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Acid-Base Status of the Oyster *Crassostrea virginica* in Response to Air Exposure and to Infections by *Perkinsus marinus*

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Abstract. Hemolymph acid-base variables were investigated in the Eastern oyster, *Crassostrea virginica,* to determine its responses to air exposure and to infections by the parasite *Perkinsus marinus.* Infected and uninfected oysters were subjected to two treatments of temperature (21° and 30°C) and air exposure (5 and 24 h). Upon exposure to air, oysters underwent a respiratory acidosis that remained uncompensated in uninfected oysters but was partially compensated in highly infected oysters at both 21° and 30°C. The acidosis was significantly greater in oysters with high infections. Hemolymph in uninfected oysters had a greater buffering capacity (−6.80 ± 0.76 SEM slykes) than hemolymph in highly infected oysters (−3.30 ± 0.50 SEM slykes). Calcium ion concentrations in hemolymph increase when the hemolymph becomes acidic, suggesting that shell decalcification plays a role in buffering the acid. During air exposure, although oysters do not visibly gape, they access air and are apparently not completely anaerobic.

Introduction

Crassostrea virginica (Gmelin), the Eastern (or American) oyster, occurs along the east coast of North America from the Gulf of St. Lawrence, Canada, to Key Biscayne, Florida, and along the shores of the Gulf States. In South Carolina estuaries, the species is essentially limited to the intertidal zone (Burrell *et al.,* 1984). In its habitat, *C. virginica* encounters harsh living conditions that may contribute to physiological stress and vulnerability to disease. Large fluctuations in natural environmental conditions such as temperature, salinity, and oxygen occur routinely in oyster habitats (Shumway and Koehn, 1982). Oysters may also encounter fluctuations in levels of anthropogenic pollutants including heavy metals, pesticide runoff, and hydrocarbons. In addition, oyster populations such as those in South Carolina endure air exposure twice daily due to tides. Periodically, they can also be exposed to the type of hypoxic and hypercapnic (high CO_2) conditions reported in tidal creeks in the Charleston Harbor estuary (Cochran and Burnett, 1996).

Although man has played a major role in the decline of oyster populations through over-harvesting, pollution, and the degradation of oyster habitat, other factors have contributed to oyster mortalities. Chief among them are diseases, notably MSX (*Haplosporidium nelsoni*) and "dermo" (*Perkinsus marinus*). Periodic epizootics have devastated oyster populations. It is not unusual for 75% of the oysters in a bed to have *P. marinus* disease in one summer, and infection rates are typically between 70% and 90% in a given year during the warm season (Andrews and Hewatt, 1957). The impact of *P. marinus* infections on oyster populations has been quantified by Mackin (1951), Ray (1953, 1954), and Ray and Chandler (1955). Associated with the disease are high incidences of annual mortality, usually exceeding 50% (Quick and Mackin, 1971).

Perkinsus marinus (Levine, 1978) is a protozoan pathogen of oysters (Schmidt and Roberts, 1989). Physiological abnormality accompanies the development of *Perkinsus* disease in oysters. The disease is distributed by the hemolymph to all parts of the body; in histological sections, infective spores (usually 2–20 μm in diameter) can be seen both intra- and intercellularly (Cheng, 1973). Progression of disease leads to extensive tissue damage,

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abscesses, and retarded gonadal development. Menzel and Hopkins (1955), Andrews (1961), and Paynter and Burreson (1991) have reported retarded oyster growth as well. Although the exact mode of death has yet to be elucidated, infected oysters that become severely emaciated typically gape and then die. Death is probably caused by tissue lysis and embolism of circulatory passages (Andrews and Hewatt, 1957). In general, however, sublethal manifestations of parasitism in estuarine organisms—such as that of *P. marinus* on oysters—have not been considered heretofore.

The acid-base physiology of oysters is of particular interest because changes in acid-base status can influence biochemical processes, including the deposition of shell that is essential to oyster growth (Booth *et al.*, 1984; Burnett, 1988). Exposure to air induces significant acidosis in the hemolymph of bivalves; this acidosis is generally not compensated (Booth *et al.*, 1984). Intertidal oysters experience hours of air exposure during which they have no access to water. Acid-base balance is also of interest since conditions of hypoxia and hypercapnia, which can contribute to changes in acid-base variables, have been observed in the Charleston Harbor estuary (Cochran and Burnett, 1996), which supports a large oyster population. The present study examines the acid-base status of hemolymph in *C. virginica:* how it is influenced by air exposure and temperature and how it responds to infections of *P. marinus*.

Materials and Methods

Two populations of *Crassostrea virginica* were examined in this study. Local South Carolina oysters from the Charleston Harbor estuary were used as experimental animals. Uninfected oysters from the Chesapeake Bay in Maryland were used as controls because so few (2 of the 200 animals examined) uninfected oysters were found in the study population.

Collection and preparation of oysters

Experimental animals were from a bed in the Folly River, Folly Beach, South Carolina (32°38.8′ N; 79°57.1′ W), where the salinity is normally greater than 25 ppt. Oysters were collected from the low- and mid-intertidal zone at low tide. Specimens had a mean shell height of 85.8 mm (SEM = 0.8) and were about 2–3 years old. All epibionts were removed in order to obtain individual oysters for physiological measurements. The oysters were scrubbed clean, placed in aquaria in well-aerated 25 ppt seawater at the Grice Marine Biological Laboratory, and allowed 24 h to recover before being prepared for experiments.

Oysters uninfected with *Perkinsus* served as controls and were collected from the Wye River in the upper Chesapeake Bay where they were tray-cultured in low-salinity water (8–10 ppt) by Chesapeake Mariculture, Queenstown, Maryland. The animals were packed in ice and shipped overnight to the Grice Marine Laboratory. There they were placed in aquaria with recirculating water and acclimated to 25 ppt salinity in a stepwise fashion. Over the course of the experiments, control oysters had a mean shell height of 60.4 mm (SEM = 0.6). They were certified free of *Perkinsus* disease by Dr. Kennedy Paynter of the University of Maryland. In addition, all oysters were assayed for *Perkinsus* in our laboratory.

Oysters were prepared for hemolymph sampling by drilling a 1-mm hole in the shell over the adductor muscle and covering the hole with dental dam cemented into place with cyanoacrylate glue. The oysters were then returned to holding tanks and allowed 48 h to recover. Oysters from Maryland were held in aquaria separate from those housing oysters collected in South Carolina.

Bioassay for Perkinsus marinus

All physiological measurements were made on oysters in the holding conditions described, after which they were immediately assayed for the presence and intensity of *Perkinsus* infections. As soon as possible after measuring total CO_2 and pH, the oysters were sacrificed and necropsies performed to assay for *P. marinus*. The fluid thioglycollate medium (FTM) technique, being specific for *P. marinus* (Ray, 1952; Mackin and Ray, 1966) was used, as modified by Quick (1972), for culturing the parasite. The rectum was excised from the oyster and placed in FTM culture medium prepared according to Howard and Smith (1983). After 7 days the tissue explant was analyzed for parasite hypnospores according to procedures by Howard and Smith (1983).

The intensity of the parasitic infection was determined according to the index developed by Ray (1953, 1954, 1966) and modified by Quick and Mackin (1971), where 0 = no hypnospores in the entire sample and 6 is $\geq$ 1001 hypnospores per 5-mm field. Oysters were placed into one of three infection categories: none (index = 0), low (index = 1, 2, or 3), or high (index = 4, 5, or 6).

Experimental design and statistical analysis

Most experiments were designed for a multiway three-factor analysis of variance (ANOVA) to differentiate among the effects of temperature, air exposure, and infection status on pH and total CO_2. The physiological responses of hemolymph pH and total CO_2 (defined as all forms of CO_2 including molecular CO_2, HCO_3^-, and $CO_3^=$) in control and experimental oysters were measured against three treatments: temperature (21° and 30°C), air exposure (immersion longer than 24 h compared with 5-h and 24-h emersion), and infection level ("None" compared with "High" levels) under controlled laboratory

conditions. In each of the treatments salinity was maintained at 25 ppt. All aquaria were well aerated and the oysters were not fed before or during the experiments. Water pH was always at or above 8.0.

The length of air exposure in oyster habitats depends upon a regular sinusoidal variation in tidal height, amplitude changes associated with spring and neap tides, and other factors (McMahon, 1988). A 5-h emersion is a typical maximum air exposure for local oysters during periods of spring tides. Because high-intertidal oysters are commonly exposed for even longer periods, and oysters have been known to tolerate days of air exposure, 24 h was chosen as the upper limit of exposure.

Most experiments were conducted over the period of July through November when *in situ* water temperatures were similar to those employed in the study (21° and 30°C). Furthermore, July, August, September, and October are the months when *P. marinus* infection levels are at their peak, coinciding with the warmest water temperatures.

Values are expressed throughout as mean ± 1 standard error of the mean (SEM). Total CO_2 and pH values were tested for variance according to Sokal and Rohlf (1981) by a three-factor ANOVA. Due to nonuniform variances in total CO_2 (heteroskedasticity), these values were natural log transformed before analysis. Statistically significant interactions were further analyzed by graphing techniques. The significance of differences for pH and total CO_2 between uninfected and highly infected oysters was determined by a Mann-Whitney rank sum test.

Differences in calcium ion concentrations between air exposure treatments within an infection group were tested with a Mann-Whitney rank sum test (uninfected group) or a Kruskal-Wallis one-way analysis of variance on ranks (low-infection and high-infection groups) (Table I). Differences in mean hemolymph pH, total CO_2, and calcium ion concentrations between oysters emersed in air and oysters emersed in nitrogen were tested using a student's *t* test or, when a test for normality failed, a Mann-Whitney rank sum test (Table II).

Hemolymph acid-base status

At the start of each experiment about 80 μl of hemolymph was anaerobically drawn, using a 1-ml glass syringe and a 23-gauge needle, from the sinus of the adductor muscle of each oyster. The samples were placed on ice until measurements were made. In all experiments, hemolymph was drawn just once from each animal.

Total CO_2 was measured by the method of Cameron (1971) or with a Capni-Con 5 total CO_2 analyzer. The pH of the oyster hemolymph was measured using a capillary pH electrode (Radiometer BMS2 Mk2) calibrated at experimental temperatures with precision Radiometer buffers.

In separate experiments to test the hypothesis that calcium ions are mobilized from the shell in response to acidification, calcium ion concentration was measured in hemolymph from oysters held at 21°C and 25 ppt salinity. Hemolymph was sampled as described above, and the concentration of free calcium ions was measured with a calcium ion electrode (Radiometer).

Because our results indicated a significant increase in hemolymph P_{CO_2} upon air exposure, we wanted to test the hypothesis that oysters were somehow accessing oxygen in air and using it metabolically to produce CO_2 even though their valves were assumed to be tightly closed. Thus, we performed additional experiments in which we compared acid-base variables (measured as above) for two groups of oysters—one emersed in an atmosphere of pure nitrogen for 24 h and the other emersed in air.

Buffering capacity of the hemolymph

Buffering properties of the oyster hemolymph were determined at 21° and 30°C by incubating hemolymph samples of highly infected oysters and uninfected oysters ($n = 4$ for each) in thermostatted tonometers (Radiometer BMS2 Mk 2) at four different CO_2 pressures (2, 4, 15, and 22 torr) provided by Wösthoff precision gas mixing pumps. Hemolymph was drawn as described above from each oyster and centrifuged for 2 min in a microcentrifuge. Hemolymph (80 μl) was incubated in a thermostatted tonometer for at least 30 min at a particular P_{CO_2}. Total CO_2 and pH were then measured for each sample as above, permitting the calculation of p*K*. Appropriate p*K* (calculated from oyster hemolymph) and CO_2 solubility constants (Truchot, 1976) for the experimental temperatures were used to create pH–bicarbonate diagrams.

The buffering capacity of each hemolymph sample was determined from the slope of the linear relationship between pH and bicarbonate ion concentrations at the different CO_2 pressures. The resulting slope is a measure of the non-bicarbonate buffers ($\Delta[HCO_3^-]/\Delta pH$ = slykes) in the hemolymph.

Results

Hemolymph acid-base status and buffering capacity

Values for hemolymph pH and total CO_2 were analyzed using pH–bicarbonate diagrams (Figs. 1 and 2). At 21°C, highly infected oysters have a pH significantly ($P = 0.0003$) different from those of uninfected oysters at 0 h. Both the highly infected and the uninfected oysters underwent a respiratory acidosis induced by air exposure (Fig. 1). The acidosis at 24 h was slightly greater for the uninfected Maryland oysters (pH = 6.607) than for the highly infected South Carolina oysters (pH = 6.653). The uninfected oysters showed no significant compensation after 24 h. The

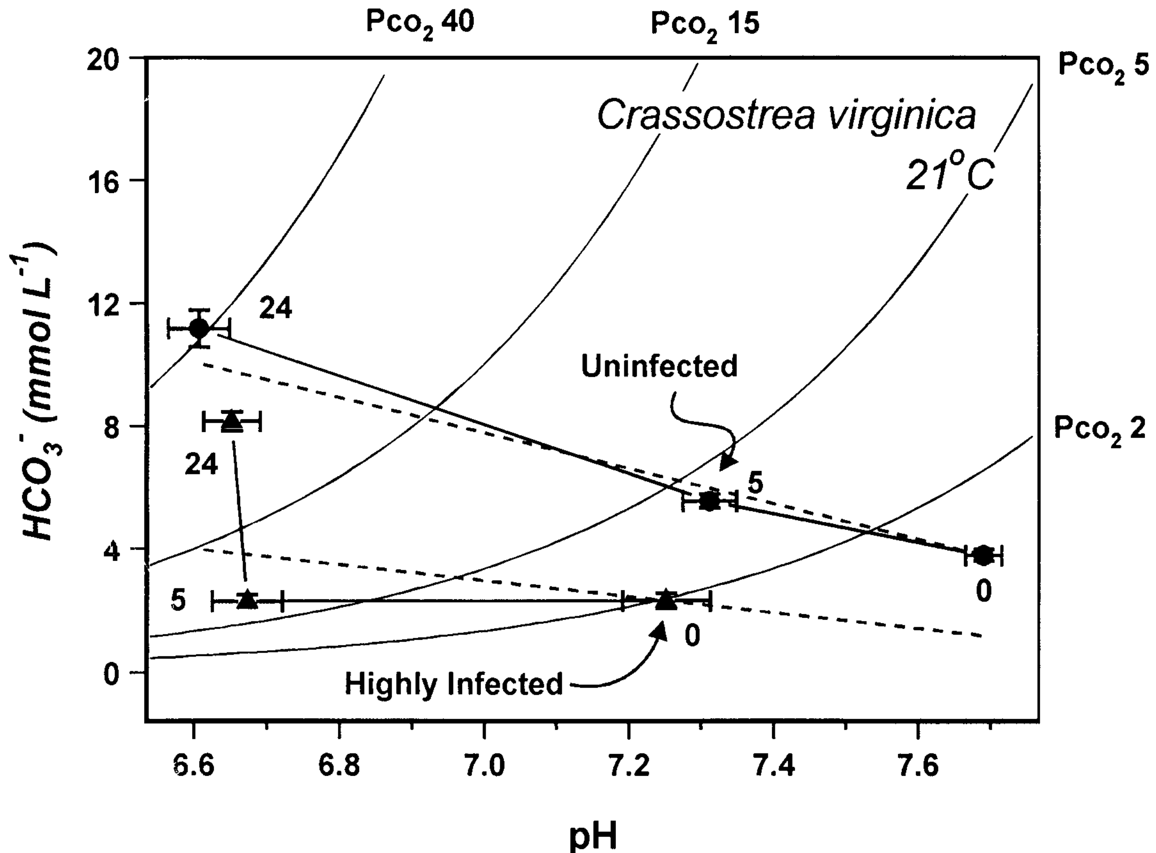

Figure 1. A pH–HCO_3^- diagram showing the acid-base status of oyster hemolymph at 0, 5, and 24 h of air exposure at 21°C. P_{CO_2} isopleths (curved lines) are given in torr. *In vitro* buffer lines are shown as dashed lines. Circles represent oysters uninfected with *Perkinsus marinus;* triangles represent oysters with "high" infections. Values are mean ± SEM; *n* for each experiment ranged from 22 to 56.

uninfected group had a higher P_{CO_2} (~42 torr) than did the highly infected group (~28 torr). The acidosis in the highly infected group was uncompensated at 5 h of air exposure, but was partially compensated at 24 h.

At 30°C, highly infected oysters had a pH significantly ($P = 0.0004$) different from that of uninfected oysters at 0 h. Both groups underwent a respiratory acidosis induced by air exposure (Fig. 2). The acidosis at 24 h was greater for the uninfected oysters (pH 6.73) than for the highly infected group (pH 6.94). The uninfected oysters showed no significant compensation after 24 h of air exposure. The uninfected group had a P_{CO_2} similar to that at 21°C (~44 torr) and greater than that of the highly infected group (~31 torr). The acidosis in the highly infected group was uncompensated after 5 h of air exposure, but was partially compensated at 24 h. The change in hemolymph pH with temperature in uninfected oysters ($\Delta pH/°C = 0.045$) was twice that predicted for passive temperature effects on pH and commonly reported for ectotherms.

Calcium ion concentration in hemolymph of uninfected oysters was lower than that in hemolymph of oysters with low or high infections ($P < 0.05$; Kruskal-Wallis one-way analysis of variance on ranks; Table I). Exposure of oysters to air resulted in an elevation of hemolymph calcium ion concentrations in all treatment groups (Table I).

Oysters emersed and exposed to nitrogen for 24 h had an acidosis level similar to that of air-exposed oysters regardless of infection level, but total CO_2 (with one exception) and calcium ion concentrations differed significantly between treatments (Table II).

The buffering properties of the hemolymph were examined by comparing the slopes of the *in vitro* buffer lines (Figs. 1 and 2). At 21°C, the slope for the *in vitro* buffer line (indicating the non-bicarbonate buffering strength of the hemolymph) was −5.56 slykes for uninfected oysters ($\Delta[HCO_3^-]/\Delta pH$) and −2.57 for highly infected oysters. At 30°C, the slope of the *in vitro* buffer line was −7.81 slykes for uninfected oysters and −4.02 for highly infected oysters. The slopes of the individual *in vitro* buffer lines for highly infected and uninfected oysters at the two temperatures were compared with *t* tests. At 21°C, the slopes were not significantly different ($P = 0.082$; df = 3).

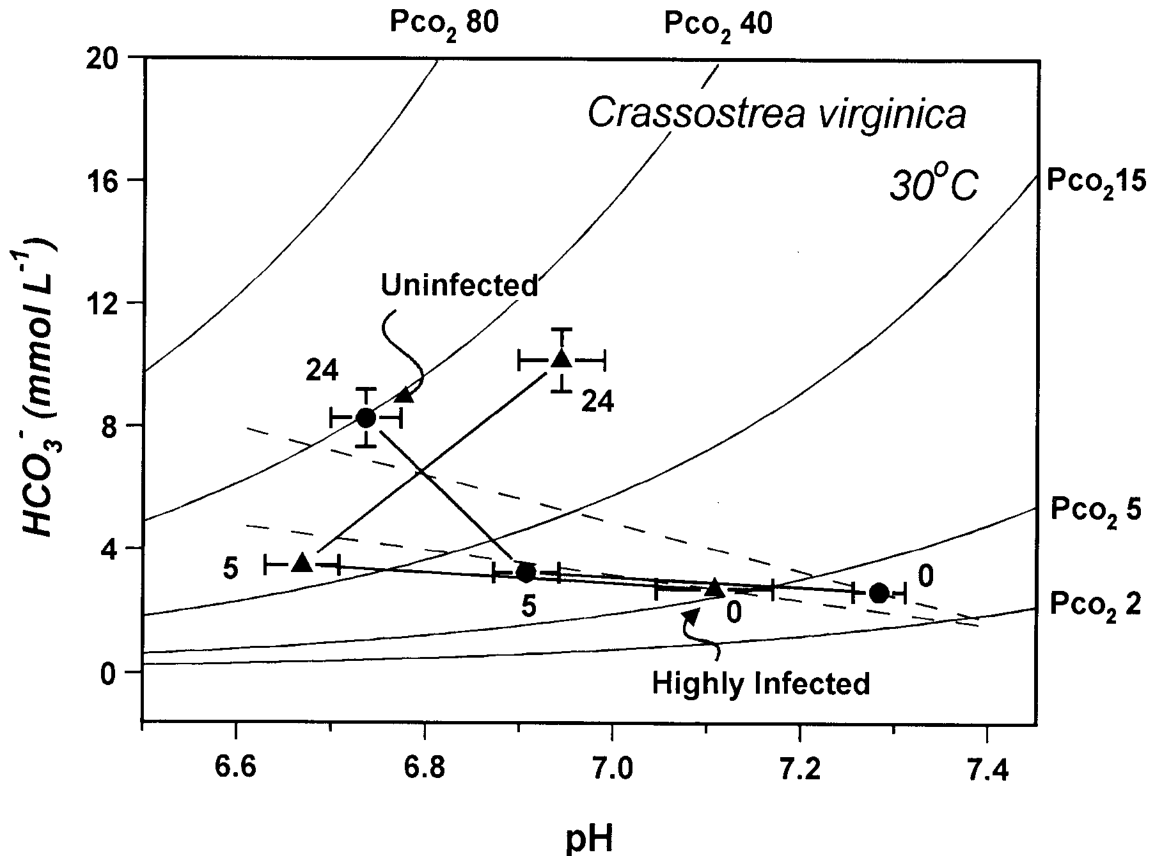

Figure 2. A pH–HCO_3^- diagram showing the acid-base status of oyster hemolymph at 0, 5, and 24 h of air exposure at 30°C. Pco_2 isopleths (curved lines) are given in torr. *In vitro* buffer lines are shown as dashed lines. Circles represent oysters uninfected with *Perkinsus marinus;* triangles represent oysters with "high" infections. Values are mean ± SEM; *n* for each experiment ranged from 22 to 56.

At 30°C, however, the slopes of the lines were significantly different ($P = 0.025$; df = 5).

Infection levels

Mean *P. marinus* infection levels of oysters collected in South Carolina in 1993 peaked in September ($\bar{x} = 3.6 \pm 0.2$). The mean level was 1.8 ± 0.1 in July 1993 and 2.0 ± 0.2 in November 1993 (Fig. 3). Throughout the study period, bioassays for the detection of *P. marinus* were performed on more than 200 South Carolina oysters. Only two oysters were found to be infection-free; thus the infection prevalence was 99%. Among all treatments, the infection level for the control Maryland oysters was zero. In fact, throughout the study, in no case was infection detected in the Maryland oysters ($n = 159$).

Discussion

The use of oysters from two different populations (Maryland and South Carolina), while not ideal, was necessary because no uninfected oysters were available from South Carolina. Nevertheless, we feel that the physiological effects we observed in this study were induced by infections of *P. marinus* and not by differences between the oysters from different sources. Most of the results presented here were obtained from uninfected (Maryland) oysters and highly infected (South Carolina) oysters. South Carolina oysters with infection intensities intermediate between these two extremes (*i.e.,* oysters with "low" infections) show intermediate responses. For example, the results in Table I suggest that intermediate (low) infections of *P. marinus* yield a result intermediate between uninfected and highly infected oysters. Furthermore, a similar pattern of intermediate responses with "low" infection intensities was found among the acid-base variables (*e.g.,* Figs. 1 and 2), but we have not presented the data. We suggest that the intermediate responses are a function of infection intensity and that the uninfected Maryland oysters fit into the pattern. We cannot, however, exclude the possibility that Maryland oysters have a different acid-base physiology from South Carolina oysters.

When the valves of an oyster are closed, the Pco_2 rises and the pH of the hemolymph declines (Crenshaw and

Table I

Hemolymph calcium ion concentration (mmol/l) as a function of air exposure in oysters uninfected with Perkinsus marinus, *oysters with low infections, and oysters with high infections at 21°C and 25 ppt salinity; statistical tests are between immersed oysters and air exposed oysters within an infection group*

Treatment		Uninfected	Low infection (Index = 1–3)	High infection (Index = 4–6)
Immersed	Mean	3.9	5.4	6.4
	SEM	0.14	0.27	0.36
	n	60	41	21
Air exposed (5 h)	Mean		9.1	9.1
	SEM		0.27	0.45
	n		34	15
	p		<0.05	<0.05
Air exposed (24 h)	Mean	10.6	11.3	13.9
	SEM	0.87	1.00	0.80
	n	5	20	26
	p	<0.0001	<0.05	<0.05
	Statistical test	Mann-Whitney rank sum	Kruskal-Wallis one-way analysis of variance on ranks	

Neff, 1969; Booth *et al.*, 1984). Some intertidal bivalves can sustain prolonged exposures to anoxia and display impressive anaerobic capacities (Grieshaber *et al.*, 1994). McMahon (1988) suggested that during valve closure many intertidal bivalves remain almost completely anaerobic to minimize evaporative water loss. Several authors (*e.g.*, Dugal, 1939; Crenshaw and Neff, 1969) have shown that the molluscan shell helps to buffer decreases in pH produced by anaerobic metabolism. It is not known, however, whether bivalves possess other mechanisms to regulate the pH of their body fluids.

Booth *et al.* (1984) observed respiratory acidosis in the mussel *Mytilus edulis* when it was exposed to air. The authors attributed the acidosis entirely to observed increases in P_{CO_2}. Although they presumed that anaerobic metabolism was occurring during air exposure, they concluded, on the basis of work by Zurburg *et al.* (1982), that no metabolic acids entered the hemolymph. Partial compensation for the acidosis was observed and attributed to shell decalcification because an increase of calcium ions was measured in the hemolymph. An increase in ammonia levels was also implicated in compensation.

The present study reveals that oysters exposed to air develop a respiratory acidosis of the hemolymph that is comparable to the pattern observed by Booth *et al.* (1984) for mussels, although more severe. These results are also similar to the acid-base responses of other intertidal invertebrates (Truchot, 1988; Grieshaber *et al.*, 1994). The primary source of the respiratory acidosis in *C. virginica* during air exposure is most likely the retention of CO_2 produced metabolically. The oysters kept their valves closed during exposure to air, when CO_2 accumulated. CO_2 is produced by aerobic pathways; however, as the limited O_2 stores within the shell become depleted, it is possible that CO_2 is produced by anaerobic means (reviewed by de Zwaan and Wijsman, 1976). On the other hand, our observations on emersed oysters exposed to an atmosphere of nitrogen indicate that oysters are not fully isolated from the aerial environment and, thus, not fully anaerobic.

It is likely that oysters, like other bivalves, lower their metabolism when they are exposed to air and that a portion of their metabolism is aerobic (Widdows and Shick, 1985). The acidosis produced in a nitrogen atmosphere was just as severe as that produced in air (Table II), but the amount of total CO_2 present in the hemolymph was generally less. In addition, the calculated P_{CO_2} of hemolymph was much lower (~20 torr) in nitrogen-exposed oysters than in air-exposed oysters (~42 torr). These results indicate that metabolic acids, possibly acetic acid, propionic acid, or succinic acid, are produced in the nitrogen atmosphere. Less CO_2 is also produced. Thus, acidosis has an obvious "metabolic" component in the absence of oxygen.

The acid-base balance in many aquatic animals is thought to depend mainly upon active transport of ions between the extracellular fluid and the environment. Both Lindinger *et al.* (1984) and Booth *et al.* (1984) demonstrated that *M. edulis* appears to buffer H^+ loads by passive dissolution of carbonates, thereby producing CO_2. Their findings were based in part upon increases in hemolymph Ca^{++} concentration; a result consistent with ours. Interestingly, the hemolymph calcium concentrations in nitrogen-exposed oysters are lower than those in air-exposed oysters (Table II). These results are in contrast to what is expected. Crenshaw and Neff (1969) demonstrated a direct relationship between the appearance of succinic acid and calcium ions in the extrapallial fluid of *Mercenaria mercenaria.* Because we sampled hemolymph, there may be a decrease in the transport of calcium between the hemolymph and the extrapallial fluid under completely anaerobic conditions. Dugal and Fortier (1941) also reported that the calcium concentration in the mantle cavities of oysters increased from 40 to 400 mg/100 ml (10 to 100 mmol/l) during air exposure, accompanied by an increase in CO_2 content. Here again, there may be a difference between changes in calcium ion concentrations in the hemolymph and other fluid compartments.

The acidosis quantified in the present study is of concern for several reasons. The data suggest that, to buffer the acid, calcium ions are released through dissolution of the $CaCO_3$ bound in the shell. The net result of shell dissolution may be retardation of growth. The effects of severe hemolymph acidosis, as observed here, are even more far-reaching. At these hemolymph pH levels, intracellular

Table II

Hemolymph acid-base variables in oysters emersed in air or a nitrogen atmosphere at 21°C and 25 ppt salinity and with infections of Perkinsus marinus; *statistical tests are between oysters emersed in air and oysters emersed in nitrogen within an infection group*

Infection level	Treatment		Total CO_2 (mmol/l)	pH	Ca^{++} (mmol/l)
Uninfected	Emersed 24 h in air	Mean	11.5	6.58	10.6
		SEM	1.00	0.095	0.87
		n	5	5	5
		Test	*t* test	*t* test	*t* test
		p-value	<0.0001	0.928	0.0073
	Emersed 24 h in N_2	Mean	4.9	6.57	7.5
		SEM	0.45	0.054	0.53
		n	10	10	10
Low infection	Emersed 24 h in air	Mean	11.9	6.58	11.25
		SEM	0.99	0.044	0.99
		n	20	16	20
		Test	Mann-Whitney rank sum test	*t* test	*t* test
		p value	0.0062	0.710	0.041
	Emersed 24 h in N_2	Mean	5.4	6.55	7.5
		SEM	0.47	0.061	0.63
		n	7	7	7
High infection	Emersed 24 h in air	Mean	9.5	6.77	13.9
		SEM	0.78	0.051	0.79
		n	26	24	26
		Test	*t* test	*t* test	Mann-Whitney rank sum test
		p value	0.0519	0.608	0.0192
	Emersed 24 h in N_2	Mean	5.9	6.83	8.35
		SEM	0.25	0.124	0.77
		n	6	6	6

pH will be low as well (Lindinger *et al.,* 1984; Walsh *et al.,* 1984). The resulting systemic acidosis may also affect cellular metabolism by decreasing the rate of glycolysis or by shifting the use of one metabolic pathway to another (see Grieshaber *et al.,* 1994, for review).

Acid-base balance in *C. virginica* is clearly affected by infections with *P. marinus.* Most protozoan parasites are ammonotelic and excrete most of their nitrogen as ammonia. Ordinarily, ammonia readily diffuses across cell membranes into the surrounding medium. This avenue may not be available to oysters during air exposure and the resultant valve closure (Schmidt and Roberts, 1989). Ammonia is also a common waste product of oysters and bivalves in general. It is possible that NH_3 contributes to the pH compensation in infected oysters by buffering H^+. CO_2, lactate, pyruvate, and short-chain fatty acids also are common waste products of these protozoans (Schmidt and Roberts, 1989). *P. marinus* is undoubtedly a source of CO_2 that accounts for a portion of the respiratory component of the acidosis.

The pH–bicarbonate diagrams (Figs. 1 and 2) show that the highly infected oysters, unlike those uninfected, compensated partially for the acidosis after 24 h of air exposure. Compensation was greater at the higher temperature (30°C). We do not know why the control (Maryland) oysters did not compensate for their respiratory acidosis. The age and size of these oysters may provide a clue. The Maryland oysters were somewhat younger and smaller

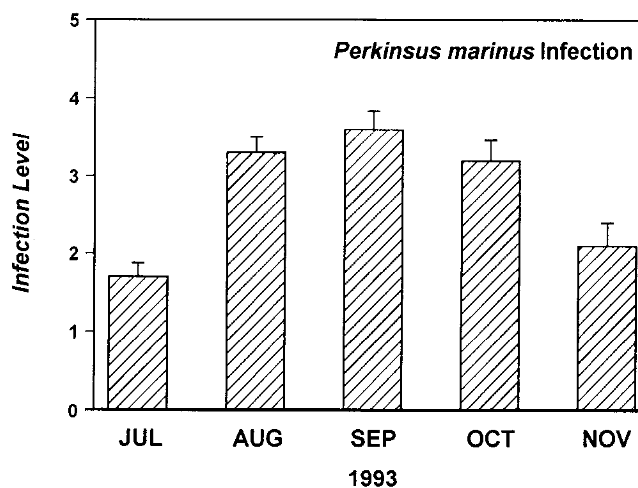

Figure 3. Bar graph depicting *Perkinsus marinus* infection levels in South Carolina oysters in the months of July through November 1993 from the Folly River collection site. Values are mean ± SEM.

than the South Carolina oysters, so there may be some adaptive value in retaining shell for growth rather than allowing dissolution of calcium carbonate to create a buffer for H^+ ions. Another possibility is that the biochemical matrix of young oyster shell is different from the shell of older oysters.

In the present study, the pattern of infection intensity (Fig. 3) peaked during warm-weather months when water temperatures are about 30°C, the experimental temperature treatment that yielded the largest acidosis. Similar findings were obtained by Burrell *et al.* (1984). All of the above findings lead us to speculate that a positive feedback mechanism may operate in conditions of high temperature and acidity that favor the propagation and development of *P. marinus* infection. The parasite may in turn contribute to the acidic environment of its host tissues and extracellular fluids, further perpetuating itself. Interestingly, in experiments where *P. marinus* was cultured, Chu and Greene (1989) observed significant but unexplained drops in the pH levels of the cultures. It is likely that the low pH was due to the metabolic production of CO_2.

This investigation into the physiological effects of *Perkinsus marinus* on oyster physiology has shown that under conditions of air exposure and high temperature, oysters with high *Perkinsus* infections undergo a severe acidosis. The acidosis is due to elevated hemolymph CO_2 and is partially compensated in infected oysters, but uncompensated in uninfected oysters. Many infected oysters remained at or below a pH of 6.7 during air exposure. Profound negative effects may result from this acidosis since pH affects, and may even regulate, physiological and biochemical processes (Grieshaber *et al.*, 1994).

Perkinsus marinus may thrive under the acidic conditions it induces in oysters. Whether environmental conditions that induce an acidosis in oysters (*e.g.*, elevated environmental CO_2 and air exposure) stimulate *P. marinus* infections is unknown but currently under investigation. Certainly, *P. marinus* is a significant problem in South Carolina waters. Infection intensities are high in warm-water months (Fig. 3; Crosby and Roberts, 1990), and prevalence may be as high as 100%.

This study also points out the need to consider the possibility and effects of parasitism in research involving an organism's physiology and biochemistry. Oysters infected with *Perkinsus* are not obviously different from those that are uninfected; thus, researchers must look for infections. Oysters that are infected and acidotic may respond to environmental challenges differently from oysters that are not infected and not acidotic. Anderson (1975) suggested that grass shrimp parasitized by epicaridean isopods have lowered rates of oxygen uptake, and that this response may reflect changes in the lipid metabolism of the host. It is not possible to generalize about the physiological and metabolic responses marine organisms have to parasitism, but it should be recognized that a parasite can have profound effects.

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