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REGULATION OF PULMONARY BLOOD FLOW AND OF BLOOD PRESSURE IN A MANGROVE CRAB (*GONIOPSIS CRUENTATA*)

BY J. L. WILKENS

Regulatory Mechanisms Research Group, Department of Biological Sciences, University of Calgary, Calgary, Canada

AND R. E. YOUNG

Department of Physiology, University of the West Indies, Kingston, Jamaica, WI

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Summary

The air-breathing mangrove crab *Goniopsis cruentata* ventilates the branchial chambers with its scaphognathites (SG). Ventilation is predominantly in the forward direction, but is punctuated by bouts of reversed pumping. Reversals are more frequent when crabs are in air than in water, and yet more frequent during respiratory stress (hypoxia or exercise). Reversed SG pumping is tightly coupled with bursts of impulses to the dorsal–ventral muscles (DVM) which span the anterolateral thorax. Phasic contractions of the DVMs increase the hemolymph pressure in the dorsal sinuses. These pressure pulses help drive hemolymph through the lungs. The coupled SG reversed ventilation and DVM-assisted increases in lung perfusion appear to be an adaptation to increase gas exchange at the lungs.

When crabs are made hyper- or hypotensive by changes in hemolymph volume, the EMG activity of the DVMs dramatically decreases or increases, respectively. The resultant expansion or constriction of the dorsal sinuses is an effective baroreceptor reflex producing short-term adjustments in hemolymph pressure.

Introduction

The supply of respiratory gases to the tissues requires coordinated functioning of the respiratory exchange apparatus and the circulatory system. Several recent reviews have focused on aspects of both the cardiovascular and respiratory physiology of air-breathing crustaceans (Burggren and McMahon, 1988*a*; Greenaway and Farrelly, 1990; Truchot, 1990). In this paper, we examine interactions of these systems in an amphibious grapsid mangrove crab, *Goniopsis cruentata*.

Terrestrial crabs have two routes for respiratory gas exchange, the gills and the

Key words: baroreceptor, branchiostegite, lung, crustacean, dorsal sinus, dorsal–ventral muscle, hypoxia, reversed ventilation, *Goniopsis cruentata*.

vascularized epithelial linings of the branchiostegites or lungs (McMahon and Burggren, 1988*a*). In the transition from aquatic to terrestrial habitats, the area of the gills becomes reduced while the area devoted to the lungs is expanded (Gray, 1957; Bliss, 1968; Diaz and Rodriguez, 1977; Cameron, 1981). The branchial chambers (BC) of both aquatic and terrestrial crabs are ventilated by the scaphognathites (SG), which pump either water or air. The SGs normally beat in the forward mode, which pumps water or air from the ventral margins of the BC, over the gills and out of the exhalant channels. The SGs can also reverse the direction of pumping. In one desert-adapted crab (Greenaway and Taylor, 1976), and possibly in others as well (Innes *et al.* 1987; Whiteley *et al.* 1990; Al-Wassia *et al.* 1989), movements of the flexible thoracic wall of the anterior portions of the BC also tidally ventilate the BCs.

In purely aquatic crabs, the gills occupy almost the entire branchial chamber. The gills have numerous delicate, closely packed lamellae which, in water, are expanded and separated, allowing both vascular perfusion and ventilation at the lamellar surfaces. In air, the gill lamellae collapse and clump together so that both perfusion and ventilation are compromised.

Terrestrially adapted crabs have fewer and smaller gills, which occupy as little as one-third of the BCs. The lamellae are more widely spaced and their outer margins are heavily cuticularized and expanded. In air, these gills do not collapse, and so may remain functional. Fully terrestrial species, however, rely almost exclusively on the lungs and may drown if submerged (McMahon and Burggren, 1988). Transitional crabs such as *Goniopsis*, capable of living either in water or in air, rely predominantly on their gills for oxygen exchange while in water, and switch to the lungs for gas exchange when in air (Maitland, 1987, 1990). When such crabs move from water to air, there may be a facultative shift in the hemolymph flow from the gills to the lungs as a result of the increased resistance of the gill vasculature caused by altered transmural pressures and a tendency for the lamellae to collapse (Taylor and Greenaway, 1984; Al-Wassia *et al.* 1989). The gills receive hemolymph from the infrabranchial sinus (IS) located at the base of the legs, while the lungs are supplied from the dorsal thoracic sinuses (DS) located in the anterolateral thorax (Greenaway and Farrelly, 1984, 1990; Taylor and Greenaway, 1979, 1984). Hemolymph dammed up in the IS during facultative switching is shunted to the pulmonary circulation by way of the thoracic sinus system (Taylor and Greenaway, 1984).

Both gills and lungs contain anastomosing capillary-like networks, which drain directly to the pericardial sinus (PS) *via* the branchio-pericardial and efferent pulmonary veins, respectively. The vasculature in both gills and lungs constitutes venous portal systems operating at relatively low blood pressures, so that any factors tending to assist or hinder flow might have quite significant effects. In aquatic crabs during forward ventilation, the negative hydrostatic pressure in the BC tends to expand blood vessels of the gills, thus facilitating hemolymph flow (Taylor, 1990). During bouts of reversed ventilation, the positive BC water pressures tend to force hemolymph from the gills, transiently increasing venous

return to the heart (Blatchford, 1971; Rajashekhar and Wilkens, 1991). In terrestrial crabs breathing air, the SG-generated branchial pressures should have little effect on the dynamics of gill or lung perfusion because of the smaller BC pressure fluctuations, the more rigid gills (Harms, 1932) and the impedance mismatch from air to hemolymph. Since the IS is below the PS, gravity would tend to hinder, not assist, gill perfusion. Movements of the legs, whose sinuses drain into the IS, produce pressure pulses in the IS and may serve as an auxiliary pumping mechanism assisting gill perfusion during activity (J. L. Wilkens and R. E. Young, unpublished observations). The DSs are at approximately the same level as the PS.

In this report, we show that the band of dorsal–ventral muscles (DVM, Pearson, 1908) functions as an auxiliary pump for the pulmonary circulation in an air-breathing mangrove crab. It is known that the DVMs in an aquatic crab, *Carcinus maenas*, may participate in the control of the DS pressure (Rajashekhar and Wilkens, 1991; Taylor and Taylor, 1991); however, in air, both *Goniopsis* and *Carcinus* utilize the DVMs in a completely opposite manner to *Carcinus* in water.

Electromyogram (EMG) recordings from the DVMs unexpectedly revealed that these muscles also play an important role in regulating overall hemolymph pressure. These findings clearly demonstrate that baroreceptors sensing hemolymph volume and/or pressure must exist, although such receptors have yet to be positively identified. The generality of these findings is substantiated by similar findings in *Carcinus* (Taylor and Taylor, 1991).

Materials and methods

Abbreviations used

BC	branchial chamber
DS	dorsal sinus
DVM	dorsal–ventral muscles
f_{DVM}	EMG spike rate in the DVM (impulses s^{-1})
f_H	heart rate (beats min^{-1})
f_R	ventilation rate (beats min^{-1})
f_{REV}	reversal rate (bouts min^{-1})
IS	infrabranchial sinus
PS	pericardial sinus
P_{DS}	hemolymph pressure in dorsal sinus
P_{PS}	hemolymph pressure in pericardial sinus
SG	scaphognathite

Mangrove crabs (*Goniopsis cruentata*) were collected from *Rhizophora* mangrove trees at the Port Royal Marine Laboratory, University of the West Indies, Jamaica. They were maintained in aquaria provided with raised platforms which allowed them to climb into the air. The shallow depth of the sea water allowed crabs in the water to elevate the exhalant ventilatory channels above the surface. Crabs were fed carrot slices and *Rhizophora* sp. leaves.

During experimental observations, the crabs were restrained by rubber bands stretched across the base of the legs. This allowed the animals to move their body slightly and to elevate the anterior 'facial' area. All experiments were performed at room temperature (25±1°C). The saline formulated for *Carcinus* (Wilkens *et al.* 1989) was used to expand hemolymph volume. *Goniopsis* tolerated multiple injections without obvious signs of distress and no loss of function in the variables measured. To estimate the fractional expansion or reduction, the normal hemolymph volume was taken to represent 30% of the body mass (Gleeson and Zubkoff, 1977).

Scaphognathite ventilation and heart rates were measured by impedance transduction (Biocom Impedance Converter, model 2991). DVM electromyograms (EMG) were recorded by Grass P15 a.c. amplifiers. Holes were drilled through the dorsal carapace at the muscle scars marking the origin of the DVMs (see Fig. 1) and 0.4 mm diameter copper or silver wires were implanted into the dorsal ends of the muscles and fixed in place using cyanoacrylate glue. Branchial air pressure was measured with a Grass PT5C transducer *via* 1.67 mm i.d. polyethylene tubing inserted through a hole drilled in the dorsal branchiostegite. The amplitude of air pressure transducer voltage deflections was converted to air volumes by calibrators supplied with the instrument. Hemolymph pressures were recorded by Statham P23Db pressure transducers coupled to Grass 7P1 d.c. preamplifiers. The transducers were connected *via* polyethylene tubes (1.14 mm i.d.) implanted through predrilled holes over the appropriate sinus. Cannulae were inserted through tight-fitting holes in squares of dental dam cemented over the predrilled holes with cyanoacrylate glue.

The recorded motor unit potentials of the DVMs were displayed directly or as a rate using a Grass 7P3 a.c. preamplifier and integrator. For integration, the spikes from the Grass P15 amplifier were further amplified by a Neurolog NL105 preamplifier, then passed through a Neurolog NL200 spike trigger/window discriminator. The BRIT signal from the window was used to trigger a Grass S9 stimulator which, in turn, supplied an acceptable signal to the Grass integrator. Integrator output therefore indexed frequency of spikes in a predetermined height window, regardless of variations in spike height or duration.

Data were stored on a Grass 7E polygraph and on a Hewlett-Packard 3960 instrumentation tape recorder. Some data were filmed from the screen of a Tektronics 5103N storage oscilloscope. Numerical data were transcribed to Lotus 1–2–3 software for analysis and graphs were constructed using SigmaPlot V.4.4. Student's *t*-test was used to test for differences between unpaired means.

Results

Behavior

Goniopsis cruentata were collected from near the water's edge up to several meters into the foliage of *Rhizophora* sp. mangrove trees. When avoiding our

attempts to capture them, crabs often ran or dropped into the water and escaped under water. In aquaria, crabs distribute themselves equally in air or in water, but when in water they orientate themselves to keep the anterior 'facial' area elevated so that the exhalant ventilatory openings are held above the surface of the water and the dorsal lung portion of the BC is filled with air. In this condition, they ventilate predominantly in the forward direction. They may also be moving air through the BCs, but this has not been directly measured. After disturbances and/or forced exercise, crabs choose to be in the water, but they always orientate with the exhalant channels in air. These crabs show frequent bouts of reversed ventilation (2–3 min^{-1}) during which bubbles of air are blown out of the back of the BCs over the fifth pereiopods. We investigated the cardiorespiratory behavior of these crabs in each of the three conditions in which they normally choose to reside: fully submerged, partially submerged with the Milne-Edwards and other inhalant channels submerged and the exhalant channels above water, and fully emerged in humid air.

Characterization of DVM activation

Having previously shown that the activation of DVMs of an aquatic crab is coupled to the SG rhythm and that contraction of the DVM is able to regulate hemolymph pressure in the DS (Rajashekhar and Wilkens, 1991), we first characterized the innervation of the DVMs of *Goniopsis*. On each side of the crab, five columns of muscle extend from their origin on the dorsal carapace 4–5 mm (50 g crab) to the flexible thoracic wall in the anterior one-third of the BCs (Fig. 1A–C).

Dissection indicated that the contraction of these muscles should squeeze the anterior thoracic area which forms the anterior–dorsal and medial walls of the BCs (the thoracic lung, Greenaway and Farrelly, 1990). All five muscles on a side are innervated by one common motoneuron, confirmed by pair-wise recordings from all five DVMs, and all are activated together during both forward and reversed ventilation (Fig. 2A and B). During reversed ventilation, activation of the DVMs changes abruptly from a tonic discharge at about 3–6 impulses s^{-1} to a phasic burst with frequencies of up to 20–30 impulses s^{-1} (Figs 2B,E, 4). The branchiostegal nerve travels across the band of DVMs and, in the example shown in Fig. 2C, the delay in arrival of impulses in DVMs from medial to fourth lateral muscle was 9 ms. The common innervation of the DVMs on each side was further confirmed by the following test. When one DVM is electrically stimulated *via* an electrode with an elongated bare tip (2–3 mm), the branch of the motoneuron to this muscle is activated and this action potential can invade the branches to the other muscles *via* an 'axon reflex' (Fig. 2D). In the example presented, 15 of 30 stimuli delivered to the first, or most proximal, DVM resulted in time-locked spikes in the fourth DVM. Not all stimuli cause impulses in adjacent muscles because many antidromic action potentials die out at some point along the axon branch to the stimulated muscle as a result of collision with centrally generated action potentials.

Reversed ventilation sessions and DVM bursts are bilaterally synchronous over 95 % of the time (Fig. 2E and F). Oscilloscope recordings at fast sweep speeds

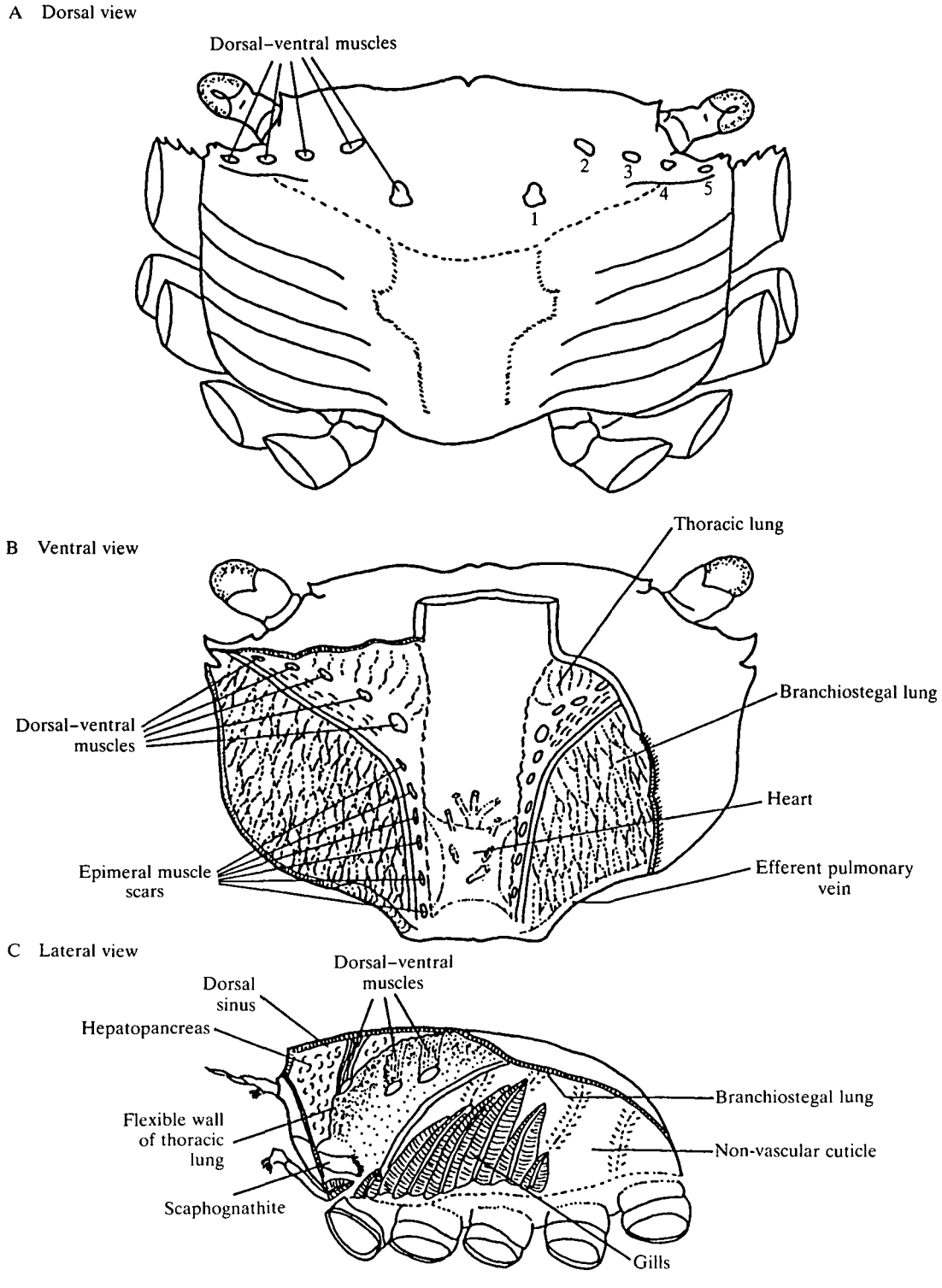
A Dorsal view
Dorsal–ventral muscles
1
2
3
4
5
B Ventral view
Thoracic lung
Dorsal–ventral muscles
Branchiostegal lung
Heart
Epimeral muscle scars
Efferent pulmonary vein
C Lateral view
Dorsal sinus
Dorsal–ventral muscles
Hepatopancreas
Flexible wall of thoracic lung
Branchiostegal lung
Non-vascular cuticle
Scaphognathite
Gills

Fig. 1

Fig. 1. Drawings of various views of *Goniopsis cruentata* to reveal the anatomy of the dorsal–ventral muscles (DVMs) and the dorsal sinus. (A) Dorsal view showing the scars where the DVMs originate on the dorsal carapace. (B) Ventral view with the sternum and viscera removed to show the inside lining of the branchial chambers (BCs). In the anterior one-third of the BC, the DVMs insert on the flexible branchial wall of the thoracic lung, while the posterior two-thirds of the BC makes up the branchiostegal lung. (C) Lateral view in which the outer half of the branchiostegite has been removed to reveal a cross section of the dorsal sinus, the DVMs, which traverse it, and the reduced gills.

show that bursts and momentary gaps in the firing pattern of bilateral pairs of DMVs are, in general, tightly coupled (Fig. 2E).

Cardiorespiratory responses to different external media

Even though these mangrove crabs prefer to be partially or completely in air, they can survive prolonged submersion in aerated sea water. To evaluate adjustments which might occur in response to these different habitats, we measured a number of cardiorespiratory variables from crabs in each of these conditions. Table 1 contains values measured beginning 1–2 h after crabs had been instrumented. Ventilation rate is lowest when crabs are in air, doubles when they are partially submerged and doubles again when they are totally submerged in aerated sea water. These crabs showed a high incidence of reversed ventilation sessions in air but almost none when completely submerged. The duration of reversal sessions was similar in all environments. Tonic DVM EMG impulse rates remain relatively constant in all three environments. Heart rate fell slightly (8%) during the transition from complete emergence to submergence.

Well-settled crabs left in the recording chamber overnight with enough sea water to cover the ventral margins of the BCs exhibit a high incidence of

Table 1. *The effects of different respiratory environments (air, partially submerged or fully submerged) on cardioventilatory and dorsal–ventral muscle performance*

Variable	Air	Partially submerged	Fully submerged
f_R (beats min^{-1})	69.1±20.8 (6)	144.3±33.3 (6)	271.8±26.4* (6)
f_{REV} (bouts min^{-1})	1.96±0.6 (5)	1.54±0.2 (5)	0.28±0.1* (5)
f_{DVM} (impulses s^{-1})	5.4±0.7 (7)	5.1±0.5 (7)	5.6±0.8 (7)
f_H (beats min^{-1})	171.0±8.9 (5)	167.8±8.0 (5)	155.6±5.2 (5)

Values are mean±S.E.M.; number of observations in parentheses.
* Significantly different from the value in air; $P<0.01$.
See Materials and methods for an explanation of the abbreviations.

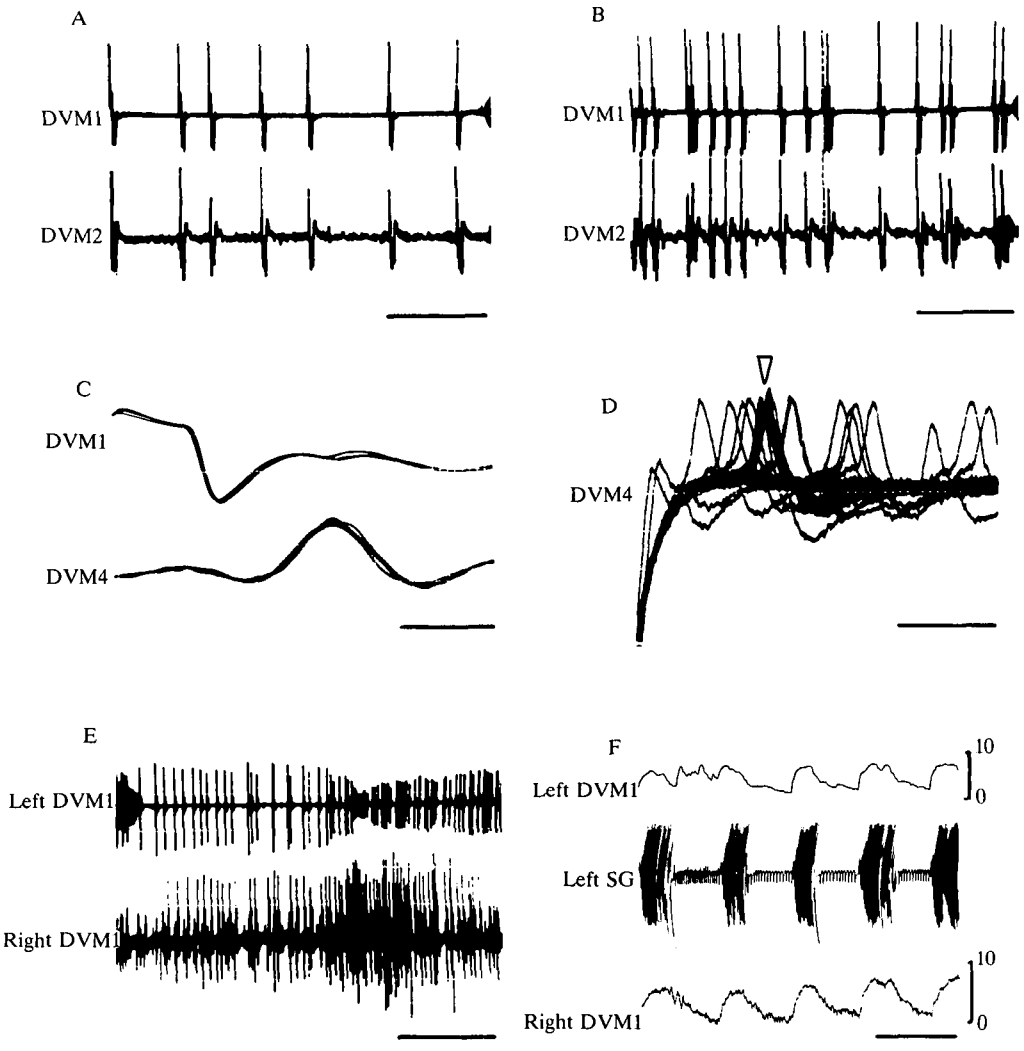

Fig. 2. EMG recordings from DVM muscles. (A) Recordings from DVM1 (most medial) and DVM2 on one side during forward ventilation, f_{DVM}=3.5 impulses s^{-1}; (B) recordings from the same pair of muscles during a scaphognathite reversal, showing that the same motoneuron is active, f_{DVM}=8.5 impulses s^{-1}. (C) Five superimposed sweeps of recordings taken from DVM1 and DVM4 during tonic activation, delay=9 ms. (D) A phase-locked spike (arrowhead) occurred in DVM4 during 15 of 30 superimposed sweeps, where each sweep was triggered by a single stimulus to DVM1. (E, F) Recordings from the right and left DVM1 showing strict bilateral coordination of EMG activity. In F, the left and right medial DVM activity is displayed as the integrated rate (in impulses s^{-1}). The middle trace displays left SG activity, the large excursions indicate intermittent reversals. Time calibration bars (A, B) 0.5 s, (C) 5 ms, (D) 12.5 ms, (E) 1.25 s, (F) 20 s.

Table 2. *Effects of different levels of hypoxia on cardioventilatory performance in partially submerged crabs*

Variable	Air/N_2 mixture		
	100/0 (≈20.3 kPa)	62/38 (≈12.8 kPa)	31/69 (≈6.4 kPa)
f_R (beats min^{-1})	70.4±31.3	72.4±22.3	98.0±28.2
f_{REV} (bouts min^{-1})	0±0	1.5±0.4*	2.9±0.8*
Reversal bout duration (s)	0±0	3.6±0.3*	7.0±1.0*
	(4)	(4)	(4)
f_{DVM} (impulses s^{-1})	5.12±1.6	6.1±0.7	5.2±1.0
f_H (beats min^{-1})	155±9.1	154±11.9	139.4±14.2

Values are mean±S.E.M. Observations are taken from five crabs except for durations of reversal bouts.

* Significantly different from the value at approximately 20.3 kPa; $P<0.01$.

See Materials and methods for an explanation of the abbreviations.

ventilatory pausing, which typically lasts for many minutes (see Fig. 6A). Ventilatory rates during bouts of ventilation, and heart rates, were lower than those shown in Table 1. These crabs showed very few reversal sessions in any well-aerated environment.

Exposure of partially or totally emerged crabs to mildly hypoxic air (P_{O_2}≈12.8 kPa, 100 mmHg) did not significantly alter DMV EMG rate, f_R or f_H, but less than 1 min of exposure caused a dramatic increase in the frequency and duration of reversal sessions (Fig. 3, Table 2). During the first 5 min of exposure to moderate hypoxia (P_{O_2}≈6.4 kPa, 50 mmHg), ventilatory rate increased, heart rate fell, and the frequency and duration of reversal sessions was further increased (Fig. 3, Table 3). During all reversal sessions, the DVMs received an intense burst of impulses lasting the duration of the session, while heart rate was unchanged. The absolute coupling between reversed SG beating and DVM bursts also occurs when large holes (4–5 mm diameter) are drilled in the dorsal BCs. When the BCs are so opened, reversed SG pumping does not cause air pressure pulses in the BC, thus excluding the possibility that the DVM bursts are a reflexive response to pressure pulses.

Role of the DVMs in pulmonary circulation

SG reversals and bursts of impulses in the DVMs are accompanied by increases in hemolymph pressure in the DS (Fig. 4). These pressure pulses increase DS pressure by about 45 %. Their magnitude was about twice as great in the DS as in the PS (Fig. 5A), and effectively doubled the pressure difference between these two sinuses (Table 3). There is close coupling between the pressures in these two sinuses at all times. These pressure pulses should accelerate hemolymph flow through the branchiostegal portion of the lung, assuming that the resistance of the vascular bed remains constant.

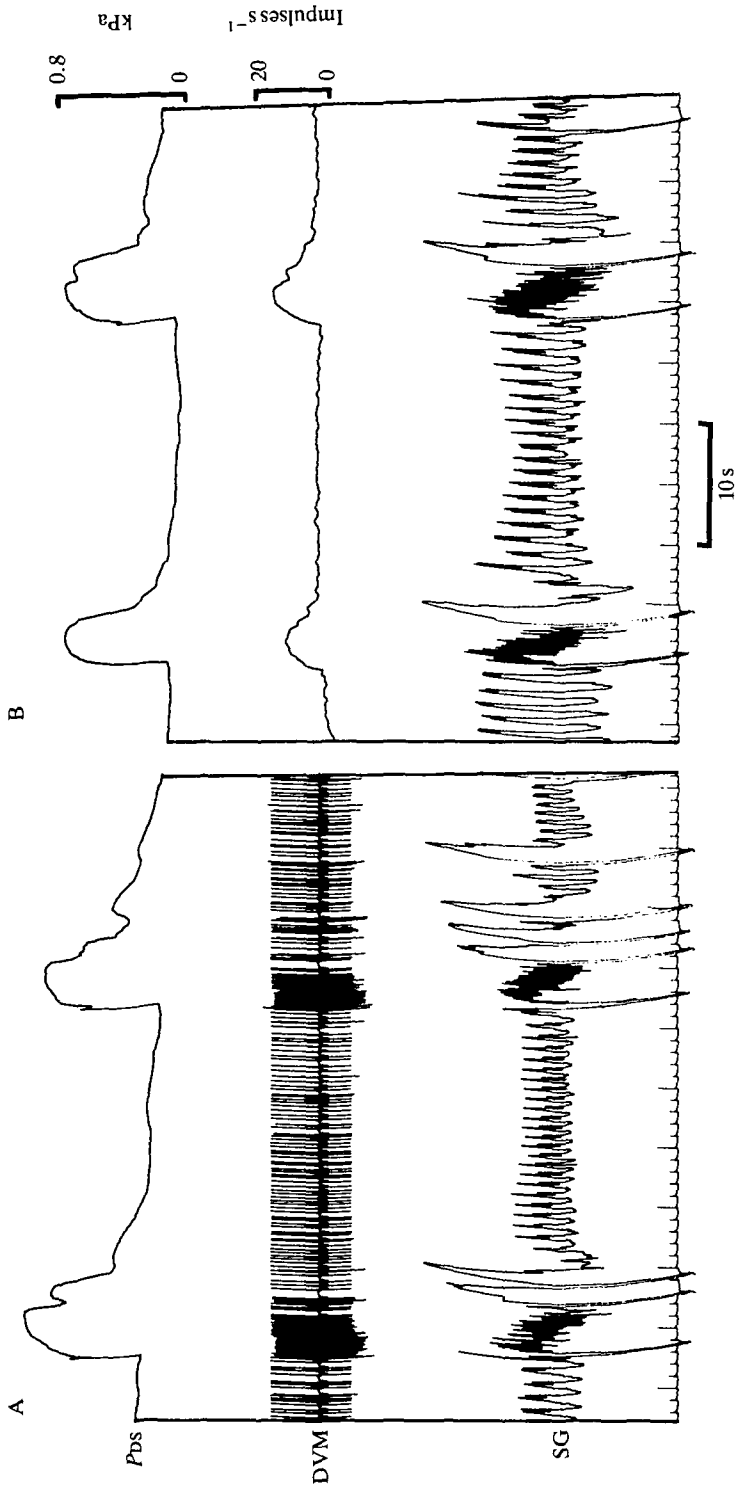

Fig. 4. Two panels showing the coupling between SG reversals, DVM bursts and dorsal sinus pressure pulses (P_{DS}). (A) DVM EMG as raw data; (B) DVM as displayed by the rate meter. The large reversed SG beating signals appear to arise from air bubbles which cause increased impedance between the recording electrodes. Time is marked at 1 s intervals.

Table 3. *Hemolymph pressures recorded from different body cavities in partially submerged crabs*

	Pressure (kPa)
Heart	
Systole	2.75±0.20 (5)
Diastole	1.17±0.08 (5)
Pericardial sinus	
Systole	1.59±0.28 (5)
Diastole	1.25±0.14 (6)
Infrabranchial sinus	1.91±0.13 (6)
Dorsal sinus	1.56±0.15 (6)
DS–PS (diastole)	0.35±0.17 (6)

Values are mean±S.E.M. Number of observations in parentheses.
DS–PS, the pressure difference between these two sinuses.

Although DVM contractions coincide with the DS pressure pulses during reversals, they need not be the sole cause of these pulses. Reversals have been likened to coughs and, as such, may involve concerted responses in several systems. To test for a causal connection between DVM contractions and the pressure pulses, electrodes were implanted into the medial four DVMs on each side and all eight were simultaneously stimulated in several crabs. Stimulation of these muscles at 20 Hz caused pressure pulses in the DS and PS of about the same magnitude as those that occurred spontaneously (Fig. 5B). Bilateral stimulation produced approximately twice the pressure produced when only one side was stimulated. The pressure increases in the DS last for the duration of stimulation, whereas those in the PS are transient. The pressure in the PS drops below pre-stimulus levels at the end of the period of stimulation. Stimulation at similar voltages with the electrodes implanted at 'neutral' sites near the DVMs did not produce any pressure pulses. It should be noted that SG pumping is slowed or momentarily inhibited during strong DVM stimulation. This SG response probably arises from stimuli spreading to the sensory axons of the branchiostegal nerve supplying the DVMs. Direct stimulation of the branchiostegal nerve in isolated thoracic ganglion preparations of *Carcinus* inhibits the motor program to the SGs (J. L. Wilkens and K. P. Rajashekhar, unpublished observations).

To evaluate the DVM-induced pressure changes in the context of the overall hemolymph pressure relationships, the hydrostatic pressures were measured from various body spaces (Table 3). The pressure drops from the heart across the arteries and capillaries to the infrabranchial and dorsal sinuses are quite similar. The effect of gravity, as predicted by Burggren and McMahon (1988*b*), may explain the slightly higher pressure in the infrabranchial sinus: the pressure in the IS exceeds that in the DS by 0.35 kPa (2.6 mmHg); the depth of these crabs was

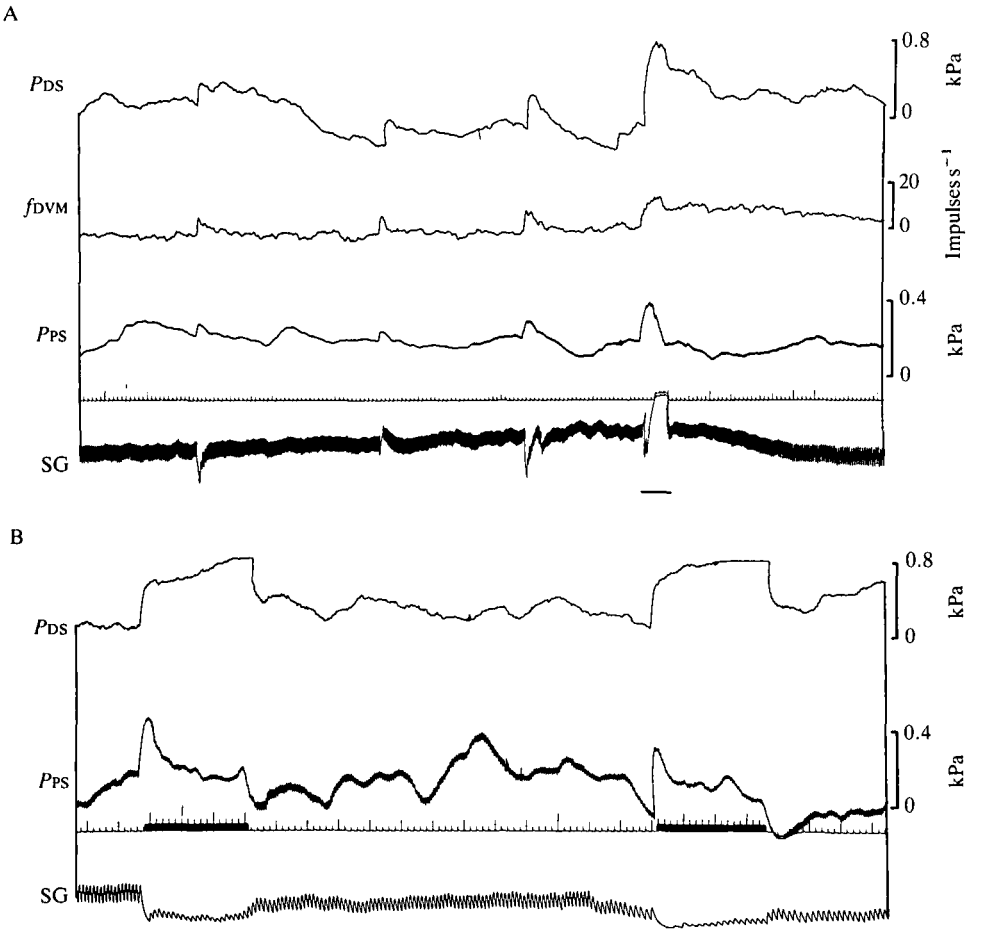

Fig. 5. (A) Records showing the reflection of pressure pulses in the dorsal sinus (P_{DS}) as pressure pulses in the pericardial sinus (P_{PS}). Three brief and one longer (7 s, underscored) reversed ventilation sessions occurred during this recorded interval. The longer event is the most typical pattern observed. (B) Simultaneous stimulation of the eight medial DVMs (black bars on time scale) causes pressure pulses in the DS and PS of equal magnitude to those arising spontaneously. Ventilation rate decreased during DVM stimulation. Time is marked at 1 s intervals.

between 3 and 5 cm. The pressure drop across the IS (gill) and DS (lung) portal systems to the pericardial sinus is much smaller than that across the various organ systems preceding them and averages 0.4–0.7 kPa.

Contractions of the DVMs cause relatively large positive pressure pulses in the DS and small decreases in the air pressure in the branchial chambers (Fig. 6A). During this recording, the SG only beat on two occasions, but not during the pressure pulses. The amount of air moved by this pressure pulse was calculated to be 0.024 ml; the air volume of this BC, measured by the volume of latex required

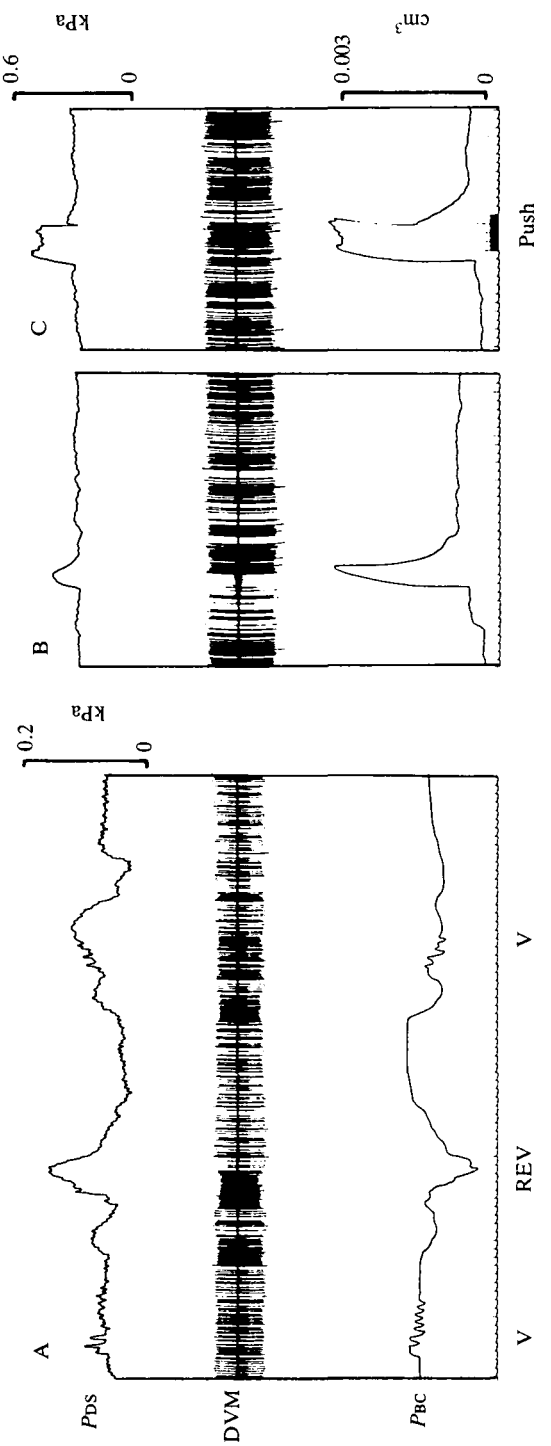

Fig. 6. (A) Records showing the inverse effects of contractions of the DVMs on hemolymph pressure in the dorsal sinus (P_{DS}) and air pressure in the branchial chamber (P_{BC}). The crab showed two brief periods of SG beating (V) during this time and one reversal bout (REV). (B) A rare record of simultaneous increases in DS and BC pressures. Note that the DVM EMG rate is reduced at the onset of this pulse and increases thereafter. (C) Lightly pressing on the middle of the dorsal carapace (Push) caused increased pressures in the DS and BC; the DVM EMG response was similar to that in B. Time is marked at 1 s intervals.

to fill it, was 1.6 ml. DVM bursts always accompany reversed SG pumping but in some cases, as in this example, they can also occur independently during ventilatory pausing.

Occasionally, positive pressure pulses are recorded from both the DS and BC and during these pulses the input to the DVM is reduced or absent (Fig. 6B). The untested explanation for this is that contraction of the epimeral muscles, which would serve to compress the dorsal carapace against the thorax, would be expected to cause increases in the pressures in both these spaces. Indeed, lightly pressing down on the carapace with the tip of a cotton swab also caused positive excursions in the pressure of both spaces (Fig. 6C).

Role of the DVMs in hemolymph pressure control

We attempted to confirm the observations made on *Carcinus* (Rajashekhar and Wilkens, 1991) that small changes in hydrostatic pressure in the DS would cause baroreceptor-like phasic changes in the impulse rate of the DVMs; however, no changes in DVM firing rate occurred when small amounts of saline (0.1 ml, 0.5 % of crab hemolymph volume) were injected or when hemolymph was withdrawn from the DS. However, the tonic DVM EMG rate and DS pressures did change inversely following increases or decreases in the hemolymph volume by larger amounts. Increasing hemolymph volume by 5 % elevated DS pressure by about 2 kPa and reduced DVM EMG rate by about 30 % (from 12.6 to 8.8 impulses s^{-1}, Fig. 7A). The initial increases in P_{DS} attenuated rapidly over the first 10 min but had not returned to control levels at 20 min. It generally required as long as 40 min before P_{DS} was fully restored. Heart rate, however, was unchanged following hemolymph volume expansion. Two of the eight crabs studied showed decreases in DVM rate lasting only 1–2 min and almost no increases in pressure. These same crabs voided urine during this time which, typical of these herbivorous crabs, stained the bath water a vivid yellow-green color. Data from these two animals were not included with those summarized in Fig. 7A.

Following a 5 % reduction in hemolymph volume, the DS pressure fell about 2 kPa while DVM EMG rate doubled (from 7.6 to 14.3 impulses s^{-1}, Fig. 7B). These pressure offsets and increased impulse rates took up to 120 min to be restored to control levels. Heart rate increased by about 5 % following the hemolymph withdrawal and remained elevated for more than 20 min.

Thus, changes in DVM discharge rate are reciprocal and closely parallel those in hemolymph pressure. Except in the case of urine loss, it is not known how or where the adaptation in hemolymph pressure occurs, but after an hour of recovery these changes were reliably reproduced upon successive injections or withdrawals of fluid.

Discussion

Cardiorespiratory behavior

Goniopsis cruentata prefers to live in the air or, when in water, to sit or stand so

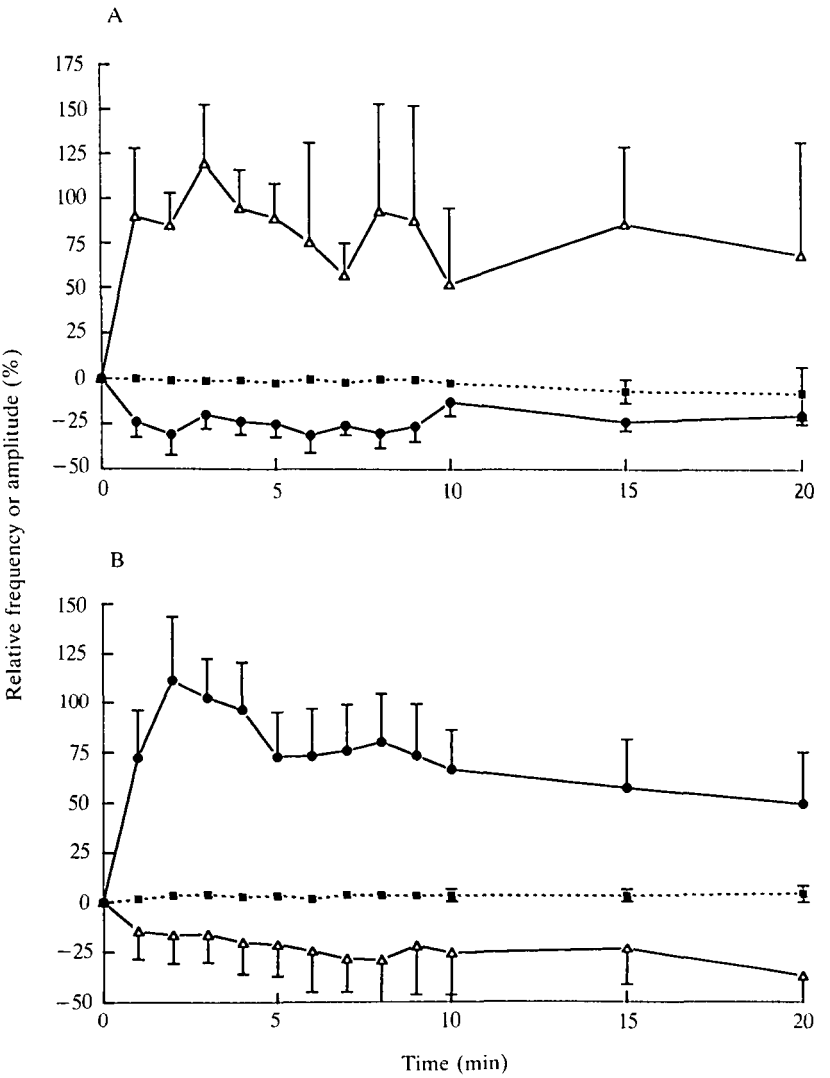

Fig. 7. (A) The effects of a 5% increase in hemolymph volume on dorsal sinus pressure (△), tonic DVM EMG rate (●) and heart rate (■). All values were normalized, with the rates during the 5 min prior to injection taken as unity. Mean±S.E.M., N=6. (B) The effects of a 5% reduction in hemolymph volume on the same variables. Mean±S.E.M., N=8.

as to keep the lung portions of the branchial chambers filled with air. Branchial ventilation rates are lowest when the lungs are filled with air and increase dramatically when the animals are submerged. As in other terrestrial crabs (Innes and Taylor, 1986; Maitland, 1987, 1990; Whiteley *et al.* 1990), respiratory gas exchange may be easier across the lungs from air than *via* the gills from water. The high rates of ventilation when partially or completely submerged may be required

for adequate respiratory gas exchange across the greatly reduced gills. During forward ventilation while the crab is submerged, the branchial water is moving countercurrent to the direction of hemolymph flow both in the gills (Hughes *et al.* 1969) and the lungs (Greenaway and Farrelly, 1990). This will maximize respiratory gas exchange with the hemolymph. Reversed ventilation was rarely observed in crabs submerged in aerated sea water.

Lung perfusion

Reversed SG pumping and DVM contractions must be considered together since their occurrence is so tightly coupled. These events are prominent only when the lungs contain air. They occur more frequently after forced activity, during exposure to hypoxic air and in mildly stressed crabs after handling, i.e. in conditions consistent with an increased demand for respiratory gas exchange across the lungs. The reversed SG pumping will replenish the air in the lungs. The bilateral phasic contractions of the array of DVMs at this time serve to elevate DS pressure. The 0.4 kPa DS pressure pulse essentially doubles the hydrostatic gradient from the DS to the PS and causes a 0.2 kPa pulse in the PS as well. This will serve to increase perfusion of hemolymph through the lung to the PS, to match the increased ventilatory flow and, perhaps, also to increase stroke volume and hence cardiac output.

In support of this hypothesis, electrically stimulating the DVMs for prolonged periods (15–20 s) causes persistent increases in P_{DS} and transient increases in P_{PS}. The fall in P_{PS} during prolonged stimulation of the DVMs probably results from increased cardiac output (stroke volume; frequency being unaffected), which removes the excess hemolymph being pushed from the DS.

In *Goniopsis*, the DVMs appear always to be synchronously activated on both sides of the animal. Perhaps these same muscles, if activated reciprocally on the two sides, could produce the shifting of the visceral mass that effects tidal ventilation in another terrestrial crab *Parathelphusa transversa* (Greenaway and Taylor, 1976). However, our calculations for *Goniopsis* indicate that the negative air pressure pulses produced in the BCs when the DVMs contract phasically would move only negligible volumes of air (one-hundredth of the branchial chamber volume) and, hence, would not be important in ventilating the BCs. The DVM dorsal sinus pump therefore constitutes primarily an auxiliary mechanism, operating in conjunction with ventilatory reversals, for ensuring ventilation/perfusion matching at the pulmonary exchange surface. This matching should enhance gaseous interchange and promote oxygen delivery to the tissues, under conditions of respiratory stress.

Hemolymph pressure control – evidence for baroreceptors

The regulation of overall hemolymph pressure is a second important function for the DVMs. The anterior one-third of the branchial chambers, including the dorsal sinuses, are soft-walled and filled with extensions of the hepatopancreas. The dorsal sinuses are the only areas available for short-duration volume

regulation since the gills, as in other terrestrial crabs (Taylor and Greenaway, 1979; Al-Wassia *et al.* 1989), do not appear to collapse or expand because of their heavy cuticularization, and all other portions of the body are encased in more rigid cuticle. The DVMs are strategically placed to regulate the volume of this space.

Changing the hemolymph volume of *Goniopsis* by small amounts (0.5 % of hemolymph volume) did not cause the marked reflexive changes in DVM EMG rate observed in *Carcinus* (Rajashekhar and Wilkens, 1991). However, when hemolymph volume was increased by 5 %, the DS pressure rose by as much as 2 kPa, and the tonic rate of discharge of the DVMs was depressed by about 30 %, gradually returning towards the control values as hemolymph pressure returned to normal, over a period of about 10–40 min. This response of the DVMs is evidently a first step in rapid compensation for hypervolemia. Very similar DVM responses have been reported for *Carcinus* (Taylor and Taylor, 1991). Urination and possibly regurgitation (Lockwood *et al.* 1974; Norfolk, 1978; Greco *et al.* 1986; Maitland, 1990; Greenaway *et al.* 1990) may act as additional short-term corrective responses. Indeed, two of the crabs in our study voided a considerable quantity of bright yellow-green urine immediately after they had been injected with saline. In both of these two special cases the changes in P_{DS} and f_{DVM} were minimal. Urine formation (and voiding) and fluid losses across the respiratory surfaces (Taylor and Butler, 1978) may constitute long-term corrective responses. Changes in f_H did not accompany these compensatory responses to hypervolemia in any of the crabs. These compensatory responses may come into play when hemolymph volume increases in crabs exposed to hypotonic media, following drinking, or when hemolymph pressure rises following the ingestion of a large meal.

Compensation in the opposite direction is seen when hemolymph volume is reduced by 5 %. The motor drive to the DVMs doubles immediately after hemolymph loss and remains elevated for up to 1 h. Again, similar responses have been documented in *Carcinus*, although the time course of recovery was not followed in that study (Taylor and Taylor, 1991). The increases in tonic contraction of the DVMs, by compressing the DSs, must minimize the fall in hemolymph pressure. In these cases, a slight increase in f_H may also contribute to the elevation of hemolymph pressure. Reductions of hemolymph volume by 5 % or more may be due to desiccation when these crabs are feeding high in trees. These compensations, therefore, may prolong the amount of time the crabs can remain away from water.

The obvious baroreceptors that must exist to sense and control the DVM, and possibly the urinary, reflex have not been positively identified; however, thoracic stretch receptors seem to be likely candidates (Pilgram, 1974; Greco *et al.* 1986). Heart rate is the least variable component of the combined cardiovascular–respiratory system, remaining almost constant during the emergence of crabs from water to air even though ventilation rates slow by many times. Heart rate rarely changes during bouts of reversed ventilation as it does in most other species (Burggren *et al.* 1985, 1990). The f_H in this quick and agile crab is higher than in most other terrestrial crabs (Burggren and McMahon, 1988*b*) and in any aquatic

crab on record (McMahon and Burnett, 1990). It would be interesting to measure stroke volume and cardiac output in this crab under the conditions employed here. In the absence of such data, it is concluded that f_H is not a critical variable for *Goniopsis*, and we suggest that stroke volume adjustments might play a major role. Clearly, however, the regulation of the tonic rate of discharge of the DVMs must provide an important means of rapid compensation for changes in volume of the body fluids in the crab.

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References

AL-WASSIA, A. H., INNES, A. J., WHITELEY, N. M. AND TAYLOR, E. W. (1989). Aerial and aquatic respiration in the ghost crab *Ocypode saratan*. I. Fine structure of respiratory surfaces, their ventilation and perfusion; oxygen consumption and carbon dioxide production. *Comp. Biochem. Physiol.* **94**A, 755–764.

BLATCHFORD, J. G. (1971). Haemodynamics of *Carcinus maenas* (L.). *Comp. Biochem. Physiol.* **39**A, 193–202.

BLISS, D. E. (1968). Transition from water to land in decapod crustaceans. *Am. Zool.* **8**, 355–392.

BURGGREN, W. W. AND MCMAHON, B. R. (1988*a*). *Biology of the Land Crabs*. Cambridge: Cambridge University Press.

BURGGREN, W. W. AND MCMAHON, B. R. (1988*b*). Circulation. In *Biology of the Land Crabs* (ed. W. W. Burggren and B. R. McMahon), pp. 298–332. Cambridge: Cambridge University Press.

BURGGREN, W. W., PINDER, A., MCMAHON, B., DOYLE, M. AND WHEATLY, M. (1990). Heart rate and hemolymph pressure responses to hemolymph volume changes in the land crab *Cardisoma guanhumi*: evidence for 'baroreflex' regulation. *Physiol. Zool.* **63**, 167–181.

BURGGREN, W. W., PINDER, A., MCMAHON, B., WHEATLY, M. AND DOYLE, M. (1985). Ventilation, circulation and their interactions in the land crab, *Cardisoma guanhumi*. *J. exp. Biol.* **117**, 133–154.

CAMERON, J. N. (1981). Brief introduction to the land crabs of the Palau Islands: stages in the transition to air breathing. *J. exp. Zool.* **218**, 1–5.

DIAZ, H. AND RODRIGUEZ, G. (1977). The branchial chamber in terrestrial crabs: a comparative study. *Biol. Bull. mar. biol. Lab., Woods Hole* **153**, 485–504.

GLEESON, R. A. AND ZUBKOFF, P. L. (1977). The determination of hemolymph volume in the blue crab, *Callinectes sapidus*, utilizing ^{14}C-thiocyanate. *Comp. Biochem. Physiol.* **56**A, 411–413.

GRAY, I. E. (1957). A comparative study of the gill area of crabs. *Biol. Bull. mar. biol. Lab., Woods Hole* **112**, 34–42.

GRECO, T. M., ALDEN, M. B. AND HOLLIDAY, C. W. (1986). Control of hemolymph volume by adjustments in urinary and drinking rates in the crab, *Cancer borealis*. *Comp. Biochem. Physiol.* **84**A, 695–701.

GREENAWAY, P. AND FARRELLY, C. A. (1984). The venous system of the terrestrial crab *Ocypode cordimanus* (Desmarest 1825) with particular reference to the vasculature of the lungs. *J. Morph.* **181**, 133–142.

GREENAWAY, P. AND FARRELLY, C. (1990). Vasculature of the gas-exchange organs in air-breathing brachyurans. *Physiol. Zool.* **63**, 117–139.

GREENAWAY, P. AND TAYLOR, H. H. (1976). Aerial gas exchange in Australian arid-zone crab, *Parathelphusa transversa* von Martens. *Nature* **262**, 711–713.

GREENAWAY, P., TAYLOR, H. H. AND MORRIS, S. (1990). Adaptations to a terrestrial existence by the robber crab *Birgus latro*. VI. The role of the excretory system in fluid balance. *J. exp. Biol.* **152**, 505–519.

HARMS, J. W. (1932). Die Realization von Genen und die consecutive Adaptation. II. *Birgus latro* L. als Landkrebs unseine Bezeihungen zu den Coenabiten. *Z. wiss. Zool.* **140**, 167–290.

HUGHES, G. M., KNIGHTS, B. AND SCAMMEL, C. A. (1969). The distribution of P_{O_2} and hydrostatic pressure changes within the branchial chambers of the shore crab *Carcinus maenas*. *J. exp. Biol.* **51**, 203–220.

INNES, A. J. AND TAYLOR, E. W. (1986). The evolution of air-breathing in crustaceans: a functional analysis of branchial, cutaneous and pulmonary gas exchange. *Comp. Biochem. Physiol.* **85**A, 621–637.

INNES, A. J., TAYLOR, E. W. AND EL HAJ, A. J. (1987). Air-breathing in the Trinidad mountain crab: A quantum leap in the evolution of the invertebrate lung. *Comp. Biochem. Physiol.* **87**A, 1–9.

LOCKWOOD, A. P. M., NORFOLK, J. R. W., BULLEID, N. C. AND TAYLOR, P. A. (1974). Urinary bladder volume and the reabsorption of water from the urine of crabs. *J. exp. Biol.* **60**, 167–181.

MAITLAND, D. P. (1987). A highly complex invertebrate lung. The gill chambers of the soldier crab *Mictyris longicarpus*. *Naturwissenschaften* **74**, 293–295.

MAITLAND, D. P. (1990). Aerial respiration the semaphore crab, *Heloecius cordiformis*, with or without branchial water. *Comp. Biochem. Physiol.* **95**A, 267–274.

MCMAHON, B. R. AND BURGGREN, W. W. (1988). Respiration. In *Biology of the Land Crabs* (ed. W. W. Burggren and B. R. McMahon), pp. 249–297. Cambridge: Cambridge University Press.

MCMAHON, B. R. AND BURNETT, L. E. (1990). The crustacean open circulatory system: a reexamination. *Physiol. Zool.* **63**, 35–71.

NORFOLK, J. R. W. (1978). Internal volume and pressure regulation in *Carcinus maenas*. *J. exp. Biol.* **74**, 123–132.

PEARSON, J. (1908). Cancer. Liverpool Marine Biological Committee Memoirs, no. 8, London: Williams and Nordgate.

PILGRAM, R. L. (1974). Stretch receptor organs in the thorax of the hermit crab, *Pagurus bernhardus* (L. 1758). *J. mar. biol. Ass. U.K.* **54**, 13–24.

RAJASHEKHAR, K. P. AND WILKENS, J. L. (1991). Control of 'pulmonary' pressure and coordination with gill ventilation in the shore crab *Carcinus maenas*. *J. exp. Biol.* **155**, 147–164.

TAYLOR, E. W. AND BUTLER, P. J. (1978). Aquatic and aerial respiration in the shore crab, *Carcinus maenas* (L.), acclimated to 15°C. *J. comp. Physiol.* **127**, 315–323.

TAYLOR, H. H. (1990). Pressure–flow characteristics of crab gills: implications for regulation of hemolymph pressure. *Physiol. Zool.* **63**, 72–89.

TAYLOR, H. H. AND GREENAWAY, P. (1979). The structure of the gills and lungs of the arid-zone crab, *Holthuisana (Austrothelphusa) transversa* (Brachuyra: Sundathelphusidae) including observations on arterial vessels within the gills. *J. Zool., Lond.* **189**, 359–384.

TAYLOR, H. H. AND GREENAWAY, P. (1984). The role of the gills and branchiostegites in gas exchange in a bimodally breathing crab, *Holthuisana transversa*: evidence for a facultative change in the distribution of the respiratory circulation. *J. exp. Biol.* **111**, 103–121.

TAYLOR, H. H. AND TAYLOR, E. W. (1991). The dorsoventral muscles of *Carcinus maenas*: evidence for hydrostatic pressure control in a crab. *Physiol. Zool.* (in press).

TRUCHOT, J. P. (1990). Respiratory and ionic regulation in invertebrates exposed to both water and air. *A. Rev. Physiol.* **52**, 61–76.

WHITELEY, N. M., INNES, A. J., AL-WASSIA, A. H. AND TAYLOR, E. W. (1990). Aerial and aquatic respiration in the ghost crab, *Ocypode saratan*. II. Respiratory gas exchange and transport in the haemolymph. *Mar. Behav. Physiol.* **16**, 261–273.

WILKENS, J. L., YOUNG, R. E. AND DICAPRIO, R. A. (1989). Responses of the isolated crab ventilatory central pattern generators to variations in oxygen tension. *J. comp. Physiol.* B **159**, 29–36.