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Whole animal and gill tissue oxygen uptake in the Eastern oyster, *Crassostrea virginica*: Effects of hypoxia, hypercapnia, air exposure, and infection with the protozoan parasite *Perkinsus marinus*[1]

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Abstract

The Eastern oyster, *Crassostrea virginica*, lives in shallow coastal waters and experiences many different environmental extremes including hypoxia, hypercapnia and air exposure and many oysters are infected with the protozoan parasite *Perkinsus marinus*. The effects of these conditions on oyster metabolism, as measured by oxygen uptake, were investigated. Mild hypercapnia had no effect on the ability of oysters to regulate oxygen uptake in hypoxic water, as measured by the B2 coefficient of oxygen regulation. The average B2 was -0.060×10^{-3} ($\pm0.01\times10^{-3}$ S.E.M.; $n=20$; low and high CO_2 treatments combined) in oysters uninfected with *P. marinus* and -0.056×10^{-3} ($\pm0.01\times10^{-3}$ S.E.M.; $n=16$; low and high CO_2 treatments combined) in infected oysters. There was no significant effect of light to moderate infections of *P. marinus* on oxygen regulation. Nor did the presence of *P. marinus* have an effect on the rate of oxygen uptake of whole animals in well-aerated water. In well-aerated conditions, oxygen uptake was significantly reduced by moderate hypercapnia in oysters when data from uninfected and infected oysters were combined. Mean oxygen uptake of infected oysters under hypercapnia (pCO_2 = 6–8 Torr; pH 7) was 9.10 μmol O_2 g ww^{-1} h^{-1} ±0.62 S.E.M. ($n=9$), significantly different from oxygen uptake under normocapnia (pCO_2 ≤1 Torr; pH 8.2) (10.71 μmol O_2 g ww^{-1} h^{-1} ±0.62 S.E.M.; $n=9$). Similar to what occurred in infected whole animals, mean oxygen uptake of uninfected gill tissues under high CO_2, low pH conditions was 9.44 μmol O_2 g ww^{-1} h^{-1} ±0.95 S.E.M. ($n=10$), significantly different from oxygen uptake under low CO_2, high pH conditions (12.30 μmol O_2 g ww^{-1} h^{-1} ±0.95 S.E.M.; $n=10$). This result is due primarily to the low pH induced by hypercapnia rather than a CO_2-specific effect. The presence of *P. marinus* had no effect on oxygen uptake in gill tissues. Intertidal oysters from South Carolina take up very little

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oxygen from the air when they are air exposed. Mean oxygen uptake in air at 25°C (5.66×10^{-4} μmol O_2 g ww^{-1} $h^{-1} \pm 2.65 \times 10^{-4}$ S.E.M.; $n = 11$) is less than 0.1% of oxygen uptake in seawater, suggesting that upon air exposure, oysters close their valves and isolate themselves from air. Oxygen uptake in air is slightly elevated at 35°C (9.28×10^{-4} μmol O_2 g ww^{-1} h^{-1} $\pm 5.57 \times 10^{-4}$ S.E.M.; $n = 11$). There was not a strong correlation between oxygen uptake and *P. marinus* infection intensity at either 25 or 35°C.

Keywords: Air exposure; Hypercapnia; Hypoxia; Oyster; *Perkinsus*; Respiration

1. Introduction

The Eastern oyster, *Crassostrea virginica*, lives in shallow coastal waters and experiences many different environmental extremes of temperature, salinity, pH and oxygen. In some parts of its range, oysters are abundant in the intertidal zone and are exposed to air on a regular basis. This remarkable ability of *C. virginica* to succeed in environments characterized by rapid changes accounts for its abundance in the past. However, at present, oyster populations along the Atlantic coast are severely depleted (MacKenzie, 1996). Factors responsible for this depletion include overharvesting (Hargis and Haven, 1988; MacKenzie, 1996), disease (Ford and Tripp, 1996), poor water quality (Seliger et al., 1985) and habitat destruction (Rothschild et al., 1994). Oyster production along the Atlantic coast, including the Chesapeake Bay, has declined, especially since 1980 (MacKenzie, 1996). The commercial harvesting of oysters is now a small fraction of what it once was. Maintenance and restoration of oyster populations in East Coast estuaries have been the thrust of coastal management efforts.

Low dissolved oxygen, or hypoxia, is a major factor limiting animal distribution and is also responsible for affecting species composition in a community (Llanso, 1992). Diurnal fluctuations in oxygen have been reported in estuarine waters along the East Coast, including the Chesapeake Bay (Breitburg, 1990; Diaz and Rosenberg, 1995) and the Southeast (Fulton et al., 1993; Cochran and Burnett, 1996; Lenihan and Peterson, 1998). Hypoxia has been well studied in the Chesapeake Bay, where dissolved oxygen can drop to less than 2 mg l^{-1} (Breitburg, 1990). In South Carolina, the dissolved oxygen in tidal salt marsh creeks can drop from approximately air saturation at high tide to significantly lower values, often close to zero, at low tide (Cochran and Burnett, 1996). Dissolved oxygen is typically at its lowest during early morning low tides (Fulton et al., 1993).

While hypoxia has been a water quality parameter used to indicate the health of estuaries (Officer et al., 1984; Breitburg, 1990; Nixon, 1993), the elevated levels of carbon dioxide, or hypercapnia, that often accompany hypoxia are often overlooked. Hypercapnia is a result of respiration and can acidify even shallow estuarine waters, especially during the night hours (Cochran and Burnett, 1996). When hypercapnia occurs, organisms experience an acidification of the body fluids and tissues, which can affect important physiological processes (Burnett, 1997). The degree to which organisms can compensate for this acidosis is often limited (Burnett, 1997). In bivalves, compensation for the hypercapnia-induced acidosis involves the dissolution of the

calcium carbonate shell, (Lindinger et al., 1984; Dwyer and Burnett, 1996; Burnett, 1997). Until recently, hypercapnia has been largely ignored as a significant factor influencing the health of estuarine organisms; it should be considered in concert with the effects of hypoxia.

In addition to fluctuating water variables, South Carolina oysters experience frequent periods of aerial exposure (Dame, 1979). Fully 95% of oysters in South Carolina are intertidal (Dame, 1979). Oysters exposed to air experience a wide range of temperatures, reaching as high as 55°C (L. Coen and D. Bushek, personal communication). These high temperatures undoubtedly influence oyster metabolism. Emersed oysters are thought to metabolize aerobically as their tissues acidify due to the accumulation of CO_2 (Dwyer and Burnett, 1996), however, the mechanisms oysters use to continue aerobic metabolism during air exposure are not clear. Although many intertidal animals utilize oxygen from the air when immersed (Widdows et al., 1979; McMahon, 1988), oxygen uptake by *C. virginica* exposed to air has not been demonstrated (Shumway, 1982). Nonetheless, despite the harsh conditions associated with air exposure, oysters are abundant in the intertidal zone in South Carolina.

The presence of oyster disease is an important additional factor limiting the abundance of oysters. The protozoan parasite *Perkinsus marinus*, also known as Dermo, has contributed greatly to oyster mortality along the Atlantic and Gulf Coasts (Mackin and Ray, 1954; Andrews and Hewatt, 1957; Mackin, 1962; Andrews, 1988). Infections spread when prezoosporangia are released from infected, dying oysters into the water column and are ingested by uninfected oysters (Ray, 1954; Mackin, 1962; Andrews, 1965, 1996). Once an oyster is infected, the parasite may spread to all tissues, causing cell lysis and eventually death (Mackin, 1951).

It is the focus of this study to explore how the environmental variables of hypoxia, hypercapnia, and air exposure, as well as infections of *P. marinus*, affect the aerobic metabolism of oysters. A number of questions are addressed in this study. First, how does elevated ambient CO_2 (hypercapnia) affect the degree to which oysters regulate their oxygen uptake as oxygen pressure declines? Secondly, what is the effect of hypercapnia on oxygen uptake of oysters in well aerated conditions ($pO_2 = 125–145$ Torr)? Thirdly, what are the effects of CO_2 and pH on oxygen uptake of gill tissues from oysters? Preliminary experiments showed that under high CO_2, low pH conditions, a decrease in oxygen uptake in gill tissues occurred when compared to low CO_2, high pH conditions. Therefore, in this study, gill tissue experiments were designed to elucidate the specific and independent effects of CO_2 and pH on the metabolism of gill tissues uninfected with *P. marinus*. Fourthly, do oysters take up oxygen upon air exposure and if so, is this influenced by temperature? Finally, how does the presence of *P. marinus* affect aerobic metabolism of oysters under a variety of environmental conditions?

2. Methods

2.1. Experimental animals

In this study, both oysters infected with *P. marinus* and uninfected oysters were used. Previous studies (Dwyer and Burnett, 1996; Bobo et al., 1997) have demonstrated that

virtually all South Carolina oysters were infected with *P. marinus*. Therefore, oysters uninfected with *P. marinus* were obtained from tray culture in June, 1996 from the Pemaquid Oyster Company, Waldoboro, Maine. Adult oysters (height = 60–78 mm) were shipped on ice overnight to the Grice Marine Laboratory. South Carolina oysters (height = 40–113 mm) were field collected regularly from Inlet Creek and on a tidal flat near the Grice Marine Laboratory, Charleston, South Carolina from September 1996 to May 1997.

In order to determine the effects of *P. marinus* on whole animal oxygen uptake, it was necessary to induce *P. marinus* infections in a subset of Maine oysters. This was done in the laboratory by placing Maine oysters in aquaria with South Carolina oysters infected with *P. marinus* for approximately 1 month. Likewise, the uninfected subset of Maine oysters were held for approximately 1 month in the laboratory as well with no South Carolina oysters and served as a control for infection. Both subsets of oysters were maintained in well-aerated unfiltered seawater (25°C, 25‰ salinity and pH 7.8–8.2) and fed three times a week with *Isochrysis galbana*. Oysters were not fed for a period of 24 h prior to experiments. All statistical calculations were performed using the statistical software Sigmastat. An alpha level of $P \leq 0.05$ was used in all tests of significant differences.

2.2. Perkinsus marinus assay

The total body burden of *P. marinus* (Bushek et al., 1994) was determined for all oysters except those used in gill tissue experiments. For gill tissue respirometry, only the gill tissues used in the experiment were assayed. Immediately following an experiment, the wet tissue from the whole animal or the gill tissues were homogenized and incubated in 5 ml of Ray's fluid thioglycollate medium (RFTM) (Ray, 1952) with 1 ml nystatin. After 5–7 days of incubation, oyster tissues were digested in 2 mol l^{-1} NaOH (20 ml g^{-1} tissue) at 60°C for 3 h. This process digests only the oyster tissue leaving the *P. marinus* hypnospores intact (Choi et al., 1989). Samples were then washed twice with distilled water, stained with Lugol's iodine, and *P. marinus* hypnospores were counted using a hemocytometer (Choi et al., 1989) and quantified as the number of cells per gram wet oyster tissue weight (g ww).

2.3. Whole animal respirometry

Whole animal oxygen uptake of Maine oysters, both those that were infected with *P. marinus* and those that were uninfected, was measured under low CO_2, high pH conditions ($pCO_2 \leq 1$ Torr; pH 8.2; $pO_2 = 155$ Torr) and high CO_2, low pH conditions ($pCO_2 = 6$–8 Torr; pH 7.0; $pO_2 = 155$ Torr). Low CO_2, high pH conditions occur typically in well-aerated seawater. High CO_2, low pH conditions mimic those that occur during environmental hypoxia and hypercapnia (Cochran and Burnett, 1996).

For each oyster (uninfected oysters $n = 10$; infected oysters $n = 9$), O_2 uptake was measured first in low CO_2, high pH and then in high CO_2, low pH conditions within a three day period. Each oyster was scrubbed with a dilute (1%) bleach solution that was neutralized to pH 7.0, and rinsed thoroughly to kill or remove any exterior micro-

organisms and residual chlorine. Oysters were placed in a respirometer (approximate volume = 450 ml) and the respirometer was filled with artificial seawater (25‰), filtered through a 0.45-μm filter prior to use. Oxygen uptake was measured continuously by monitoring the decline of pO_2 within the respirometer from 155 Torr to less than 10 Torr. It took from 2 to 8 h for the oysters to reduce the oxygen pressure within the respirometer to 10 Torr, with most oysters taking approximately 6 h. Temperature was held at 25±0.1°C. pO_2 was measured with a Yellow Springs Instrument (YSI) model 58 oxygen meter and a YSI model 5730 probe with a self stirring sensor tip. Output from the meter was recorded using a Sable Systems Data Acquisition system. Throughout the experiment, pH was monitored with a Corning combination pH electrode place directly in the respirometer. Once the pO_2 in the respirometer fell to less than 10 Torr, the oyster was removed and the seawater was gassed briefly with O_2 to raise the pO_2 to approximately 100 Torr. Oxygen uptake of the seawater without the oyster was measured and found to be negligible, demonstrating that oxygen uptake is attributed solely to the oyster and associated parasites and bacteria within the shell. Also, oxygen uptake of oyster shells (with the oyster removed) was measured and found to be negligible. The volume of the seawater within the respirometer was also measured. When oxygen uptake of a single oyster had been determined under both experimental conditions the oyster was shucked, weighed and the oyster tissue assayed for *P. marinus* (see *P. marinus* assay).

To analyze the data, oxygen uptake was averaged (Cochran and Burnett, 1996) over each decade of oxygen pressure for each treatment (e.g. 140–149 and 150–159 Torr). These data were then normalized and analyzed using the quadratic (or second-degree) polynomial model to determine the degree to which the animal is an oxygen regulator or an oxygen conformer over a range of oxygen pressures (Mangum and van Winkle, 1973). From this model, the quadratic coefficient, B2 was calculated for each O_2 uptake curve. The greater degree to which an organism regulates its O_2 uptake, the more negative the B2 value. A two-factor repeated measures analysis of variance was used to determine the fixed effects of hypercapnia and the presence of *P. marinus* infection on the B2 value. In this analysis, the presence of *P. marinus* is considered a fixed factor, since only the presence or absence, rather than infection intensity, was considered.

The effects of hypercapnia and *P. marinus* infection on O_2 uptake in whole animals under well-aerated conditions (125–145 Torr) were also analyzed. For each oyster, oxygen uptake between pO_2 125 and 145 Torr was determined. A two-factor repeated measures analysis of variance was performed to determine the fixed effects of hypercapnia and *P. marinus* infection on oxygen uptake.

2.4. Low CO_2, high pH experiments

Low CO_2, high pH seawater conditions were obtained by gassing artificial seawater with nitrogen to remove CO_2 ($pCO_2 < 1$ Torr) resulting in a pH between 8.0 and 8.2. The water was then gassed briefly with O_2 to achieve a pO_2 slightly greater than air saturation (i.e. >155 Torr). As the oyster consumed oxygen, CO_2 accumulated in the respirometer, gradually lowering the pH of the seawater. When low CO_2, high pH conditions were desired, the respirometer was flushed with low CO_2, high pH seawater

when the pH within the respirometer fell below 7.8 to raise the pH. The pO_2 of the filtered seawater used to flush the respirometer was adjusted to approximate the pO_2 within the respirometer. In this way, the pH of the water within the respirometer was held above 7.8.

2.5. High CO_2, low pH experiments

To obtain high CO_2, low pH conditions (pCO_2 = 6–8 Torr; pH 7.0; $pO_2 > 155$ Torr), seawater was gassed with a combination of O_2 and 1% CO_2 to achieve a pO_2 between 155 and 160 Torr and a pH between 6.9 and 7.0. Gas mixtures were supplied by Wösthoff gas mixing pumps. Under high CO_2, low pH conditions the pH never fell below 6.9 and no flushing of the respirometer was necessary.

2.6. Gill tissue respirometry

Two separate experiments were performed using gill tissues from oysters. First, gill tissues from Maine oysters uninfected with *P. marinus* were used to determine the independent effects of CO_2 and pH on oxygen uptake. Secondly, gill tissues from South Carolina oysters infected with *P. marinus* were used to determine the effects of infection intensity and CO_2 on oxygen uptake.

In Maine oysters uninfected with *P. marinus*, oxygen uptake by gill tissues was measured between pO_2 100 and 150 Torr under four different seawater conditions ($n = 10$ gill tissue segments per treatment) to determine the specific and independent effects of pH and CO_2. The mean weight of the gill segments from both Maine and South Carolina oysters was 0.26 g$\pm$0.02 S.E.M. In general, it took approximately 20 min for the gill tissues to deplete the oxygen in the respirometer from 150 to 100 Torr. Two seawater conditions are identical to the conditions under which whole animal O_2 uptake was determined: low CO_2, high pH and high CO_2, low pH. To obtain these conditions, artificial seawater was prepared as described above in whole animal experiments. Oxygen uptake of gill tissues was also measured under low CO_2, low pH and high CO_2, high pH conditions. To obtain low CO_2, low pH conditions ($pCO_2 \leq 1$ Torr; pH 7.0; $pO_2 = 155$), seawater buffered with 10 mmol l^{-1} HEPES was gassed with a combination of nitrogen and oxygen to remove CO_2 and achieve a pO_2 of approximately 155 Torr. With continuous gassing, pH was titrated to 7.0 with 5 mmol l^{-1} HCl. HEPES buffer was necessary with this treatment in order to maintain the pH at 7.0. To obtain high CO_2, high pH conditions (pCO_2 = 6–8 Torr; pH 7.8; $pO_2 = 155$ Torr), the buffered seawater was titrated with 5 mmol l^{-1} NaOH while gassing with a combination of O_2 and 1% CO_2 to achieve a pO_2 of approximately 155 Torr and a pH of 7.8. Data from this experiment were analyzed using a two way analysis of variance to determine the fixed effects of pH and CO_2 on oxygen uptake.

Oxygen uptake was also measured in the gill tissues of oysters infected with *P. marinus* (South Carolina oysters) to determine the effects of *P. marinus* infection intensity and CO_2 on oxygen uptake. Oxygen uptake by gill tissues was measured between pO_2 100 and 150 Torr under low CO_2, high pH and high CO_2, low pH conditions ($n = 56$ gill tissue segments per treatment). Artificial seawater was prepared

as described in whole animal experiments. A two way analysis of variance was performed to determine the fixed effects of *P. marinus* infection intensity (intensity quantified using Mackin groupings) and CO_2 on oxygen uptake. Oxygen uptake values were arranged in five groups corresponding to the levels of *P. marinus* infection categorized by the Mackin scale (Gauthier and Fisher, 1990).

The procedure for both gill tissue experiments (Maine oysters uninfected with *P. marinus* and South Carolina oysters infected with *P. marinus*) was identical. Gill tissue from each oyster was excised (average weight of gill segment = 0.26 ± 0.02 g). Oxygen uptake by gill tissues was measured in a manner similar to that described above for whole animals as the tissues depleted oxygen from filtered seawater. pO_2 was measured in a respirometer using a (YSI) model 53 meter with a YSI model LN1532 probe. Output from the meter was recorded on a Sable Systems Data Acquisition system. Temperature was maintained at 25 ± 0.1°C. The seawater was gassed to the appropriate pCO_2, pH and pO_2 levels for each treatment as described above. Prior to the oxygen uptake measurement, each gill segment was rinsed with filtered seawater and incubated separately for approximately 30 min under the appropriate treatment condition. Each gill segment was then rinsed again with filtered seawater and placed in a closed respirometer (approximate volume = 12 ml) with seawater identical to the conditions under which the tissue was incubated. The rate of decline of pO_2 between 150 and 100 Torr in the closed respirometer was recorded. When pO_2 in the respirometer dropped below 100 Torr, the oxygen probe was removed and pH of the water was measured immediately. Gill tissue wet weight and the water volume of the respirometer were measured. Each gill segment was assayed for *P. marinus* as described above.

2.7. Aerial oxygen uptake

In air exposure experiments, only South Carolina oysters were used. Intertidal South Carolina oysters were collected and held in well-aerated artificial seawater (25°C, 25‰ salinity and pH 7.8–8.2). Oysters were not fed and measurements of aerial oxygen uptake were made within 1 week of collection.

Oxygen uptake of air exposed oysters was measured at 25 and 35°C (±1°C). Oxygen uptake in the ribbed mussel, *Geukensia demissa*, was also measured for purposes of comparison. Since there were no values in the literature for aerial oxygen uptake for *C. virginica*, the methodology was validated by comparing oxygen uptake in the ribbed mussel to known values in the literature.

Prior to each experiment, oysters ($n = 11$ oysters at each temperature) were scrubbed with a dilute (1%) bleach solution that was neutralized to pH 7.0, and rinsed thoroughly to remove or kill any exterior microorganisms. To minimize the disturbance of the oyster upon exposure to air, a single oyster was placed in an open respirometer within a tank of well-aerated seawater (25°C) and allowed to remain overnight. The following morning, the respirometer containing the oyster was gently removed from the tank and the water was slowly siphoned out of the respirometer, exposing the oyster to air. An air-tight lid, containing a port for sampling air, was fitted on top of the respirometer and the respirometer was placed in a water bath at either 25 or 35°C (±1°C). Oysters exposed to 35°C were acutely exposed.

Oxygen uptake was measured in air exposed oysters by monitoring oxygen depletion in the respirometer. Initially, 2 ml of air was sampled from the respirometer and analyzed for oxygen using an oxygen analyzer (Applied Electrochemistry S-3A/II). Thereafter, gas was sampled every 2 h for a period of 8 h. Each 2-ml gas sample removed from the respirometer was replaced with 2 ml filtered seawater. Oysters were positioned above the water in the respirometer. Oysters were then shucked, weighed, and the whole animal assayed for *P. marinus*. Oxygen uptake of oyster shells was also measured and found to be negligible. To compare oxygen uptake of whole oysters at 25 and 35°C, a one-way repeated measures analysis of variance was used.

3. Results

3.1. Whole animal respirometry

Following all whole animal experiments, oysters were analyzed for the total body burden of *P. marinus*. The subset of Maine oysters that were maintained in aquaria with no South Carolina oysters were found to be uninfected with *P. marinus*. Maine oysters held in aquaria with South Carolina oysters to induce *P. marinus* infection had light to moderate levels of *P. marinus*. Infection intensities ranged from 250 to 778 162 *P. marinus* cells per gram wet weight^{-1} (g ww^{-1}). On the Mackin Scale (Gauthier and Fisher, 1990), these infection levels ranged from near 0 to slightly above 4.

Oxygen uptake decreases as oxygen pressure declines (Fig. 1). The B2 coefficient indicates the degree of oxygen regulation over a range of oxygen pressures (Mangum and van Winkle, 1973), with more negative B2 values indicating greater regulation. A two-way repeated measures analysis of variance, comparing B2 coefficients, shows that there is no effect of hypercapnia ($P = 0.912$) and no effect of infection with *P. marinus* ($P = 0.771$) (Table 1). The average B2 was -0.060×10^{-3} ($\pm 0.01 \times 10^{-3}$ S.E.M.; $n = 20$; low and high CO_2 treatments combined) in oysters uninfected with *P. marinus* and -0.056×10^{-3} ($\pm 0.01 \times 10^{-3}$ S.E.M.; $n = 16$; low and high CO_2 treatments combined) in infected oysters.

Oxygen uptake of oysters in well-aerated conditions ($pO_2 = 125$–145 Torr) is unaffected by infection with *P. marinus* (two-way repeated measures analysis of variance; $P = 0.251$), but is significantly affected by hypercapnia ($P = 0.017$) (Fig. 2). Moderately high CO_2 conditions significantly lower oxygen uptake (9.10 μmol O_2 g ww^{-1} h^{-1} ±0.62 S.E.M., $n = 9$) compared with low CO_2 conditions (10.71 μmol O_2 g ww^{-1} h^{-1} ±0.62 S.E.M., $n = 9$) (Figs. 1 and 2), but the effect is not large and occurs only when infection status is ignored.

3.2. Gill tissue respirometry

Two separate experiments were performed using gill tissues from oysters. In gill

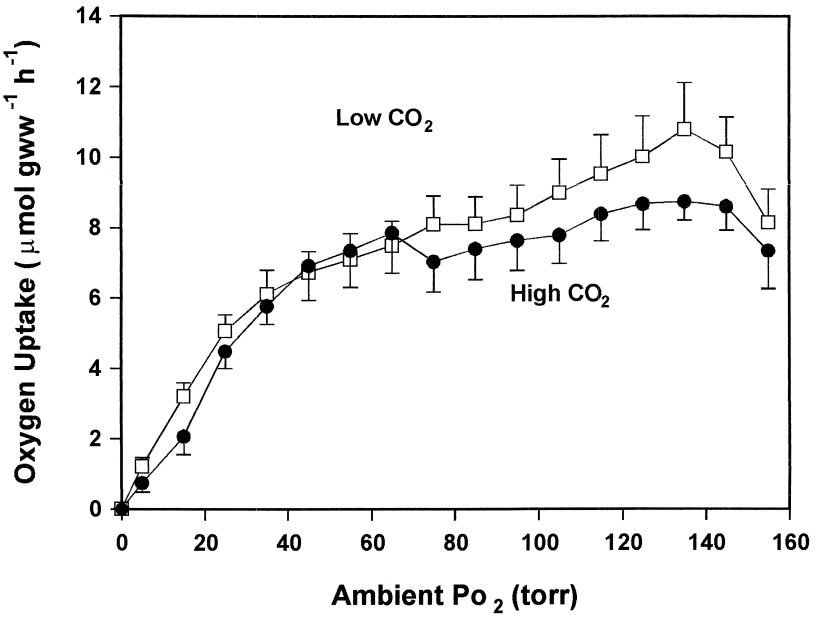

Fig. 1. Mean oxygen uptake (μmol g ww^{-1} h^{-1}) for each decade of pO_2 in *Crassostrea virginica* uninfected with *Perkinsus marinus* and subjected to both low (< 1 Torr) and high (6–8 Torr) CO_2 pressures at 25‰ salinity and 25°C. Error bars show + S.E.M. for low CO_2 and − S.E.M. for high CO_2.

Table 1
Comparison of B2 coefficients of several species of bivalved molluscs[a]

Species	B2 coefficient ($\times 10^{-3}$)		Conditions	*Perkinsus marinus* infection status	Reference
	Mean±S.E.M. (*n*)	Range			
Crassostrea virginica	−0.054±0.01 (10)	−0.002 to −0.132	Low CO_2, High pH	Uninfected	Present study
	−0.066±0.01 (10)	−0.014 to −0.135	High CO_2, Low pH	Uninfected	Present study
	−0.060±0.02 (8)	−0.026 to −0.167	Low CO_2, High pH	Infected; 250 to 778 162 cells g ww^{-1}	Present study
	−0.052±0.02 (8)	−0.005 to −0.100	High CO_2, Low pH	Infected; 250 to 778 162 cells g ww^{-1}	Present study
	−0.063		14‰, 30°C	Unknown	Shumway and Koehn (1982)
	−0.110		28‰, 20°C		
	−0.097		28‰, 30°C		
	−0.12		24.5°C	Unknown	Galtsoff and Whipple (1931)
Geukensia demissa (= *Modiolus demissus*)	−0.2280	−0.0831 to −0.7810		Unknown	Mangum and van Winkle (1973)
Rangia cuneata	−0.0695	−0.0271 to −0.1300		Unknown	Mangum and van Winkle (1973)

[a] Low CO_2, high pH ($pCO_2 \leq 1$ Torr; pH 8.2) and high CO_2, low pH (pCO_2 = 6–8 Torr; pH 7.0) at 25‰ salinity and 25°C are indicated for the present study.

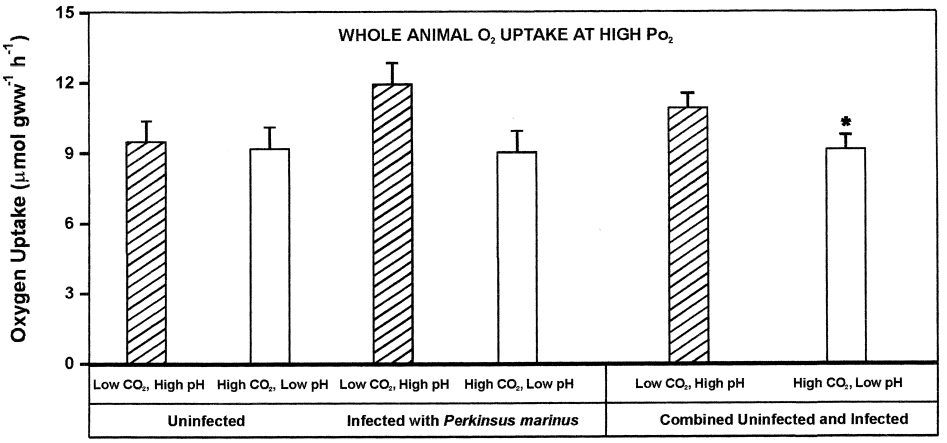

Fig. 2. Mean (+ S.E.M.) oxygen uptake (μmol g ww^{-1} h^{-1}) in *Crassostrea virginica* uninfected ($n = 9$) with and infected ($n + 9$) with *Perkinsus marinus* subjected to low CO_2, high pH ($pCO_2 \leq 1$ Torr; pH 8.2) and high CO_2, low pH ($pCO_2 = 6$–8 Torr; pH 7.0) at $pO_2 = 125$–145 Torr, 25‰ salinity and 25°C. Infection intensities ranged from 250 to 778 162 *Perkinsus marinus* cells per gram wet weight (g ww^{-1}). * Denotes a significant difference ($P < 0.05$, two-way RM ANOVA) and found only when data from uninfected and infected oysters are combined ($n = 18$ for each treatment).

tissues uninfected with *P. marinus*, a two-way ANOVA comparing oxygen uptake at high pO_2 (100–155 Torr) indicated that there was no effect of CO_2 ($P = 0.603$), but a significant effect of pH ($P = 0.041$) (Fig. 3). When data for low and high CO_2 treatments

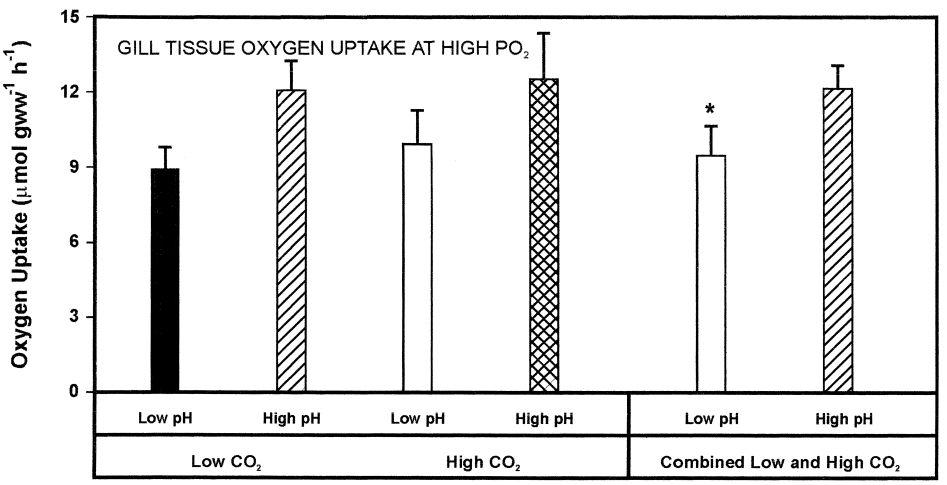

Fig. 3. Mean (+ S.E.M.) oxygen uptake (μmol g ww^{-1} h^{-1}) in gill tissues from *Crassostrea virginica* at high pO_2 (100–155 Torr), 25‰ salinity, 25°C and four treatments: low CO_2, low pH ($pCO_2 \leq 1$ Torr; pH 7.0), low CO_2, high pH ($pCO_2 \leq 1$ Torr; pH 7.8–8.0), high CO_2, low pH ($pCO_2 = 6$–8 Torr; pH 7.0), and high CO_2, high pH ($pCO_2 = 6$–8 Torr; pH 7.8). * Denotes a significant difference between low and high pH treatments ($P < 0.05$, two-way ANOVA) and found only when data from low and high CO_2 treatments are combined.

were combined, oxygen uptake decreased at low pH (9.44 μmol O_2 g ww^{-1} h^{-1} ±0.95 S.E.M., $n = 20$, pH 7.0) when compared with high pH conditions (12.30 μmol O_2 g ww^{-1} h^{-1} ±0.95 S.E.M., $n = 20$, pH 7.8) (Fig. 3).

In gill tissues infected with *P. marinus*, oxygen uptake under low CO_2, high pH conditions was compared with that under high CO_2, low pH conditions. Infection levels ranged from 2×10^3 to 6.0×10^7 *P. marinus* cells g ww^{-1}. A two-way ANOVA, comparing oxygen uptake, showed no effect of infection ($P = 0.24$) but a significant effect of hypercapnia ($P = 0.03$) when infection levels are combined (Fig. 4). Oxygen uptake was depressed under high CO_2, low pH conditions (11.94 μmol O_2 g ww^{-1} h^{-1} ±0.71 S.E.M., $n = 56$) compared with low CO_2, high pH (14.20 μmol O_2 g ww^{-1} h^{-1} ±0.71 S.E.M., $n = 56$) (Fig. 4). Pairwise comparisons showed a significant difference between oxygen uptake at high CO_2 at infection level 3 and high CO_2 at infection level 1 ($P < 0.05$, *t*-test).

3.3. Air exposure

Oxygen uptake of oysters in air is less than 0.1% of their oxygen uptake in well aerated seawater (Table 2). A one-way repeated measures analysis of variance indicated that there was there was a significant effect of temperature on oxygen uptake in air exposed oysters ($P = 0.024$). Aerial oxygen uptake measured at 25°C in the ribbed mussel, *Geukensia demissa*, is similar to values in the literature (Coleman, 1976; Widdows et al., 1979), thereby validating the methodology used in this study (Table 2).

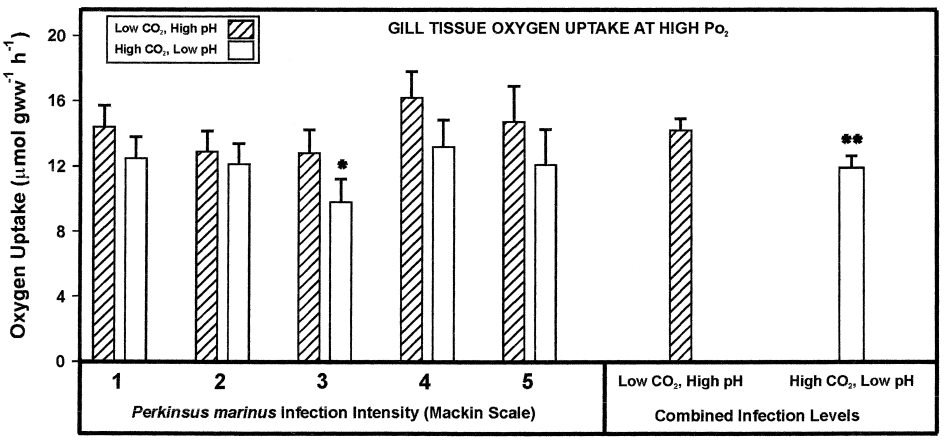

Fig. 4. Mean (+ S.E.M.) oxygen uptake (μmol g ww^{-1} h^{-1}) of gill tissues from *Crassostrea virginica* at low CO_2, high pH ($pCO_2 \leq 1$ Torr; pH 8.2) and high CO_2, low pH ($pCO_2 = 6$–8 Torr; pH 7.0) at $pO_2 = 120$–140 Torr, 25‰ salinity and 25°C. Infection intensities of *Perkinsus marinus* in gill tissues ranged from 1 to 5 on the Mackin Scale (Gauthier and Fisher, 1990) or from 2300 to 60 million *Perkinsus marinus* cells per gram wet weight (g ww^{-1}). * Denotes a significant difference between oxygen uptake at high CO_2 at infection level 3 and oxygen uptake at high CO_2 at infection level 1 ($P < 0.05$, *t*-test). ** Denotes a significant difference between oxygen uptake at low CO_2, high pH and high CO_2, low pH when data from all infection levels are combined ($P < 0.05$, two-way ANOVA).

Table 2
Comparison of aerial oxygen uptake in several species of bivalve molluscs (grams dry weight is abbreviated g dw^{-1})

Species	°C	O_2 uptake			Reference
		Original values	μmol g dw^{-1} h^{-1}		
			Mean	Range	
Crassostrea virginica	25	0.00025–0.0013 μmol g ww^{-1} h^{-1}; $n = 11$	0.002	0.00098 to 0.005	Present study
	35	0.00036–0.00204 μmol g ww^{-1} h^{-1}; $n = 11$	0.004	0.0015 to 0.008	Present study
Geukensia demissa (= *Modiolus demissus*)	25	1.26–4.77 μmol g ww^{-1} h^{-1}; $n = 3$	9.5	4.8–18.2	Present study
	20	0.23 ml O_2 g dw^{-1} h^{-1}	10.3		Widdows et al. (1979)
	21.4	0.2–0.8 ml O_2 g dw^{-1} h^{-1}		9.0–35.7	Coleman (1976)
Mytilus edulis	20	0.025 ml O_2 g dw^{-1} h^{-1}	1.1		Widdows et al. (1979)
	26.4	0.4–0.8 ml O_2 g dw^{-1} h^{-1}		17.9–35.7	Coleman (1973)
Cardium edule	20	0.35 ml O_2 g dw^{-1} h^{-1}	15.6		Widdows et al. (1979)
Crassostrea rhizophorae	25	0.315 ml O_2 g dw^{-1} h^{-1}	14.1		Littlewood (1989)

4. Discussion

Oysters are profoundly affected by changes in ambient oxygen and, to a lesser extent, carbon dioxide. Many investigators have examined the physiological responses of bivalves to hypoxia (Bayne, 1967; Mangum and van Winkle, 1973; Taylor and Brand, 1975; Shumway and Koehn, 1982; Booth et al., 1984; Walsh et al., 1984; de Zwaan et al., 1991; Wang and Widdows, 1993). However, the effects of hypercapnia (i.e. high CO_2), which often accompanies hypoxia (Cochran and Burnett, 1996), have been largely ignored. In fact, it has been suggested that hypercapnia may play a critical role in metabolic regulation (Lindinger et al., 1984). The present study indicates that both low oxygen and, to a smaller extent, moderate hypercapnia (Figs. 1 and 2) act separately to depress the rate of oxygen uptake, and, therefore, the aerobic metabolic rate of oysters. The effects of these variables on anaerobic metabolism were not addressed in the present work.

One advantage of lowering the aerobic metabolic rate is that energy demand can be decreased, thereby conserving substances necessary to carry out physiological processes (Grieshaber et al., 1994). However, metabolic rate depression is not without its disadvantages. A lower aerobic metabolic rate likely explains the detrimental effects of hypoxia on oyster larval settlement and development (Baker and Mann, 1992, 1994), juvenile oyster growth (Baker and Mann, 1992), oyster immune responses (Boyd and Burnett, 1999), adult oyster growth (Osman and Abbe, 1995) and adult survival (Lenihan and Peterson, 1998).

The rate of oxygen uptake by oysters, under well-aerated conditions, as determined in the present study was similar to that found by others with one exception (Table 3). Shumway and Koehn (1982) report significantly lower oxygen uptake, a difference that

Table 3
Comparison of studies measuring the oxygen uptake of *Crassostrea virginica* in well aerated conditions (grams dry weight is abbreviated g dw^{-1})

	°C	O_2 uptake			Reference
		Original values	μmol g dw^{-1} h^{-1}		
			Mean	Range	
Whole animals					
(anatomic size)					
66–75 mm in length; 0.362 g dw^{-1}	25	6.5–15.2 μmol O_2 g ww^{-1} h^{-1}	82	49–116	Present study; low CO_2, high pH
66–75 mm in length; 0.362 g dw^{-1}	25	4.5–14.4 μmol O_2 g ww^{-1} h^{-1}	69	34–110	Present study; high CO_2, low pH
1.68–3.0 g dw^{-1}	24.5	1.72–2.77 mg O_2 g dw^{-1} h^{-1}		54–87	Galtsoff (1964)
0.01–1.0 g dw^{-1}	20	400–2000 μl O_2 g dw^{-1} h^{-1}		18–90	Dame (1972)
0.03–0.70 g dw^{-1}	20	0.1 ml O_2 0.4 g dw^{-1} h^{-1}	11		Shumway and Koehn (1982)
100 × 70 mm	25	0.303 ml O_2 g^{-1} h^{-1}	103		Hammen (1969)
Gill tissues					
(conditions)					
	20	1850–3000 μl O_2 g dw^{-1} h^{-1}		83–134	Percy et al. (1971)
	25	1200 μl O_2 g dw^{-1} h^{-1}	54		Bass (1977)
	20–28	1202–1281 μl O_2 g dw^{-1} h^{-1}		54–58	Scott et al. (1985)
Low pH; 25‰	25	2.7–19.7 μmol O_2 g ww^{-1} h^{-1}	53	15–110	Present study
High pH; 25‰	25	5.9–23.1 μmol O_2 g ww^{-1} h^{-1}	69	33–129[c]	Present study

[a] To determine level of *P. marinus* infection, it was not possible to determine dry weight of oyster tissues. Original values are μmol O_2 g ww^{-1} h^{-1}.
[b] Dry weight for whole animal oysters is 13.1% of the wet weight.
[c] Dry weight for gill tissues is 17.9% of the wet weight.

may be due to a 2–3-week period of starvation for their oysters. In contrast, oysters used in the present study were fed three times a week and deprived of food 24 h prior to the experiment. Other factors that may affect oxygen uptake of bivalves include season and reproductive stage (Bayne, 1976).

Adult oysters regulate oxygen uptake over a wide range of oxygen tensions (Mitchell, 1914; Galtsoff and Whipple, 1931; Galtsoff, 1964; Shumway and Koehn, 1982; present study). We employed a quantitative approach (Mangum and van Winkle, 1973) to determine the degree of regulation. The quadratic coefficient, B2 calculated from the quadratic model (Mangum and van Winkle, 1973), most often has a negative value indicating the degree of oxygen regulation, with more negative B2 coefficients indicating a higher degree of oxygen regulation. Although the B2 coefficient has no real biological meaning it is useful when comparing results from different treatments. The average B2 coefficient of oysters under low CO_2, high pH conditions is $-0.054 \pm 0.01 \times 10^{-3}$, indicating a moderate regulatory response. Under the different experimental treatments mean B2 values ranged from -0.052×10^{-3} to -0.066×10^{-3} (Table 1), although no statistical differences were detected. These values are lower than those reported by Shumway and Koehn (1982) ($B2 = -0.212 \times 10^{-3}$ at 20°C and 28‰ salinity). However, these authors appeared to have analyzed their data using oxygen values as percent air saturation instead of partial pressure in units of Torr, as prescribed by

Mangum and van Winkle (1973). We analyzed their data using oxygen partial pressure, and the resulting B2 values are much closer to the values reported here, but still indicate a slightly greater degree of regulation (Table 1). We also calculated a B2 of -0.12×10^{-3} using the data of Galtsoff and Whipple (1931). The reasons for these differences are unclear but nutritional state and season may be factors. Other bivalves, such as the ribbed mussel, *Geukensia demissa* and the clam, *Rangia cuneata* demonstrate stronger regulatory responses than *C. virginica* with mean B2 values of -0.228×10^{-3} and -0.070×10^{-3}, respectively (Mangum and van Winkle, 1973) (Table 1). However, it is clear in all cases that at oxygen pressures less than 60 Torr oysters decrease oxygen uptake (Fig. 1).

Although moderate hypercapnia depresses the oxygen uptake of oysters under well aerated conditions (Fig. 2), the ability of oysters to regulate oxygen uptake over a wide range of oxygen tensions is unaffected by moderately high CO_2 (Table 1). These results are similar to those found for other estuarine organisms such as the grass shrimp, *Palaeomonetes pugio*, and the mummichog, *Fundulus heteroclitus*, where elevated CO_2 does not influence oxygen regulation (Cochran and Burnett, 1996). Interestingly, Galtsoff and Whipple (1931) were concerned about the effects of metabolite accumulation in the respirometer in their studies of *C. virginica*. They investigated the specific effects of CO_2 and, like the results of the present study, found no influence of CO_2 on oxygen uptake at water pH down to 6.6.

Oxygen uptake of isolated tissues may differ markedly from those observed in whole animals (Percy et al., 1971). However, the results of the present study demonstrate similar rates of oxygen uptake by whole animals and gill tissues and similar responses of each to hypercapnia (Figs. 2 and 3). If slight differences in size and experimental conditions are accounted for, oxygen uptake by gill tissues of oysters in the present study are similar to gill tissues in oysters from other studies (Percy et al., 1971; Bass, 1977; Scott et al., 1985) (Table 3). In gill tissues, high CO_2, low pH conditions lower oxygen uptake significantly (Fig. 3), but this is due specifically to low pH. A similar metabolic depression occurs in oysters infected with *P. marinus*, where high CO_2, low pH conditions significantly lower oxygen uptake when all levels of infection are considered (Fig. 4). If each infection level is considered separately, no significant difference in oxygen uptake is found between CO_2 treatments, with the exception of infection level 3. This is likely due to a smaller sample size for each infection level. Clearly gill tissues decrease their metabolism in response to low pH, suggesting that these tissues may be sensitive to acidic end-products of anaerobic metabolism as well as to low environmental pH. These results are similar to those found in the marine worm, *Sipunculus nudus*, where low extracellular pH depresses aerobic metabolism in body wall musculature under both hypercapnic and normocapnic conditions (Reipschläger and Pörtner, 1996). These authors have also shown that pCO_2 is not specifically responsible for depression of metabolism in *S. nudus*.

The depression of metabolism observed in oyster gill tissues may be due to a reduction in ciliary motion that occurs under high CO_2, low pH conditions (Galtsoff and Whipple, 1931). Reipschläger and Pörtner (1996) suggest that on a cellular level, ion channels and transport proteins may be sensitive to changes in pH, thereby causing a

decrease in metabolic rate. They also suggest that when the pH is low, there may be a decrease in the energy demand for intracellular pH regulation and thus, a decrease in overall tissue metabolic rate (Reipschläger and Pörtner, 1996).

The depression of aerobic metabolism by low pH may be an important mechanism in oysters. Using microcalorimetry, Stickle et al. (1989) showed that oysters maintained 75% of their normoxic energy consumption in extreme hypoxia ($<5\%$ air saturation). At this same oxygen level, oxygen uptake is approximately 10% of that in well-aerated water (present study) accounting for approximately 15% of the overall metabolism, the balance being anaerobic.

Oxygen uptake appears to be unaffected by *P. marinus*. Newell et al. (1994) came to a similar conclusion, although they did not measure oxygen uptake directly. However, only light to moderate infections were present in oysters used in the present study. With heavier infection levels differences may be detected. The lack of an influence of *P. marinus* on oxygen uptake in whole oysters or gill tissues is not entirely unexpected. Measurements of oxygen uptake by *P. marinus* in culture suggest that the oxygen demand by the parasite is only 0.3% of total oxygen uptake in an infected oyster (Milardo and Burnett, 1997). Thus, *P. marinus* does not appear to be competing with the oyster for available oxygen.

In many areas along the East Coast, *C. virginica* is primarily subtidal (Wallace and Lunz, 1968), however, oysters in South Carolina are primarily intertidal (Lunz, 1950) and therefore, experience periods of air exposure. Intertidal bivalves demonstrate a variety of responses to immersion. While some bivalves simply isolate themselves by closing their valves until they are submerged again, others utilize the aerial environment as a source of oxygen to maintain a partially, albeit reduced, aerobic metabolism (Widdows et al., 1979; McMahon, 1988) (Table 3). McMahon (1988) suggested that strategies used by bivalves are largely determined by their position in the intertidal zone. Upon immersion, low and mid-littoral bivalve species generally close their valves and rely primarily on anaerobic pathways coupled with a large reduction in metabolic rate (Widdows et al., 1979; see review by McMahon, 1988). Conversely, high littoral bivalves, such as *Geukensia demissa*, open their shells and obtain oxygen from the air (Lent, 1968). Tropical bivalves also demonstrate gaping and take up oxygen from the air (Littlewood, 1989). The results of the present study indicate that intertidal oysters from South Carolina do not demonstrate an observable shell gape and take up only minuscule amounts of oxygen from the air at 25 and 35°C (Table 3).

It is likely that low pH during immersion (Dwyer and Burnett, 1996) plays a critical role in metabolic depression and may induce a switch to anaerobic metabolism (Reipschläger and Pörtner, 1996). The low levels of pH used in this study precipitate a small, but significant decrease in aerobic metabolism. The more severe acidosis that develops during immersion (pH 6.58; Dwyer and Burnett, 1996) could reduce aerobic metabolism further and facilitate the switch to anaerobic metabolism.

Oyster metabolism is clearly capable of changing as a function of oxygen and pH. The flexibility of the oyster in responding to environmental conditions by regulating its aerobic metabolism in mild hypoxia and shifting to anaerobic metabolism has no doubt contributed to its ability to survive under harsh conditions. It will be interesting to see

how the pH and oxygen levels within the oyster tissues change with hypercapnic hypoxia and air exposure and to understand how the importance of these variables in regulating anaerobic metabolism.

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References

Andrews, J.D., 1965. Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. Chesapeake Sci. 6 (1), 60–67.

Andrews, J.D., 1988. Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. Am. Fish. Soc. Spec. Publ. 18, 47–63.

Andrews, J.D., 1996. History of *Perkinsus marinus*, a pathogen of oysters in Chesapeake Bay 1950–1984. J. Shellfish Res. 15, 13–16.

Andrews, J.D., Hewatt, W.G., 1957. Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. Ecol. Monogr. 27, 1–26.

Baker, S.M., Mann, R., 1992. Effects of hypoxia and anoxia on larval settlement, juvenile growth, and juvenile survival of the oysters *Crassostrea virginica*. Biol. Bull. 182, 265–269.

Baker, S.M., Mann, R., 1994. Description of metamorphic phases in the oyster *Crassostrea virginica* and effects of hypoxia on metamorphosis. Mar. Ecol. Prog. Ser. 104, 91–99.

Bass, E.L., 1977. Influences of temperature and salinity on oxygen consumption of tissues in the American Oyster (*Crassostrea virginica*). Comp. Biochem. Physiol. 58A, 125–130.

Bayne, B.L., 1967. The respiratory response of *Mytilus perna* L. (Mollusca: Lamellibranchia) to reduced environmental oxygen. Physiol. Zool. 40, 307–313.

Bayne, B.L. (Ed.), 1976. Marine Mussels: Their Ecology and Physiology, Cambridge University Press, Cambridge, p. 506.

Bobo, M.Y., Richardson, D.L., Coen, L.D., Burrell, V.G. (Eds.), 1997. A report on the protozoan pathogens *Perkinsus marinus* (Dermo) and *Haplosporidiumnelsoni* (MSX) in South Carolina shellfish populations, South Carolina Marine Resources Division, p. 50, Technical Report No. 86.

Booth, C.E., McDonald, D.G., Walsh, P.J., 1984. Acid–base balance in the sea mussel, *Mytilus edulis*. I. Effects of hypoxia and air-exposure on hemolymph acid–base status. Mar. Biol. Lett. 5, 347–358.

Boyd, J.N., Burnett, L.E., 1999. Reactive oxygen intermediate production by oyster hemocytes exposed to hypoxia. J. Exp. Biol. 202, 3135–3143.

Breitburg, D.L., 1990. Near-shore hypoxia in the Chesapeake Bay: Patterns and relationships among physical factors. Estuar. Coast. Shelf Sci. 30, 593–609.

Burnett, L.E., 1997. The challenges of living in hypoxic and hypercapnic aquatic environments. Am. Zool. 37, 633–640.

Bushek, D., Ford, S.E., Allen, Jr. S.K., 1994. Evaluation of methods using Ray's fluid thioglycollate medium for diagnosis of *Perkinsus marinus* infection in the eastern oysters, *Crassostrea virginica*. Annu. Rev. Fish Dis. 4, 201–217.

Choi, K.S., Wilson, E.A., Lewis, D.H., Powell, E.N., Ray, S.M., 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: Quantification of the thioglycollate method. J. Shellfish. Res. 8, 125–131.

Cochran, R.E., Burnett, L.E., 1996. Respiratory responses of the salt marsh animals, *Fundulus heteroclitus*, *Leiostomus xanthurus*, and *Palaemonetes pugio* to environmental hypoxia and hypercapnia and to the organophosphate pesticide, azinophosmethyl. J. Exp. Mar. Biol. Ecol. 195, 125–144.

Coleman, N., 1973. The oxygen consumption of *Mytilus edulis* in air. Comp. Biochem. Physiol. 45A, 393–402.

Coleman, N., 1976. The aerial respiration of *Modiolus modiolus*. Comp. Biochem. Physiol. 54A, 401–406.

Dame, R.F., 1972. The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica*. Mar. Biol. 17, 243–250.

Dame, R.F., 1979. The abundance, diversity, and biomass of macrobenthos on North Inlet, South Carolina, intertidal oyster reefs. Proc. Natl. Shellfish. Assoc. 69, 6–10.

de Zwaan, A., Cortesi, P., van den Thillart, G., Roos, J., Storey, K.B., 1991. Differential sensitivities to hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. Mar. Biol. 111, 343–351.

Diaz, R.J., Rosenberg, R., 1995. Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr. Mar. Biol. Annu. Rev. 33, 245–303.

Dwyer, III J.J., Burnett, L.E., 1996. Acid–base status of the oyster *Crassostrea virginica* in response to air exposure and to infections by *Perkinsus marinus*. Biol. Bull. 190, 139–147.

Ford, S.E., Tripp, M.R., 1996. Diseases and defense mechanisms. In: Eble, A.F., Kennedy, V.S., Newell, R.I.E. (Eds.), The Eastern Oyster, *Crassostrea virginica*, Maryland Sea Grant, College Park, Maryland, pp. 581–642.

Fulton, M.H., Scott, G.I., Fortner, A., Bidleman, T.F., Ngabe, B., 1993. The effects of urbanization on small high salinity estuaries of the Southeastern United States. Arch. Environ. Contam. Toxicol. 25, 476–484.

Galtsoff, P.S., 1964. The American oyster *Crassostrea virginica* Gmelin. US Fish Wild. Serv. Fish. Bull. 64, 1–480.

Galtsoff, P.S., Whipple, D.V., 1931. Oxygen consumption of normal and green oysters. Fish. Bull. US Natl. Mar. Fish. Serv. 46, 489–508.

Gauthier, J.D., Fisher, W.S., 1990. Hemolymph assay for diagnosis of *Perkinsus marinus* in oysters *Crassostrea virginica* (Gmelin, 1791). J. Shellfish. Res. 9, 367–371.

Grieshaber, M.K., Hardewig, I., Kreutzer, U., Pörtner, H.-O., 1994. Physiological and metabolic responses to hypoxia in invertebrates. Rev. Physiol. Biochem. Pharmacol. 125, 44–147.

Hammen, C., 1969. Metabolism of the oyster, *Crassostrea virginica*. Amer. Zool. 9, 309–318.

Hargis, W.J., Haven, D.S., 1988. Rehabilitation of the troubled oyster industry of the lower Chesapeake Bay. J. Shellfish. Res. 7, 271–279.

Lenihan, H.S., Peterson, C.H., 1998. How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. Ecol. Appl. 8 (1), 128–140.

Lent, C.M., 1968. Air gaping by the ribbed mussel, *Modiolus demissus* (Dillwyn). Effects and adaptive significance. Biol Bull. 134, 60–73.

Lindinger, M.I., Lauren, D.J., McDonald, D.G., 1984. Acid–base balance in the sea mussel, *Mytilus edulis*. III. Effects of environmental hypercapnia on intra- and extracellular acid–base balance. Mar. Biol. Lett. 5, 371–381.

Littlewood, D.T.J., 1989. Aquatic and aerial respiration of *Crassostrea rhizophorae*. Comp. Biochem. Physiol. 93A, 773–775.

Llanso, R.J., 1992. Effects of hypoxia on estuarine benthos: the Lower Rappahannock River (Chesapeake Bay), a case study. Estuar. Coast. Shelf Sci. 35, 491–515.

Lunz, R.G. (Ed.), 1950. Production and yield of the oyster canning industry of South Carolina, Contributions of the Bears Bluff Laboratory No. 9.

MacKenzie, C.L., 1996. Management of natural populations. In: Eble, A.F., Kennedy, V.S., Newell, R.I.E. (Eds.), The Eastern Oyster, *Crassostrea virginica*,, Maryland Sea Grant, College Park, Maryland, pp. 707–719.

Mackin, J.G., 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier. Bull. Mar. Sci. Gulf Car. 1, 73–87.

Mackin, J.G., 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publ. Inst. Mar. Sci. Univ. Tex. 7, 132–229.

Mackin, J.G., Ray, S.M., 1954. Studies on the effect of infection by *Dermocystidium marinum* on ciliary action in oysters (*Crassostrea virginica*). Proc. Natl. Shellfish. Assoc. 45, 168–181.

Mangum, C., van Winkle, W., 1973. Responses of aquatic invertebrates to declining oxygen conditions. Am. Zool. 13, 529–541.

McMahon, R.F., 1988. Respiratory response to periodic emergence in intertidal molluscs. Am. Zool. 28, 97–114.

Milardo, C.S., Burnett, L.E., 1997. The effect of pH on the respiration of the protozoan parasite, *Perkinsus marinus* in vitro. Am. Zool. 37, 146A.

Mitchell, P.H., 1914. The oxygen requirements of shellfish. Bull. US Bur. Fish. 32, 207–222.

Newell, R.I.E., Paynter, K.T., Burreson, E.M., 1994. Physiological effects of protozoan parasitism on the eastern oyster *Crassostrea virginica*: feeding and metabolism. J. Shellfish Res. 13, 294.

Nixon, S.W., 1993. Nutrients and coastal waters: too much of a good thing? Oceanus 2, 39–47.

Officer, C.B., Biggs, R.B., Taft, J.L., Cronin, L.E., Tyler, M.A., Boynton, W.R., 1984. Chesapeake Bay anoxia: Origin, development, and significance. Science 223, 22–27.

Osman, R.W., Abbe, G.R., 1995. Post-settlement factors affecting oyster recruitment in the Chesapeake Bay, USA. In: Dyer, K.R., Orth, R.J. (Eds.), Changes in Fluxes in Estuaries, Olsen and Olsen Press, Fredensborg, Denmark, pp. 335–340.

Percy, J.A., Aldrich, F.A., Marcus, T.R., 1971. Influence of environmental factors on respiration of excised tissues of American oysters, *Crassostrea virginica* (Gmelin). Can. J. Zool. 49, 353–360.

Ray, S.M., 1952. A culture technique for the diagnosis of infections with *Dermocystidium marinum* Mackin, Owen, and Collier in oysters. Science 116, 360–361.

Ray, S.M. (Ed.), 1954. Biological studies of *Dermocystidium marinum* a fungus parasite of oysters, Rice Inst. Pamph. Monogr. Biol. Spec. Issue, p. 114.

Reipschläger, A., Pörtner, H.-O., 1996. Metabolic depression during environmental stress: The role of extracellular versus intracellular pH in *Sipunculus nudus*. J. Exp. Biol. 199, 1801–1807.

Rothschild, B.J., Ault, S., Goulletquer, P., Heral, M., 1994. Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. Mar. Ecol. Prog. Ser. 111, 29–39.

Scott, G.I., Middaugh, D.P., Sammons, T.I., 1985. Interactions of chlorine-produce oxidants (CPO) and salinity in affecting lethal and sub-lethal effects in the eastern or American oyster, *Crassostrea virginica* (Gmelin), infected with the protistan parasite, *Perkinsus marinus*. In: Vernberg, F.J., Thurberg, F.P., Calabrese, A., Vernberg, W.B. (Eds.), Marine Pollution and Physiology: Recent Advances, University of South Carolina Press, Charleston, pp. 351–376.

Seliger, H.H., Boggs, J.A., Giggley, W.H., 1985. Catastrophic anoxia in the Chesapeake Bay in 1984. Science 228, 70–73.

Shumway, S.E., 1982. Oxygen consumption in oysters: An overview. Mar. Biol. Lett. 3, 1–23.

Shumway, S.E., Koehn, R.K., 1982. Oxygen consumption in the American Oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 9, 59–68.

Stickle, W.B., Kapper, M.A., Liu, L.-L., Gnaiger, E., Wang, S.Y., 1989. Metabolic adaptations of several species of crustaceans and molluscs to hypoxia: Tolerance and microcalorimetric studies. Biol. Bull. 177, 303–312.

Taylor, A.C., Brand, A.R., 1975. Effects of hypoxia and body size on the oxygen consumption of the bivalve *Arctica islandica* (L.). J. Exp. Mar. Biol. Ecol. 19, 187–196.

Wallace, E.M., Lunz, G.R., 1968. The oysters . . . a shellfish delicacy. In: Marine Resources of the Atlantic Coast, Atlantic States Marine Fisheries Commission, Leaflet No. 11.

Walsh, P.J., McDonald, D.G., Booth, C.E., 1984. Acid–base balance in the Sea Mussel, *Mytilus edulis*. II. Effects of hypoxia and air exposure on intracellular acid–base status. Mar. Biol. Lett. 5, 359–369.

Wang, W.X., Widdows, J., 1993. Calorimetric studies on the energy metabolism of an infaunal bivalve, *Abra tenuis*, under normoxia, hypoxia and anoxia. Mar. Biol. 116, 73–79.

Widdows, J., Bayne, B.L., Livingstone, D.R., Newell, R.I.E., Donkin, P., 1979. Physiological and biochemical responses of bivalve molluscs to exposure to air. Comp. Biochem. Physiol. 62A, 301–308.