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Immune Defense Reduces Respiratory Fitness in *Callinectes sapidus*, the Atlantic Blue Crab

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Abstract. Crustacean gills function in gas exchange, ion transport, and immune defense against microbial pathogens. Hemocyte aggregates that form in response to microbial pathogens become trapped in the fine vasculature of the gill, leading to the suggestion by others that respiration and ion regulation might by impaired during the course of an immune response. In the present study, injection of the pathogenic bacterium *Vibrio campbellii* into *Callinectes sapidus,* the Atlantic blue crab, caused a dramatic decline in oxygen uptake from 4.53 to 2.56 μmol g^{-1} h^{-1}. This decline in oxygen uptake is associated with a large decrease in postbranchial Po_2, from 16.2 ($\pm$0.46 SEM, n=7) to 13.1 kPa ($\pm$0.77 SEM, n=9), while prebranchial Po_2 remains unchanged. In addition, injection of *Vibrio* results in the disappearance of a pH change across the gills, an indication of reduced CO_2 excretion. The hemolymph hydrostatic pressure change across the gill circulation increases nearly 2-fold in *Vibrio*-injected crabs compared with a negligible change in pressure across the gill circulation in saline-injected, control crabs. This change, in combination with stability of heart rate and branchial chamber pressure, is indicative of a significant increase in vascular resistance across the gills that is induced by hemocyte nodule formation. A healthy, active blue crab can eliminate most invading bacteria, but the respiratory function of the gills is impaired. Thus, when blue crabs are engaged in the immune response, they are less equipped to engage in oxygen-fueled activities such as predator avoidance, prey capture, and migration. Furthermore, crabs are less fit to invade environments that are hypoxic.

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Introduction

Callinectes sapidus (Rathbun), the Atlantic blue crab, like many crustaceans, has gills that are highly functional in exchanging gases (Mangum *et al.,* 1985) and maintaining osmotic balance (Towle and Burnett, 2006). In addition, gills serve an important role in the immune response (Johnson, 1976; White *et al.,* 1985; Martin *et al.,* 1993, 2000; Burgents *et al.,* 2005b). However, it is the role in the immune response that may compromise the ability of the gill to exchange gases and take up ions.

Particles such as bacteria and ink are rapidly removed from the circulating hemolymph of crustaceans, with the gills and hepatopancreas often implicated as major sites for removal of these foreign bodies (Fontaine and Lightner, 1974; Smith and Ratcliffe, 1980b; White and Ratcliffe, 1982; Martin *et al.,* 1993). The number of hemocytes also declines rapidly after injection of bacteria in crustaceans (Smith and Ratcliffe, 1980a; Martin *et al.,* 1993; van de Braak *et al.,* 2002) including the blue crab (Holman *et al.,* 2004); within 10 min, loose aggregates of hemocytes and bacteria, called nodules, begin to appear in hemolymph (reviewed by Smith, 1991). The nodules increase in size in the hemolymph, becoming lodged in narrow hemolymph spaces, including and especially the narrow channels of the gill circulation, where the nodules become melanized (White *et al.,* 1985; Martin *et al.,* 1998). Martin *et al.* (2000) found that the melanized nodules persist for a month or longer, during which time they are externalized between the gill epithelium and the exoskeleton and eliminated on the following molt.

Johnson (1976) provided histological evidence that nodule formation in response to stress-induced bacteremia in blue crabs might have adverse effects on gill function, including distention of gill lamellae and disruption of he-

molymph flow. In *Carcinus maenas* injected with bacteria, Smith and Ratcliffe (1980a) observed the formation of compact hemocyte clumps or diffuse hemocyte networks that appeared to occlude the lumen of the lamellar sinus, leading several authors, including White *et al.* (1985) and Martin *et al.* (2000), to suggest that hemocyte aggregates/nodules could interfere with respiration and ion regulation. Interference with either function could greatly impact the ability of the blue crab to live in coastal and estuarine waters with highly variable oxygen and salinity conditions.

We have shown that a dose (2.5×10^4 g^{-1} crab) of the bacterium *Vibrio campbellii* injected into the ventricle of the blue crab is removed from the hemolymph in less than 1 h (Holman *et al.,* 2004). Within 10 min of bacterial injection, the number of hemocytes circulating in the hemolymph dramatically decreases to one-third of the original number and remains low for at least 2 hours (Holman *et al.,* 2004). Within 20 to 40 min the number of culturable *Vibrio* circulating in the hemolymph is reduced to nearly undetectable levels.

In the present study, we test the hypothesis that the elimination of the pathogen *Vibrio campbellii* interferes with the respiratory function of the gills of the blue crab. We confirmed the presence of hemocyte aggregations within the gills of the blue crab and assessed the overall ability of quiescent blue crabs to take up oxygen from the ambient environment. We also made some specific measures of gas exchange across the gills. Certain elements of cardiovascular function specifically relating to the gill circulation were monitored; we quantified heart and scaphognathite function, and measured the hemolymph hydrostatic pressure change across the gill circulation in both saline- and *Vibrio*-injected crabs.

Materials and Methods

Blue crabs were collected locally in Charleston, South Carolina, and held at 25 °C and 25 psu salinity for at least 3 days prior to use. Crabs weighed between 92 and 236 g and were held for a minimum of 3 days prior to experimentation but no longer than 10 days. Crabs were initially assessed for the presence of bacterial infections within their hemolymph by culturing 150 μl of hemolymph, freshly drawn and diluted (1:10 in 10 mmol l^{-1} HEPES, pH 7.6, 2.5% NaCl) in marine agar overlays on tryptic soy agar. Only crabs with no evidence of culturable bacteria in the hemolymph were used for these studies. Crabs were prepared for the injection of saline (= control) or bacteria into the ventricle and for hemolymph sampling from the pericardium as described by Holman *et al.* (2004). Injections of saline or *Vibrio* did not exceed 300 μl in volume.

A 1-mm hole was drilled in the carapace directly over the heart, creating a port through which saline or saline containing *Vibrio* could be injected directly into the heart. Bacteria injected into the heart could then be rapidly distributed by the circulatory system throughout the hemocoel. Two similar holes were drilled over the pericardium adjacent to the heart through which hemolymph was withdrawn from the pericardium. A thin layer of latex rubber was glued in place over each hole with cyanoacrylate glue. A needle could be inserted into each hole through the rubber diaphragm and easily withdrawn without causing bleeding. These procedures were performed 2 days prior to experimentation.

The bacteria used in the present study were *Vibrio campbellii* 90-69B3 transformed with pMSB6, a plasmid that bears genes encoding green fluorescent protein (GFP) and resistance to the antibiotic kanamycin (kan), as described by Burgents *et al.* (2005b). The injection dose of live, culturable bacteria was prepared as described by Holman *et al.* (2004). Crabs were injected with 2.5×10^4 colony-forming units of bacteria g^{-1} crab weight, to achieve a circulating dose of 1×10^5 ml^{-1} of hemolymph, assuming a hemolymph volume of 25 ml 100 g^{-1} body weight (Gleeson and Zubkoff, 1977). This injection dose is below the LD_{50} for *C. sapidus* (6.21×10^5 CFU g^{-1}; Thibodeaux and Burnett, unpubl. data).

Oxygen uptake

Oxygen uptake was determined by measuring the rate of decline of oxygen in a closed respirometer. A crab was placed in the respirometer for at least 1.5 h prior to an injection treatment to allow the crab to become quiescent. During this time the respirometer was flushed continuously with filtered (0.45 μm) and well-aerated water at 25 °C and 25 psu salinity. The lid of the respirometer was removed and the crab injected directly into its ventricle with a dose of either saline (control) or *Vibrio.* The respirometer was then closed and the respirometer was once again flushed with well-aerated seawater for 30 min. To begin measurements of oxygen uptake, seawater flow through the respirometer was stopped and the oxygen decline was measured using an oxygen electrode (Yellow Springs Instruments, Yellow Springs, OH) and Sable Systems International (Las Vegas, NV) data acquisition system. Measurements of oxygen uptake were made at $Po_2 > 18.6$ kPa from 30 min to 1.5 h after injection.

Hemolymph variables

Crabs were held overnight in vigorously aerated seawater in individual containers lined with gravel. A dose of either saline (control) or *Vibrio* was injected directly into the ventricle at time zero. After 30 min, about 0.5 ml of hemolymph was sampled from both the infrabranchial sinus, and the pericardium without removing the crab from the water and with minimal disturbance to the crab. Po_2 and pH were measured using a Radiometer blood gas analyzer (BMS2

Mk2; Radiometer, Copenhagen, Denmark) thermostatted to 25 °C. Hemocyanin concentrations were measured in hemolymph samples by diluting the hemolymph with a saline solution containing 2.5% NaCl and 10 mmol l^{-1} EDTA at pH 10 (this causes the hemocyanin subunits to dissociate), measuring absorbance at 345 nm, and using hemocyanin extinction coefficients (Nickerson and Van Holde, 1971).

Hemolymph and branchial chamber pressures

Hemolymph pressure was monitored in the infrabranchial and pericardial sinuses using indwelling catheters (polyethylene, PE 190; OD = 1.7 mm, ID = 1.19 mm) connected to strain gauge pressure transducers (Gould P23ID; Gould-Statham, Oxnard, CA). Transducer output was amplified and digitally acquired (Biopac MP100; Biopac Systems, Inc., Goleta, CA). The transducers were statically calibrated with a common pressure source. All pressures were referenced to tank water level.

For infrabranchial sinus pressures, the catheter tip was inserted to a depth of about 1 mm through the arthrodial membrane at the base of the fifth leg. A small patch of latex dental dam was fixed to the membrane surface to provide stability. For pericardial sinus pressures, the catheter tip was inserted through a 1-mm-diameter hole drilled just posterolateral to the ventricle and on the same side of the crab where infrabranchial sinus and branchial chamber pressures were measured. Heart rate was determined from pulsatile pericardial sinus pressure data. The pressure drop across the gill circuit was calculated from mean hemolymph pressure data as [infrabranchial sinus pressure − pericardial sinus pressure].

Hydrostatic pressure in the branchial chamber was measured similarly. Hook-shaped catheter tips fashioned from PE 190 tubing were inserted through the Milne-Edwards opening, gently pushed between the first and second gills, and positioned in the hypobranchial space. The catheter was seated in the corner of the Milne-Edwards opening opposite the mouth so that the opening was not occluded. Care was taken to avoid catheter contact with the gill raker and scaphognathite. All parameters were monitored for at least 1 h prior to either saline or *Vibrio* injection.

Gill microscopy

Individual gills were removed by cutting at the base of the efferent vessel. A gill was placed in 20 ml of fixative (2.5% glutaraldehyde in 0.1 mol l^{-1} phosphate buffer, adjusted to pH 7.4 and an osmotic concentration of 894 mmol kg^{-1}), where it remained for at least one day. The tissue was dehydrated in ascending concentrations of acetone, cleared in toluene, embedded longitudinally into paraffin, cut into 8-μm sections, and mounted onto glass slides. Slides were stained using hematoxylin and trichrome (Humason, 1972). Permount was used to mount coverslips to the slides. Slides were observed using a BH-2 Olympus microscope and photographed using a Spot RT digital color camera (Diagnostics Instruments, Inc., Sterling Heights, MI).

Statistical analysis

Statistical tests were performed using SigmaStat ver. 3.0 statistical software. Pressure and heart rate data were analyzed using R, a language for statistical computing (R Foundation for Statistical Computing, 2005).

Results

Hemocytes aggregate to form nodules (Martin *et al.,* 2000) in the gills of blue crabs within 30 min of injection with *Vibrio* (Fig. 1). Nodules form at the bases of lamellae where they branch from the afferent and efferent vessels. Nodules also form within the lamellae.

The uptake of oxygen from the ambient medium is rapidly and significantly reduced by the injection of *Vibrio* ($P < 0.011$, Student's t test). Mean oxygen uptake in saline-injected control crabs was 4.53 μmol g^{-1} h^{-1} ($\pm$0.59 SEM, $n = 6$) and 2.56 μmol g^{-1} h^{-1} ($\pm$0.23 SEM, $n = 6$) in *Vibrio*-injected crabs.

Large and significant differences between postbranchial and prebranchial oxygen pressures were found 30 min after injecting either saline or *Vibrio* (Fig. 2, $P < 0.001$ for both treatments using a paired t test). Postbranchial Po_2 differences between the treatments were highly significant ($P = 0.006$, 14 df using a t test); however, there was no difference between treatments in prebranchial Po_2 ($P = 0.768$, 14 df, t test). A significant difference was found between postbranchial pH and prebranchial pH in saline-injected crabs (Fig. 2, $P = 0.049$, paired t test), but not *Vibrio*-injected crabs ($P = 0.91$, using a Wilcoxon signed rank test, since the data-failed the test for normality).

Hemolymph pressure in crabs is relatively variable, particularly in the sinuses where we made measurements: the pericardial space (downstream from the gill circulation) and the infrabranchial sinus (upstream from the gill circulation). Pressure drop across the gill circulation in crabs prior to either *Vibrio* or saline injection ranged from 214 to 763 Pa (Fig. 3; mean values: controls = 497 ± 89 SEM, $n = 4$; *Vibrio* = 451 ± 64, $n = 5$). The pressure drop changed little 30 and 60 min after injection with saline (3% decrease at 30 min and an additional 6% decrease at 60 min). The gill circulation pressure change in *Vibrio*-injected crabs increased nearly 2-fold at 30 min post-injection, and this elevated level was maintained through the 60-min time period (Fig. 3).

Branchial chamber hydrostatic pressure was more negative immediately post-catheterization as the crabs were disturbed and scaphognathite activity was higher. Over the 1- to 2-h period prior to injection with saline or *Vibrio,* branchial chamber pressure became less negative as scapho-

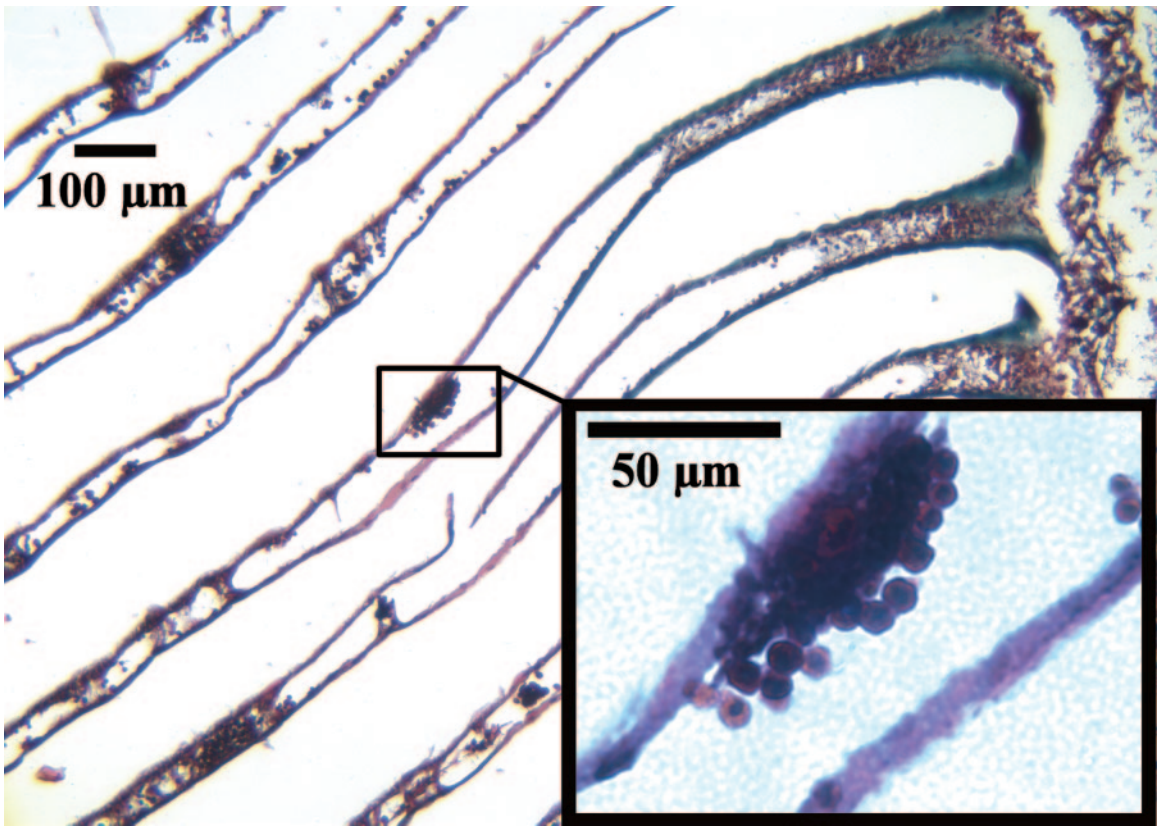

Figure 1. A cross-section of gill #7 of blue crab, 30 min after injection with *Vibrio campbellii* at a concentration of 2.5×10^4 g^{-1} crab into the ventricle. Individual lamellae are shown arising from the main vessel along the right side. Hemocyte aggregates, or nodules, are most easily observed within the lamellae, but also occur at the base of the lamellae. These nodules are thought to interfere with hemolymph flow, resulting in loss of respiratory function.

gnathite activity decreased, and settled at about −100 Pa (Fig. 3; mean values: controls = −90.1 ± 17.9 SEM; *Vibrio* = −105 ± 11.4 SEM). By 30 min post-injection in control crabs, branchial chamber pressure had increased (*i.e.,* become less negative) by only 5%; by 60 min, it had decreased by only 6% compared to pre-injection values. In *Vibrio*-injected crabs, branchial chamber pressure changed less than 2% over 60 min (Fig. 3). We suggest these changes to be within the range of variability for ventilatory function in quiescent crabs. We consider branchial chamber pressure to be the crucial variable in our experiments because it is the transmural pressure (the pressure difference between the gill circulation and branchial chamber spaces) that could have an effect on the hemolymph pressure drop across the gill circulation. However, we determined scaphognathite rate from the pressure records, noting that there was less than 5% change in rate throughout the experiments for both saline- and *Vibrio*-injected crabs (mean pre-injection scaphognathite rates: controls = 111.9 ± 20.8 SEM min^{-1}; *Vibrio* = 139.7 ± 15.8 SEM).

Heart rates remained relatively constant (changing by less than 10% in control crabs and less than 3% in *Vibrio*-

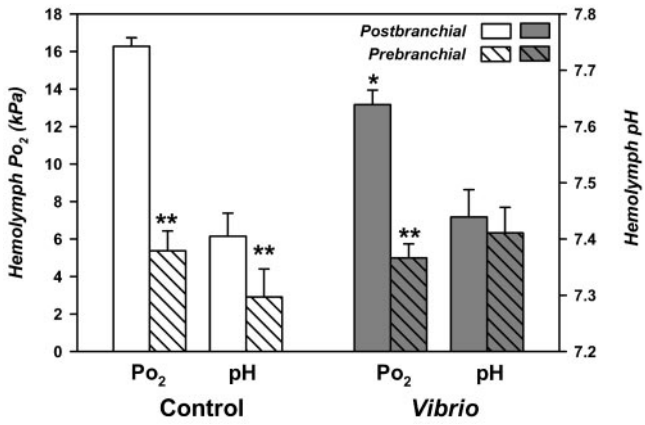

Figure 2. Prebranchial and postbranchial hemolymph oxygen pressures (Po_2) and pH in crabs 30 min after injection with either saline (control) or *Vibrio.* Values are means ± SEM; $n = 7$ for control and $n = 9$ for *Vibrio*-injected crabs. Significant differences between prebranchial and postbranchial variables within a treatment are indicated by **. Significant differences of a variable between treatments are shown by *. In comparing prebranchial and postbranchial values in *Vibrio*-injected crabs, the test for normality failed and a Wilcoxon signed rank test revealed no difference between median values.

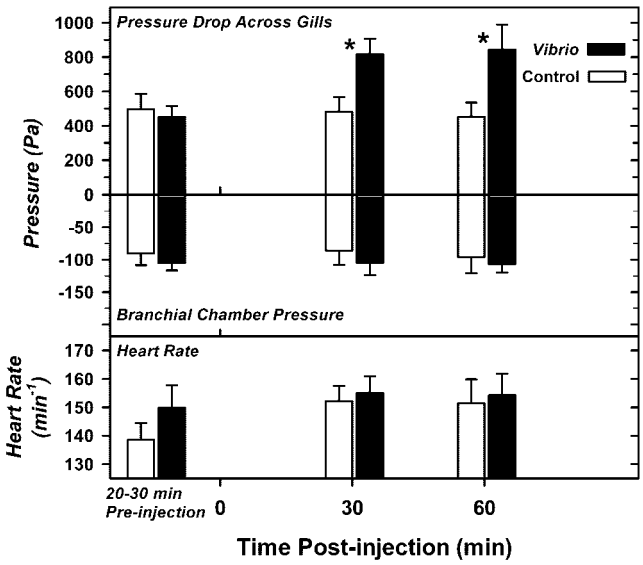

Figure 3. Pressure drop across the gills increases significantly (*) in *Vibrio*-injected crabs (black bars). Branchial chamber pressures, which are negative pressures, and heart rate remain unchanged. The increase in the pressure drop across the gills indicates an increase in vascular resistance following injection of *Vibrio.* All data are reported as mean ± SEM; control $n = 4$, *Vibrio*-injected $n = 5$.

challenged crabs) over the experimental period (Fig. 3; mean pre-injection values: controls = 139 ± 6 SEM min^{-1}; *Vibrio* = 150 ± 7.8 SEM).

We analyzed the pressure and heart rate data using a single-level mixed model with the fixed effects of time and treatment (Pinheiro and Bates, 2000), finding a significant effect ($P = 0.0116$) of *Vibrio* injection on pressure changes across the gills with time, but no effects on branchial chamber pressure ($P = 0.83$), heart rate ($P = 0.15$), or scaphognathite rate ($P = 0.50$).

To exclude the possibility that differences in hemolymph oxygenation are due to differences in the amount of the respiratory pigment, hemocyanin, we measured hemocyanin concentration (Nickerson and Van Holde, 1971) in both saline-injected (mean = 41.6 mg ml^{-1} ± 4.1 SEM, $n = 7$) and *Vibrio*-injected (mean = 40.3 mg ml^{-1} ± 3.7 SEM, $n = 9$) crabs, finding no statistical difference ($P = 0.8$, *t* test).

Discussion

Our findings indicate a significant disruption of gas exchange across the gills of the blue crab following injection of the bacterium *Vibrio campbellii.* These findings are consistent with the suggestion that the formation of hemocyte aggregates or nodules in the gills of a crab (Fig. 1) interferes with respiratory function (Johnson, 1976; Smith and Ratcliffe, 1980a; White *et al.,* 1985; Martin *et al.,* 2000). A decline in the overall flux of oxygen into the crab by 43% is associated with a reduction in oxygenation of hemolymph at the gills (Fig. 2).

A large decline in postbranchial Po_2 induced by *Vibrio* injection and the subsequent formation of nodules is consistent with the blockage of hemolymph flow through the gills. Quiescent blue crabs injected with saline achieve high levels of oxygenation at the gills (Po_2 = 16.2 kPa, Fig. 2). This value is slightly higher than those previously reported for *C. sapidus,* 15 kPa (Mangum *et al.,* 1985; deFur *et al.,* 1988), but may reflect the high degree of water oxygenation (>20 kPa) in our treatments and the quiescence of our crabs. Injection of *V. campbellii* caused a 19% decline in Po_2, and this is consistent with a substantial blockage of the flow of hemolymph through the gills. Prebranchial Po_2 is higher in both control and experimental crabs than reported elsewhere for this species. Our experimental manipulations of the crabs required that we inject either saline or saline containing *Vibrio,* 30 min before removing hemolymph samples. We observed that when we instrumented the crabs for pressure-measuring catheters there was a hyperventilation of the branchial chamber (elevated scaphognathite beat frequency) for a short time (see below). An elevated prebranchial Po_2 might be due to this hyperventilation on an otherwise quiescent crab. Hyperventilation might also explain the high postbranchial Po_2.

Similar indications of a disruption of gill hemolymph flow are seen in the differences in pH between prebranchial and postbranchial hemolymph. These differences are normally small but measurable in crabs (McDonald *et al.,* 1979; McMahon *et al.,* 1984), reflecting the efflux of CO_2 across the gills; prebranchial hemolymph contains more CO_2 and is more acidic. The saline-injected control crabs had a significant pH difference of 0.1, and this difference disappeared in *Vibrio*-injected crabs. An overall reduction of CO_2 excretion might result in a slight acidification of the well-buffered hemolymph; however, because of a decline in aerobic metabolism in *Vibrio*-injected crabs, CO_2 excretion would also decrease, resulting in a hemolymph pH that would be stable or slightly alkaline compared with controls. Indeed, a decline in the overall production of CO_2 might also explain the lack of a difference between postbranchial and prebranchial pH.

In one final assessment of the impacts of bacterial challenge on respiratory performance, we measured the hemolymph pressure drop across the gill circulation. The crab circulatory system is arranged such that the gill circuit is located in series with, and downstream from, the systemic circuit. Venous hemolymph pools in the infrabranchial sinus (the high-pressure end of the gill circulation), just upstream from the gills, moves through the gill hemolymph channels, and collects in the pericardial sinus (the low-pressure end) in which the ventricle is suspended. Hemolymph then flows into the ventricle, which pumps it into the systemic circuit (McMahon and Burnett, 1990). We mea-

sured pressures in the pericardial sinus and the infrabranchial sinus, using indwelling catheters before and after injections of either saline or *Vibrio campbellii.*

If hemocyte nodules embolize in the circulatory channels of the gills, resistance to hemolymph flow would increase and should be reflected in a greater hemolymph pressure change across the gills. The hydraulic analog of Ohm's Law states that a hydrostatic pressure drop across a fluid-filled tube or tube network is the product of total fluid flow and resistance to flow—that is, pressure drop and resistance are directly related. We suggest that an increased pressure change across the gill circulation is induced by *Vibrio* injection and indicates an increased resistance to hemolymph flow through the circulatory channels in the gills, assuming relatively constant total hemolymph flow through the gill circuit. Cardiac output (and by extension, hemolymph flow through the gill circulation) in crustaceans is a function of both heart rate and cardiac stroke volume. The small changes in heart rate observed in both control and *Vibrio*-injected crabs (Fig. 3) cannot account for the near doubling of pressure drop across the gill circulation in *Vibrio*-injected animals. We cannot rule out differences in cardiac stroke volume that could account for the observed pressure differences, but the almost 2-fold increase in pressure drop (given the relative constancy of our heart rate data) would require a near doubling of cardiac stroke volume. This is unlikely. Moreover, we were able to obtain relatively clean pulsatile hemolymph pressure measurements in the pericardial space in most experiments for both control and *Vibrio*-injected crabs. We noted little change in pericardial pulse pressure (difference between maximum and minimum pressure for a cardiac cycle) throughout the experiments. It would be expected that a substantial increase in cardiac stroke volume would be reflected in a change in pericardial pulse pressure.

During these experiments, crabs appeared to be quiescent, if not resting. Heart rate values fell in the range of the 110 to 210 min^{-1} previously reported for blue crabs at a variety of experimental temperatures (deFur and Mangum, 1979; Booth *et al.,* 1982; Rantin *et al.,* 1996; McGaw and Reiber, 1998) when adjusted to 25 °C using $Q_{10} = 2$ (deFur and Mangum, 1979). A resting crab would be expected to show periods of cardiac pausing. We did not observe cardiac pausing in our crabs, but we did note that, within 20–30 min of catheterization, they would typically burrow into the sediment and remain quiescent throughout the experimental period. We therefore believe that they were not unduly stressed.

Our scaphognathite rate data fall in the range previously reported for blue crabs at a variety of experimental temperatures, with values ranging from 70 to 200 min^{-1} (Batterton and Cameron, 1978; Booth *et al.,* 1982; Rantin *et al.,* 1996) adjusted to 25 °C using $Q_{10} = 2$ (deFur and Mangum, 1979). We noted higher scaphognathite rates immediately after catheterization, with the rates settling to our reported pre-injection levels within about 30 min. Again, we believe this indicates that our crabs were quiescent and not unduly stressed.

While our scaphognathite data are important for comparative purposes, we believe that the more important variable is branchial chamber pressure, because we also make the assumption that total hemolymph flow through each gill set is relatively constant. Crab gills are compliant (McMahon and Burnett, 1990), and the hemolymph channels coursing through the gill lamellae are likely to be responsive to transmural pressure differences across the gill epithelium (*i.e.,* the pressure difference between the hemolymph space within the gill and the water-filled space — the branchial chamber — into which the gills extend). So, we measured the hydrostatic pressure in the branchial chamber concurrently with hemolymph pressure. Our results show that branchial chamber pressure is relatively constant throughout both control and *Vibrio* experiments; therefore, the pressure changes following *Vibrio* injection are not the result of pressure changes outside the gills but indicate increased resistance to hemolymph flow through the gill circulatory channels. This observation is consistent with occlusion of gill circulation caused by nodules collecting in those channels. Moreover, the time course of the hemodynamic changes coincides with the decline in respiratory performance.

The results presented here offer strong circumstantial evidence that the immune response launched by *C. sapidus* against *V. campbellii* compromises the ability of the gills to take up oxygen. This result could be due to the formation of nodules within the vasculature of the gills in response to a microbial pathogen, as demonstrated by others (Johnson, 1976; Smith and Ratcliffe, 1980a; White *et al.,* 1985; Martin *et al.,* 2000). However, we cannot rule out the possibility that the observed depression of respiration is mediated by other direct or indirect effects of *V. campbellii,* its exotoxins or endotoxins, leading to vasoconstriction within the gills. For example, isolated lipopolysaccharide (LPS), an integral component of the outer membrane in gram-negative bacteria, can induce hemocytopenia in crustaceans, mimicking the effects of intact bacterial cells (Martin *et al.,* 1993; Lorenzon *et al.,* 1999). In addition, LPS can induce the release of crustacean hyperglycemic hormone (CHH) from the sinus gland in decapod crustaceans (Lorenzon *et al.,* 1997, 2004). CHH has complex effects on carbohydrate metabolism, osmoregulation, ecdysis, and reproduction, any one of which might alter hemodynamics in the blue crab following injection of *V. campbellii* (reviewed by Fanjul-Moles, 2006).

Clearly we do not know if the decline in aerobic metabolism is due purely to the reduction in the performance of the oxygen delivery system. Other factors such as those mentioned above can influence metabolism. Even though

we did not measure hemolymph lactate in the present study, in a parallel study on the shrimp *Litopenaeus vannamei* (Scholnick *et al.,* 2006) oxygen uptake declined and only a small amount of lactate was produced in response to *Vibrio* injection, indicating that a major shift to anaerobic metabolism did not occur.

For a quiescent blue crab in well-aerated water and at moderate temperatures, the decrease in respiratory fitness associated with mounting an immune defense is likely to have minimal impact. However, demands on the oxygen delivery system of the blue crab under conditions of environmental hypoxia and or high activity may be exacerbated by the process of removing or inactivating a bacterial pathogen. Hypoxia is common in estuaries and coastal waters (Rabalais and Turner, 2001); it may be severe and last for many hours or days. The occurrence of hypoxia in shallow coastal waters appears to be increasing globally, and it is thought to be linked to human activity (Diaz, 2001). Blue crabs are well-known for their ability to cope with hypoxia. Like many aquatic organisms, they compensate for low oxygen by increasing ventilation rate and heart-beat frequency. They supplement their aerobically driven energy supply using anaerobic pathways that lead to the production of lactic acid (deFur *et al.,* 1990), indicating that the oxygen delivery system cannot meet the energy demands of the resting crab. In view of our findings, hypoxic conditions would only exacerbate the already-compromised oxygen uptake and delivery capacity of a crab fighting bacterial infection.

The second circumstance that would pose a serious challenge to the blue crab would be that of activity. Blue crabs are active and aggressive animals. They are also swimming crabs and are known to migrate over long distances (Aguilar *et al.,* 2005). High levels of activity are supported by the oxygen delivery system, but as with hypoxia, the oxygen transport system fails to meet all of the demands for energy during locomotion, and lactic acid is produced (Booth *et al.,* 1982). Interference with oxygen uptake from the ambient medium, as occurs when the crab is injected with *Vibrio* or becomes infected in the field (Davis and Sizemore, 1982), could place burdens on the oxygen transport system such that the crab could not sustain its normal level of swimming or other kinds of activity. We are currently investigating how bacterial challenges affect overall locomotory performance in crustaceans.

Finally, in all the situations presented above, the levels of oxygen circulating in the hemolymph are lower than in a quiescent blue crab that has no trace of bacterial infection and is immersed in well-oxygenated water. Low oxygen itself has been shown to suppress the immune responses of a variety of marine organisms (Boyd and Burnett, 1999; Boleza *et al.,* 2001) including crustaceans (Mikulski *et al.,* 2000; Holman *et al.,* 2004; Burgents *et al.,* 2005a). The decline of oxygen levels in coastal ocean environments is cause for concern, as organisms are clearly limited in their ability to mount effective responses to routine bacterial and viral challenges, and at least in crustaceans, the process of eliminating these pathogens has its own cost on the respiratory fitness of the organism.

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