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Does feeding limit cardiovascular modulation in the Dungeness crab *Cancer magister* during hypoxia?

Iain J. McGaw

Department of Biological Sciences, UNLV, 4505 Maryland Parkway, Las Vegas, NV 89154-4004, USA and *Bamfield Marine Sciences Centre, Bamfield, British Columbia, Canada VOR 1BO*

e-mail: imcgaw@ccmail.nevada.edu

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Summary

Decapod crustaceans inhabit aquatic environments that are frequently subjected to changes in oxygen content. The physiological mechanisms that allow them to cope with periodic episodes of hypoxia have been well documented. Most crustaceans exhibit a bradycardia coupled with diversion of haemolymph from digestive organs towards ventral structures. However, all these experiments were conducted on animals that were starved prior to experimentation in order to avoid increases in metabolism associated with digestive processes. The present study sought to determine how the Dungeness crab *Cancer magister* balances the demands of physiological systems when they feed and digest in hypoxia. Cardiac parameters and haemolymph flow rates through each arterial system exiting the heart were measured using a pulsed-Doppler flowmeter. Scaphognathite beat frequency (ventilation rate) was calculated by recording changes in pressure in the branchial chamber. There was an increase in both cardiac and ventilatory parameters following feeding. Digestive processes were facilitated by an increase in haemolymph flow rates through the anterior aorta, hepatic arteries and sternal artery. *Cancer magister* showed a typical bradycardia during hypoxia (3.2 kPa). However, food intake caused a significant reduction in this response. Likewise, ventilation rate also showed effects of addivity, increasing in response to both food intake and hypoxia. Digestion during hypoxia was associated with a decrease in both stroke volume and cardiac output. Blood was diverted away from digestive structures, suggesting that blood flow events are prioritized during hypoxia. The changes in haemolymph flow rates paralleled those in previous reports on reductions in protein synthesis in the hepatopancreas during hypoxia. Haemolymph flow rates through the anterior aorta did not change; thus the blood supply to the supraoesophageal ganglion was maintained during feeding in hypoxia. The results show that the nutritional state of an animal is important in modulating its physiological responses to environmental perturbations. This underscores the importance of an integrative approach, studying physiological responses at the organismal level.

Key words: *Cancer magister,* cardiovascular system, crab, digestion, feed, hypoxia, physiology, ventilation.

Introduction

Episodes of hypoxia are common in the marine environment, especially in shallow coastal zones. Mobile organisms such as fish and crustaceans may migrate away from areas of sustained low oxygen (Diaz and Rosenburg, 1995). Nevertheless, in areas where escape responses are not possible, many organisms have evolved physiological mechanisms that allow them to cope with acute hypoxic exposure.

The physiological responses of decapod crustaceans to hypoxia are well documented. Most species exhibit a pronounced bradycardia in conditions of dissolved oxygen decrease (McMahon, 2001). In a number of species, cardiac output is maintained or even increases, driven by an increase in stroke volume (McMahon and Wilkens, 1975; Wilkes and McMahon, 1982; Airriess and McMahon, 1994; Reiber, 1995; Reiber and McMahon, 1998). Regional haemolymph flows are more variable among species; individual organ perfusion may increase or decrease depending on the species and the severity of the hypoxic regime (Airriess and McMahon, 1994; Reiber and McMahon, 1998). Oxygen uptake is usually maintained down to a critical oxygen tension (P_{crit}), initially by an increase in ventilation rate and later by internal compensatory mechanisms (Wilkes and McMahon, 1982).

All the crustaceans used for the above mentioned studies were starved prior to and/or were not fed during experiments. This protocol is adopted since the stimulatory effect of food ingestion on metabolic processes is well known (Wang, 2001). Decapod crustaceans are no exception; oxygen uptake increases immediately after feeding, reaching maximal levels within 2.5–4 h (Houlihan et al., 1990; McGaw and Reiber,

2000; Robertson et al., 2002; Mente et al., 2003). Oxygen uptake can remain elevated for up to 48 h (Legeay and Massabuau, 1999; McGaw and Reiber, 2000). Blood flow is diverted to the muscles while feeding and to the digestive organs thereafter (McGaw and Reiber, 2000). However, in nature, organisms do not starve themselves before encountering environmental perturbations. The question then arises as to how an animal balances the simultaneous demands of these physiological systems.

The Dungeness crab *Cancer magister* is a commercially important species along the Pacific coast of North America. It inhabits sandy and muddy bays and estuaries, where it can encounter hypoxic water as low as 1.2 kPa (Airriess and McMahon, 1994; Bernatis and McGaw, 2004). Its cardiovascular responses to hypoxia follow the typical decapod pattern, with heart rate decreasing from 75 beats min^{-1} to 45 beats min^{-1} in severe hypoxia. This bradycardia is coupled with an increased stroke volume, leading to a slight increase in cardiac output (Airriess and McMahon, 1994). A decreased heart rate aids diastolic filling time, the trade off between reducing heart rate and increasing stroke volume may be energetically advantageous as well allowing increased perfusion pressures of arterial systems (Reiber and McMahon, 1998). The majority of this maintained cardiac output is shunted via the sternal artery to the scaphognathite muscles, which aids the increase in ventilatory frequency. The redistribution of blood through the sternal artery may also protect the CNS from the effects of hypoxic exposure (Airriess and McMahon, 1994). Thus, these alterations in cardiovascular variables enhance the ability of *Cancer magister* to cope with hypoxia (Airriess and McMahon, 1994). However, these experiments were performed on starved animals. Digestive processes will pose an additional burden on animals already attempting to supply adequate amounts of oxygen to the tissues. Therefore, the present study sought to determine how feeding and subsequent digestion affect the ability of *Cancer magister* to maintain cardiac function and balance tissue perfusion in low oxygen tension environments.

Material and methods

Adult male intermoult Dungeness crabs *Cancer magister* (authority?), of 400–500 g were trapped in Barkley Sound, British Columbia, Canada, from May to August 2003. They were transferred to the Bamfield Marine Sciences Centre and held in running seawater (32‰) of 11±1°C at dissolved oxygen levels of 18±1 kPa for a week prior to experimentation. Crabs were fed fish every other day, but were isolated from the general population and starved for 3 days prior to experimentation. This time period allowed all food to be evacuated from the digestive system, but avoided large-scale physiological changes associated with starvation (Wallace, 1973). A 545C pulsed-Doppler flowmeter (University of Iowa-Bioengineering, Iowa City, State, USA) was used to measure haemolymph flow rates in each of the major arterial systems exiting from the heart. Piezo-electric Doppler flow probes were either implanted directly above the arteries in grooves abraded in the carapace (anterior and posterior aortae, anterolateral arteries) or guided to lie adjacent to the artery *via* internal catheter-mounted probes (hepatic arteries, sternal artery). The probes were manoeuvred to obtain maximal signal and were held in place with dental wax and super glue. Heart rate was obtained by counting the peaks on the phasic traces; summation of all arterial flows (paired arteries were doubled) gave a value for cardiac output, and division of this value by heart rate yielded cardiac stroke volume. A detailed description of the set-up and methods is covered elsewhere (Airriess et al., 1994). Changes in pressure were also measured in the branchial chambers allowing calculation of scaphognathite beat frequency (ventilation rate). Holes drilled into the carapace above the branchial chamber were covered with dental dam and a chronically implanted polythene catheter (PE160; Becton Dickinson, Sparks, MD, USA) was held in place with wax and super glue. Each catheter was filled with seawater and connected to a disposable blood pressure transducer (MLT0698, ADInstruments, Mountain View, CA, USA).

During experiments, crabs were held in a circular tank of 32 cm×30 cm diameter×depth in aerated running seawater at 10–12°C, with a layer of sand lining the bottom. The tips of the chelae were glued together to prevent the crabs from cutting the catheters, other than this they were able to move freely. The crabs were allowed to settle for 12 h in the chamber before experimentation. Data for cardiac and ventilatory parameters were recorded continuously using an ADInstruments data acquisition system. All recordings were carried out in constant dim light, which helped reduce any nocturnal activity. The entire apparatus was surrounded by black plastic sheeting to avoid visual disturbance to the animal. Hypoxic conditions (3.2±0.2 kPa) were initiated by bubbling a mixture of nitrogen and air into the water using a GF3/MP gas mixing pump (Cameron Instruments, Port Aransas, TX, USA); oxygen levels were checked with an oxygen meter (YSI 55, Yellow Springs, OH, USA). New steady states of dissolved oxygen were reached in the experimental apparatus within 15–20 min and did not vary by more than 0.2 kPa during the experiments. A hypoxic regime of 3.2 kPa was used since it approximated to the lowest oxygen tension at which the crabs would feed (Bernatis and McGaw, 2004). For feeding, a polyethylene tube (PE160) was inserted into the oesophagus and held in place with dental dam. This allowed a liquefied fish meal, 2% of the crab's body mass, to be administered at a rate of approximately 5 ml min^{-1}. It also helped reduce changes in physiological parameters (apparent specific dynamic action) associated with food handling (Carefoot, 1990).

Three separate experiments were carried out. In the first experimental series, cardiovascular parameters of eight Dungeness crabs were monitored for 3 h in normoxia. The animals were then fed and monitored for a further 12 h in normoxia. In a second series of experiments, cardiovascular

parameters of eight crabs were monitored for 3 h control period in normoxia. The animals were fed and then 1 h after feeding, hypoxia (3.2 kPa) was initiated for a total time of 6 h. Normoxic conditions were then restored for a further 6 h. In a final series of experiments eight crabs were monitored in control conditions for 3 h. Hypoxia (3.2 kPa) was then initiated and 3 h later the crabs were fed. Changes in cardiovascular parameters were recorded for a further 6 h in hypoxia, after which, normoxic conditions were restored for an additional 6 h. This time course of hypoxic exposure was chosen to emulate naturally occurring conditions based on the tidal cycle in Barkley Sound, British Columbia.

Haemolymph L-lactate levels (N=7) were measured during feeding in hypoxia. At set intervals the crabs were quickly removed from the tank (separate crabs were used at each time interval). Within 10 s, a 50 µl sample of haemolymph was withdrawn from a pereiopod artery at the base of the walking legs using needle and syringe. The sample was immediately frozen at –80°C to avoid degradation of compounds. L-Lactate levels of a 6 µl sample were later analysed using a Pointe Scientific (Canton, MI, USA) UV lactate test kit, with absorbance read at 550 nm (Spectra-Max Plus, Molecular Devices, Sunnyvale, CA, USA).

One-way ANOVA with repeated measures (RM) design was used to test for significant differences in cardiovascular and ventilatory parameters. Data showing a significant effect, were further analysed by a Fisher's LSD multiple comparison test (P<0.01) to determine at which time periods significant effects were observed.

Results

Feeding only

Heart rate (Fig. 1A) increased significantly from mean values of 74 beats min^{-1} to 84 beats min^{-1} during feeding (F=11.7, P<0.001). Heart rate remained elevated over pre-feeding levels for 5–6 h. Thereafter, there was a slow decrease in heart rate, reaching control levels 10 h after feeding. There was a significant increase in cardiac stroke volume (Fig. 1B) during feeding (F=3.6, P<0.001). This increase was only transient, and stroke volume returned to pre-feeding values within an hour. An increase in both heart rate and stroke volume during feeding lead to a significant increase in cardiac output (F=5.6, P<0.001). Cardiac output increased from approximately 47±9 ml min^{-1} to over 85±16 ml min^{-1} during feeding. Cardiac output dropped sharply after feeding to between 58–60 ml min^{-1}; but these values remained significantly elevated for 6 h, before returning to pre-feeding levels (Fig. 1C).

Ventilation rate (Fig. 1D) increased significantly from between 56–59 beats min^{-1} to 70 beats min^{-1} during feeding (F=4.9, P<0.001). These rates were sustained above pre-feeding values for 10 h, before dropping to levels that were not significantly different from those measured pre-feeding (Fisher's LSD test, P<0.001).

There were also significant changes in haemolymph flow rates. Haemolymph flow through the anterior aorta (Fig. 2A), increased 3 h after feeding (F=5.1, P<0.001) and remained elevated for a further 7 h, at which time flow rate decreased to pre-feeding levels. There was an increased perfusion of the hepatopancreas via the hepatic arteries (Fig. 2C): a steady increase in flow rates occurred during feeding, becoming significantly higher than control values 3 h after feeding (F=2.8, P<0.001). Haemolymph flow rates through the hepatic arteries remained elevated for 4 h before decreasing to levels that were not significantly different from those measured pre-feeding. Haemolymph flow through the sternal artery (Fig. 2E) increased substantially from approximately 28 ml min^{-1} to over 60 ml min^{-1} during feeding (F=5.8, P<0.001). Thereafter, sternal flow rates decreased to levels that were not significantly different from those measured pre-feeding.

Despite an apparent increase in flow rates through the anterolateral arteries (Fig. 2B)

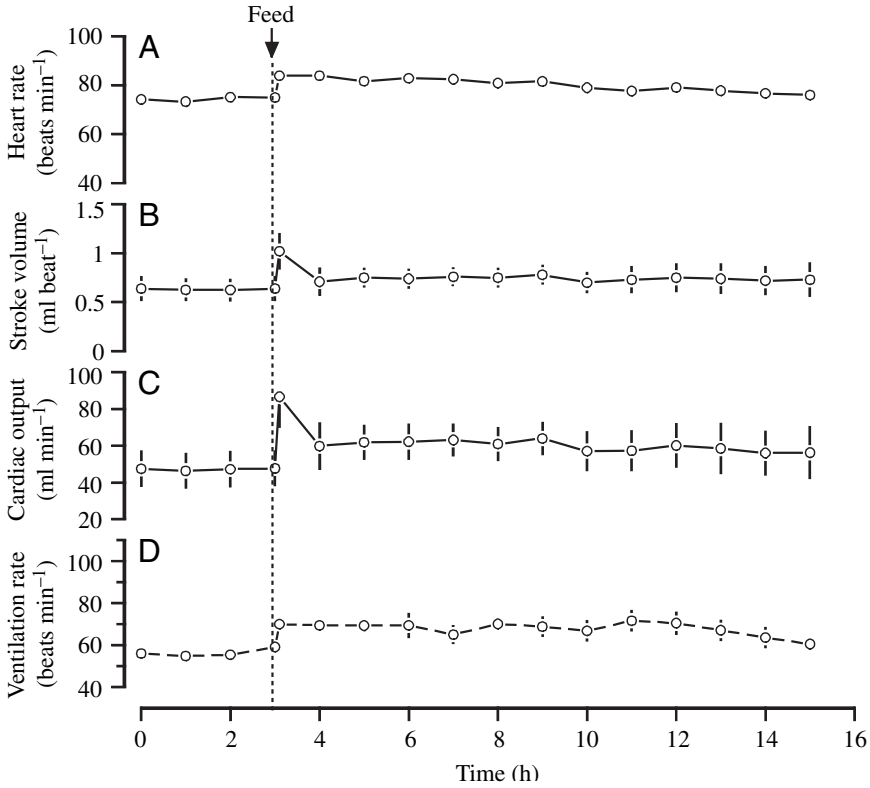

Fig. 1. Changes in cardiac and ventilatory parameters (means ± S.E.M.) of eight *Cancer magister* during a 3 h control period in normoxia, food was then administered and changes followed for a further 12 h in normoxia. (A) Heart rate (beats min^{-1}), (B) stroke volume (ml beat^{-1}), (C) cardiac output (ml min^{-1}) and (D) ventilation rates (beats min^{-1}). In some cases standard errors were very small and do not show clearly on the figure.

and the posterior aorta (Fig. 2D) following feeding, no statistically significant change could be demonstrated (F=1.4 and 1.1, respectively, P>0.1).

Feeding followed by hypoxia

Heart rate (Fig. 3A) increased significantly by approximately 6 beats min^{-1} during feeding and remained elevated thereafter (F=8.1, P<0.001). Once hypoxia was administered there was a steady decrease in heart rate; after 1 h in hypoxia, heart rate had dropped to approximately 71 beats min^{-1}, which was 12 beats min^{-1} lower than rates measured during feeding. On return to normoxia there was significant increase in heart rate and pre-feeding levels were rapidly regained. Stroke volume (Fig. 3B) also increased significantly during feeding (F=8.0, P<0.001), however, this increase was transient and stroke volume rapidly decreased to pre-feeding levels following administration of food. When hypoxia was initiated there was a further decrease in stroke volume, dropping approximately 0.3 ml beat^{-1} below pre-feeding levels. When the oxygen tension was restored to normoxic levels, stroke volume increased, reaching values similar to those measured during pre-feeding. Cardiac output (Fig. 3C) also increased transiently during feeding, reaching 91±28 ml min^{-1} before dropping back to pre-feeding levels of 62–69 ml min^{-1}. Once hypoxia was initiated there was a further decrease (F=11.1, P<0.001) in cardiac output to 40–50 ml min^{-1}; this was maintained throughout the hypoxic exposure period. As with heart rate and stroke volume, initiation of normoxia was associated with a rapid but transient increase in cardiac output; pre-feeding levels were regained within an hour.

Ventilation rate varied between 60 and 63 beats min^{-1} during control conditions (Fig. 3D). There was a significant increase in rate (F=17.5, P<0.001) to 77 beats min^{-1} during administration of food. Rates remained significantly elevated thereafter. Upon exposure to hypoxic conditions there was a further significant increase; ventilation rate approached 90 beats min^{-1}. There was a steady decline in rate when normoxic conditions were restored; ventilation rates reached levels that were similar to those measured during the initial feeding phase in normoxia.

There was no significant change in haemolymph flow through the anterior aorta

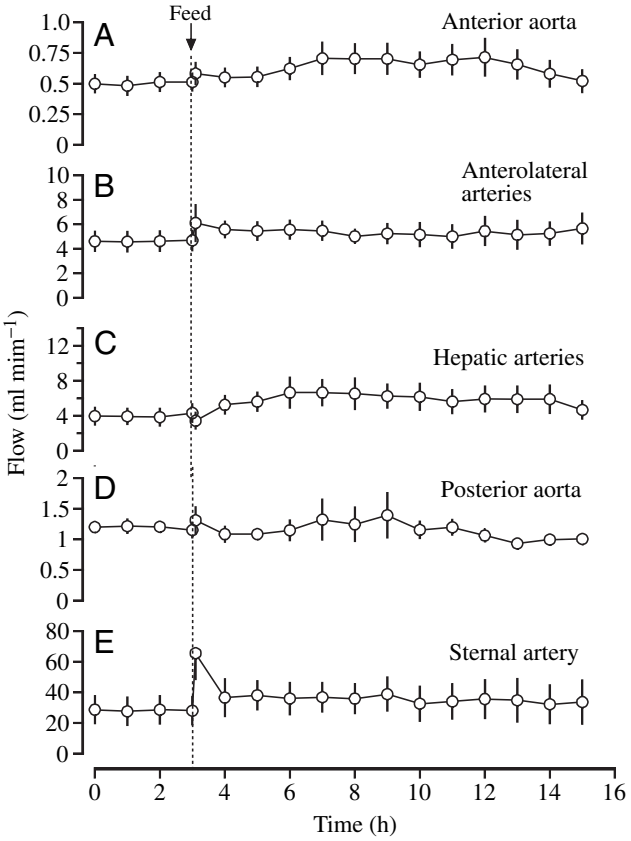

Fig. 2. Haemolymph flow rates (ml min^{-1}) through the (A) anterior aorta, (B) left anterolateral artery, (C) right hepatic artery, (D) posterior aorta and (E) sternal artery of eight *Cancer magister* during a 3 h control period at which point food was administered and changes followed for a further 12 h. Values are means ± S.E.M.

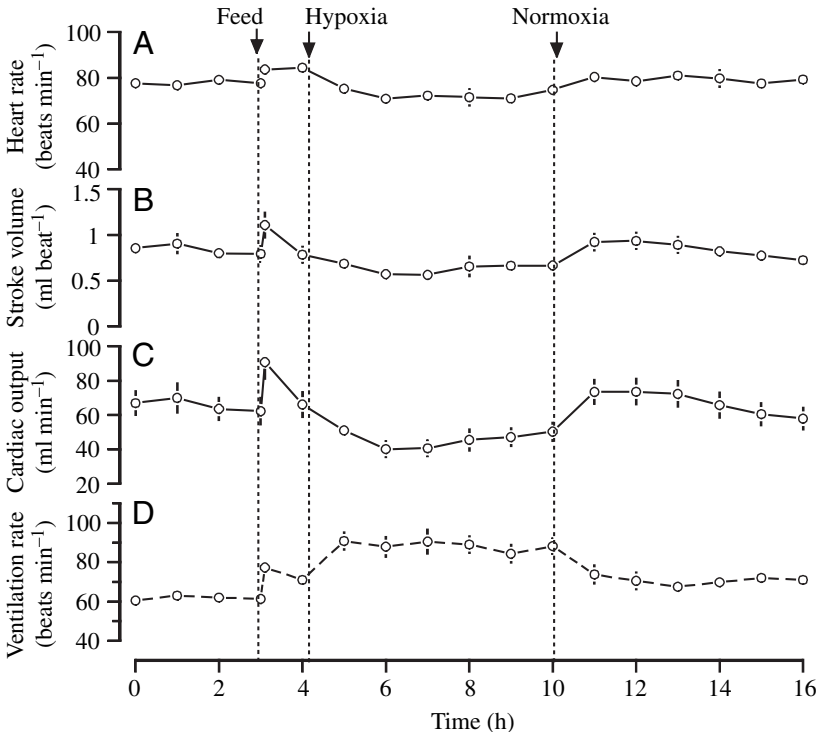

Fig. 3. (A) Heart rate (beats min^{-1}), (B) stroke volume (ml beat^{-1}) (C) cardiac output (ml min^{-1}) and (D) ventilation rates (beats min^{-1}) of eight *Cancer magister*. Crabs were monitored during a 3 h control period in normoxia, food was administered and cardiac parameters followed for a further 1 h in normoxia after which, hypoxic conditions of 3.2 kPa were initiated for 6 h. Normoxic conditions were then restored for an additional 6 h. Values are means ± S.E.M.

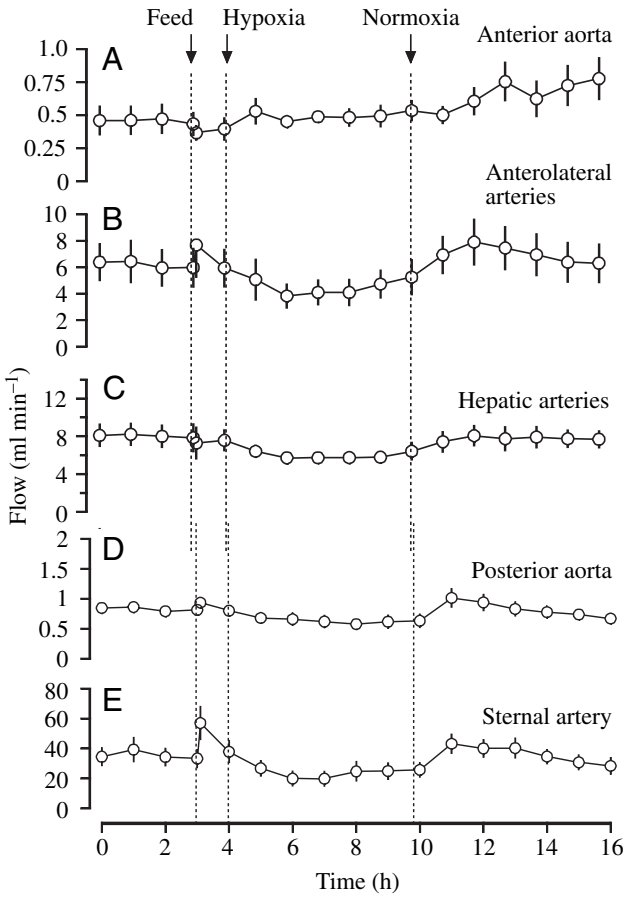

Fig. 4. Haemolymph flow rates (ml min^{-1}) through the (A) anterior aorta (B) left anterolateral artery (C) right hepatic artery (D) posterior aorta and (E) sternal artery of eight *Cancer magister*. Crabs were monitored during a 3 h control period in normoxia, food was administered and cardiac parameters followed for a further 1 h in normoxia after which, hypoxic conditions of 3.2 kPa were initiated for 6 h. Normoxic conditions were then restored for an additional 6 h. Values are means ± S.E.M. The beginning of the feeding, hypoxic and normoxic periods are marked. In some cases standard errors were very small and do not show clearly on the figure.

(Fig. 4A) in response to feeding or hypoxia. Haemolymph flow rates through this artery only started to increase significantly (F=3.6, P<0.001) towards the end of the recovery period in normoxia (13–16 h). Although haemolymph flow appeared to increase through the anterolateral arteries (Fig. 4B) during feeding, no statistical significance could be demonstrated (Fisher's LSD test, P>0.05). However, when hypoxia was initiated flow rates decreased below levels measured during both feeding and pre-feeding (F=4.5, P<0.001). When oxygen levels were restored there was a significant increase in flow rates through the anterolateral arteries; control levels were regained within an hour.

There was no significant change in perfusion of the hepatopancreas, *via* the hepatic arteries (Fig. 4C), while the crabs were feeding. However, when hypoxia was initiated there was a slight, but significant drop in flow rates (F=2.3, P<0.01). There was no significant change in flows when normoxia was restored (Fishers LSD P>0.05).

There was no significant change in flow rates through the posterior aorta while the crabs were feeding (Fig. 4D). After 2 h in hypoxia, a significant decrease in flow rates occurred (F=3.9, P<0.001). There was an overshoot in flow rates when normoxic conditions were restored; these subsided after 2 h to levels similar to pre-treatment levels.

During feeding, haemolymph flows through the sternal artery (Fig. 4E) increased significantly (F=7.4, P<0.001), before quickly returning to pre-feeding levels. This was followed by a further decrease in haemolymph flow rates during hypoxia. On return to normoxia there was a rapid increase in flow rates, such that during the first hour they increased over pre-feeding levels. Thereafter they decreased to levels that were not significantly different from those measured during pre-feeding.

Hypoxia followed by feeding

A pronounced bradycardia occurred during hypoxic exposure (Fig. 5A). Heart rate dropped significantly from between 79–82 beats min^{-1} to 48–52 beats min^{-1}. This bradycardia was sustained for the duration of hypoxia (F=26.9, P<0.001). When the crabs were fed there was a significant increase in heart rate to 69–72 beats min^{-1}. This was still significantly lower than heart rates recorded during control conditions. On return to normoxia there was a further increase and heart rate reached control levels within an hour. An immediate increase in stroke volume (Fig. 5B) occurred during hypoxia, however, this increase was only sustained for 1 h before declining to control levels (F=5.9, P<0.001). An additional small, but significant, decrease in stroke volume occurred an hour after feeding; this rate was sustained until oxygen levels were restored. In normoxia, stroke volume quickly returned to pre-treatment levels. Changes in cardiac output (Fig. 5C) were predominately affected by heart rate. Cardiac output decreased significantly in hypoxia (F=5.6, P<0.001). When the crabs were fed in hypoxia there was an increase in cardiac output and pre-treatment levels were regained. Nevertheless, this increase was not sustained and during the following 6 h there was a significant decrease in cardiac output, reaching levels comparable to those measured during pre-feeding in hypoxia. On return to normoxia there was a significant rise in cardiac output and pre-treatment levels were rapidly regained.

Ventilation rates (Fig. 5D) were maintained between mean values of 57–62 beats min^{-1} during control conditions. There was a rapid increase in ventilation rate when hypoxia was initiated; rates reached over 90 beats min^{-1} (F=23.6, P<0.001). Although there appeared to be a further increase in rate following administration of food, this proved to be statistically insignificant (Fisher's LSD test, P<0.05). On return to normoxic conditions there was a significant decrease in ventilation rates. However, these rates still remained elevated over pre-treatment levels.

There were also changes in regional haemolymph flow

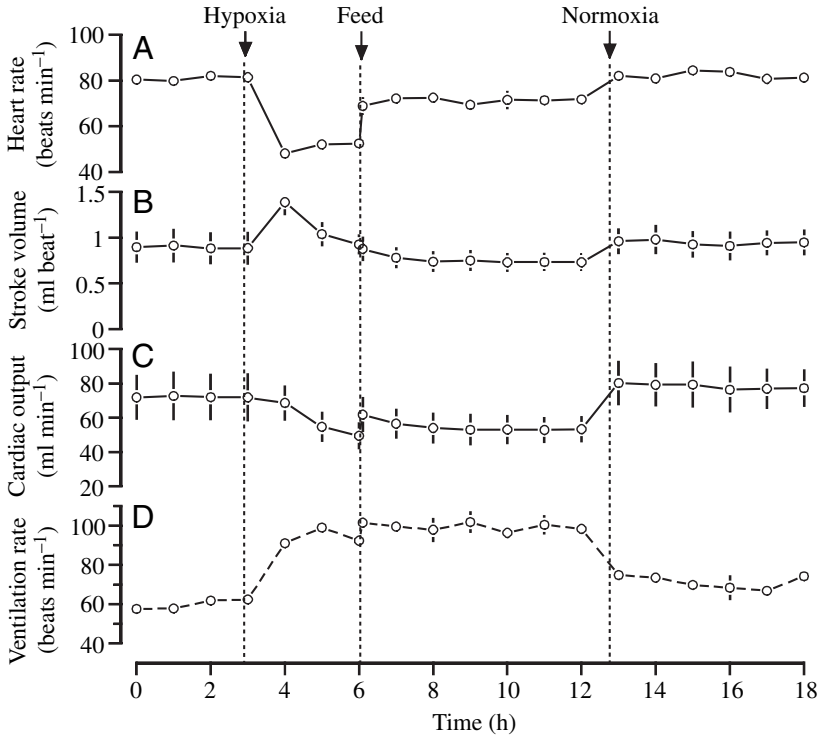

Fig. 5. Changes in (A) heart rate (beats min^{-1}), (B) stroke volume (ml $beat^{-1}$), (C) cardiac output (ml min^{-1}) and (D) ventilation rate of the branchial chambers (beats min^{-1}) of eight *Cancer magister*. Crabs were monitored during a 3 h control period in normoxia, before hypoxic conditions of 3.2 kPa were initiated for 3 h. Crabs were then fed and monitored for a further 6 h in hypoxia after which normoxic conditions were restored for an additional 6 h. Values ARE means ± S.E.M. In some cases standard errors were very small and do not show clearly on the figure.

associated with food intake during hypoxia. An immediate increase in haemolymph flow through the anterior aorta (Fig. 6A) occurred when the crabs fed in hypoxia (F=3.0, P<0.001). This increase was only sustained during the feeding phase, after which, flow rates dropped to levels that were not significantly different than pre-feeding values. On return to normoxia there was a slight, transient increase in flow rate through the anterior aorta before returning to pre-treatment levels. A decrease in haemolymph flows through the hepatic arteries (Fig. 6C) occurred when the crabs fed in hypoxia (F=3.6, P<0.001). This decrease was sustained for the duration of the postprandial period in hypoxia. Pre-treatment flow rates were regained within 1 h of transfer to normoxic conditions. Haemolymph flows through the sternal artery (Fig. 6E) exhibited the greatest changes (F=3.7, P<0.001). After 3 h in hypoxia, a slight decrease in flow was apparent. A transient increase in flow rates occurred during feeding before dropping back to levels that were not significantly different to those measured pre-treatment. On return to normoxia, pre-treatment levels were rapidly regained.

Despite apparent changes in flow rates through the anterolateral arteries (Fig. 6B) and the posterior aorta (Fig. 6D) no statistically significant difference could be demonstrated in either case (F=0.9 and F=1.4, respectively, P>0.1).

L-Lactate levels

Haemolymph lactate levels varied between 0.56 and 1.12 mmol l^{-1} in starved crabs (Fig. 3D) in normoxia (Table 1A,B). When crabs were fed in normoxia (Table 1A) there was no significant change in lactate levels. After 6 h exposure to hypoxic conditions, there was a statistically significant rise in haemolymph lactate, reaching 1.22±0.21 mmol l^{-1} (F=4.71, P<0.01). When normoxic

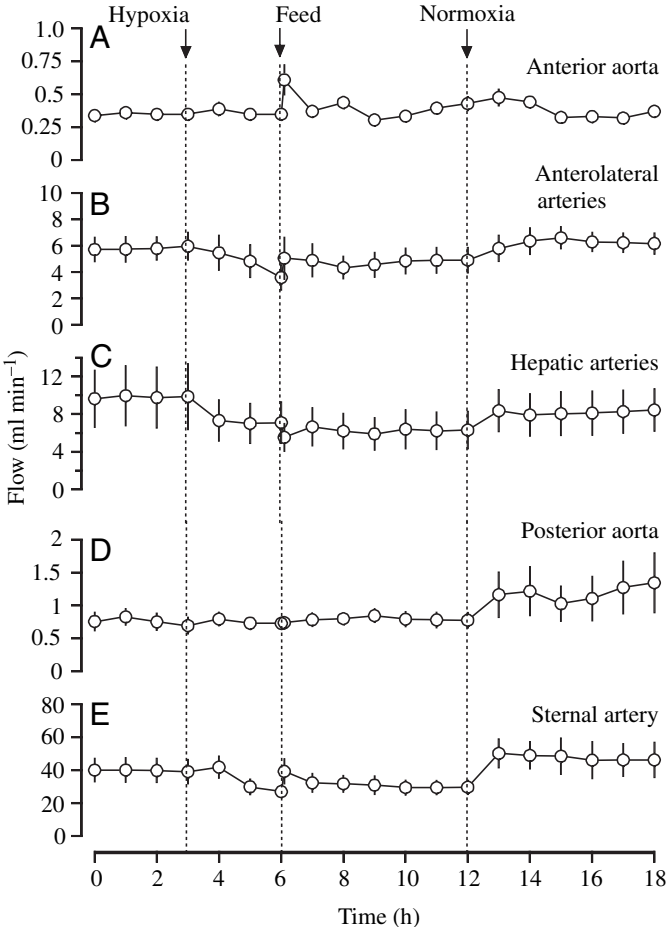

Fig. 6. Haemolymph flow rates (ml min^{-1}) through the (A) anterior aorta, (B) left anterolateral artery, (C) right hepatic artery, (D) posterior aorta and (E) sternal artery of eight *Cancer magister*. Crabs were monitored during a 3 h control period in normoxia, after which hypoxic conditions of 3.2 kPa were initiated for 3 h. Food was then administered and cardiac parameters followed for a further 6 h in hypoxic conditions. Normoxic conditions were then restored for an additional 6 h. Values means ± S.E.M.

Table 1. *Haemolymph L-lactate levels in* Cancer magister

Time (h)	Treatment	[L-Lactate] (mmol l^{-1})
A		
0	Normoxia: control	0.56±0.01
2	Normoxia: 2 h postprandial	0.55±0.12
8	Hypoxia: 6 h postprandial	1.22±0.21*
11	Normoxia: 9 h postprandial	0.63±0.12
B		
0	Normoxia: control	1.12±0.10
3	Hypoxia: 3 h preprandial	1.61±0.28*
9	Hypoxia: 6 h postprandial	0.95±0.12
12	Normoxia: 9 h postprandial	0.56±0.12

(A) Crabs were fed in normoxia, then 2 h after feeding they were exposed to 6 h of hypoxia (3.2 kPa). Normoxic conditions were then restored for a further 3 h.

(B) Crabs were fed following 3 h exposure to hypoxia (3.2 kPa). Haemolymph samples were taken after an additional 6 h in hypoxia. Normoxic conditions were then restored for an additional 3 h.

Values are mean ± S.E.M. (N=7).

conditions were restored, haemolymph lactate levels dropped to pre-feeding values (Table 1A).

After 3 h exposure to hypoxia, lactate levels increased slightly (Table 1B), but these levels were not significantly higher than those measured during normoxia (Fisher's LSD test, P>0.01). There was no significant change in lactate levels 6 h after feeding in hypoxia (Fisher's LSD test, P>0.01). When normoxic conditions were restored, lactate levels dropped to 0.56±0.12. These were significantly lower than those measured after 3 h exposure to hypoxia (F=5.54, P<0.01).

Discussion

In decapod crustaceans, oxygen uptake and protein synthesis increase rapidly during the first few hours after feeding (Barker and Gibson, 1977; Houlihan et al., 1990; Mente et al., 2003). Although the physiological effects of intracellular digestion (post 24 h) during hypoxia have been investigated in some detail (Legeay and Massabuau, 1999, 2000a,b; Mente, 2003), there is much less information on the interactions between physiological systems during the first stages of digestion (Mente, 2003).

Cardiac and ventilatory parameters increased immediately in *Cancer magister* following feeding. These responses were a direct action of food on metabolism, since administration of an equal amount of saline caused only slight and transient increases (not shown). The immediate increase in cardiac and ventilatory parameters were associated with food handling and food processing in the foregut (Carefoot, 1990). Following this initial increase, cardiac and ventilatory parameters remained elevated for up to 10 h, reflecting their involvement in protein synthesis (Houlihan et al., 1990; Mente, 2003; Mente et al., 2003). A standardised amount of food was delivered to each crab to reduce variation associated with meal size (Mente, 2003) and to synchronise feeding time. This feeding method also aimed to reduce some of the activity associated with feeding (apparent specific dynamic action; Carefoot, 1990). Nevertheless, the crabs still moved the mouthparts and chelae when food was administered: this was seen as an increase in flow through the sternal artery (Fig. 2E), which supplies these structures (McGaw, 2005). Haemolymph flow rates through the hepatic artery (which supplies the hepatopancreas) increased 3 h after feeding, which corresponds closely with the time that protein synthesis increases in the hepatopancreas (Houlihan et al., 1990).

The magnitude of changes in heart rate during hypoxia (Fig. 5A) were similar to those reported previously for unfed *Cancer magister* (Airriess and McMahon, 1994). However, these researchers reported the bradycardia was associated with a sustained increase in stroke volume, resulting in maintained cardiac output. In the present study the increase in stroke volume was only transient and not large enough to affect cardiac output (Fig. 5B,C). This difference may have arisen because of differences in experimental design. Previous experiments were performed on restrained animals (McMahon and Wilkens, 1975; Reiber, 1995; Reiber and McMahon, 1998) or in chambers that allowed minimal movement (Wilkes and McMahon, 1982; Airriess and McMahon, 1994). When crabs are restrained during such experiments they will continue to struggle (personal observation). The untethered approach used in this study reduces stress associated with instrumentation (McDonald et al., 1977; Hassall and McMahon, 1980) and the layer of sand in the tank minimizes sensory stimulation to the crabs (Florey and Kriebel, 1974). These assertions are upheld by observations that heart rates recorded *in situ*, in the natural environment, may differ from responses observed in the lab (Styrishave et al., 2003). The changes in stroke volume and cardiac output in the present study (Fig. 5B,C) are also emulated by behavioural observations: *Cancer magister* was active during the initial oxygen reduction period, but became quiescent, exhibiting little or no movement thereafter. The crabs remained inactive until oxygen levels dropped to 1.5 kPa, but below these levels they became agitated and attempted to escape (J. L. Bernatis and I.J.M., unpublished observation). Therefore, the data obtained in the present study may be the result of behavioural differences associated with experimental design.

Food intake during hypoxia clearly affected heart rate, reducing but not abolishing, the bradycardic response. Therefore, in the case of heart rate, addivity of effects occurs, rather than prioritization of one or other of the systems (Bennett and Hicks, 2001). Because the bradycardic response was reduced by feeding, its physiological role may be diminished. Increased mortality occurs in the shore crab *Carcinus maenas* when sufficient oxygen cannot be supplied following feeding in hypoxia (Legeay and Massabuau, 2000b). However, both starved and postprandial *Cancer magister* survived 48 h exposure to 2.1 kPa water (unpublished observation). This suggests that although bradycardia may aid

survival of *Cancer magister* in low oxygen environments (Airriess and McMahon, 1994), its role is only minor and other compensatory mechanisms are involved.

Although, both heart and ventilation rates showed addivity as a result of the effects of hypoxia and food intake, blood flow events tended to be prioritized. Following a transient increase in haemolymph flow rates to the mouthparts, via the sternal artery and anterolateral arteries (Figs 4B,E, 6B,E) there was a trend towards a decrease in arterial flow rates. The anterior aorta was the only artery in which flow was maintained (Figs 4A, 6A). This artery supplies the supraoesophageal ganglion of the crab (McGaw and Reiber, 2002; McGaw, 2005) so it is important to maintain a blood supply to this organ. The general decrease in haemolymph flow is the opposite to the pattern occurring in feeding alone (Fig. 2); thus digestion must be slowed, prioritizing blood flow for other processes. This assertion is substantiated by an overall decrease in whole animal protein synthesis in shore crabs in 3 kPa hypoxia (Mente, 2003; Mente et al., 2003). Since protein synthesis is an energetically costly process, accounting for over 50% of oxygen uptake, then a decrease in protein synthesis may be vital to the reduction of a hypoxic crabs energy budget (Mente, 2003). Most notably during hypoxia, a steady decline in protein synthesis occurs in hepatopancreas 2 h after feeding (Mente, 2003). This time period correlates closely with decreased blood flow to this organ (Figs 4C, 6C). There was also a concomitant decrease in flow through the anterolateral arteries (Fig. 4B), which supply the foregut region. Decreasing oxygen levels modulate foregut contraction causing uncoupling of gastric and pyloric rhythms and a slowing of food filtering in the pyloric stomach (Massabuau and Meyrand, 1996; Clemens et al., 1998). This response is opposite to the sea bass *Dicentrarchus labrax,* which maintain gut blood flow during hypoxia and is thus committed to digestion following feeding (Axelsson et al., 2002). It appears that *Cancer magister* can delay digestion after feeding, sparing oxygen for other systems.

In contrast to the present observations, Legeay and Massabuau (1999) reported a *calculated* increase in blood flow (measured using the Fick principle), facilitating oxygen delivery in postprandial *Carcinus maenas* in hypoxia. Measurement of blood flow using the Fick principle involves disturbing the animal and does not record instantaneous changes in flow (Airriess and McMahon, 1994). Pulsed-Doppler measurements used here have an advantage, they allow second by second changes to be monitored in untethered, undisturbed animals (Airriess et al., 1994). In the Dungeness crab, since haemolymph flows (and hence cardiac output) decreased after the crabs fed (Fig. 4B–E), alterations in cardiac output cannot account for increased oxygen delivery. The benefit of decreased cardiac output would be a higher haemolymph residence time in the gills, coupled with an increased ventilation rate (Figs 3D, 5D) this could allow a higher saturation of the branchial excurrent haemolymph, as well as an increased oxygen extraction from the circulating haemolymph (Larimer, 1964). Other mechanisms could be used to compensate for changes in cardiac parameters. Ventilation rates showed additivity of effects, increasing in response to both food intake and hypoxia, which would help in extra oxygen delivery. In addition to physiological changes, internal compensatory mechanisms will take over (McMahon, 2001). Changes in acid-base balance can enhance oxygen carrying capacity of the haemolymph (Legeay and Massabuau, 1999). Oxygen binding affinity of haemocyanin can also be enhanced by increasing lactic acid levels, such as those occurring during mild anaerobic metabolism (Truchot, 1980).

Cardiac and ventilatory parameters rapidly returned to control levels when normoxic conditions were restored. Since no overshoot in physiological parameters was observed, this suggests no oxygen debt was incurred during the hypoxic period (Herried, 1980). The low level of L-lactate production during experiments (Table 1) also indicates anaerobic respiration was negligible. For comparison, during strenuous walking activity, lactate levels in *Cancer magister* increase from 0.7 mmol l^{-1} up to 11.1 mmol l^{-1} (McDonald et al., 1979). The L-lactate levels for *Cancer magister* are much lower than those reported for postprandial green crabs, *Carcinus maenas*, in similar hypoxic conditions (Mente et al., 2003). This suggests that *Cancer magister* is more tolerant of hypoxia than *Carcinus maenas*: *Cancer magister* is commonly found in muddy bays where dissolved oxygen levels drop below 1 kPa (Bernatis and McGaw, unpublished observation), whereas *Carcinus maenas* typically inhabits rocky shorelines. Indeed the P_{crit} for *Cancer magister* lies below 1.3 kPa (I.J.M. and J. L. Bernatis, unpublished observation).

In some instances behavioural regulation may be employed by crustaceans as a means of avoiding hypoxia, thus negating the use of physiologically costly processes (Taylor and Spicer, 1988). For example, in the laboratory, postprandial Dungeness crabs will select higher oxygen regimes to digest food (Bernatis and McGaw, 2004). However, in Barkley Sound, areas of widespread hypoxia are common and behavioural avoidance of such areas may not be possible (Bell et al., 2003), therefore physiological mechanisms will be used.

The present study has shown the nutritional status of an animal can alter physiological mechanisms. Consequently, 'controlled' laboratory experiments, where animals are starved prior to experimentation, may not be wholly representative of physiological processes in the natural environment. This underscores the importance of an integrative approach, studying physiological responses at the organismal level.

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