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Integration of physiological responses of crustaceans to environmental challenge

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Brachyuran crustaceans are useful models for physiological studies because of their intermediate size and since they occupy a spectrum of habitats requiring widely varied behaviour. In this paper we examine the physiological responses to environmental fluctuations, extremes of habitat and consequent behaviours, with special emphasis on the adoption of air-breathing. It is established that metabolic end products such as lactate, intermediates including urate, and monoamine and peptide neurohormones can have important regulatory roles. These include effects on ventilation and heart function, blood perfusion, respiratory gas transport, as well as water and salt homeostasis providing an integrated suite of control mechanisms to regulate responses to environmental or behaviourally induced stress. A separate body of work has long suggested that the regulation of energy metabolism and provision of metabolic fuel for glycolysis is influenced by similar effectors. Most recently, metabolic end-products have been implicated as effectors of behaviour and thereby metabolic state. Thus, there is strong, emerging evidence for integration of physiological control mechanisms at the organismal level. We present new information, both mechanistic and from eco-physiological laboratory simulations, and from field studies of terrestrial crabs, that strengthens and extends the scope of this integration. Branchial chamber ventilation, cardiovascular function, relative perfusion of gills *v.* lungs, gas transport in the blood, the mobilisation of energy reserves, ion transport and water balance are all apparently influenced by similar messengers which coordinate and optimise these functions to meet specific requirements.

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Introduction

The integration of physiological function is essential to the lives of all animals. To accomplish the most seemingly simple process a large number of physiological control mechanisms must be regulated in a manner consistent with the current demands placed on an organism. The integration of physiological processes must be sufficiently sophisticated and plastic to respond to a range of behavioural and environmental challenges. Loss of regulation, usually by failure of part of the integrated response under extreme conditions, leads to increasing physiological disturbance and ultimately death.

Crustaceans provide an important and valuable study model for investigation of regulation and integration in physiology. These animals occupy a large range of available habitats, from abyssal ocean to montane terrestrial, and thus exhibit a plethora of adaptations to environment. The central nervous system (CNS) is sophisticated enough to allow generation of general animal models but discrete enough to facilitate experimentation. Similarly, the complex but accessible nature of the respiratory and the cardiovascular systems encourages study of their control mechanisms and integration in responding to stress. Amphibious and terrestrial crustaceans possess both lungs and gills, permitting investigation of physiological integration with twin gas exchange organs and during the transition between water and air-breathing. Crustaceans are special amongst the invertebrates in that energy metabolism during anaerobiosis is similar to that of vertebrates during stress and results in lactacidosis. They possess a sophisticated respiratory gas transport pigment, regulated by a suite of modulators. The transition from seawater to fresh water, and from water to land, requires significant adaptations and changes in the control of water and salt balance. The ideal nature of crustaceans for experimental physiology notwithstanding, this is perhaps the most eco-physiologically diverse and rewarding group of animals available to the comparative and evolutionary physiologist.

The objective of this review is to illustrate by example the wealth and the breadth of information available on physiological control in crustaceans. Furthermore, to go beyond this to illustrate how these disparate data might be brought together to give a more complete picture of (a) how these physiological responses can be integrated with respect to both environmental and behavioural challenges to physiological processes, and (b) how much more must yet be determined. Future work clearly must integrate biochemistry, cell biology and ecology into the physiology of the animals, some directions to which are outlined in this review.

Integration of the control of physiological responses requires a range of receptors (external and internal) via which signals can be transduced to a variety of diverse end effects. The CNS is, by definition, paramount in physiological integration but a detailed examination of the CNS is outside the scope of this review. The interface between primary (intercellular) and secondary (intracellular) messengers in cell-communication systems is a pivotal aspect to address. This review is thus primarily concerned with the suite of physiological mechanisms which respond to, and are potentially controlled by, signalling molecules transported in the haemolymph and second messenger cascades within target cells (Figure 1). Among the putative primary messengers are biogenic neuroamines and peptide hormones. However, intermediate and terminal metabolites are increasingly appreciated to be

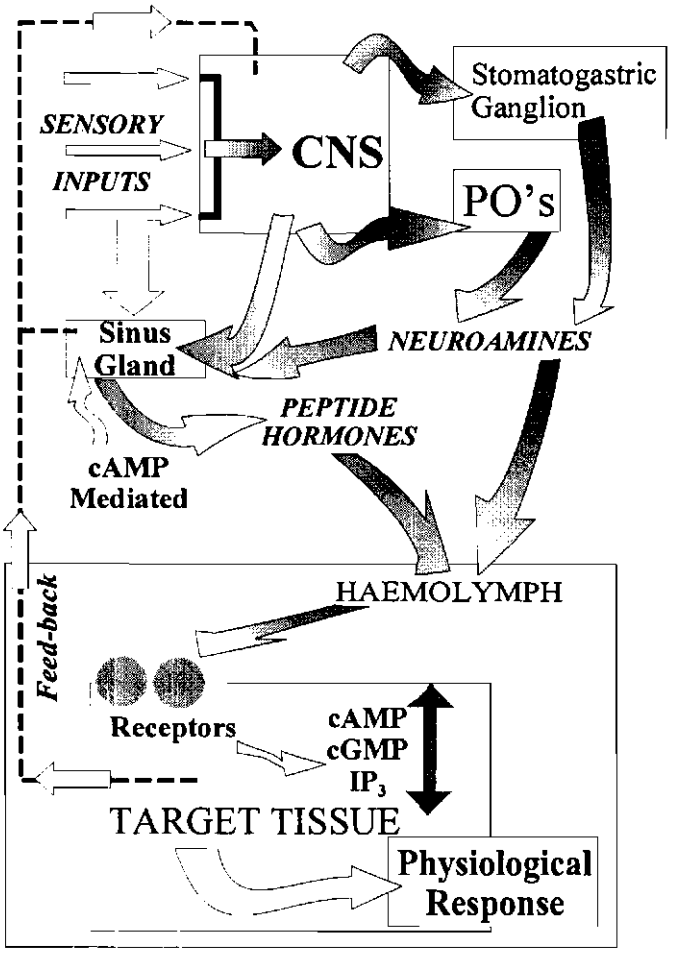

Figure 1 Sensory input from either internal or external receptors is integrated within the CNS. Primary messengers (monoamines and peptides) are released from the pericardial organs (POs) and stomatogastric ganglion (STG) complex into the haemolymph. The sinus gland similarly releases peptide hormones, either under control of the CNS or modulated by circulating neuroamines. Current hypotheses suggest that receptors associated with the Y- and X-organs of the eyestalk system are linked to cAMP 2nd messenger systems. A variety of target tissues including the heart, arterial valves, ventilatory muscles, branchial ATPases, locomotory muscles, fuel stores and the haemocyanin are either affected directly or possibly *via* local nervous receptors and transmission.

important physiological signals. In addition, the concept that respiratory gases function as signals controlling and integrating metabolism and physiological responses in crustaceans is examined in some detail.

Contemporary reports, taken collectively, suggest that a relatively small suite of messengers regulates and integrates a wide variety of physiological processes. The paramount question is, how can this be? How do crustaceans generate specific responses to the same, widely dispersed messenger molecules in separate systems? How are these systems modulated to respond variably but in concert? For example, groups of modified nerve terminals in crustaceans, including the pericardial organs, release biogenic amines and peptides, and the stomatogastric nervous system, can respond to these same biogenic amines. Other neurohaemal structures, in the eyestalk for example, are suggested to be responsive to neuroamines and to release complexes of peptide hormones. Despite the volume of study the suggestion that intracellular concentrations of cAMP, cGMP and IP_3 are modified by activation of G-protein linked receptors, thus regulating cellular responses to neuroamines and peptides, remains undemonstrated in all but a few cases.

Another level of integration of physiological function, which will be considered in some detail in the relation to metabolism, oxygen delivery and thermoregulation, involves the role of metabolic intermediates and end-products as intercellular messengers. This is a conceptually very different system, where compounds such as glucose, urate and lactate arising from metabolic processes can influence other physiological and behavioural response systems—an 'autoregulation' of sorts.

Experimentally, hypoxia, exercise, and changes in temperature and salinity are frequently applied challenges and, therefore, are widely used in interpretation of physiological capability. The literature provides material on the regulation of respiratory gas exchange, modification of cardiovascular responses, the provision of metabolic fuels, the maintenance of salt and water balance and the inter-dependence of physiology and behaviour. Additionally, circadian and circannual changes are superimposed on these as are the demands of reproduction. This review draws on examples of each, including previously unpublished work, and attempts to delineate some of the required but as yet wanting information.

Neuroendocrine regulation

There is a large body of information available on the neuroendocrine pathways of physiological regulation in crustaceans. One of the more developed knowledge bases exists for the cardiorespiratory system, including heart function [whole heart (McGaw, Wilkens, McMahon & Airriess 1995; Wilkens, Kuramoto & McMahon 1996), cardiac ganglion (Berlind 1989; Cooke 1988), contractile properties (Wilkens *et al.* 1996], haemolymph redistribution mechanisms (Kuramoto & Ebara 1984, 1989; Wilkens 1997)], aorto-arterial valves (Wilkens, Davidson & Cavey 1997), venous valves (Taylor & Taylor 1986), respiratory gas uptake and transport in the haemolymph (Morris 1991). Much of this work has been done on relatively well-defined models, although there is often a gap between investigations of different levels of control since various laboratory groups historically and inevitably specialise in different preparations and species. There has been a strong tendency to assume that mechanisms functioning in one species or subgroup will also be present in other less-studied species and while not necessarily wrong, such generalisations should be treated with caution since pronounced species-specific responses to neurohormone signals do occur.

Arguably one of the best studied and 'classical' endocrine control systems in the Crustacea involves the eyestalk sinus glands (Figure 1). These glands release a variety of peptide hormones such as crustacean hyperglycaemic hormone (CHH), gonad inhibiting hormone, moult inhibiting hormone and red pigment concentrating hormone (Keller 1992) which are primarily concerned with growth and reproduction. CHH is thought to be the chief regulator of metabolic fuel availability in crustaceans, and the release of this peptide from the sinus glands is apparently modulated by the biogenic

monoamines (Lüschen, Willig & Jaros 1993; Kuo, Hsu & Lin 1995; Sarojini, Nagabhushanam & Fingerman 1995). The numerous peptide modulators of reproductive state will not be considered in detail here although a strong integrating influence of these compounds seems likely, for example in the context of the seasonal nature of locomotory energetics of reproductive migrations.

Neurohormones, amine- and peptide-signalling molecules released into the haemolymph from neurosecretory neurons, are stored in and released from, for example, the pericardial organs (PO). Substances released from the POs include 5-hydroxytryptamine (5-HT), octopamine (OA), dopamine (DA), proctolin (PR), crustacean cardioactive peptide (CCAP) and several FMRF-amide related peptides (Beltz & Kravitz 1986; Keller 1992). The POs consist of numerous neurosecretory terminals formed from the peripheral ramifications and anastomoses of the second dorsal roots (segmental nerves) of the thoracic ganglion (Alexandrowicz 1953) and can be stimulated both electrically and pharmacologically to release their products (Cooke & Sullivan 1982). The POs are situated on the inner, lateral walls of the pericardial sinus, spanning the openings of the branchiopericardial veins and thus bathed by venous haemolymph returning to the heart. Their capacity to produce and store cardioactive amines and peptides means the POs of decapod crustaceans are well suited for a role in neurohumoural coordination of physiological function (Figure 1). Included in this capacity for physiological coordination is the possibility that amine or peptide neurohormones released from the POs, acting through receptors in the central nervous system (below), might provide feedback regulation of tonic levels of their own circulating concentrations.

The stomatogastric ganglion (STG) responds to monoamines as well as electrical stimulation, resulting in intracellular cAMP production and transport in the neurites of the STG, and ultimately electrical activity in the ventral nerves (Figure 2; Hempel, Vincent, Adams, Tsien & Selverston 1996). The nerves of the STG are associated with the oesophageal ganglia (Massabuau & Meyrand 1996), again illustrating the tight coupling of the CNS and neuroendocrine regulatory pathways. The location of the STG in the lumen of the anterior aorta means it will receive circulating hormones from the POs and sinus glands (Christie, Skiebe & Marder 1995). Thus there is a demonstrated capacity for various parts of the nervous system to communicate *via* haemolymph-borne messengers and feed back into specific fibres and the CNS. For much of our discussion, the origin of these substances is of little importance but the fact that they are released into the haemolymph from some neurohaemal organ in response to input from the CNS, whence they can be transported to distal target tissues and organs, is of crucial consideration. Equally important, the crustacean open circulatory system allows neurohormones released into the haemolymph to be brought into proximity with all tissues in the animal. This allows organism-wide coordination of physiological function, but also suggests the existence of mechanisms at the tissue and cellular level capable of modifying local responses to circulating neurohormones (if one assumes a general response is to be avoided in most instances).

The types of challenge that might bring about a release of

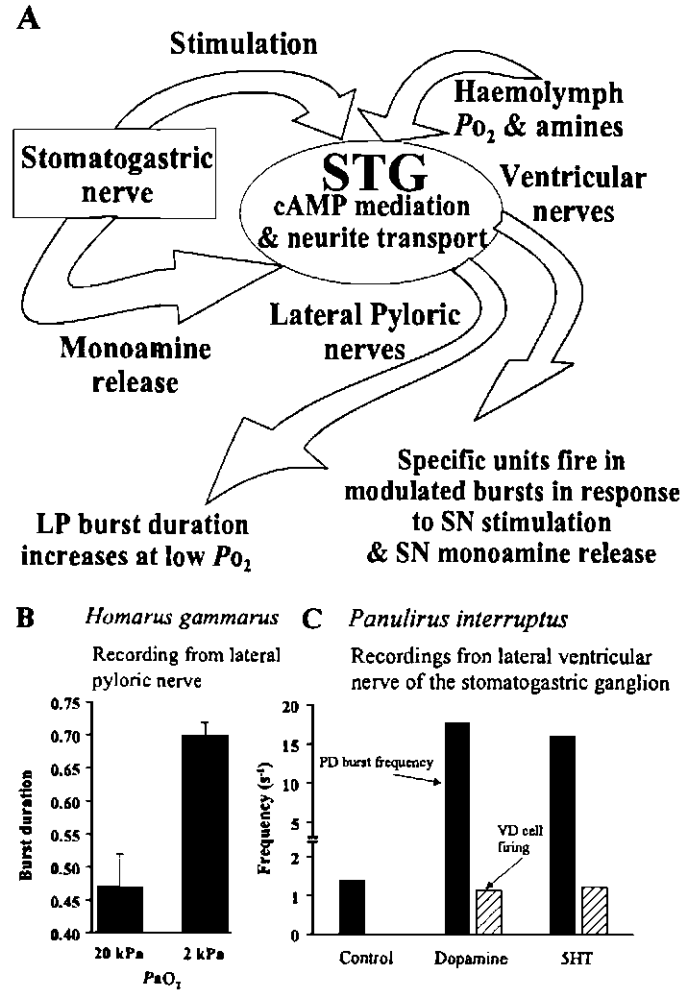

Figure 2 (A) The stomatogastric ganglion (STG) of crustaceans receives electrical input from the stomatogastric nerve, which also releases biogenic monoamines. Dopamine and 5-HT elicit increased cAMP levels in different cells of the STG. Elevated cAMP causes intracellular transport of cAMP within affected neurites. **(B)** Monoamine stimulation of the STG promotes increased electrical activity in at least two different units of the ventricular nerves. Located within the arterial lumen the STG will be sensitive to circulating monoamines released remotely from the stomatogastric nervous system (after Massabuau & Meyrand 1996). **(C)** Reducing the PO_2 of the haemolymph bathing the STG also promotes changes in bursting patterns, but of specific cells of the lateral pyloric nerve. Thus the STG is sensitive to haemolymph-borne monoamines, input from the nervous system and to internal hypoxia, and responds by specific motor directed outputs and likely feed-back to the CNS (after Hempel *et al.* 1996).

neurohormonal modulators into the haemolymph include environmental hypoxia, the functional (internal) hypoxia associated with exercise or exposure to the inappropriate respiratory medium (e.g. air exposure of water breathers), changing energetic requirements, disturbance to water balance/ion-homeostasis and changes in temperature.

Sites of action of the monoamine and peptide neurohormones are numerous. They include the myocardium, cardiac ganglion, cardioarterial valves, aorto-arterial valves, haemocyanin, scaphognathites, sinus glands, glucose storage sites in muscle and other organs, stomatogastric ganglion, branchial

ion pumps and reproductive organs to list a few. Obviously there must be neurohormone receptors in each of these target tissues and organs, although these are not yet well known in crustaceans, the impermeant nature of the neurohormones dealt with here requires membrane-bound receptors as intermediaries to the subsequent activation of intracellular signalling pathways. There is a great deal of published work on receptor types, localisation and second messenger activation in insects, and receptors for many of the same signalling molecules (particularly the amines) also exist in vertebrates. Comparable work on receptor classification has yet to be carried out on crustaceans.

Of the biogenic amines stored in crustacean POs, the indolamine 5-HT and the phenolamine OA are known to be released in suffcient quantity *in vivo* to trigger cardiovascular responses in some decapods (Florey & Rathmayer 1978; Sullivan 1978; Livingstone, Schaeffer & Kravitz 1981). The catecholamine DA is also found at a high concentration in the POs of several crab species (Cooke & Goldstone 1970). All three of the pericardial monoamines possess excitatory properties in studies of isolated decapod hearts (Beltz & Kravitz 1986; Wilkens *et al.* 1996), but each has markedly different effects, for example, on the electrical activity of the stomatogastric ganglion (Hempel *et al.* 1996). Neurogenic peptides are also released from the POs and can have different excitatory effects, for example, on the cardiac ganglion (Lemos & Berlind 1981). The peptides proctolin, F1 and F2 markedly influence heart rate and stroke volume in decapods (McGaw *et al.* 1995).

Many of the actions of 5-HT on cardiovascular targets in decapods are likely to be brought about *via* a cyclic AMP (cAMP) mediated second messenger system (Battelle & Kravitz 1978; Lemos & Berlind 1981). Lemos & Berlind (1981) concluded that the involvement of cAMP in the cardiac ganglion (CG) response to 5-HT is probably much lower than in the myocardium, since the increase in cAMP in the CG was smaller. Experiments investigating the effects of cAMP on ion transport have suggested that this intracellular messenger is also involved in stimulation of Na^+-uptake by the gills of *Callinectes sapidus* (Lohrmann & Kamemoto, 1987).

Cooke & Goldstone (1970) demonstrated that 5-HT and DA are sequestered in separate nerve terminals within the POs of brachyuran crabs. DA is produced in cells of the POs from its precursors, L-dihydroxyphenylalanine (L-DOPA) and tyrosine (Barker, Molinoff & Kravitz 1972) but is stored in smaller quantities than 5-HT (25–33%) in the POs of both *Panulirus* (Sullivan, Friend & McCaman 1976) and *Carcinus* (Cooke & Sullivan 1982). DA acts primarily on the small pacemaker cells of the crab (*Portunus sanguinolentus* and *Podophthalmus vigil*; Miller, Benson & Berlind 1984). The threshold for DA effects on the isolated CG is approximately 10 nmol.l^{-1} and, interestingly, at near-threshold concentrations, CG burst duration tends to increase in conjunction with a decrease in burst frequency. DA has been shown to increase intracellular cAMP levels (Hempel *et al.* 1996) and also to enhance neuromuscular communication by either pre- or post-synaptic mechanisms (Lingle 1981). A role in modification of the courtship behaviour of *Callinectes sapidus* is also suggested for DA, in conjunction with proctolin (Wood, Gleeson & Derby 1995). Although DA has obvious excitatory effects on the function of the stomatogastric system (Lingle 1981; Harris-Warrick, Coniglio, Barazangi, Guckenheimer & Gueron 1995), it appears to have less striking and more ambiguous influences on the cardiovascular system at likely physiological concentrations. There are indications that DA exerts its excitatory effects on branchial Na^+/K^+-ATPase *via* changes in intracellular cAMP levels (Morris & Edwards, 1995; see below), and that increases in stomatogastric ganglion activity are brought about by DA-mediated decreases in membrane K^+ permeability (Harris-Warrick *et al.* 1995).

Synthesis of OA and DA from tyrosine occurs in separate cell types (Barker *et al.* 1972). Experimental release of OA from the POs can be induced by either electrical stimulation (Sullivan, Friend & Barker 1977) or incubation in very high K^+ (Evans, Kravitz & Talamo 1976) and is strongly Ca^{2+}-dependent (Sullivan *et al.* 1977). Much more is known about the actions of OA as an invertebrate neurohormone than about the equivalent roles of 5-HT or DA, owing in part to its ubiquity in arthropod neuromuscular systems (Nathanson & Greengard 1973, Florey & Rathmayer 1978; Benson 1984; Morton 1984; Rane, Gerlach & Wyse 1984; Whim & Evans 1988; O'Gara & Drewes 1990). In the isolated CG of the crab *Portunus sanguinolentus*, OA caused a decrease in burst frequency together but with increased burst duration at concentrations up to 1 μmol.l^{-1} (Benson 1984). At higher concentrations (5-10 μmol.l^{-1}) this inhibition of burst frequency was overshadowed by excitation of the CG and higher than control rates of bursting in some cases. The action of OA on the CG of crabs is thought to be a result of inhibition of the motor neurones since bursting continues unabated in the pacemaker cells. In the lobster *P. japonicus*, the burst frequency of the CG pacemaker cells is inhibited by OA under conditions of minimal myocardial stretching (Kuramoto & Ebara 1991) but at high internal pressures it caused an increase in burst frequency. Elevated cAMP levels occurred in the myocardium of both crab and lobster in response to OA treatment (Sullivan & Barker 1975; Battelle & Kravitz 1978).

Proctolin, a pentapeptide hormone found in the pericardial organs and in the sinus glands, as well as within the input of the stomatogastric ganglion (Keller 1992; Christie *et al.* 1995) has excitatory effects on isolated crustacean hearts, increasing both the rate and force of contraction (McGaw *et al.* 1995; Wilkens *et al.* 1996). However, proctolin is cardioinhibitory when applied to intact animals (McGaw, Airriess & McMahon 1994a; McGaw *et al.* 1995), supporting the suggestion of modulation of peptide hormone effects by the CNS or other circulating neurohormones. Proctolin has pronounced effects on the cardioarterial valves of decapods, causing strong rhythmic contraction of the sternal valve (Kuramoto & Ebara 1989; Kuramoto, Wilkens & McMahon 1995). When applied subsequent to red pigment concentrating hormone to the stomatogastric nervous system of the lobster *Panulirus interruptus*, proctolin activates the cardiac sac motor pattern (Dickinson, Fairfield, Hetling & Hauptman 1997) but has no effect when applied alone in this preparation. Reproductive and swimming behaviours appear to be altered by proctolin in *C. sapidus* (Wood 1995). The mode of action of proctolin at target cells is not known in crustaceans, but in insects the peptide is thought to elevate intracellular

(Ca^{2+}) *via* the production of inositol 1,4,5-triphosphate (IP_3) and inositol 1,3,4,5-tetrakisphosphate (IP_4) (Hinton & Osborne 1996).

The physiological responses of crustaceans to experimental infusion of the pericardial monoaminos implies considerable complexity in the interaction of various control systems. With potential targets in the CNS, in the various neurohaemal organs and in distributed effector systems, the modulation of neurohormone activity by other neuromodulators is probable, and results in a multi-tiered system with potential feedback regulation at several possible levels of control. In addition, it seems very likely that most effects of neurohormones are modulated or overridden by neural regulatory mechanisms in whole animals, such that responses *in vivo* are quite different to those recorded *in vitro*. The most effective dosages of monoamines utilised experimentally are probably much higher than those required for activity under natural conditions. Lower circulating levels of effector substances would allow finer adjustment of physiological performance to meet specific metabolic requirements, but such fine adjustments may not be detectable in investigations which rely on the analysis of grouped data from many individuals.

Oxygen demand, availability and delivery

The cardiovascular system is responsible for the conduction of haemolymph and the transport of O_2, thus the optimisation of the circulatory system is important in maximising O_2 delivery for minimum cost. The multitude of effects of the pericardial neurohormones on the decapod crustacean heart, taken together with the proximity of the pericardial organs and heart, strongly suggest an important role in cardiovascular regulation and respiratory gas transport for the POs. In addition, the concentrations of neurohormones released from the POs must be highest at the heart, before dilution in the circulating haemolymph has occurred. Electrical stimulation of the segmental nerves of *Panulirus interruptus* brings about the sudden, quantal release of enough pericardial 5-HT to elevate the circulating concentration of this amine to 1.2 nmol.l^{-1} (Sullivan 1978); the concentration of 5-HT at cardiac targets must be many fold higher.

The regulation of heart beat frequency and cardiac stroke volume by the Crustacea is dependent on the unique innervation of the neurogenic crustacean heart. The cardiac ganglion (CG), consisting of nine neurones arranged in a characteristic pattern on the inner dorsal wall of the heart (Alexandrowicz 1932), generates rhythmic burst discharges which induce myocardial contraction. In addition to nervous regulation *via* synaptic input from the cardioaccelerator and cardioinhibitor nerves, which arise from the central nervous system, the frequency of CG bursting appears to be altered by neurohormonal agents (Guirguis & Wilkens 1995).

In decapod crustaceans, seven major systems of arteries originate at or near the heart and supply haemolymph to distinct body regions and organs (Pearson 1908). The exact origin of each artery is variable among sub-groups, but the same seven vessels occur in all species and deliver haemolymph to the same morphological regions (Miller 1895; Pearson 1908; Baumann 1921; Belman 1975). The anterior aorta is a median dorsal vessel which supplies haemolymph to the eyestalks and their associated sinus glands, and to the antennules and the supraoesophageal ganglion. The paired anterolateral arteries arise lateral to the anterior aorta and supply haemolymph to the testes, digestive gland, integument and antennae. Haemolymph delivered *via* branches of these vessels also perfuses the supraoesophageal ganglion, the optic ganglia and the oculomotor muscles (Baumann 1921; Sandeman 1967). The hepatic arteries, too, are paired vessels originating slightly medial and ventral to the anterolateral arteries and delivering haemolymph to the gut and digestive gland. The sternal artery is a large non-paired vessel arising at the ventral side of the heart. This vessel runs anteroventrally before branching to form the ventral thoracic artery which supplies haemolymph to the cephalic appendages, the anterior portion of the ventral nerve cord and the digestive gland; and the ventral abdominal artery, which delivers haemolymph to the posterior region of the ventral nerve cord and the ventral side of the abdominal musculature. The thoracic ganglion of the CNS is perfused with haemolymph from branches of the pedal arteries. These vessels arise from the ventral thoracic and ventral abdominal arteries and also deliver haemolymph to the pereiopods. The median posterior aorta originates in close proximity to the sternal artery, and supplies haemolymph to the musculature of the abdomen and pereiopods.

Until recently, the absence of vasoconstrictive musculature in the walls of decapod crustacean arteries was considered indisputable (Maynard 1960; Shadwick, Pollock & Stricker 1990). Striated muscle has now been described, however, in the posterior aorta of the prawn *Sicyonia ingentis* and the lobster *Homarus americanus* (Martin, Hose & Corzine 1989; Wilkens *et al.* 1997). In *H. americanus* vascular resistance to haemolymph flow can be modulated by a variety of neurotransmitters and neurohormones (Figure 3; Wilkens 1997) and in the abdominal aorta these changes in resistance are thought to be brought about by valves at the origin of lateral

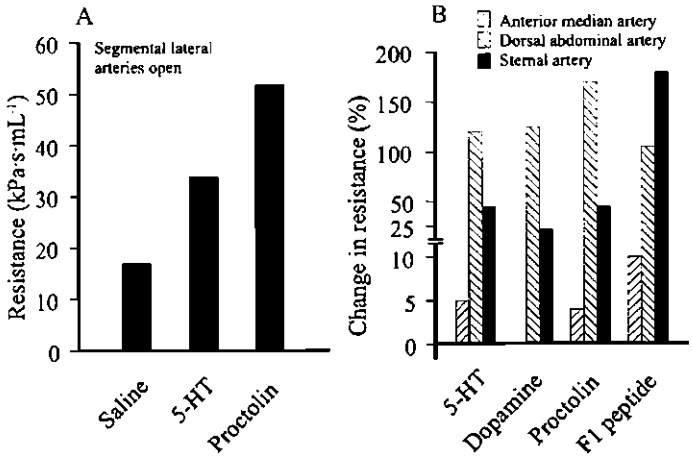

Figure 3 Effects of pericardial neurohormones on arterial resistance to haemolymph flow in the lobster *Homarus americanus* (after Wilkens 1997). **(A)** Increased resistance to flow through the abdominal aorta with the segmental arteries open in response to 5-HT and proctolin. **(B)** Relative increases in resistance to haemolymph flow through vessels supplying diverse regions in response to biogenic amines and peptides. These changes in resistance occurred down-stream from the heart and cardioarterial valves, suggesting other active mechanisms to control the redistribution of haemolymph pumped by the heart.

arterial branches. Work on *Homarus* and *Panulirus* has shown muscular, innervated semilunar valves are present at the origin from the heart of each of the anterior arteries; the sternal artery and posterior aorta share a common valve in *Panulirus* (Alexandrowicz 1932; Kuramoto & Ebara 1989; Kuramoto, Hirose & Tani 1992). Each of the valves receives innervation *via* fibres originating from the CNS (Alexandrowicz 1932), and can contract, in isopods, in response to neural (Kihara & Kuwasawa 1984; Kihara, Kuwasawa & Yazawa 1985) and, in lobsters, to neurohormonal stimulation *in vitro* (Kuramoto & Ebara 1984, 1989; Kuramoto *et al.* 1992). Independent changes in haemolymph flow through separate arterial systems have also been demonstrated *in vivo* in response to enforced activity, environmental hypoxia, emersion, salinity and temperature changes, and neurohormone treatment (Airriess & McMahon 1992, 1994, 1996; Bourne & McMahon 1989; DeWachter & McMahon 1996a,b; McGaw *et al.* 1994a; McGaw & McMahon 1996; McMahon 1992; Reiber 1994, 1995); therefore, it appears that the cardioarterial valves may play an active role in the diversion of haemolymph from one arterial system to another in response to changes in local tissue demand.

The monoamine (5-HT, OA, DA) and peptide (PR, F1, F2) neurohormones all increase the beat frequency of decapod crustacean hearts in numerous *in situ* and isolated heart preparations (Florey & Rathmayer 1978; Wilkens & Mercier 1993; Wilkens *et al.* 1996). However, differences between the effects of the pericardial peptides *in vivo* and *in vitro* indicate that comparisons among studies must be made with caution (McGaw *et al.* 1995). Concentrations of neurohormones lower than those employed in experimental manipulations might be more effective when released from the pericardial organs under natural conditions owing to the close proximity of target receptors in the cardiac ganglion and myocardium.

At the cardioarterial valves, 5-HT causes hyperpolarisation of all valve muscles, leading to relaxation of the valves and unimpeded haemolymph flow. OA relaxes the muscle of the anterior cardioarterial valves whilst causing the muscle of the posterior valve to contract (Figure 4a; Kuramoto & Ebara 1984), so is potentially capable of redirecting haemolymph flow toward cephalic structures in intact animals. DA hyperpolarises the posterior cardioarterial valve muscle in *H. americanus* (Kuramoto *et al.* 1992), allowing maximum haemolymph flow through the sternal artery and posterior aorta. Proctolin causes depolarization and contraction of all of the cardioarterial valves (Figure 4a) and also induces rhythmic contractions of the posterior valve (Kuramoto & Ebara 1984, 1989) although the latter effect can be overcome by inhibitory nerve stimulation (Kuramoto *et al.* 1995). Apparently contradictory effects on arterial haemolymph flow *in vivo* and in different species (Figure 4b) as well as down-stream modulation of arterial resistance (Wilkens 1997) clearly indicate that the physiological role of the cardioarterial valves and other mechanisms of haemolymph redistribution need considerable further investigation.

The spontaneous alternation of haemolymph delivery *via* the sternal artery and anterolateral arteries of control animals which occurs between brief periods of cardiac arrest (McGaw, Airriess & McMahon 1994b) may be an energy-saving mechanism supplementary to the well-defined periods

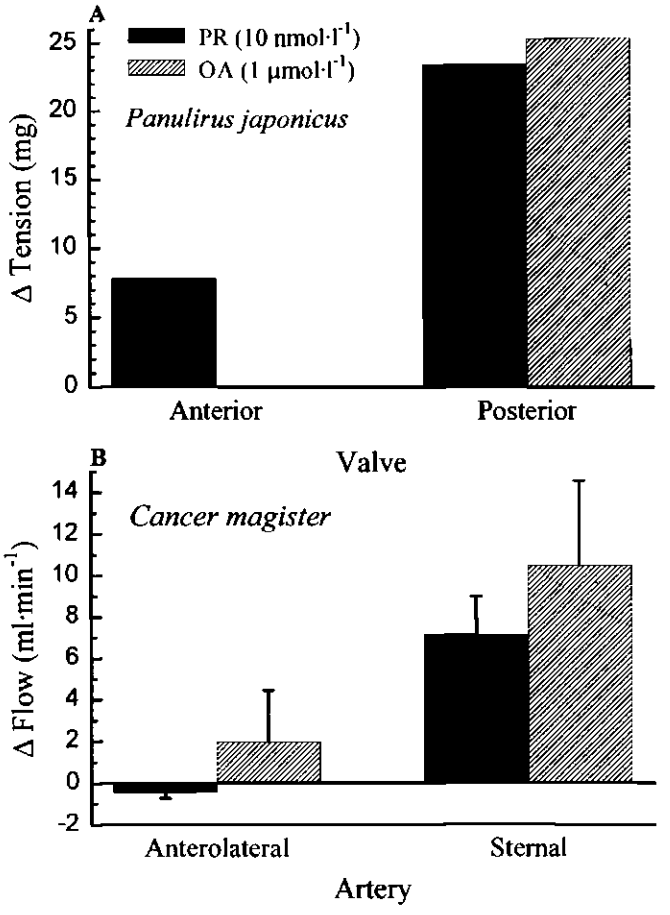

Figure 4 (A) In *Panulirus japonicus* proctolin (PR) and octopamine (OA) increase tension production in the muscle of the posterior cardioarterial valves. Proctolin also increases tension in the anterior valve muscle while octopamine has no effect (after Kuramoto & Ebara 1984). **(B)** Haemolymph flow through the anterolateral arteries of *Cancer magister* is decreased slightly following proctolin infusion but is increased by octopamine. Both neurohormones increase flow through the sternal artery (after Airriess 1994 & McGaw *et al.* 1994a).

of apnoea and cardiac arrest which have been described for decapods resting in well-aerated seawater (Burnett & Bridges 1981). Cyclic patterns of arterial and venous perfusion are seen in the terrestrial brachyuran *Gecarcoidea natalis*, and changes in these patterns are observed following treatment with pericardial monoamines (Figure 5). Such cyclic changes and diversions in flow suggest sensors and control systems responding to thresholds in O_2 availability. The reconfiguration of pattern generators in the stomatogastric ganglion by neurohormones and oxygen (Hempel *et al.* 1996; Massabuau & Meyrand 1996; Figure 2b) provides one mechanism by which peripheral and haemolymph O_2 changes can be transduced to changes in perfusion.

In responding to environmental hypoxia, it is crucial that uptake of O_2 from the ventilatory medium be maintained without compromising off-loading at the tissues. General responses to hypoxic exposure are well known in Crustacea, and much work has been devoted to understanding the ability of aquatic decapod crustaceans to maintain respiratory function independent of environmental O_2 partial pressure above certain, species-dependent, critical levels. As a result of the

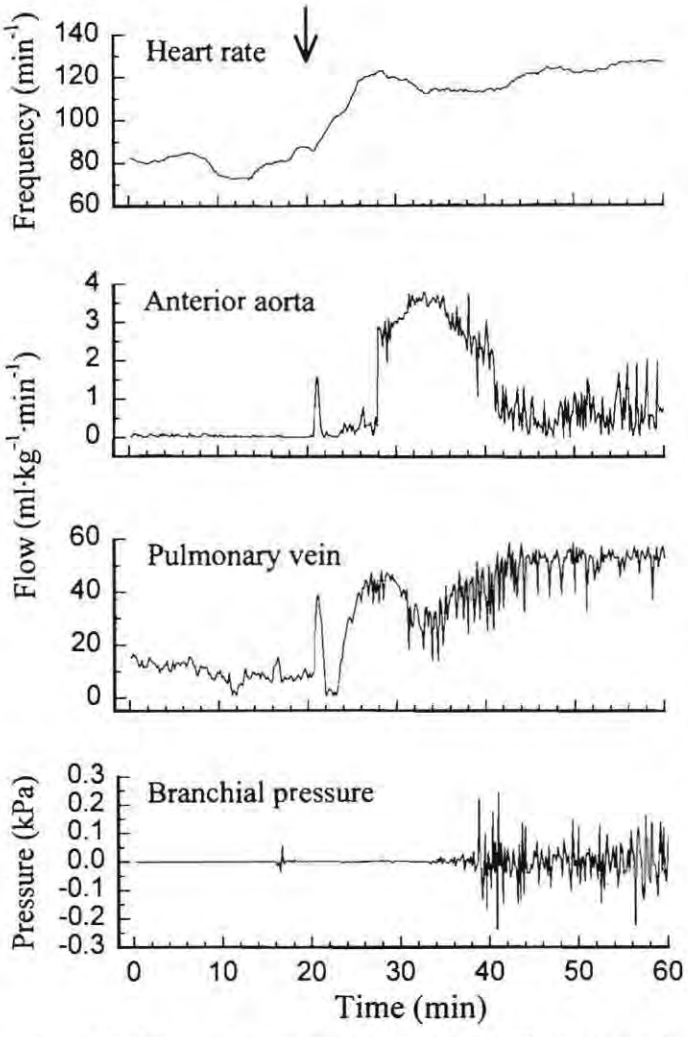

Figure 5 In *Gecarcoidea natalis* pericardial 5-HT infusion (1 µmol. l^{-1}; arrow) causes an increase in heart beat frequency, branchial chamber pressure and haemolymph flow through the anterior aorta and pulmonary veins. Superimposed on the changes in haemolymph flow are spontaneous fluctuations which become more pronounced after 5-HT treatment. Note that flow through the anterior aorta declines after approximately 10 min whilst heart rate and flow through the pulmonary vein remain elevated. Haemolymph flow was measured using a pulsed-Doppler flowmeter, heart beat frequency was determined from the mean duration between systolic flow peaks.

much lower O_2 solubility in water than air, water-breathing species must ventilate their gas exchange organs at a higher rate than air breathers in order to maintain comparable arterial O_2 levels (e.g. Morris 1990). Along with the greater viscosity of the respiratory medium, this presents special problems to aquatic crustaceans faced with environmental hypoxia which must expend as much as 30% of resting oxygen consumption for ventilation of the gills (Burggren & McMahon 1983; Wilkens, Wilkes & Evans 1984) and to these species matching of gill ventilation and perfusion is critical.

In general, gill ventilation increases as PO_2 declines below normal environmental levels and oxygen consumption (MO_2) is maintained although heart beat frequency may decline as O_2 delivery to the myocardium is compromised (Wilkens 1993). Along with a decrease in heart beat frequency there is an increase in cardiac stroke volume, allowing cardiac output to be maintained at near-normal levels during acute hypoxic exposure (Airriess & McMahon 1994; Reiber 1995). Increased perfusion of the sternal artery of crabs (Airness & McMahon 1994) and crayfish (Reiber, McMahon & Burggren 1992) augments the supply of haemolymph to the scaphognathites, supporting a 50–200% increase in pumping frequency (Figure 6a). Below the critical partial pressure of O_2 (Pc) the work of the scaphognathites and heart requires more energy than can be harnessed using aerobic metabolism; MO_2 can no longer be maintained and the venous O_2 reserve is depleted. Anaerobic metabolism may take over in some species although the scope for this, as well as the ability to maintain oxygen consumption despite environmental O_2 depletion, is highly variable among species (McMahon 1988).

There is some evidence of neurohormonal responses to hypoxia. Of the neurohormonal modulators with cardioactive properties, proctolin is the most likely effector involved in the responses to hypoxia observed in crabs and crayfish. Increases in scaphognathite pumping rate have been reported following administration of this peptide (Wilkens, Mercier & Evans 1985), as have concurrent bradycardia and increased cardiac stroke volume *in vivo* (McGaw *et al.* 1994a; 1995). The shift of a greater proportion of haemolymph into the sternal artery of *Cancer magister* during hypoxic exposure is also

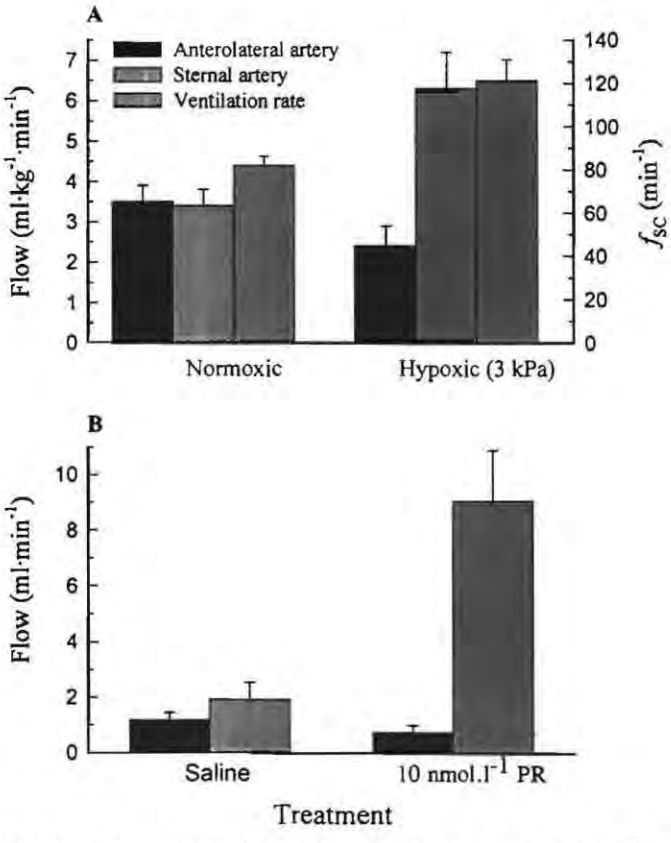

Figure 6 (A) Changes in ventilation rate and haemolymph flow through the anterolateral and sternal arteries of *Cancer magister* as a result of decreased environmental PO_2 (after Airriess & McMahon 1994). **(B)** Haemolymph flow through the anterolateral and sternal arteries was similarly affected by pericardial infusion of proctolin (after McGaw *et al.* 1994a).

seen following proctolin infusion (Figure 6b; Airriess & McMahon 1994; McGaw *et al.* 1994a). Attempts to measure circulating levels of neurohormones in crustaceans using HPLC have met with limited success, owing to the minute concentrations involved and problems in sample purification. The strong circumstantial evidence linking proctolin to the physiological effects of hypoxia would be substantiated if measurements of increased circulating concentration of this peptide during hypoxia could be obtained.

The cardiorespiratory demands imposed by exercise are similar to those experienced during environmental hypoxia, except that the availability of ambient O_2 is not limiting. Therefore, as long as cardiac output can be increased to supply tissue O_2 demand, internal, functional hypoxia does not develop. Increased cardiac output must be brought about by increased heart rate and/or stroke volume; in some cases there may be an increase in both variables (Adamczewska & Morris 1994; DeWachter & McMahon 1996b). Along with increased perfusion of gas exchange structures, there is a corresponding elevation of ventilation rate during exercise. Increased haemolymph supply to the scaphognathites and locomotory appendages *via* the sternal artery is therefore required, and has been demonstrated in two brachyuran species (DeWachter & McMahon 1996; McMahon, Airriess & Airriess 1996) along with a relative decrease in perfusion of other arterial branches. Based on our knowledge of cardiorespiratory modulation it is probable that a single neurohormone is not responsible for the diverse changes in cardiac function, scaphognathite beat frequency and arterial perfusion during exercise. As suggested for environmental hypoxia, proctolin could bring about an increase in scaphognathite frequency, cardiac stroke volume and perfusion of the sternal artery; this, however, does not account for the massive increase in heart rate which occurs during exercise (e.g. McMahon *et al.* 1996). The involvement of more than one neuromodulator, or direct nervous stimulation by the cardioaccelerator nerves of the CNS thus seems likely.

It would be simplistic to believe that haemolymph distribution and thus the transport of O_2 and CO_2 is affected only in times of 'stress' or extreme physiological challenge. A model whereby respiratory gas transport is optimised by the effects of biogenic, cardiovascular-active amines and peptides is seductive to the crustacean physiologist. A finely graded system that can respond, in a sophisticated fashion, to minor changes in respiratory requirements on a routine basis is clearly implicated. For example, the concept of 'sleeping' crustaceans suggests that large and significant changes in mechanisms supporting tissue O_2 provision occur during the transition between various resting conditions. Forgue *et al.* (1992a,b) suggested that haemolymph O_2 was typically very low only in truly quiescent animals, close to the threshold of anaerobiosis. The slightest disturbance stimulated the ventilatory and cardiovascular systems to raise haemolymph O_2 levels. Logically, this is acceptable since low PaO_2 maximises the gradient for diffusion of O_2 into the haemolymph and minimises ventilatory costs. The magnitude of the cardiovascular response will depend on, and be integrated with, haemocyanin O_2 capacity. Important corroboratory data come from the terrestrial Christmas Island red crab (*G. natalis*) which utilises both lungs and gills for air breathing. This species is diurnally active and 'sleeps' at night. In the field, sleeping red crabs direct very little haemolymph through the pulmonary pathway of venous return (Figure 7); but during the day awake, resting, crabs show much more equal perfusion of the pulmonary and branchial circuits. Thus, without any real respiratory challenge large changes in patterns of perfusion occur and must reflect altered priorities of the gills and lungs, presumably to optimise oxygenation of the arterial haemolymph. Putative increases in circulating neuroamines might modulate and integrate the various components of this

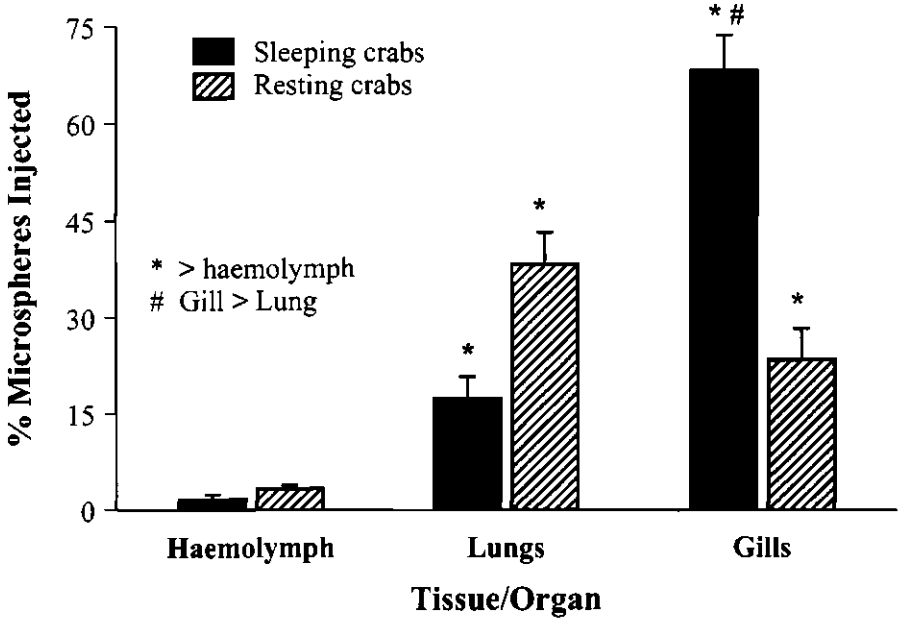

Figure 7 Christmas Island red crabs, *Gecarcoidea natalis*, were injected in the field with ^{57}Co labeled microspheres (15µm; as per Taylor & Greenaway 1984). Sleeping crabs were injected between 4.15 and 4.30 am, 2 h prior to sunrise; the resting crabs at least 3 h after sunrise. The microspheres were allowed to circulate and lodge for at least 20 min after injection (Airriess, C.N. & Morris, S., in prep.). Major changes in the relative perfusion of gills and lungs are obvious when comparing these two quiescent states.

response but there have been insufficient attempts to resolve this, compounded by the very real difficulties in measuring the very small changes in modulator concentrations likely to be involved.

It seems axiomatic that crustaceans must be provided with receptors to control the drive of ventilation and perfusion. Ventilation is primarily O_2 driven in water breathers but there is some evidence to suggest that CO_2 acts as a ventilatory stimulus in air-breathing decapods (McMahon & Burggren 1988; Morris 1991 for reviews). Some evidence points to internal receptors closely associated with the CNS (e.g. Wilkens, Young & DiCaprio 1989). However, oxygen sensitive receptors were described by Ishii, Ishii, Massabuau & Dejours (1989) in the branchiopericardial veins of crayfish suggesting peripheral O_2 sensors drive ventilation, as corroborated by Zinebi, Simmers & Truchot (1990) for *Carcinus maenas*. While Reiber (1997a) makes a similar conclusion, suggesting that branchial O_2 receptors influence cardiovascular responses to hypoxia, he suggests that ventilation responds to more centrally situated receptors. These drives provide the proximal stimulus for neurohormone release and thereby the coordinated response in ventilation, cardiac function and perfusion patterns. Recent pivotal work by Massabuau & Meyrand (1996) implicates centrally mediated drivers and has provided direct evidence of the transduction of haemolymph PO_2 into nervous input to the CNS. Lowering PaO_2 to 2 kPa in lobsters (*Homarus gammarus*), just above the anaerobic threshold, significantly increased the duration of burst impulses in the stomatogastric nervous system (Figure 2b).

That O_2 and CO_2 also act as signals and modulators of respiratory status, prompting responses that optimise their transport, is an important concept. The regulation of haemocyanin transport of O_2 and CO_2 is now understood to be as complex as that of vertebrate haemoglobins. Oxygen transport must be optimised for both loading and unloading, and most frequently has been assessed in crustaceans with respect to either environmental hypoxia or exercise (Morris 1990, 1991; Burnett 1992; Truchot 1992; Morris & Bridges 1994 for reviews). The ventilatory and cardiovascular adjustments to hypoxia (above) serve to match O_2 delivery to demand (i.e. maintain O_2 constant as a signal) but the reciprocal changes in CO_2 markedly influence haemocyanin function and thereby O_2 delivery. This is possible since in normal aerobic metabolism O_2 and CO_2 are linked by respiration and in their transport (Bridges & Morris 1989, review). Thus a hyperventilatory response to environmental hypoxia promotes CO_2 excretion and haemolymph alkalosis, leading to an adaptive increase in oxygen affinity. Since metabolic demand is not otherwise greatly increased the primary effect is to facilitate loading from an O_2-poor environment.

In contrast, during the initial phases of exercise, O_2 demand is increased and CO_2 production elevated promoting a hypercapnic acidosis, reduced haemolymph pH and thereby a lowered Hc-O_2 affinity (Figure 8). However, in these circumstances the environment is most frequently well oxygenated and arterial saturation maintained by hyperventilation; thus, the

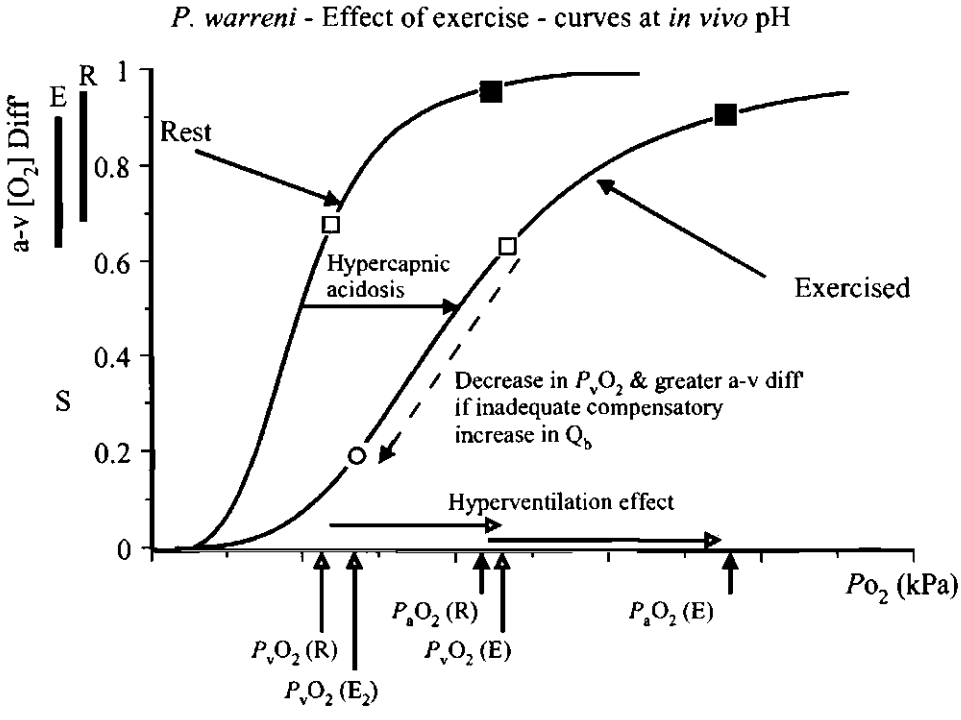

Figure 8 Model for integration of ventilation, perfusion and haemocyanin oxygen affinity during aerobic exercise. Resting (R) P_aO_2 and P_vO_2 lie on the O_2 equilibrium curve such that ~25% of bound O_2 is released to the tissues. A respiratory acidosis induces a Bohr shift and reduces Hc-O_2 affinity. The hyperventilatory response to exercise sustains O_2 loading (P_aO_2) and provides for a higher P_vO_2 (E) and assists in O_2 diffusion into the tissues. The a-v [O_2] difference can be maintained similar to that in resting animals providing cardiac output is increased proportionally. Increased or sustained exercise, or a failure of perfusion, must lead to increased a-v [O_2] difference and thus a decrease in P_vO_2 (E_2). Therefore, while hyperventilation is integrated with Hc-O_2 affinity to maintain loading, the P_vO_2 must be integrated with and dependent on the capacity to increase haemolymph flow and redirect perfusion. Curves for resting and exercised *Potamanautes warreni* (Adamczewska, van Aardt & Morris 1997).

limitations on O_2 supply are primarily at the site of unloading, and facilitated by the lowered O_2 affinity. While this decrease in Hc-O_2 can assist O_2 diffusion into the tissues by raising the P_vO_2, the diffusion gradient and protection of the O_2 venous reserve depend on the extent to which cardiac output can be increased and perfusion pattems optimised. Logically, there must be central O_2 receptors (above), or perhaps CO_2 receptors in air-breathers, that sense respiratory status and initiate the necessary cardiovascular and ventilatory compensations, *via* neurohormone release, matching these to the O_2 and CO_2 transport characteristics of the haemocyanin.

In addition to promoting and co-ordinating mechanical adjustments of the cardiorespiratory system, haemolymph-borne neurohormones are very likely to influence the function of crustacean haemocyanins (Morris 1990, 1991; Morris & Bridges 1994; for reviews). Some monoamines, especially dopamine, act directly on haemocyanin to increase oxygen affinity (Morris & McMahon 1989) and thus provide a reciprocal signal, whereby the Hc-O_2 affinity can potentially be fine tuned for closer integration with ventilation and circulation. There is some suggestion, however, that monoamine binding by haemocyanin may be an indication of transport/enzymatic function of the protein (Morris & McMahon 1989). The bases of integration between cardiovascular function, haemocyanin function and respiration requires careful investigation under realistic ecophysiological circumstances.

As the duration and extent of respiratory challenge increase, either ventilatory compensation or more likely haemolymph perfusion will fail to maintain O_2 delivery at the required rate. Lowered P_aO_2 and especially low P_vO_2 are clear indications that the feedback between O_2 as a signal and O_2 supply has exceeded the capacity of mechanical and biochemical compensations. Decapod crustaceans, at least, possess a further suite of effectors that operate under these circumstances.

Urate has been identified as modulator of Hc-O_2 binding, increasing the affinity for O_2 (Morris, Bridges & Grieshaber 1985, 1986; Lallier, Boitel & Truchot 1987; Nies, Zeis, Bridges & Grieshaber 1992; Zeis, Nies, Bridges & Grieshaber 1992). Urate is an intermediate in the purinolytic pathway to urea and NH_3, and is oxidised by urate oxidase (UO) requiring O_2 as co-substrate. When circulating and tissue O_2 decline, especially during hypoxia the rate of urate catabolism, depending on the affinity of UO for O_2, will slow and urate will accumulate (Figure 9; Dykens 1991). Thus under conditions in which the O_2 signal is not so low as to promote anaerobiosis, there is a progressive increase in urate concentration, a consequent improvement Hc-O_2 affinity and thus in O_2 loading, providing some support for maintaining O_2 supply. Changes in tissue urate occur relatively slowly and seem more important in the long term than during episodic exercise.

Rapid or sustained exercise often increases O_2 demand beyond the capacity of the delivery system. Haemolymph O_2 then quickly declines and anaerobiosis is initiated. L-lactate is the end product of anaerobiosis in crustaceans, and a progressive lactacidosis results from O_2 shortfall. While the concomitant acid production will reduce Hc-O_2 affinity and facilitate O_2 unloading, this is of little utility under extreme hypoxia, functional or environmental, if O_2 loading at the gills or lungs

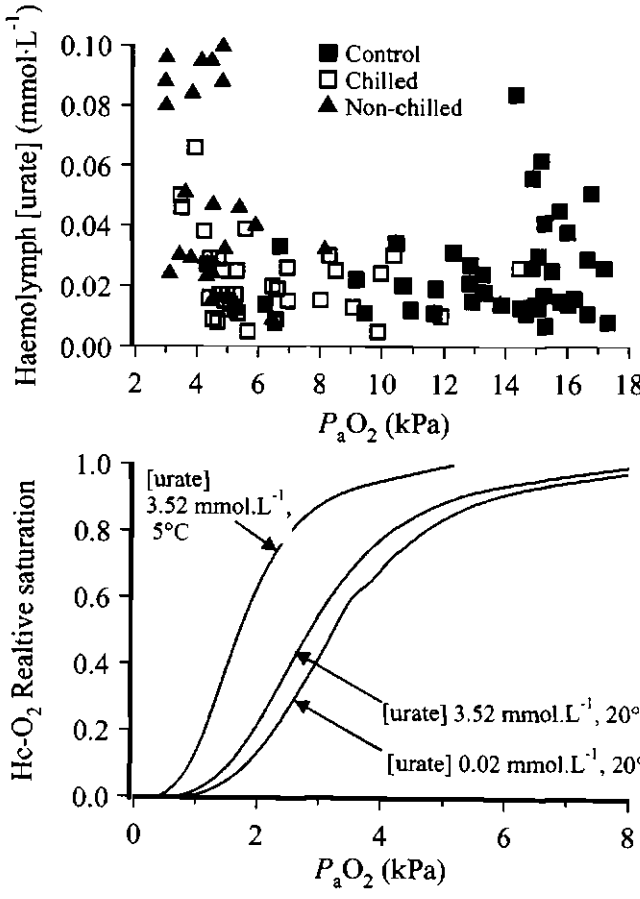

Figure 9 Circulating [urate] increases when PaO_2 falls below that required to support urate oxidase activity. Top panel shows effect of hypoxia on haemolymph urate in southern rock lobsters, *Jasus edwardsii*. Control animals were held in normoxic water at 18°C and hypoxia was induced by air exposure at both 18°C (non-chilled) and 5°C (chilled). Note that chilling ameliorates the increase in urate at the same PaO_2. The lower panel shows O_2 equilibrium curves from *Jasus edwardsii* haemolymph at high and low temperature, demonstrating the direct effect of temperature on Hc-O_2 affinity. In *Jasus*, urate also markedly improves the O_2 affinity of the Hc (Morris, S. & Olliver, S., in prep.).

is simultaneously impaired. However, in many crustaceans that routinely experience hypoxia, L-lactate has a specific effect, partially opposing the reduction in affinity caused by lowered pH (Truchot 1980; Morris 1990; 1991). Thus, as the ventilatory and cardiovascular adjustments become progressively inadequate in matching O_2 supply to demand, specific mechanisms to sustain O_2 transport become important. L-lactate has most pronounced effects during the crucial, initial stages of the recruitment of anaerobiosis into energy metabolism, reducing further 'right-shift' of the Hc-O_2 equilibrium curve and thereby assisting to some extent the hyperventilatory response in support of O_2 uptake.

Of some interest is the recent suggestion that both monoamines and L-lactate may be important in inducing and regulating behavioural hypothermia in decapods (DeWachter, Sartoris & Pörtner 1997). This is an intriguing response whereby hypoxia encourages animals to seek out lower ambient temperatures, lowering the metabolic rate and therefore O_2 demand and maintaining high internal O_2 levels. This behavioural response might well enable crustaceans to avoid

a number of costly compensatory responses (see below).

Aquatic crustaceans transferred into air suffer extreme functional hypercapnic hypoxia, largely owing to the failure of the gills in respiratory gas exchange. Conversely, species adapted to life on land frequently drown in water. In both of these groups exposure to the alien environment results in a rapid switch to anaerobiosis. However, some crustaceans regularly move between air and water, with some ability to breathe both media. Air-breathing crabs have lungs but retain gills for ion regulation and nitrogen excretion (see below). Taylor & Greenaway (1984) showed that *Holthuisana transversa* increased perfusion of the gills while in water and the lungs during air-breathing. Similarly, the Christmas Island blue crab, *Cardisoma hirtipes*, exhibits quite different perfusion of the gills and lungs while in air compared to when breathing water (Airriess, C.N. & Morris, S., in prep). Evidence from perfusion studies under other circumstances suggests that such changes in perfusion may be under active control or be passive responses to alterations in venous resistance. In the former case there must be a mechanism to sense the change in respiratory media and respond with a switch to utilisation of the appropriate gas exchange organ. Changes between air and water breathing would tend to promote large respiratory acid-base changes. However, in truly amphibious species, for example some grapsid crabs, these disturbances are transitory and minor (e.g. Morris, Greenaway & McMahon 1996; Morris & Butler 1996) and thus CO_2 excretion can be managed and integrated with the ventilatory and perfusion adjustments for O_2 uptake and delivery. Considerable further work is required to properly elucidate the interrelationships between ventilatory and cardiovascular adjustments, perfusion of twin gas exchange organs, and respiratory status and acid base balance in amphibious crustaceans as well as their fully aquatic and terrestrial counterparts.

Energetics and Metabolism

Glucose is the major circulating carbohydrate in crustaceans and levels of this energy source must be regulated in response to metabolic status and requirements. Increases in circulating glucose levels, frequently accompanied by elevated muscle glucose, have been demonstrated for numerous decapod species in response to a variety of physiological challenges [*Callinectes sapidus* (Kleinholz, Havel & Reichart 1950; Santos & Colares 1990), *Chasmagnathus granulata* (Schmitt & Santos 1993), *Carcinus maenas, Orconectes limosus* (Santos & Keller 1993a), *Gecarcoidea natalis* (Adamczewszka & Morris 1994) and *Cardisoma hirtipes* (Adamczewszka & Morris 1996)]. Such hyperglycaemia is a common response to environmental or functional hypoxia, and is thought to be triggered in many cases by the action of crustacean hyperglycaemic hormone (CHH) on various target tissues (Figure 1). Evidence suggests both monoamine stimulated CHH release leading to hyperglycaemia and also direct hyperglycaemic effects of 5-HT and OA independent of CHH elevation (Lüschen *et al.* 1993).

CHH is synthesized within the X-organ of the optic ganglion and transported axonally to the sinus gland, located beneath the ganglionic sheath on the surface of the medulla terminalis in the base of each eyestalk (Cooke & Sullivan 1982). There are no significant extra-eyestalk sources of CHH (Keller, Jaros & Kegel 1985), although mRNA encoding two CHH isoforms have been localised in other regions of the CNS (de Kleijn, Leeuw, van den Berg, Martens & van Herp 1995). The amino acid sequence of CHH is highly homologous with moult-inhibiting hormone (MIH), another product of the sinus gland in crustaceans, and high affinity binding sites for CHH are found in the Y-organ (Webster 1993) indicating possible involvement in the control of moulting and reproduction. The mechanism of action of CHH is not well established but available evidence suggests that it stimulates a G-protein coupled receptor in the membrane of target cells, leading to increases in intracellular cyclic nucleotides (Sedlmeier 1987) and IP_3 (Smullen, David & Pitman 1996) and promoting increased intracellular glucose levels. It has been demonstrated that the digestive gland, abdominal musculature, gonads, gills, integument and haemolymph are all targets for CHH (Keller & Andrew 1973; Santos & Stefanello 1991; Kummer & Keller 1993), but there is great species-specificity among the target tissues (Keller *et al.* 1985; Santos & Keller 1993a).

CHH, and also the other amine and peptide products of the sinus glands, are released into the haemolymph which is delivered to the optic ganglia *via* the optic and oculomotor arteries. These vessels arise as paired branches from the anterior aorta and anterolateral arteries respectively (Baumann 1921; Sandeman 1967), implying that preferential changes in arterial perfusion, shifting haemolymph into or away from the anterior dorsal arteries could affect the distribution — and thus modulate the activity — of hormones from the sinus glands. Monoamines such as 5-HT and OA, which have effects on cardiac output and the redistribution of haemolymph (see above), could therefore affect haemolymph glucose concentration by altering the delivery of CHH as well as through direct effects on the sites of carbohydrate storage. Furthermore, there are strong suggestions that 5-HT, OA and DA act on dedicated receptors at the sinus glands to alter hormone release (Kuo *et al.* 1995; Lüschen *et al.*, 1993; Sarojini *et al.* 1995) and thus modulate indirectly the concentration of glucose in the haemolymph.

Although the relationship between CHH and haemolymph glucose concentration has been well documented, the role of CHH in blood glucose regulation under physiological conditions is relatively poorly understood. A diurnal peak in CHH concentration and corresponding elevation of haemolymph glucose levels have been reported in *O. limosus* (Kallen, Abrahamse & van Herp 1990), and the time course of these peaks suggests a causal relationship. *Cancer pagurus* experiences a peak in haemolymph CHH concentration during the first 30 min of air exposure which precedes a more slowly developing hyperglycaemic response (Webster 1996) and again the temporal relationship indicates that the hyperglycaemia is CHH dependent. In *C. magister* there is an abrupt increase in perfusion of the anterior aorta at the onset of emersion (Airriess & McMahon 1996) which reflects the increase in haemolymph flow through the same vessel after pericardial infusion of OA (Figure 10; Airriess & McMahon 1992). Hyperglycaemia also develops throughout a 6 h emersion period. Given that subsidiaries of the anterior aorta provide the primary haemolymph supply to the sinus glands, it is tempting to suggest that changes in arterial perfusion brought

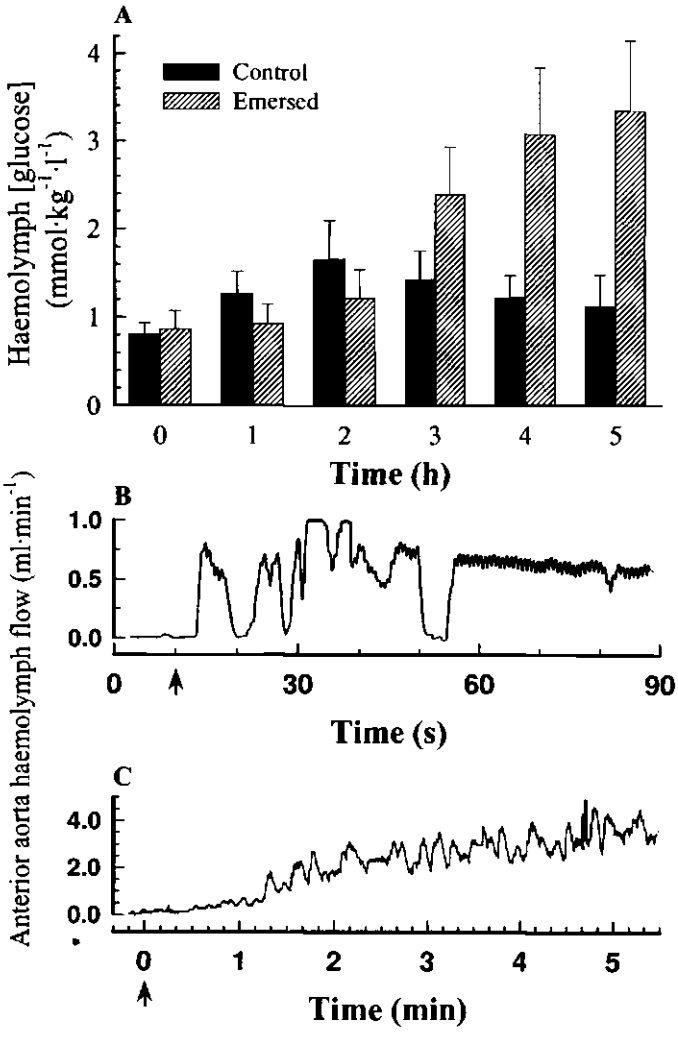

Figure 10 (A) Haemolymph [glucose] increases over 5 h of experimental emersion in *Cancer magister* (Airriess 1994). Haemolymph flow through the anterior aorta increases rapidly during (B) emersion and (C) following pericardial infusion of 10 µmol.l^{-1} octopamine (indicated by arrows; after Airriess & McMahon 1992, 1994). The anterior aorta carries the primary supply of haemolymph to the eyestalk sinus glands, and thus would preferentially direct monoamines released from the pericardial organs to these glands.

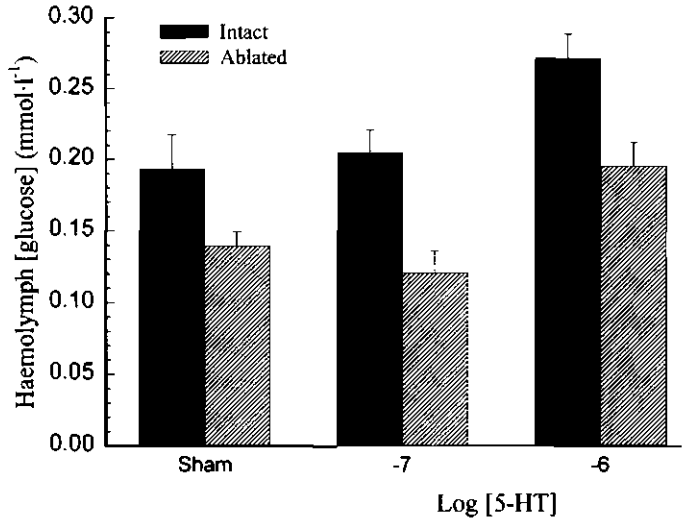

Figure 11 In *Gecarcoidea natalis* 5-HT increases haemolymph [glucose] both in the presence and absence of the eyestalks, suggesting that this amine can indirectly affect liberation of intracellular carbohydrate stores, mediated by factors released from the sinus glands and also has direct effects on glucose mobilisation. 5-HT and saline control solutions were injected into the venous haemocoel at the base of one of the fourth walking legs; after 30 min a venous haemolymph sample was obtained and assayed for [glucose]. Eyestalks were removed from cold-anaethsetised crabs at least 72 h before experimental use of ablated animals (Kilham, B.G., Airriess, C.N. & Morris, S. in prep).

about by OA might be part of an integrative response, facilitating the release of CHH from the sinus glands and (or) its distribution to distal target organs and leading to general hyperglycaemia.

There are conflicting reports on the role of monoamines in regulation of the hyperglycaemic response in crustaceans. For example, recent reviews claim that dopamine acts both to reduce blood glucose by inhibiting CHH release (Sarojini *et al.* 1995) and, conversely, that it promotes hyperglycaemia (Lüschen *et al.* 1993). The ability of monoamine neurohormones to raise circulating glucose levels in bilaterally eyestalk-ablated decapods also seems controversial, or at least species-specific. Recent evidence suggests that 5-HT is capable of increasing the haemolymnph glucose (Figure 11) by mobilising muscle glycogen of *G. natalis* in the absence of the eyestalk sinus glands. However, the 5-HT induced hyperglycaemia is more pronounced in non-ablated crabs (Kilham, B.G, Airriess, C.N. & Morris, S. in prep). Thus it seems likely that there are monoamine receptors in the crustacean sinus glands that promote CHH release but also receptor populations associated with target tissues containing carbohydrate stores which can, when activated, liberate intracellular stores and contribute to changes in circulating glucose levels. It is logical, therefore, that the interaction between neurohormones and CHH must be important both in determining normal tonic levels of blood glucose, and in regulating glycaemia in response to the metabolic demands imposed by physiological challenge.

Artificial elevation of haemolymph glucose decreases circulating levels of CHH but increases in lactate increase haemolymph CHH concentration (Figure 12; Santos & Keller 1993b). CHH-containing neurones in the X-organ of *Cancer borealis* are hyperpolarised by glucose, with a K_m of 0.25 mmol.l^{-1} suggesting sensitive negative feedback regulation of haemolymph glucose levels (Glowik, Golowasch, Keller & Marder 1997). Not only do these studies imply that glucose and lactate independently influence CHH release but also that the provision of fuel for routine aerobic metabolism (driven by blood glucose) and for the accelerated glycolysis of anaerobiosis (responding to lactate) are under separate but interactive control. Extreme hyperglycaemia seen in response to physiological stress may be a passive result of the elevation of intracellular glucose levels to maximise the gradient supporting glycolysis; however, the resulting hyperglycaemia should eventually lead to down-regulation of glucose mobilisation, both into the blood and in the cytosol.

Muscle and mid-gut gland stores of lipid and protein may be energetically important during different seasons depending on diet and food availability. Lipids comprise a major portion

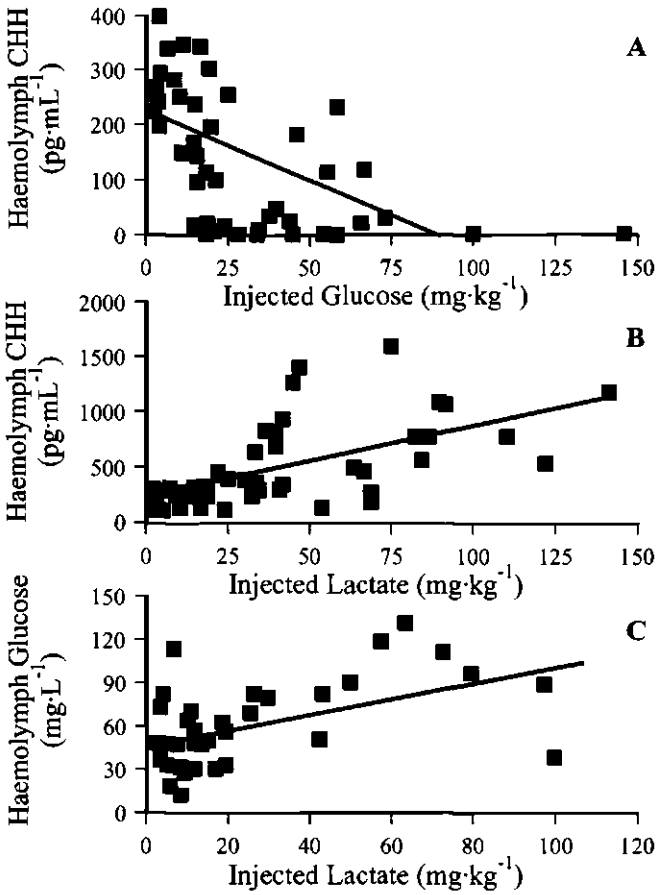

Figure 12 Data abstracted from Santos & Keller (1993b) implicating circulating lactate and glucose as regulators of CHH and thus haemolymph hyperglycaemia in *Carcinus maenas*. Glucose and lactate were injected into crabs, and [CHH] and [glucose] then determined. Panel A demonstrates that [CHH] is elevated in response to lowered haemolymph glucose; panel B that increasing [L-lactate] (simulated anaerobiosis) elevates [CHH]; and panel C that increasing [L-lactate], most likely *via* CHH, promotes hyperglycaemia.

of the mid-gut gland with the highest values occurring in terrestrial crabs where neutral lipids constitute 60% of the total (Morris, S., Hughes, J. & Thompson, M., in prep.). Lipid mobilisation occurs during moulting and gametogenesis and thus MIH might also have a role in the control of energy metabolism (Kummer & Keller 1993). Patterns of lipid and protein utilisation are variable but generally carbohydrate is used before either. Land crabs which are on nitrogen-limited diets, and therefore can not afford to use protein unless critically starved, must accumulate non-protein stores to support migratory activity. If CHH is also involved in the control of lipid metabolism (Santos, Nery, Keller & Goncalves 1997) this implies a further level of integration by the family of sinus gland peptides.

Temperature and behavioural thermoregulation

The body temperature of crustaceans (T_b), since they are ectothermic, is dependent on the ambient temperature (T_a). Thus metabolism and activity tend to be directly affected by changes in T_a, although the effect of temperature is usually of different magnitude on various components of metabolism. Species in thermally variable habitats must have an integrated suite of compensatory responses to temperature fluctuation.

It is well known that changes in temperature are associated with alterations in heart beat frequency and ventilation rate in crustaceans. Until recently, however, there has been a lack of detailed knowledge of the responses and associated mechanisms mediating cardiovascular adjustments to temperature stress. However, in semi-isolated heart experiments the tachycardia associated with increased temperature was small in comparison to that in intact animals, while a concurrent decrease in stroke volume leads to an overall decline in cardiac output (DeWachter & Wilkens 1996). This is in contrast to the increase in cardiac output observed in intact crabs, often despite a decrease in stroke volume (DeWachter & McMahon 1996a; DeWachter & Wilkens 1996), and suggests the involvement of exogenous modulators in the superficially simple cardiovascular responses to temperature change in whole animals.

Neuroamines, particularly octopamine, have been implicated in the response to acute temperature decrease (Kuramoto & Tani 1994). Interestingly, increased electrical activity in the segmental nerves, leading to neurohormone release from the pericardial organs, was greatest at the onset of experimental temperature decrease. In a longer duration protocol no clear changes in neurohormone concentration were detectable in response to a decreased temperature (DeWachter *et al.* 1997). Under hypoxic conditions there was, however, a significant decrease in OA along with a large increase in DA concentration associated with hypothermia, indicating that a modulation of the suite of responses to temperature fluctuation is required to deal with the superimposed challenge of environmental hypoxia. As with segmental nerve activity, changes in haemolymph flow through the sternal artery are most pronounced at the onset of experimental changes in temperature (DeWachter & McMahon 1996a), suggesting that sensitive temperature receptors exist in the aquatic species studied. In terrestrial crustaceans the effects of temperature changes are more acute, owing to the poor thermal capacity of air relative to water. This problem is offset somewhat by the normally stenothermal habitats of land crabs but may pose an additional burden to amphibious crustaceans already challenged by the switch between ventilatory media (e.g. Greenaway, Morris, McMahon, Farrelly & Gallagher 1996; Morris *et al.* 1996).

Overall, cardiovascular responses to changes in T_a must compensate for the associated changes in respiratory (metabolic) demands if they are to be considered adaptive. Importantly, direct effects of increased T_b and thus increased metabolism include acidosis (the neutral point of water varies -0.014 pH units.K^{-1}), facilitating O_2 unloading at the tissues, changes in protein configuration resulting in decreased Hc-O_2 affinity (Figure 8) with similar results and reduced solubility of respiratory gases. The latter may decrease O_2 capacity of the haemolymph but for a given amount of O_2 unloaded will encourage a higher PO_2 and facilitate diffusion into the tissues. Integration at this level must be achieved through evolutionary changes accommodating these phenomena rather than through short-term physiological compensations, although the latter surely occur in response to acute temperature change.

Behavioural hypothermia, whereby an animal selects a lower T_a, has the important potential advantage of lowering

metabolic demand in poikilotherms. Where O_2 supply becomes limiting, moderate hypothermia can be beneficial in (i) reducing O_2 demand, (ii) increasing the oxygen affinity of the respiratory pigment, and (iii) avoiding metabolically expensive increases in cardiac and ventilatory activity.

The crayfish *Cambarus* clearly selects a lower T_a under hypoxic conditions (Dupré & Wood 1988) as does the shore crab, *Carcinus maenas* (DeWachter *et al.* 1997). The O_2 threshold for stimulating 'hypoxia induced behavioural hypothermia' (HIBH) appears to relate to the functioning of the respiratory pigment. Progressively more extreme hypoxia (decline of O_2 signal) can be considered the proximal cause and the selection of a lower T_a the effect, but the relationship between decreasing blood O_2 and the translation into initiation of hypothermia remains largely obscure, possibly involving O_2 chemoreceptors (Massabuau & Meyrand 1996) (Figure 2). As the ventilatory medium becomes depleted of oxygen (mild hypoxia) the arterial oxygen saturation is initially maintained but further reduction in inspired O_2 compromises oxygen delivery. The progressive hypoxia would normally elicit increased ventilation and often blood perfusion to sustain O_2 uptake. Decreasing T_b not only reduces O_2 demand but also increases Hc-O_2 affinity; preserving P_aO_2. The mechanism translating these circumstances (i.e. environmental hypoxia) into a behavioural response, the search for lower T_a, integrating with ventilatory and cardiovascular responses remains unclear but attempts to unravel this mechanism have begun.

Injection of isosmotic Na-lactate into *C. maenas* apparently induced behavioural hypothermia (DeWachter *et al.* 1997); however, such L-lactate levels are usually associated with pronounced hypoxia and significant acidosis. Lactate is attractive as an integrative signal since it responds to the O_2 signal, but the involvement of lactate as a primary signal in HIBH requires persistent hypoxia, with O_2 levels well below those stimulating behavioural hypothermia. In addition the lactacidosis reduces Hc-O_2 affinity, opposite to temperature. Indeed, this may explain the effect of lactate in *Carcinus*, the Hc of which is lactate sensitive. Introducing neutral lactate simulates no physiological condition and large increases in Hc-O_2 affinity will occur; consequently greatly lowering the P_vO_2 required to unload sufficient O_2, thereby lowering the O_2 signal. This is consistent with the equilibrium curve model (above).

If lactate were a signal in HIBH, the re-oxidation of lactate to pyruvate becomes a limiting process and would be slowed by the selection of a lower temperature, thus perpetuating the O_2 debt and the required duration of HIBH. It seems intuitively inappropriate that the product of anaerobiosis should be the signal to initiate HIBH for the purposes of avoiding a systemic shortfall in O_2 (and consequent anaerobiosis); instead, the suggestion of Tattersall & Boutilier (1997) that HIBH functions to retard lactic acid release seems more likely. Clearly the nature of the HIBH stimulus, the transduction systems, and the ecophysiological benefits of this behaviour remain to be resolved. For example, DeWachter *et al.* (1997) suggest that changes in circulating neuroamine concentrations might be involved but the data remain preliminary. Since these monoamine hormones influence cardiac and ventilatory function in crustaceans under hypoxic conditions (above) they remain good candidates not only as messenger/transducer molecules but as integrative elements of a complex behavioural response. Recent findings of O_2 and monoamine sensitive nerve cells in ganglia of crustaceans (Hempel *et al.* 1996; Massabuau & Meyrand 1996; Figure 2) suggest an integrative mechanism requiring some considerable further investigation.

Despite the deficiencies in the models there are strong cases presented suggesting that the balance between 100% aerobiosis and the initiation of anaerobiosis is a crucial condition and may provide some signal for HIBH. However, if it can be shown that HIBH is initiated before recruitment of anaerobiosis and/or that amines can elicit HIBH in the absence of L-lactate then a different but more informative model will result.

Ion regulation and water balance

There are yet further systems that can be influenced by the suite of effectors discussed above, suggesting that many elements of neurohaemal integration are yet to be elucidated. For example, neuroendocrine control of osmoregulation and ion balance has been established in principle for many years (e.g Kamemoto & Ono 1969; Kamemoto 1976; Charmantier, Charmantier-Daures & Aiken 1984.; Mantel 1985), and apparently uses primary messengers similar to those purported to be involved in respiratory, cardiovascular and metabolic control. Extracts of the pericardial organs (PO) of marine crabs such as *C. maenas* have marked potentiating effects on ion and osmoregulatory activity (Sommer & Mantel 1988). DA increases both Na uptake and Na^+/K^+-ATPase activity in *C. maenas* (Sommer & Mantel 1988, 1991) and stimulates Na^+/K^+-ATPase in the supratidal *Leptograpsus variegatus* (Figure 13; Morris &: Edwards 1995). In both of these species cAMP has been implicated as a 2nd messenger transducing the up-regulation of transport within the gill epithelial cells, and the ATPase activity to increase in response to lowered salinity (Sommer & Mantel 1991; Cooper & Morris 1997) implicating salinity activated 2nd messenger systems. Work on isolated perfused gills using membrane permeable cAMP derivatives has linked increased (cAMP) directly to increased Na uptake in *Callinectes sapidus* (Lohrmann & Kamemoto 1987) and similarly in *Eriocheir sinensis* (Bianchini & Gilles 1990). Importantly, neuroamines stimulate not only the Na^+/K^+ATPase in *Eriocheir sinensis* gills but also phosphorylation of membrane proteins, possibly the ATPase (Trausch *et al.* 1989). Thus, it appears that neurohormones such as DA, and possibly others including peptides, bind to receptors on the gill epithelial cell surface and, *via* a G-protein linked transduction system, stimulate adenylate cyclase to elevate intracellular cAMP. A common consequence of elevated (cAMP) is the activation of protein kineses and thereby stimulation of protein phosphorylation, consistent with the suggestions of Trausch *et al.* (1989). While it is possible that a membrane protein adjacent to the ATPase rather than the enzyme itself may be the target of phosphorylation, the end result is the activation of Na^+/K^+-ATPase and Na transport. In terrestrial decapods such as *Birgus latro* it appears that DA and cAMP act on the same pathway to bring about a decrease in both ATPase activity and ion transport (Morris, Greenaway & Adamczewska, unpub.). The

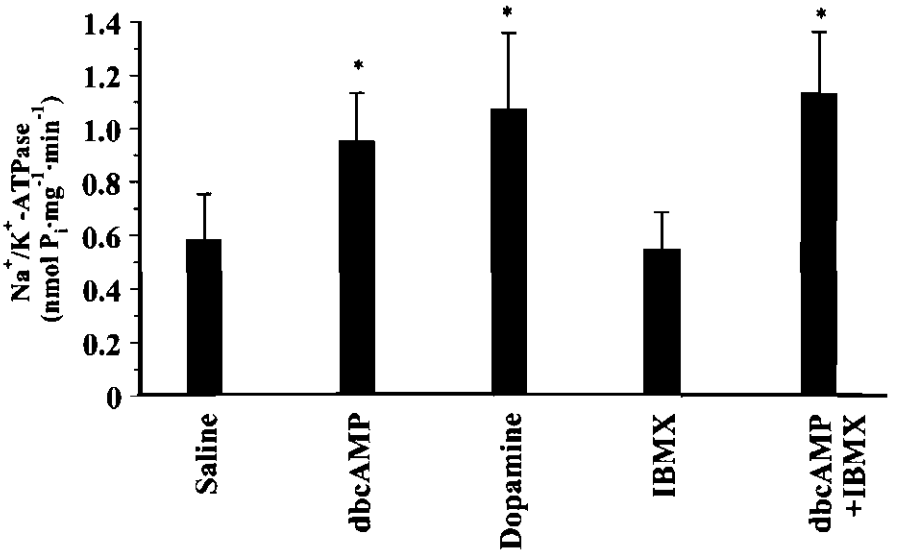

Figure 13 Intracellular transduction of the Na^+ transport response to dopamine in the gills of the marine supra-tidal crab *Leptograpsus variegatus*. The increase in Na^+/K^+-ATPase activity caused by dopamine is mimicked by the cell-permeant cAMP analogue dibutyryl-cAMP (dbcAMP). Isobutyl-methyl-xanthine (IBMX) does not affect Na^+/K^+-ATPase on its own, but in conjunction with dbcAMP this phosphodiesterase inhibitor magnifies the response to cAMP (after Morris & Edwards 1995).

uncertainty in the details of mechanisms in integration of salt transport is highlighted by Riestenpatt, Zeiske & Onken (1994) who concluded that cAMP stimulation of branchial ion transport occurred by an increase in the number of Na^+ channels, and not the ATPase. Additionally, that an increase in apical V-ATPase activity drove Cl^- uptake *via* the HCO_3^-/Cl^- exchanger. Furthermore, recent information for terrestrial crabs show DA stimulation depresses NaCl transport (below). Nonetheless, the implied integrative possibilities remain.

These possibilities must incorporate, however, the likelihood of other neuroendocrine effectors of hydromineral balance–calmodulin mediated modulation of NaCl transport, for example (Pequeux & Gilles 1992). The sinus glands of the crustacean eyestalks produce a variety of peptide hormones and extracts from the sinus gland of *Pachygrapsus marmoratus* are implicated in osmoregulatory control of isolated perfused gills (Pierrot, Eckhardt, Van Herp, Charmantier-Daures, Charmantier, Trilles & Thuet 1994). More recently, this stimulatory effect of sinus gland extract has been attributed to a peptide factor (Eckhardt, Pierrot, Thuet, Van Herp, Charmantier-Daures, Trilles & Charmantier 1995). Elevated Na uptake is an essential part of adaptation to freshwater; and in the Australian crayfish *Cherax destructor*, this eyestalk factor appears crucial in the pumping response to lowered water (Na) and the maintenance of haemolymph (Na) (Sithigorngul, Sithigorngul & Morris, unpub.)

Therefore, while there is good evidence that biogenic amines may directly influence gill epithelium function *via* a cAMP 2nd messenger system the strong possibility that amines may mediate the release of a peptide that also stimulates ion transport (see energetics section) must now be investigated. A more sophisticated and diffuse regulatory system would be consistent with the observation that some effects can be elicited in intact animals but not isolated tissues (Morris & Edwards 1995).

When placed in dilute (50%) seawater, the marine crab *Cancer magister* experiences an increase in heart beat frequency commensurate with a decline in cardiac stroke volume and, consequently, cardiac output (McGaw & McMahon 1996). Scaphognathite beat frequency increased during hyposaline exposure, as did haemolymph flow through the posterior aorta. Flow through all other major arteries decreased. The effects of DA on cardioarterial valve contractility (Kuramoto *et al.* 1992) are consistent with redirection of haemolymph flow favouring perfusion of the posterior aorta, and are particularly interesting given the involvement of this amine in the ionoregulatory responses of aquatic crustaceans to hyposmotic exposure.

The available evidence strongly supports the suggestion that marine and especially freshwater crustaceans utilise neuroendocrine systems to up-regulate ion pumping in response to exposure to dilute media — as an 'on switch'. New data from terrestrial crab models suggest a quite different system. Gecarcinid land crabs and terrestrial anomuran crabs of marine ancestry frequently have only freshwater to drink and must conserve both body water and minimise salt loss in the urine (Greenaway 1988 for review). To this end these crabs both drink part of their urine and pass the remainder over their gills so that required salts can be actively reabsorbed across the branchial epithelium. Under these circumstances it seems reasonable that branchial ion pumping must frequently run at near maximal rates but may slow if the animal becomes salt replete from occasional access to salt water. A combination of branchial perfusion studies (Morris, Taylor & Greenaway 1991 for methods) and determination of gill Na^+/K^+-ATPase in the terrestrial anomuran *Birgus latro* (Figure 14; Morris, S., Greenaway, P. & Adamczewska, A.M., in prep) have revealed the appropriate 'offswitch'. Dopamine and cAMP (10^{-6} mol.l^{-1} circulating) have marked depressing effects on branchial ion uptake and the associated ATPase activity.

In terrestrial crabs it would clearly be advantageous to regulate water turnover independently of salt reclamation *Gecarcoidea natalis* experiences both wet and dry seasons and thus

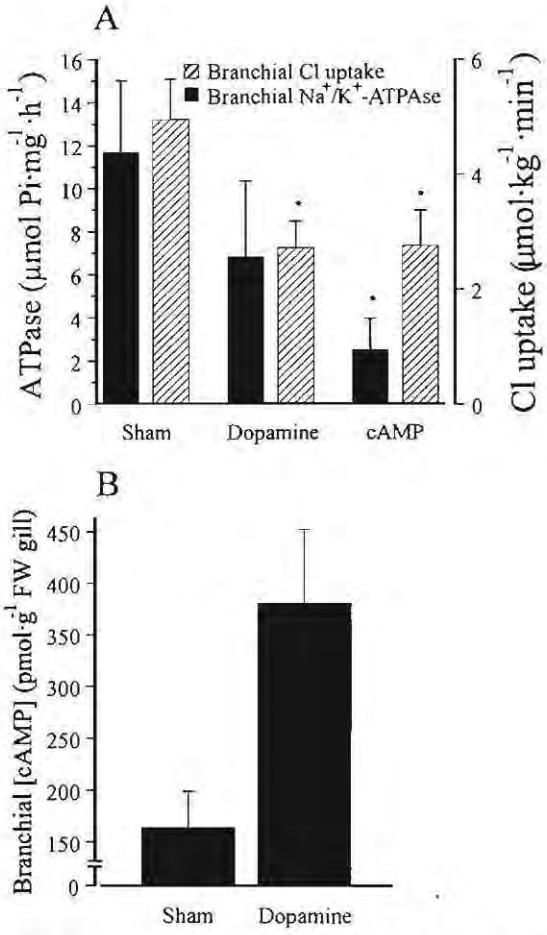

Figure 14 (A) The branchial Na^+/K^+-ATPase in the terrestrial anomuran *Birgus latro* is inhibited by circulating dopamine and dbcAMP (10^{-6} mol.l^{-1}) as is the uptake of Cl^- in experiments in which the branchial chambers were artificially irrigated. **(B)** Importantly, the injection of dopamine elevates endogenous branchial [cAMP] by more than two-fold, confirming cAMP levels respond to extracellular dopamine and are involved in the down regulation of ion reclamation in land crabs.

variable water availability. While the availability of drinking water has marked effects on urine clearance rate of red crabs (e.g. Greenaway 1994), 5-HT (10^{-7} mol.l^{-1} circulating) significantly reduces urine clearance and promotes water retention (Morris, S. & Ahern, M.D., in prep.).

Higher level integration

Throughout this review we have selected examples to illustrate how various components of the physiological responses to behavioural and environmental challenge may be initiated, controlled and integrated. Obviously these separate physiological responses must be further integrated and modified within the whole animal. Furthermore, this integration must itself be modulated with respect to fluctuating daily requirements, season, reproductive state and developmental stage, and all will likely differ between species. Each of these is worthy of review but they can not be considered in detail here.

For example, the annual migratory behaviour of some crustaceans must produce severe physiological demands and influence both behaviour and physiology. The Christmas Island red crab breeding migration is an obvious illustrative model of how all of the various physiological systems must be coordinated and integrated during the migration. Seasonal changes in this species include important modifications to both the hyperglycaemia factor within the eyestalks and tissue sensitivity to this factor (Figure 15). Further levels of sophistication must exist in the integrative mechanisms since these seasonal changes are imposed on diel changes in numerous aspects of metabolism and behaviour.

There is tantilising evidence for ontological changes in sensor, messenger, receptor and transduction mechanisms (e.g. Reiber 1997b; Spicer & Morritt 1996) which surely must occur but extend beyond the scope of the present discussion. A more comprehensive understanding of these systems is a necessary step in future detailed investigation of changes with development.

It is attractive to hypothesise that variation in the circulating levels of a suite of neurohormones may act to regulate the function of many systems. Consideration of specific segments of the physiological literature, in isolation of other aspects of crustacean physiology, tends to lead to the development of oversimplified models. Such simple models can not persist since they require the same primary messengers, at similar concentrations, to influence differentially a wide variety of disparate physiological processes. The specificity of neurohormone effects in crustaceans, and particularly the possibility of tissue-level modification of neurohormone activity, is an area requiring immediate and substantial attention. Clearly, equipped with a contemporary perspective, the investigation of all the discussed aspects of crustacean physiology must henceforth be conducted under physiological conditions. Importantly, new information is beginning to show how neurohormonal function may be integrated by and

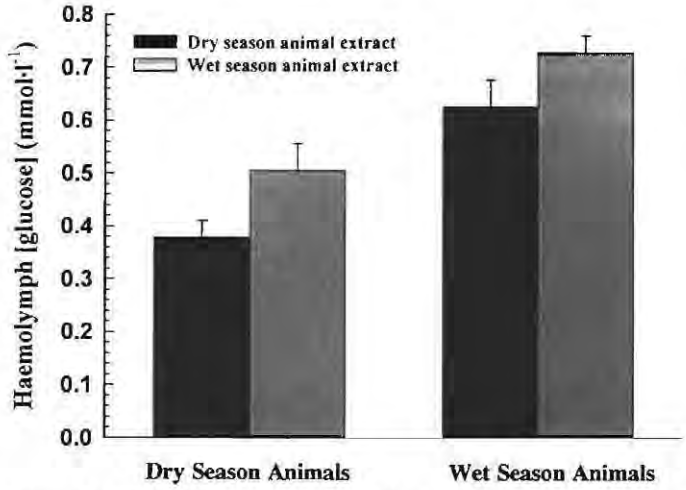

Figure 15 The effectiveness of eyestalk extracts in eliciting a hyperglycemia response in *Gecarcoidea natalis* is dependent on seasonal variation in environment. Eyestalk extract (one eyestalk per crab) was injected into the venous haemocoel of crabs collected during the wet season (February 1997) and dry season (June 1997). After 30 min haemolymph samples were obtained and assayed for haemolymph [glucose] (Kilham, B.G., Airriess, C.N. & Morris, S. in prep). Wet-season animals are clearly more sensitive to eyestalk factors than dry-season animals, whilst the eyestalks of the dry-season animals have less efficacy in inducing hyperglycaemia in both groups of crabs.

together with peripheral receptors and the CNS. Thus while, of necessity, increasing both the species database and the available experimental models it is clear that resolution of physiological integration in the Crustacea will require the marriage of whole animal biology with cellular and biochemical methodologies.

The examples emphasised in this review are a small subset of the many investigations covering most of the major aspects of crustacean physiology. However, the literature on these topics is diffuse and fractured, often contradictory and largely uncollated outside of narrow specific fields of study. The synthesis of existing information with new, and different types, of data on crustacean physiology will improve our appreciation of the biology of these animals and provide general models that will contribute significantly to our understanding of animal adaptation to environment.

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