THE EFFECTS OF ENVIRONMENTAL VARIABLES ON THE HEART RATES OF INVERTEBRATES

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Abstract—1. The effects of temperature, salinity and declining O_2 on the heart rates of nine species representing four animal phyla have been investigated in relation to other respiratory paramters.

2. The effect of temperature on heart rate is at least the same as, and often greater than, the effect of temperature on O_2 consumption, thus providing no evidence that adaptations of the cardiovascular system facilitate metabolic compensations for a temperature change.

3. Responses to reduced acclimation salinity are very diverse among the various species. permitting no general conclusions about the role of the cardiovascular system in adaptations to estuarine habitats.

4. At low PO_2 the typical response is bradycardia, which is especially notable in species with a high capacity for anaerobic metabolism. Compensatory tachycardia, the expected response in vertebrates, is very rare in other animal groups.

5. Estimates of cardiac output from these data generally agree with those obtained according to the Fick principle from blood gas tensions.

6. The estimates of cardiac output are evaluated in terms of body size, temperature and the design of cardiac muscle, which is fundamentally different in various animal phyla.

INTRODUCTION

The effects of an unstable environment on the respiratory performance of different kinds of circulatory systems are poorly known. Until recently, studies were conducted on isolated hearts or exposed preparations from molluscs and a few decapod crustaceans, and *in vivo* preparations of only the smaller crustaceans (Cladocera, Amphipoda, Isopoda, etc.) (Maynard, 1960b; Hill & Welsh, 1966). While the *in vitro* data may elucidate control mechanisms, their contribution to an understanding of *in vivo* function is uncertain. Furthermore, most investigators have examined acute rather than more meaningful long term responses to variables in aquatic environments such as temperature and salinity. Thus, the respiratory consequences of cardiovascular responses to environmental change cannot be accurately predicted from the available information.

The cardiac responses of intact invertebrates to reduced salinities have been described for only one mollusc (Bayne, 1973) and four decapods (Spaargaren, 1973, 1974; Taylor *et al.*, 1977) and these data do little to explain the relationship between circulatory and respiratory responses. It is clear that some crustacean cardiovascular systems are sensitive to salinity (Spaargaren, 1973, 1974; Hume & Berlind, 1976), despite conflicting data in the literature (Spaargaren, 1974; Taylor *et al.*, 1977). Temperature coefficients for heart rate in the molluscs and crustaceans have been widely reported (see Maynard, 1960b; Hill & Welsh, 1966), but only a few recent measurements have been made on acclimated, intact animals (Ahsanullah & Newell, 1971; Taylor *et al.*, 1973; Widdows, 1973; Earll, 1975). The effect of low or declining oxygen on heart rate in intact bivalves and crusta-ceans has received some attention; the characteristic response seems to be a decrease in heart rate during hypoxia, but the degree of bradycardia is highly variable (Larimer, 1962; Lowe & Trueman, 1972; Florey & Kriebel, 1973; Taylor *et al.*, 1973; Uglow, 1973; McMahon *et al.*, 1974; Hill & Koopowitz, 1975; McMahon & Wilkens, 1975; Belman & Childress, 1976; Coleman, 1976; Coyer, 1977; Deaton & Mangum, 1978). In the bivalves, a compensatory tachycardia may or may not precede the ultimate slowdown, depending on species (Bayne, 1971; Brand & Roberts, 1973; Taylor & Brand, 1975; Booth & Mangum, 1978). Virtually no information is available for other groups.

Heart rates of nine species representing four phyla were measured under one or more of three experimental conditions: (1) long term exposure to normal and reduced salinity, (2) long term exposure to high and low temperature, and (3) brief exposure to declining oxygen. An attempt is made to reach physiological conclusions using known respiratory and anatomical parameters. Heart volumes were also measured and the results used to predict cardiac output independently of the Fick principle, which has yielded estimates for various species of crustaceans that differ by a factor of five (Johansen *et al.*, 1970; Belman, 1975). In an effort to clarify the physiological basis of the high heart rates observed in the crustaceans, the ultrastructure of cardiac muscle was examined.

METHODS AND MATERIALS

Most of the animals (Table 1) were collected from the lower Chesapeake Bay. The annelid *Nereis virens* was obtained from Woods Hole, and the crab *Hemigrapsus nudus* from Charleston, OR. Animals were maintained in aerated, recirculating water using either natural seawater or York River water (16-20‰, or increased to 32‰ salinity with Dayno Co. sea salt).

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Table 1. Control heart rates (= 1.0 in Figs. 2 and 3)—one to three measurements per animal per day

Species	Temp. (°C)	PO₂ (mmHg)	Salinity (o/oo)	Body Weight (gm)	Mean Heart Rate (beats/min.) ± S.E.	No. Animals	Maximum Observation Period (da)
Xiphosura							
Limulus							
polyphemus (L.)	25	120–140	30–35	210–857	37.5 ± 3.5	12	30
Crustacea							
Callinectes							
sapidus Rathbun	25	140–150	30–35	80–192	127 ± 9.0	20	50
Hemigrapsus							
nudus(Dana)	18	159 (air)	30–35	18–25	235 ± 12.8	3	8
Libinia							
emarginata Leach	25	140–150	30–35	77–221	81.5 ± 8.9	8	39
Panopeus							
herbsti Milne-Edw.	20	159	26–32	11–23	131.7 ± 11	6	8
Mollusca							
Bivalvia							
Spisula							
solidissima (Dillwyn)	7–10	159	29–33	104–158	8.9 ± 0.2	15	45
Noetia							
ponderosa (Say)	25	159	34	15.5 ± 3.2	14.5 ± 0.8	5	12
Gastropoda							
Busycon							
canaliculatum (L.)	20	159	26–35	40.3 – 60.7	14.1 ± 1.1	4	34
Annelida							
Nereis succinea	15	159	32	0.8–1.2	7.4 ± 0.4	13	6
(Frey & Leuckhart)							
N. virens Sars	15	159	34–35	3.1–7.9	6.8 ± 0.1	12	12

Heart rates of animals other than the annelids were monitored according to Trueman (1967) and Trueman et al. (1973). Small holes were drilled in the shell or carapace and thin silver, platinum or gold electrodes inserted and held in place with dental wax (Surgident). Using an impedance pneumograph (E & M Instrument Co. MK IV) a small (5 μA) current was passed between the electrodes, the changes in impedance converted to a voltage signal and the resulting signal displayed on a multi-channel pen recorder (E & M Instrument Co. Physiograph Six). The amplitude of the recording was set for each animal individually and then held constant.

All experiments were conducted in the dark to avoid visual stimulation. Animals were initially held under the conditions specified in Table 1 for 5–12 days. During this time the experiments on declining oxygen were conducted, and then either the salinity or temperature was changed and heart rates measured for the following 6–12 days.

Blood pulse rates in the dorsal vessel of N. virens and N. succinea were visually observed with the worms in glass tubes (initial temperature of 15°C). The elapsed time for 10 blood pulses was measured with a stop watch to the nearest 0.5 sec. This procedure was repeated daily for 6 days prior to and for 2 days following the declining oxygen experiment. The temperature was changed to 5 or 25°C and pulse rates were counted over the next 4 consecutive days. The observations at low salinity were made 2–4 days after the change.

Measurements of heart rates in declining oxygen were conducted in closed vessels, or in aquaria with plastic wrap placed on the surface of the water. Once the heart rate stabilized after placing an animal in the vessel (1–12 hr), the vessel was sealed, the air flow stopped, and the animal allowed to deplete the oxygen. Oxygen concentrations were measured with a polarographic electrode (Yellow Springs Instrument Co., Model 54). Recordings of heart rates (1 min duration) were made every 0.5 ppm as the oxygen declined at a rate of 2–4 ppm/hr. In the case of Spisula solidissima, nitrogen gas was used to reduce the oxygen at a rate of 3.0 ppm/hr.

Recordings were made at least once daily, frequently two or three times. The records (0.5–5.0 min duration) for a particular set of environmental conditions were analyzed in entirety unless mechanical or electrical interference occurred.

Although the data are presented below as mean values for all of the individuals of a species, they were in fact taken and analyzed as paired observations (according to Student's t-test) on a particular individual before and after an environmental change. Changes in heart rates were considered significant if $P < 0.05$.

Several techniques were used to estimate heart volumes. The exterior dimensions of intact, exposed hearts were measured with calipers, the figures reduced by the mean width of the heart wall and the volume calculated. Sea water or latex were injected and withdrawn with a syringe and the volume to distension noted. Estimates obtained by injecting excised hearts were conspicuously larger than any of the others, and the data were discarded. Finally, stroke volume (ml) in the crustaceans was calculated as a percentage of body weight. In N. virens stroke volume was estimated with an ocular micrometer by measuring the length of the blood pulse in the dorsal vessel and the diameter of the vessel before and during a pulse. Treating the vessel as a cylinder, the difference between the volumes before and during the passage of a blood pulse was taken as the stroke volume.

Heart tissue from P. herbsti was fixed in 2% glutaraldehyde, the pH (7.4) and osmolality (500 mOsm) of which were adjusted with seawater. Tissue was post-fixed in osmium tetroxide, stained in uranyl acetate, imbedded in Epon 812, sectioned with a Porter-Blum MK 2 B Ultramicrotome. Lead acetate was used in final staining and sections were examined with a Zeiss 9 electron microscope.

RESULTS

General observations

As shown in Table 1, heart rates are less variable in the molluscs and annelids than in the arthropods. In the three crustaceans and the horseshoe crab Limulus polyphemus, sudden increases in heart rate were associated with spontaneous increases in motor activity or with external stimuli. Florey & Kriebel (1973) have shown that external stimulation has a direct positive effect on heart rate of the crustacean Cancer magister, and Hill & Kopowitz (1975) found that heart rates in Scylla serrata declined 45% during

Table 2. Relationship of locomotor activity and heart rate (mean ± S.E.) in *Libinia emarginata* and *Limulus polyphemus*

Body Weight (gm)	Activity Level	Heart Rate (beats/min.) 15°C	N	25°C	N	Q$_{10}$
Limulus polyphemus						
856.6	active	19.2 ± 2.0	4	33.8 ± 9.5	4	1.75
	inactive	8.0 ± 2.4	3	13.8 ± 2.3	4	1.73
750	active	34.9 ± 3.3	4	42.9 ± 7.3	4	1.23
	inactive			22.3 ± 2.6	3	
	unresponsive			11.7 ± 2.4	3	
720	active	18.2 ± 3.6	5	32.9 ± 7.7	13	1.81
	unresponsive			7.6 ± 1.4	5	
823	active			24.3 ± 2.6	5	
	inactive			16.2 ± 3.1	6	
	unresponsive	4.6 ± .4	5	8.7 ± 1.5	3	1.88
Libinia emarginata						
116.5	active	32.4 ± 2.8	5	59.5 ± 2.5	4	1.84
	inactive	13.6 ± 1.2	5	28.3 ± 4.7	4	2.07

sensory deprivation. The greater variability in the arthropods was found within individuals as well as between individuals. Variations within an individual held under constant conditions were as great as 40 beats/min in the blue crab *Callinectes sapidus*, 35 beats/min in the spider crab *Libinia emarginata*, and 20 beats/min in *L. polyphemus*.

L. polyphemus was the only species to exhibit discrete and regular activity levels (Table 2). Johansen & Petersen (1975) also observed different activity levels with correspondingly different levels of oxygen consumption, taking the form of a six-fold difference between extremes.

Cyclic variations in heart rates not associated with activity level were observed in *C. sapidus* and, on rare occasions, in *L. emarginata*. These cycles of 1–4 min typically consisted of a sudden bradycardia followed by a gradual recovery of the initial rate (Fig. 1A). The cycles, which had no apparent behavioral correlates, may be similar to the cardiac arrests reported in other crustaceans (McMahon & Wilkens, 1972, 1977; Florey & Kriebel, 1973).

Cardiac arrest occurred only in *C. sapidus*, *L. emarginata* and the mud crab *Panopeus herbsti*. It could not be induced by mechanical or visual stimulation, which is effective in other species (McMahon & Wilkens, 1972; Florey & Kriebel, 1973). Figure 1(B) illustrates the abruptness with which cardiac arrest is initiated and terminated. Arrests usually last about 30 sec in *L. emarginata* and 1 min in *C. sapidus*. *P.*
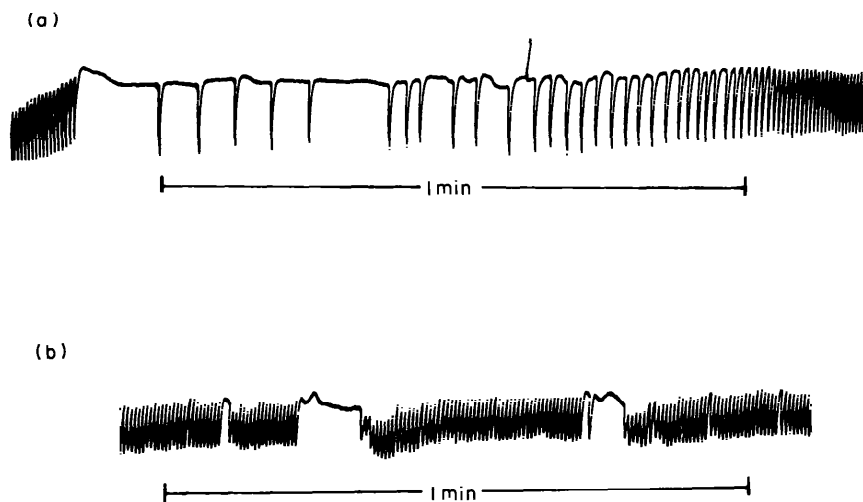
(a)

1 min

(b)

1 min

Fig. 1. (A) Record of the heartbeat of a female blue crab, *Callinectes sapidus* (25°C, 35‰, PO$_2$ = 140 mm Hg), demonstrating cyclic variations in heart rate. (B) Record of the heartbeat of a male mud crab, *Panopeus herbsti* (25°C, 35‰, PO$_2$ = 159 mm Hg), exhibiting cardiac arrest.
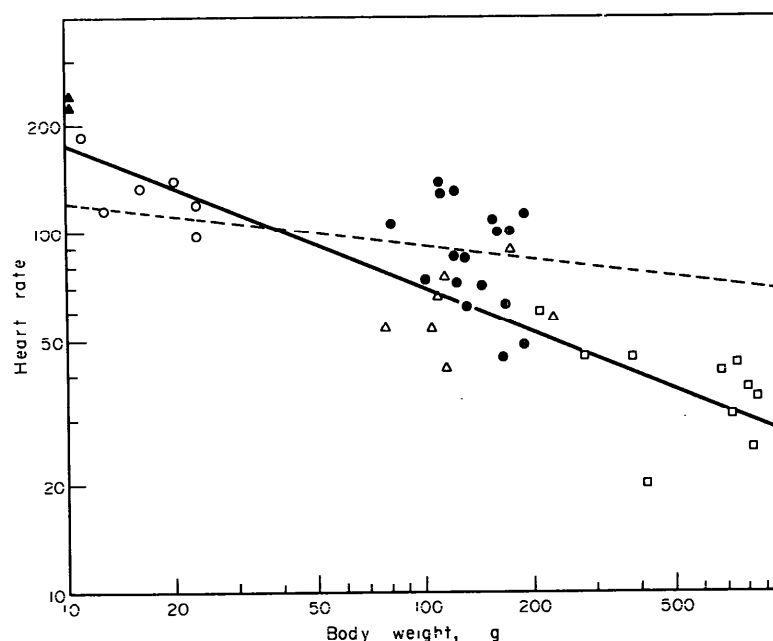
Fig. 2. Regression line (——) describing data for heart rate as a function of body weight in *L. poly-phemus* (□), *L. emarginata* (△), *P. herbsti* (O), *Hemigrapsus nudus* (▲). $r = 0.92$, $P < 0.001$, slope $(b - 1) = -0.39$. ● data for *C. sapidus*, which fit poorly. Dotted line taken from Schwartzkopff (1955), 20–22°C.

herbsti did not exhibit cardiac arrest as often as the other species and the arrests were of shorter duration (5–10 sec).

In the arthropods, heart rate is clearly correlated with body weight (Fig. 2). This relationship was first demonstrated in 1955 by Schwartzkopff; the effect of body weight is even greater in Fig. 2. The blue crab *C. sapidus* has an exceptionally high heart rate for its body size (Fig. 2), and also an exceptionally high rate of oxygen consumption (Mangum & Weiland, 1975). The data for this species were not used in calculating the regression line shown in Fig. 2.

In contrast to the arthropods, no relationship could be discerned between heart rate and body size in the molluscs ($r = 0.19$; $P > 0.10$).

Effects of temperature

In general, temperature coefficients (Q_{10}) for heart rate (Table 3) either approach or fall within the range regarded as typical of physiological processes, 2.0–2.5 (Prosser, 1973). There were no cases of markedly reduced temperature sensitivity ($Q_{10} < 1.8$), and no apparent relationship between temperature sensitivity and motor activity (Table 3).

With the exception of the surf clam *Spisula solidis-sima* and two of the four individuals of *L. emarginata*, the cardiac adjustments to a temperature change were completed prior to the first observation at a new temperature, which varied from 8 to 24 hr; no further change was ever found.

The temperature sensitivity of heart rate in the two

Table 3. The effect of acclimation temperature on heart rates (mean ± S.E.)

Species	No. Animals	Heart Rate (beats/min.) 5°C	15°C	25°C	Q_{10}
Limulus polyphemus -- active	4		23.0 ± 4.0	39.0 ± 3.4	1.77 ± 0.02
inactive	2		10.3 ± 2.4	19.3 ± 5.5	2.03 ± 0.30
unresponsive	1		4.60 ± 0.4	8.67 ± 1.5	1.88
Callinectes sapidus	6		62.3 ± 10.0	120.5 ± 15.0	2.01 ± 0.10
	1	8.1 ± 1.9		143.0 ± 3.0	4.20
Libinia emarginata	4		38.0 ± 4.0	72.4 ± 8.8	1.92 ± 0.19
Spisula solidissima	4	8.5 ± 0.62(7°C)	14.0 ± 0.76(12°C)		2.88 ± 0.56
Noetia ponderosa	5	0	5.0 ± 0.24	14.5 ± 0.79	2.92 ± 0.22
Busycon canaliculatum	1	0	9.43 ± 0.11	20.7 ± 0.58	2.19
Nereis virens	6		6.8 ± 0.42	12.8 ± 1.4	1.86 ± 0.14
	6	3.33 ± 0.16	6.8 ± 0.27		2.04 ± 0.08

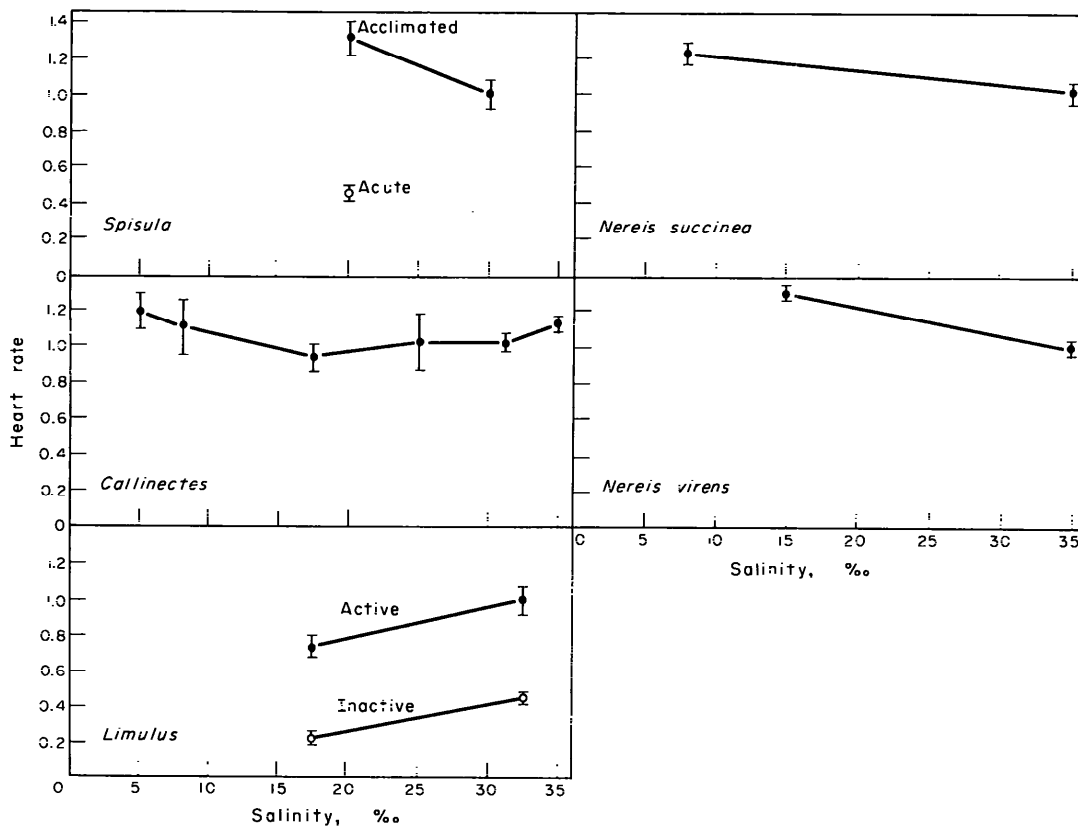Q_{10} values are the means for paired observations on single individuals.

Fig. 3. Responses of the heart rates of five species to acclimation salinity. $PO_2 = 130$–159 mm Hg. Control rates (= 1.0) and experimental temperatures given in Table 1.

bivalves is greater than in the other species (Table 3). In *S. solidissima* transferred from 7.5 to 12.5°C, a negative acclimation took place at some time between 6 and 12–24 hr at the new temperature: at 6 hr the Q_{10} was only 2.0, whereas heart rate declined further at 12 and 24 hr.

Repeated observations on three individuals of the ark *Noetia ponderosa* over a 3 day period indicates that heartbeat ceases completely at 5°C. When the animals were reacclimated to 15°C, the heart rate returned to the initial values at that temperature. The same phenomenon was observed in the single individual of *Busycon canaliculatum* in which the preparation was successful. Heartbeat never ceased completely in the crabs (maximum interbeat interval of 12.0 sec), but the rate in the one individual of *C. sapidus* studied at 5°C was extremely low (Table 3).

Effects of salinity

The responses to decreased salinity are quite diverse (Fig. 3). In *L. polyphemus*, heart rate declines an average of 25% when the animals are transferred from 30 to 20‰ (Fig. 2). The period required for acclimation is 3 days, after which the rates no longer change. The activity level does not influence the response, which does not differ significantly in active and inactive animals.

The response of *S. solidissima* is more complex; (Fig. 3) an initial bradycardia (58%) at low salinity is followed by a lasting tachycardia (30%) which represents a significant increase of 2.7 beats/min over the initial rate of 8.9 beats/min. It should be pointed

out that 20‰ approaches the lower limit of salinity tolerance in this species (Castagna & Chanley, 1973). The only other bivalve for which data have been reported (*Mytilus edulis*) shows no change in heart rate in the salinity range 16.3–32.3‰ (Bayne, 1973); *M. edulis* is a far more euryhaline species.

In the two specimens of *B. canaliculatum* for which data were obtained, the response to decreased salinity was inconsistent and therefore not shown in Fig. 3. In one animal, heart rate increased from 11.1 (± 0.2) ($N = 6$) beats/min at 35‰ to 12.5 (± 0.7) ($N = 6$) beats/min after 4 days at 20‰. The other animal showed bradycardia (14.9 $\pm$ 1.0, $N = 6$, to 9.67 $\pm$ 0.33, $N = 6$) beats/min after 3 days at 20‰, but the observation period does not permit complete behavioral acclimation (Polites & Mangum, in preparation).

When *C. sapidus* is exposed to reduced acclimation salinities, heart rate does not change in the range 8–20‰, but it increases at 5‰ (Fig. 3). The elevation is significant and it occurs regardless of the direction of the salinity change. The response parallels that of oxygen uptake, which does not change in the range 10–25‰, but increases at 5‰ (Mangum, 1976). Such a correlation between changes in heart rate and oxygen consumption at low salinity was not observed by Taylor *et al.* (1977) in the related species, *Carcinus maenas*.

The responses in the two species of *Nereis* are very similar, despite the fact that *N. succinea* maintains a hyperosmotic condition of body fluids at the low salinity tested and *N. virens* does not. The tachycardia
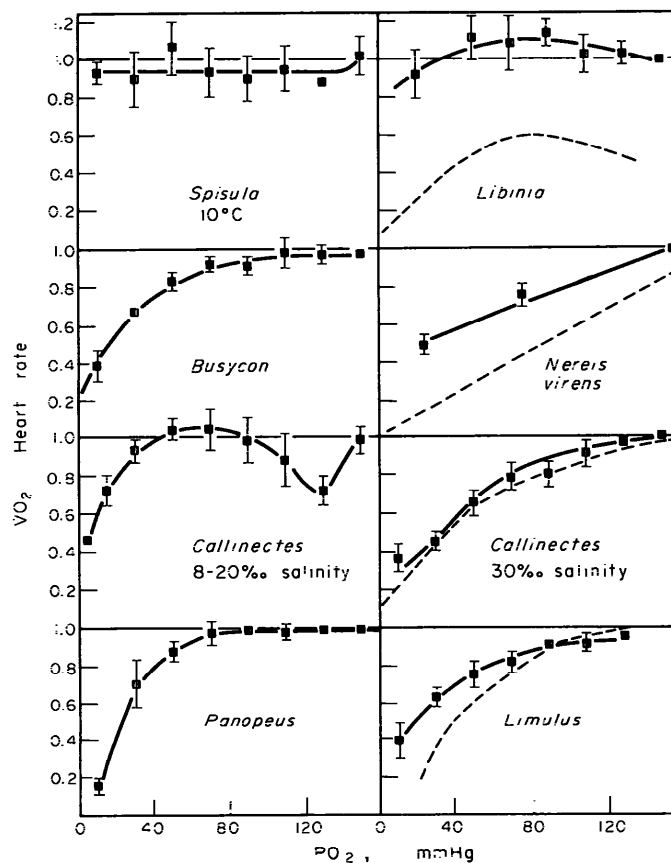
Fig. 4. The acute response to ambient PO_2 (reduced during period of 1–4 hr) of heart rate (mean $\pm$ S.E., ——) and oxygen consumption (.....). Salinity 35‰ and temperature 25°C unless otherwise noted. Heart rate (beats/min) and oxygen consumption (VO_2 μl/g hr) normalized by setting the initial value at 1.0 and expressing all subsequent values as fractions of 1.0. Oxygen consumption data for *Callinectes* from Mangum (1976), for *Nereis* from Mangum & Van Winkle (1973), for *Limulus* from Mangum *et al.* (1976) and for *Libinia* from Burnett (1977).

in these species is also accompanied by an increase in O_2 uptake (Oglesby, 1975).

Effects of declining O_2

Most of the species showed bradycardia at low PO_2, usually when the O_2 level fell below 80 mm Hg (Fig. 4); the response was often delayed. In no case did the heart of an animal cease to beat, even in anoxia for periods up to 6 hr. Compensatory tachycardia, which was significant, was observed only in *L. emarginata*, although *S. solidissima* strongly regulated its heart rate in response to the acute decrease in PO_2 (Fig. 5). It should be noted that PCO_2 and pH change very little in the high salinity waters used in the experiments except at very low PO_2, far below 80 mm Hg. Moreover, changes in pH or PCO_2 are often difficult to detect in shelled molluscs until the animal has experienced anoxia for periods far greater than the length of the present experiments (Booth & Mangum, 1978; Polites & Mangum, in preparation).

The response was highly variable in three species (*C. sapidus* at low salinity, *L. emarginata* and *S. solidissima*), both within and between individuals. The variability was especially notable in *C. sapidus*, because it contrasted sharply with the very consistent response to hypoxia at high salinity. In this species

acidification of the medium can be easily detected (Mangum *et al.*, 1976b) and therefore differences in pH or PCO_2 may be responsible for the variability. Other differences found at the experimental salinity are the critical PO_2, which is lower, and the magnitude of the response, which is twice as great (Fig. 4).

Like the response of oxygen uptake to declining oxygen levels (Mangum & Van Winkle, 1973) the response of heart rate does not appear to be correlated with environmental or taxonomic classification. When data describing the relationship of oxygen uptake to PO_2 are superimposed upon the heartbeat data, however, a strong correlation between the two is readily apparent (Fig. 4). This correlation supports the contention that the performance of the cardiovascular system in many species has a simple and direct influence on oxygen consumption, a point which has been elegantly argued by Coulsen *et al.* (1977) on quite different grounds. We should note, however, that without an oxygen carrier the blood may have little or no respiratory significance (Booth & Mangum, 1978).

Heart volumes

The various techniques for measuring heart volume yielded different results. The volumes calculated as exterior dimensions less the mean width of the heart

PO$_2$ = 137 mm Hg

PO$_2$ = 83 mm Hg

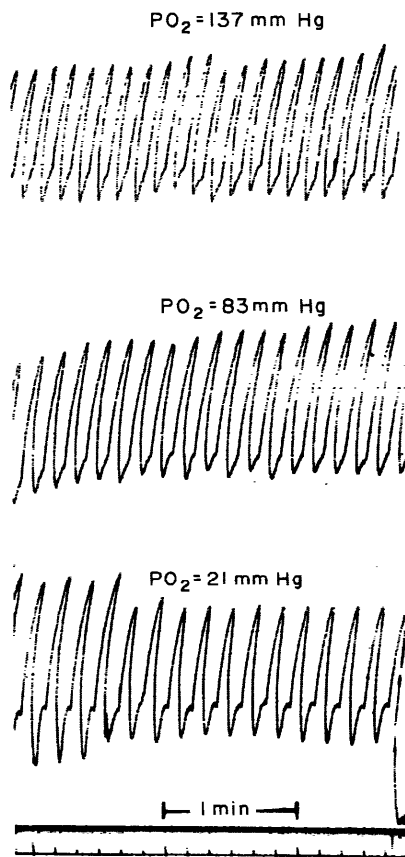PO$_2$ = 21 mm Hg

|— I min —|

Fig. 5. Record of the heartbeat of individual surf clam, *Spisula solidissima*, in declining oxygen tensions. Wet weight, 143 g; temp., 10°C; salinity, 30‰.

wall were always the greatest, and those obtained by filling and emptying the hearts with latex or seawater were consistently smaller. The discrepancy is probably due to incomplete emptying of the trabecular sinuses and thus imperfect displacement of the blood by the injection. While the dimensions may give an accurate estimate of total heart volume, the volumes injected are probably closer to stroke volume. In order to obtain conservative estimates of cardiac output, the heart volumes given in Table 4 were calculated as 50% (total heart volume + volume injected/2). Fifty per cent, the lower limit of the stroke volume in mammals (Rushmer, 1970), was chosen to maintain the conservative character of the results, even though Belman's (1975) studies indicate that 70% is the correct value for decapod crustaceans.

Heart size has also been calculated in the past as a percentage of total body weight, the factor ranging from about 0.1% (Maynard, 1960a) to 0.3% (Burger & Smythe, 1953). Using the higher figure the volumetric equivalents are always greater than the values given in Table 4, thus emphasizing the minimal magnitude of the calculations of cardiac output.

Cardiac output

In Table 4, cardiac output is calculated as (heart rate) (mean heart volume) (1/2) and compared with independent estimates obtained from the Fick equation. The agreement between the two sets of data is

very good, and the present results provide independent evidence of the diverse capabilities of the circulatory systems in different taxa (see also DeJours, 1970; Mangum 1977). Using a similar procedure. Ansell (1973) estimated cardiac output in a 200 g *Cancer pagurus* at 11°C to be 44–76 ml/kg min, which would be about 157–274 ml/kg min at 25°C if $Q_{10} = 2.5$.

Fine structure of cardiac muscle

The structure of the bivalve cardiac myofiber is discussed by Heyer *et al.* (1973). The sarcomeres are often described as rather loosely organized, with no M-bands, incomplete Z-lines and often no H-zones. They lack transverse tubules and, although no measurements of the density of sarcoplasmic reticulum have been reported, it appears to be rather small ($< 1\%$) and often difficult to resolve (Koch & Greenberg, 1977. personal communication).

The cardiac myofiber of *Panopeus herbsti* is illustrated in Fig. 6. The fiber is striated and the sarcoplasmic reticulum is enriched relative to that in other types of cardiac fibers. As shown in Fig. 6(B), the sarcoplasmic reticulun: occupies approx 6% of the cross-sectional area of the fiber.

DISCUSSION

Effects of temperature

In general, the results suggest that blood flow slows greatly at low temperature and that compensatory adaptations of heart rate do not offset depression of the rates of metabolic reactions by increasing oxygen convection to deep tissue. Indeed, in the molluscs *N. ponderosa* and *B. canaliculatum*, the temperature coefficients for heart rate in the range 10–25°C are more than twice those for oxygen uptake (Deaton & Mangum, 1976; Polites & Mangum, in preparation). In *N. ponderosa*, the respiratory properties of the blood appear to be highly adapted for functioning at temperatures below the summer maxima (Freadman & Mangum, 1976), and the low temperature sensitivity of oxygen consumption in that range is more closely related to adaptations of the carrier molecule than those of the heart. At 5°C, cardiac arrest in these species must effectively inhibit oxygen uptake by the blood, and one would expect a large drop in total aerobic metabolism in the interval 5–10°C. Data on oxygen uptake support this conclusion for *B. canaliculatum* (Q_{10} at 5–10°C = 55; Q_{10} at 10–23°C = 1.0) (Polites & Mangum, in preparation), but the response of oxygen uptake below 10°C in *N. ponderosa* is unknown.

In *Mytilus edulis*, a bivalve mollusc with no oxygen carrier in the blood, the temparature coefficients for oxygen uptake and heart rate are similar and low (Widdows, 1973). The relationship may be correlative and not causal, however, because the respiratory role of the blood in mussels appears to be very small (Booth & Mangum, 1978).

In the annelid *N. virens* blood pulse rate has a lower temperature coefficient (≤ 2.0) than oxygen uptake ($Q_{10} = 2.98$; Scott *et al.*, 1975). Econimides & Wells (1975) reported that the temperature dependence of its HbO_2 affinity (ΔH) is -17.97 kcal/mole, which is unusually large. Unless some other respiratory parameter such as ventilation results in a sub-

Table 4. Cardiac output, as calculated from the present results and from the
Fick equation, using data from the literature cited

Species	Temp. (°C)	Mean Heart Rate (bts/min)	Mean Body Wt. (gm.)	Mean Heart Vol. (ml.)	Cardiac Output (ml/kg-min) present results	Cardiac Output Fick	Source
Xiphosura							
Limulus							
polyphemus	25	37.5	601	3.3	103	--	--
	20	--	--	--	--	105	Mangum et al. (1976)
	15	23	601	3.3	63	78	Mangum et al. (1975)
unresponsive	18-20	11.7	601	3.3	32	--	--
Crustacea							
Callinectes							
sapidus	22-25	127	141	0.42	189	175	Mangum (1976b)
	15	62	141	0.42	92	--	--
Carcinus							
maenas	15	90	60-80	--	--	70-80	Taylor (1976b)
Cancer							
magister	8-10	--	1000	--	--	29	Johansen et al(1970)
	8-10	70	1000	--	--	--	Florey & Kriebel(1973)
	7-9	56	811	--	--	95	McDonald (1977)
Homarus							
americanus	18-20	--	400-600	--	--	88	Redmond (1955)
	13.5	92	400-600	--	--	121	McMahon & Wilkens(1975)
Libinia							
emarginata	25	81.5	135	0.33	100	--	--
Panulirus							
interruptus	16	60	600	1.95	--	128*	Belman (1975)
Mollusca							
Bivalvia							
Noetia							
ponderosa	25	14.5	22.5	0.062	20.0	25	Deaton & Mangum(1976)
	15	5.0	22.5	0.062	6.9	--	--
Spisula							
solidissima	10	9.9	141	0.40	12.0	--	--
Gastropoda							
Busycon							
canaliculatum	20	14.1	49	0.18	25.9	--	--
Annelida							
Nereis							
virens	15	6.8	4.63	0.01	14.7	--	--

* Direct estimate of flow.

stantial decrease in burrow PO_2, it is unlikely that blood PO_2 would fall enough to allow unloading of the carrier at low temperature, and oxygen uptake would be expected to decline sharply.

Heart rates in the arthropods decrease as expected ($Q_{10} \sim 2$) in the interval 15–25°C, but at 5°C the heartrate in *C. sapidus* slows to a very low value. In this species the response of oxygen uptake is very similar (Mauro, 1977), and it is correlated with a behavior pattern known as "hibernation", which appears abruptly at 10°C. Its respiratory basis is presently under investigation. The sharp decrease in heart rate at low temperature apparently occurs in other portunid crabs as well (Ashanullah & Newell, 1971; Taylor et al., 1973).

Effects of salinity

The scope of the available data on cardiac responses to a salinity change is still too limited to reveal general trends among the various animal groups. Among the crustaceans, the group for which there are the most data, the common response to any salinity change is tachycardia (Spaargaren, 1973, 1974), although Taylor et al. (1977) found no change in heart rate in *Carcinus maenas* upon 50% dilution of the medium. Nonetheless, the present results con-

sidered with other respiratory responses can elucidate the role of the cardiovascular system in adaptation to estuarine habitats.

The responses observed at low salinity in *L. polyphemus* and *C. sapidus* were predicted by Fick calculations of cardiac output (Mangum et al., 1976a; Mangum & Towle, 1977). In *L. polyphemus* the bradycardia permits adjustments of blood PO_2 that enhance the respiratory role of hemocyanin in the altered ionic environment in the blood. The onset of tachycardia in *C. sapidus* below, and not above, 10‰ is additional evidence that the adjustment to oligohaline waters in this extremely euryhaline crab demands special adaptations that are not utilized in less dilute habitats.

The transient bradycardia in *S. solidissima* immediately following transfer to a dilute medium is not surprising. In bivalves, volume readjustment at low salinity requires 48 hr (Pierce, 1971). Swelling may influence the mechanical properties of the muscle, and the ionic change could decrease excitability. The ensuing tachycardia at a reduced salinity, which apparently has not been reported previously, is not easily explained by a direct mechanical or ionic effect on the muscle. It could involve the nervous system, either the cardioregulator nerves directly, or a neuroendocrine secretion.

Fig. 6. (A) Longitudinal section through a cardiac myofiber of *Panopeus herbsti*. showing prominent striations. Especially evident are the light I zone. (I) and H zone. (H) with the central M line. (M). Z line, (Z); sarcoplasmic reticulum. (SR); sarcolemma. (S); sarcolemma invagination. (SI); mitochondria. (Mt); basement membrane. (Bm). (B) Cross-section through a cardiac myofiber of *Panopeus herbsti*, showing the extent of sarcoplasmic reticulum. Myofibril, (Mf.) × 30.000.

In the two nereids, *N. succinea* and *N. virens*, the increase in blood pulse rate at low salinity presumably enhances oxygen transport to vascularized tissue, and a parallel increase in ventilation (M. A. C. Russell, unpublished data) provides additional oxygen to unvascularized tissue. Thus, the observed increase in oxygen uptake (Oglesby, 1975; Oglesby & Mangum, in preparation) involves both the cardiovascular and ventilatory systems.

Effects of hypoxia

Together with the results of previous investigations (Bayne, 1971; Brand & Roberts, 1973; Taylor, 1976a; Booth & Mangum, 1977), the present findings suggest that the most common response of heart rate to hypoxia is bradycardia. It occurs in bivalve molluscs (Bayne, 1971; Brand & Roberts, 1973; Taylor, 1976; Booth & Mangum, 1978), in gastropod molluscs (Fig.

4), in decapod crustaceans (Stiffler & Pritchard, 1972; Taylor *et al.*, 1973; Hill & Koopowitz, 1975; McMahon & Wilkens. 1975; Belman, 1976; Coyer, 1977; also Fig. 4) and in xiphosurans (Fig. 4). It may take the form of a high degree of regulation followed by a sharp decline at some critical PO_2 (*e.g. Panopeus* in Fig. 4), or imperfect regulation with no definite critical PO_2 (e.g. *Busycon* in Fig. 4). In several decapods. bradycardia at very low PO_2 is preceded by a slight tachycardia at intermediate oxygen levels (McMahon *et al.*. 1974; Hill & Koopowitz. 1975; *Libinia* in Fig. 4). In *Libinia*, the tachycardia is significant. and its effect can be seen in an increased oxygen uptake (Burnett, 1977). which may involve an increase in ventilation as well. as in other crustaceans (McMahon *et al.*, 1974). The only species in which heart rate declines linearly with PO_2, with no regulatory phase are the annelids *Nereis virens* (Fig. 4) and *N. succinea*.

Two exceptions, species that maintain a constant heart rate despite a decline in ambient PO_2, are known: the surf clam *Spisula* (Fig. 4) and the lobster *Homarus vulgaris* (Butler *et al.*, 1978). As pointed out by Bayne (1976), heart rate in the bivalves slows only after the cessation of oxygen uptake, and presumably a decrease in blood PO_2, which may mean that the heart responds directly to blood oxygen levels without the involvement of the central nervous system. If this hypothesis is correct, then the highly regulatory response of *Spisula* implies either a reduced O_2 sensitivity of the heart muscle, or a regulatory response of another respiratory mechanism that results in the maintainance of a constant blood PO_2. Little is known about O_2 uptake and transport in this species, which inhabits subtidal waters off sandy beaches where it does not experience hypoxia.

Perhaps the most important generality is the scarcity of the compensatory increases in heartbeat that are typical of the vertebrate response to hypoxia. We should emphasize that the prevalent response, significant bradycardia, is not associated with distress or mortality; it occurs at oxygen levels which are well within the range tolerated for indefinite periods.

Cardiac output

In Table 4, cardiac output was calculated on the assumption of a constant value for stroke volume. The factor for stroke volume as a fraction of total volume has been investigated only in the crustaceans, and indirect evidence suggests that it may not be constant in an individual, even under stable environmental conditions (McMahon & Wilkens, 1977). The assumptions are made to obtain estimates of blood flow that can be compared with calculations from the Fick equation. We do not intend to imply that stroke volume in fact remains unchanged throughout the animal kingdom, regardless of environmental conditions.

The performance of different kinds of circulatory systems has received more attention as blood gas tensions are determined. It is increasingly clear that the simpler circulatory systems are not built for high velocity blood flow, but that earlier skepticism regarding the performance of the crustacean heart is unwarranted. The effects of body size and temperature have not always been considered. Assuming that stroke volume is not influenced by either, the present results suggest that apparent disagreement between the Fick predictions of cardiac output are due to these variables. For example, if the Q_{10} for heart rate is 2.0 (Table 3), then blood flow at 23–26°C (Redmond, 1955) should be 4 times that at 8–10°C (Johansen *et al.*, 1970). If the coefficient ($b - 1$) describing the logarithmic relationship between heart rate and body size is −0.39 (Fig. 2), then heart rate in a 0.75 kg crab (Redmond, 1955) should be 1.5 times that in a 1.0 kg crab (Johansen *et al.*, 1970). In sum, the two sets of data would be expected to differ by a factor of six; the actual discrepancy is a factor of five (Redmond, 1955; Johansen *et al.*, 1970). We should point out that body size and temperature are not known to influence blood gas tensions, on which the data in question were based. Our point is simply that very high rates of blood flow in crustaceans are real, a conclusion which is strongly supported by direct
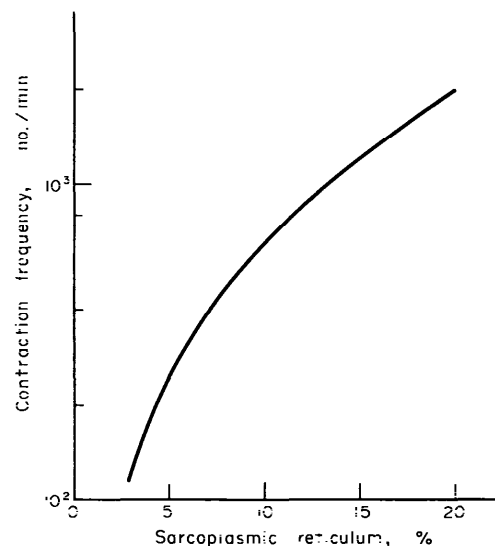

Fig. 7. The relationship between contraction frequency and density of the sarcoplasmic reticulum in arthropod fast muscles. Modified from Josephson (1975), by taking the reciprocal of twitch duration (crest to 50% recovery at 25°C) as an index of the maximum rate at which a muscle can contract and still give tension oscillation of appreciable amplitude.

measurements (Belman, 1975; Belman & Chidness, 1976).

The basis of the performance capability of the crustacean heart lies in part in the distinctive properties of the cardiac myofibers, which differ fundamentally from those in vertebrates or bivalve molluscs. In crustaceans the fibers are striated, and the shortening velocity is directly proportional to sarcomere length and fiber length, while the brevity of individual contractions is related to the development of the sarcoplasmic reticulum, which determines the distance for the diffusion of Ca^{-2} between sites of activation and sequestering. The distance is a critical factor limiting the refractory period of the muscle and possibly other aspects of excitation–contraction coupling. Maximum contraction velocities and frequencies of the myofiber should be greater in crustaceans than in vertebrates, due to the differences in all three variables of functional organization. To illustrate the point, the capability of the *Panopeus* heart can be predicted from Fig. 6 and from the relationship between twitch duration and the development of the sarcoplasmic reticulum shown by Josephson (1975). This relationship is reproduced in Fig. 7, with the ordinate transformed to equal heart rate. At 6% sarcoplasmic reticulum, the twitch duration of the fiber should be 0.18 sec, and the heart rate 315 beats/min. The mean heart rate is only 132 beats/min and the maximum observed was 240 beats/min. Far from exceeding its expected capability, the heart in this crab does not equal the performance levels of other arthropod fast muscles.

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