandica* was significantly higher in burrowed clams compared with non-burrowed clams, but these differences were not significant between GB_{normox} and GB_{anox} (Table 3). Succinate accumulation was significantly higher in IC clams that had burrowed for 3.5 days than GB clams (Table 3).

DISCUSSION

Burrowing behaviour in *A. islandica* varied greatly with season and likely depends on seasonal feeding conditions and temperature. In June, when phytoplankton concentrations at the study site in Eyjafjördur, North Iceland, are 30 times higher than in February (Kaasa and Gumundsson, 1994), 50% of all bivalves burrowed directly beneath the sediment surface. Siphons of shallow burrowing bivalves are in direct contact with the overlying seawater to take up oxygen and food. Predation has been suggested to be a major burrowing elicitor in bivalves (Griffith and Richardson, 2006), but the massive shells of adult *A. islandica* apparently provide sufficient protection from predators such as fish (Arntz and Weber, 1970). Thus, low food conditions in winter appear to be a major environmental cause, which can explain greater seasonal burrowing depth. Further, low water temperatures in the cold season support metabolic arrest in bivalves (Morley et al., 2007).

MRD in *A. islandica* is an intrinsically controlled behaviour in bivalves. This is illustrated by the mantle cavity P_{O_2} measurements and the ANCOVA model of P_{O_2} residuals over time in spontaneously 'hibernating' ocean quahog. Depression of metabolism in the ocean quahog is not exclusively linked to, or a consequence of, burrowing into deeper sediment horizons, but it is also self-induced when the bivalves are deprived of their sedimentary retreat and, instead, maintained in normoxic seawater (see also Abele et al., 2010). Accordingly, more than 30% of GB (5 out of 13 animals) and IC (5 out of 16 animals) clams did not respire for >24 h during the measurements of whole-animal respiration at 10°C (J.S. and D.A., unpublished), indicating the downregulation of the metabolism in *A. islandica*. An intrinsic and species-specific burrowing pattern, independent of water temperature and feeding, was visible in both populations during laboratory experiments, and individual periods of constant burrowing lasted between 1 day and 6 days. In preparation for their short term 'hibernation', *A. islandica* show a distinct breathing behaviour several hours before entering the metabolically depressed state. Within the period 2300–800 min P_{O_2} of the MRD animals is not significantly higher than P_{O_2} of the normoxic animals (fully factorial ANCOVA model), i.e. the increase of the residual curve reflects a real increase in P_{O_2} in the MRD animals above the level seen in the normoxic animals. Enhanced oxygen uptake may increase ATP production just before closing the shell or burrowing down, presumably to meet energy requirements that may not yet be downregulated during the first hours of shell closure or during burrowing exercise. Bivalves that merely closed their shells for 24 h accumulated no succinate whereas *A. islandica* that burrowed into the sediment for longer periods than 3.5 days had increased succinate levels in the adductor muscle. In contrast, within one day of anoxic exposure (=forced anoxia) at seawater temperatures comparable to the present study (9.5±0.5°C), succinate accumulation occurred in tissues of the ocean quahog (Oeschger, 1990; Oeschger and Storey, 1993).

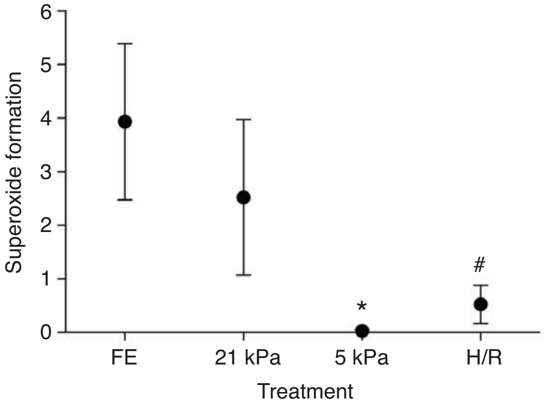

Fig. 6. Superoxide formation [in % stained after 20 min of incubation with dihydroethidium (DHE)] in the gill tissues of Iceland *Arctica islandica* immediately following dissection [freshly excised (FE), N=5] after 2 h of incubation under normoxic partial pressure of oxygen (P_{O_2}) conditions (21 kPa, N=3) or hypoxic P_{O_2} conditions (5 kPa, N=3), or after hypoxia/reoxygenation (H/R, N=11), means ± s.d., incubation temperature=8°C. *Significant difference between 5 kPa *vs* FE and 21 kPa; [#]significant difference between H/R and FE (Kruskal–Wallis test P<0.001, Dunn's test P<0.05).

Arctica islandica are well adapted to hypoxia and maintain low internal oxygenation also under normoxic environmental conditions. Mean mantle cavity P_{O_2} in GB specimens was <5 kPa during siphon opening and closure, and the same was found in a parallel experimental study with Kiel Bight *A. islandica* (Abele et al., 2010). Steady control of mantle cavity oxygenation may be instrumental in achieving low and protective P_{O_2} levels in cells of hypoxia-tolerant and oxygen-sensitive animals (Massabuau, 2003). However, *A. islandica* can be distinguished from other bivalves such as *Mya arenaria* through the specific 'rhythms' of mantle cavity P_{O_2} oscillations. Whereas the deep burrowing soft-shell clam *M. arenaria* maintains a mantle cavity P_{O_2} almost constant between 0 kPa and 2.6 kPa (Abele et al., 2010), GB *A. islandica* in the present study oscillated P_{O_2} between 2 kPa and 21 kPa for a period of 50 min. Kiel Bight animals measured by Abele et al. oscillated in the same

Table 2. Adenylate concentrations and citrate synthase (CS) activity in gill and mantle tissues of German Bight (GB) *Arctica islandica* with changing partial pressure of oxygen ($P_{\mathrm{O_2}}$) in the mantle cavity water ($\mathrm{GB_{normox}}$) or with ≥24 h of 0% $\mathrm{O_2}$ in the mantle cavity water ($\mathrm{GB_{anox}}$)

	Age (years)	ATP+ADP+AMP (nmol g^{-1} wet mass)	ATP (nmol g^{-1} wet mass)	ADP (nmol g^{-1} wet mass)	AMP (nmol g^{-1} wet mass)	AMP/ATP (nmol g^{-1} wet mass)	CS (U g^{-1} wet mass)
Gill $\mathrm{GB_{normox}}$	28–62	352.06±67.37†	213.96±42.42†	86.81±27.59†	51.87±24.54	0.26±0.17	1.75±0.68
Gill $\mathrm{GB_{anox}}$	43–83	292.08±71.13	134.24±39.32**	83.81±23.17	74.03±33.40	0.60±0.36	1.81±0.80
Mantle $\mathrm{GB_{normox}}$	28–62	191.17±55.68	109.18±41.65	37.22±11.73	44.77±25.33	0.47±0.39	2.47±0.59
Mantle $\mathrm{GB_{anox}}$	43–83	290.33±82.09*	119.40±34.87	88.12±31.62**	82.81±28.25*	0.73±0.30	2.40±0.67

Asterisks indicate significant differences in gill and mantle tissues between $\mathrm{GB_{normox}}$ and $\mathrm{GB_{anox}}$ clams (*one-way ANOVA $P<0.05$, *Tukey's test $P<0.05$; **one-way ANOVA $P<0.001$, **Tukey's test $P<0.01$). †Significant differences between gill and mantle tissues within the same state (one-way ANOVA $P<0.001$, Tukey's test $P<0.01$).

Data are means ± s.d., $N=9$, experimental temperature=10°C, assay temperature of CS measurements=20°C.

$P_{\mathrm{O_2}}$ range but more slowly with periods of 96 min and 250 min (Abele et al., 2010). Thus, there may be population-specific patterns of shell water ventilation in *A. islandica*: with more frequent ventilation in bigger North Sea specimens and less frequent ventilation in smaller Baltic Sea specimens. However, both ventilation patterns achieve the same mean $P_{\mathrm{O_2}}$ level in mantle cavity water, which seems to be species specific and adaptive for *A. islandica*'s shallow burrowing lifestyle. Indeed, mean shell water $P_{\mathrm{O_2}}$ achieved by the ocean quahog is not as low as in the deep burrowing (±50 cm) soft-shell clam *M. arenaria* and not as high as in epibenthic swimming scallops (Abele et al., 2010).

The mitochondrial and energetic capacities, represented by the CS activity and ATP or whole adenylate concentrations, are low in *A. islandica* compared with other bivalves. ATP concentrations of 214 nmol g^{-1} wet mass in gills and 110 nmol g^{-1} wet mass in mantle are less than 10% of the ATP values in *Mytilus edulis* mantle tissue (2 µmol ATP g^{-1} wet mass) (Wijsman, 1976) or oyster gills (2.2 µmol ATP g^{-1} wet mass) (Sokolova et al., 2005). These low values are in keeping with the extraordinarily low metabolic rate of the ocean quahog (Begum et al., 2009). CS activities remained constant in the mantle and gill tissues of GB bivalves between normoxia and the first 24 h of MRD. Nevertheless, ATP utilisation in the gills seems to outrun ATP production, possibly due to continued ciliary movements when the shells are closed and anaerobic energy production has not yet started. Thus, ATP content decreased in the gill tissue, and succinate, which is the first intermediate signalling the onset of mitochondrial anaerobiosis in the ocean quahog (Oeschger, 1990; Strahl et al., 2011), remained low during ≥24 h of 0% $\mathrm{O_2}$ in the mantle cavity water of *A. islandica*. The gills of GB *A. islandica* are metabolically much more active than the mantle. A somewhat higher overall adenylate content as well as higher SOD and CAT activities were detected in gill tissue compared with the mantle tissue. This is consistent with higher tissue respiration and cell turnover rates in gills of *A. islandica* than in mantle (Tschischka et al., 2000; Strahl and Abele, 2010; Strahl et al., 2011). Maintenance of ATP concentrations in the generally less active mantle tissue of *A. islandica* after >24 h of 0% $\mathrm{O_2}$ in the mantle cavity water can be attributed to a coordinated slow down of metabolism. This was also found in the limpet *Patella vulgata* which even increased ATP content in the foot muscle after 6 h of air exposure compared with control conditions (=immersed), without inducing anaerobiosis (Brinkhoff et al., 1983). The AMP/ATP ratio in MRD bivalves increased in both gill and mantle tissues, which, at a certain threshold, activates glycolysis, lipid oxidation and anaerobic metabolism *via* the AMP-activated protein kinase (AMPK) pathway (Taylor, 2008). AMPK downregulates energy-consuming anabolic processes and supports hypoxic survival in a metabolically depressed state.

The transient reduction of metabolically derived ROS formation during MRD may have life prolonging effects in the ocean quahog (Abele et al., 2010; Buttemer et al., 2010). Although *A. islandica* already features very low *in vitro* ROS formation under normoxic states 3 and 4 (Buttemer et al., 2010), ROS production in isolated gill tissue was found to be drastically reduced under low oxygen conditions of 5 kPa compared with 21 kPa and supposedly fully subsides as cellular respiration stops at 0 kPa. The crucial point is that oxidative burst may be happening as the bivalves surface and cells are flooded with oxygen. Our data on enzymatic antioxidants, as well as on glutathione levels during hypoxic exposure of *A. islandica* in a previous paper (Strahl et al., 2011), indicate that no anticipatory antioxidant response takes place. In agreement, a ROS burst was absent in isolated gill tissue of IC *A. islandica* (present study) and of GB clams (S. Hardenberg and D.A., unpublished) after hypoxia/reoxygenation, and ROS levels were much lower than under constant normoxic exposure. An alternative oxidase pathway in mitochondria of *A. islandica* during reoxygenation may act as respiratory protection by increasing the rate of cellular oxygen

Table 3. Succinate content in the adductor muscle of non-burrowed ($\mathrm{GB_{non\text{-}bur}}$, $\mathrm{IC_{non\text{-}bur}}$) or 3.5-days-burrowed ($\mathrm{GB_{bur}}$ / $\mathrm{IC_{bur}}$) German Bight (GB) and Iceland (IC) *Arctica islandica*, and of GB clams with changing $P_{\mathrm{O_2}}$ in the mantle cavity water ($\mathrm{GB_{normox}}$) or with ≥24 h of 0% $\mathrm{O_2}$ in the mantle cavity water ($\mathrm{GB_{anox}}$)

	Age (years)	Duration of MRD	Succinate (nmol mg^{-1} wet mass)
$\mathrm{GB_{non\text{-}bur}}$	33–98	–	5.65±1.44
$\mathrm{GB_{bur}}$	33–99	3.5 days	65.49±19.07**
$\mathrm{IC_{non\text{-}bur}}$	29–141	–	7.92±3.13
$\mathrm{IC_{bur}}$	29–142	3.5 days	90.71±25.34$^{**,\#}$
$\mathrm{GB_{normox}}$	28–62	–	5.69±3.11
$\mathrm{GB_{anox}}$	43–83	24 h	10.09±4.24

Data are means ± s.d., each group $N=7$–10, experimental temperature=10°C, assay temperature=20°C. **Significant differences in the GB and IC population between burrowed and non-burrowed clams (one-way ANOVA, $P<0.0001$; Tukey, $P<0.001$). $^{\#}$Significant differences between $\mathrm{GB_{bur}}$ and $\mathrm{IC_{bur}}$ clams (one-way ANOVA, $P<0.0001$; Tukey, $P<0.01$). MRD, metabolic rate depression.

consumption, and thereby lowering the tissue P_{O_2} and minimising the risk of oxygen radical formation (Tschichka et al., 2000). Thus, there is little need to protect tissues in surfacing clams. The high superoxide level in gills immediately after dissection can be attributed to ROS formation as a stress response in FE samples.

In conclusion, *A. islandica* spontaneously induce a metabolically depressed and energy-saving state at all times of the year, but this behaviour seems to be distinctive during winter when food availability and water temperature are low. Biogeographical acclimation seems to have a minor impact on this behaviour, and North Sea bivalves burrow at similar rates as bivalves from North Iceland when maintained under the same conditions. We conjecture that external factors such as climate change-induced warming and intensive bottom trawling in the North Sea reduce *A. islandica* lifespan in the GB (see Strahl and Abele, 2010). Furthermore, shorter annual feeding periods and colder annual temperatures in Icelandic waters may lead to longer burrowing periods in the IC population and indirectly support a longer MLSP in this population. Changes of adenylate concentrations, especially in the AMP/ATP ratio, seem instrumental in regulating metabolism under MRD. Avoiding accumulation of anaerobic metabolites in the burrowed state limits the need for enhanced recovery respiration during surfacing and helps to prevent an oxidative burst reaction during reoxygenation. Consequently, neither the levels of enzymatic nor low molecular antioxidants such as glutathione are enhanced in preparation for an oxidative burst, at least on the protein and activity level. Investigation of the expression level (mRNA) of antioxidant genes will provide further information of the extent to what this species is prepared for reoxygenation.

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