The Journal of Experimental Biology **199**, 569–578 (1996)
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JEB0004

SHORT-TERM EMERSION AFFECTS CARDIAC FUNCTION AND REGIONAL HAEMOLYMPH DISTRIBUTION IN THE CRAB *CANCER MAGISTER*

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Accepted 6 November 1995

Summary

Changes in cardiac function and arterial haemolymph flow associated with 6 h of emersion were investigated in the crab *Cancer magister* using an ultrasonic flowmeter. This species is usually found sublittorally but, owing to the large-scale horizontal water movements associated with extreme tides, *C. magister* may occasionally become stranded on the beach. Laboratory experiments were designed such that the emersion period was typical of those that might be experienced by this crab in its natural environment.

The frequency of the heart beat began to decline sharply almost immediately after the start of the experimental emersion period. Cardiac stroke volume fell more gradually. The combined reduction in these two variables led to a maximum decrease in cardiac output of more than 70 % from the control rate. Haemolymph flow through all the arteries originating at the heart, with the exception of the anterior aorta, also declined markedly during emersion. As the water level in the experimental chamber fell below the inhalant branchial openings, a stereotypical, dramatic increase in haemolymph flow through the anterior aorta began and this continued for the duration of the emersion period.

The rapid time course of the decline in heart-beat frequency and the increase in haemolymph flow through the anterior aorta suggest a neural mechanism responding to the absence of ventilatory water in the branchial chambers. These responses may be adaptations, respectively, to conserve energy by reducing the minute volume of haemolymph pumped by the heart and to protect the supply of haemolymph to cephalic elements of the central nervous system. The decline in cardiac stroke volume, which occurs more slowly over the emersion period, may be a passive result of the failure to supply sufficient O_2 to meet the aerobic demands of the cardiac ganglion.

Key words: heart, blood flow, emersion, cardiac output, haemolymph, crab, *Cancer magister*.

Introduction

A wide range of tolerance to atmospheric air exposure (emersion) has been documented for brachyuran crabs. Fully aquatic species may survive only very short periods of emersion, whilst many land crabs venture near water only to drink or to breed. The latter organisms may drown when forcibly immersed for extended periods (von Raben, 1934; Taylor and Davies, 1982; Santos and Costa, 1993). The anatomical adaptations which permit prolonged survival of this group in terrestrial habitats are largely involved with the shift from aquatic to aerial gas exchange (McMahon and Burggren, 1988); therefore, in many land crabs, the branchial chambers have been enlarged and their internal membranes are highly vascularised to serve as functional lungs across which O_2 uptake occurs. Gill surface area is markedly reduced and these organs are utilized largely for CO_2 excretion and ionoregulation.

Other, less dramatically adapted species, such as those frequenting the intertidal zone, may be capable of lengthy periods of activity on land but must either return to the water or retain water in the branchial chambers for osmoregulation and to eliminate CO_2 (Wheatly and Taylor, 1979; Burnett and McMahon, 1987). The gills of these forms may have a relatively reduced surface area compared with those of fully aquatic species (Gray, 1957) and they are generally more rigid (Taylor and Butler, 1978). This allows O_2 uptake to take place across the gills in both air and water.

Subtidal crabs typically possess few anatomical adaptations for aerial gas exchange: the closely spaced, flexible gill lamellae clump together when water drains from the branchial chambers (deFur *et al.* 1988) and the branchiostegal linings are poorly vascularized (von Raben, 1934). Hence, gas exchange

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is severely limited during periods of emersion. These animals are usually confined to the aquatic realm, but crabs in near-shore habitats may face occasional emersion due to extreme tidal fluctuations. In the absence of anatomical adaptations, the ability to survive natural periods of air exposure must rest on physiological adaptations which mitigate the deleterious effects of aerial exposure.

Although usually found subtidally, the Dungeness crab (*Cancer magister* Dana) may occasionally become stranded on the beach by the receding tide. Cardiovascular responses apparently contribute to the ability of this species to survive periods of severe hypoxic exposure (Airriess and McMahon, 1994) and may also be important factors conferring emersion-tolerance. Good baseline data are available for the respiratory and cardiovascular performance of quiescent *C. magister* under the least confining laboratory conditions possible (McDonald *et al.* 1977; McGaw *et al.* 1994*a*), allowing comparisons with the responses to experimental treatment.

The use of ultrasonic flowmeters, based on the Doppler principle, with miniature transducer crystals has recently allowed long-term, minimally invasive recordings of blood flow in crustaceans (Bourne and McMahon, 1989; Reiber *et al.* 1992; Airriess and McMahon, 1994; McGaw *et al.* 1994*a*,*b*; Reiber, 1994). The ability to quantify simultaneously haemolymph flow through all of the arteries originating at the heart allows direct determination of cardiac output; from this value and the cardiac beat frequency the calculation of stroke volume is straightforward. In the past, only estimates of cardiac output and stroke volume have been available for crustaceans, and their relative importance in cardiovascular responses has been a matter of debate. Because the pulsed-Doppler flowmeter system allows accurate determination of both components of cardiac output simultaneously, it enables accurate comparison of their relative importance during responses to experimental manipulations.

In the present study, the changes in cardiac function (both heart-beat frequency and stroke volume) as well as haemolymph flow and its redistribution were examined in order to elucidate the strategies utilised by *C. magister* to enable survival during episodic emersion.

Materials and methods

Adult male *Cancer magister* Dana (673±33 g; range 490–940 g) were purchased from local native fishermen and held in flowing seawater aquaria at the Bamfield Marine Station, British Columbia, Canada. Seawater temperature in both the holding and experimental aquaria was 12±1 °C and salinity was 33 ‰. Crabs were exposed to ambient light and fed fresh or frozen fish twice weekly but were starved for 2 days prior to use in experiments.

The rates of haemolymph flow through the anterior aorta, left anterolateral artery, right hepatic artery, posterior aorta and sternal artery (see Airriess and McMahon, 1994) were measured using a pulsed-Doppler flowmeter. For a full description of the calibration and use of these instruments for the measurement of haemolymph flow in crabs, see Airriess and McMahon (1994) and Airriess *et al.* (1994). Briefly, piezoelectric-crystal probes (Iowa Doppler Systems or Crystal Biotech Inc.) connected to a pulsed-Doppler flowmeter (University of Iowa Bioengineering 545C-4) were either mounted in abrasions in the carapace dorsal to the arteries of interest or, in the case of the sternal artery, inserted through a polyethylene catheter which terminated in close proximity to the vessel. The focus range and rotational orientation of the probes were adjusted to obtain a measure of centre-stream haemolymph velocity in each artery. Heart-beat frequency (f_H) was determined from the frequency of systolic peaks in the arterial flow records. Since any of the arteries under investigation could provide this information, it was possible to determine f_H at all times, despite intermittent cessation of flow through some arterial systems.

Measurements of scaphognathite pumping frequency (f_{SC}) were obtained using a seawater-filled polyethylene catheter (PE 200) implanted in the right epibranchial chamber (after Hughes *et al.* 1969) and connected to a pressure transducer (Statham/Gould, PB23Db). Owing to sensitivity limitations, f_{SC} could only be determined using this method when the crabs were immersed, as the absence of water in the branchial chambers precluded the detection of pressure fluctuations. These measurements were obtained primarily as an indicator of the physiological state of the animal prior to and following experimental emersion, rather than with the intention of assessing respiratory capabilities; for this reason, only unilateral pressure recordings were obtained. High rates of bilateral scaphognathite pumping are typical of physiologically stressed crustaceans (McMahon and Wilkens, 1983) and thus would be expected in crabs just re-immersed following 6 h of emersion.

Following surgical implantation of the recording instruments, crabs were placed in a 3 l plastic chamber which, prior to and following emersion, was supplied with sea water flowing at approximately 200 ml min^{-1}. A water jacket was used to maintain the temperature of the experimental chamber. A black plastic cover prevented visual disturbance of the crabs, but light was allowed to enter through the rear of the chamber to avoid disruption of usual circadian rhythms. Normoxic conditions (P_{O_2}≈18 kPa) were maintained within the experimental chamber, during control and recovery periods, using a large air stone situated at the seawater inflow and connected to a high-capacity aquarium pump. The chelae of the animals were immobilised using rubber bands, but the crabs were not otherwise restrained.

Experimental protocol

Subsequent to 24 h of post-operative recovery, ventilatory and cardiovascular variables were recorded continuously during a 1 h control period using a six-channel oscillograph (Gould). Three control measurements were obtained at 30 min intervals during this time. At 0 h, the seawater inflow was disconnected, allowing the water to drain from the experimental chamber (which took approximately 10 min). All variables were measured every 5 min for the first 30 min, and

then every 30 min thereafter for the duration of the emersion period. Following 6 h of air exposure, a period approximately equivalent to the longest tidal exposure likely to occur in the natural habitat of *C. magister*, the seawater supply to the experimental chamber was reconnected and measurements of cardiovascular and ventilatory variables were obtained over a 24 h recovery period.

Data analysis

Measurements of all variables were obtained by increasing the chart speed for a 30 s period, during which the average heart and scaphognathite pumping frequencies and the areas under the mean haemolymph velocity traces could be determined. The latter areas correspond to voltage output from the pulsed-Doppler flowmeter and are linearly related to haemolymph flow (Airriess *et al.* 1994). Cardiac output ($\dot{V}$b) was determined by summation of arterial haemolymph flow rates for experiments in which all arterial systems were successfully instrumented. Heart stroke volume (VS) was calculated by dividing $\dot{V}$b by fH. All rates except fH and fSC were normalised to crab mass.

The data for each variable were analyzed, to test the null hypothesis that 6 h of emersion does not affect cardiovascular function in *C. magister*, using analysis of variance with repeated measures (ANOVAR). Samples obtained during the first 25 min of the 6 h emersion period, while the chamber was draining and the initial responses were developing, were not included in these analyses. ANOVARs showing significant differences between 6 h control and emersed groups (P<0.05) were further analyzed, using specific comparisons (F-tests), to determine at what time during the emersion period significant deviations from the initial control values occurred. The relationships among cardiac variables were examined using least-squares regression analysis after the data had been transformed, to remove serial correlation, using the first difference method (Gujarati, 1978). The error bars associated with mean data points represent 1 S.E.M.

Results

Within 5–6 min after the water began to drain, most crabs were emersed to the level of the inhalant (Milne-Edwards) openings of the branchial chambers. It took approximately 10 min to drain the experimental chamber completely after the seawater inflow was removed. Any water remaining on the bottom of the chamber after this time was aspirated by a vacuum pump connected to the former inflow tube.

By the time that half of the water had drained from the experimental chamber (5 min after the inflow was disconnected), fH began to decrease (Fig. 1). fH continued to decline over the next 2 h, before stabilising, and then remained at this depressed level for the duration of the air exposure period (ANOVAR, F=47.99, P<0.001, d.f.=9, 270). Upon re-immersion, fH regained its control rate within 30 min.

$\dot{V}$b also decreased significantly in response to enforced emersion (ANOVAR, F=5.45, P<0.001, d.f.=9, 189), but the onset of this response was slightly delayed in comparison with the rapid change in fH (Fig. 1). $\dot{V}$b decreased sharply over the first hour of emersion and then continued to decline more gradually for the duration of the exposure period. In contrast, cardiac VS remained stable for the first 30 min of air exposure, then gradually declined until the water supply was restored (Fig. 1). VS reached a level significantly different from the control value after 5 h of emersion (ANOVAR, F=2.48, P<0.05, d.f.=9, 54). Following the return of sea water to the experimental chamber, both $\dot{V}$b and VS increased dramatically, overshooting control values and reaching maxima within 1 h.

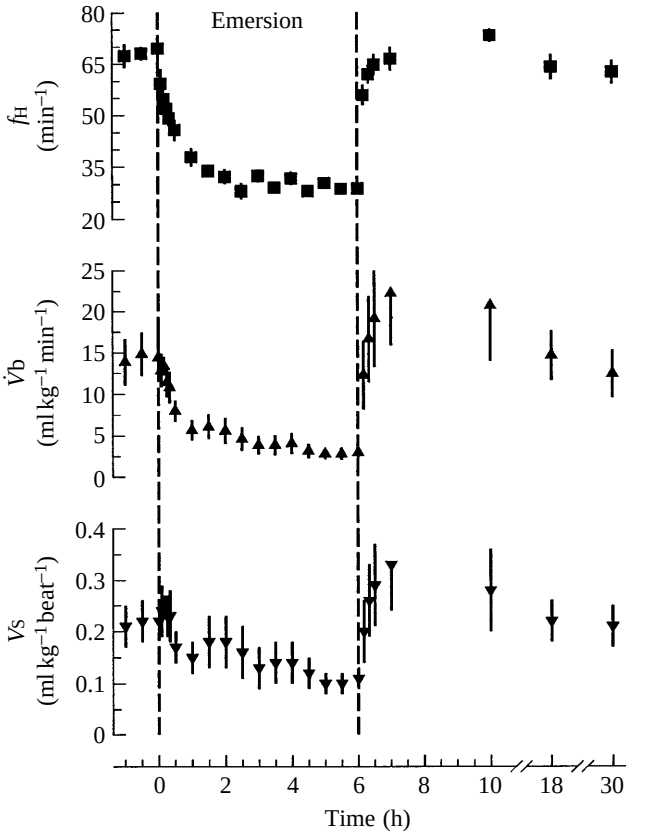

Fig. 1. The changes in cardiac variables over time prior to, during and following 6 h of enforced air exposure (emersion) in *Cancer magister* at 12 °C. At time 0 h, the seawater inflow to the experimental chamber was disconnected, allowing the chamber to drain within 10 min. Heart-beat frequency (fH, ■, N=16) started to decline within approximately 5 min, with the water level still above the inhalant branchial openings, and continued to fall for the next 2 h (ANOVAR, P<0.001). Cardiac output ($\dot{V}$b, ▲, N=7) also decreased in response to air exposure (ANOVAR, P<0.001), although the onset of this response was not quite as rapid as that of the emersion-induced bradycardia. The decrease in cardiac stroke volume (VS, ▼, N=7) occurred more slowly and was of smaller magnitude than the changes in either fH or $\dot{V}$b, and was determined to be significant only after 5 h of emersion (ANOVAR, P<0.05). Following the return of water to the experimental chamber at 6 h of emersion, fH regained its initial rate while both VS and $\dot{V}$b overshot their respective control values and remained elevated for several hours. All values are shown as mean ±1 S.E.M.

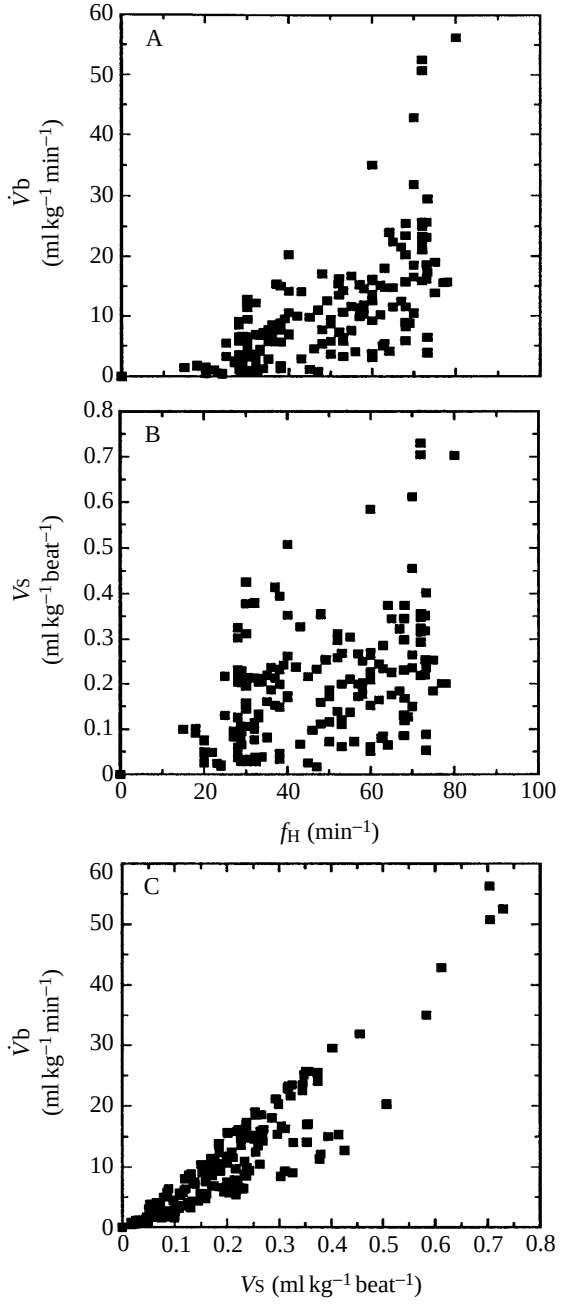

Fig. 2. The relationships among cardiac variables in *Cancer magister* (N=7) during emersion. (A) $\dot{V}$b was positively correlated with f_H in this study (r^2=0.44, P<0.001). (B) There was no significant correlation between VS and f_H (r^2=0.02, P>0.05) in emersed crabs. (C) $\dot{V}$b and VS were highly correlated, however (r^2=0.61, P<0.001), showing a positive dependency of $\dot{V}$b on VS under these experimental conditions. Data were transformed to remove serial correlation using the first difference method (Gujarati, 1978); each point represents an individual value. See Fig. 1 for definition of abbreviations.

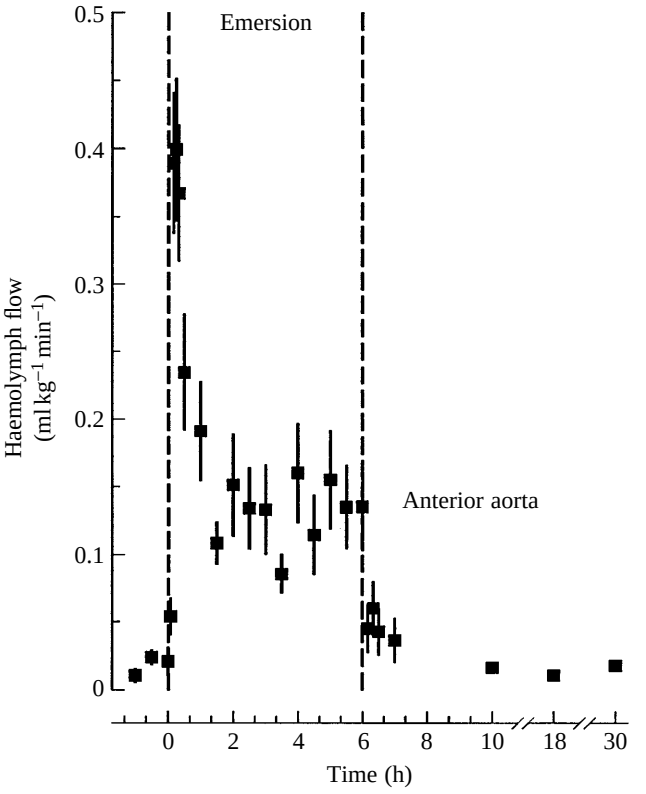

Fig. 3. There was a dramatic and significant increase in haemolymph flow through the anterior aorta of *Cancer magister* during air exposure (ANOVAR, P<0.001). The onset of this response was very rapid, occurring between 5 and 10 min after the water began to drain from the experimental chamber. The initial, high level of perfusion of this vessel induced by air exposure began to decline within 30 min, but flow remained significantly elevated above the control rate for the duration of the emersion period. All data are shown as mean ±1 S.E.M., N=16.

In *C. magister* subjected to 6 h of emersion, the highest correlation among cardiac variables was that between $\dot{V}$b and VS (Fig. 2C), which showed a very rigid dependency (r^2=0.61, F=155.54, P<0.001). $\dot{V}$b was less highly correlated with f_H in emersed crabs (r^2=0.44, F=90.61, P<0.001), but the relationship between these variables was generally conserved (Fig. 2A). The least dependent relationship among the indicators of cardiac performance was between VS and f_H (Fig. 2B). There was no significant correlation between these variables (r^2=0.02, F=2.70, P>0.05) although the highest values of VS were associated with the highest heart rates and *vice versa*.

The most distinctive vascular response to emersion was a dramatic increase in haemolymph flow through the anterior aorta, which occurred in all crabs within several seconds of the water level falling below the inhalant branchial openings (Figs 3, 4). In quiescent crabs resting in normoxic sea water, the rate of sustained haemolymph flow through this vessel was uniformly very low and often undetectable. As the water level receded below the Milne-Edwards openings, however, sporadic pulses of flow through the anterior aorta occurred with increasing frequency and quickly gave way to continuous perfusion (Fig. 4A). The average rate of haemolymph flow through this system reached a peak within the first 10 min of the emersion period (Fig. 3), but significantly elevated levels of flow were sustained until the animals were re-immersed

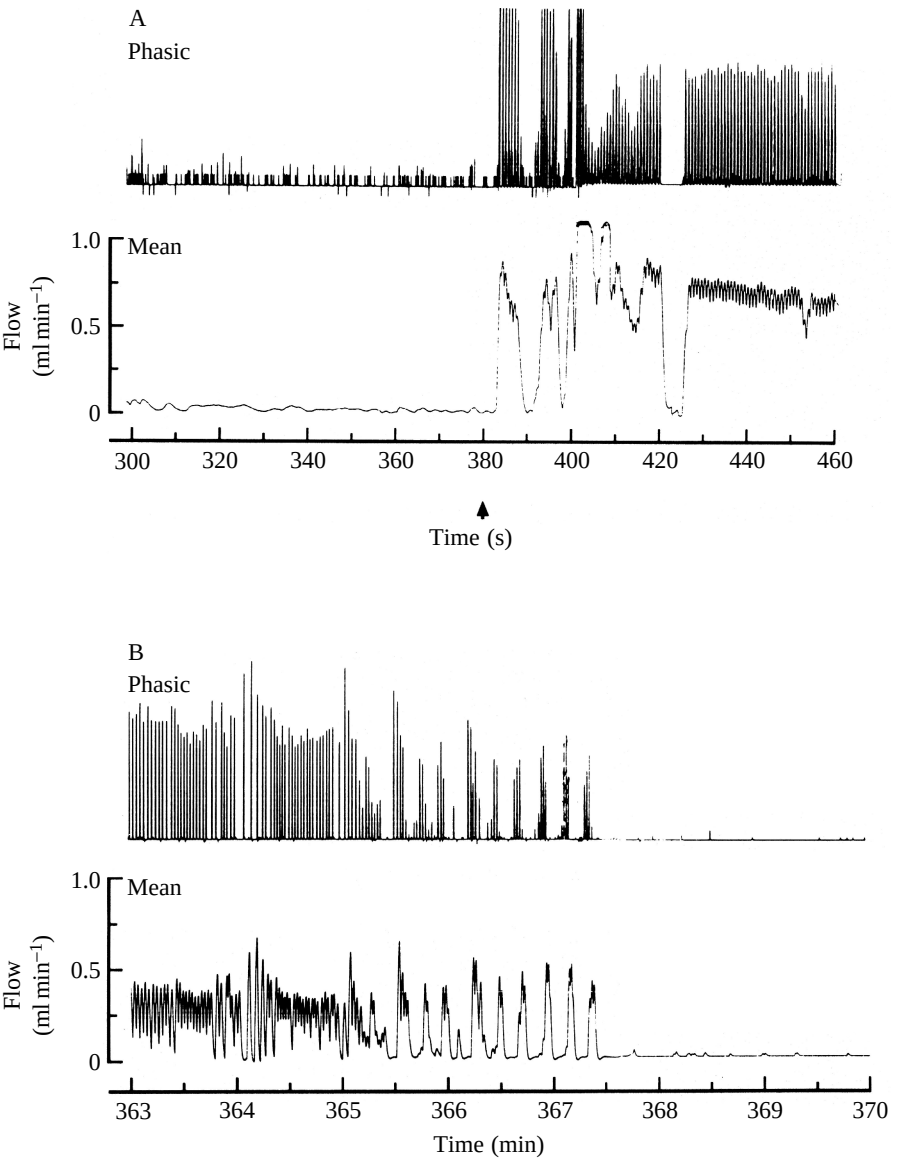

Fig. 4. Chart recording showing the changes in pulsatile and mean haemolymph flow through the anterior aorta of a single *Cancer magister* at the start (A) and end (B) of 6 h of air exposure. Tick labels on the *x*-axes (note different time scales in A and B) show the elapsed time after sea water began to drain from the experimental chamber. (A) The rate of haemolymph flow through the anterior aorta of non-stressed crabs in normoxic sea water was generally very low. As soon as the water level in the experimental chamber fell below the inhalant branchial openings (arrow at 380 s), however, the anterior aorta began to receive heavy perfusion. This was a stereotypical response which occurred in all crabs tested. (B) Following 6 h (360 min) of emersion, the seawater supply to the experimental chamber was reconnected. Within 5 min, the water level had risen above the level of the inhalant branchial openings and shortly thereafter the steady flow of haemolymph through the anterior aorta began to subside. Short bursts of perfusion followed for approximately 2 min, but eventually these also ceased.

(ANOVAR, F=9.23. P<0.001, d.f.=9, 270). During refilling of the experimental chamber, the water level reached the inhalant branchial openings within 4–5 min. Perfusion of the anterior aorta gradually began to decline at this time, and continuous perfusion was replaced by short bursts of flow (Fig. 4B). In most animals, this sporadic perfusion also ceased to occur within 10 min.

Concurrent with the increase in perfusion of the anterior aorta, significant decreases in haemolymph flow through the left anterolateral artery (ANOVAR, F=4.58, P<0.001, d.f.=9, 261) and the right hepatic artery (ANOVAR, F=6.74, P<0.001, d.f.=9, 207) occurred during air exposure (Fig. 5). The decreases in the rates of flow through both of these vessels occurred over approximately the same time period, and both rates remained stable after the first 2 h of emersion until the water supply to the experimental chamber was restored. Upon re-immersion, haemolymph flow through these two vessels rapidly returned to the respective control level. In the sternal artery, also (Fig. 6), haemolymph flow declined dramatically during the first 2 h and then remained stable at a significantly reduced level for the duration of the emersion period (ANOVAR, F=5.78, P<0.001, d.f.=9, 261). In contrast to flow through the anterior vessels, however, which returned to control rates within 30 min of re-immersion, haemolymph flow through the sternal artery increased to almost double its original rate over the first 4 h after the return of water to the experimental chamber, then slowly declined over the next 20 h. The rate of haemolymph flow through the posterior aorta was much more variable than the flow rates in the other arteries (Fig. 6) and, although the general change in flow appeared to be similar to that in the sternal artery, there was no significant response to enforced emersion in this vessel (P>0.05).

There was an immediate 40 % increase in f_{SC} at the start of the emersion period (Fig. 7). This rate had decreased somewhat by the time water flow was restored to the experimental chamber, but it again increased upon re-immersion and reached a maximum within 1 h. The control rate of f_{SC} was regained over 12 h of recovery.

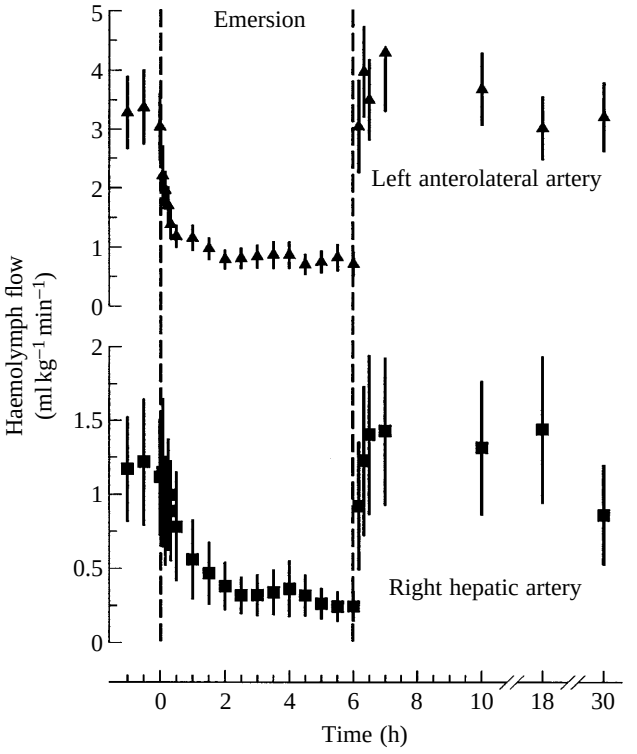

Fig. 5. The rate of haemolymph flow through the left anterolateral artery (▲, N=15) and right hepatic artery (■, N=9) of *Cancer magister* prior to, during and after 6 h of enforced emersion at 12 °C. Flow through the anterolateral artery began to decrease within 5 min of the start of seawater removal from the experimental chamber and remained depressed for the duration of the emersion period (ANOVAR, P<0.001). Haemolymph flow through the right hepatic artery did not decline as rapidly as that through the anterolateral artery, but there was a significant decrease in flow through this vessel in response to air exposure (ANOVAR, P<0.001). Data are shown as mean ±1 S.E.M.

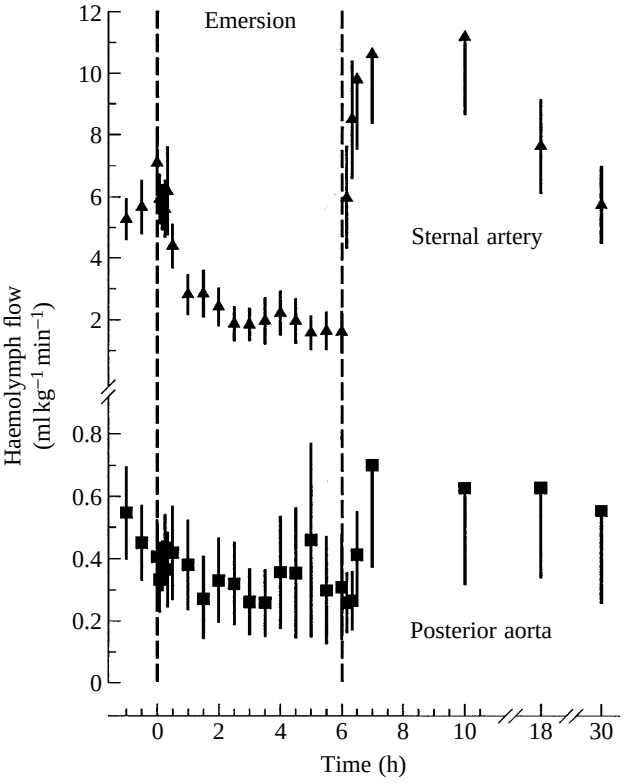

Fig. 6. Changes in haemolymph flow through the posterior arterial systems of *Cancer magister* in response to enforced air exposure at 12 °C. Flow through the sternal artery (▲, N=15) remained stable for the first 25 min of emersion, but then fell dramatically (ANOVAR, P<0.001) and remained at this low level for the duration of air exposure. The rate of haemolymph flow through the posterior aorta (■, N=12) did not change markedly in response to emersion (P>0.05). Following the return of sea water to the experimental chamber, flow through the sternal artery increased to 200 % of the pre-emersion value and remained elevated for over 12 h. Values are shown as mean ±1 S.E.M.

Although total $\dot{V}$b declined, the percentage of $\dot{V}$b distributed to the sternal artery increased sharply (from 38±7 to 54±5 %) as the water was draining from the experimental chamber and then gradually declined over the balance of the emersion period (Fig. 8). Following re-immersion, the proportion of $\dot{V}$b delivered *via* this vessel again increased to over 50 %. The changes in the percentage of haemolymph allocated to the sternal artery closely corresponded to the changes in f_{SC} observed at the start and end of the emersion period (Fig. 7) although the two variables were not significantly correlated (P>0.05). The percentage of $\dot{V}$b delivered *via* the anterolateral arteries decreased initially, then gradually increased over the emersion period. Together, the sternal artery and the anterolateral arteries received the highest percentage of the total haemolymph pumped by the heart at all times in this investigation. The percentage of $\dot{V}$b distributed to the anterior aorta increased from less than 0.5 % prior to emersion to 7±3 % within 10 min and remained at this level for the duration of air exposure. The hepatic arteries and posterior aorta received a relatively constant percentage of the total $\dot{V}$b during control and emersion periods, but the allocation of haemolymph to the hepatic arteries decreased somewhat upon re-immersion.

Discussion

Strategies employed by decapod crustaceans to minimize the impact of emersion-induced internal hypoxia may include general metabolic depression and either the mobilisation of stored glucose reserves or the inhibition of glycogen synthesis (Depledge, 1984; Burnett and McMahon, 1987; Santos and Keller, 1993). These strategies both minimize the amount of energy required to sustain vital metabolic processes and maximise the amount of substrate available to fuel glycolysis *via* the lactate pathway, the chief anaerobic pathway utilised by crustaceans (Livingstone, 1983).

The shore crab *Carcinus maenas* is often active in the littoral zone during low tide and can sustain rates of O_2 uptake ($\dot{M}_{O_2}$) in air which are equal to or higher than those achieved during aquatic ventilation (Taylor and Butler, 1978). During

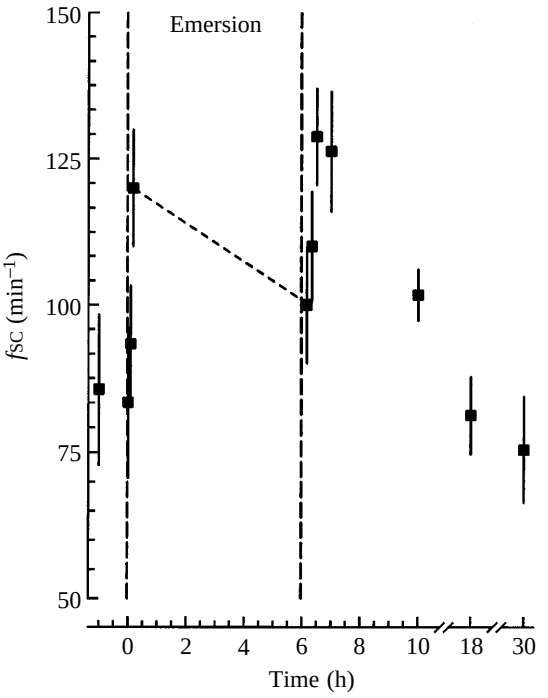

Fig. 7. The frequency of scaphognathite pumping (f_{SC}) in the right branchial chamber of *Cancer magister* immediately prior to and following 6 h of emersion at 12 °C. f_{SC} could not be measured during air exposure because the recording method utilised depends on the presence of water in the branchial chamber. During the first 10 min after the sea water began to drain from the experimental chamber, there was a dramatic increase in f_{SC}, but this rate had declined somewhat by the end of the emersion period. Subsequent to re-immersion, f_{SC} again increased sharply and remained elevated for up to 12 h. All values are shown as mean ±1 S.E.M., N=4.

experimental emersion, both f_H and $\dot{M}_{O_2}$ remain constant while arterial P_{O_2} decreases (Taylor and Butler, 1978). P_{CO_2} of the haemolymph increases with a concomitant decrease in pH (Truchot, 1975; Taylor and Butler, 1978), indicating that the elimination of CO_2 across the gills may be less efficient in air than in water, but the absence of L-lactate accumulation in the haemolymph (Taylor and Butler, 1978) suggests that anaerobic pathways of metabolism are not utilised.

Cancer productus, a crab more closely related to the species used in the present study, undergoes an age-specific change in metabolic responses to emersion as well as a shift in intertidal zonation (deFur and McMahon, 1984*a*). Small (=young, <100 g) individuals are often found on sheltered beaches during low tides (deFur *et al.* 1983), but these crabs are usually buried in the substratum or under rocks and are not active during emersion. Large (>200 g) *C. productus* are only rarely observed in the littoral zone (deFur, 1980). Small crabs of this species show a decrease in both f_H and $\dot{M}_{O_2}$ during experimental air exposure, but no change in f_{SC} (deFur and McMahon, 1984*a*). Arterial P_{O_2} (Pa_{O_2}) declines markedly and arterial P_{CO_2} (Pa_{CO_2}) increases, resulting in a haemolymph acidosis (deFur, 1980). Large crabs respond differently to enforced emersion, showing no change in f_H but a significant increase in f_{SC} (deFur and McMahon, 1984*a*). $\dot{M}_{O_2}$ and Pa_{O_2} both declined in large *C. productus* during emersion, but the decrease in Pa_{O_2} was more severe than that observed in small crabs (deFur and McMahon, 1984*a*). Haemolymph lactate concentration increased in both size classes during emersion (deFur and McMahon, 1984*b*); however, this response was most pronounced in small *C. productus*.

The present investigation demonstrates that *C. magister* undergoes a depression in cardiovascular function during

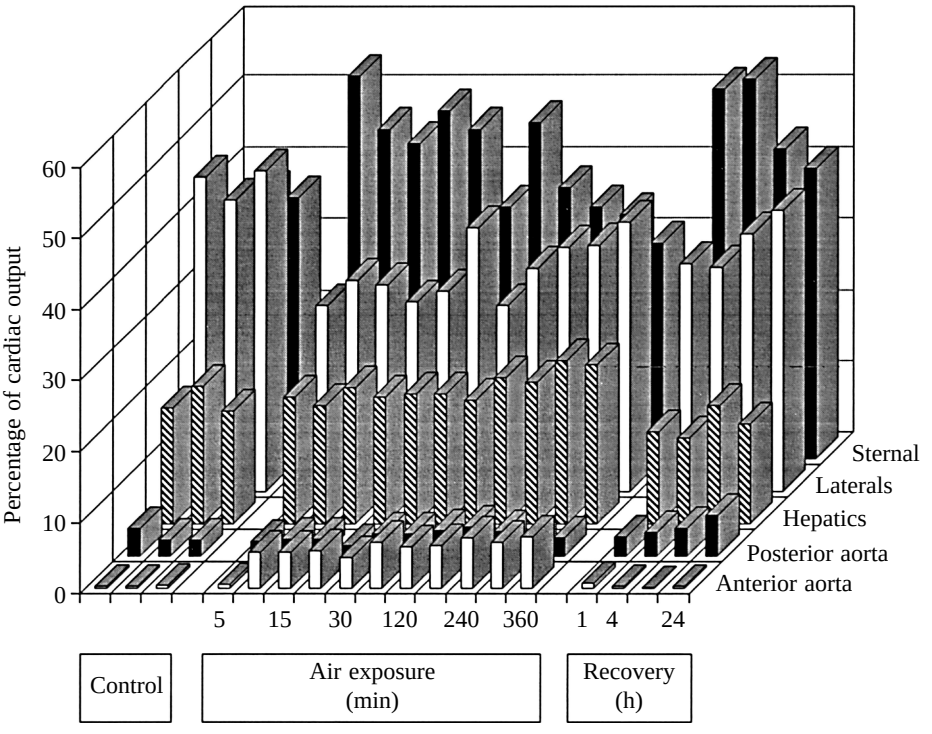

Fig. 8. The mean percentage of cardiac output ($\dot{V}$b) distributed to each arterial system of *Cancer magister* (N=7) during control, emersion and recovery periods at 12 °C. 80 % of the total $\dot{V}$b was distributed approximately equally between the paired anterolateral arteries and the sternal artery prior to emersion. The distribution of $\dot{V}$b initially shifted to favour the sternal artery after the experimental chamber had been drained, but by the end of the emersion period the percentage of $\dot{V}$b delivered to each of these systems had again reached equal proportions. The anterior aorta received less than 0.5 % of the total $\dot{V}$b during the control and recovery periods, but during air exposure this vessel received 7±3 % of the haemolymph pumped by the heart. The percentage of $\dot{V}$b distributed to the hepatic arteries increased slightly over the emersion period, whilst the proportion of $\dot{V}$b pumped through the posterior aorta remained constant.

emersion which is similar to the bradycardia observed in small *C. productus* (deFur and McMahon, 1984*a*). The early ventilatory response was more reminiscent of the tachypnea which occurs in large *C. productus* during emersion, although it appears, on the basis of the ventilation rate recorded at the end of the emersion period in the present study, that elevated rates of ventilatory pumping could not be sustained by *C. magister* for the duration of the experimental emersion. The rates of haemolymph flow through all of the arterial systems except the anterior and posterior aortae decreased significantly during air exposure, as did f_H, $\dot{V}b$ and V_S. These results suggest that general metabolic depression, which is one of the key strategies employed by facultative anaerobes in order to conserve energy during periods of low O_2 availability (Hochachka and Somero, 1984), is employed by air-exposed *C. magister*. The maintenance of f_H and $\dot{V}b$ as well as arterial flow rates at low, stable levels after the first 2 h of emersion is consistent with this interpretation and suggests that the rates of metabolic processes are adjusted to a new set point dictated by the severity of internal hypoxia.

In a previous report on the effects of emersion on respiratory function in *C. magister* (McDonald, 1977), it was demonstrated that following 2 h of air exposure both $\dot{M}_{O_2}$ and haemolymph lactate concentration were elevated while arterial pH declined. Unfortunately, no cardiovascular responses to air exposure were reported. Both the level of lactate production and the haemolymph acidosis were of similar magnitude to the changes reported for *C. productus* after the same length of air exposure (deFur and McMahon, 1984*b*). Post-emersion $\dot{M}_{O_2}$ values for *C. magister* could not be compared with values from *C. productus*, however, because of the longer emersion period utilised with the latter species (deFur and McMahon, 1984*a*).

Determination of $\dot{M}_{O_2}$ was beyond the scope of this investigation, and the present data are insufficient either to support or to reject the contention of McDonald (1977) that *C. magister* is able to sustain aerobic metabolism during emersion. The relationships among cardiac variables (Fig. 2), however, and particularly the reduction of V_S associated with declining f_H, suggest that there was not enough O_2 uptake during emersion to supply the aerobic needs of the cardiac ganglion, the function of which is very susceptible to hypoxic conditions (Wilkens, 1993). In contrast, *C. magister* subjected to 6 h of hypoxic exposure (minimum P_{O_2}=2.96 kPa) were able to maintain the control rate of $\dot{V}b$, despite a dramatic decrease in f_H, owing to a compensatory increase in V_S (Airriess and McMahon, 1994).

One of the most interesting responses of *C. magister* to enforced air exposure was the stereotypical increase in perfusion of the anterior aorta, which occurred as soon as the water level in the experimental chamber fell below the inhalant branchial openings. The immediacy of this response suggests a neural mechanism, which is feasible owing to the unique innervation of the anterior cardioarterial valve by fibres arising from the supraoesophageal ganglion (Steinacker, 1978). Relaxation of the anterior aorta valve could also be mediated neurohormonally, by octopamine or 5-hydroxytryptamine, both of which are present in the pericardial organs of decapods (reviewed in Cooke and Sullivan, 1982) and cause hyperpolarization of the anterior cardioarterial valves of isolated lobster (*Panulirus interruptus*) hearts (Kuramoto and Ebara, 1984). For a more detailed explanation of the possible mechanisms of haemolymph redistribution, see Airriess and McMahon (1994) and McGaw *et al.* (1994*a*,*b*).

At least three possible explanations for the emersion-induced increase in perfusion of the anterior aorta can be suggested. The simplest of these is that during emersion, when haemolymph flow through the other anterior systems is reduced, O_2 supply to the supraoesophageal ganglion of the central nervous system (CNS) must be protected. The supraoesophageal ganglion (brain, cephalic ganglion) of decapod Crustacea is the main centre of sensory integration (Sandeman, 1982). It receives haemolymph distributed *via* a system of capillaries and sinuses originating from the cerebral artery, which is a branch of the anterior aorta (Sandeman, 1967; McLaughlin, 1983). A collateral supply of haemolymph may also reach the supraoesophageal ganglion *via* the occulomotor arteries, which arise from the external ramus of the paired anterolateral arteries (Baumann, 1921). The occulomotor arteries, which ultimately deliver haemolymph to the occulomotor muscles and portions of the optic ganglion (Baumann, 1921; Sandeman, 1967), first give off several branches which penetrate the neuropile of the supraoesophageal ganglion (Baumann, 1921). Assuming that the blood distribution vessels of crayfish (*Astacus*), *Carcinus* and *C. magister* are similar, it seems likely that only a fraction of the haemolymph supply to the brain is delivered *via* the anterior aorta of non-stressed *C. magister* resting in normoxic sea water. The majority of the haemolymph reaching this nervous centre must therefore originate from the anterolateral arteries which, in the present study, were perfused at more than 50 times the rate of the anterior aorta in resting, immersed crabs.

During air exposure, the rate of haemolymph flow through the anterolateral arteries declined by a maximum of 78 % after 4.5 h. After the same length of emersion, haemolymph flow through the anterior aorta was more than double its control rate, and over 7 % of the total $\dot{V}b$ was distributed *via* this vessel. Supply of haemolymph to the brain *via* the anterolateral arteries may be energetically inefficient since it involves concurrent perfusion of the integument, stomach, digestive gland and testes (Pearson, 1908). Thus, when O_2 resources are extremely low, the specific perfusion of the anterior elements of the CNS *via* the anterior aorta may be favoured. The origination of the motor nerves to the cardioarterial valve of the anterior aorta from the supraoesophageal ganglion suggests an autoregulatory mechanism by which requirements for additional perfusion of the brain can be fulfilled.

Another possible explanation for the increased minute volume of haemolymph flowing through the anterior aorta is switching of the venous return pathway to favour perfusion of the branchiostegal linings, which receive haemolymph primarily from the eye sinuses in species adapted for aerial gas

exchange (Greenaway and Farrelly, 1984; Farrelly and Greenaway, 1987). In aquatic crabs lacking the branchial and pulmonary adaptations which facilitate uptake of atmospheric O_2, the vascularization of the branchiostegal linings is comparatively simple (von Raben, 1934). However, a route for venous haemolymph return *via* these structures does exist in such species. It is possible that the increase in perfusion of the anterior aorta observed during emersion in the present investigation results in greater venous return to the heart *via* the branchiostegal linings. Even so, such a shift is unlikely to play a large role in promoting O_2 uptake from atmospheric air, since branching of the afferent vessels within the branchiostegal linings is minimal in comparison with that of air-breathing species and there are no surface modifications of these membranes to increase surface area. If perfusion of the branchiostegal linings does increase as a result of increased haemolymph delivery to the eye sinuses *via* the anterior aorta, this may represent a pre-adaptation allowing a shift towards heightened venous return *via* the lung during aerial respiration in more highly adapted forms.

Of the general strategies outlined above, which may be used by sublittoral crustaceans to minimize the impact of emersion-induced internal hypoxia, metabolic depression is the component most obviously demonstrated by *C. magister* during experimental emersion. The rapid onset of bradycardia at the start of the emersion period as well as the associated reduction in V_S and the rates of haemolymph flow through several arterial systems clearly indicate a reduction in overall metabolic rate. Low levels of lactate production in conjunction with a reduction of $\dot{M}_{O_2}$, previously reported for *C. magister* subjected to 2 h of undisturbed emersion (McDonald, 1977), provide further evidence for a decrease in metabolic rate. The second component of this general strategy, augmentation of the quantity of glucose available for use in anaerobic pathways of metabolism, also occurs in *C. magister* subjected to experimental emersion (C. N. Airriess and B. R. McMahon, unpublished observations).

Hyperglycaemia in crustaceans is thought to be triggered in many cases by the action of crustacean hyperglycaemic hormone (CHH) on various target tissues (Keller *et al.* 1985). CHH is synthesized within the X-organ of the optic ganglion and transported axonally to the sinus gland, which is located beneath the ganglionic sheath on the surface of the medulla terminalis in the base of each eyestalk (Cooke and Sullivan, 1982). Haemolymph is delivered to the optic ganglia *via* the optic arteries, which arise as paired branches from the anterior aorta, as well as the occulomotor arteries which originate from the external ramus of each anterolateral artery in decapods (Baumann, 1921; Sandeman, 1967). The dramatic increase in perfusion of the anterior aorta at the onset of air exposure in the present investigation is particularly intriguing, given that subsidiaries of this vessel provide the primary haemolymph supply to the sinus glands. Thus, increased perfusion of the anterior aorta may be an important factor in the release of CHH and/or the distribution of this hormone throughout the cardiovascular system.

Although lacking adaptations of the gas-exchange organs for aerial O_2 uptake, *C. magister* appears to employ cardiovascular measures during emersion which protect as far as possible the supply of O_2 to the vital anterior regions of the CNS while limiting the total metabolic cost of haemolymph circulation. The contrasting cardiac responses to hypoxic exposure and emersion suggest that, in the former situation, enough O_2 can be obtained from the ventilatory medium to supply the metabolic demands of ventricular contraction; in the case of emersion, however, when the natural ventilatory medium has been replaced by air, O_2 uptake and delivery appear to be insufficient to meet the aerobic requirements of the heart.

The authors would like to acknowledge the following people for their contributions to this study: Dr G. B. Bourne, for the use of equipment and helpful discussion of results; Dr I. J. McGaw for helpful discussion during the experimental work; R. A. Airriess and Drs W. W. Burggren, J. L. Wilkens, J. V. Tyberg and W. A. Whitelaw for critical reading of early stages of this manuscript; Dr F. E. R. McCauley for consultation with regard to experimental design and the statistical analysis of data; and the Director and Staff of the Bamfield Marine Station for facilitating the experiments described herein. Financial support was provided by NSERC operating grant A5762 to B.R.M. and fellowships to C.N.A. from NSERC, AHFMR and the Bamfield Marine Station.

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