

THE EFFECTS OF ENVIRONMENTAL VARIABLES ON THE
HEART RATES OF INVERTEBRATES

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Peter Lee deFur

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This thesis is submitted in partial fulfillment of
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Peter L. de Tur
- Author

Approved, May 1977

Charlotte P. Mangum
Charlotte P. Mangum

Eric L. Bradley
Eric L. Bradley

Gregory M. Capelli
Gregory M. Capelli

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TABLE OF CONTENTS

	page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	vii
INTRODUCTION	2
METHODS AND MATERIALS	5
RESULTS	8
DISCUSSION	23
SUMMARY	36
LITERATURE CITED	37
VITA	42

LIST OF TABLES

Table	page
1. Control heart rates (=1.0 in Figs 2 & 3).	9
2. Relationship of locomotor activity and heart rate (mean \pm S.E.) in <u>Libinia emarginata</u> and <u>Limulus</u> <u>polyphemus</u>	10
3. The effect of acclimation temperature on heart rates (mean \pm S.E.). Q_{10} values are the means for a set of individuals.	14
4. Cardiac output as calculated from 1) the Fick equation, using data from the literature, and 2) results obtained by the present author	22

LIST OF FIGURES

Figure	page
1. Recording of the heart rate of a female blue crab, <u>Callinectes sapidus</u> , (25°C, 35 o/oo; P _O ₂ = 140 mmHg) demonstrating cyclic variations in heart rate . . .	12
2. Recording of the heart rate of a male mud crab, <u>Panopeus herbsti</u> , exhibiting cardiac arrest (25°C, 35 o/oo; P _O ₂ = 159 mmHg).	13
3. Responses of the heart rates of three species to acclimation salinity (25°C for <u>Callinectes sapidus</u> ; 10°C for <u>Spisula solidissima</u> ; 23°C for <u>Limulus polyphemus</u>). P _O ₂ = 130-159. Control rates (= 1.0) given in Table 1	17
4. The acute effect of ambient P _O ₂ (reduced over period of 1-4 hr) on heart rate (mean ± S.E., solid line) and oxygen consumption (dotted line). Salinity 35 o/oo and temperature 25°C unless otherwise noted	19
5. Recordings of the heart rate of a single clam, <u>Spisula solidissima</u> , in declining oxygen tensions. Body weight = 143 gm; T = 10°C; salinity = 30 o/oo . . .	20
6. Regression line (solid) describing present data for heart rate as a function of body weight in <u>Limulus</u> (open squares), <u>Libinia</u> (open triangles), <u>Panopeus</u> (open circles), and <u>Hemigrapsus</u> (closed triangles). Data for <u>Callinectes</u> (closed circles) is excluded. R = 0.92, P < .001, slope (b-1) = -1.39. Dotted line taken from Schwartzkopff (1955), 20-22°C	32
7. Longitudinal section through a cardiac myofiber of <u>Panopeus herbsti</u> , indicating the 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. X 19,000 .	34
8. Cross section through a cardiac myofiber of <u>Panopeus herbsti</u> , showing the extent of sarcoplasmic reticulum. Myofibril, Mf. X 30,000	35

ABSTRACT

The effects of temperature, salinity and declining oxygen on heart rates of eight invertebrates have been measured. The heart rates of all species are temperature sensitive and in the range 5-15°C some species have elevated sensitivities. Thus, compensatory mechanisms other than heart rate must be evoked to explain the general temperature sensitivities of oxidative metabolism. Cardiac responses to reduced salinity provide evidence that circulation plays an important role in respiratory adaptations to low salinity. All species but one exhibit bradycardia in declining oxygen and no species compensates by increasing heart rate as do the vertebrates.

The diverse capabilities of crustacean hearts are emphasized by the data. Estimates of cardiac output which exceed those in fish and approach values for warm-blooded vertebrates are explainable in terms of muscle structure.

THE EFFECTS OF ENVIRONMENTAL VARIABLES ON THE
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INTRODUCTION

The question of how environmental changes affect the respiratory performance of different kinds of circulatory systems has remained for the most part unanswered. Until recently, studies of invertebrate hearts have employed isolated or exposed preparations from molluscs and a few decapods, and in vivo preparations of only the smaller crustaceans (Cladocera, Amphipoda, Isopoda, etc.) (Maynard, 1960; Hill & Welsh, 1966). In vitro data supply information on control mechanisms, yet contribute little to an understanding of physiological function. Furthermore, most investigators have examined the acute rather than the more meaningful long term responses to environmental variables such as temperature and salinity. Thus, the respiratory role of cardiovascular adaptations cannot be accurately predicted from the available information.

With the one exception of one mollusc (Bayne, 1973), there are no data on the cardiac responses of intact animals after adjustment to the reduced salinities regularly encountered by many species. And yet studies on the acute effects of ionic or osmotic change suggest that crustacean cardiovascular systems may respond to salinity change (Maynard, 1960; Hume & Berlind, 1976).

Although temperature coefficients for heart rate in the molluscs and crustaceans have been widely reported (see Maynard, 1960; Hill & Welsh, 1966), only a few recent measurements have been made on acclimated, intact animals (Ahsanullah & Newel, 1971; Taylor et al., 1973; Widdows, 1973; Earll, 1975). In fact, studies on the same species yield

data which are not in complete agreement, for example in the crab, Carcinus maenas (Ahsanullah & Newel, 1971; Taylor et al., 1973; Taylor, 1976).

The response of heart rate to low or declining oxygen has received some attention in intact bivalves and crustaceans; characteristic response is a decrease in hypoxic waters. At least seven species of bivalves exhibit some form of bradycardia during hypoxia, but the degree is highly variable (Lowe & Trueman, 1972; Coleman, 1976; Deaton & Mangum, 1977). A compensatory tachycardia may or may not precede the ultimate slowdown, depending on species (Bayne, 1971; Brand & Roberts, 1973; Taylor & Brand, 1975; Booth & Mangum, 1977). Similarly, crustaceans generally exhibit bradycardia in declining oxygen or hypoxia, although the extent varies greatly from 50 to 75 % (Larimer, 1962; Florey & Kriebel, 1973; Taylor et al., 1973; McMahon et al., 1974; Hill & Koopowitz, 1975; McMahon & Wilkens, 1975; Belman & Childress, 1976; Coyer, 1977). Other groups of invertebrates are not well known.

In addition to the scarcity of information on the influence of important environmental variables, fundamental aspects of invertebrate cardiovascular systems are poorly understood. For example, conflicting data exist on the cardiac output capabilities of the crustacean heart (Johansen et al., 1970; Belman, 1975), with estimates differing by factors of 5.

Heart rates of eight species representing four phyla were measured under one or more of three experimental conditions: long term exposure to normal and reduced salinity, long term exposure to high and low

temperature, and brief exposure to declining oxygen. An attempt is made here to reach physiological conclusions in light of known respiratory and anatomical parameters.

METHODS AND MATERIALS

Most of the animals were collected or purchased locally in the lower Chesapeake Bay. Nereis virens was obtained from the Supply department, Marine Biological Laboratory, Woods Hole, Mass. Animals were maintained in aerated, recirculating water, using either natural seawater or York River water (16-20 o/oo) made up to 32 o/oo.

Heart rates of animals other than Nereis virens were monitored using techniques described by Trueman (1967) and Trueman et al. (1973). Small holes were drilled in the shell or carapace, thin silver, platinum, or gold electrodes were inserted and held in place with dental wax (Surgident). Using an impedance pneumograph (E & M, MK IV) a small (5 μ a) current was passed between the electrodes, the changes in impedance converted to a voltage signal, and the resulting signal was 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 held constant throughout. After surgery, the animals were held in an incubator (Lab Line) prior to experiments.

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, following which, either salinity or temperature was changed and heart rates measured for the following 6-12 days.

Blood pulse rates in the dorsal vessel of the annelid Nereis virens were visually observed while the worms were 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 for 6 days prior to the declining oxygen experiment, then for 2 days following. The temperature was then changed to 5 or 25°C and pulse rates were counted over the next 4 consecutive days.

Several techniques were used to measure heart volumes. All intact, exposed hearts were measured with calipers and the volume calculated. A 1.0 ml syringe was then used to inject and withdraw seawater and the volume noted. Latex was also injected and the volume determined by displacement. Finally, in the case of Limulus polyphemus, Libinia emarginata and Callinectes sapidus, other hearts were ligated, excised, and filled with water.

Experiments on the heart rates in declining oxygen were conducted in closed respiratory vessels, or in aquaria with plastic wrap placed on the surface of the water. Once the heart rate stabilized after the animal was placed in the vessel (1-12 hours), 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). Measurements of heart rates of 0.5-1.0 min. duration were made every 0.5 ppm as the oxygen declined. In the case of Spisula solidissima, nitrogen was used to reduce the oxygen at the rate of 1.0 ppm/20 min.

Since most recordings during the entire study were relatively short (0.5-5.0 min.), the entire record was used whenever possible in determining individual rates. When only part of a recording was used,

either the initial portion or that portion free from mechanical or electrical interference was used. Recordings were not used if electrodes were found to be loose.

Although the data are presented below as mean values (\pm Standard Error) for all of the individuals of a species, they were in fact taken and analyzed as paired observations on a particular individual before and after an environmental change. Data were analyzed according to Student's t for paired observations.

RESULTS

GENERAL OBSERVATIONS ON SENSITIVITY

The data in Table 1 show that heart rates are less variable in the molluscs and Nereis virens than in the three crustaceans and Limulus polyphemus. In all likelihood, the variability results from sensory induced modulation of heart rate. In the three crustaceans and Limulus, it was casually observed that sudden increases in heart rate were associated with increased activity, increased lighting and other visual stimuli, or prodding. Florey & Kriebel (1973) have shown that the degree of external tactile stimuli has a direct positive effect on the heart rate of Cancer magister, and Hill & Koopowitz (1975) found that heart rates in Scylla serrata declined 45 % during sensory deprivation. The greater variability in the decapods and Limulus is true within species as well as between species. Variations within an individual crab were as great as 40 beats/min in Callinectes, 35 beats/min in Libinia, and 20 beats/min in Limulus. Most of the variation in Libinia and Callinectes was not closely related to activity as opposed to Limulus in which heart rate seemed related to the discrete activity levels which were observed.

Limulus polyphemus was the only species to exhibit discrete activity levels on a regular basis (Table 2). Johansen & Petersen (1975) also observed different activity levels with corresponding levels of oxygen consumption and they found a six-fold difference between the extremes. In the present study, a similar relationship between activity

TABLE 1

CONTROL HEART RATES (=1.0 IN FIGS 2 & 3)

Species	Temp. (°C)	PO ₂ (mmHg)	Salinity (o/oo)	Body Weight (gm)	Mean Heart Rate (beats/min.) ± S.E.	No. Animals	N	Maximum Observation Period (da)
Xiphosura								
<u>Limulus</u>								
<u>polyphemus</u>	25	120-140	30-35	210-857	37.5 ± 3.5	10	80	30
Crustacea								
<u>Callinectes</u>								
<u>sapidus</u>	25	140-150	30-35	80-192	127 ± 9.0	20	120	50
<u>Libinia</u>								
<u>emarginata</u>	25	140-150	30-35	77-221	81.5 ± 8.9	8	51	39
<u>Panopeus</u>								
<u>herbsti</u>	20	159	26-32	11-23	131.7 ± 11	6	15	8
Mollusca Bivalvia								
<u>Spisula</u>								
<u>solidissima</u>	7-10	159	29-33	104-158	8.87 ± 0.19	15	131	45
<u>Noetia</u>								
<u>ponderosa</u>	25	159	34	15.5 ± 3.2	14.5 ± 0.8	5	20	12
Gastropoda								
<u>Busycon</u>								
<u>canaliculatum</u>	20	159	26-35	40.3-60.7	14.1 ± 1.14	4	29	34
Annelida								
<u>Nereis</u>								
<u>virens</u>	15	159	34	3.1-7.9	6.82 ± 0.06	12	96	12

TABLE 2

RELATIONSHIP OF LOCOMOTOR ACTIVITY AND HEART

RATE (MEAN \pm S.E.) IN LIBINIA EMARGINATA AND LIMULUS POLYPHEMUS

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

level and heart rate was observed, but there was only a four-fold difference between extremes. The discrepancy between parameters measured during maximum activity is likely due to greater activity during burrowing, a phenomenon not studied here. Johansen & Petersen (1975) also recorded the lowest rates after the animals were buried, which is consistent with the present observations (Tables 2 and 3).

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

Cardiac arrest occurred only in the three species of decapods and could not be induced by occasional mechanical or visual stimulation, an effective mechanism in other species of decapods (McMahon & Wilkens, 1972; Florey & Kriebel, 1973). Fig. 2 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. herbsti did not exhibit cardiac arrest as often as the other species and they were of a shorter duration (5-10 sec).

TEMPERATURE

The responses of heart rate to a change in acclimation temperature suggest that cardiovascular adaptation does not mitigate the temperature sensitivity of oxidative metabolism (Table 3). The Q_{10} values either approach or fall within the range 2.0-2.5 which is regarded as

Fig 1. Recording of the heart rate of a female blue crab, Callinectes
sapidus, (25°C, 35 o/oo; PO₂ = 140 mmHg) demonstrating cyclic
variations in heart rate.

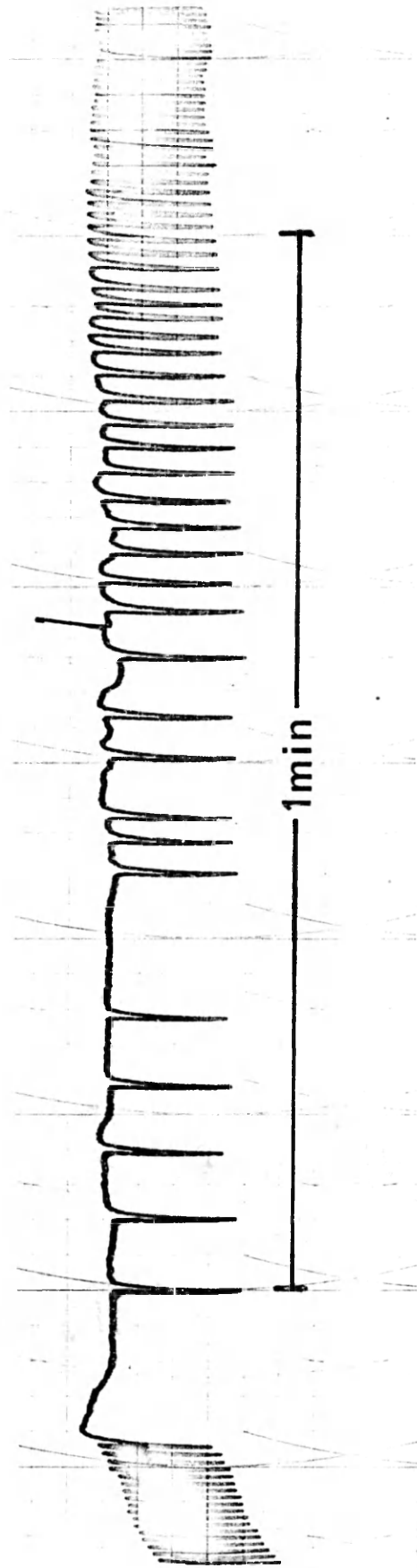


Fig. 2. Recording of the heart rate of a male mud crab, Panopeus
herbsti, exhibiting cardiac arrest (25°C, 35 o/oo; $PO_2 =$
159 mmHg).

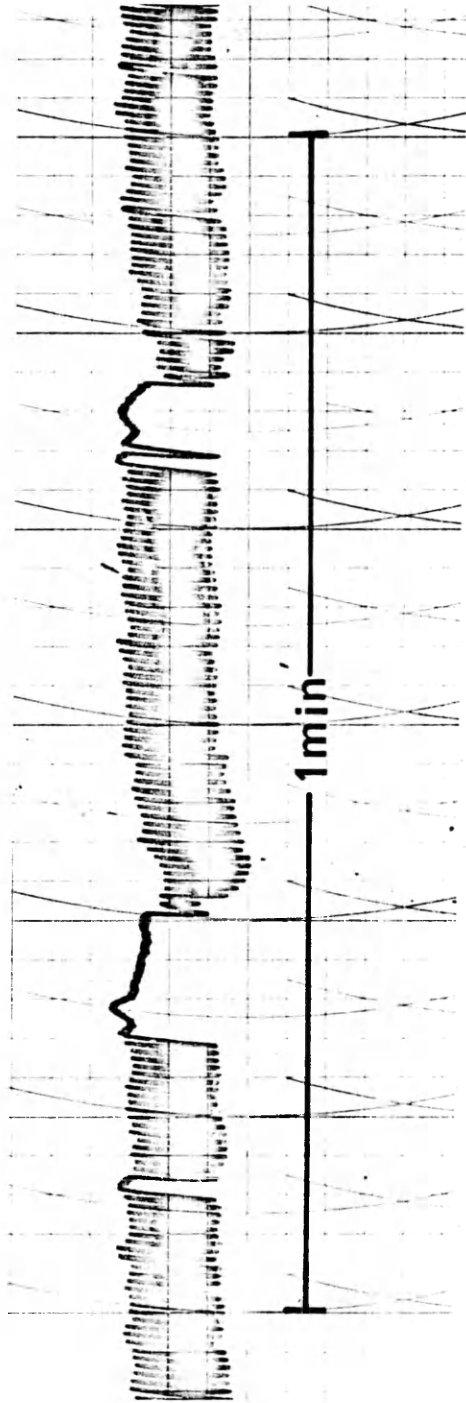


TABLE 3

THE EFFECT OF ACCLIMATION TEMPERATURE ON

HEART RATES (MEAN \pm S.E.). Q_{10} VALUES ARE THE

MEANS FOR A SET OF INDIVIDUALS.

Species	No. Animals	5°C	Heart Rate (beats/min.) 15°C	25°C	Q_{10}
<u>Limulus polyphemus</u> -- active	4		23.0 \pm 4.0	39.0 \pm 3.4	1.77 \pm .02
inactive	2		10.3 \pm 2.4	19.3 \pm 5.5	2.0 \pm .3
unresponsive	1		4.60 \pm 0.4	8.67 \pm 1.5	1.88
<u>Callinectes sapidus</u>	6		62.3 \pm 10	120.5 \pm 15	2.0 \pm 0.1
	1	8.1 \pm 1.9		143.0 \pm 3.0	4.2
<u>Libinia emarginata</u>	4		38.0 \pm 4.0	72.4 \pm 8.8	1.92 \pm 0.19
<u>Spisula solidissima</u>	4	8.5 \pm .62(7°C)	14.0 \pm .76(12°C)		2.88 \pm 0.56
<u>Noetia ponderosa</u>	5	0	5.0 \pm .24	14.5 \pm .79	2.92 \pm 0.22
<u>Busycon canaliculatum</u>	1	0	9.43 \pm .11	20.7 \pm .58	2.19
<u>Nereis virens</u>	6		6.8 \pm .42	12.8 \pm 1.4	1.86 \pm 0.14
	6	3.33 \pm .16	6.8 \pm .27		2.04 \pm 0.08

typical of physiological processes (Maynard, 1960; Prosser, 1973; Bayne, 1976). With the exception of two Libinia emarginata, the cardiac acclimations to temperature were completed within the first 24 hours, and there was no further change. There were no cases of markedly reduced temperature sensitivity ($Q_{10} < 1.8$) in any species. There was no indication that the temperature sensitivity of heart rate is either influenced or paralleled by changes in motor activity (Table 2), although the question was not systematically investigated.

The temperature sensitivity of heart rate is greater in the two bivalves (Table 3) than in members of the other phyla. In the same temperature range, the Q_{10} for oxygen uptake in Noetia ponderosa, however, is only 1.72 (Deaton & Mangum, 1976), suggesting that other respiratory parameters, such as the conspicuously reduced temperature sensitivity of hemoglobin oxygen affinity, more than compensates for the reduction in heart rate (Freadman & Mangum, 1976). The thermal sensitivity of oxygen consumption in Spisula solidissima, having no oxygen carrier in the blood, is unknown.

Repeated observations over a 3 day period on three N. ponderosa indicate that heart beat 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 a single Busycon canaliculatum studied. Although heart beat never ceased completely in the crabs, in the same range, the temperature coefficient in one individual C. sapidus is very high (4.2).

SALINITY

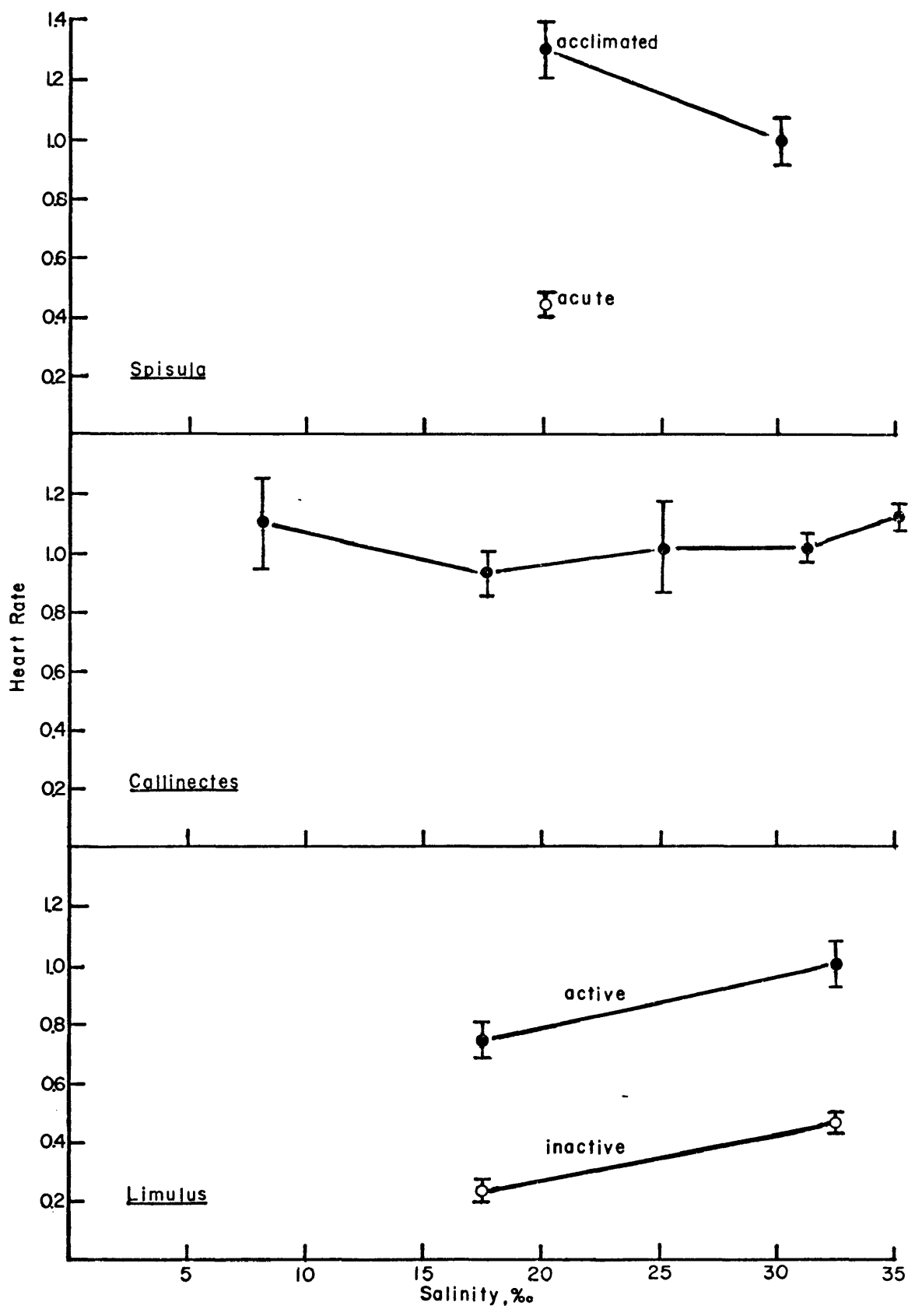
The responses of heart rate to decreased salinity differs in each of the three species tested (Fig. 3).

The heart rate of Limulus polyphemus declines an average of 24.7 % when the animals are transferred from 30 o/oo to 20 o/oo (Fig. 3). The period required for stabilization is three days, after which the rates no longer change. Active and inactive rates are affected similarly by salinity, and the per cent changes are not significantly different ($P > .05$ according to Student's t). The observed response agrees quantitatively with that predicted from Fick calculations (Mangum et al., 1976).

The response of S. solidissima is more complex than that in other species (Fig. 3); an initial bradycardia (58 %) is followed by a lasting tachycardia. The increase in rate (30 %) is significant ($P < .05$, Student's t for paired observations), and represents an increase of 2.7 bts/min over an initial rate of 8.9 bts/min. It should be pointed out that one of the six animals in this study failed to survive the dilution, consistent with the conclusion that 20 o/oo is close to the lower limit of salinity tolerance in this species (Castagna & Chanley, 1973). The only other bivalve species for which data have been reported (Mytilus edulis) shows no change in heart rate in reduced salinities (Bayne, 1973).

In the two specimens of Busycon 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 declined initially from 11.1 (± 0.2) bts/min at 35 o/oo to 6.5 (± 0.3) bts/min at 26 o/oo over 3 days then increased to 12.5 (± 0.7) bts/min at 20 o/oo (4 da).

Fig 3. Responses of the heart rates of three species to acclimation salinity (25°C for Callinectes sapidus; 10°C for Spisula solidissima; 23°C for Limulus polyphemus). $PO_2 = 130-159$. Control rates (= 1.0) given in Table 1.



The other animal showed bradycardia (14.9 ± 1.0 to 9.67 ± 0.33 bts/min) upon transfer from 35 to 20 o/oo, but the observation period (3 da) was too short to permit osmotic acclimation (G. Polites, unpublished data).

Heart rate of C. sapidus does not change at reduced environmental salinity (Fig. 3). Although there appears to be a slight bradycardia at 15-20 o/oo and a slight tachycardia at 8 o/oo, the differences are not significant ($P > 0.05$, Student's *t* for paired observations).

DECLINING OXYGEN

The most common response to an acute decrease in PO_2 is bradycardia, often delayed (Fig. 4). Compensatory tachycardia occurred only in the crab Libinia emarginata. In no case was cardiac arrest observed, even at $PO_2 = 0$ mmHg for 1-6 hours. In three species (Callinectes sapidus acclimated to low salinity, Libinia emarginata, and Spisula solidissima) the response was highly variable both among and within individual animals.

Greatest variability of heart rate occurred in C. sapidus at low salinity (8-20 o/oo). It is especially noticeable when compared to the response at high salinity. The variability may be a result of increased activity, since crabs were more active and excitable at low salinity. Other differences related to the salinity are the PO_2 threshold for the decline in heart rate (Fig. 4). The bradycardia at low salinity is twice as great, 10 vs. 20 % decrease in rate per 10 mmHg decline.

Although there appear to be no patterns which separate animals by phylum or habitat, several points should be noted. S. solidissima, the only species studied which has no blood pigment, is the only one that fails to show bradycardia (Fig. 5). Nereis virens, with the most primitive

Fig 4. The acute effect of ambient PO_2 (reduced over period of 1-4 hr) on heart rate (mean \pm S.E., solid line) and oxygen consumption (dotted line). Salinity 35 o/oo and temperature 25°C unless otherwise noted. Heart rate (in bts/min) and oxygen consumption (in μ l/gm/hr) were normalized by setting the initial value at 1.0 and expressing all subsequent values as fractions of 1.0.

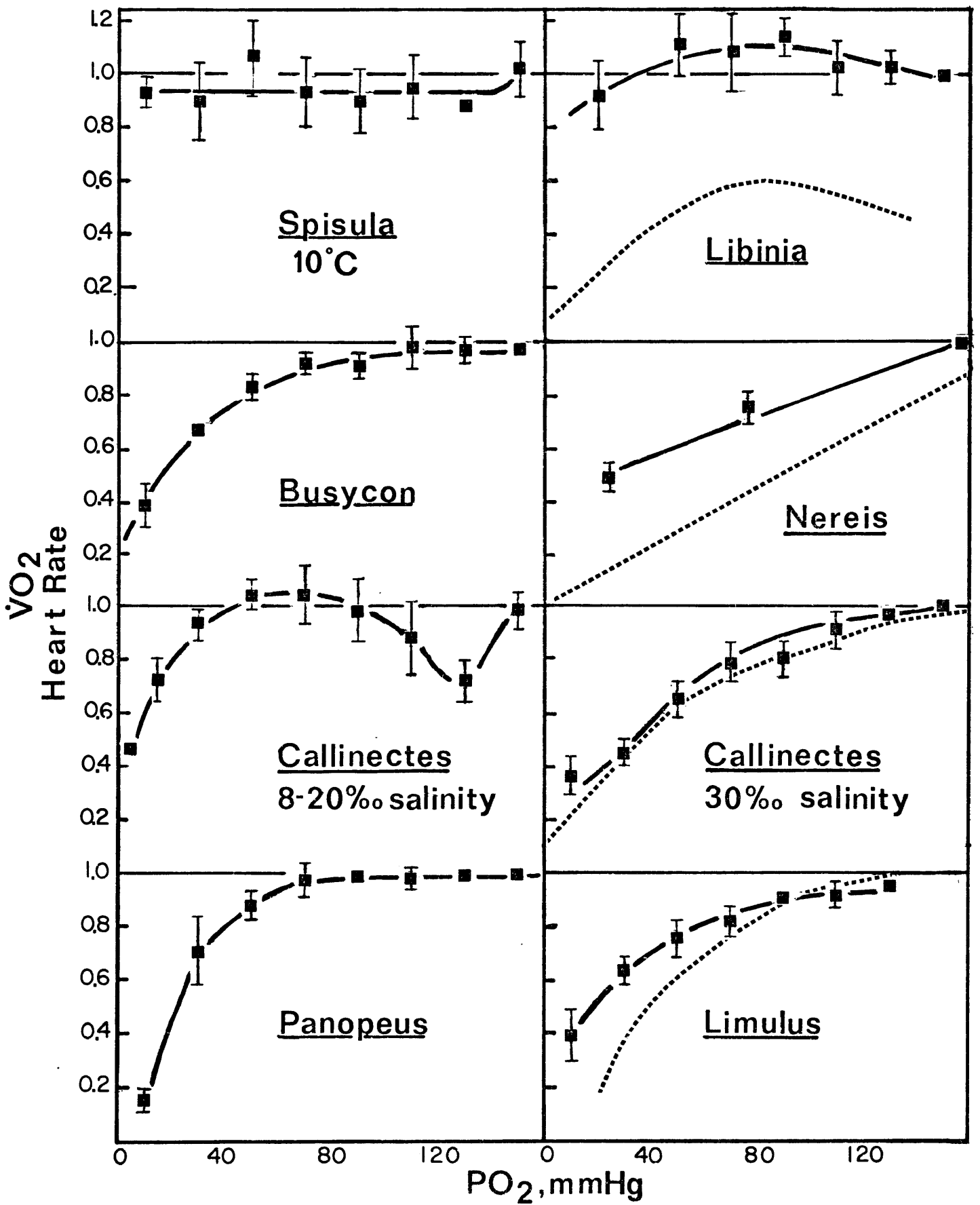
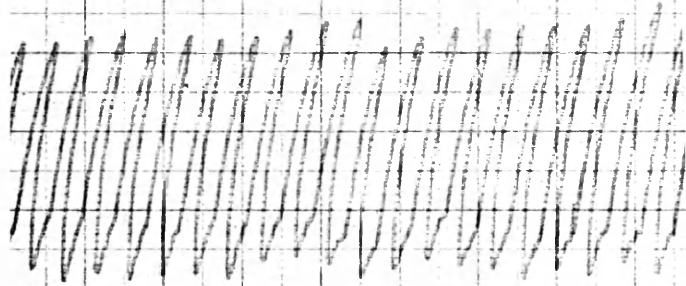
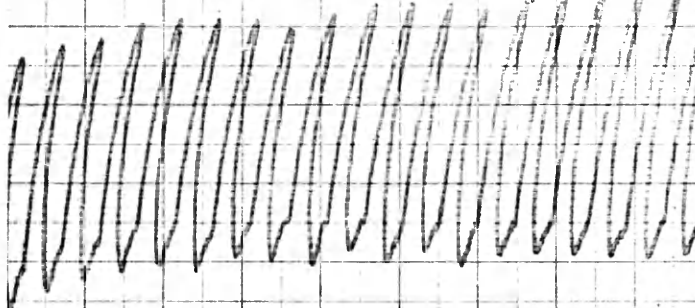


Fig. 5. Recordings of the heart rate of a single clam, Spisula
solidissima, in declining oxygen tensions. Body weight =
143 gm; T = 10°C; salinity = 30 o/oo.

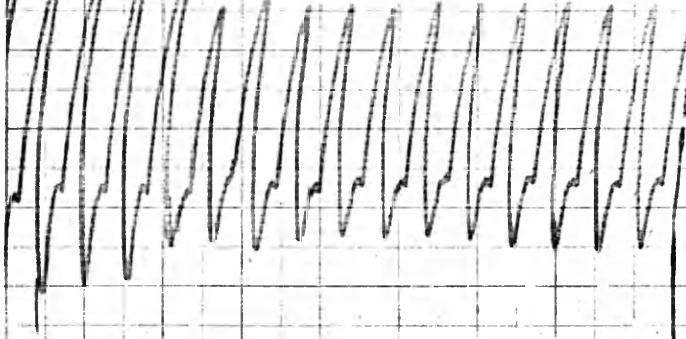
$PO_2=137\text{mmHg}$



$PO_2=83\text{mmHg}$



$PO_2=21\text{mmHg}$



— 1 min —



"heart" among the species studied, exhibits no regulation of heart rate, also true for oxygen uptake in nereids (Mangum & Van Winkle, 1973). This correlation between oxygen uptake and heart rate supports the contention that the performance of the cardiovascular pump has a direct and simple influence on total oxidative metabolism, a point which has been elegantly argued by Coulsen et al. (1977) on quite different grounds.

HEART VOLUMES

Measurements of heart volume yielded conflicting data, depending on the technique used. If the heart was ligated and excised, very large volumes were obtained by filling (e.g., 10-25 ml in L. polyphemus). If the heart was left in situ, the volume estimated by injecting either water or latex were very similar to one another and much smaller than those obtained by alternative procedures. Finally, the volumes calculated from outer measurements in situ were close to those obtained by filling excised hearts. The estimates obtained from excised hearts and from exterior dimensions are probably too great; in the former case the elasticity of the muscle allows distention beyond physiological limits. The latter technique assumes the heart does not allow for the space occupied by the heart wall. The latex volume is probably too small, due to leakage, contraction of the muscle and constriction by ligatures. The error of in situ injection, however, is probably less than the error of alternative methods, and therefore these values are used to estimate cardiac output (Table 4).

TABLE 4
 CARDIAC OUTPUT AS CALCULATED FROM 1) THE FICK EQUATION, USING
 DATA FROM THE LITERATURE, AND 2) RESULTS OBTAINED BY THE PRESENT AUTHOR.

Species	Temp. (°C)	Heart Rate (beats/min.)	Body Wt. (gm.)	Heart Vol. (ml.)	Cardiac Output (ml./kg.-min.)		Source
					present results	Fick	
Xiphosura							
<u>Limulus</u>							
<u>polyphemus</u>	25	37.5	210-857	0.8-5.0	90-150	--	--
	20	--	--	--	--	105	Mangum et al. (1976)
	15	23	--	--	55-92	78	Mangum et al. (1975)
unresponsive	18-20	11.7	--	--	28-47	17	Johansen & Petersen (1975)
Crustacea							
<u>Callinectes</u>							
<u>sapidus</u>	22-25	125	80-200	0.1-0.3	228	175	Mangum & Weiland (1975)
	15	62	--	--	113	--	Mangum (1976b)
<u>Carcinus</u>							
<u>maenas</u>	15	90	60-80	--	--	70-80	Taylor (1976)
<u>Cancer</u>							
<u>magister</u>	8-10	70	1000	--	--	29	Johansen et al. (1970); Florey & Kriebel (1973)
<u>Homarus</u>							
<u>americanus</u>	13.5	--	400-600	--	--	88	Redmond (1955)
	18-20	92	--	--	--	--	McMahon & Wilkens (1975)
<u>Libinia</u>							
<u>emarginata</u>	25	81.5	77-221	0.16	99	--	--
<u>Panulirus</u>							
<u>interruptus</u>	16	60	600	1.95	--	148	Belman (1975)
Mollusca Bivalvia							
<u>Noetia</u>							
<u>ponderosa</u>	25	14.5	12-30	0.055-0.064	33.6	25	Deaton & Mangum (1976)
	15	5.01	--	--	12.0	--	--
<u>Spisula</u>							
<u>solidissima</u>	10	8.9	104-158	0.18	15.0	--	--
Gastropoda							
<u>Busycon</u>							
<u>canaliculatum</u>	20	14.1	40-60	0.10	28.2	--	--
Annelida							
<u>Nereis</u>							
<u>virens</u>	15	6.8	3-11	0.01	8.5	--	--

DISCUSSION

In order to understand the respiratory role of the cardiovascular system the results must be considered in light of other respiratory parameters.

TEMPERATURE

The thermal sensitivity of heart rate in the conch, Busycon canaliculatum and in the two bivalves, Noetia ponderosa and Spisula solidissima, is greater at low acclimation temperatures (5-7.5°C). This pattern was also reported in the sea mussel, Mytilus edulis, even though the temperature coefficients were lower throughout the range (Widdows, 1973). Increased sensitivity may be widespread among the molluscs, although data on acclimated responses of other species are lacking.

The temperature sensitivity of total oxygen uptake in N. ponderosa and B. canaliculatum is less than expected (Deaton & Mangum, 1976; G. Polities, unpublished) and the temperature sensitivity of oxygen uptake in Mytilus edulis is reduced ($Q_{10} = 1.3-1.7$) in the temperature range 10-25°C (Widdows, 1973). In N. ponderosa a large reduction in blood flow may be offset by a passive response of the oxygen carrier in the blood which is virtually temperature insensitive ($\Delta H = -2.12$ Kcal/mole) in the range 10-23°C (Freadman & Mangum, 1976). The respiratory role of the blood is believed to be very small in species with no blood oxygen carrier (Booth & Mangum, 1977), and therefore a large change in blood flow may have little respiratory importance. The thermal sensitivity of

oxygen uptake in the range 10-23°C in B. canaliculatum also seems unrelated to the performance of the heart and its basis is not yet clear. However, the cessation of heartbeat at lower temperatures is accompanied by a large drop in oxygen consumption ($Q_{10} = 55$ in the range 6-10°C; G. Politics, unpublished).

Since the temperature (5°C) at which heart beat ceases in N. ponderosa is well within the range experienced in nature, it seems that the respiratory role of the blood is severely curtailed at low temperatures. If this conclusion is correct, blood PO_2 and total oxygen uptake must drop sharply at some threshold between 5 and 15°C. The finding of a relatively low Q_{10} for total oxygen uptake in the interval 10-23°C (Deaton & Mangum, 1976) suggests that the threshold is between 5 and 10°C.

The temperature response of heartbeat in Callinectes sapidus, is also of special interest. At 10°C these animals terminate locomotion and become buried in sand, a habit known to fishermen as "hibernation". Although the metabolic consequences of this response are unknown, the Q_{10} for total oxygen uptake in the range 10-17.5°C is quite high (Laird & Haefner, 1976). The Q_{10} for heart rate (5-15°C) is even greater, suggesting that the respiratory role of the blood at low temperature is reduced in crustaceans also. A depression of heart rate at low temperatures also occurs in Carcinus maenas (Ahsanullah & Newel, 1971; Taylor et al., 1973) a member of the same family (Portunidae).

One of the intriguing questions raised by the data reported here is the mechanism of rate modulation in the bivalves. If the heart responds passively to temperature, then there may be some adaptation of structure or excitability which modulates rate changes in different temperature

regimes. Alternatively, as Trueman & Lowe (1971) suggest, there may be nervous control of heart rate. This question might be decided by data on denervated preparations, which are presently unavailable. On the basis of very rapid responses to temperature, also true in Spisula solidissima, Trueman & Lowe (1971) favor the hypothesis of nervous control.

In the annelid, Nereis virens, the temperature coefficient for heart rate is 2.0; blood PO_2 would not be expected to decline precipitously. The oxygen affinity of its hemoglobin increases ($\Delta H = -3.06$; Economides & Wells, 1975) and it is likely that less oxygen is supplied to deep tissues. This conclusion is supported by the temperature sensitivity ($Q_{10} = 2.98$) of oxygen uptake in N. virens (Scott et al., 1975). It predicts a decreased role of hemoglobin at low temperature unless a large decrease in ventilation permits burrow PO_2 to drop to the functional range.

SALINITY

The cardiac responses to salinity change have not been studied widely enough either here or previously to permit predictive generalizations about different animal groups. Comparisons of the present results with other respiratory data can, however, yield important conclusions regarding the role of the cardiovascular system in osmotic adaptations.

The bradycardia induced by low salinity in the horseshoe crab, Limulus polyphemus, was predicted from blood gas tensions and hemocyanin oxygenation properties (Mangum et al., 1976). The response is functional in reducing blood PO_2 , permitting effective oxygen transport even though

the equilibrium properties of the carrier are altered by the loss of blood salts.

In contrast, the respiratory response of Callinectes sapidus does not necessitate the hypothesis of a cardiovascular change between 10 and 35 o/oo. The reduction in blood salt is opposed by an increase in pH, and the oxygenation properties at the pigment are perturbed very little (Weiland & Mangum, 1975; see also Mangum & Towle, 1977). At salinities below 10 o/oo, however, prebranchial blood PO_2 increases (Weiland & Mangum, 1975), possibly resulting from decreased equilibration at the tissue, implying a higher rate of blood flow. Although Laird & Haefner (1976) found no change in oxygen uptake in different individuals measured at 10 and 30 o/oo, Mangum (1977) found a 25 % increase at 5 o/oo relative to 25 o/oo. Her data were collected as paired observations on the same crabs and the difference occurred regardless of the direction of change. Considered together, the observations of higher blood PO_2 , higher rates of oxygen uptake and higher heart rate suggest a coordinated respiratory response to low salinity.

The immediate bradycardia in Spisula solidissima following transfer to dilute medium is not surprising. In bivalves, volume readjustment at low salinity requires 48 hours (Pierce, 1971). If swelling occurs in S. solidissima, it could cause the acute bradycardia as a result of loss of contractility or elasticity. The ionic changes could also decrease the excitability of the heart. Alternatively, rate modulation via cardio-regulatory nerves may be affected by salts. Again, this question might be further evaluated by using similar studies on denervated heart preparations. The ensuing tachycardia at a reduced salinity is more difficult

to explain, and it apparently has not been reported previously. It is possible that the myogenicity or cardio regulatory nerves are directly affected by one or more ions. The adaptive significance of such a response may be the removal of osmotic effectors or metabolites entering the blood during low salinity adaptation.

Blood flow in the two nereids, N. succinea and N. virens increases significantly ($P < 0.01$) at low salinity (8 and 15 o/oo respectively, M. A. Russell, unpublished). Also, oxygen uptake increases at 8 o/oo in N. succinea when ion regulation is occurring (Mangum & Oglesby, in prep.), and from 35 to 8 o/oo in N. virens. The increase in oxidative metabolism when blood salts drop may be mediated by higher circulation rates or may be a result of combined effects of changes in ionic composition on the blood pigment and other components of the respiratory system.

DECLINING OXYGEN

In the bivalves and decapods the cardiac responses to declining oxygen reported here are, for the most part, within the range of those in the literature. Heart rates in the xiphosurans, annelids, and gastropods have not previously been studied. Bivalves commonly maintain constant heart rates in declining oxygen to some PO_2 , at which bradycardia occurs (Bayne, 1971; Brand & Roberts, 1973; Taylor, 1976; Booth & Mangum, 1977). Unlike many of the species examined previously, none of the molluscs studied here exhibit a brief tachycardia immediately preceding the bradycardia. The general pattern among decapods is bradycardia preceded by some period of no change (Stiffler & Pritchard, 1972; Taylor et al., 1973; Hill & Koopowitz, 1975; McMahon & Wilkens, 1975; Belman, 1976; Coyer, 1977). Only the decapod, Scylla serrata, at intermediate

oxygen levels, exhibits any distinct tachycardia (Hill & Koopowitz, 1975). In the annelid, Nereis virens, pulse rate declines linearly with PO_2 , and no regulation is apparent. Most importantly, heart rate and probably blood flow in all of the groups studied respond to hypoxia in a fashion which is very different from that in vertebrates. A decrease in heart rate at low PO_2 occurs not only in the sedentary and benthic species, which have great capacities for anaerobiosis (Mangum & Van Winkle, 1973; Hochachka et al., 1973), but also in the very mobile and active crustaceans whose anaerobic capacities are believed to be less. It is important to note that the response is not associated with distress or mortality; it occurs at oxygen levels which are well within the range of tolerance.

One of the two exceptions to this pattern is the bivalve, Spisula solidissima. Bayne (1976) pointed out that heart rate in several bivalves slows down only after oxygen uptake, and presumably blood PO_2 drop. He concludes that heart rate responds directly to blood oxygen levels and not to sensors activated by external oxygen levels. If Bayne's hypothesis is correct, then either the heart of S. solidissima is less sensitive to low oxygen than other bivalves, or S. solidissima has the ability to maintain high blood oxygen levels via compensatory circulation and/or ventilation. The adaptive significance of the constant heart rate, and presumably blood flow, is not clear. It should be pointed out that the species is confined to subtidal coastal waters off sandy beaches, where it does not experience prolonged periods of low oxygen; its anaerobic capacity may simply not be very great.

The other exceptional species is the crab, Libinia emarginata, which

is not restricted to well oxygenated habitats. Unless stroke volume decreases excessively, thus offsetting the increase in heart rate, then blood flow increases at reduced oxygen levels, oxygen uptake increases proportionately less than heart rate (L. Burnett, unpublished), and thus the blood convection requirement (cardiac output/oxygen consumption) increases. Acute circulatory compensation has been reported previously in Scylla serrata (Hill & Koopowitz, 1975) and in the crayfish, Oronectes virilis (McMahon et al., 1974). In the former case other respiratory parameters are unknown and in the latter case, the response seems coupled with ventilation. The net respiratory result of low oxygen depends on the oxygenation properties of the carrier as well as the internal and external flow of the oxygen supplying medium. Since ventilation and circulation in decapods appear to be neurally coupled (Coyer, 1974; Wilkins et al., 1974), the residual unknown in relating the present results to total oxygen consumption is the performance of the respiratory pigment.

No evidence has been presented that ventilation and circulation are functionally coupled in other taxa. All possible combinations of responses seem possible in the bivalves; the relationship between the two parameters may be direct (Lowe & Trueman, 1972; Earll, 1975) or opposite (Bayne, 1976; Booth & Mangum, 1977; Deaton & Mangum, 1977). In Limulus polyphemus, the data on ventilatory responses do not agree. Johansen & Petersen (1975) found that the ventilatory movements of the book gills increase during gradual hypoxia, while Crabtree & Page (1974) found a decrease in ventilation and corresponding firing of oxygen sensitive units. The time factor of the PO_2 change in the two studies,

which was different, may be the critical factor determining the response; the discrepancy can be resolved only by additional data. There are no data on the two parameters for a single species of annelid.

CARDIAC OUTPUT

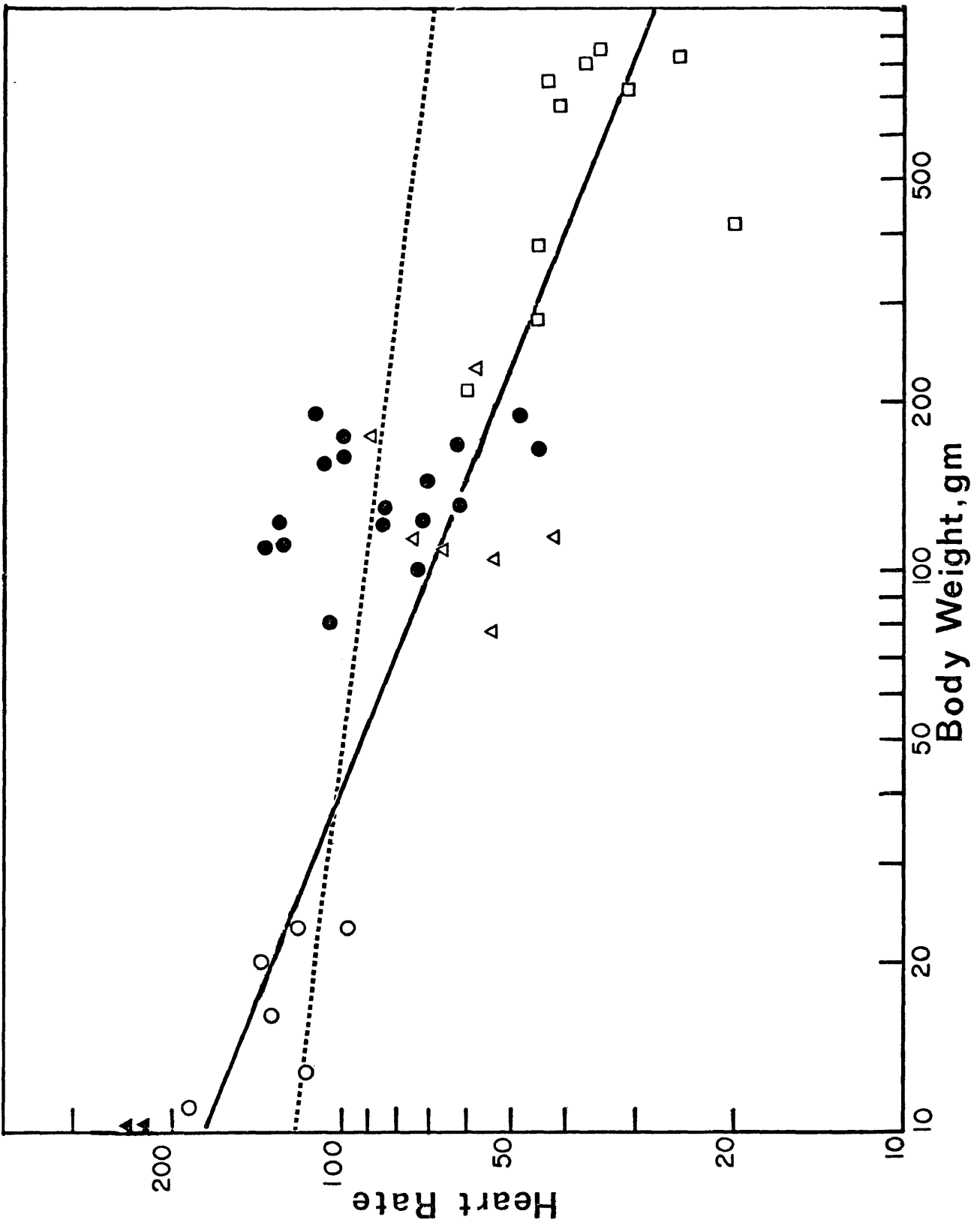
The performance of circulatory systems has received more attention as data for blood gas tensions became available for different animals (see Dejours et al., 1970; Mangum, 1977). Direct measurements of blood flow and blood pressure in the crustaceans illustrate the fallacy of the notion that open circulatory systems must be sluggish (Belman, 1975, 1976, 1977). The data presented here provide further evidence of the diverse capabilities of circulatory systems in different taxa.

Estimates of blood flow based on the Fick principle (blood flow = oxygen consumption/volume of oxygen transported by the blood) compare well with estimates based on the assumption that blood flow is heart rate X heart volume (Table 4). While stroke volume is unlikely to equal total heart volume, the lowest estimate of heart volume was used in these calculations, to minimize the error. The results support the previous conclusion that blood flow rates are very small in the annelids and molluscs (see also Mangum, 1976a, 1977). However, they also support the conclusion that very high flow rates may occur in crustaceans. Cardiac output estimates for crustaceans (Table 4) exceed those for fish by almost an order of magnitude and approximate those in warm-blooded vertebrates (Prosser, 1973). Somewhat higher values were termed excessive by Johansen et al (1970), on the basis that the blood PO_2 data, and hence the denominator in the Fick equation, were erroneously low. McMahon (1975) contends that PO_2 values reported by Johansen et al (1970)

are too high due to incomplete laboratory acclimation. It is not clear which values best represent the natural situation, but two important factors have not been considered in the discussion of cardiac output: 1) heart rate varies with both body weight and experimental temperature, and 2) crustacean cardiac muscle is fundamentally different from vertebrate cardiac muscle (Huddard, 1975). Thus, the values for cardiac output at 8-10°C (Johansen et al., 1970) should be about 4 times smaller than those at 23-26°C (Redmond, 1955). Furthermore, heart rate for a 0.1 kg crab should be (Schwartzkopff, 1955) greater than that for a 1 kg crab. The relationship of heart rate and body size in the animals studied here is shown in Fig. 6. In general the fit of data to the Schwartzkopff (1955) curve describing species is good. It is not surprising that the data for Callinectes sapidus do not fit; both oxygen consumption and cardiac output are greater than expected for an animal of its body size (Mangum & Weiland, 1975). The relationship between heart rate and body weight⁶ in the molluscs is not expressed by the general expression Heart rate = a X Body weight⁶ (r=0.19).

In addition to body size and temperature, the inherently different properties of vertebrates and crustacean cardiac muscle must be appreciated. Crustacean cardiac fibers are striated and, as Josephson (1975) discusses in depth, their rate of contraction is directly proportional to 1) sarcomere length, 2) fiber length and 3) the extent of sarcoplasmic reticulum. The development of the sarcoplasmic reticulum influences the diffusion of Ca⁺⁺ from the store to the activation site, a critical factor in excitation-contraction coupling. Maximum contraction velocity

Fig. 6. Regression line (solid) describing present data for heart rate as a function of body weight in Limulus (open squares), Libinia (open triangles), Panopeus (open circles), and Hemigrapsus (closed triangles). Data for Callinectes (closed circles) excluded. $R = 0.92$, $P < .001$, slope $(b-1) = -1.39$. Dotted line taken from Schwartzkopff (1955), 20-22°C.



should be much greater in crustacean myocardial fibers than in vertebrates, due to the development of the tubule systems and the length of fibers and sarcomeres (Figs. 7 & 8; Smith, 1972; Huddart, 1975). Vertebrate cardiac myofibers lack well developed tubule systems and they are indistinctly, striated, if at all (Page & Neidergerke, 1972; Huddart, 1975). According to the relationship shown by Josephson (1975), the heart of Panopeus herbsti (fibers with 6 % SR) should be capable of at least 375 bts/min. Even though the beat frequency is higher than predicted for its body range, the actual performance does not attain its capability.

Fig 7. Longitudinal section through a cardiac myofiber of Panopeus herbsti, indicating the 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; Mitrochondria, Mt; Basement membrane, Bm. X 19,000.

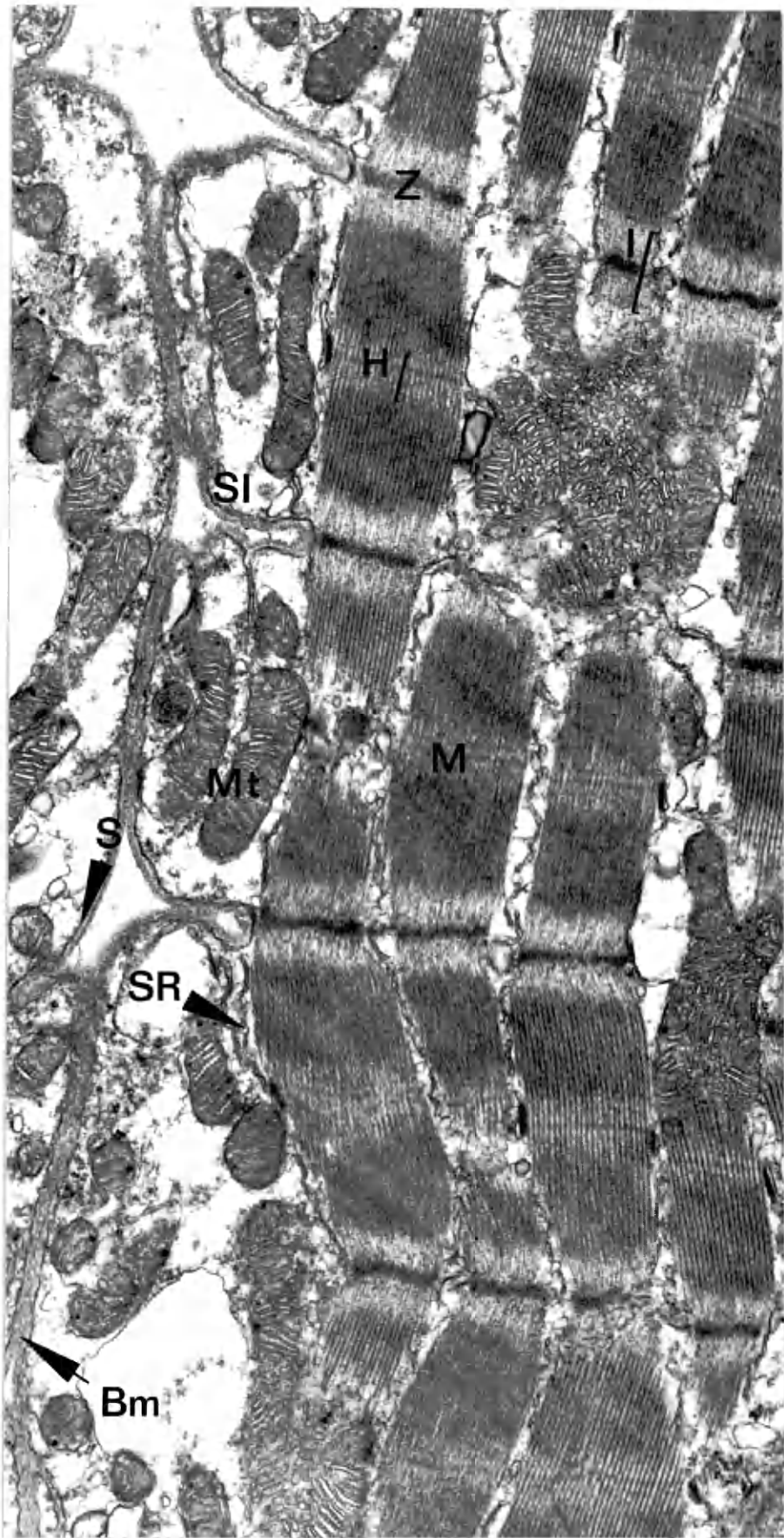
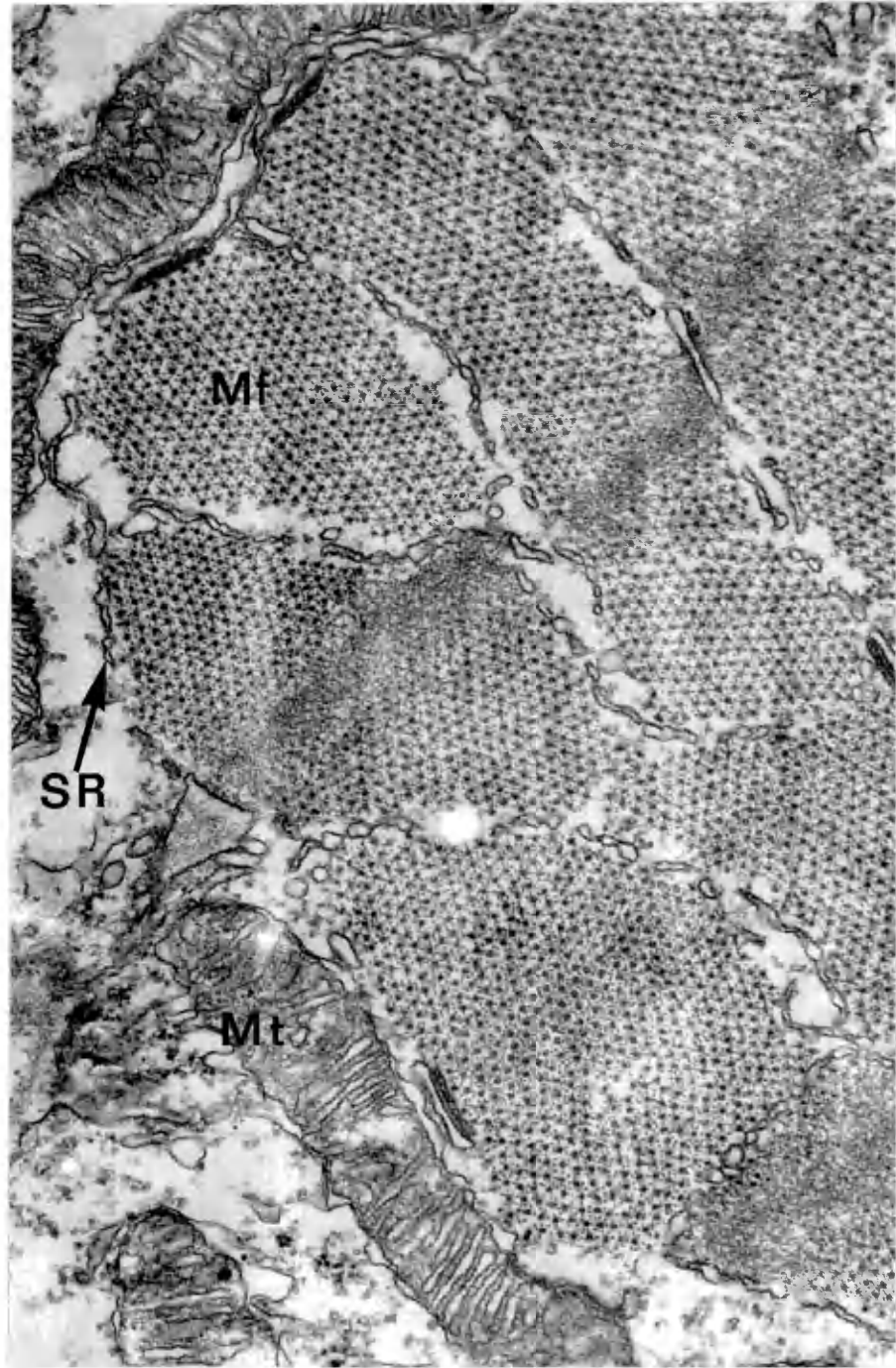


Fig. 8. Cross section through a cardiac myofiber of Panopeus herbsti, showing the extent of sarcoplasmic reticulum. Myofibril, Mf. X 30,000.



SUMMARY

- 1) The effects of temperature, salinity and declining oxygen on the heart rates of animals representing four phyla have been investigated in light of other respiratory parameters.
- 2) In all species, heart rates respond positively to temperature between 15 and 25°C. The bivalves and several species exhibit elevated sensitivity ($Q_{10} = 3.0$) at low temperatures (5-15°C).
- 3) Responses to reduced acclimation salinity differ among the species, but the data yield cardiac output values that agree with predictions.
- 4) The clam, Spisula solidissima, does not exhibit bradycardia in hypoxia and the crab, Libinia emarginata, shows tachycardia at intermediate PO_2 . Bradycardia occurs in all other species during hypoxia.
- 5) Cardiac output estimates for crustaceans exceed earlier predictions and approach those for warm-blooded vertebrates, contrary to the concept of sluggish open circulatory systems.
- 6) The capabilities of crustacean circulation are assessed in terms of body size, temperature and the fundamentally different design of cardiac muscle. There is no reason to doubt estimates of cardiac output in excess of 100 ml/kg-min.

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VITA

Peter Lee deFur

Born February 11, 1950 in Norwalk, Connecticut. Graduated from Wilton Senior High School, Wilton, Connecticut in June, 1968. Received a B.S. in biology from The College of William and Mary, Williamsburg, Virginia, June, 1972. Worked for the Johns Hopkins University, Baltimore, Maryland as a Research Assistant from June, 1972 until June, 1974. From June, 1974 until June, 1975 was employed by Ecological Analysts, Inc., as an Associate Research Scientist. Entered graduate school in the Department of Biology at The College of William and Mary in September, 1975, and served as a graduate teaching assistant through May, 1977. Currently a candidate for the degree of Master of Arts in Biology.

