Mantle cavity water oxygen partial pressure (P_{O_2}) in marine molluscs aligns with lifestyle

Doris Abele, Melanie Kruppe, Eva E. R. Philipp, and Thomas Brey

Abstract: Marine invertebrates with open circulatory system establish low and constant oxygen partial pressure (P_{O_2}) around their tissues. We hypothesized that as a first step towards maintenance of low haemolymph and tissue oxygenation, the P_{O_2} in molluscan mantle cavity water should be lowered against normoxic (21 kPa) seawater P_{O_2}, but balanced high enough to meet the energetic requirements in a given species. We recorded P_{O_2} in mantle cavity water of five molluscan species with different lifestyles, two pectinids (*Aequipecten opercularis*, *Pecten maximus*), two mud clams (*Arctica islandica*, *Mya arenaria*), and a limpet (*Patella vulgata*). All species maintain mantle cavity water oxygenation below normoxic P_{O_2}. Average mantle cavity water P_{O_2} correlates positively with standard metabolic rate (SMR): highest in scallops and lowest in mud clams. Scallops show typical P_{O_2} frequency distribution, with peaks between 3 and 10 kPa, whereas mud clams and limpets maintain mantle water P_{O_2} mostly <5 kPa. Only *A. islandica* and *P. vulgata* display distinguishable temporal patterns in P_{O_2} time series. Adjustment of mantle cavity P_{O_2} to lower than ambient levels through controlled pumping prevents high oxygen gradients between bivalve tissues and surrounding fluid, limiting oxygen flux across the body surface. The patterns of P_{O_2} in mantle cavity water correspond to molluscan ecotypes.

Résumé : Les invertébrés marins à système circulatoire ouvert maintiennent une P_{O_2} faible et constante autour de leurs tissus. Notre hypothèse veut que, comme première étape vers le maintien d'une oxygénation faible de l'hémolymphe et des tissus, la P_{O_2} dans l'eau de la cavité du manteau des mollusques devrait être réduite par rapport à la P_{O_2} de l'eau de mer normoxique (21 kPa), mais maintenue à un équilibre suffisamment élevé pour satisfaire les besoins énergétiques de l'espèce en question. Nous avons enregistré la P_{O_2} dans l'eau de la cavité du manteau de cinq espèces de mollusques à modes de vie différents, deux pectinidés (*Aequipecten opercularis*, *Pecten maximus*), deux moules de vase (*Arctica islandica*, *Mya arenaria*) et une patelle (*Patella vulgata*). Toutes les espèces maintiennent l'oxygénation de l'eau de la cavité du manteau sous la P_{O_2} normoxique. Il y a une corrélation positive entre la P_{O_2} moyenne de la cavité du manteau et le taux métabolique standard (SMR) qui est maximal chez les pétoncles et minimal chez les moules de vase. Les pétoncles montrent une distribution de fréquence typique des P_{O_2} avec des pics entre 3 et 10 kPa, alors que les moules de vase et les patelles maintiennent surtout des valeurs <5 kPa. Seuls *A. islandica* et *P. vulgata* possèdent des patrons temporels discernables dans leurs séries chronologiques de P_{O_2}. L'ajustement de la P_{O_2} de la cavité du manteau à un niveau inférieur aux valeurs ambiantes par pompage contrôlé empêche l'établissement de forts gradients d'oxygène entre les tissus des bivalves et le liquide environnant, ce qui limite le flux d'oxygène à travers la surface corporelle. Les patrons de P_{O_2} dans l'eau de la cavité du manteau correspondent aux écotypes des mollusques.

[Traduit par la Rédaction]

Introduction

In contrast to most terrestrial habitats, oxygenation in the marine environment can be very variable in space and time. P_{O_2} in water is a function of physical factors such as temperature, currents, and surface mixing, with lowest P_{O_2} in stagnant, low-salinity, warm water bodies. Further, light can influence the water P_{O_2} through the initiation of macro- and micro-algal photosynthesis. In intertidal pools and shallow areas, algal photosynthesis can produce hyperoxic conditions reaching as high as 300% of superoxygenation on very sunny days (1980; Bridges et al. 1984), causing bubble formation as the water warms and oxygen solubility decreases, which can lead to the formation of hydrogen peroxide through photo-oxidation of light-absorbing dissolved organic matter (Abele-Oeschger et al. 1997). In marine systems, chemical and microbial oxygen demand, as well as heterotrophic respiration of plants and animals, are the major oxygen sinks. Microbial respiration and the chemical oxidation of its reduced end products such as hydrogen sulphide, especially in the sediment–water interface, can lead to complete consumption of the available oxygen (De Wit et al. 1989; Fenchel and Finlay 1995).

Marine invertebrates colonize environments of both high and low oxygen concentrations, and many species tolerate large and rapid fluctuations of environmental oxygenation and even prolonged exposure to hypoxic and anoxic condi-

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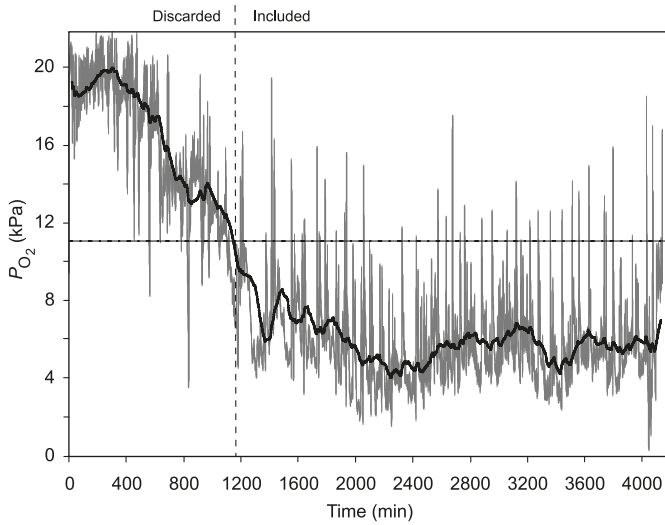**E.E.R. Philipp.** Institute of Clinical Molecular Biology, University Kiel, Schittenhelmstrasse 12, 24105 Kiel, Germany.

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Table 1. Shell size and body mass of the five species.

Species	N	Shell length (cm; mean ± SD)	Shell-free wet body mass (g)		
			Mean	Mini-mum	Maxi-mum
Aequipecten opercularis	12	5.5±0.4	5.8	3	14
Arctica islandica	11	5.3±0.4	8.1	5	10
Mya arenaria	12	4.8±0.3	17.5	14	20
Patella vulgata	11	4.4±0.5	1.6	1.1	2.3
Pecten maximus	12	11.1±1.2	28.7	18	40

Fig. 1. Elimination of biased initial data, example showing one *A. opercularis* time series. The shaded line represents the measurements, the bolded line represents the moving average over 220 measurements (MA_{220}). The horizontal line is the ninth percentile (P_9) of virtually unbiased data (9.76 kPa, all data > 1500 min). The broken vertical line indicates the intersection of MA_{220} and P_9.

tions in sedimentary habitats (Dries and Theede 1974; Oeschger 1990; Theede 1973). Many hypoxia-tolerant benthic organisms are sensitive to hyperoxic conditions, and some species even avoid exposure to normoxic P_{O_2} (Abele et al. 1998; Corbari et al. 2005). In particular, soft-bodied animals from sedimentary habitats, which take up a major proportion of O_2 via the body surface (Schmidt-Nielsen 1975), suffer oxidative stress when exposed to normoxic and higher O_2 concentrations. Many hypoxia- and anoxia-tolerant marine invertebrates respire in an oxyconforming manner (respiration positively correlated with P_{O_2}) above a low critical level of P_{O_2} (= P_{crit}:P_{O_2} below which mitochondrial respiration is limited by cellular O_2 availability), presumably to consume excess oxygen and keep tissue oxygenation low (Abele et al. 1998; Buchner et al. 2001; Pörtner and Grieshaber 1993). Tissue and cellular P_{O_2} in several marine animal ectotherms, including crustaceans and molluscs, were found to range mostly below 3 kPa and to be stabilized at this low level against the environmental fluctuations, comparable with tissue oxygenation in vertebrates (for review, see Massabuau 2001). Oxygen levels ranging considerably above 3 kPa within a tissue can accelerate the formation rate of hazardous reactive oxygen species (ROS), and oxidative stress is assumed to increase linearly with tissue O_2 concentration. Over 90% of cellular ROS generation results from mitochondrial respiratory activity (Balaban et al. 2005; Turrens 2003), in addition to a smaller proportion of ROS that originates from cytosolic and peroxisomal redox reactions, as well as cellular NADPH oxidases (Lambeth 2004). Higher tissue oxygenation (P_{O_2}), higher site-specific redox potential (E_{OXsite}) of mitochondrial electron transporters, and higher numbers of mitochondria and mitochondrial chain units are all bound to increase the release rates of ROS (Q_{ROS}) from the mitochondrial compartment as a first-order kinetic function (see eq. 1 in Balaban et al. 2005). ROS (superoxide anions, H_2O_2, OH·) are highly potent chemical oxidants and, if not immediately detoxified, interact with oxidizable lipids, proteins, and DNA and jeopardize cellular structure and metabolism (Barja 2004; Brunk and Terman 2002). Although the vast majority of generated mitochondrial ROS are detoxified by mitochondrial antioxidant systems (Balaban et al. 2005), hyperoxic tissue P_{O_2} increases the risk of oxidative stress in cells and tissues. On the other hand, too low tissue oxygenation leads to an increased reduction state of electron transporters (E_{OXsite}), and when oxygen is re-introduced into the tissue, ROS formation rates become elevated during the onset of reoxygenation compared with stable control P_{O_2} conditions (Boutilier 2001; Li and Jackson 2002; Nohl et al. 1993). Therefore, it is important for marine invertebrates with open circulatory systems and high body surface oxygen uptake to establish low and constant P_{O_2} levels around and within their tissues (Abele et al. 2007).

Various strategies are employed by marine invertebrates to maintain tissue P_{O_2} low and within tolerable margins (see the "low tissue oxygen strategy" proposed by Massabuau 2003). A prime behavioural strategy of many infaunal sediment dwellers is the retreat to low O_2 sediment horizons in search of a constant low P_{O_2} environmental atmosphere (Corbari et al. 2004), preferably between 3 and 7 kPa. In the confined spaces of sediment burrows often lined with mucus, animals manage to lower the P_{O_2} by collective or individual respiration to levels that they can tolerate. The same applies for epifauna: Davenport and Irwin (2003) documented maintenance of low mantle cavity water P_{O_2} in three different barnacle species against a normoxic environment. Shell water P_{O_2} in Antarctic intertidal snails is maintained below 6 kPa in fully oxygenated seawater (Weihe and Abele 2008). Control of arterial P_{O_2} to low levels is affected by ventilation in larger crustaceans and also in animals with closed circulatory systems such as fish (Itazawa and Takeda 1978; Forgue et al. 1989; Massabuau and Burtin 1984).
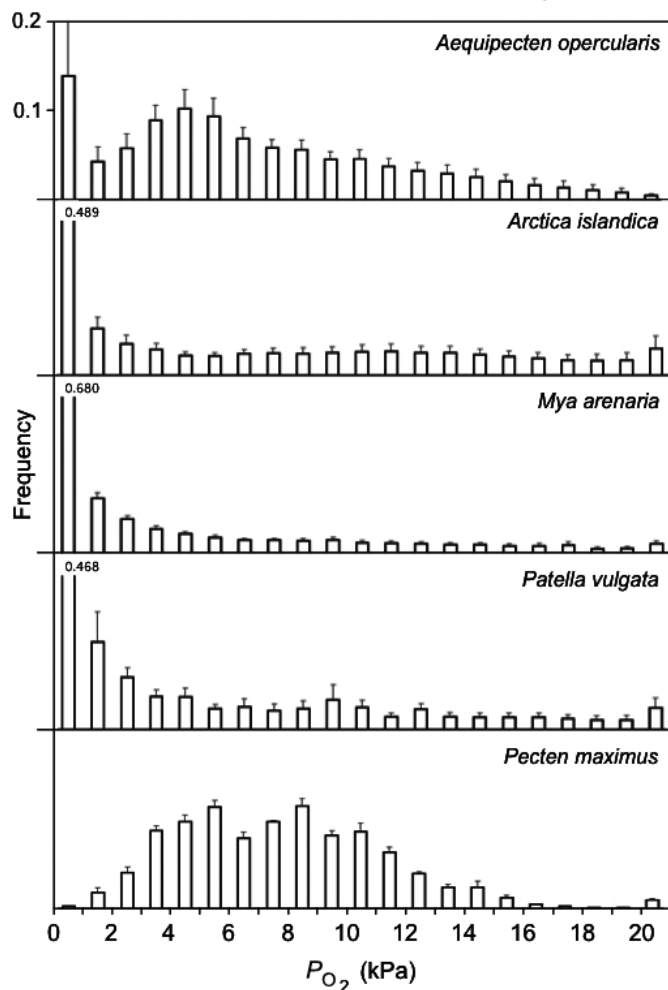
In the present study, we recorded the P_{O_2} in mantle cavity

Table 2. Can the different species be identified by average P_{O_2} values? Comparison of median mantle cavity water P_{O_2} values between species by analysis of variance (ANOVA) and post hoc Tukey's honestly significant different (HSD) test on differences between least squares means.

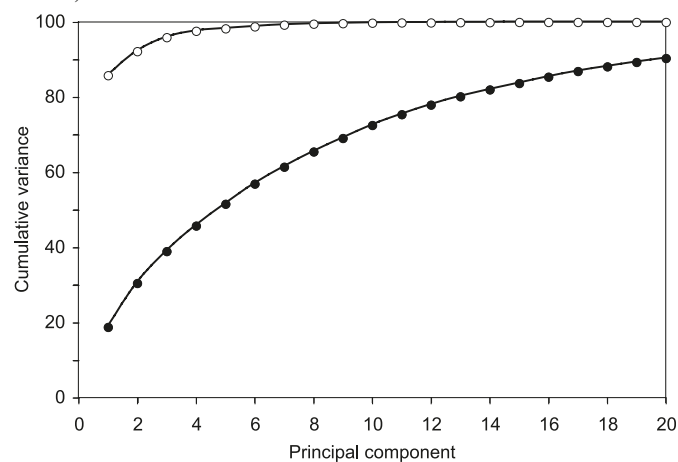|	Median P_{O_2}			Post hoc test		
Species	Group mean	Mini-mum	Maxi-mum	Group A	Group B	Group C
Aequipecten opercularis	6.18	0.16	13.37	A	B	—
Arctica islandica	3.62	0	15.92	—	B	C
Mya arenaria	0.37	0	2.62	—	—	C
Patella vulgata	2.62	0	8.33	—	B	C
Pecten maximus	8.30	2.57	13.82	A	—	—

Note: Overall level of significance is $P < 0.001$. Groups differ significantly at $P < 0.05$.

Fig. 2. Frequency distribution of measured P_{O_2} values across 1 kPa classes (21 kPa = 100% saturation) for each species. Note that *y* axes are of the same scale for all species. Numbers above columns indicate maximum values outside the *y*-axis range.

Fig. 3. Principal component analysis of P_{O_2} frequency data (21 classes from 0–1 kPa to <20 kPa) and of P_{O_2} time series data (149 spectral density classes from 0.0008 to 0.060 min^{-1}). The plot shows cumulative variance explained by the first 20 principal components for frequency data (open circles) and time series data (solid circles).
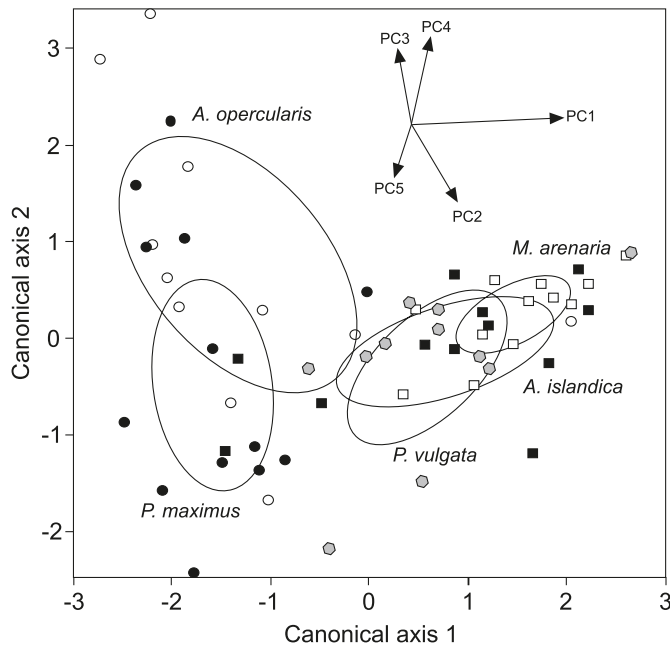
We further assume that both the extent and the temporal pattern of P_{O_2} reduction are specific to a particular ecotype; in particular, swimming scallops might preserve higher and perhaps more fluctuating P_{O_2} levels. Accordingly, we analyze (*i*) whether ecotypes differ in average mantle cavity P_{O_2} (even under conditions of locomotory restriction), (*ii*) whether there are ecotype-specific preferred P_{O_2} ranges, and (*iii*) whether we can detect specific, behaviour-related rhythms and periodicities in the P_{O_2} recordings.

Materials and methods

Animal collection and maintenance

The limpet (*Patella vulgata*) and the queen scallop (*Aequipecten opercularis*) were provided by the Station Biologique de Roscoff, France (48°43.7′N, 3°59.2′W) in summer 2006. Limpets were collected from the intertidal zone near the station, and *A. opercularis* were fished north of Roscoff at a water depth of 60 m. Both species were maintained for less than 2 weeks in constant-flow aquaria in the Roscoff Biological Station until sent to the Alfred Wegener Institute (AWI), Bremerhaven, Germany. Great Jacques scallops (*Pecten maximus*) had been maintained in the aquarium of AWI since October 2005. These animals came originally

water of five marine molluscs under normoxic conditions in experimental systems (21 kPa). Because we used optical oxygen sensors, animal movements within the aquarium had to be restrained. However, the animals could perform normal shell clapping and contraction movements in site. We hypothesize that as a first step towards maintenance of low haemolymph and tissue oxygenation, the P_{O_2} in the shell water should be reduced against the outside oxygen levels.

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Fig. 4. Canonical plot of the quadratic discriminant analysis (QDA, on the covariance matrix) based on the frequency distribution of the individual P_{O_2} time series. QDA is applied to the 58 individuals × 5 categories matrix (principal components 1 to 5 of the 58 P_{O_2} frequency distributions). Ellipses indicate 95% confidence limits of the multivariate mean of the corresponding species. Arrows indicate direction and strength of each variable, i.e., principal component 1 to 5. Species: ■, *A. islandica*; ●, *P. maximus*; □, *M. arenaria*; ○, *A. opercularis*; shaded hexagon, *P. vulgata*.

from the "Bay of Morlaix", Bretagne, France (48°40.5′N, 3°53.3′W) from a water depth of 10 m. The salinity around the sampling sites of *P. vulgata*, *A. opercularis*, and *P. maximus* varies seasonally between 33‰ and 35‰. Soft-shelled clams (*Mya arenaria*) were dug in June 2006 on a Wadden Sea intertidal mud flat near Bremerhaven. The animals dwell in sediment depths of 30–50 cm, where summer ambient water temperatures reach up to 15°–18° in July and August (Abele et al. 2002) and down to 0 °C in winter. Salinity is lower than in the open North Sea and ranges from 24‰ to 28‰. The ocean quahog (*Arctica islandica*) was dredged in Kiel Bight (Süderfahrt 54°32.6′N, 10°42.1′E) in summer 2006. The annual ambient water temperature ranges from 2 to 16 °C, with an average temperature of 8 °C. Salinity at this station varies between 18‰ and 26‰ due to the water flowing through the Skagerrak, with an average salinity of 21.8‰.

Aequipecten opercularis, *P. maximus*, and *P. vulgata* were maintained at AWI in aerated, 9 °C seawater at 32‰–33‰ salinity with a 12 h day – 12 h night rhythm. *Mya arenaria* and *A. islandica* were kept in plastic containers with aquarium gravel in aerated seawater of 25‰ salinity. Except for *P. maximus*, maintenance time prior to experimentation never exceeded four weeks. Filter-feeding species were fed ad libitum with live phytoplankton composed of *Nannochloropsis oculata*, *Chlorella*, and *Phaeodactylum tricornutum*, with cell sizes ranging from 2 to 12 μm (DT's Plankton Farm, Sycamore, Illinois, USA) twice a week. *Patella vulgata* grazed the epiphytes of red algae, shipped from

Roscoff, together with the animals. Water quality was checked at weekly intervals with quick tests of Nanocolor®, Germany. Water quality limits were 0.4 mg·L^{-1} ammonium and 0.2 mg·L^{-1} nitrite. Water was exchanged whenever these limits were reached.

Measurements of oxygenation (P_{O_2}) in the mantle cavity water

Measurements of the P_{O_2} in the animals' mantle cavity water were carried out using a fibre-optic oxygen measurement system (i.e., optode) of PreSens (Precision Sensing GmbH, Regensburg, Germany). We used single channel Microx TX-3 oxygen meters equipped with oxygen needle optodes (PSt1-L5-TF) that had fluorescein-coated flat tips of 140 μm diameter. The oxygen-sensitive tips of the optical fibers were protected inside a stainless-steel needle (12 × 0.04 mm) for implantation into the animals. Prior to insertion into the mantle cavity, optodes were calibrated to 100% air saturation with aerated seawater and to 0% using water saturated with nitrogen at 9 °C. The optical tip was gently pushed into the shell water after the needle had been placed and fixed within the mantle cavity.

Animals were kept in aquaria in a cool room at 9 °C and fully aerated seawater at the salinity typical for each animal. The experimental aquaria were laminated with black foil and covered during the experiments to minimize disturbance of the animals by movements in the aquarium.
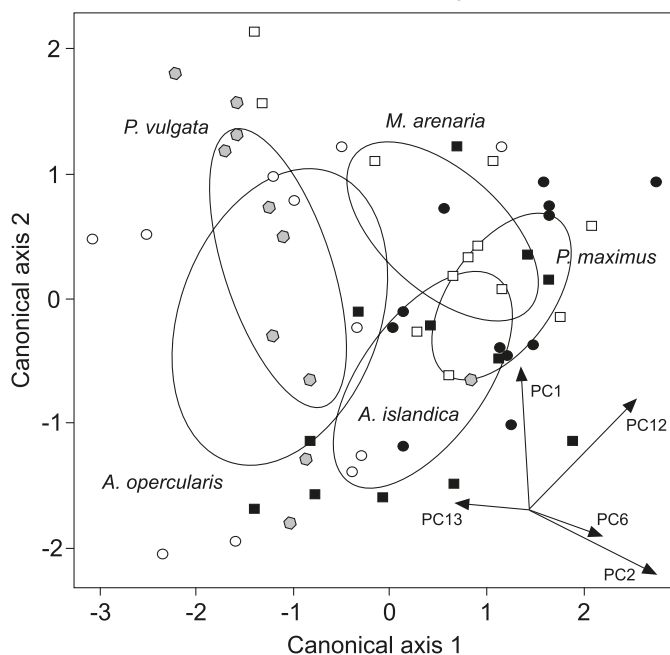
To avoid damage of the optodes, the mobility of the animals was restricted. A Teflon nut was glued to the bivalves lower shell at least 24 h before an experiment so that the animals could later be screwed onto the experimental setup in the aquarium. To insert the fibre-optic oxygen sensor into the mantle cavity of *A. islandica* and *P. vulgata*, a 1 mm hole was drilled into the top of the shell in limpets and into the side of the shell about 1 cm from the edge in *A. islandica* one day before beginning the experiment. The hole was covered with thin elastic latex foil (Rubber Dam, Heraeus Kulzer, Germany). This foil was covered with isolation material (Armaflex, Armacell, Germany) to avoid exchange with the outside water after inserting the optode. A hole was pinched through the isolation material using an injection needle, and the optode was gently introduced into the animal. In *A. opercularis* and *P. maximus*, the sensor could easily be introduced into the mantle cavity during a shell-opening phase. As both mantle edges of *M. arenaria* are mostly grown together, the sensor was inserted through the foot opening. Air saturation was recorded at 30 s intervals using the TX3_v520 software of PreSens. Continuous recordings were carried out over three days and nights for each animal.

Each animal was equipped with the sensor at least 1 h before measurements were started. We observed high intraspecific variability of shell-opening behaviour, affecting the patterns of mantle cavity water oxygenation. Therefore, a minimum of 12 animals per species was studied. On average, *P. vulgata* individuals were smallest and *P. maximus* were largest. Mean shell length ranged from 4.4 to 11.1 cm, whereas mean shell-free body mass covered more than one order of magnitude from 1.6 to 28.7 g. Within each species, body mass covered a range of approximately 50% to 100% of the lightest animal (Table 1).

Table 3. Can the different species be identified by their P_{O_2} frequency? Match–mismatch matrix derived from the quadratic discriminant analysis of P_{O_2} frequency data, applied to the 58 individuals × 5 categories matrix (principal components 1 to 5 of the 58 P_{O_2} frequency distributions). Thirty-eight of 58 animals (65%) have been classified correctly.

	Predicted species				
Actual species	Aequipecten opercularis	Arctica islandica	Mya arenaria	Patella vulgaris	Pecten maximus
Aequipecten opercularis	4	1	1	0	5
Arctica islandica	0	8	2	0	2
Mya arenaria	0	0	12	0	0
Patella vulgata	1	3	1	6	0
Pecten maximus	2	2	0	0	8

Fig. 5. Canonical plot of the quadratic discriminant analysis (QDA, on the covariance matrix) based on the spectral density distribution of the individual P_{O_2} time series. QDA is applied to the 58 individuals × 5 categories matrix (principal components 1, 2, 6, 12, and 13 of the 149 spectral density classes from 0.0008 to 0.060 min^{-1}). Ellipses indicate 95% confidence limits of the multivariate mean of the corresponding species. Arrows indicate direction and strength of each variable, i.e., principal components 1, 2, 6, 12, and 13. Species: ■, *A. islandica*; ●, *P. maximus*; □, *M. arenaria*; ○, *A. opercularis*; shaded hexagon, *P. vulgata*.

Statistical analysis of the P_{O_2} data

Two individuals, one *A. opercularis* and one *P. vulgaris*, appeared to be distinctly stressed during measurements: they were extremely active and maintained fully oxygen-saturated shell water P_{O_2} throughout the whole measurement. These measurements were discarded, reducing the overall data set to 58 animals. Oxygen saturation data (%) were converted to oxygen partial pressure (kPa) prior to further analysis. We carefully checked the initial phase (first 24 h) of each measurement for signs of incomplete adaptation of the individual to the experimental conditions, visible as a distinct downtrend in P_{O_2} into the range maintained during the remaining experimental time. In data sets that we con-

sidered to be biased by this type of stress response (two *A. opercularis* and two *P. maximus*), we applied a simple threshold triggered cutoff procedure: We defined the ninth percentile (P_9) of the right-hand (>24 h), apparently unbiased part of the time series as the threshold and the moving average over 220 measurements (MA_{220}) as the representative of the long-term trend. All data to the left of $MA_{220} = P_9$ were discarded (see example in Fig. 1).

P_{O_2} average level and frequency distribution

We computed the median P_{O_2} of each individual time series and analysed differences between species by means of one-way analysis of variance (ANOVA) with subsequent Tukey's honestly significant difference (HSD) post hoc tests on differences between means (Sokal and Rholf 1995). To compare the P_{O_2} distribution between species, data were converted to frequencies corresponding to 1 kPa classes ranging from class 0–1 kPa to class >20 kPa. The resulting matrix of 58 individuals × 21 P_{O_2} frequency classes was subjected to principal component analysis (on the covariance matrix) to reduce the data set to lower dimensions (Shaw 2003). Subsequently, we applied discriminant analysis (Huberty 1994) to the set of principal components to see how well the five species were separated by means of the P_{O_2} frequency distribution. We used quadratic discriminant analysis, which allows unequal covariance across frequency classes.

P_{O_2} level temporal patterns

To reduce noise for determination of the temporal patterns in the original P_{O_2} time series, we reduced temporal resolution from 30 s to 5 min by averaging across 10 subsequent values. This series was then interpolated with a stiff cubic spline (lambda = 108, e.g., Pollock 1999) that was considered to represent long-term trends (ca. > 500 min range) in the P_{O_2} time series. To eliminate such trends, we used the residuals of the cubic spline interpolation for further analysis. Spectral densities (periodograms) were computed from the coefficients of the Fourier series (Pollock 1999) using the standard settings of the software package JMP (SAS Institute Inc., Cary, North Carolina). Visual inspection indicated that the spectra contained negligible information at frequencies > 0.060 min^{-1} (i.e., periods < 16.7 min) and at frequencies < 0.0008 min^{-1} (i.e., periods > 1250 min). Therefore, we reduced the data set to the frequency space between these limits, i.e., 149 spectral density classes with a resolution of 0.0004 min^{-1} on the frequency axis. Each of

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Table 4. Can the different species be identified by mantle cavity water P_{O_2} pattern? Match–mismatch matrix derived from the quadratic discriminant analysis of P_{O_2} time series data, applied to the 58 individuals × 5 categories matrix (principal components 1, 2, 6, 12, and 13 of the 149 spectral density classes from 0.0008 min^{-1} to 0.060 min^{-1}). Thirty-eight of 58 animals (65%) have been classified correctly.

	Predicted species				
Actual species	Aequipecten opercularis	Arctica islandica	Mya are-naria	Patella vulgaris	Pecten maximus
Aequipecten opercularis	5	2	1	3	0
Arctica islandica	0	10	0	0	2
Mya arenaria	0	2	6	1	3
Patella vulgata	0	0	0	10	1
Pecten maximus	0	1	4	0	7

the 58 spectral density series was adjusted to mean = 0 and standard deviation (SD) = 1 to facilitate comparability. The resulting matrix of 58 individuals × 149 P_{O_2} spectral density classes was subjected to principal component analysis and discriminant analysis as described above.

Relationship between P_{O_2} level and respiration rate

To relate mantle cavity water P_{O_2} to ecotype specific respiration rates, we analysed the effects of body mass (M; joules, range 13 – 474 130 J), temperature (T; Kelvin, range 273.15–303.15 K), and bivalve family on mass-specific respiration rate (MSR; J·J^{-1}·day^{-1}). We choose family as ecotype representative, because of insufficient available data at higher taxonomic resolution in scallops and in gastropods. All available data on Arcticidae (*A. islandica*, *N* = 233), intertidal Myidae (*M. arenaria*, *N* = 198), Pectinidae (eight species, *N* = 629), and Patellidae and Nacellidae combined (three species, *N* = 118) were extracted from a large literature-based databank (Brey 2001, 2010; Supplemental Table S1[2]) and subjected to a full factorial analysis of log(MSR) versus log(M), 1/T, and family.

Results

P_{O_2} average level and frequency distribution

We detected significant differences between median P_{O_2} levels of the different species (ANOVA, *P* <0.001). The post hoc test identified three overlapping groups (Table 2). The two scallops *P. maximus* and *A. opercularis* form one group (A), which has the highest median P_{O_2} (8.30 kPa and 6.18 kPa, respectively). *Aequipecten opercularis*, *P. vulgata*, and *A. islandica* form a second group (B) of intermediate P_{O_2} (6.18–2.62 kPa), and *P. vulgata*, *A. islandica*, and *M. arenaria* constitute the third, low P_{O_2} group (C: 2.62–0.37 kPa). The most obvious difference in the P_{O_2} frequency distribution is in the 3–10 kPa range, where the two scallops show consistently higher frequencies than the other three species (Fig. 2). Unique to *P. maximus* are the low frequencies in the P_{O_2} classes ≤ 2 kPa, as well as in the classes between 16 and 20 kPa.

The first five principal components of the data set of the 58 individuals × 21 frequency classes account for about 98% of cumulative variance in the frequency data (Fig. 3), indicating a high degree of common patterns.

The discriminant analysis based on these components separated the five species very well. The canonical plot indicates a clear separation into scallops and "non"-scallops, but indicates also that some species such as *M. arenaria* and *P. maximus* are very well defined (i.e., low interindividual variability) whereas variability is distinctly higher in species such as *P. vulgata* and *A. opercularis* (Fig. 4). *Mya arenaria* is discriminated best, with 12 out of 12 correctly matched individuals. *Aequipecten opercularis* is discriminated least, with just 4 out of 11 individuals classified correctly (Table 3).

P_{O_2} level temporal patterns

Analysis of the periodograms of P_{O_2} temporal patterns (Fig. 3) indicates less common features and much higher diversity persisting in the time patterns across all 58 individuals than in the frequency distributions. In other words, much less overall variance is explained by the first principal components (Fig. 3). Nevertheless, discriminant analysis based on the five components with highest discriminative power (components 1, 2, 6, 12, 13) separated the five species quite clearly (Fig. 5), although we cannot see the same separation between scallops and non-scallops as in the P_{O_2} frequency analysis above. Twenty of the 58 individuals (<35%) do not match the predictions made by the model (Table 4). *Patella vulgata* and *A. islandica* are discriminated best, with 10 out of 12 individuals classified correctly. *Mya arenaria* is discriminated least, with just 6 out of 12 individuals correctly classified. *Arctica islandica* is characterized by distinct peaks at 0.0040 min^{-1} (period 250 min) and 0.0104 min^{-1} (96 min), whereas *P. vulgata* shows a high frequency peak at 0.0018 min^{-1} (period 555 min) and a second distinct peak at 0.0096 min^{-1} (105 min). *Aequipecten opercularis* exhibits a very "noisy" spectrogram, with peaks all over the frequency range (Fig. 6), corresponding to the scattered distribution of data in the canonical plot (Fig. 5).
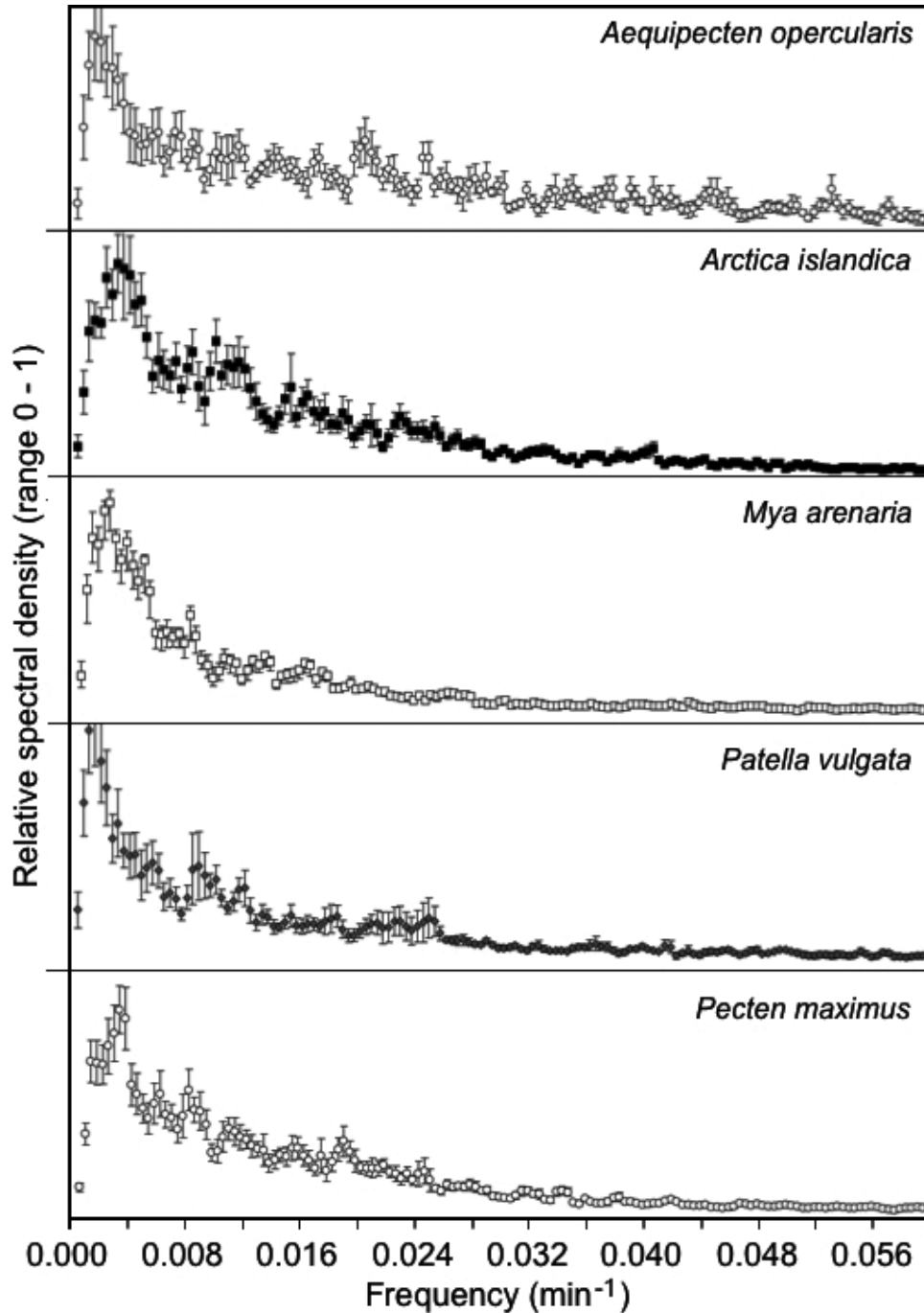
Relationship between P_{O_2} level and respiration rate

Family or ecotype mass-specific respiration rate (MSR) was explained best by the ANCOVA model (reduced to significant terms):

$$\log(\text{MSR}) = 6.621 - 0.259 \cdot \log(M) - 2224.983/T + D1 + D2 \cdot (\log(M) - 4.015)$$

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[2] Supplementary data for this article are available on the Journal Web site (http://cjfas.nrc.ca).

Fig. 6. Average spectral densities (periodograms) of the P_{O_2} time series for each of the five species in the frequency range of 0.0008 to 0.060 min^{-1} (corresponding to periods between 16.7 and 1250 min). Vertical bars represent standard error.

where $D1$ and $D2$ take on the taxon- or ecotype-specific values –0.027, 0.039, 0.144, and –0.156 and –0.009, –0.072, 0.034, and 0.046 for Arcticidae, Myidae, Pectinidae, and Patellidae–Nacellidae, respectively (J·J·day^{-1}, J, K; $N = 1178$, $r^2 = 0.757$).
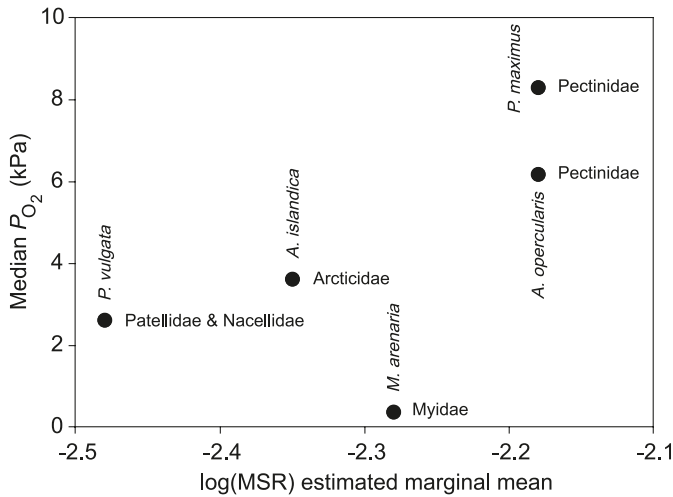
The model indicates that MSR is highest in Pectinidae, does not differ between Myidae and Arcticidae, and is significantly lower in Patellidae–Nacellidae than in all other groups if effects of body mass and experimental temperature are accounted for. This correlates well with mantle cavity P_{O_2} (Sperman rank correlation coefficient = 0.707; Fig. 7).

Only *Mya arenaria* does not fit into the general scheme: in this species, mantle cavity water P_{O_2} is maintained at extremely low levels, or respiration rates reported in the literature are overproportionally high.

Discussion

The P_{O_2} patterns that we recorded and the resulting models support the hypothesis that ecotypes are characterized by, and can be grouped according to, the P_{O_2} dynamics in their mantle cavity water. The separation of scallops from clams and limpets is particularly obvious.

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Fig. 7. Median P_{O_2} versus estimated marginal mean of log(mass-specific respiration rate), i.e., group mean derived from the analysis of covariance (ANCOVA) model, after eliminating the influence of the covariates body mass and temperature.

Scallops maintain significantly higher average P_{O_2}, even under nonswimming conditions, and a frequency distribution of P_{O_2} values with emphasis on the 3–10 kPa range, clearly discriminative against the other species. *Arctica islandica* and *P. vulgata* are distinguished from the other species, including *Mya arenaria*, less through their specific P_{O_2} frequency distribution but more through their P_{O_2} temporal patterns, meaning that P_{O_2} oscillates with specific "rhythms". *Mya arenaria* maintains the lowest average mantle cavity P_{O_2} and also shows a unique P_{O_2} distribution pattern with progressively declining frequencies at higher P_{O_2}s, clearly discriminating this species from the rest.

The positive correlation between median mantle water P_{O_2} and family-specific respiration, calculated from diverse literature data to be more representative of the whole species, supports our hypothesis that P_{O_2} levels represent a lifestyle-specific trait. In the very active epibenthic scallops, adapted to a well-aerated environment, high metabolic rates coincide with high mantle cavity water P_{O_2}, whereas the less active, burrowing mud clams and snails had low P_{O_2} and low respiration rates. Only *M. arenaria* does not fully fit this picture: very low mantle cavity water P_{O_2}, as expected for such a semisessile burrowing animal, contrasts with its disproportionally high MSR. Intertidal *M. arenaria* seem most clearly adapted to life under hypoxic conditions within the sediment (Auffrey et al. 2004). This species colonizes sediment depths between 15 and 25 cm, where the animals respire through long siphons extended through a permanent burrow towards the sediment surface. Specimens of *M. arenaria* remain constantly burrowed and only migrate towards the sediment surface when experiencing complete acute and permanent hypoxia (e.g., under dense algal mats). In so doing, they increase siphon diameter and ventilation volume to counteract the hypoxic situation (Auffrey et al. 2004). The soft-shelled clams in our experiments came from Wadden Sea intertidal mud flats where environmental conditions are highly variable. Many infaunal bivalves are known to reduce or even stop respiration during tidal flat, when P_{O_2} at the sediment surface can be high or low, depending on the

time of day and other oxygen sinks in the system. In our experiments, specimens of *M. arenaria* almost always maintained mantle cavity P_{O_2} as low as 0.4 kPa against fully oxygenated experimental conditions. This results in low mantle cavity water to tissue P_{O_2} gradients and limits the oxygen flux over the mantle surface, avoiding high tissue overoxygenation and increased formation of ROS (hazardous reactive oxygen species). Such low mantle cavity P_{O_2} can only be achieved by control of ventilation rates and siphon diameter, as described by Auffrey et al. (2004), and by elevated rates of respiration once P_{O_2} does increase during ventilation bouts. Indeed, we observed *M. arenaria* rapidly lowering mantle water P_{O_2} after only occasionally occurring peaks by closely controlling the size of the siphon opening and water exchange following these short bouts of intense respiration.

In contrast to *M. arenaria*, *A. islandica* comes from subtidal environments in the Baltic Sea where environmental P_{O_2} in the sediment–water interface is much less variable compared with intertidal conditions. However, during warm summer months and especially as an effect of eutrophication, bottom waters in the Baltic can be extremely low in oxygen and even sulphidic for short periods (Babenerd 1991), and *A. islandica* is among the few survivors in these transiently anoxic zones (Weigelt and Rumohr 1986). These animals live close to the sediment surface and ventilate through short siphons. Periodically they self-induce hypometabolism by closing the shells and burrowing into the sediment and, in the burrowed state, drastically reduce heart beat and tissue energy demand (Taylor 1976). This burrowing behaviour is not only connected to energy saving, but also leads to a reduction of metabolic ROS production and as such may be part of the long lifespan strategy of *A. islandica* (Abele et al. 2008), a species that can easily reach an age of more than 200 years (Schöne et al. 2005). The present investigation confirms this indirectly by showing that when deprived of their sedimentary retreat, *A. islandica* do not maintain low and protective mantle cavity water P_{O_2} comparable with that of the soft-shelled clam or even the limpets over extended stretches of time. This only happens when individuals enter a state of metabolic dormancy, when shells are closed and P_{O_2} rapidly falls to 0 kPa (see Supplemental Fig. S1,[2] original tracks of three *A. islandica* mantle cavity water P_{O_2} recordings).

By contrast, in the mobile scallops, adjustment of mantle cavity water oxygenation at higher P_{O_2} values obviously serves an elevated oxygen demand for higher routine activity and exercise swimming. Bigger *P. maximus* (shell length (SL), 11 ± 1.2 cm (mean ± SD); adductor muscle weight (AMW), 8.8 ± 3.5 g; N = 12) keep mantle cavity water and presumably also haemolymph oxygenation higher than smaller *A. opercularis* (SL, 5.5 ± 0.4 cm; AMW, 2.0 ± 0.5 g; N = 10) to support respiration in their larger muscle mass by maintaining a sufficiently high extra- to intra-cellular P_{O_2} gradient and compensate for the body size related capacity limits of the haemolymph transportation system (Brown et al. 2004). It is interesting to observe, and again may represent a protective mechanism against tissue overoxygenation, that in spite of being dependent on high extracellular oxygen levels, even the scallops maintain mantle cavity water P_{O_2} mostly below 10 kPa, with only occasionally occurring

higher values. The controlling mechanism is obviously behavioural: shell clapping and pumping movements cause an increase in mantle water P_{O_2}, whereas shell closure leads to a decrease. In separate experiments, we triggered shell clapping in scallops by mimicking a sea star attack. Mantle water P_{O_2} increased during escape swimming and rapidly returned to a basal level of 5 kPa within minutes after terminating the response.

Patellid snails are epibenthic like scallops but with respect to P_{O_2} level and frequency patterns cluster together with the mud clams. The P_{O_2} frequency spectrum that we found in limpet mantle cavity water (P_{O_2} mostly < 10 kPa, but higher P_{O_2}s and even 20 kPa can occur) and the more accentuated time pattern indicate again a strong behavioural control of mantle cavity water oxygenation comparable with that of scallops. It remains unknown what triggers the ventilation behaviour, but the mechanism itself also seems to be conserved under prolonged maintenance in capture. Mantle cavity water exchange in limpets depends on water flow and shell-lifting movements (Santini et al. 2000) that produce a kind of ram ventilation. Water is transported through the ventral (inhalant) mantle cavity by ciliary movements into the upper (exhalant) dorsal mantle cavity chamber (see Brusca and Brusca 1990), from where it again leaves the limpets' respiratory system. The low mantle cavity water P_{O_2} may also be a remnant of their early evolutionary history (Ordovicium) in an environment of variable and slowly rising oxygen concentration (Canfield and Teske 1996) that favoured slowly performing lifestyles. Similar P_{O_2} was determined in the postbranchial haemolymph of the prosobranch abalone *Haliotis iris* (77 torr (10 kPa) in the efferent ctenidial vein) by Ragg and Taylor (2006). Work carried out in parallel with the Antarctic limpet (*Nacella concinna*; Weihe and Abele 2008) documented P_{O_2} in limpet mantle cavity water to be maintained at a low level (mean value < 3 kPa). We could show that animals that are able to fully contract to the experimental "rock" surface during aerial exposure lowered the mean P_{O_2} value to below 0.1 kPa, whereas animals that did not contract completely maintained between 1 and 3 kPa P_{O_2} in shell water. Thus, during low tides, limpets that tightly contracted their shell over the body surface not only reduced water loss, but also exposed part of their soft tissue transiently to severely lower oxygen partial pressure. As patellides are also able to respire over their ctenidial fringe during air exposure, the animals do not run the risk of asphyxiation under these conditions. In contrast, the *N. concinna* study indicates that the subtle but significant reduction in shell water P_{O_2} in air-exposed limpets could be instrumental in switching on hypoxia-activated gene transcription (E. Weihe, C. Held, M. Lucassen, and D. Abele, unpublished data).

To summarize, of the marine invertebrates, bivalve and gastropod molluscs are most clearly adapted to low metabolic performance. They are famous for their capacities to survive without oxygen and employ specialized anaerobic pathways, avoiding a Pasteur effect under environmental hypoxia (Oeschger 1990). Here we have shown that even in the fully oxygenated experimental setup, they control the oxygen levels in their mantle cavity water at low and protective levels (see also Massabuau 2003) by the ventilator pattern. Apparently, these P_{O_2} patterns were optimized to match ecotype-dependent energy demand with the environmental oxygen availability. This strategy is found in evolutionarily early prosobranch limpets and is still the same in later-evolved eulamellibranch bivalves. Even actively swimming scallops do not allow much more than 10 kPa oxygen in the mantle cavity water, although one would imagine that their mantle cavity water would approach external ambient oxygen concentration during clapping movements, which the animals could perform in the experimental setup. As most bivalves are relatively small animals and their body fluids are in direct exchange with the ambient water, it seems to be a prime protective function of the shell to isolate the animals from too high environmental oxygenation next to the shielding effect against predators. The shells and, in some cases, the mantle margins help the animals to establish and control the desired P_{O_2} in the confined environment surrounding the tissues, and shell-closing behaviour may form part of an antioxidant strategy, supporting slow aging, negligible senescence, and long life expectancy in several bivalve molluscs (Abele et al. 2008; Strahl et al. 2007).

Acknowledgements

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