ENVIRONMENTAL PHYSIOLOGY OF THE INTERTIDAL LIMPETS *PATELLA* (PROSOBRANCHIA) AND *SIPHONARIA* (PULMONATA), WITH PARTICULAR REFERENCE TO THEIR METABOLIC ADAPTATIONS.

by

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THESIS

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CONTENTS

6: PROGRESSIVE AERIAL EXPOSURE OF *PATELLA GRANULARIS* AND *SIPHONARIA OCULUS*: OXYGEN CONSUMPTION, HEART RATE AND WATER RELATIONS

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PREFACE

The experimental chapters in this thesis have been structured on the format of scientific papers. The set of references generally differs among the chapters, thus for easy referral, reference lists have been kept separately, and appear at the end of each chapter. Some references are, however, common to more than one chapter, and there is unavoidable repetition of these in the various reference lists. Publications have arisen using limited data presented in this thesis and these are included at the end.

ABSTRACT

Physiological response to environmental change is examined in species of the intertidal limpets, *Patella* (Prosobranchia) and *Siphonaria* (Pulmonata). Characteristics of heart beat were determined using impedance pneumography and these are described. Heart rate of *P. granularis* is related to temperature and body size, and cardiac arrest in this limpet is apparently stress- related. *Siphonaria oculus* may exhibit a temperature-independent, extreme, and often prolonged bradycardia (<10 beats/min). When measured shortly after aerial exposure, heart rate and oxygen consumption of the above limpet species are closely correlated.

The relationships of aerial oxygen consumption with body weight and ambient temperature were determined for the above high shore species of limpet. Both have low aerial rates of oxygen consumption relative to low shore limpet species, and their Q_{10} values decrease with increasing temperature. Diel field recordings of heart rate of *S. oculus*, taken during summer and winter, suggest absence of temperature acclimation, and this was also shown for oxygen consumption at high aerial temperatures (30°C) in laboratory experiments. Oxygen consumption of *P. granularis* is partially temperature compensated (temperature acclimation).

In air, even though *S. oculus* loses water faster, it shows greater tolerance of water loss and survives longer than *P. granularis*. Prolonged aerial exposure of *S. oculus* leads to depression of heart rate and of $\dot{V}O_2$ (down to 18% of the pre- exposure rate), responses interpreted as representing adaptive metabolic rate depression. In *P. granularis* aerial heart rate remains constant and $\dot{V}O_2$ never falls below

38% of pre-exposure rate. This reduction in $\dot{V}O_2$ in air is considered as being stress-related, resulting from impairment of oxygen uptake.

In declining oxygen tension *S. capensis* shows a better capacity for oxyregulation than *P. granularis*. On exposure to hypoxia, sand-inundation and hyposalinity, *S. capensis* may show typical bradycardia (<10 beats/min), suggesting depression of aerobic metabolism, and on return to pre-exposure conditions there is no overshoot of heart rate, suggesting absence of oxygen debt. The effect of hypoxia, sand-inundation and hyposalinity on heart rate of *P. granularis* is variable; this becomes depressed and is often interspersed with extended cardiac arrest. When conditions are normalized, this species exhibits a clear overshoot of heart rate.

The significance of differences in physiological response between patellid and siphonariid limpets is discussed with regard to their habitat segregation, particularly in the upper-shore zone (open rock and tidal pools) and sand-inundated rock substrata, where only siphonariid limpets may be found. While not previously characterized in marine gastropods, metabolic rate depression by *Siphonaria*, through facilitating isolation and conserving food reserves, is suggested as a key factor determining their distribution in physico-chemically more extreme and variable intertidal habitats.

CHAPTER 1

GENERAL INTRODUCTION

All three sub-classes of gastropod mollusc, the Prosobranchia, Opisthobranchia and Pulmonata, are represented in the marine environment. Most marine gastropods are prosobranchs. The opisthobranchs, an small entirely marine group, and the pulmonates, a predominantly terrestrial/freshwater group, together, have relatively few marine representatives. Along the coast of South Africa both the prosobranch and pulmonate groups are represented by a rich intertidal limpet fauna. There occur seventeen patellid (prosobranch) species (Kilburn and Rippey 1982), and four siphonariid (pulmonate) species (Allanson 1959), although an earlier study described twelve (Hubendick 1946). All marine pulmonates are essentially intertidal, and most, as suggested by the distributions of *Melampus bidentatus*, *Amphibola crenata*, *Benhamina obliquata* and *Kerguelenella stewartiana*, appear to be restricted to the upper intertidal zone (see Knox 1955; McMahon and Russell-Hunter 1981; Shumway 1981; Innes, Marsden and Wong 1984). However, the pulmonate limpet *Siphonaria*, survives continual submergence in tidal pools (Allanson 1958; Branch and Cherry 1985; pers. obs.), and *Trimusculus reticulatis* occurs subtidally, although it may depend on air pockets in this habitat (Haddock 1989).

Apart from its existence in tidal pools, *Siphonaria* is remarkable in the range of intertidal habitats it exploits, co- occurring with prosobranch limpets in some, and extending into others to the exclusion of prosobranchs, such as regularly sand- inundated rocky substrata (Dower 1989; Marshall and McQuaid 1989). Habitat segregation between prosobranch and pulmonate limpets may also be seen in the upper-intertidal zone, where representatives of these sub-classes have different vertical limits. Along the coastline of South Africa, *S. oculus* extends its vertical distribution on wave-exposed rocky shores into the supralittoral fringe or *Littorina* zone, well above the uppermost prosobranch limpet, *Patella granularis*, which extends only into the upper- balanoid zone (see Stephenson 1939, for zonation nomenclature).

Intertidal distribution is influenced by numerous factors, including those pertaining to reproduction (e.g., dispersal and tolerance by larval stages) and biological interaction (e.g., predation and competition). However, morphological, physiological and behavioural adaptations facilitating tolerance of the physico- chemical conditions of a specific habitat, are a pre-requisite for existence in that habitat. Central to the theme of this investigation was an examination of the physiological adaptations of prosobranch and pulmonate limpets, in an attempt to explain discrepancies in their intertidal distributions.

The biology of patellids has been well documented (see Branch 1981 for review). Most investigations on siphonariid limpets have examined their activity patterns (Cook 1969; Cook 1971; Cook and Cook 1978; Cook and Cook 1981; Verderber, Cook and Cook 1983; Branch and Cherry 1985), other aspects of their ecology (Black 1979; Cook 1980; Creese 1981; Garrity and Levings 1983; Garrity 1984; Levings and Garrity 1984; Santelices and Correa 1985; Sutherland and Ortega 1986; Quinn 1988a), and reproduction (Creese 1980, Quinn 1988b). Although interesting because of their terrestrial links, very little is known about the adaptational physiology of siphonariids (Innes, Marsden and Wong 1984; Dye 1987), and in fact marine pulmonates in general.

A common feature of many pulmonate snails is the development of a lung which facilitates aerial respiration. *Siphonaria* characteristically bears a lung in the form of a vascularized lining of the mantle cavity (Fig 1.1; Yonge 1952). Within the mantle cavity of *Siphonaria* is housed a secondary gill which presumably allows efficient aquatic respiration. Although the pulmonate mantle cavity is primarily designed for functioning in air, movement of water through this, and its role in aquatic respiration, have been examined for some other pulmonates (Yonge 1958; Pilkington, Little and Stirling 1984). Respiration by the prosobranch limpet, *Patella*, is facilitated by a ring of external secondary gills lining the pallial groove (Fig. 1.1; Yonge 1947; Nuwayhid, Davies and Elder 1978). Given these morphological differences, this investigation focuses on the respiratory/metabolic physiology of these limpet types. Two basic physiological parameters, heart rate and rate of oxygen consumption, were measured.

Chapter 2 describes the methodology for determination of heart beat, and examines heart beat in response to acute change in temperature. Chapter 3 investigates the relationship between measurements of aerial heart rate and oxygen consumption. For comparison with laboratory measurements, heart rate of *S. oculus* was measured *in situ*, dielly, during summer and winter (Chapter 4). Special attention is given to the respiratory/metabolic adaptations for upper-shore existence of *S. oculus* and *P. granularis* (Chapters 5 and 6). Another siphonariid limpet, *S. capensis*, is a prominent inhabitant of habitats where patellid limpets do not occur, particularly shallow upper-shore pools covered by the crustose coralline alga *Lithothamnion* (Allanson 1958; pers. obs.) and shores subjected to regular sand-inundation (Dower 1989). Aspects of the respiratory physiology of this limpet species, and of *P. granularis*, were compared under conditions similar to those prevailing in upper-shore pools and sand-inundated regions (Chapter 7).

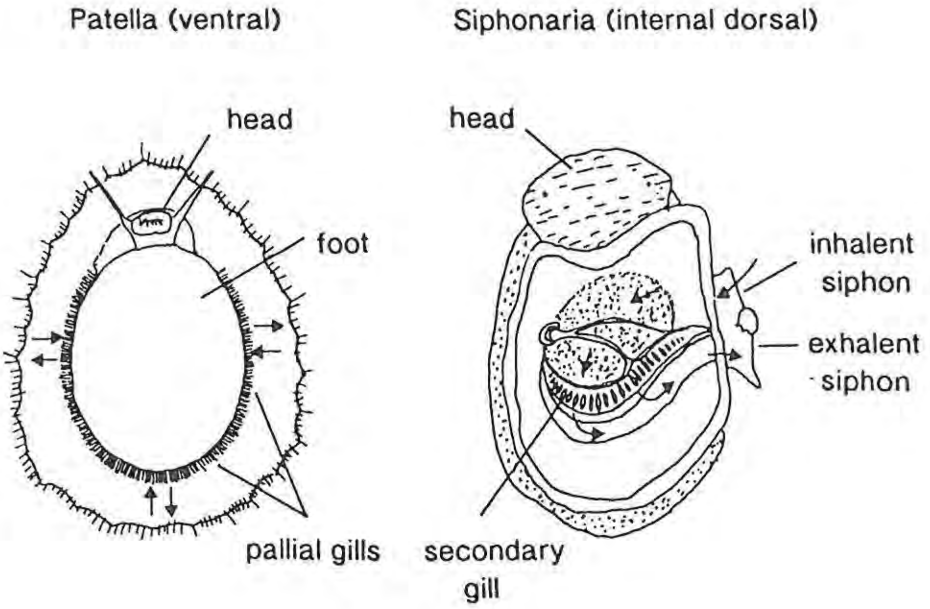

Fig. 1.1. Diagrams of *Patella* and *Siphonaria* showing position of the respiratory organs after Yonge (1947) and Yonge (1952). Arrows indicate ventilatory currents.

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CHAPTER 2

IMPEDANCE PNEUMOGRAPHY, HEART BEAT CHARACTERISTICS AND EFFECT OF TEMPERATURE ON HEART RATE

2.1 INTRODUCTION

In studies on environmental physiology of marine invertebrates, heart rate is an attractive physiological rate function to measure, because the procedure is simple, and it usually mirrors metabolic rate (Lowe and Trueman 1972; Spaargaren 1973; Coleman 1974). One means of recording heart beat is by impedance pneumography. This enables detection of pulsatile variations in volume of the heart. Early investigations using impedance pneumography, include those on bivalves (Trueman 1967) and limpets (Jones 1968). Recently, this technique has been used to record heart rate in land snails (Bailey and Lazaridou-Dimitriadou 1991; Wunnenberg 1991). While impedance pneumography is reliable for the determination of heart beat frequency, this is not the case for other heart beat characteristics, such as, stroke volume (Wells 1979). In some instances where there is no penetration of the tissue, this technique has been considered non-invasive (Depledge and Phillips 1986).

This chapter describes the impedance methodology and the nature of impedance recordings acquired, during investigations on physiological response to environmental change of intertidal limpets (*Patella* species and *Siphonaria oculus*). Specifically, variability of heart rate, and the relationship of heart rate to acute change in ambient temperature of *P. granularis* Linne and *S. oculus* Krauss, are presented.

2.2 MATERIAL AND METHODS

2.2.1 Collection and maintenance of specimens

Specimens of *Patella granularis, P. oculus Born, P. concolor* Krauss, and *Siphonaria oculus* (100-500 mg dry weight) were collected from intertidal rocks at Port Alfred (33°36'S; 26°53'E) and near Fish River Mouth (33°30'S; 27°08'E), eastern Cape Province. Limpets, prized from rocks, were immediately transferred onto polythene covered perspex plates, and returned, air-exposed, to the laboratory within 5 h. The polythene sheeting was readily peeled from the limpet foot, allowing later detachment without further trauma.

All limpets (n = 60 for each *P. granularis* and *S. oculus*) were kept unfed in controlled environment rooms for at least 5 days prior to use. Temperature (15±0.5° or 20±0.5°C), salinity (35‰) and subdued illumination were held constant. A tidal cycle of 12:12 h, air:water was maintained in 150 L plastic aquaria. Limpets held in the laboratory for longer than 6 weeks were considered unsuitable for experiments. While *P. granularis* and *S. oculus* were used in most investigations presented here, impedance recordings for *P. oculus* and *P. concolor* are also given.

2.2.2 Impedance technique for measuring heart rate

Heart beat characteristics (e.g., regularity and frequency (f_h)) were determined by use of impedance pneumography (Trueman 1967). Recording traces were obtained by coupling an impedance transducer (Z135/Bioscience) and a strain gauge coupler (FC 135/Bioscience), with a twin-channel George Washington oscillograph (MD 400). In preparation for experiments, two holes were drilled through the shell on either side of the heart, and a fine platinum electrode (0.3 mm diameter) soldered to one strand of twin core screened wire was inserted into each hole. Electrodes were set in position with dental wax and epoxy glue. Limpets were allowed to recover from handling for 12 h in seawater, and only limpets which had firmly re-adhered to a watch glass (or plastic disc), were used in experiments. Limpets with or without electrodes survived equally well (for months) under laboratory conditions.

2.2.3 Characteristics of impedance recordings

During the various experiments undertaken, unusual impedance recordings were at times observed. These were, where possible, monitored continuously for some time. They were later compiled and related to behavioural patterns, temperature, and emersion/immersion.

2.2.4 Heart rate and acute temperature change

Heart beat, in response to acute change in temperature, was determined for a wide range of aerial temperatures (10^o-30^oC). Intertidal limpets generally become inactive in air. Aerial heart rate therefore represents standard, rather than active metabolism (see Newell 1979). Firstly, temporal variability in aerial heart rate was examined by taking recordings at 15^o, 20^o, 25^o and 30^oC, hourly, for 5 h. In a subsequent experiment, temperature was abruptly raised or lowered (usually by 5^oC over 30 min) and recordings were taken after stabilization of temperature for 30 min. Relative humidity was kept above 80% by placing lids on the empty aquaria in which limpets were held. Between experiments limpets were kept overnight in seawater at 15^o or 20^oC. Aerial and aquatic temperatures were controlled, by controlling the aerial temperature in constant environment rooms.

2.3 RESULTS

2.3.1 Impedance recordings

Examples of impedance recordings for *Patella* species and *Siphonaria oculus* are shown in Fig. 2.1. Placement of electrodes was such that in addition to detecting changes in volume of the heart, body movements were also detected. Gross body movements interfered with the recording signal causing irregular drift (Fig. 2.1 b), and often off-scale deflections. Interference patterns of a regular nature were also commonly observed (Fig. 2.1 c-g). These consisted of regular vertical deflections of the entire recording trace, of various frequencies and amplitudes (Fig. 2.1 c,d,e), or vertical shifting of successive heart beats with only subtle displacement of the recording trace (Fig. 2.1 f,g). While regular surging of the impedance trace, thought to be the result of raising and lowering of the shell, was restricted to periods of immersion in seawater (30°C) in the *Patella* species, this occurred in air and seawater in *S. oculus*. Beat to beat displacement patterns in *Patella* species (Fig. 2.1 f,g) were suggestive of superimposition of heart beat on some other regular body movement, such as radula activity, which was indeed noted in some limpets displaying such impedance patterns.

Patella granularis occasionally underwent transient bradycardia (reduction in heart rate) and/or cardiac arrest (absence of heart beat; Figs. 2.2 and 2.3). Cardiac arrest was preceded by, or independent of, brief bradycardia (Fig. 2.2 c,d); this occurred at low (15°C) and high temperatures (30°C), and varied from a few seconds to seldom longer than 2 minutes (Fig. 2.3 a,b). Cardiac arrest was observed interrupting vertical surging (Fig. 2.3 c).

Siphonaria oculus exhibited a well-developed capacity for bradycardia, and a heart rate of below 10 beats/minute was frequently observed in this limpet (Fig.

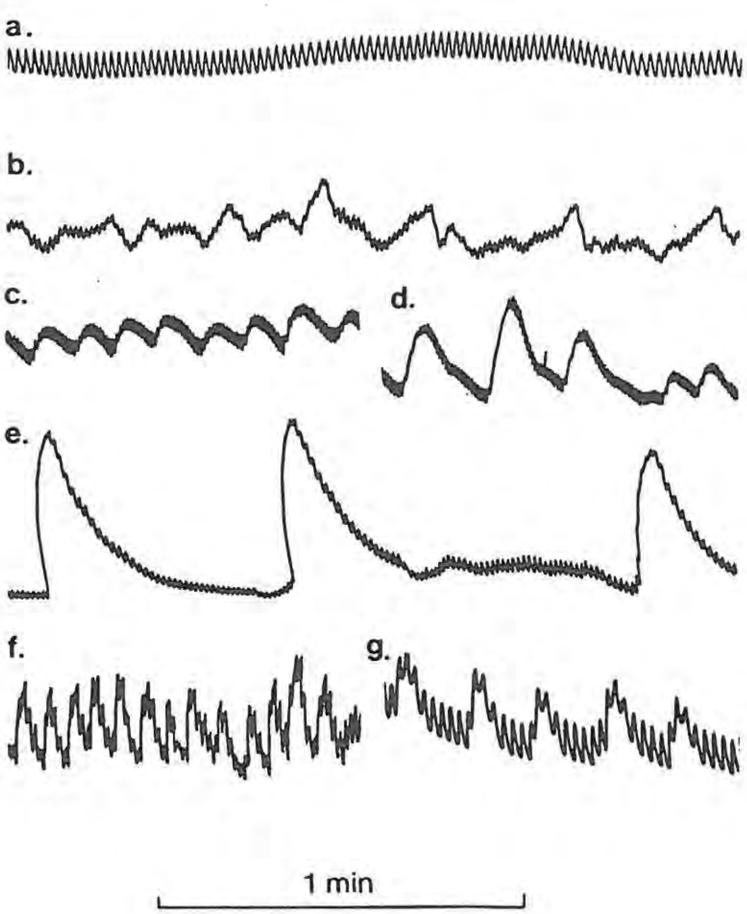

Fig. 2.1. Examples of impedance recording traces of *Patella* species and *Siphonaria oculus*. (a) No deflection of recording trace. (b) Erratic deflections (*S. oculus*; 25°C; water). (c,d) Regular vertical surging, varying in frequency and amplitude for *P. granularis* (30°C; water), and (e) for *S. oculus* (25°C; water). (f,g) Superimposition of "radula activity" on heartbeat of *P. concolor* (20°C; water) and *P. oculus* (15°C; water), respectively.

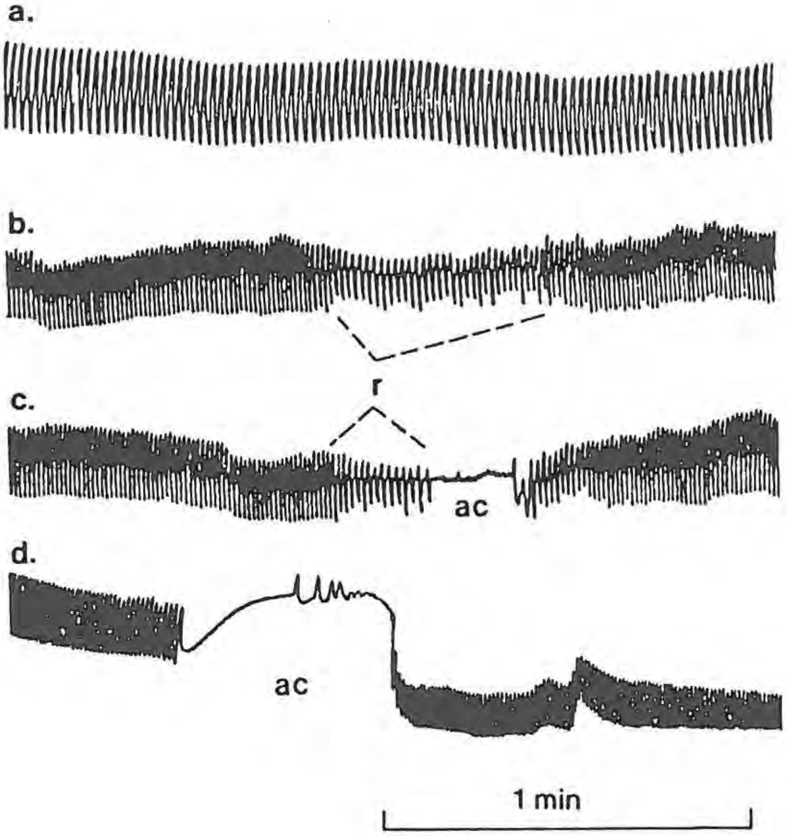

Fig. 2.2. Cardiograms of an individual *Patella granularis* taken when aerial temperature was raised from (a) 20°C through (b,c) 25°C to (d) 30°C, showing the development of bradycardia (r) and cardiac arrest (ac).

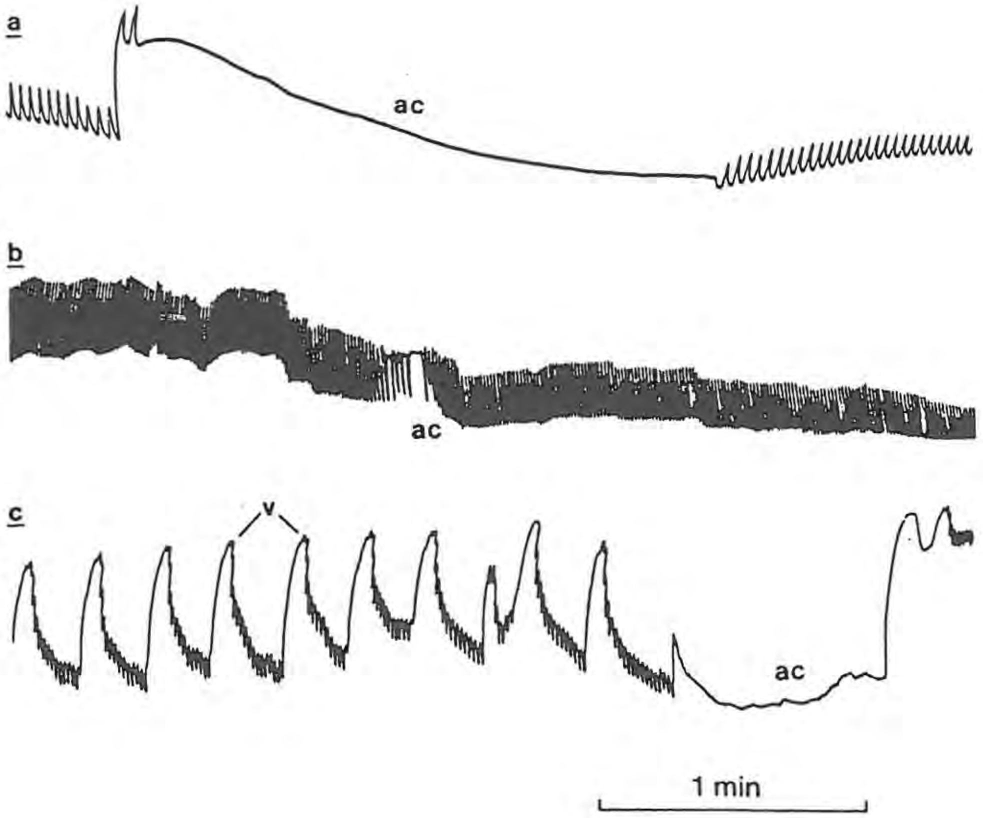

Fig. 2.3. Cardiograms of *Patella granularis* in air at (a) 15°C and (b) 30°C. Cardiac arrest (ac) varies from a few seconds to over two minutes. (c) Cardiac arrest associated with impedance surging (v) at 30°C in water.

2.4). Bradycardia could be sustained for hours (Fig. 2.5), and possibly even days. Bradycardia (f_h of between 1-10 beats/minute) was often associated with acardia (f_h of less than 1 beat/minute) (Fig. 2.4) and, at times no distinction is made between these, together being referred to as acardia/bradycardia. Continuous recordings taken for up to 12 h showed that acardia may be initiated rapidly (within minutes; Fig 2.4 a), or over a longer period (hours; Fig. 2.5), and may be preceded by oscillations in heart activity (Fig. 2.5). On one occasion, surging of the recording trace was found to continue during acardia (Fig. 2.4 a).

Variability in impedance amplitude between individuals in many cases is likely to be the result of differences in positioning (on the shell), and distance apart, of the electrodes. A change in position of the body relative to the shell, oxidation of, and growth of non-conducting tissue over the electrodes, are factors likely to alter impedance amplitude with time. For these reasons differences in impedance amplitude between and within individuals are difficult to interpret. Loss of impedance signal, often observed for *S. oculus* in air, was presumably the result of a break in contact between mantle tissue and the electrodes.

2.3.2 Heart rate and temperature

Aerial heart rate of *P. granularis* was closely linked to ambient temperature (Fig. 2.6). Heart rates recorded over 5 h in air at constant temperature were found to be consistent, and these data are not shown. Different individuals in identical situations showed considerable (Fig. 2.6), but consistent differences in rate from day to day. Individual variability was apparently size-dependent with larger limpets displaying slower heart rates (Fig. 2.7). For this reason, in some other experiments, limpets of a narrow size range were selected.

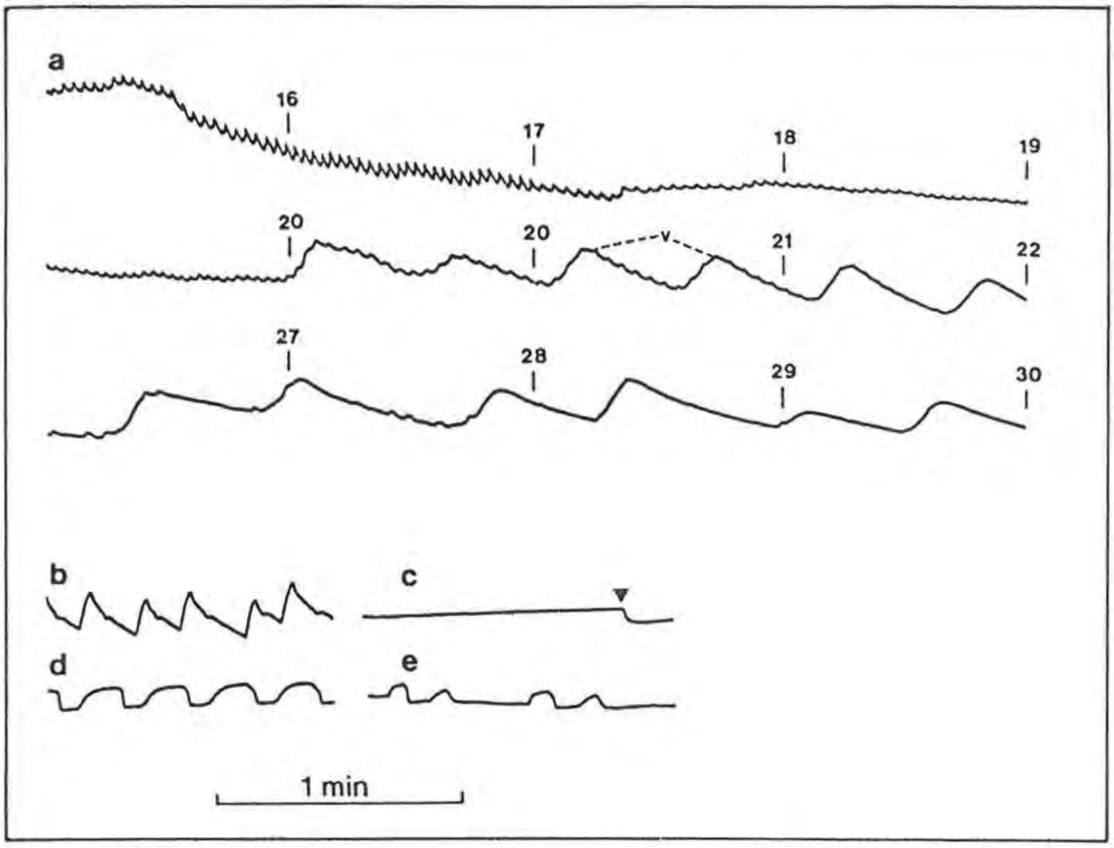

Fig. 2.4. (a) Continuous impedance recording of *Siphonaria oculus* (15°C; water), showing the entering into acardia (recorded for up to 6 h) preceded by bradycardia. Infrequent heart contractions and impedance surging (v) (although observed on this occasion only) continue during acardia. Numbers indicate time (min) after commencement of the recording. (b,c,d,e) Examples of heart contractions during bradycardia (b, 15°C, air; d,e, 15°C, water; c, 25°C, air). Arrow indicates contraction.

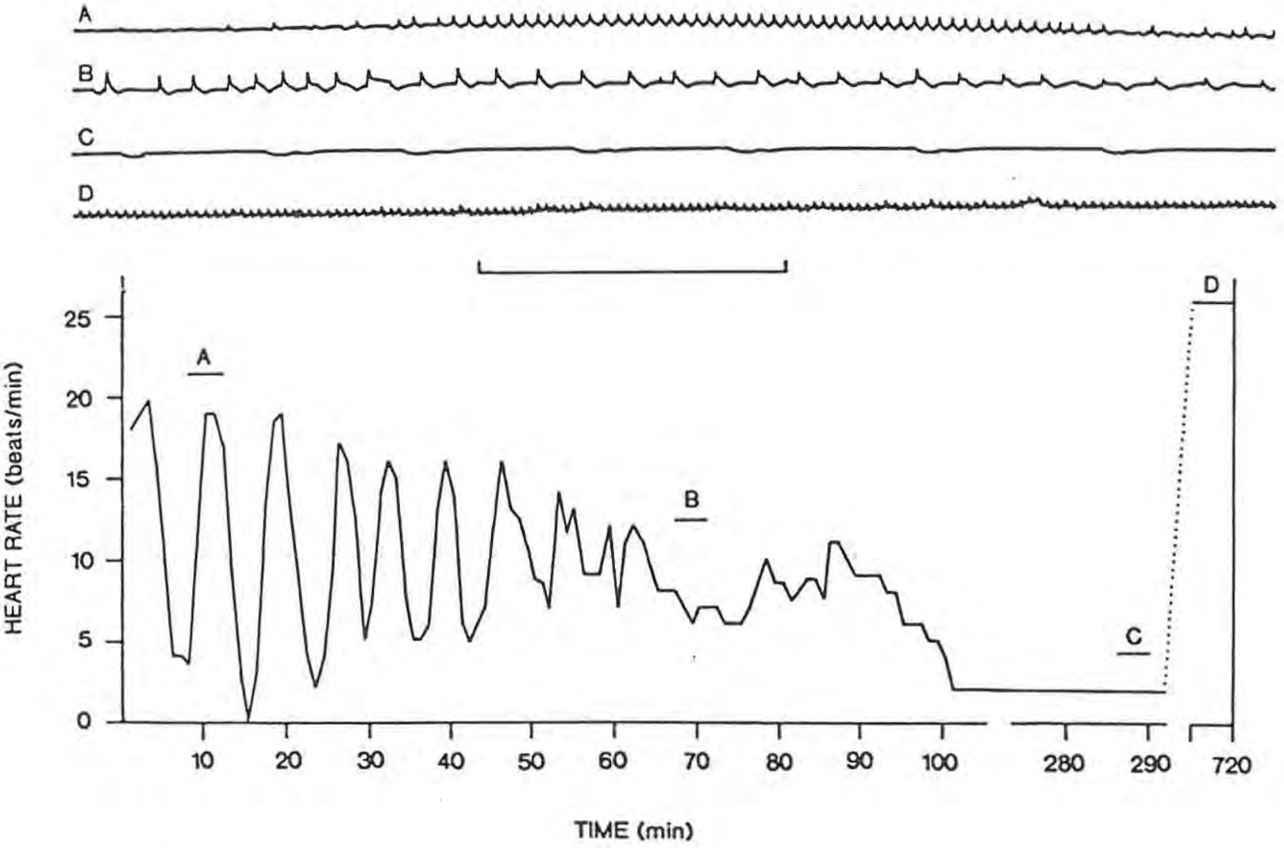

Fig. 2.5. Heart rate of *Siphonaria oculus* (15°C; air) determined from a continuous impedance recording, showing oscillations of rate for some time (± 100 min) prior to the entering into acardia/bradycardia. Cardiograms A, B, C, and D represent stages of the continuous recording, as indicated in the figure below.

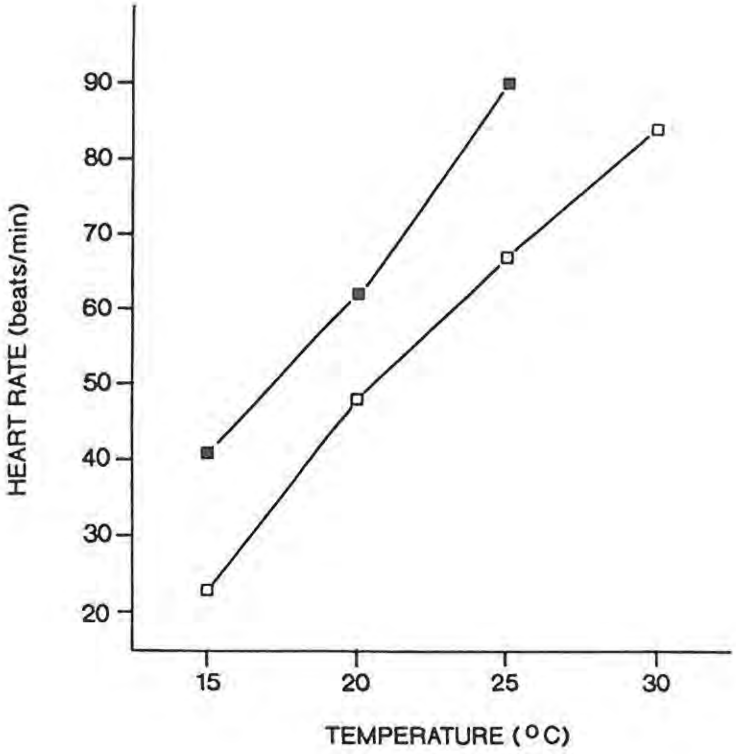

Fig. 2.6. Aerial heart rate of two individuals of *Patella granularis* in response to acute change in temperature, demonstrating individual differences.

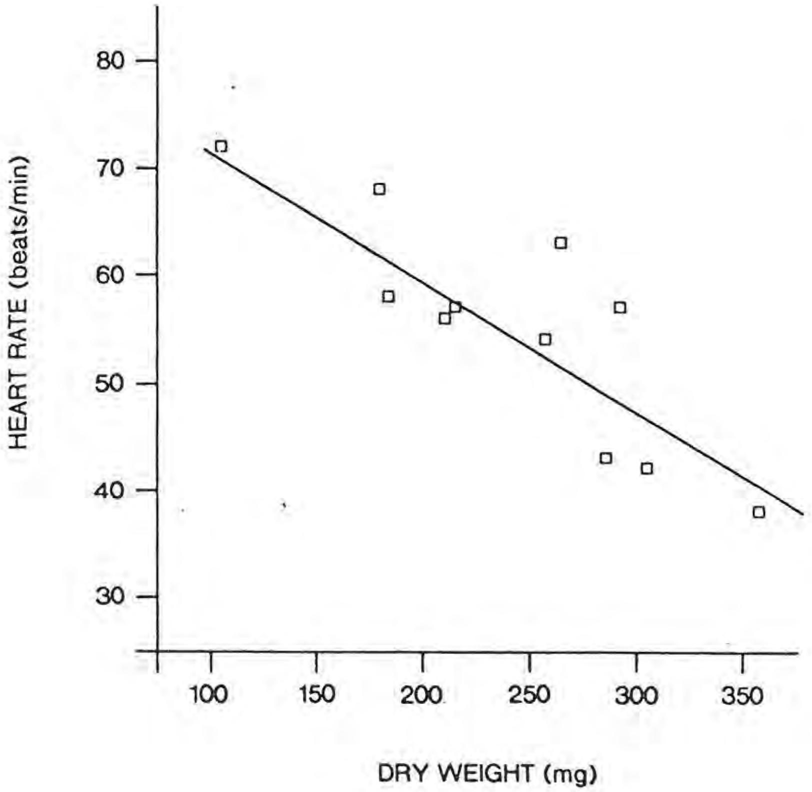

Fig. 2.7. Relationship of heart rate with dry tissue weight for *Patella granularis* (air; 25°C). Equation for linear regression is $Y = 83.54-0.12X$ ($r = -0.84$; $n = 11$).

When exposed to air at a constant temperature for 5 h, heart rate varied temporally and among individuals of *S. oculus*, being particularly variable at 30°C. For one individual, heart rate varied by 25 beats/min over 5 h at 30°C (7, Table 2.1). There was however, no general pattern of increase or decrease in heart rate within the 5 h period of aerial exposure (Table 2.1). Cases where one standard deviation of the mean exceeded 7.5, an arbitrary figure, were considered to show excessive variability and were excluded from the data set.

There was considerable variability in the effect of aerial temperature on heart rate, among individuals, and for the same individual of *S. oculus* on different days (Figs. 2.8 and 2.9). The greatest variability occurred at 30°C, at which temperature heart rate varied from 5 to 80 beats/minute (Fig. 2.8). However, acardia was responsible for much of the variability. Because acardia represents a response different from the "normal" heart rate- temperature response, it is considered separately below. Even excluding acardial rates, variability in heart rate was particularly high at 30°C (from around 40 to 80 beats/min; Figs. 2.8 and 2.9; Table 2.1).

Statistical analyses using "normal" heart rate for *S. oculus*, were performed for comparison with *P. granularis* the change in heart rate with change in temperature (Table 2.2). Initial bradycardia, bradycardia during the experimental run, or a profound decline in heart beat with increase in temperature, were the criteria used for excluding data from the "normal" sample set. *Patella granularis* showed a higher mean heart rate than *S. oculus* at all temperatures (Table 2.2). Rate-temperature relationships are usually expressed semi-logarithmically. The change in rate with temperature for poikilotherms is constant, and this forms the basis of Van't Hoff's rule which considers a 10°C temperature change:

$$Q_{10} = R_{t+10}/R_t \,;$$

and if applied to any temperature range, the equation takes on the form:

Table 2.1

Heart rate (beats/min) of 7 individuals of *Siphonaria oculus* recorded initially in water at 20°C (w20), then hourly for 5 h during aerial exposure (30°C). Mean (± 1 SD) aerial heart rate is given.

Limpet		Aerial Exposure Time (30°C)					
	w20	1h	2h	3h	4h	5h	$\bar{X}$±1 SD
1	34	58	50	46	44	47	49.0±4.9
2	46	55	72	69	63	75	66.8±7.1
3	34	54	60	53	56	52	55.0±2.8
4	--	71	73	82	81	86	78.6±5.7
5	31	47	57	40	41	43	45.6±6.2
6	29	56	63	47	54	54	54.8±5.1
7	42	59	68	76	84	80	73.4±8.9

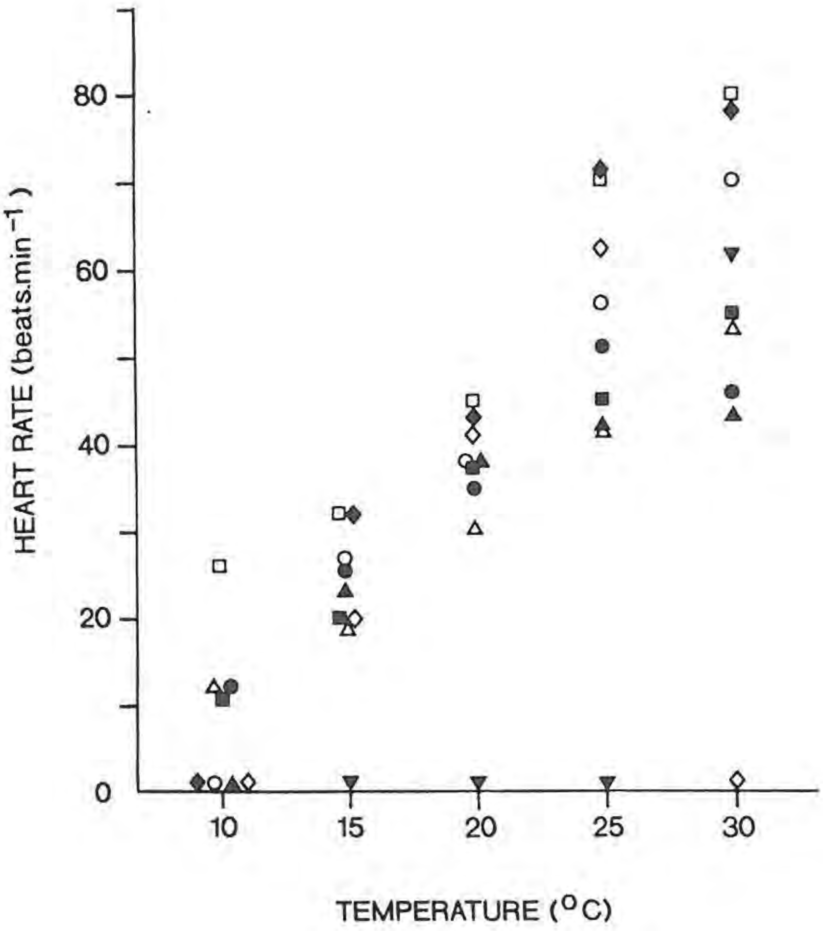

Fig. 2.8. Response of aerial heart beat of individuals (indicated by different symbols) of *Siphonaria oculus* (n = 9) to acute change in temperature. Heart rates of below 10 beats/min (acardia/bradycardia) are placed on the abscissa.

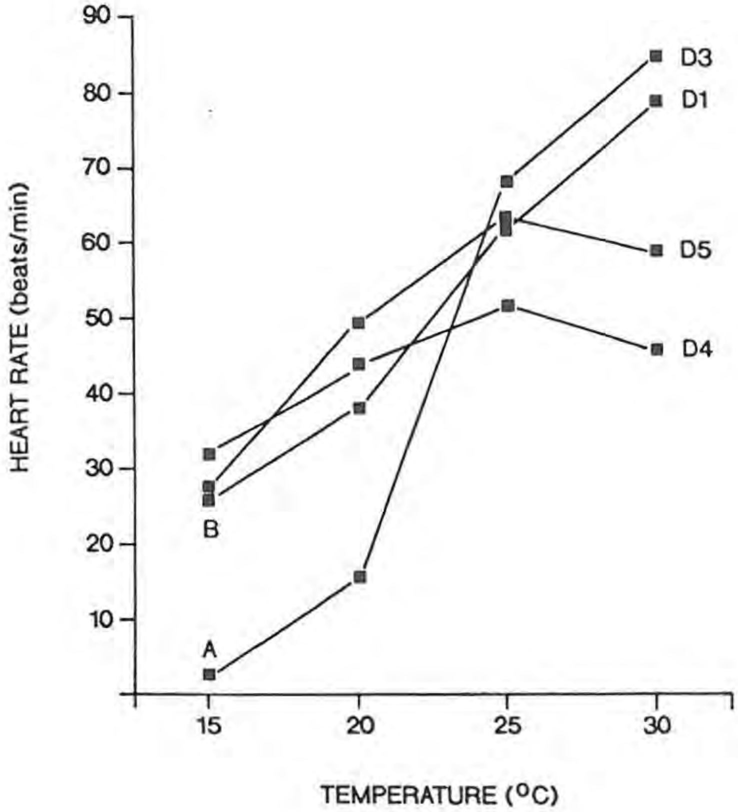

Fig. 2.9. Response of aerial heart beat of an individual *Siphonaria oculus* to acute change in temperature, recorded on consecutive days (D1-D5).

Table 2.2

Mean heart rate (beats/min) and Q_{10} for *Patella granularis* and *Siphonaria oculus* at different temperature exposures.

Temperature	Heart rate (*P. granularis*) $\overline{X}\pm1$ SD (n)	Q_{10}
15	29.86±5.10 (22)	
		2.35
20	45.75±5.21 (20)	
		1.92
25	62.01±7.30 (21)	
		1.71
30	81.07±6.30 (15)	

Temperature	Heart Rate (*S. oculus*) $\overline{X}\pm1$ SD (n)	Q_{10}
15	25.48±5.07 (23)	
		2.22
20	36.55±6.61 (20)	
		1.88
25	50.05±8.53 (21)	
		1.45
30	60.35±10.89 (20)	

$Q_{10} = (R_1/R_2)^{10/(T_2-T_1)}$; (R) rate at (T) temperature. Paired *t*- tests showed that heart rates for each species at different temperatures were significantly different $P<0.01$.

$$Q_{10} = (R_1/R_2)^{10/(T_2-T_1)};$$ (Cossins and Bowler 1987)

where R is rate and T is temperature. For most biological rate functions the Q_{10} is around 2, indicating the doubling of rate with a 10°C increase in temperature. Temperature coefficients (Q_{10}) for both species were similar (Table 2.2), they were relatively low, and decreased with an increase in temperature. The notably low Q_{10} of 1.45 between 25° and 30°C coupled with the high variability in heart rate at 30°C of *S. oculus*, can be explained by the occasionally observed temperature-independent individual responses (D4 in Fig. 2.9).

The effects of environmental conditions (temperature, aerial exposure) on acardia/bradycardia of *S. oculus* were examined retrospectively, using heart recordings taken for experiments with alternative objectives. Examples of conditions causing emergence from acardia/bradycardia are given in Figs. 2.10, 2.11 and 2.12. Generally, limpets which exhibited bradycardia in both air and water emerged from this when environmental temperature was raised (particularly above 25°C), and/or when the medium (air/water) was changed. Opposite conditions, the lowering of temperature (below 15°C) and maintaining limpets in either air or water, induced bradycardia. Emergence from bradycardia (induced by exposure to air) was usually slower at lower temperatures (5 h at 10°C; Fig. 2.10) than at higher temperatures. However, recovery of normal heart rate following emergence was variable, taking at times over 2 h (Fig. 2.11). On full recovery there was no clear overshoot, above that of normal heart rate (Fig. 2.12). In a few limpets, acardia/bradycardia was maintained regardless of temperature and submergence/emergence regime. A single observation was made of the induction of bradycardia by an increase in temperature (Fig. 2.13).

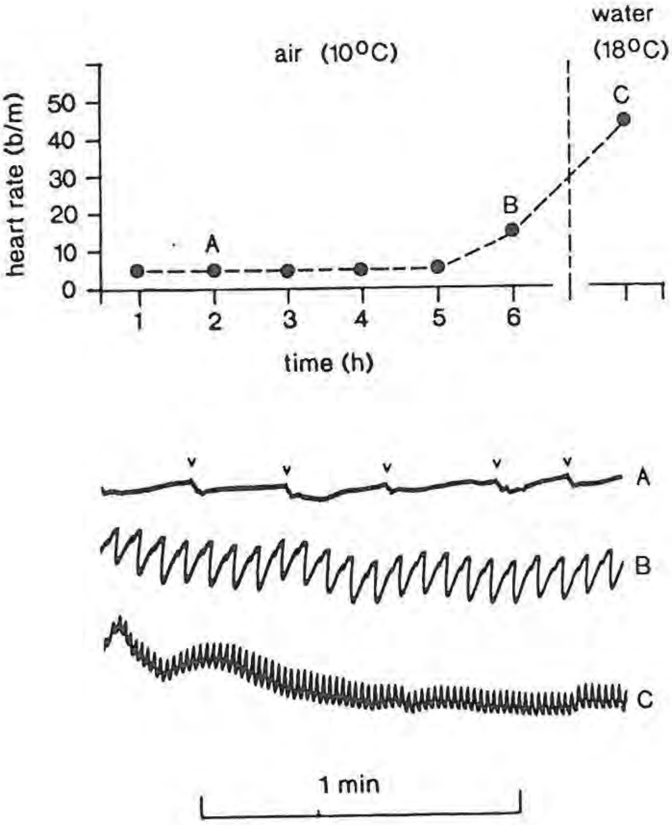

Fig. 2.10. Emergence from bradycardia of an individual *Siphonaria oculus* after 6 h aerial exposure (10°C), and heart rate recorded later in water (18°C). Cardiograms of events indicated on the graph are given below.

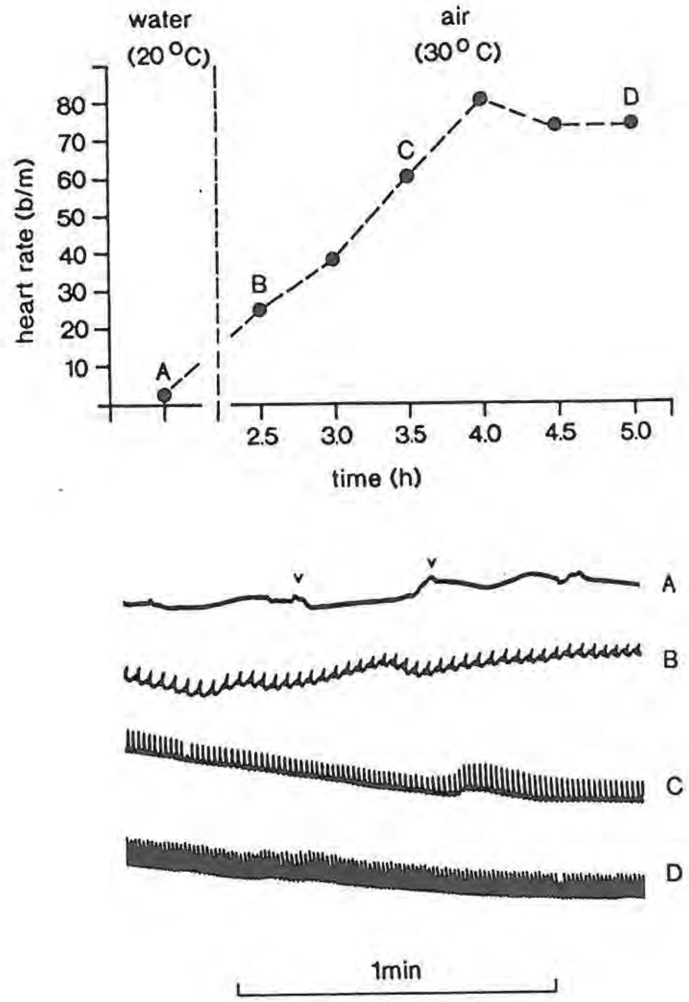

Fig. 2.11. Emergence from bradycardia of an individual *Siphonaria oculus* following aerial exposure and an increase in temperature (from 20°-30°C). Recovery of normal heart rate is observed after 4 h. Cardiograms of events indicated on the graph are given below.

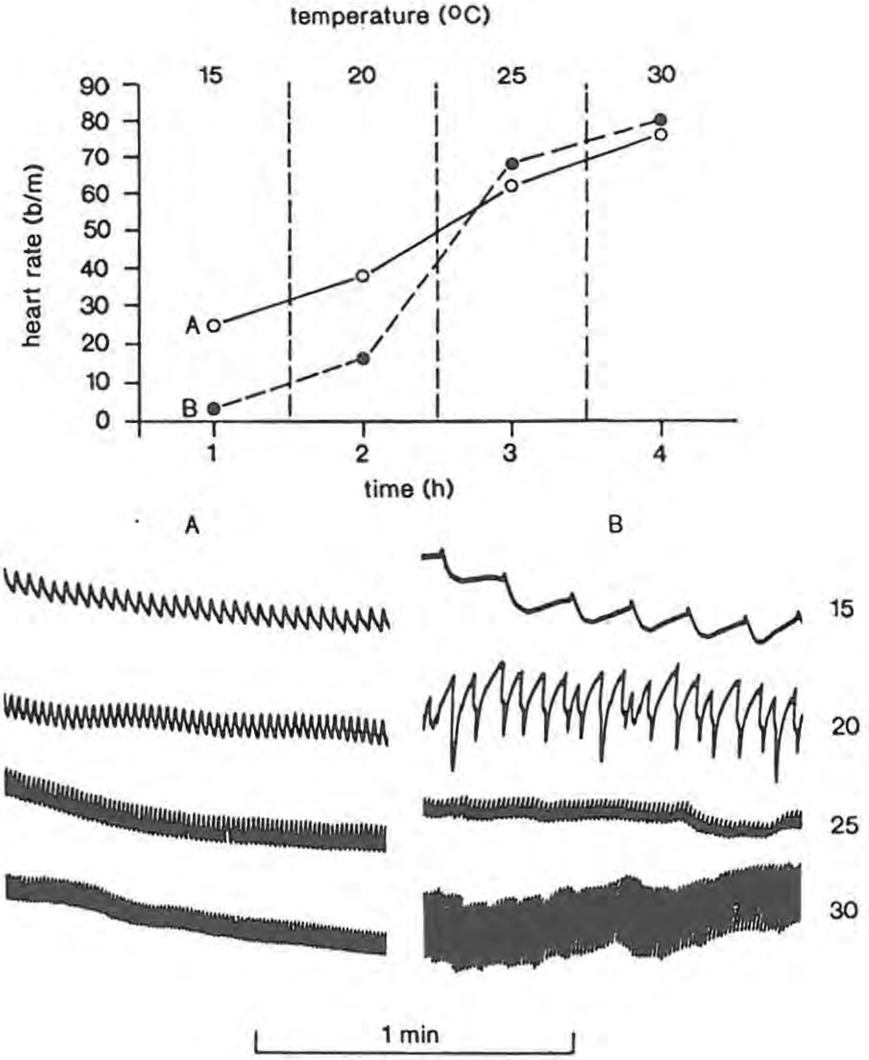

Fig. 2.12. Aerial heart rate responses for the same individual of *Siphonaria oculus* to a change in temperature, recorded on different occasions. (A) Represents the "normal" response, and (B) that incorporating bradycardia. Cardiograms of the respective responses are given below.

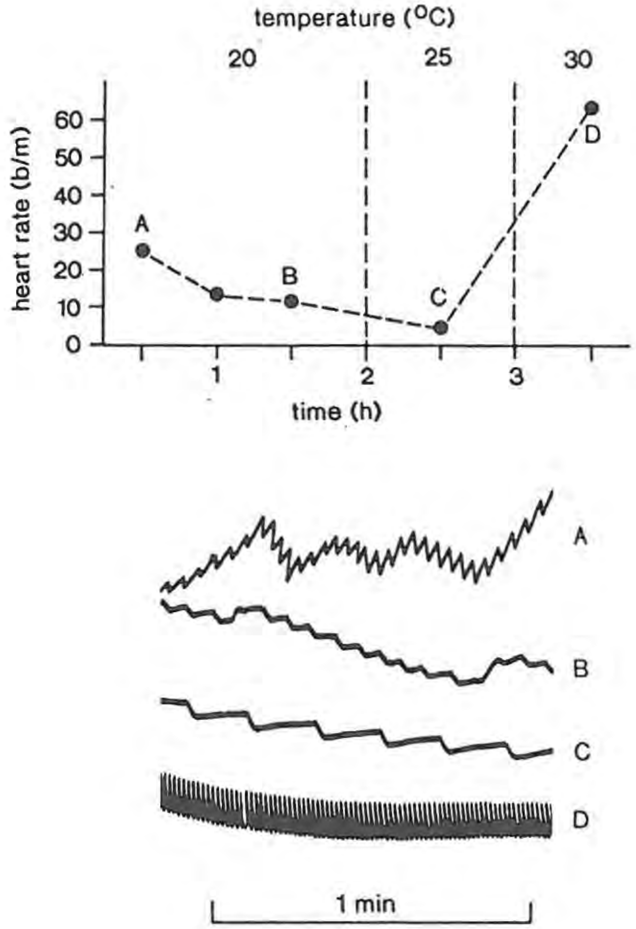

Fig. 2.13. Example of the induction of bradycardia in *Siphonaria oculus* by increasing aerial temperature. Cardiograms of events indicated on the graph are given below.

2.4 DISCUSSION

In limpets, the regular raising and lowering of the shell is suggested as enhancing ventilation of the respiratory organs (Daniel 1980). Such movements of the shell were considered to be responsible for the regular vertical surging of the impedance recording trace both in *Patella granularis* and *Siphonaria oculus* (Fig. 2.1 c,d,e).

Normally, the gills of *P. granularis* are ventilated by a flow of water, opposite in direction to the flow of blood, generated by ciliary movement (Nuwayhid, Davies and Elder 1978). Impedance surging by *P. granularis* was observed only in seawater at 30°C, a condition never experienced in the natural environment. At high temperatures (for example, 30°C) the oxygen holding capacity of water is reduced, compared to at lower temperatures (Graham 1990). Metabolic rate of limpets, as for poikilotherms in general, is raised at higher temperatures. Thus, additional ventilatory behaviour may compensate for the raised metabolism under conditions of lowered oxygen availability. In support of this idea, mushrooming (excessive lifting of the shell), an apparently stress- induced behaviour, was observed during ventilatory surging. In air, the shell of *P. granularis* is held close to the substratum, primarily to reduce water loss, which may explain the absence of ventilatory surging in this medium. However, under high aerial temperatures mushrooming by this limpet has been observed (C.D. McQuaid pers. com.)

Water flow across the internal gill of *Siphonaria* is generated by cilia located on the dorsal ridge of the mantle cavity (Yonge 1952), and on the gill (de Villiers and Hodgson 1987). In air, the mechanism of ventilation of the mantle cavity is presumably similar to that for pulmonate snails in general; pressure within the mantle cavity is increased by raising the floor, resulting in the flushing of air out of this cavity. Lowering of the mantle cavity floor has an opposite effect. Contrary to the viewpoint of Yonge (1952), impedance recordings suggest contractility of mantle cavity muscle and, dilation and contraction of the pneumostome is readily observed in *Siphonaria*. The raising and lowering of the shell would similarly alter internal air pressures, shell movements presumably supplementing normal respiratory ventilation.

Jones (1968) found no correlation between radula activity and regular drift of the impedance trace for *Patella vulgata*. However, the impedance effect described by Jones (1968) may have been the result of shell lifting rather than radula activity. In the present study, radula activity was only detected when electrodes, although inserted for heart beat recordings, came to lie near the radula. This was frequently observed in the relatively flat-shelled limpets, *P. oculus* and *P. concolor*, but not in the dome-shelled, *P. granularis* or laboratory-held *S. oculus*.

Bradycardia in response to environmental conditions inhibiting normal respiratory functioning (for example, hypoxia), is common in many marine invertebrates (Bayne 1971; deFur and Mangum 1979; Shick, Gnaiger, Widdows, Bayne and de Zwaan 1986). Some crustaceans (de Fur and Mangum 1979), bivalves (Earll 1975) and gastropods (Bourne, Redmond and Jorgensen 1990; Arshavsky, Deliagina, Gelfand, Orlovsky, Panchin, Pavlova and Popova 1990)), also exhibit brief cessation of heart beat (cardiac arrest) in air or normoxic water. Spontaneous, transient cardiac arrest, usually lasting for less than 2 minutes, was occasionally observed by *P. granularis* (Figs. 2.2 and 2.3). In the gastropods, *Haliotis, Polinices, Archidoris* spontaneous cardiac arrest has been suggested as being associated with extension of a region of the body (Bourne et al. 1990). Cessation of heart beat in the swimming pteropod, *Clione*, was linked with periods of locomotory inactivity (Arshavsky et al. 1990), and in the bivalve *Scrobicularia*, with a decline in pumping activity (Earll 1975). In *P. granularis* body movement caused the recording trace to be deflected (frequently off-scale). Heart beat, and cardiac arrest were observable in association with, and without deflection of the recording trace, respectively, suggesting no relationship between cardiac arrest and movement in this limpet. While the functional significance of cardiac arrest in *P. granularis* is unclear, the absence of heart beat has at times been associated with the stressful "ventilatory" surging (Fig 2.3).

Siphonaria oculus exhibited spontaneous acardia/bradycardia which, unlike that of *P. granularis*, could be sustained for hours and probably days, and was occasionally preceded by oscillations of heart rate (Figs. 2.4 and 2.5). The opisthobranch, *Aplysia* has also been reported to undergo bradycardia and prolonged acardia from a few minutes to hours (Koester, Mayeri, Liebeswar and Kandel 1974; Feinstein, Pinsker, Schmale and Gooden 1977). However, acardia/bradycardia in *S. oculus* represents a different response from that of *Aplysia*, which is apparently related to respiratory stress. Bradycardia in *Aplysia* occurred in response to hypoxia, or aerial exposure (Feinstein et al. 1977; Koester, Dieringer and Mandelbaum 1979; Dieringer, Koester and Weiss 1978) and prolonged acardia was shown to be coupled with a gill withdrawal reflex (Koester et al. 1974). Under identical environmental conditions, *S. oculus* shows either acardia/bradycardia or normal heart rate (Fig. 2.9). Acardia/bradycardia in *Siphonaria* does not appear to be linked with respiratory stress, or body movement, for similar reasons to those given for *P. granularis.*

Like most other physiological rate functions of poikilotherms, heart rate is generally related to ambient temperature. Specific studies concerning heart rate change with temperature change of limpets, include those for the prosobranch *Acmaea (Collisella) limatula* (Segal 1956; Segal 1962; Markel 1974). Other than temperature, factors such as food intake, reproductive status, and body size, also influence metabolic rate (Newell 1973), and are particularly responsible for variability of metabolic rate among individuals. In marine invertebrates in general, there exists a good relationship between body size and oxygen consumption (Zeuthen 1953; Newell and Roy 1973). While heart rate and body size are correlated in crustaceans, this has not always been found in molluscs (de Fur and Mangum 1979). As for the gastropods *Acmaea* and *Hemifusus* (Segal 1956; Depledge and Phillips 1986), heart rate and body mass are closely related in *P. granularis* (Fig. 2.7), but not in *S. oculus*. The relationship for *S. oculus* was partly obscured by a narrow size range.

"Normal" heart rates of *S. oculus* were lower than those of *P. granularis* for a range of temperatures (Table 2.2). While a change in heart rate for an individual is likely to represent a corresponding change in metabolic rate, inter-specific differences in heart rate, cannot be interpreted as differences in metabolic rate between the species. The temperature coefficient (Q_{10}), however, allows for interspecific comparison of dependence of rate on temperature. Both species responded similarly, showing a decline in Q_{10} (to below 2) with increasing temperature (Table 2.2). The Q_{10} for *S. oculus* between 25° and 30°C was a very low value of 1.45, and, rate-temperature responses by individual limpets of this species occasionally showed even greater independence than predicted by the population Q_{10} (extreme responses by the same individual are given in Fig. 2.9; D4 and D1 represent temperature- independence and temperature-dependence, respectively). Branch and Newell (1978) showed depression of Q_{10} of metabolic rate at high temperatures by some upper-shore intertidal gastropods, and suggested this as a means of conserving energy by these species.

Prolonged bradycardia, which occurred randomly among laboratory- held individuals of *S. oculus*, was prevalent at low temperatures (below 15°C), and when the medium of either air or water was kept constant. Figs. 2.10-2.13 give examples of environmental conditions causing cessation or induction of bradycardia. While temperature and constancy of medium appear to be environmental cues stimulating bradycardia, this response may largely depend on deprivation of food. Whereas bradycardia was seldom observed in limpets freshly returned to the laboratory, it occurred in 10 of 14 limpets held for 5 weeks without food (15°C). Food deprivation induces dormancy in land snails (Machin 1975), characterized by metabolic rate depression (Barnhart 1986). The conditions given above, stimulating bradycardia in *S. oculus*, suggest that this response may be associated with metabolic rate depression (see Storey and Storey 1990).

2.5 SUMMARY

1. In addition to heart beat, body movements were detected by impedance pneumography. Certain impedance recordings suggested movements such as shell lifting and radula movements.

2. *Patella granularis* occasionally exhibited transient bradycardia and brief cardiac arrest (lasting for a few minutes only). In *Siphonaria oculus* acardia/bradycardia could be sustained for long periods (hours) before resumption of normal heart rate.

3. In *P. granularis*, variability in heart rate between individuals was related to dry tissue weight. This relationship for *S. oculus* was not clear. "Normal" heart rate (that is, not considering bradycardia) of *S. oculus* was relatively variable between individuals, and for the same individual at different times, particularly at relatively high temperatures (25° and 30°C).

4. Both *P. granularis* and *S. oculus* showed depression of temperature coefficient values for heart rate (Q_{10} fell below 2) when ambient temperature increased.

5. The onset of, or emergence from, acardia/bradycardia in *S. oculus* was influenced by temperature, a change in medium (from air to water and visa versa), and food deprivation.

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CHAPTER 3

RELATIONSHIP BETWEEN HEART RATE AND OXYGEN CONSUMPTION IN *PATELLA GRANULARIS* AND *SIPHONARIA OCULUS*

3.1. INTRODUCTION

Aerobic metabolic rate (oxygen consumption) is probably the most commonly measured rate function in ecophysiological studies. There are, however, constraints when measuring this using conventional differential volume respirometry, in that it requires stability of temperature, pressure and medium (air or water). The measurement of heart rate, a physiological parameter thought to reflect metabolic rate, is not limited by such constraints, and is thus advantageous in allowing the determination of physiological change during changing environmental conditions (see Chapters 4 and 7). Unlike oxygen consumption, which is determined over a unit time period, heart rate can be measured directly, enabling the instantaneous detection of a physiological change.

Among invertebrates, however, the relationship of these two physiological rate functions is quite variable. In insects, for example, the circulatory system has no respiratory function and, predictably a poor relationship exists between these parameters. There is also controversy as to the role of the circulatory system in some bivalve molluscs (Booth and Mangum 1978; Famme 1981).

Even if the circulatory system does play a role in delivery of oxygen to the tissues, heart rate may still not be a reliable indicator of metabolic rate. While oxygen delivery is related to cardiac output, which is most certainly increased by an increase in heart rate, cardiac output may be altered independently of heart rate by a change in stroke volume. Thus, heart rate and cardiac output (and therefore oxygen delivery) are not necessarily always correlated. Another potential reason for a poor correlation of these parameters is that, in soft-bodied molluscs, oxygen uptake may occur directly through the integument, thereby by-passing the circulatory system (Brown 1984; Graham 1988).

The correlation between aerial heart rate and oxygen consumption is analysed for *Patella granularis* and *Siphonaria oculus* in this chapter. Experiments were performed allowing simultaneous measurement of these parameters under sets of environmental conditions known to alter aerial metabolism (i.e., temperature and desiccation). In addition to determining the predictability of one parameter from measurement of the other, a broader understanding of the functioning of the respiratory system can perhaps be gained from this relationship.

3.2. MATERIAL AND METHODS

Patella granularis (n = 40; 170-220 mg dry weight) and *Siphonaria oculus* (n = 40; 170-220 mg dry weight) were collected and maintained as outlined in Chapter 2, as is the method for determination of heart rate. Aerial oxygen consumption was measured with a Gilson Differential Respirometer (Gilson Medical Electronics) using standard manometric techniques. A 20% KOH solution was used to absorb the evolved CO_2. Before implanting impedance electrodes, shells of the limpets were thoroughly scrubbed to remove encrusting algae. After electrode implantation, limpets were allowed a 12-16 h recovery period in seawater. Prior to respirometry, limpets with electrodes intact, were encouraged to settle on watch glasses by holding them in air of a high humidity (80% R.H.) for 1 h. After this, they were briefly immersed in seawater, then blotted to remove superficial water. Limpets, on their watch glasses, were placed in modified respirometer flasks, having a small hole to allow impedance leads to pass through to the oscillographs. The point of exit of the impedance lead was sealed with dental wax, rendering flasks airtight.

Aerial heart rate and rate of oxygen consumption were measured simultaneously for both species of limpet at 15°, 20°, 25° and 30°C. Following initial measurements at 15°C, measurements at 20°, 25° and 30°C were determined after raising the temperature by 5°C intervals. Prior to taking recordings (over 2 h), each experimental temperature was held constant for 1 h.

As intertidal animals may experience prolonged aerial exposure, an experiment was also performed to examine the relationship between heart rate and oxygen consumption following a period of continuous aerial exposure. After an initial set of readings, taking less than 12 h, limpets (still in their respirometer flasks) were held overnight for a further 12 h under controlled aerial conditions (80% R.H.; 20°C). Total aerial exposure was thus 24 h. Then $\dot{V}O_2$ and heart rate were measured again over the same temperature range.

A further experiment considered the simultaneous measurement of f_h and oxygen consumption of *S. oculus*, after being rapidly desiccated, by holding them for various periods in a silica gel dried air-stream (20°C). In all but this experiment, oxygen consumption (at STP) was converted to weight-specific values. Regression lines were fitted to the relationship for $\dot{V}O_2$ against heart rate by the method of least squares (Statgraphics Ver. 4.0, Statistical Graphics Corporation).

3.3 RESULTS

Weight-specific aerial oxygen consumption was strongly, linearly correlated with heart rate for *Patella granularis* over a range of 15°C ($r = 0.97$; $P < 0.001$; $n = 30$; Fig. 3.1). Correlation was also high when measurements were taken for the same individuals of *P. granularis* after 24 h aerial exposure ($r = 0.87$; $P < 0.001$; $n = 12$;

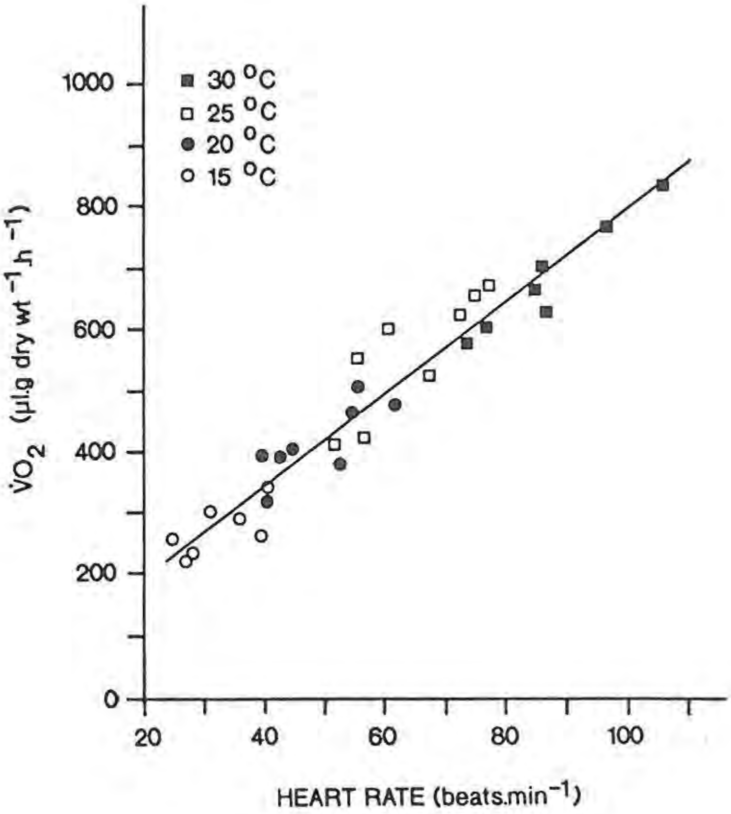

Fig. 3.1. Relationship between heart rate and $\dot{V}O_2$ for *Patella granularis* measured within 12 h aerial exposure at four experimental temperatures (n = 8 individuals; Y = 48.04 + 7.41X, r = 0.97, n = 30).

Fig. 3.2). The regression determined after 24 h aerial exposure, although not significantly different with regard to slope (Fig. 3.2; $P > 0.05$; $F = 0.4$; df = 1, 23), was shifted significantly below the 12 h exposure regression (Fig. 3.2; $P < 0.05$; $F = 22.9$; df = 2, 23).

There was a significant correlation ($r = 0.88$; $P < 0.001$; $n = 16$) between $\dot{V}O_2$ and heart rate for *Siphonaria oculus* over a range of 15°C (Fig. 3.3). Relationships for the ≤12 h and 24 h aerial exposures were not significantly different (Fig. 3.4; $P > 0.05$; $F = 1.55$; df = 2, 38), and the slightly weaker correlation for the combined data was 0.78 (Fig. 3.4; $P < 0.001$; $n = 41$). When considering specific data given in Fig. 3.4, two largely different heart rates (77 and 36 beats.min^{-1}) for similar oxygen consumption rates (around 420 uL.g dry weight^{-1}.h^{-1}) provide some indication of the level of correlation. As these heart rates represent the ≤12 h and 24 h aerial exposure treatments, respectively (Fig. 3.4), there is suggestion that better oxygenation of the blood follows moderate aerial exposure, a trend apparent for other measurements as well (see Fig. 3.4). In contrast to initial measurements in air (Fig. 3.3), temperature-dependence of heart rate at 25° and 30°C of *S. oculus* after prolonged aerial exposure was variable; being either closely linked to temperature or, as in most cases, depressed to between 35-50 beats.min^{-1} (Fig. 3.4). $\dot{V}O_2$ and heart rate were also significantly correlated ($r = 0.91$; $P < 0.001$; $n = 16$) for *S. oculus* when physiological parameters were altered by desiccation (Fig. 3.5).

When comparing regressions of $\dot{V}O_2$ against heart rate for animals of the two species, slopes were not significantly different (Fig. 3.6; $P > 0.05$; $F = 0.40$; df = 1,23), but the curve for *P. granularis* was significantly higher than for *S. oculus* (Fig. 3.6; $P < 0.05$; $F = 22.9$; df = 2,23).

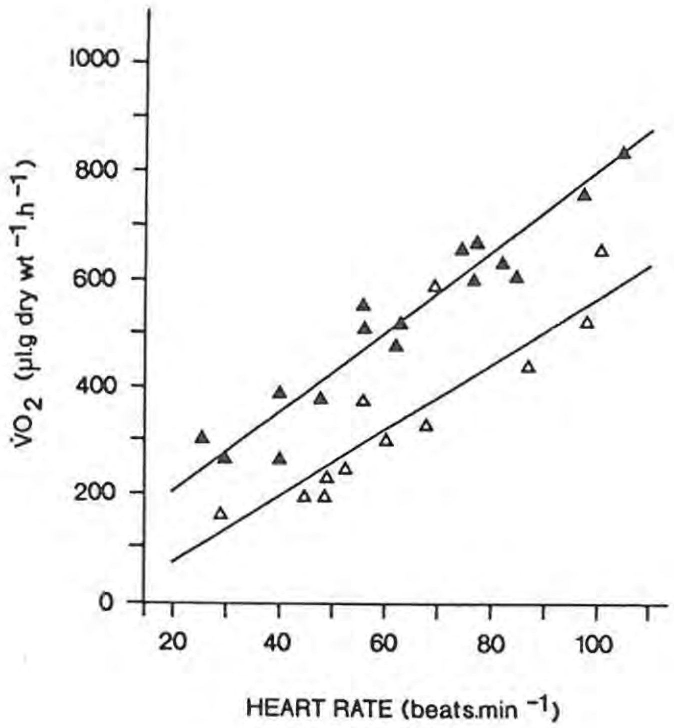

Fig. 3.2. Comparison of the relationship between heart rate and $\dot{V}O_2$ for ***Patella granularis*** measured after 12 h aerial exposure (solid triangles; $Y = 54.44 + 7.38X$, $r = 0.97$, $n = 15$, Fig 3.1) and after 24 h aerial exposure (open triangles; $Y = -71.41 + 6.63X$, $r = 0.87$, $n = 12$). The physiological parameters were varied by altering temperature, as in Fig. 3.1.

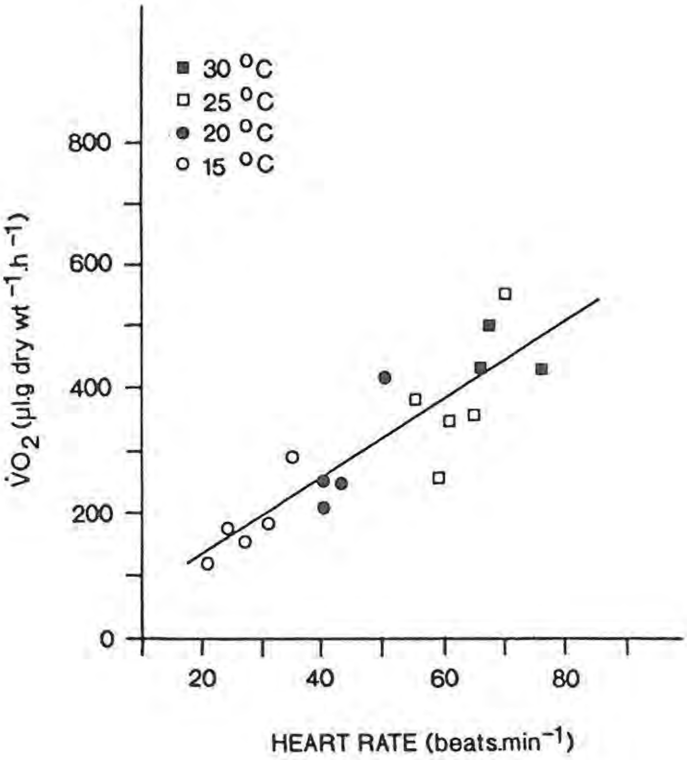

Fig. 3.3. Relationship between heart rate and $\dot{V}O_2$ for *Siphonaria oculus* measured within 12 h aerial exposure (n = 5 individuals; Y = 5.41 + 6.36X, r = 0.88, n = 17).

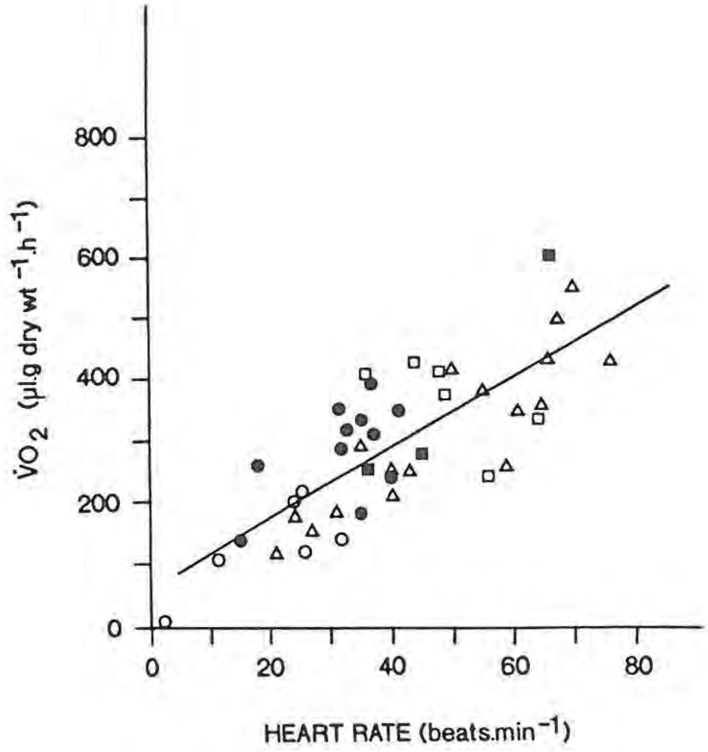

Fig. 3.4. Relationship between heart rate and $\dot{V}O_2$ for *Siphonaria oculus* measured after 12 h aerial exposure (open triangles) and after 24 h exposure. The combined regression is: Y = 64.35 + 5.64X, r = 0.78, n = 42.

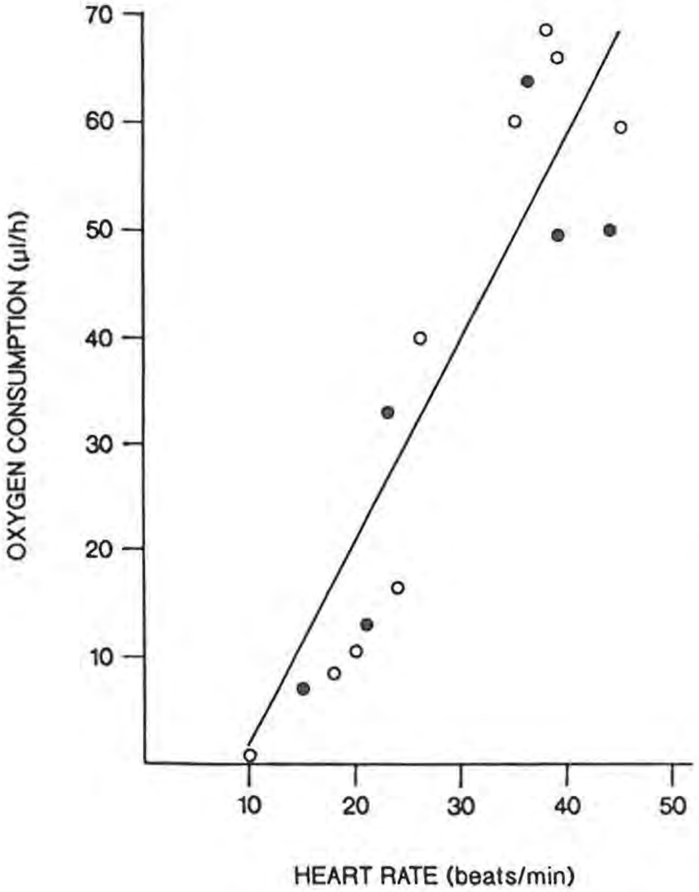

Fig. 3.5. Relationship between heart rate and oxygen consumption for two individuals of *Siphonaria oculus* (shown by open or closed circles; $Y = -28.69 + 2.28X$, $r = 0.91$, $n = 15$). Physiological parameters were varied by progressive desiccation.

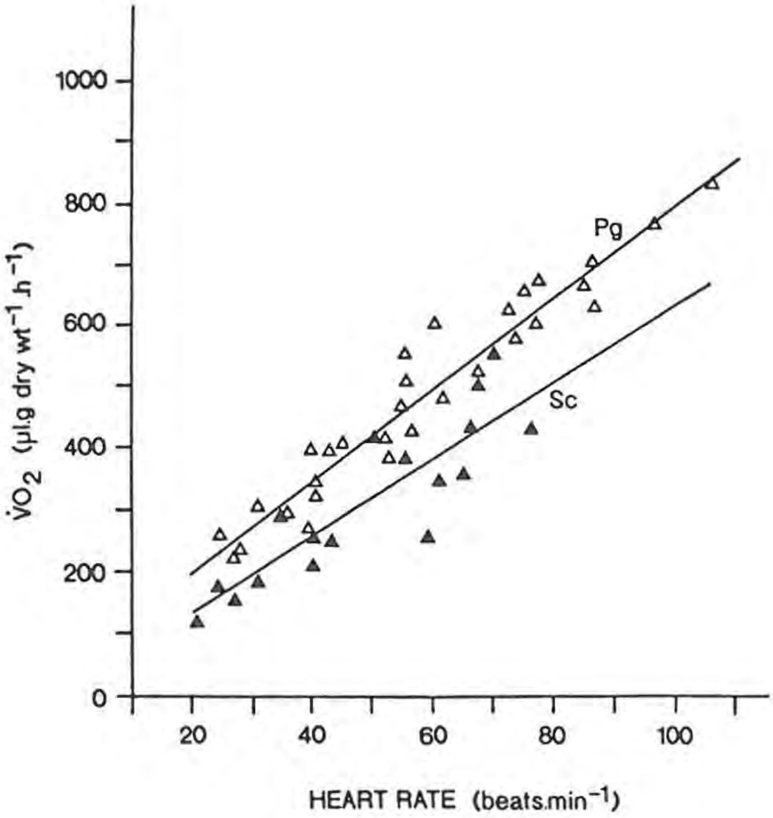

Fig. 3.6. Comparison of heart rate/VO2 regressions of *Patella granularis* and *Siphonaria oculus* shown previously in Figs. 3.1 and 3.3, respectively.

3.4 DISCUSSION

In gastropod molluscs the circulatory system performs two major functions: hydraulic functions and transport functions (Bourne, Redmond and Jorgensen 1990). The transport of respiratory gases is essentially controlled by cardiac output (a product of heart rate and stroke volume) and properties of the blood (e.g., respiratory pigment type, concentration, and functioning; Mangum 1980). As both stroke volume and blood properties may vary independently of heart rate, oxygen delivery to the tissues via the circulatory system may not necessarily be a function of heart rate. Another reason that there may be no clear relationship between oxygen consumption and f_h, is that, in soft-bodied molluscs some oxygen enters the body across the integument and arrives at the tissues by diffusion, thereby illuding the circulatory system (Brown 1984, Graham 1988).

Even though there are no known studies specifically examining the correlation between oxygen consumption and f_h of marine invertebrates, metabolic rate is frequently inferred from heart rate. Studies in which such inference has been made, include those by Lowe and Trueman (1972), Spaargaren (1973) and Depledge (1978). In an analysis of the reliability of heart rate as an index of metabolic rate, in red squirrels, Pauls (1980) showed that the correlation for $\dot{V}O_2$ against f_h varied between individuals, for each individual from day to day, seasonally, and depending on level of exercise/rest. There was no obvious variability for this correlation between individuals of *P. granularis* (Fig. 3.1; n = 8 individuals). Although heart rate for *P. granularis* (Chapter 2), and $\dot{V}O_2$ for invertebrate poikilotherms in general (Zeuthen 1953), are weight-dependent, both being inversely related to weight, variability was reduced by selecting individuals of a narrow-size range. Other data not represented here, suggest little individual variability from day to day providing pre-experimental conditions were similar. Prolonged aerial exposure however, shifted the regression of *P. granularis* below that determined shortly after exposure (Fig. 3.2). Normal gaseous exchange is impaired when water is lost from the gills by evaporation, and this may explain transposition of the regressions. However, the aerial condition in this study was such (of a relatively high humidity), that evaporative water loss was relatively slow, suggesting an effect due to aerial exposure rather than desiccation.

Aerial exposure in amphibious invertebrates usually leads to an acid-base disturbance, specifically, a hypercapnic (respiratory) acidosis (see review by Truchot 1990). This may be compensated for, and compensatory mechanisms of decapod crustaceans, and to a lesser extent bivalve molluscs, have been documented (Burnett 1988). A single study examines the buffering capacity of the tissues of a gastropod (Eberlee and Storey 1984), with no known studies on their to ability to compensate for acid-base disturbances. Assuming absence of a significant compensation, a Bohr shift resulting from acidification of the haemolymph will lower oxygen affinity of the haemocyanin, providing another possible explanation for the shifting of the 24 h aerial exposure regression in *P. granularis*.

For *S. oculus*, although highly significant, there was a relatively weak relationship between f_h and $\dot{V}O_2$ (Figs 3.3 and 3.4). Temperature independence of aerial heart rate (Figs. 3.3 and 3.4), particularly at high temperatures, is also shown previously (Chapter 2). Along with some other marine gastropods, *Buccinum, Haliotis, Busycon* (Mangum and Lykkeboe 1979; McMahon 1988), a closely-related species to *S. oculus*, *S. zelandica*, shows a reverse Bohr shift (Wells and Wong 1978), i.e., an increase in oxygen affinity with an increase in H^+ (Brix, Lykkeboe and Johansen 1979; Mangum 1980). The similarity of the relationships of f_h and $\dot{V}O_2$ for short and long term aerial exposure, and even in some cases an apparent enhancement of

$\dot{V}O_2$ after prolonged exposure, would be explained by a reverse Bohr shift by *S. oculus*.

Aponea (no detectable oxygen uptake) was associated with the bradycardia (<10 beats.min^{-1}) by *S. oculus* but, was recorded on two occasions only here (Figs. 3.4 and 3.5), and whether it is characteristic of bradycardia would require further investigation. It is elaborately argued that metabolic rate (oxygen consumption) is dependent on the amount of oxygen arriving at the tissues (Coulson, Hernandez and Herbert 1977). Therefore, as oxygen consumption is higher in *P. granularis* for any particular heart rate, than in *S. oculus* for the same heart rate (Fig. 3.6) more oxygen is transported by the blood of *P. granularis*. This may be due to a greater stroke volume in *P. granularis* which generally had a stronger pulse (indicated by stronger impedance signal). However, impedance signal may not be reliable in determining of the stroke volume (Chapter 2; Wells 1979). Also, nothing is known about the blood properties of these limpets to derive conclusions concerning this difference between the species.

Because closely-related species, *P. caerulea, P. lusitanica* and *S. zelandica* possess blood pigment (Bannister, Bannister and Micallef 1968; Wells and Wong 1978; Bonaventura and Bonaventura 1983), the circulatory system of *P. granularis* and *S. oculus* is likely to function in the transport of oxygen. However, a relationship between heart rate and oxygen consumption is not evidence for this, as both parameters may be similarly related to temperature. Even so, should causality not exist, a good correlation nevertheless allows prediction of one parameter from measurement of the other. Considering the good and significant correlations for both *P. granularis* and *S. oculus*, it would seem reasonable to make inference about their aerial metabolic rate from heart rate, providing the period of exposure is taken into account, especially in the case of *P. granularis*.

3.5 SUMMARY

1. Aerial heart rate and $\dot{V}O_2$ were linearly correlated for *Siphonaria oculus* and *Patella granularis* when these parameters were varied by changing temperature. This relationship for *P. granularis* was significantly transposed above that for *S. oculus.*

2. While prolonged aerial exposure (+ 24 h) lowered the curve for heart rate against $\dot{V}O_2$ for *P. granularis* below that determined over short-term exposure, it had no significant effect on this relationship for *S. oculus.*

3. Oxygen consumption and heart rate of *S. oculus* were correlated when these parameters were altered by desiccation.

4. The correlations of heart rate and oxygen consumption are discussed in terms of functioning of the circulatory system and the predictability of one parameter from the other.

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CHAPTER 4

SEASONAL AND DIEL VARIATION IN HEART RATE OF *SIPHONARIA OCULUS* MEASURED *IN SITU*

4.1 INTRODUCTION

Fluctuations and extremes in physico-chemical conditions characterize the marine intertidal environment. Animals inhabiting this environment show diverse behavioural and physiological adaptations. Their physiology in response to environmental change is usually examined under controlled laboratory conditions, whereby environmental variables can be considered separately (see Davenport 1982, for review on environmental simulation experiments on marine animals). However, normal behaviour and feeding patterns are potentially impeded in laboratory-held animals, in turn influencing their physiology.

Field recordings of physiological parameters are often difficult to interpret as they represent responses to an interacting set of environmental variables. By measuring key environmental variables, simultaneously, along with physiological parameters, this problem is to some extent overcome. Such field recordings are useful for comparison with laboratory recordings of the same physiological parameters. Of the various physiological rate functions, heart rate is perhaps the most practical and simplest to monitor *in situ*, for marine invertebrates. Impedance pneumography has been used to measure field heart rate in sedentary intertidal bivalves (Trueman 1967; Coleman 1974). Recently, the heart rate of the actively swimming prawn, *Palaemon elegans* has been monitored *in situ* using this, and computer-aided data acquisition techniques (Morris and Taylor 1984).

The laboratory-determined response of heart rate of *Siphonaria oculus* to acute change in ambient temperature is described in Chapter 2. In this chapter heart rate of *S. oculus* was recorded in the field, dielly, during summer and winter. Some environmental variables (temperature, period of aerial exposure, salinity, and relative humidity) were also monitored. An attempt is made to relate heart rate patterns to immersion/emersion, day/night, and short-/long-term change in temperature. *Patella granularis* does not naturally occur in estuaries, therefore it was not considered in this investigation.

4.2 MATERIAL AND METHODS

Siphonaria oculus colonises both the open rocky shore and many estuaries along the Southern African coast. Field excursions were undertaken to the Kariega River, eastern Cape Province (33°41'S/26°41'E), during summer (February 1989) and winter (July 1989). This is a tidal, marine dominated estuary having virtually no freshwater inflow, thus the salinity remains relatively constant (35‰-38‰; Allanson and Read 1987). Water temperature of the Kariega has been shown by Taylor (1988) to fluctuate throughout the year, with a range of 14°-33°C in January 1984 (mean of about 22°C) and 7°-22°C in July 1984 (mean of about 15°C). However, due to the effect of upwelling, unusually low water temperatures (14°C) are on occasions recorded in the summer months. Aerial temperatures are dielly variable, with mean winter temperatures being lower than summer temperatures.

Limpets (180±40 mg dry weight; $\overline{X} \pm 1$ SD), detached from boulders at the mid-intertidal zone along the bank of the Kariega estuary, were prepared for heart beat recordings (see Chapter 2 for method of electrode implantation). After electrode implantation the limpets were returned to their original home scars on the boulders, and allowed 12-16 h for recovery. An impedance recording trace (5 min) for each limpet (summer, n = 8; winter, n = 11) was then obtained hourly, over 24 h (2 tidal cycles). Heart rate (beats/min) was determined manually, from the recording traces. Temperature of air or water, and salinity, were recorded hourly just prior to taking heart rate recordings. Emersion or immersion of limpets was noted throughout the 24 h period.

4.3 RESULTS AND DISCUSSION

Diel field recordings of heart rate of *Siphonaria oculus*, and temperature, are shown in Figs. 4.1 and 4.2, for summer and winter, respectively. In general, heart rate recorded over 24 h was closely linked to temperature (Figs. 4.1 and 4.2). Some limpets however, underwent bradycardia (Fig. 4.3), confirming that this heart beat characteristic occurs naturally, rather than being an artifact of laboratory conditions (Chapter 2). During bradycardia, heart rate, which was independent of temperature, declined to very low levels (<10 beats/min). Although some limpets were noted to have moved from their home scars when immersed, there was no detectable difference in heart rate recorded during presumed periods of activity, and inactivity. Heart rate of the land snail, *Helix pomatia* shows a diurnal rhythm, declining slightly during daytime (Wunnenburg 1991). However, no obvious diel variation in heart rate was observed for *S. oculus*. Likewise there was no obvious difference in heart rate between aerial and aquatic exposure. As expected there was little variation in the salinity of the estuarine water, which varied between 34‰-36‰. While this may be a wide salinity range for many marine animals, it is not for intertidal animals, and the effect was therefore deemed negligible.

Irregular drift, presumably associated with body movement, was characteristic of aquatic recordings; this stabilized after a period of aerial exposure (Fig. 4.4). Impedance patterns pertaining to ventilatory surging and radula activity (although not

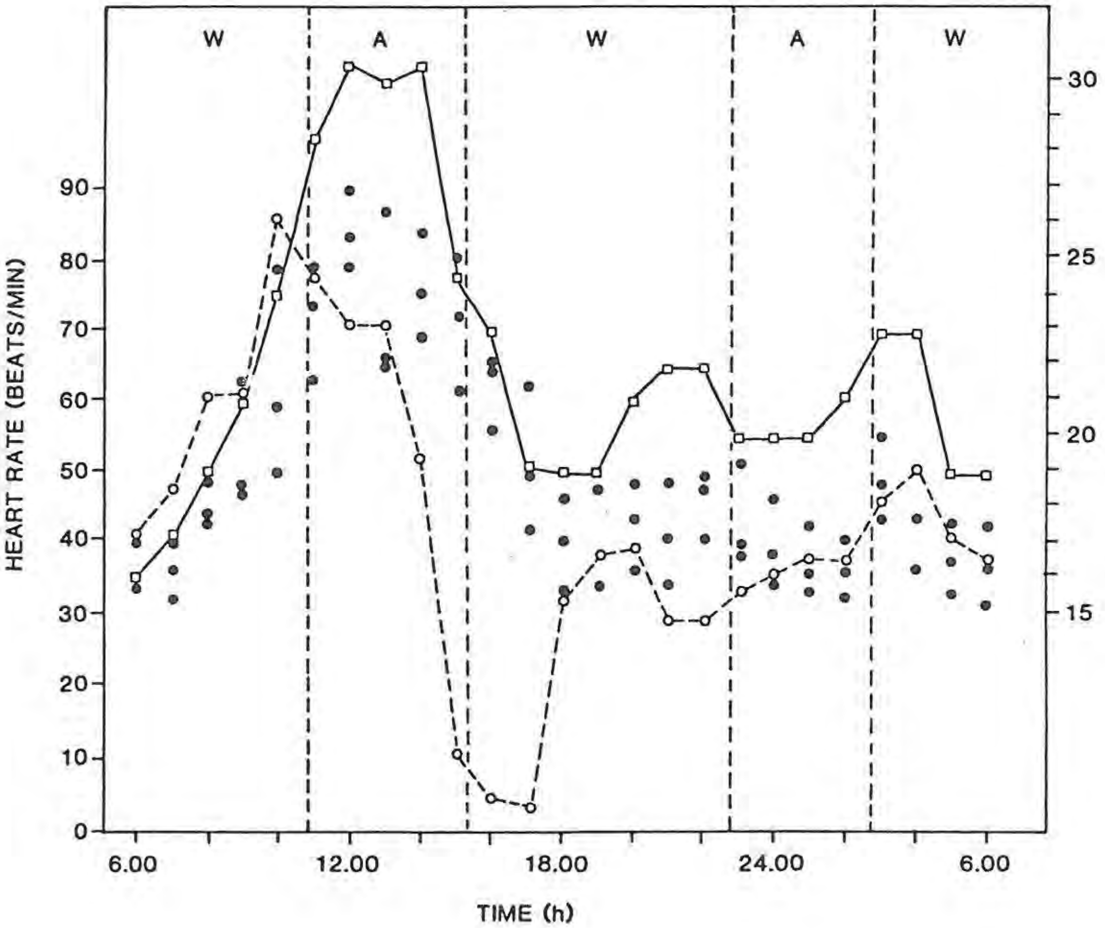

Fig. 4.1. Field recordings of heart rate of four individuals of *Siphonaria oculus* (circles) and ambient temperature (squares) taken over 24 h, in summer. Heart rate of one individual (open circles) showed temperature independence and bradycardia between 14.00 and 17.00h. W, immersion in water; A, emersion in air.

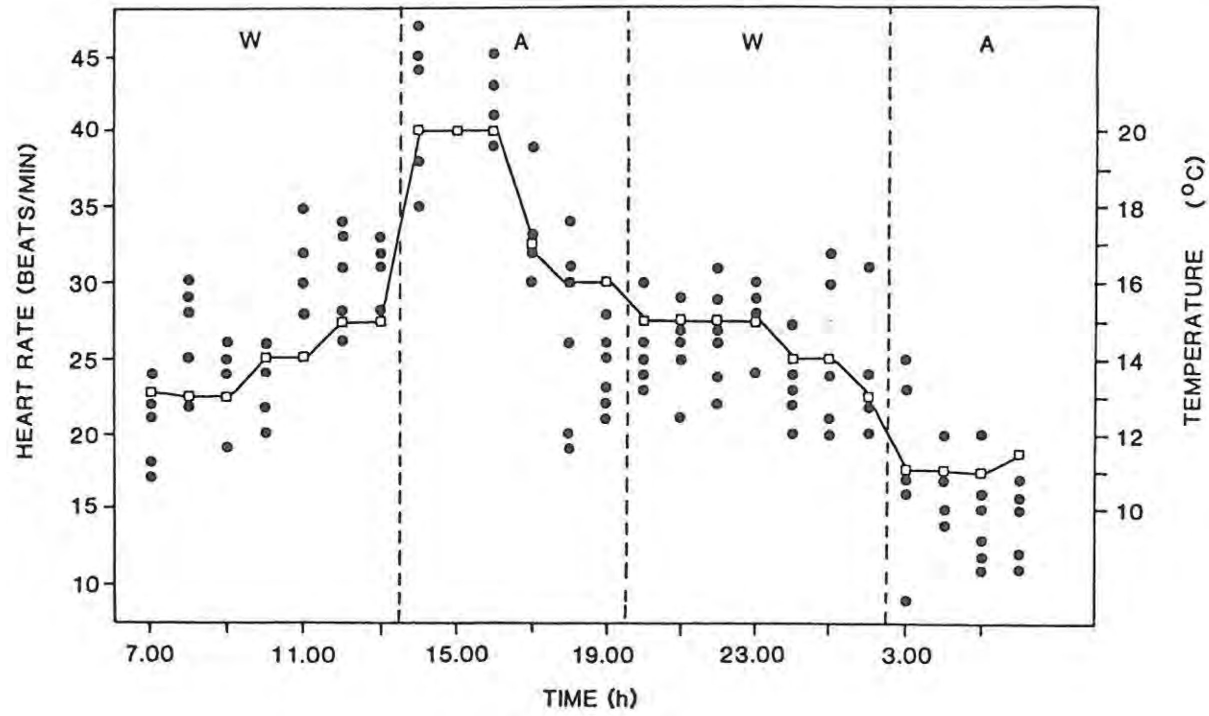

Fig. 4.2. Field recordings of heart rate of five individuals of *Siphonaria oculus* (circles) and ambient temperature (squares) taken over 24 h, in winter. W, immersion in water; A, emersion in air.

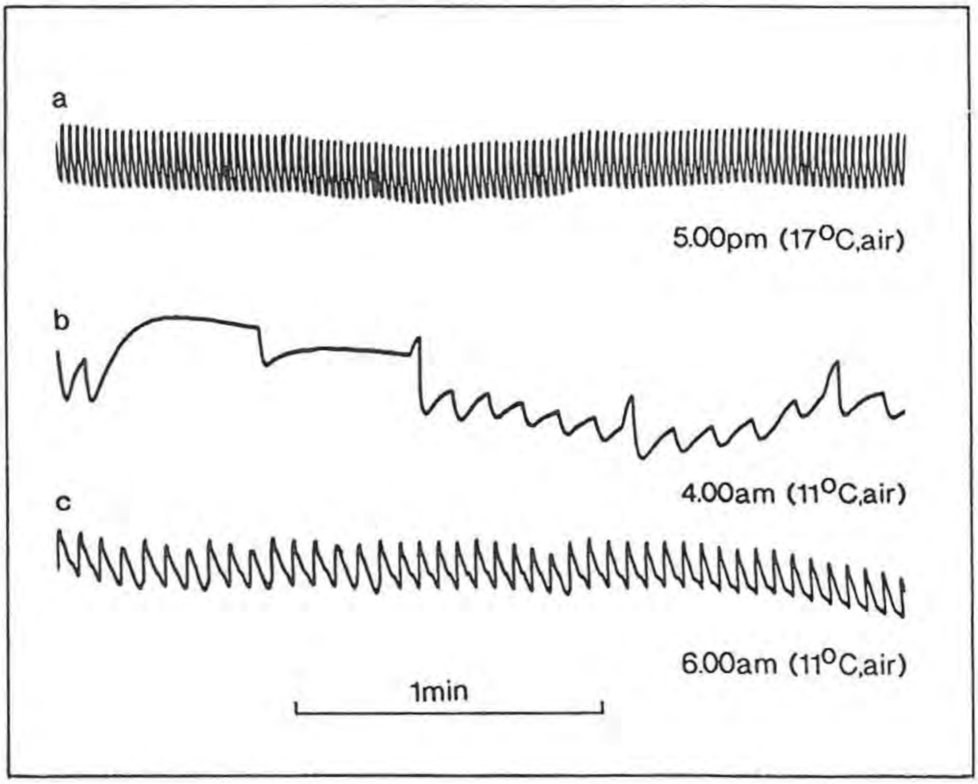

Fig. 4.3. Cardiograms of an individual *Siphonaria oculus* taken at the indicated times during a 24 h field recording in winter. Bradycardia at 11°C is shown in (b), and (c) shows later recovery to normal heart rate at this temperature.

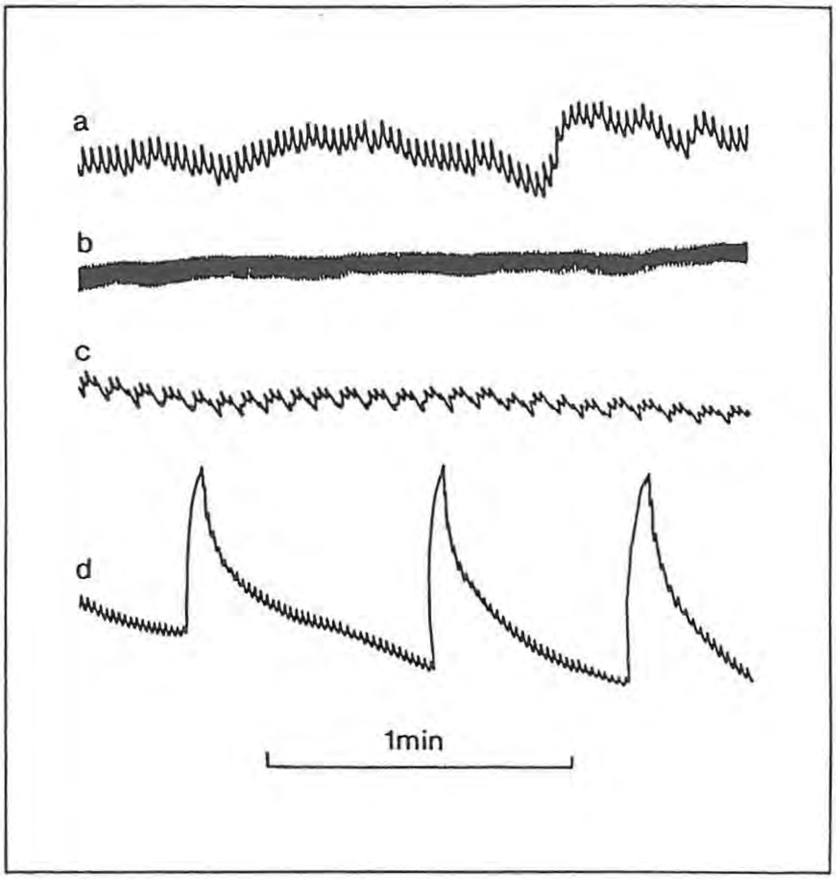

Fig. 4.4. Cardiograms of *Siphonaria oculus* recorded in the field showing (a) drift during immersion in water (17°C); (b) absence of drift in air (30°C); (c) radula movements (water, 19°C); (d) ventilatory surging (water, 19°C).

observed for this limpet in the laboratory), were recorded in the field (Fig. 4.4). To consider the medium-term effect of electrode implantation, a few limpets were left in the field with electrodes intact for four days after the 24 h monitoring exercise. Heart beat characteristics during these repeat recordings (four days later) were similar to those taken initially.

Relationships between field heart rate and ambient temperature, for summer and winter, were derived (Fig. 4.5). Because aerial and aquatic heart rates were apparently similar at the same temperature they were pooled, and heart rates associated with ventilatory surging, radula activity, and bradycardia, were excluded from the data set. Rate functions usually vary exponentially with temperature, thus by convention the relationship is expressed as the exponential equation:

$$R = ab^{T} \quad \text{or} \quad \log(R) = \log(a) + T\log(b)\ ;$$

where R is heart rate and T is temperature (Fig. 4.5). Results for the respective seasons are consistent with the generalized expectation for poikilotherms that rate (e.g., heart rate, filtration rate, metabolic rate) is to a greater or lesser extent dependent on temperature, following a short-term change in temperature (Fig. 4.5).

There was no marked depression of normal heart rate (i.e., excluding bradycardia) around 30°C in the field, as observed in the laboratory (Fig. 4.5; Chapter 2). Independence of heart rate at this temperature may be linked with altered activity patterns and reduced food availability in the laboratory. There was however, a general decline in field heart rate with increased exposure time to the higher aerial temperatures (12.00 h, 1.00 h, 2.00 h; Fig. 4.1).

Fitted regressions for the summer and winter relationships differed significantly ($P < 0.05$; $F = 21.65$; df = 1, 176). The winter regression was translated below, and rotated counterclockwise to the summer regression (Fig. 4.5). The Q_{10} values for

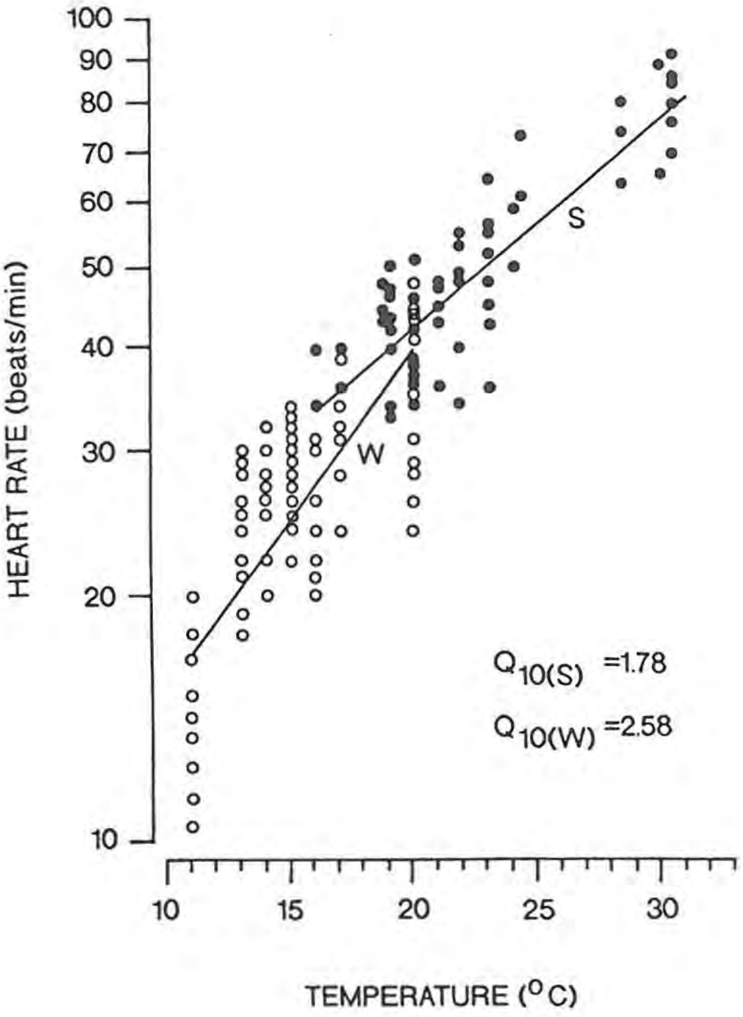

Fig. 4.5. Relationship between heart rate and ambient temperature for summer (S: log rate = 1.121 + 0.0252(temperature); r = 0.86; n = 68) and winter (W: log rate = 0.783 + 0.0409(temperature); r = 0.83; n = 112). Values of Q_{10} calculated as $(R_1/R_2)^{10/(T2-T1)}$ where (R) is rate at (T) temperature, are indicated in the figure.

the summer and winter heart rate responses were 1.78 and 2.58, respectively (Fig. 4.5).

Because heart rates at 11°C seemed to be depressed below the other winter rates, an additional winter regression excluding these values was derived (log rate = 1.015 + 0.0275(temperature); r = 0.72; n = 86). The slope of this regression was shown not to differ significantly from the summer regression ($P < 0.05$; F = 0.442; df = 1, 148). Thus, rotation of the entire winter regression (and elevation of the winter Q_{10}) results from the exaggerated lowering of heart rate around 11°C. Some of these low values may represent the onset of bradycardia.

Rate functions of most poikilotherms compensate for long-term change in temperature (Cossins and Bowler 1987). Adjustments are such that a long-term decrease in temperature, results in an increase in the temperature-related rate, and visa versa. This (thermal acclimation) optimizes rate throughout the year. Gastropods showing this type of compensation include, the freshwater snail *Lymnaea stagnalis* (Harrison 1977a; Harrison 1977b) and the intertidal limpet *Acmaea (Collisella) limatula* (Markel 1974). An opposite compensation (inverse or paradoxical acclimation) is also observed in poikilotherms (Cossins and Bowler 1987) including, many freshwater gastropods (McMahon 1983) and a land snail (Bailey and Lazaridou-Dimitriadou 1991).

A number of studies have been undertaken on intertidal limpets examining seasonal variation in heart rate and/or metabolic rate (Segal 1956; Markel 1974; Parry 1978; Parry 1984). Winter heart rates and metabolic rates for *Acmaea (Collisella) limatula* (Segal 1956; Markel 1974), and *Cellana tramoserica* (Parry 1978; Parry 1984), respectively, were elevated above summer rates. Summer metabolic rates were higher for low-shore *Patella aspera* and *P. vulgata*, but there was no difference between summer and winter rates of high-shore *P. vulgata* (Davies 1965; Davies 1967).

However, whether seasonal variation in rate functions, is temperature-mediated is a matter of controversy, as these may be influenced by variation in food availability, reproduction, and growth (Davies 1967; Markel 1974; Parry 1978; Parry 1984). Parry (1984) showed that experimental food deprivation eliminated elevated winter metabolism in *Cellana tramoserica*. Lowered metabolic rates by upper-shore, compared to low-shore individuals of the limpets, *Acmaea (Collisella) limatula* (Segal 1956), *Patella vulgata* (Davies 1967) and *P. granularis* (Branch and Newell 1978; Branch, Borchers, Brown and Donnelly 1988), is presumably related to scarcity of food in the upper-shore. Similarly, seasonal changes in food availability could be expected to alter metabolic rates in these limpets.

In *Acmaea (Collisella) limatula*, an increase in seasonal reproductive activity (indicated by a higher gonad index) corresponded with an elevated winter heart rate (Markel 1974). Some siphonariid limpets showed peak reproductive output during spring and summer (Abe 1941; Quinn 1988). In the present investigation, reproductive activity may not be of importance, as periods of maximal reproductive output (spring and early summer; R. Chambers pers. com.) do not coincide with those during which recordings were taken.

To summarise, although the lowering of winter heart rate in *S. oculus* may represent inverse (non) acclimation, as seasonal metabolic rates are influenced by factors other than temperature, this is not conclusively shown by recordings taken seasonally. Characteristics of thermal acclimation are therefore best examined under controlled laboratory conditions. The only known laboratory investigation examining thermal acclimation of an intertidal limpet, is that on *Acmaea (Collisella) limatula* (Markel 1974; Markel 1976).

4.4 SUMMARY

1. Diel variation in heart rate of *Siphonaria oculus* recorded in the field over 24 h, was closely linked to changes in ambient temperature.

2. Spontaneous bradycardia, and impedance characteristics pertaining to radula activity and ventilatory surging, observed in the field, were comparable to laboratory observations.

3. The winter regression for the relationship between field- recorded heart rate and temperature is translated below and rotated anticlockwise to that of the summer regression. The thermal acclimation status of *S. oculus* can however not be concluded from these seasonal patterns of heart rate.

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CHAPTER 5

OXYGEN CONSUMPTION IN *PATELLA GRANULARIS* AND *SIPHONARIA OCULUS*: TEMPERATURE AND BODY SIZE

5.1 INTRODUCTION

Intertidal animals are frequently exposed to extreme temperature changes. Generally, aquatic temperature remains relatively constant (upwelling however causes a rapid and extreme decline in temperature), whereas aerial temperature may vary considerably, dielly. Aerial temperatures may rise to high levels during the heat of the day (above 35°C), and are exasperated by heating of the rocky substratum. Because duration of periodic aerial exposure increases with vertical height on rocky shores, upper-shore species in particular, are exposed to unstable high temperatures.

Upper-shore gastropods must therefore either facilitate evaporative cooling, or physiologically tolerate elevated temperatures (some upper-shore littorinids have heat coma temperatures of the order of 45°C; see McMahon 1990). Though, limited thermoregulation occurs in some intertidal gastropods (Lewis 1963), including limpets (Garrity 1984), an earlier study shows that limpets had a poor capacity for this (Vermeij 1971). In addition to physiologically withstanding high temperatures, upper- shore species are faced with hypothetical energetic problems; a high energetic turnover (as metabolic rate of poikilotherms in general, is to some degree temperature-dependent) in a region of the shore permitting only low energetic input (Branch, Borchers, Brown and Donnelly 1988).

Investigations on the metabolic rate-temperature responses of some South African *Patella* species (Branch and Newell 1978; Branch 1979) have led to theoretical considerations on metabolic adaptations facilitating distribution on the shore (Branch et al. 1988). In brief, metabolic adjustments by upper-shore species are such that they reduce energetic losses. These investigations consider the metabolic rate response to short-term change in temperature only (Branch and Newell 1978; Branch 1979). Other studies on intertidal prosobranch limpets have considered long-term temperature effects, by examining seasonal variations in metabolic rate and heart rate (Segal 1956; Davies 1967; Markel 1974; Parry 1978; Parry 1984), but, whether these findings are temperature- related, requires in most cases confirmation by laboratory experiments (see Chapter 4). Metabolic rate in relation to acute temperature change has also been examined for some species of *Siphonaria* (Innes, Marsden, and Wong 1984; Dye 1987). In the previous chapter (Chapter 4), seasonal measurements of heart rate are suggestive of inverse (or non) temperature acclimation by *S. oculus.*

In this chapter the acute metabolic rate-temperature responses of *P. granularis* and *S. oculus* are compared. Because these limpets are vertically separated (*S. oculus* occurring higher on the shore), this investigation further examines the hypothesis that upper-shore species show temperature-related energy conserving mechanisms (Branch et al. 1988). In order to examine energy conservation over the long-term, metabolic rate (oxygen consumption) was determined for warm- and cold-acclimated limpets. As body weight is an important factor influencing metabolic rate, this relationship was also examined for both species of limpet.

5.2 MATERIAL AND METHODS

5.2.1 Collection and laboratory acclimation of specimens

A size-range of *Patella granularis* and *Siphonaria oculus* (60-300 mg dry weight) were collected from upper-intertidal rocks at Port Alfred (33°36'S; 26°53'E) (eastern Cape Province) during mid-August 1988 (austral winter). Limpets were collected and returned to the laboratory as explained in Chapter 2. They were kept unfed in 150 L plastic aquaria in controlled environment rooms. A tidal cycle of 12:12 h air:water was maintained. Aerial and water temperatures, salinity (35‰) and subdued illumination were held constant.

Limpets were subjected to two temperature acclimation treatments; a relatively low temperature (10°C), or a relatively high temperature (25°C), for at least 14 d prior to measuring oxygen consumption. This period is necessary to induce isoenzyme production (Cossins and Bowler 1987). A third group of limpets referred to as having been non-acclimated, were held for 5 d at 20°C before taking measurements. No measurements were made on limpets held for longer than 30 d in the laboratory.

5.2.2 Measurement of oxygen consumption

Aerial oxygen consumption of acclimated and non-acclimated limpets of a variety of sizes was determined at 15°, 20°, 25° and 30°C. All temperatures, both experimental and acclimation, were considered not to be stressful and falling well within the temperature tolerance ranges of both limpet species. Oxygen consumption was measured with a Gilson Differential Respirometer (Gilson Medical Electronics) using standard manometric techniques as outlined in Chapter 3. Prior to taking readings at a particular temperature, the equipment was left to equilibrate for 1 h. Readings were usually taken over 2 h and manometric adjustments made every 15 min. Temperatures were either raised or lowered, and there was no obvious difference in rates of oxygen consumption at any particular temperature during the 12 h period over which measurements were taken, for different pre-temperature conditions or period of aerial exposure.

Before placing limpets in specially modified respirometer flasks to allow for their size, their shells were thoroughly scrubbed and washed. They were then gently blotted to remove superficial water, and allowed to settle on watch glasses for 1 h in air of a high R.H.(around 80%). While a period of aerial exposure reduces limpet activity during respirometry, it is known to cause a slight reduction in oxygen consumption through loss of mantle water (Houlihan 1979; Houlihan, Innes and Dey 1981). Measurements for the very occasional limpet which had moved off its watch glass were discarded. Likewise, the occasional very low rates of oxygen consumption for *S. oculus*, which were presumably associated with bradycardia (see Chapter 2).

After measuring oxygen consumption, each limpet was removed from its shell and the tissue dried to constant weight at 60°C. Regression lines for relationships between oxygen consumption (corrected to STP ($uL.h^{-1}$)) and dry weight (mg), were fitted by the method of least squares (Statgraphics Ver.4.0, Statistical Graphics Corporation).

5.3 RESULTS

5.3.1 Body weight and oxygen consumption

The relationship between body weight and rate of oxygen consumption is usually expressed as the power equation:

$$M = aW^{b} \quad \text{or} \quad \log(M) = \log(a) + b\log(W) \;;$$

where M is metabolic rate and W is weight. Graphical representations of this relationship for non-acclimated (20°C) *P. granularis* and *S. oculus* for the experimental temperatures: 15°, 20°, 25° and 30°C are given in Figs. 5.1. This relationship for acclimated limpets (either 10° or 25°C) is represented graphically in Figs. 5.2 and 5.3. An analysis of regression equations for the above relationships is

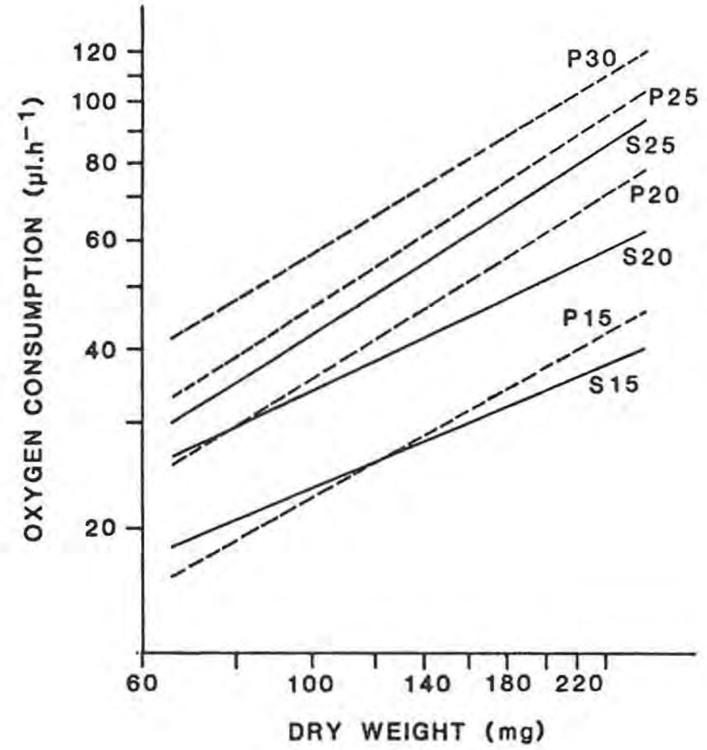

Fig. 5.1. Linear regressions (M = aW^b) of oxygen consumption (uL.h^{-1}) against dry weight (mg) for non-acclimated *Patella granularis* (P) and *Siphonaria oculus* (S). Relationships were determined at 15°, 20°, 25° and 30°C, as indicated in the figure.

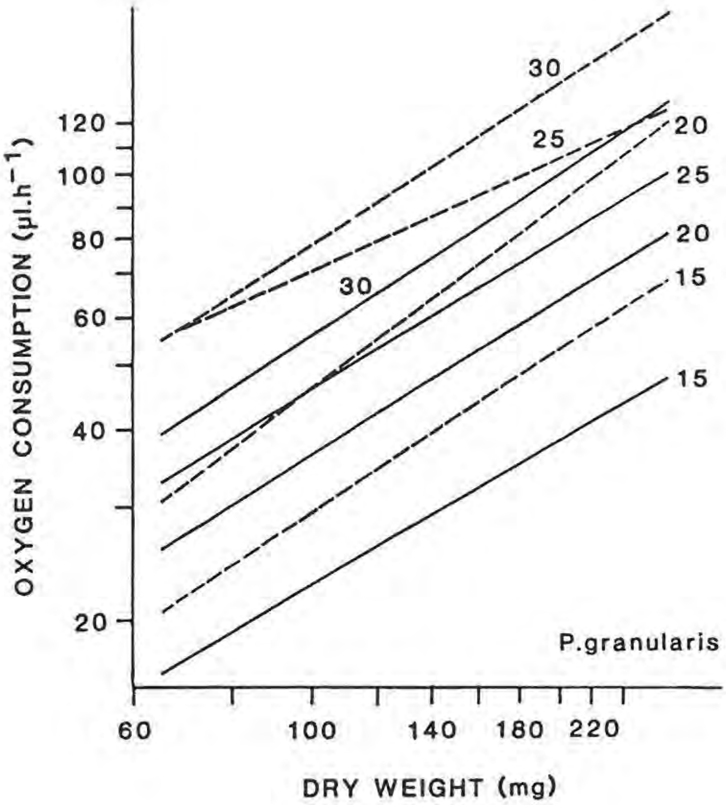

Fig. 5.2. Linear regressions (M = aW^b) of oxygen consumption ($uL.h^{-1}$) against dry weight (mg) for *Patella granularis* acclimated at 10°C (dashed line) and at 25° (solid line). Relationships were determined at 15°, 20°, 25° and 30°C, as indicated in the figure.

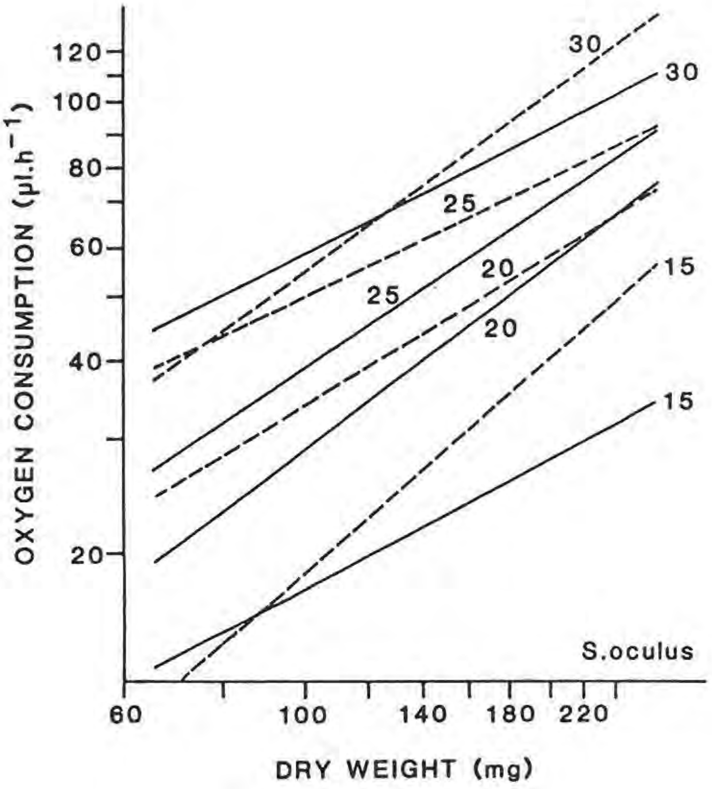

Fig. 5.3. Linear regressions (M = aW^{b} of oxygen consumption (uL.h^{-1}) against dry weight (mg) for *Siphonaria oculus* acclimated at 10°C (dashed line) and at 25°C (solid line). Relationships were determined at 15°, 20°, 25° and 30°C, as indicated in the figure.

given in Table 5.1. Values of the weight exponent (*b*) for all regressions for *P. granularis* were relatively similar, varying between 0.71 and 0.98. None however, were significantly different from the approximation of 0.75 (*t*-test; $P < 0.05$), suggested for poikilotherms in general (Zeuthen 1953). Weight exponents for *S. oculus* were more variable; nevertheless, the calculated mean for these values was 0.76, and all except two sets of temperature conditions, AT10, ET15 ($b = 1.08$) and AT10, ET30 ($b = 0.95$), were not significantly different from 0.75 (*t*-test; $P < 0.05$).

5.3.2 Oxygen consumption and temperature

Metabolic rates are expressed either as weight specific rates (per unit gram dry or fresh tissue weight), or as rates per standard- sized animal. All values of oxygen consumption were transformed to that of a standard-sized animal of 150 mg dry weight, using the formula after Newell (1979):

$$\dot{V}O_2\,(150\text{mg}) = \frac{150^{0.75}}{W} . \dot{V}O_2\,(\text{exp})\ ;$$

where $\dot{V}O_2$ (exp) is the oxygen consumption (uL) of the individual per hour and W the dry weight (mg). Weight exponents (*b*) were derived for the purpose of transforming oxygen consumption rates for each experimental and acclimation temperature condition to those for a standard-sized animal. However, as most values were not significantly different from the generalised value of 0.75, this was used throughout (Zeuthen 1953).

Patella granularis showed temperature acclimation of metabolic rate. For all experimental temperatures (15°, 20°, 25° and 30°C), standardized oxygen consumption of cold-acclimated *P. granularis* was significantly higher (*t*-test; $P < 0.05$) than warm-acclimated limpets (Fig. 5.4 and Table 5.2). There was no difference between the oxygen consumption of warm- and non-acclimated *P. granularis* (Table 5.2). In contrast, temperature acclimation was not observed at all experimental temperatures

Table 5.1.

Statistical information (r, correlation coefficient; N, sample number) and parameters of the regression equations ($M = a.W^b$) for the relationship between oxygen consumption ($uL.h^{-1}$) and dry weight (mg). AT, acclimation temperature; ET, experimental temperature.

		P. granularis				*S. oculus*			
AT (°C)	ET (°C)	a	b	r	N	a	b	r	N
NA	15	-1.48	0.71	0.67	21	5.24	0.53	0.54	21
	20	1.35	0.75	0.85	21	4.60	0.62	0.66	24
	25	1.38	0.80	0.91	23	1.35	0.78	0.81	19
	30	3.71	0.75	0.87	21	--	--	--	--
10	15	-2.68	0.83	0.81	20	-123	1.08	0.93	20
	20	-4.59	0.98	0.78	18	-1.02	0.76	0.89	19
	25	41.6	0.56	0.77	18	177	0.61	0.83	18
	30	5.62	0.78	0.85	16	-2.34	0.95	0.95	17
25	15	-1.54	0.73	0.90	16	-2.18	0.69	0.65	31
	20	-1.38	0.81	0.88	19	-6.29	0.91	0.89	18
	25	1.38	0.79	0.95	18	-1.42	0.83	0.74	20
	30	1.82	0.82	0.94	16	13.80	0.63	0.85	17

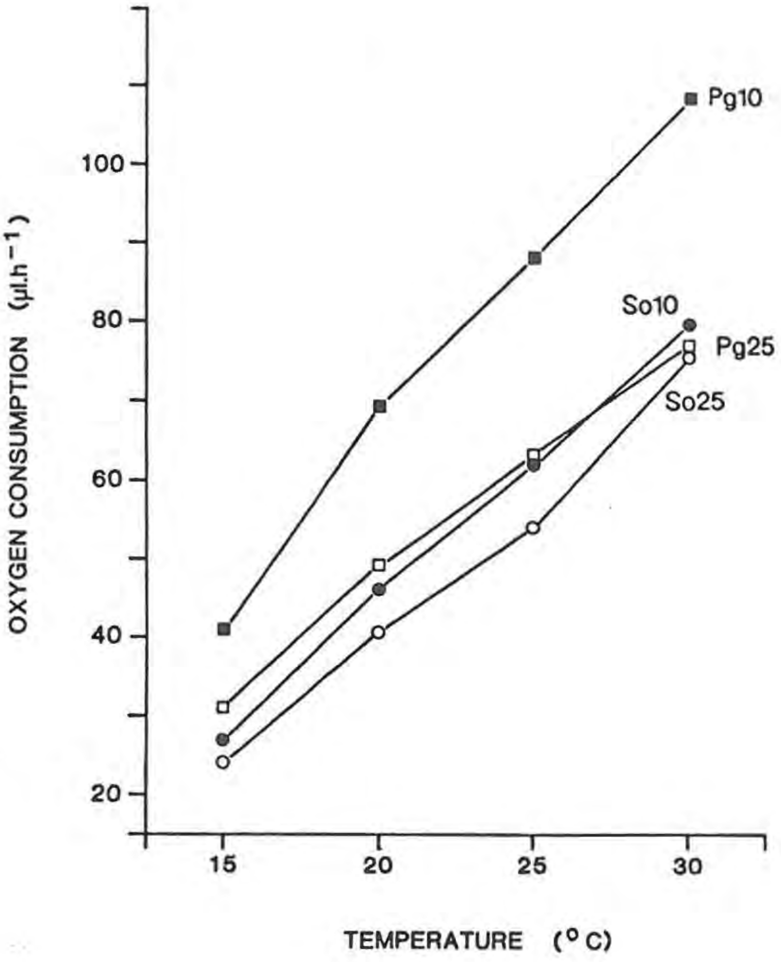

Fig. 5.4. Relationship between oxygen consumption (uL.h^{-1}) and temperature (°C) calculated for standard weight (150 mg) *Patella granularis* and *Siphonaria oculus* acclimated at 10° and 25°C.

Table 5.2.

Statistical data for the relationship of acclimation/experimental temperature (^{0}C) and oxygen consumption (uL.h^{-1}) for a standard 150 mg (dry weight) individual. NA represents non-acclimation.

Experimental Temperature (^{0}C)	Acclimation Temperature (*P. granularis*)		
	NA	10	25
15	30.73±4.7 (21)A	41.09±4.7 (20)B	31.15±2.4 (16)A
20	49.79±4.8 (21)A	69.13±8.5 (18)B	49.26±6.1 (19)A
25	64.28±4.3 (23)A	88.99±8.9 (18)B	63.16±5.1 (18)A
30	77.96±5.4 (21)A	108.54±6.0 (16)B	77.28±6.9 (16)A
Experimental Temperature (^{0}C)	Acclimation Temperature (*S. oculus*)		
	NA	10	25
15	29.64±4.7 (21)A	27.63±4.0 (20)A	24.31±5.5 (31)B
20	43.33±5.7 (24)AB	46.23±5.5 (19)B	40.52±5.4 (18)A
25	57.82±5.1 (19)A	63.81±23.1 (18)B	54.00±10.0 (20)A
30	--	79.52±8.2 (17)A	76.25±4.9 (17)A

Values are mean ± 1 SD (n), and dissimilar letters indicate significant differences (*t*-test; $P < 0.05$) between values in rows.

contrast, temperature acclimation was not observed at all experimental temperatures for *S. oculus*. Cold-acclimated *S. oculus* showed significantly higher oxygen consumption rates (*t*- test; $P < 0.05$) than warm-acclimated limpets at 15°, 20°, and 25°C (Table 5.2). This difference was however only slight, and at 20°C neither treatment was significantly different from the non- acclimation treatment (Table 5.2). At 30°C, there was no significant difference in the oxygen consumption of *S. oculus*, between acclimation treatments (Fig. 5.4, Table 5.2).

Q_{10} values for oxygen consumption of *P. granularis*, for 5°C intervals between 15° and 30°C, were remarkably similar among all pre-experimental temperature treatments. There was a general decline in these values from 2.63, 2.83 and 2.50, for the various acclimation treatments for the lowest temperature range (15°-20°C), to 1.47, 1.48 and 1.49 for the highest temperature range (25°-30°C) (Table 5.3). A similar trend was seen in *S. oculus* with temperature coefficient values for the lowest temperature range of 2.13, 2.80 and 2.78, and those for the highest temperature range of 1.55 and 1.99 (Table 5.3). The trend of decline in Q_{10} with an increase in temperature was, however, not so clear for warm-acclimated *S. oculus* (Table 5.3).

5.4 DISCUSSION

The influence of body size and temperature on metabolism is reviewed for intertidal animals in general, by Newell (1979), and Newell and Branch (1980), and for limpets specifically, by Branch (1981). The generalized weight exponent (*b* or slope) for the logarithmic relationship between oxygen consumption and body weight for poikilotherms is 0.75 (Zeuthen 1953). This *b*-value is however variable among species, and may also depend on temperature (Xie and Sun 1990). They have shown, that in the catfish *Silurus meridionalis*, *b* decreases significantly with in-

Table 5.3.

Q_{10} determined by $(R_2/R_1)^{10/(T2-T1)}$, where R is rate at T, temperature for rate of specific oxygen consumption for the acclimation and experimental temperatures indicated in in the figure. NA represents non-acclimation.

Experimental Temperature (°C)	Acclimation Temperature (*P. granularis*)		
	NA	10	25
15 - 20	2.63	2.83	2.50
20 - 25	1.67	1.66	1.64
25 - 30	1.47	1.48	1.49
Experimental Temperature (°C)	Acclimation Temperature (*S. oculus*)		
	NA	10	25
15 - 20	2.13	2.80	2.78
20 - 25	1.76	1.90	1.78
25 - 30	--	1.55	1.99

creasing temperature (opposite to the prediction from a statistical model by Newell and Roy (1973)). There was no clear temperature-dependent variation in the weight exponent (*b*) among some South African patellid limpets, except for *Patella granatina* (Branch and Newell 1978; Branch 1979). This limpet has a large range of body size which may be a determining factor for this relationship. The present study confirms the findings by Branch and Newell (1978) that no relationship between *b* and temperature holds for *P. granularis*, and also shows this for *Siphonaria oculus* (Table 5.1). The values of b for these limpet species, in most cases, were not significantly different from the generalised value of 0.75, irrespective of acclimation or experimental temperature (Table 5.1). A similar observation was made on some British species of *Patella*, where *b* conformed to the generalised value, and was the same in summer as in winter (Davies 1966). Limited data, however, show *b* as being lower in aquatic than in aerial metabolism (Branch 1980). Possible differences in *b*-values between upper- and lower- shore limpets, have been interpreted as indicating relatively size-independent metabolism by upper-shore limpets; their metabolism presumably being more closely related to food availability (Branch 1981).

In addition to experiencing extreme physico-chemical conditions, upper-shore species are faced with energetic problems. Energetic expenditure by upper-shore relative to low-shore species, is likely to be high, because of longer exposure to high aerial temperatures (for poikilotherms metabolic rate is generally temperature-dependent). Energetic input however, may be relatively low, as time available for feeding is reduced, as activity/feeding is curtailed during aerial exposure when the potential for desiccation is high, and, less food is available in the upper-shore (Newell 1979). Newell (1979) and Newell and Branch (1980) have hypothesised that the vertical limits of intertidal grazing gastropods, particularly in the upper-shore, may be largely influenced by their capacity for maintenance of energetic balance.

In the present investigation, rates of oxygen consumption for the respective species of limpet were lower than in other studies on *P. granularis*, and closely related siphonariid limpets (Fig. 5.4; Table 5.2; see Branch and Newell 1978; Dye 1987). Such differences are probably the result of different pre-experimental treatments, and methods. In comparison to low-shore individuals, and some other South African patellid limpets, upper-shore individuals of *P. granularis* have relatively low rates of oxygen consumption (Branch and Newell 1978). Relatively low metabolic rates were also observed, intraspecifically, for upper-shore individuals of *P. vulgata* (Davies 1966). While these may be a consequence of food deprivation in the upper-shore (Newell and Bayne 1973), the ability of *P. granularis* to sustain a low energy turnover, and low growth rate and reproductive output in comparison to some other patellids, is considered adaptive (Branch et al. 1988). A low energy turnover would reduce energetic losses (Branch et al. 1988). As *S. oculus* has a level of oxygen consumption similar to *P. granularis* (Table 5.2, NA15, NA20), it may also conserve energy by its low metabolic rate.

Some intertidal invertebrates show lowered temperature dependence of metabolic rate over certain temperature ranges (Newell and Pye 1971; Newell and Roy 1973; Brown and da Silva 1979). The temperature range over which intertidal winkles exhibit metabolic independence is seasonally variable (Newell and Pye 1971; Newell and Roy 1973). Some patellids show a level of independence over the temperature range they most frequently experience (Davies 1966). An indication of metabolic indepedence can be gained from the Q_{10} value (usually 2). Q_{10} values for oxygen consumption for *P. granularis*, and to a lesser extent for *S. oculus*, showed a trend of decline with an increase in temperature between 15° and 30°C (Table 5.3). Q_{10}

for heart rate of these limpets also declined with an increase in temperature (see Chapter 2). Branch and Newell (1978) observed a similar pattern for *P. granularis* in their study and, low Q_{10} values were correlated with high environmental temperatures in the upper-shore *P. vulgata* (Davies 1966). Whereas an apparent acclimation of Q_{10} was observed for *P. vulgata*, with a decrease in Q_{10} during warmer months, this was not observed in the present study (Table 5.3). Both *P. granularis* and *S. oculus* will benefit from lowered metabolic dependence, in that it reduces energetic losses, particularly at high temperatures, when energetic losses are potentially greatest.

Many poikilotherms alter cellular activity in response to long- term temperature change, such that, whole animal metabolic rate remains relatively temperature-independent over the long-term (Johnston and Dunn 1987). When measured at the same temperature, cold-acclimated animals will thus have a higher metabolic rate than those acclimated at higher temperatures. Such long-term metabolic rate compensation (referred to as temperature acclimation) has been known for some time, and is the subject of early reviews (Bullock 1955; Segal 1961; Kinne 1963; Newell and Bayne 1973). Acclimation by intertidal invertebrates, which are exposed to dielly fluctuating temperature throughout the year, follows long-term changes in mean temperature (Vernberg 1969).

Because compensation of whole animal metabolism is the summation of various degrees of compensation at lower levels (molecular and cellular), it is seldom complete. That is, following a change in temperature, metabolism does not always revert to exactly the same level. Precht (1958; in Cossins and Bowler 1987) has classified different levels of compensation, and according to this, *P. granularis* exhibits partial acclimation of oxygen consumption (Precht Type III) over the entire range of environmental temperatures examined (15^{o}-30^{o}C; Fig. 5.4). Acclimation by *S. oculus* was not observed at 30°C. While cold-acclimated oxygen consumption rates were significantly raised at 15°, 20° and 25°C, this was slight and probably of little biological significance (Fig. 5.4; Table 5.2).

Although less typical, the absence of acclimation, or inverse acclimation of metabolic rate, is observed throughout the poikilotherms (Cossins and Bowler 1987). While commonly observed among terrestrial and freshwater gastropods (McMahon 1973; Calow 1975; McMahon 1983), this metabolic adjustment is suggested for some marine bivalves (Newell, Johnson and Kofoed 1977), and intertidal gastropods, including *P. vulgata* (Davies 1966), *Bullia digitalis* (Brown, Ansell and Trevallion 1978; Brown and da Silva 1979), *Melampus bidentatus* (McMahon and Russell-Hunter 1981) and *Littorina irrorata* (Paul, Hatch, Jordan and Stein 1989). Interpretation of the findings by Davies (1966) and Brown and da Silva (1979) is, however, limited by seasonal determinations of oxygen consumption (see Chapter 4). In this study temperature was laboratory- controlled, providing another conclusive example of non-acclimation by a marine gastropod.

Nutritional status influences temperature relationships of metabolism (Newell and Bayne 1973; Newell 1979). During the acclimation period in the laboratory food intake was restricted. However, both limpet species may experience reduced food levels in the natural environment. Furthermore, comparison was made between the limpet species, which were held together under these food- restricted conditions. Also, oxygen consumption of non-acclimated limpets which experienced only minor food deprivation was of a level comparable to acclimated limpets (Table 5.2).

Normal thermal acclimation ensures metabolic constancy, and thus optimal functioning of physiological processes throughout the year. The maintenance of a high winter metabolic rate is however, energetically costly. In contrast, inverse acclimation in freshwater pulmonates is hypothesised as being adaptive by facilitating "conservation of overwintering energy stores" (McMahon 1983). Thus, by its poor and often absent acclimatory response, *S. oculus* must derive energetic benefit over *P. granularis* in the long-term. The metabolic rate of animals exhibiting non-acclimation could fall to very low levels when ambient temperature declines during the winter months. Implicit in this is the maintenance of cellular functioning at very low energetic levels. Interestingly, Bailey and Dimitriadou (1991) suggested inverse acclimation as forming part of the hibernation mechanism in land snails. Thermal acclimation by *P. granularis* may result from their possible dependence on a relatively high cellular energetic turnover (at least in comparison to animals showing metabolic rate depression, such as, hibernation).

In conclusion, both *P. granularis* and *S. oculus* reduce energetic losses by maintaining relatively low overall metabolic rates, and showing lowered temperature dependence at high temperatures, which may facilitate their existence in the upper-intertidal zone (Branch et al. 1988). Should food availability vary with season, being reduced in winter, *S. oculus* will gain additional benefit by showing inconsequential, or lack of, temperature acclimation. This may explain its distribution further up the shore than *P. granularis.*

5.5 SUMMARY

1. This chapter examines the relationships of oxygen consumption with body weight, and temperature, in *Patella granularis* and *Siphonaria oculus.*

2. In both species the slope (*b*) for the relationship between oxygen consumption and dry weight, for various experimental and acclimation temperatures, was not significantly different from 0.75. There were, however, two exceptions for *S. oculus* (Figs. 5.1, 5.2 and 5.3; Table 5.1).

3. Q_{10} values of both species, generally, became lowered with an increase in ambient temperature, indicating suppression of metabolic rate at relatively higher temperatures (Table. 5.3).

4. *Patella granularis* exhibited partial compensation (Precht Type 3) of its aerial oxygen consumption over a range of experimental temperatures (15°-30°C), when acclimated at 10°C and 25°C. Metabolic compensation was not observed in *S. oculus* at one experimental temperature (30°C), and was slight and probably inconsequential at the other experimental temperatures (15°, 20° and 25°C; Fig. 5.4; Table 5.2).

5. These metabolic rate responses are discussed with regard to their potential for reducing energetic loss, and thus facilitating upper-shore extension by the species.

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CHAPTER 6

PROGRESSIVE AERIAL EXPOSURE OF *PATELLA GRANULARIS* AND *SIPHONARIA OCULUS*: OXYGEN CONSUMPTION, HEART RATE AND WATER RELATIONS

6.1 INTRODUCTION

Water conservation during aerial exposure by limpets is limited, and their water relation properties have been the subject of numerous studies (Davies 1969; Wolcott 1973; Branch 1975; Boese and Pritchard 1981; Verderber, Cook, and Cook 1983; Innes 1984; Branch and Cherry 1985). There is also an extensive literature on the metabolic responses of marine intertidal gastropods during aerial exposure (for review, see McMahon 1988), including *Patella* (Davies 1966; Branch and Newell 1978; Branch 1979) and *Siphonaria* (Innes, Marsden, and Wong 1984; Dye 1987). In most studies, measurements of aerial oxygen consumption are used for comparison with aquatic oxygen consumption, and are taken shortly after aerial exposure. Such studies have attempted to test the hypothesis that the aerial:aquatic ratio of metabolism is correlated with vertical height on the shore, by increasing with distance upshore (the reasoning being that upper-shore species are presumably more efficient gaseous exchangers in air than their low-shore counterparts; see McMahon 1988). However, even though desiccation potentially increases with vertical height, the relationship between metabolism and desiccation of limpets has hardly been considered (Boese and Pritchard 1981; Innes 1984).

In the previous chapter (Chapter 5) it is shown that metabolic adaptations to temperature change may explain upper-shore distributions by *Patella granularis* and *Siphonaria oculus*, and extension of distribution of the latter limpet further up the shore. This study compares the water relations (rate of water loss, LD_{50}, LT_{50}) and metabolic characteristics (heart beat, oxygen consumption) of these limpet species during progressive aerial exposure, in an attempt to elucidate further reasons for differences in their "within-shore" distributions.

6.2 MATERIAL AND METHODS

6.2.1 Collection and maintenance of specimens

Specimens were collected from intertidal rocks at Port Alfred (33°36'S; 26°53'E) and near Great Fish River Mouth (33°30'S; 27°08'E), eastern Cape Province. As mentioned in Chapter 2, limpets, prised from rocks, were, immediately transferred to polythene-covered perspex plates, and returned, air-exposed, to the laboratory within 5 h. They were kept unfed in controlled environment rooms, at constant temperature, salinity (35‰) and subdued illumination, for between 5 to 14 d prior to experimentation. The maintenance temperature was 20 ± 0.5° or 25 ± 0.5°C, the same as that used during experimental procedures. A tidal cycle of 12:12 h air:water was maintained in 150 L plastic aquaria. Before experiments, individual limpets were induced to settle on watch glasses in air (± 1 h), and then briefly immersed in seawater, allowing them to recover pallial water.

6.2.2 Shell shape and rate of water loss

An index of shell shape is given by the relationship of circumference to volume. Shell circumference was measured directly, and volume by filling with ethanol.

Water loss from limpets of similar size (combined mean dry weight: 160 ± 31 mg; n = 60) was determined gravimetrically. After blotting, the initial total weight (including shell and watch glass) for individual limpets was determined. Species of limpet were then held together, in a controlled environment room, in calm air (covered plastic basins) at relative humidity of 80% ± 5% (± S D) and temperature of $20^0 \pm 0.5^0$C (± S D) for 90 h. Further measurements of total weight were taken every 12 h. Evaporative water loss, the difference between initial and desiccated total weight, is expressed as percentage initial water weight. Initial water weight was calculated by subtracting shell, watch glass and dry flesh weights from initial total weight.

6.2.3 Survival of desiccation

Mortality of limpets (n = 30; for each species) held in calm air ($20^0 \pm 0.5^0$C; 80% ± 5% R.H.) was recorded 12 hourly, by visual examination of the mantle skirt. In both species the mantle skirt contracts excessively on death. Mortality determined visually, was in all cases confirmed by lack of revival following 4 h submergence in seawater. Non-linear regressions for % survival against % water loss, and % survival against aerial exposure time, were used to derive LD_{50} and LT_{50} values.

6.2.4. Heart rate (f_h) during prolonged aerial exposure

Method of electrode implantation for impedance recording is previously described (Chapter 2). Limpets (n = 13 for *P. granularis*; n = 12 for *S. oculus*) were then exposed to calm air (25^0C; 80% R.H.) for 72 h while heart rate was recorded at 12 h intervals. Further measurements were made on return to water. Mean heart rates were calculated from paper traces over 1 min, a period shown from previous long-term, continuous recordings to be reliable when heart rate was regular. In cases of arhythmicity, however, longer time intervals were used.

6.2.5 Oxygen consumption in relation to evaporative water loss

Aerial oxygen consumption was determined before and after desiccation for various periods (no longer than 12 h) in an air- stream, dried through a column of activated silica-gel (20° and 25°C). Oxygen consumption was measured with a Gilson Differential Respirometer (Gilson Medical Electronics) using modified respirometer flasks to allow for limpet size. Dried shells became hygroscopic, disturbing the vapor pressure balance in the respirometer chambers, so shells were moistened prior to respirometry. Measurements were taken at 15 or 30 min intervals for 2 h (20° and 25°C). Limpets left in the Gilson Respirometer at 100% R.H. for up to 12 h, showed no noticeable difference in $\dot{V}O_2$ throughout this period (see method in Chapter 5), thus changes in $\dot{V}O_2$ relate to water loss rather than period of aerial exposure.

Each limpet was removed from its shell and the tissue dried to constant weight at 60°C to calculate weight-specific oxygen consumption ($\dot{V}O_2$) corrected to STP. To minimise individual differences, oxygen consumption rates are expressed as a fraction of the initial pre-desiccated rate (n = 44 and n = 52 for *P. granularis* and *S. oculus*, respectively). Percentage water loss was determined as above.

6.2.6. Oxygen consumption following reimmersion in water

Oxygen consumption in water (25°C) was measured prior to, and after, desiccating *S. oculus* (n = 4-7), for 12 and 24 h, using a dry air-stream (25°C). Oxygen concentration of seawater (35‰ salinity) inside a closed chamber (40 mL) in which a single limpet was held, was determined using a YSI polarographic electrode, coupled to an oxygen analyser (YSI model 58) and pen recorder (Marshall and McQuaid 1989). Seawater was agitated by a magnetic stirrer. Determinations were made at 5 min intervals for 15 min, after which the chamber water was flushed and the experiment repeated. Change in oxygen concentration of the chamber water was converted to weight-specific oxygen consumption (given as $\dot{V}O_2$ uL(g dry weight.h)$^{-1}$ and $\dot{M}O_2$ umoles (g dry weight.h)$^{-1}$).

Regressions were fitted to data in some experiments according to the method of least squares (Statgraphics Ver. 4.0, Statistical Graphics Corporation).

6.3 RESULTS

6.3.1 Shell shape and rate of water loss

Multiplicative regressions ($V = aC^b$) for the relationship between shell volume (V) and shell circumference (C) of the two limpet species, differed significantly ($P < 0.05$; $F = 19.06$; df = 2, 67; Fig. 6.1). Within the size range used (55-85 mm circumference), the shell of *Patella granularis* has a greater volume, indicating that it is more highly domed.

t-tests showed that *Siphonaria oculus* lost significantly more water at all time intervals than *P. granularis* ($P < 0.05$; Fig. 6.2). For both limpet species there was an initial (0-24 h) high rate of water loss (attributable to loss of superficial water), after which the rate became almost constant.

6.3.2 Survival of desiccation

Siphonaria oculus ($LD_{50} = 59\%$) survives nearly twice the degree of water loss that *P. granularis* does ($LD_{50} = 34\%$; Fig. 6.3). This is total water loss, including extravisceral water (between body and shell). The shell of *P. granularis* is more domed and presumably holds more extravisceral water than that of *S. oculus*. Thus tolerance of loss of tissue water by *S. oculus* may be even greater than suggested by comparison of LD_{50} values.

Although *S. oculus* tolerates greater water loss, it loses water at a greater rate than *P. granularis*. Tolerance of water loss was therefore measured in terms of period of aerial exposure (LT_{50}), which has ecological significance. LT_{50} for *P. granularis* was 89 h, and 158 h for *S. oculus* (Fig. 6.4).

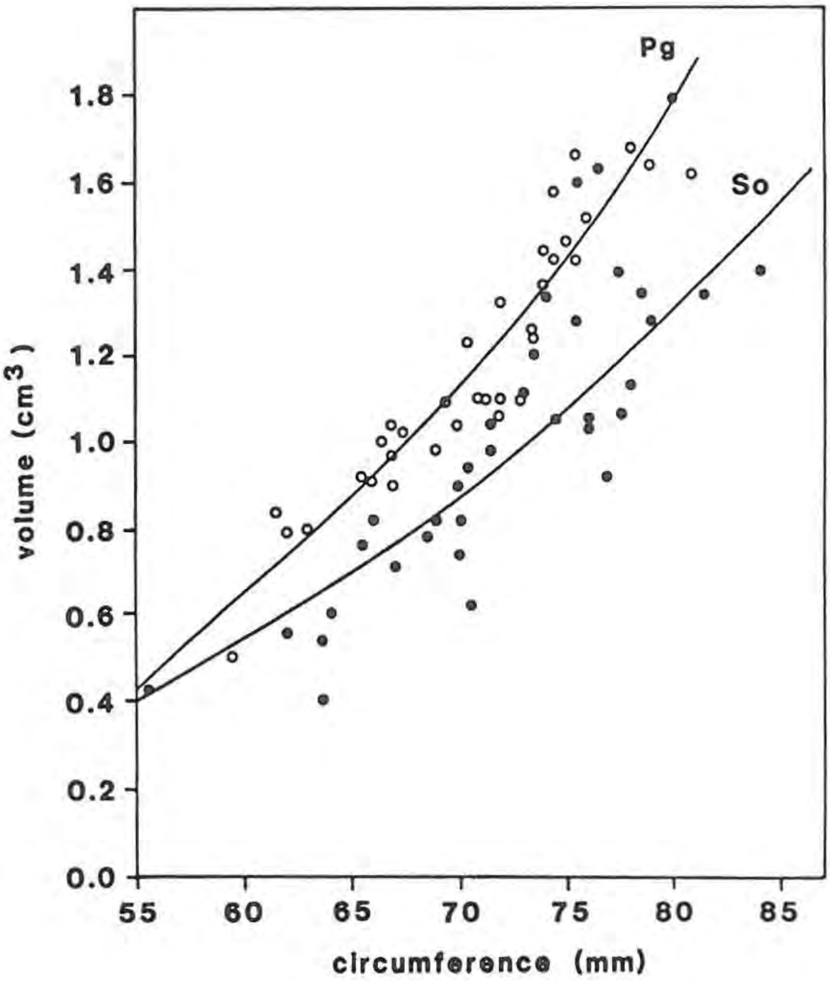

Fig. 6.1. Relationship between shell volume (V) and circumference (C) for *Patella granularis* (open circles) and *Siphonaria oculus* (closed circles). For *P. granularis*, $V = -2.647C^{1.280}$, $r = 0.94$, $n = 34$ and for *S. oculus*, $V = -2.647C^{1.026}$, $r = 0.83$, $n = 35$.

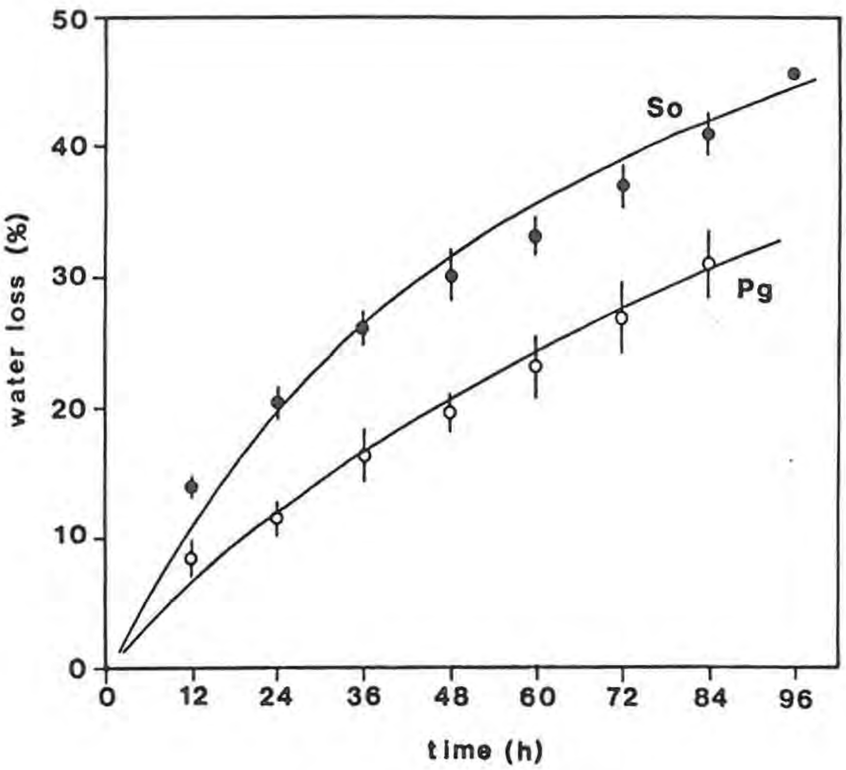

Fig. 6.2. Rate of water loss ($\overline{X} \pm 1$ S D) expressed as percentage initial water weight for *Patella granularis* (n = 30, open circles) and *Siphonaria oculus* (n = 30, closed circles) during aerial exposure ($20^{o} \pm 0.5^{o}C$; 80% ± 5% R.H.). Non-linear regressions for each species are fitted to means (*P. granularis*: $Y = 1.4574 + 0.4422X - 0.0012X^2$, $r^2 = 0.99$, n = 7; *S. oculus*: $Y = 3.0402 + 0.7057X - 0.0029X^2$, $r^2 = 0.98$, n = 8)

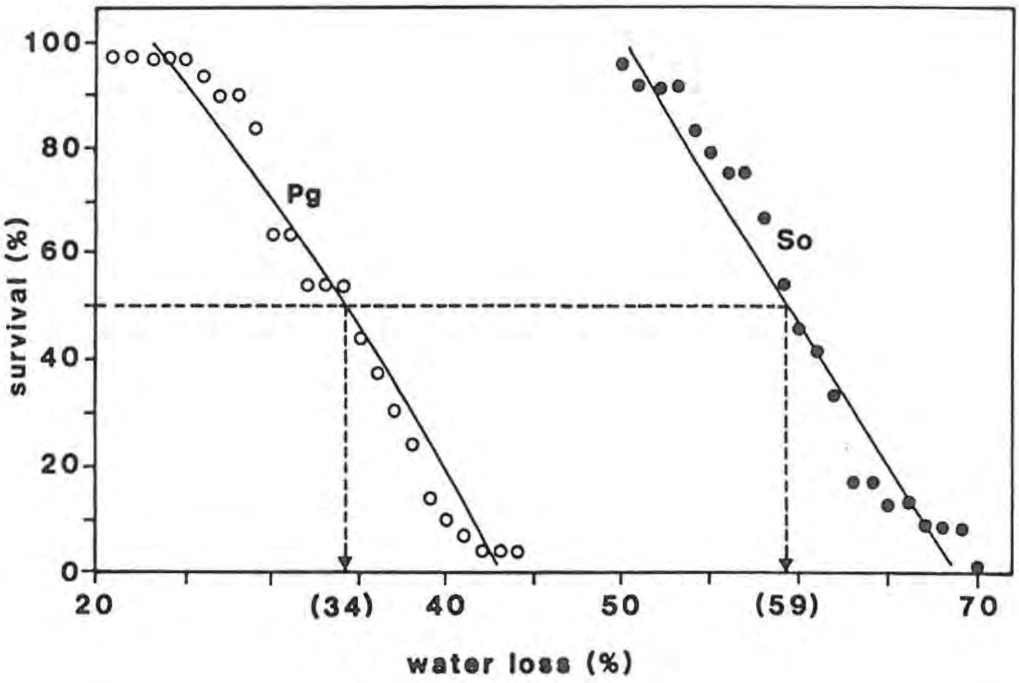

Fig. 6.3. Relationship between survival and water loss (as a percentage of initial water weight), for *Patella granularis* (Pg) and *Siphonaria oculus* (So). LD_{50} values determined from fitted non- linear regressions are indicated. Regression equations for survival of water loss are: *P. granularis*: $Y = 173.35 - 2.3293X - 0.0386X^2$, $r^2 = 0.97$, $n = 24$; *S. oculus*: $Y = 462.53 - 8.3860X + 0.0244X^2$, $r^2 = 0.96$, $n = 21$.

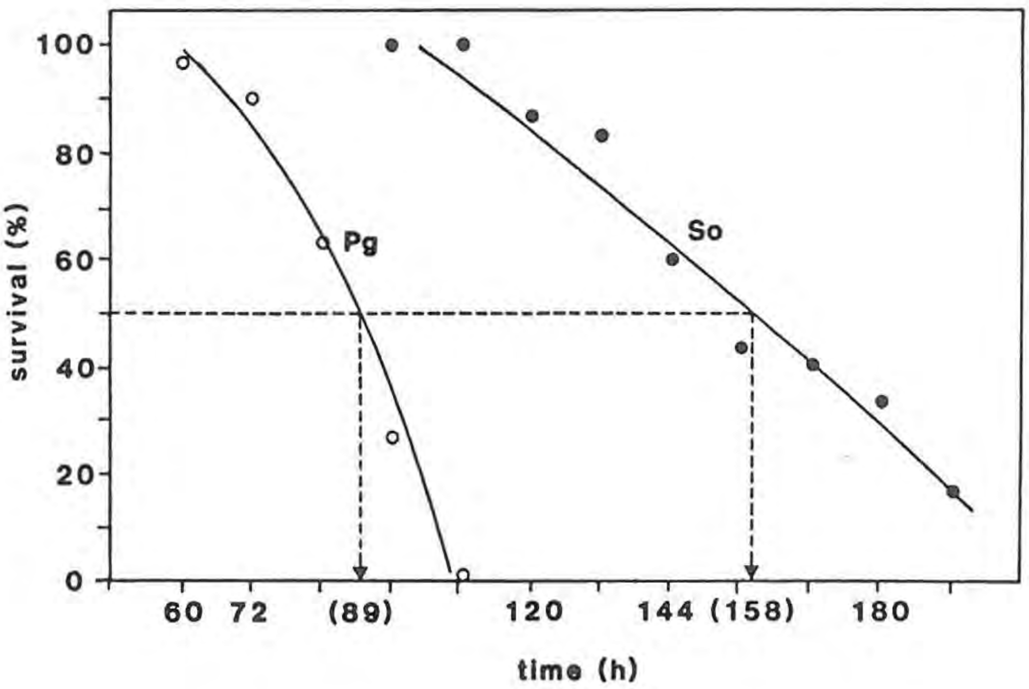

Fig. 6.4. Relationship between survival and duration of aerial exposure for *Patella granularis* (Pg) and *Siphonaria oculus* (So). LT_{50} values determined from non-linear regressions are indicated. Regression equations are: *P. granularis*: $Y = 67.67 + 2.0158X - 0.0247X^2$, $r^2 = 0.99$, $n = 5$; *S. oculus*: $Y = 172.03 - 0.5778X - 0.0012X^2$, $r^2 = 0.97$, $n = 9$.

6.3.3 Heart beat and behavioural responses during prolonged aerial exposure

Mean heart rate of *P. granularis* remained constant (50-60 beats/min) during aerial exposure (Fig. 6.5). Heart rates which fell below 35 beats/min in particular, were characterized by cardiac arrest (less than 2 min) and variation in beat to beat amplitude (Fig. 6.6). These low heart rates were followed by death within 12 h. Periods of cardiac arrest and heart rates of dying limpets were not included in the analysis of the data.

Mean aerial heart rate for *S. oculus* declined rapidly from 48 to 29 beats/min after 12 h in air (20^{o}C), and stabilized around 20 beats/min for the duration of exposure (Fig. 6.5). However, heart rates were highly variable between individuals of *S. oculus*, and for single individuals on subsequent recordings (Fig. 6.5). Bradycardia (heart rate below 10 beats/min) was occasionally observed, and was usually characterized by a loss of regularity of heart beat (Fig. 6.6). On reimmersion in water, heart rate recovered (usually within 4 h), becoming similar to initial values in air (Fig. 6.5).

Additional experiments showed that metabolic and behavioural responses of *S. oculus* to prolonged aerial exposure, are reversible. Response of heart rate to introduction of water- saturated air after 12-16 h exposure in a dry air-stream, was variable. Heart rate either increased immediately by 50%-100%, or, with more severe desiccation, showed no immediate change. Elevated heart rates then either declined after about 10-30 s (Fig. 6.7), or remained elevated for some hours. During desiccation the mantle skirt frequently became contracted. Except in cases of severe desiccation, it expanded rapidly again in water-saturated air. The pneumostome usually opened completely with the re-expansion of the mantle skirt, but otherwise showed no pattern of opening/closing during exposure to air.

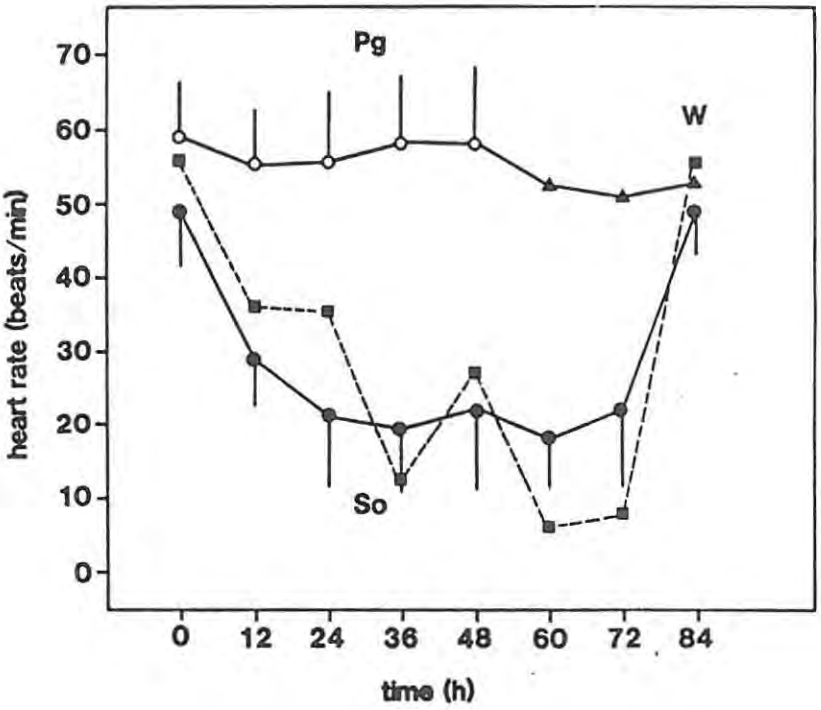

Fig. 6.5. Heart rate ($\overline{X} \pm 1$ S D) for *Patella granularis* (Pg) (n = 8-13, except for triangles where n = 1) and *Siphonaria oculus* (So) (n = 12), during continuous exposure in air (25° ± 0.5°C, 80% ± 5% R.H.) and following 12 h reimmersion in water (w). The response of a single *S. oculus* is represented by closed squares.

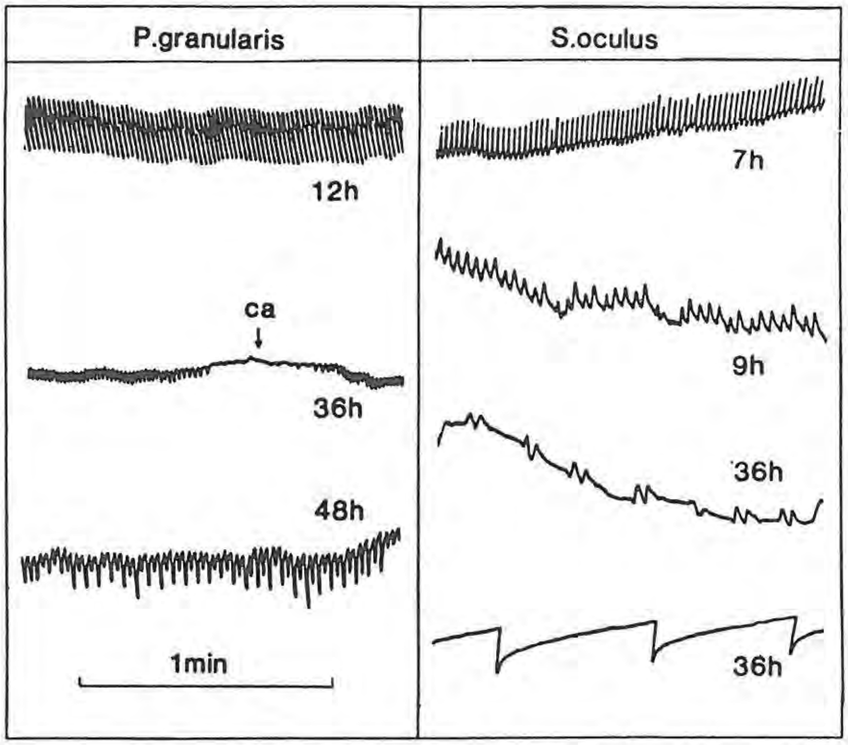

Fig. 6.6. Heart trace recordings for the two species of limpet after various periods of aerial exposure (20° ± 0.5°C; 80% ± 5% R.H.). Prolonged aerial exposure induced cardiac arrest (ca; 36 h) and beat to beat variation in amplitude (48 h) in *Patella granularis*. Bradycardia was observed in *Siphonaria oculus* (36 h).

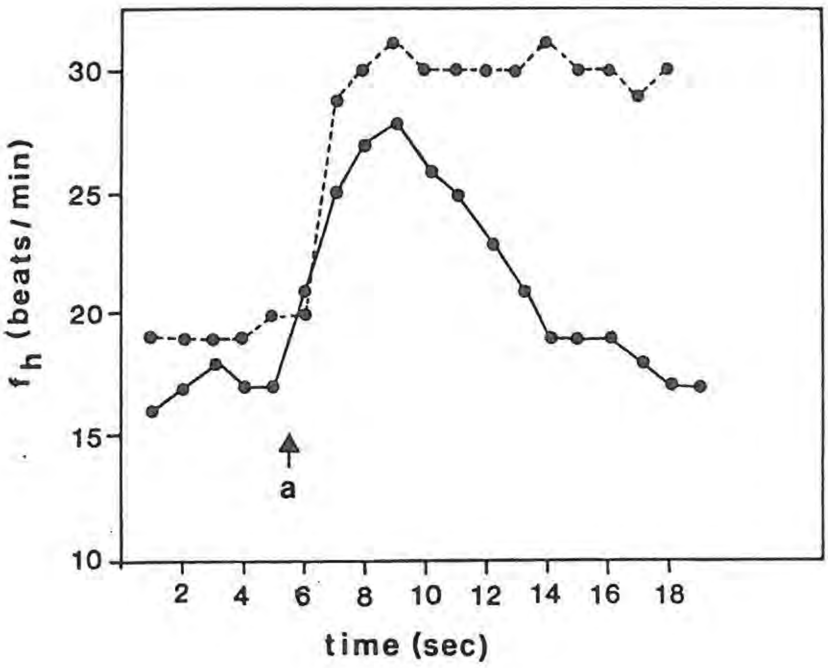

Fig. 6.7. Responses of heart rate of two desiccated individuals of *Siphonaria oculus*, to the introduction of water-saturated air (a).

6.3.4 Oxygen consumption in relation to evaporative water loss

Increasing water loss leads to a declining oxygen consumption for both species. Slopes of linear regressions for the two species differed significantly ($P < 0.05$; $F = 10.82$; df = 1, 94; Figs. 6.8 and 6.9). Oxygen consumption of *S. oculus* expressed as a fraction of that measured initially, was occasionally above 1, following a small amount of water loss (Fig. 6.9). This elevated aerobic response (heart activity also increased) may relate to: the limpet becoming more active; energy used to expel mantle cavity water; or possible optimal aerial respiration after expulsion of mantle cavity water.

Following severe desiccation (between 40%-60%) oxygen consumption of *S. oculus* declined to <20% of the initial rate, and <15% of the highest recorded rate (Fig. 6.9). Oxygen consumption of *P. granularis* never fell below 38% of the initial rate (Fig. 6.8). Oxygen consumption after desiccation was variable between individuals, and for the same individual of *S. oculus* in repeat experiments. For example, in successive experiments using the same individual, a 40% water loss resulted in a change in $\dot{V}O_2$ to 0.23 and 0.60 of the initial $\dot{V}O_2$. Temperature (20^o and 25^oC) had no apparent effect on the relationship of oxygen consumption and evaporative water loss (Figs. 6.8 and 6.9).

6.3.5. Oxygen consumption during reimmersion

There was no significant difference (*t*-test; $P < 0.05$) in the aquatic oxygen consumption of inactive *S. oculus* measured before, and after 12 h of exposure to a flow of dried air (Table 6.1). Respiration following reimmersion after 24 h aerial exposure, was variable and these data are not represented. When the mantle skirt remained contracted, oxygen consumption was 50% below pre- desiccation levels. Once the

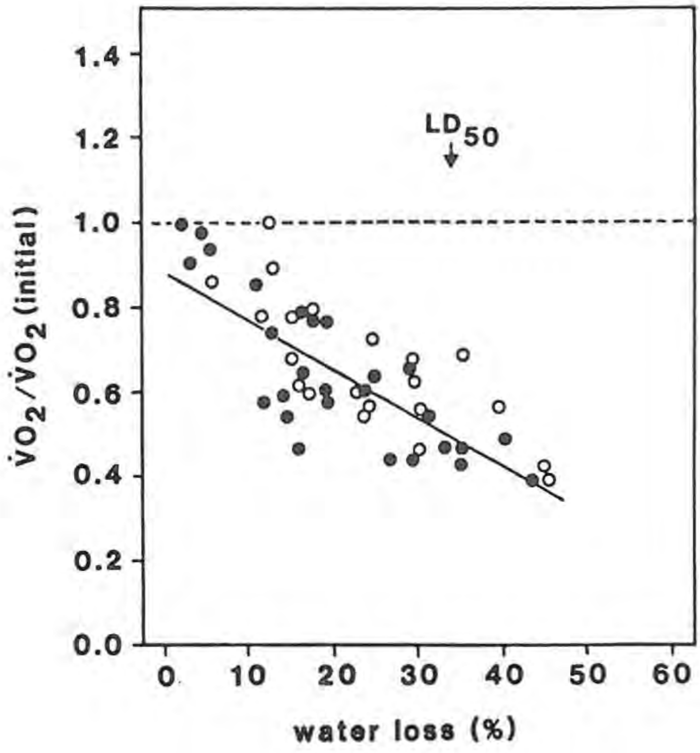

Fig. 6.8. Effect of water loss on oxygen consumption of *Patella granularis*. Ordinate shows the rate of oxygen consumption after water loss, expressed as a fraction of that measured initially, in the absence of water loss (dashed line). Lethal water loss (LD_{50}) is indicated. Oxygen consumption measured at 20° and 25°C is represented, by closed and open circles, respectively. Fitted regression is $Y = 0.86677 - 0.01154X$, $r = -0.752$, $n = 44$.

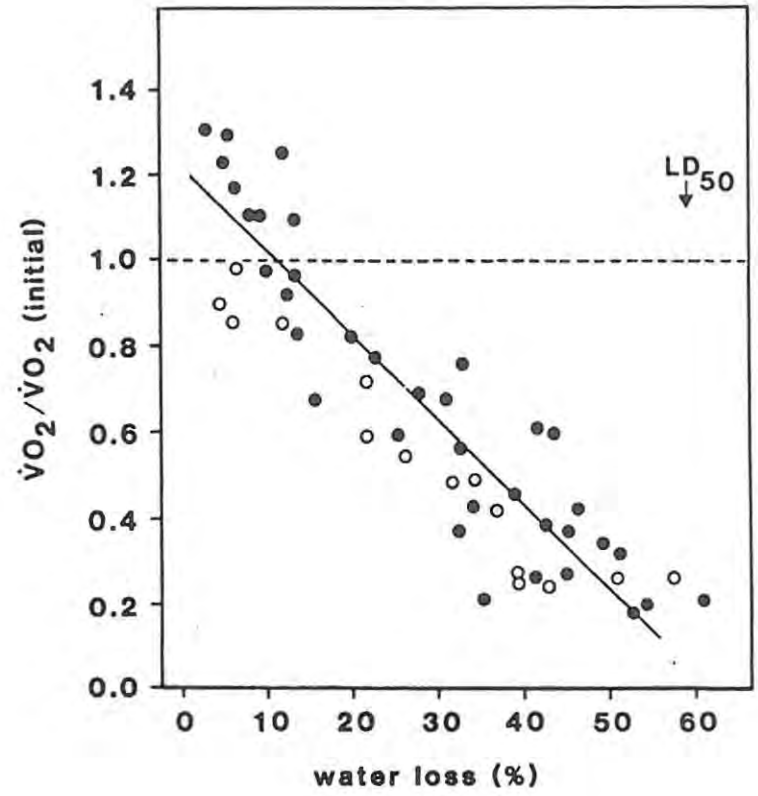

Fig. 6.9. Effect of water loss on oxygen consumption of *Siphonaria oculus*. Ordinate shows the rate of oxygen consumption after water loss, expressed as a fraction of that measured initially, in the absence of water loss (dashed line). Lethal water loss (LD_{50}) is indicated. Oxygen consumption measured at 20° and 25°C is represented by closed and open circles, respectively. Fitted regression is $Y = 1.16413-0.018737X$, $r = -0.895$, $n = 52$.

Table 6.1.

Rate of oxygen consumption (25°C; $\overline{X}$ ± SD) of *Siphonaria oculus* measured initially in water, and on reimmersion after 12 h aerial exposure.

Aerial exposure	$\dot{M}O_2$ µmoles (g dry wt.h)$^{-1}$	$\dot{V}O_2$ µL (g dry wt.h)$^{-1}$
initial	27.6±2.897	618.3±64.9 (n = 7)
re-immersed	31.5±3.225	705.6±72.1 (n = 4)

mantle skirt expanded, limpets desiccated for 24 h usually became active, so oxygen consumption exceeded initial measurements.

6.4 DISCUSSION

The patelliform shell of limpets with its wide aperture offers comparatively little protection against desiccation. Settling on a home scar (homing behaviour) where the shell margin matches the substratum (Verderber et al. 1983; Branch and Cherry 1985), or formation of a mucous sheath sealing off the marginal space (*Collisella (Acmaea) digitalis*; Wolcott 1973), have been described as mechanisms reducing rate of evaporative water loss. During aerial exposure, the shell is held slightly above the substratum, a behaviour perhaps allowing free movement of gases between the respiratory surfaces and the environment. Even water under the shell for limpets on home scars is therefore lost along the entire shell margin, as a function of the area between the shell margin and substratum (Lowell 1984). Both species in the present study exhibit irregular homing behaviour (Branch 1981; pers. observations), thus rates measured in the laboratory may be inflated in comparison to field rates on home scars, but not off home scars.

The less-domed shell of *Siphonaria oculus* may explain a more rapid water loss, as the circumference (marginal area) relative to volume is increased (Figs. 6.1 and 6.2; Lowell 1984). In addition to losing water faster than *Patella granularis*, because the extravisceral water store under the flatter shell is reduced, a greater proportion of the water lost from *S. oculus*, is tissue water. While this limpet withstands rapid water loss from the body tissue, it also tolerates a relatively higher level of water loss (LD_{50} = 59%; Fig. 6.3). Similar high lethal levels of water loss have been recorded for Hong Kong eulittoral and eulittoral fringe mangrove mesogastropods (in McMahon 1990).

Numerous physiological changes occur during prolonged aerial exposure and desiccation (e.g., acid-base disturbances), any of which may potentially cause death. Wolcott (1973) suggested that osmotic change was the reason for death following desiccation in intertidal limpets. In *P. granularis*, water lost at the time of death is low in comparison to *S. oculus* (Fig. 6.3), suggesting that osmotic stress may not be the determinant factor for death. At their LD_{50}, VO_2 was about 50% of the pre-desiccated rate, which is comparatively high (the value for *S. oculus* being <18% of the initial rate of oxygen consumption; Figs. 6.8 and 6.9). It is therefore conceivable that *P. granularis* depends on a relatively high energetic level for cellular maintenance, and death following prolonged aerial exposure results from energetic decline below the minimum maintenance requirement. The tolerance of a rapid and extreme loss of water, indicates that *S. oculus* has the capacity to tolerate rapid and extreme changes in osmolarity.

Desiccation resistance (rate of water loss) does not always correlate with vertical position on the shore (Broekhuysen 1940; Brown 1960), and it is shown here that the upper-distributed *S. oculus* loses water faster than *P. granularis* (Fig. 6.2). However, the higher LT_{50} (an indicator of the integrated effect of rate and tolerance of water loss) most certainly shows the capacity by *S. oculus* to survive higher on the shore. Because of the relationship between LT_{50} and LD_{50} (both were higher for *S. oculus*), vertical separation may be influenced by differences in the tolerance of water loss by the species. McMahon (1990) suggests little correspondence between tolerance of water loss and vertical distribution for eulittoral and eulittoral fringe gastropods (excluding limpets), but rather that control of water loss influences their distribution. The LD_{50} for the uppermost South African intertidal gastropod, *Littorina knysnaensis* (33.17%; Brown 1960), is considerably lower than that of *S. oculus* (59%). The discrepancy between limpets and other intertidal gastropods may be explained by their relatively poor ability to regulate water loss, and findings of the present study are consistent with those for other intertidal limpets, which show tolerance of water loss corresponding with vertical height (Davies 1969; Branch 1975).

Respiratory stress during aerial exposure arises from impairment of gas exchange, caused by a reduction in area and/or drying of, the primary respiratory surface. For aerial respiration *P. granularis* and *S. oculus* rely on external pallial gills and a rudimentary lung, respectively. Ventilation of the lung of *Siphonaria* occurs by diffusion through a closeable pore, the pneumostome. Having a lung, *S. oculus* could be expected to maintain a higher rate of oxygen consumption during progressive aerial exposure. And indeed this was so during initial water loss when oxygen consumption of *P. granularis* remained lower than that of *S. oculus*, following similar levels of water loss. Regression lines relating oxygen consumption and water loss intercept at about 40% water loss (Figs. 6.8 and 6.9). A rapid decrease in $\dot{V}O_2$ during aerial exposure has been ascribed to the clumping together of pallial gill filaments in another patellid, *Cellana* (Innes 1984). Because there is no ventilation of water around the gills during aerial exposure, lowered $\dot{V}O_2$ may also result from the depletion of oxygen from the gill water (Graham 1990). Also, aerial exposure usually leads to acidification of the haemolymph, causing a Bohr shift and thus lowered oxygen affinity (Burnett 1988).

Whereas impairment of gas exchange will certainly influence oxygen consumption, similar effects will be observed should the limpets lower oxygen demand by altering their metabolic status. Examples of alternative metabolic states are aerobic rate depression in land snails during aestivation (Barnhart 1986; Barnhart and McMahon 1987; Churchill and Storey 1989), and environmental anaerobiosis during valve closure in bivalves (De Zwaan and Wijsman 1976; Shick, Gnaiger, Widdows, Bayne and De Zwaan 1986).

Whereas environmental anaerobiosis has been examined in detail in bivalve molluscs (De Zwaan and Wijsman 1976; Shick et al. 1986), conditions under which intertidal gastropods utilize this are poorly understood. Many investigations on intertidal gastropods assume metabolic homeostasis, thus when comparing aerial and aquatic oxygen consumption imply any deficit in either rate as involving the incorporation of anaerobiosis (see McMahon, 1988). While this may be so, there is, however, no biochemical or calorimetric evidence for anaerobiosis by intertidal gastropods in air, when oxygen availability is not reduced. Rather, the absence of anaerobiosis in air-exposed intertidal gastropods has been demonstrated both biochemically (Wieser 1980; Brinkhoff, Stockmann, and Grieshaber 1983; Livingstone and De Zwaan 1983), and by direct calorimetry (Pamatmat 1978; Hammen 1980).

Desiccation potentially lowers tissue oxygen levels through the impairment of oxygen uptake, and has been shown to induce anaerobiosis in barnacles (Barnes, Finlayson, and Piatigorsky 1963). However, desiccation-induced anaerobiosis by *P. granularis* seems unlikely, as this limpet was shown not to accumulate succinate (an anaerobic end product) during exposure to anoxia (Marshall and McQuaid 1989). Although *Siphonaria* utilizes anaerobiosis under conditions of anoxia (Marshall and McQuaid 1989), desiccated *S. oculus* returned to water did not exhibit an elevation of aerobic rate, characteristic of repayment of "oxygen debt" incurred during anaerobiosis (Shick et al. 1986; Table 6.1). While absence of repayment of "oxygen debt" suggests the absence of anaerobiosis, it is not conclusive evidence for this, as anaerobic end products may be excreted rather than re-incorporated into the metabolic processes (Livingstone and De Zwaan 1983).

The decline in $\dot{V}O_2$ by *P. granularis* can be interpreted as being stress-related, resulting from impairment of oxygen uptake at the gills through a reduction in respiratory surface area and/or acid- base disturbance. Although constancy of heart rate in air by this limpet, to some extent supports this interpretation of reduced oxygen loading, comparison between heart rate and oxygen consumption is limited in that two different sets of experimental conditions are represented (i.e., rapid and slow levels of evaporation).

The regular, very reduced heart rate seen in *S. oculus* is suggestive of an adaptive response to aerial exposure, such as adaptive depression of aerobic metabolic rate. Other features are also indicative of this (for review, see Storey and Storey 1990), including: the reversibility of physiological (Fig. 6.7) and behavioural responses (withdrawal of the mantle skirt, and closure of the pneumostome); the exceedingly low levels to which oxygen consumption declines, levels similar to those observed for aestivating freshwater and land snails (Fig. 6.9; von Brand, McMahon, and Nolan 1957; Barnhart 1986; Barnhart and McMahon 1987); and the variability in individual responses.

Although it is usually suggested that survival of intertidal animals, when exposed in air, is related to osmotic (Wolcott 1973) (and/or acid-base) stress, it is shown here that this may have an energetic basis. It would appear that *P. granularis* is dependent of relatively high level of metabolism for cellular maintenance (LD_{50} occurred at about 50% of normal $\dot{V}O_2$). During prolonged aerial exposure and/or desiccation, this metabolic demand may no longer be met, because of impairment of oxygen uptake, thus death results. In contrast, *S. oculus* may withstand prolonged aerial exposure by its capacity for metabolic rate depression. In the long term metabolic rate depression would also have an energy conserving role. Both, the better ability to survive prolonged aerial exposure and, conservation of energy during metabolic rate depression, would be beneficial for the extension of vertical distribution by *S. oculus*, above that of *P. granularis*.

6.5 SUMMARY

1. When exposed to air, *Siphonaria oculus* lost water faster (probably relating to its less domed shell), and exhibited a higher lethal exposure time and lethal water loss (LT_{50} = 158 h; LD_{50} = 59%) than *Patella granularis* (LT_{50} = 89 h; LD_{50} = 34%).

2. In *S. oculus*, prolonged aerial exposure led to a lowered mean heart rate (from 48 to 29 beats/min), and depression of oxygen consumption ($\dot{V}O_2$) to 18% of initial non-desiccated rate. Exposure to water-saturated air caused reversal of these physiological, and behavioural (mantle skirt contraction) responses. This limpet showed no "oxygen debt" when re-immersed after 12 h aerial exposure.

3. In air, mean heart rate of *P. granularis* was constant (55 beats/min), and $\dot{V}O_2$ never fell below 38% of initial rate, being 50% of initial rate at its LD_{50}.

4. The reduction in $\dot{V}O_2$ in *P. granularis* is interpreted as being stress-related, whereas in *S. oculus* this is suggested as representing adaptive metabolic rate depression.

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CHAPTER 7

EXPOSURE TO HYPOXIA, SAND-INUNDATION AND HYPOSALINITY OF *PATELLA GRANULARIS* AND *SIPHONARIA CAPENSIS*: OXYGEN CONSUMPTION AND HEART RATE

7.1 INTRODUCTION

In the previous chapters *Patella granularis* and *Siphonaria oculus* were shown to differ physiologically in their responses to temperature and aerial exposure. These differences may explain the higher vertical distribution, on rocky-shores, of *S. oculus* (Chapters 5 and 6). Another siphonariid limpet *S. capensis*, co- occurs with *P. granularis* in the mid-tidal zone (balanoid/lower- balanoid) along the South African coastline. While the distribution of *P. granularis* within this tidal zone is essentially limited to regions of exposed rock, *S. capensis* extends its distribution into adjacent tidal pools (Allanson 1958; Branch and Cherry 1985), and regions of the rocky shore prone to periodic inundation by sand (Marshall and McQuaid 1989). Recently, it has been realised that sand deposition is a regular occurrence of many rocky shores, and these regularly sand-inundated shores constitute 31% of the coastline of South Africa (Bally, McQuaid and Brown 1984). Both tidal pools and sand-inundated shores impose respiratory stresses on their inhabitants, as availability of dissolved oxygen may become reduced. Tidal pools become hypoxic during low tide at night through plant and animal respiration, while hypoxia usually develops in the stagnant interstitial water of sand sediments (pers. obs.; Andersen and Helder 1987). Oxygen availability may also be reduced through physical effects of sand, e.g., smothering.

Tolerance of hypoxia/anoxia is variable among marine molluscs (Kapper and Stickle 1987; Stickle, Kapper, Liu, Gnaiger and Wang 1989). Some species are extremely resistant of anoxia with LT_{50} values of up to 35 days (Theede, Ponat, Hiroki and Schlieper 1969). Differences in tolerance of hypoxia between *P. granularis* and *S. capensis* have been suggested as an important determinant of their local distributions (Marshall and McQuaid 1989).

Aspects of the physiology of *P. granularis* and *S. capensis* in hypoxia are compared in this study, in an attempt to explain the underlying physiological mechanisms for tolerance of hypoxia by the latter (Marshall and McQuaid 1989). More specifically, heart rate, a likely indicator of metabolic rate, was examined during exposure to, and following recovery from, reduced oxygen tension and sand-inundation. While respiratory studies on limpets have centered around oxygen consumption in relation to body weight, temperature, food intake (Branch and Newell 1978; Branch 1979; Innes, Marsden and Wong 1984; Branch, Borchers, Brown and Donnelly 1988) and, comparisons between aerial and aquatic consumption rates (Bannister 1974; Branch and Newell 1978; Dye 1987), the effect of reduced oxygen tension on consumption has not been examined.

In addition to fluctuations in oxygen tension during low tides, tidal pools are extremely variable with regard to other physico- chemical conditions such as $_{p}H$, temperature and salinity (Morris and Taylor 1985; Huggett and Griffiths 1986; McMahon 1988; A.C. Taylor 1988). Cardiac responses of these limpet species to hyposalinity, a phenomenon arising from freshwater runoff, was also examined.

7.2 MATERIAL AND METHODS

7.2.1 Collection and maintenance of limpets

Similar sized individuals (15 mm shell length; n = 60) of ***Patella granularis*** and *Siphonaria capensis* were collected from a site of co-habitation in the rocky shore balanoid zone, at Kenton-on-Sea (33°41'S, 26°40'E), eastern Cape Province. Procedure of collection and return of limpets to the laboratory is given in Chapter 2. Experiments were started 24 h after collection and run at 37‰ salinity and 18°C (mean winter sea temperature for the area; D.I. Taylor 1988). For experiments in which heart rate was monitored, limpets were prepared as described in Chapter 2.

7.2.2 Oxygen consumption during declining oxygen tension

The effect of lowered oxygen tension on oxygen consumption may be modified by body size (Bayne 1971; Bayne 1973; Taylor and Brand 1975; Murdoch and Shumway 1980), acclimation and experimental temperatures (Bayne and Livingstone 1977; Newell, Johnson and Kofoed 1978; McMahon and Russell-Hunter 1981) and acclimation tensions (Kushins and Mangum 1971; Taylor and Brand 1975; McMahon and Russell-Hunter 1978). Experiments were therefore undertaken using a narrow size range of limpets (270.0 ± 41.9 mg dry tissue weight, n = 12), held under identical conditions, 2 days after collection.

Oxygen consumption and oxygen tension were determined concurrently, by measuring depletion of the oxygen from a 40 ml perspex respiratory chamber (similar to that illustrated by Crisp, Davenport and Shumway 1978; also given in Chapter 6). The respiratory chamber was immersed in a controlled-temperature water bath (15 L), placed above a magnetic stirrer. A YSI polarographic electrode (YSI model 5739), coupled to an oxygen meter (YSI model 58) and pen recorder, was inserted in a tapered hole in the wall of the chamber. Single animals that had clamped onto a 2 mm thick polythene surface, were allowed to settle for 30 min in the respiratory chamber, before closing the chamber door and monitoring depletion of the oxygen in the dark for up to 2 h. Oxygen consumption was determined for 10 mm Hg intervals for oxygen tensions (PO_2) between 10 and 110 mm Hg (n = 6 for each limpet species). Oxygen consumption for each individual was converted into a weight-specific rate ($\dot{V}O_2$; mL. g shell-free dry weight^{-1}. h^{-1}) after drying limpets at 60°C for 48 h.

7.2.3 Effect of acute hypoxia on heart beat

Following electrode implantation, limpets were left to settle on inverted watch glasses for 16-24 h in seawater (37‰ ; 18°C). By the time the experiments were performed, some limpets had moved from their watch glasses and settled on the side of the glass aquarium. Heart beat traces (3 X 1 min recordings) were obtained initially, for limpets (*P. granularis*, n = 13; *S. capensis*, n = 15) in air-saturated seawater (PO_2 = 148 mm Hg). The PO_2 was then lowered to 50 mm Hg, and further traces were obtained at this tension, hourly for up to 9 h. At times throughout exposure to hypoxia, heart beat was monitored continuously. The PO_2 of 50 mm Hg was attained by pumping N_2 into the seawater (for about 15 min). The decline in tension was monitored using a polarographic electrode (YSI model 5739), coupled to an oxygen meter (YSI model 58), which had been clamped in the aquarium above a stirrer bead. To reduce exchange of atmospheric gases during the experimental exposure period, the oxygen electrode was removed from the aquarium and the water surface covered with polythene sheeting. Measurements of PO_2 after 9 h showed this to have remained relatively constant at between 55-45 mm Hg. After hypoxic exposures of 3h, 6h or 9h, the PO_2 was returned to the saturation level (148 mm

Hg) by flushing with air for 15-20 min. Further heart beat recordings were taken during aeration, and up to 30 min after the return to normoxia.

7.2.4 *Effect of sand-inundation on heart beat*

Heart beat recordings (3 X 1 min) were initially taken for limpets (*P. granularis*, n = 4; *S. capensis*, n = 4) held in air-saturated seawater. Further recordings were made after covering the limpets with sand (20 mm layer) for 3 h. The sand was then removed, rapidly, by inverting the experimental aquarium (2 L; containing the limpets and sand) in a larger seawater aquarium (15 L), and the recovery heart beat on return to sand-free conditions was monitored for 30 min.

7.2.5 *Effect of hyposalinity on heart beat*

Heart beat traces (3 X 1 min) were obtained for limpets (*P. granularis*, n = 7; *S. capensis*, n = 8) under normal salinity, after 3 h hyposalinity, and following the return to normal salinity. Hyposaline conditions (20‰) were attained by diluting the normal seawater (37‰), and regularly checking the dilutions using an Atago Hand Refractometer. Several continuous recordings of heart beat were also taken.

7.3 RESULTS

7.3.1 *Oxygen consumption during declining oxygen tension*

Polynomial regressions fitted to data relating $\dot{V}O_2$ to PO_2 for individual limpets clearly differ between the two species (Fig. 7.1). $\dot{V}O_2$ for *Siphonaria capensis* was more constant over the range of external oxygen tensions used. The B2 coefficient of the polynomial for this type of data has been used as an indicator of dependence of $\dot{V}O_2$ on PO_2, with a more negative value indicating better independence (Mangum and Van Winkle 1973). However, when, as in this case, initial oxygen con-

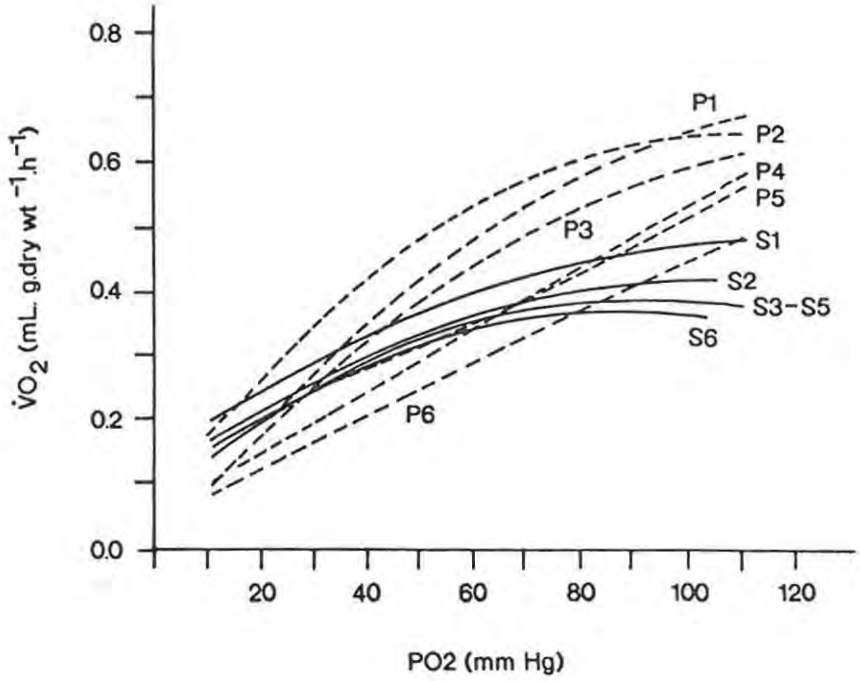

Fig. 7.1. Weight-specific rate of oxygen consumption ($\dot{V}O_2$) as a function of external oxygen tension (PO_2) for six individuals of *P. granularis* (P1-6) and *S. capensis* (S1-6). Individual polynomials are given below.

P1: $-0.046 + 0.012X - (0.0493 X 10^{-3})X^2$; $r^2 = 0.99$ (n = 10)
P2: $0.078 + 0.011X - (0.0491 X 10^{-3})X^2$; $r^2 = 0.98$ (n = 11)
P3: $0.012 + 0.009X - (0.0359 X 10^{-3})X^2$; $r^2 = 0.98$ (n = 11)
P4: $0.068 + 0.005X - (0.0027 X 10^{-3})X^2$; $r^2 = 0.98$ (n = 11)
P5: $0.165 + 0.002X + (0.0099 X 10^{-3})X^2$; $r^2 = 0.98$ (n = 11)
P6: $0.037 + 0.004X - (0.0043 X 10^{-3})X^2$; $r^2 = 0.99$ (n = 10)
S1: $0.130 + 0.006X - (0.0265 X 10^{-3})X^2$; $r^2 = 0.99$ (n = 11)
S2: $0.084 + 0.007X - (0.0356 X 10^{-3})X^2$; $r^2 = 0.94$ (n = 10)
S3: $0.075 + 0.006X - (0.0372 X 10^{-3})X^2$; $r^2 = 0.97$ (n = 11)
S4: $0.111 + 0.006X - (0.0368 X 10^{-3})X^2$; $r^2 = 0.92$ (n = 11)
S5: $0.096 + 0.006X - (0.0339 X 10^{-3})X^2$; $r^2 = 0.96$ (n = 11)
S6: $0.103 + 0.007X - (0.0383 X 10^{-3})X^2$; $r^2 = 0.90$ (n = 10)

sumption rates (at 110 mm Hg) differ considerably, the B2 value is unreliable (Fig 7.1).

Hence, the data for each individual were standardized by dividing $\dot{V}O_2$ values at each tension by the $\dot{V}O_2$ at 110 mm Hg [$\dot{V}O_2$ (initial)] and calculating the mean value for each species (Fig. 7.2). The change in $\dot{V}O_2/\dot{V}O_2$ (initial) between 110 and 50 mm Hg for *S. capensis* is approximately 0.2 unit while that for *Patella granularis* is >0.4 unit, a difference of >100%. Between 50 and 10 mm Hg, the change in $\dot{V}O_2/\dot{V}O_2$ (initial) for both limpet species is slightly >0.4 unit. Although there is no obvious point of inflexion of the curve to indicate a change from regulation to conformation, this clearly demonstrates a superior regulatory ability by *S. capensis* down to about 60 mm Hg.

7.3.2 Effect of acute hypoxia on heart beat

Acute hypoxia (50 mm Hg) induced a rapid decline in the heart rate of *P. granularis*. Although the initial response was variable between individuals, heart rate fell from around 50 to 15-30 beats/min (n = 8) within 30 min of hypoxic exposure (Figs. 7.3 and 7.4). Further exposure led to cardiac arrest which was interrupted by bursts (2 min - 30 s) of heart activity (Fig. 7.4), thus quantification of heart rate was of little meaning. The frequency of heart beat during these bursts of cardiac activity and the initial bradycardia were always above 10 beats/min. Shell lifting and corresponding drift of the recording trace, were observed for all individuals of *P. granularis* under hypoxia (Fig. 7.4). On re- establishing normoxia, heart rate steadily increased, and that of all individuals became elevated by >40% of the initial pre-hypoxic rate (Table 7.1; Fig. 7.4). This overshoot of heart rate was noted in limpets for both the 3 and 6 h hypoxic exposures.

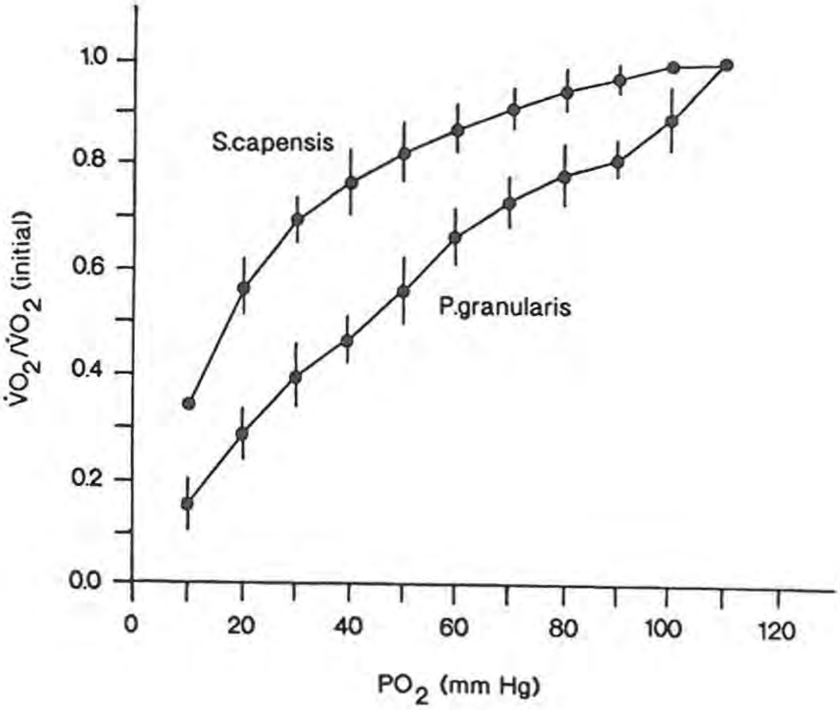

Fig. 7.2. Mean $\dot{V}O_2/\dot{V}O_2$ (initial) ± SD as a function of PO_2 for both limpets. For each species n = 6.

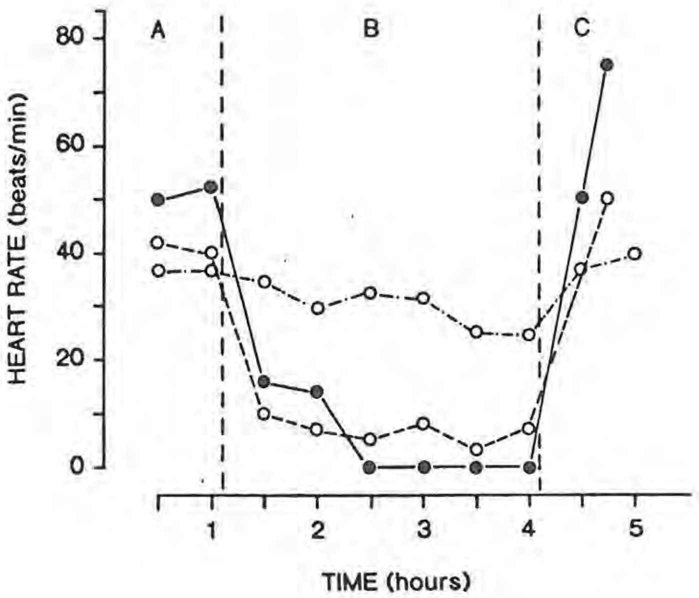

Fig. 7.3. Heart rate of individuals of *Patella granularis* (solid circles) and *Siphonaria capensis* (open circles) showing typical responses when measured initially in normoxia (A), during hypoxic exposure (B), and on return to normoxia (C). Note the two different types of response by *S. capensis*. Ambient temperature was 18°C.

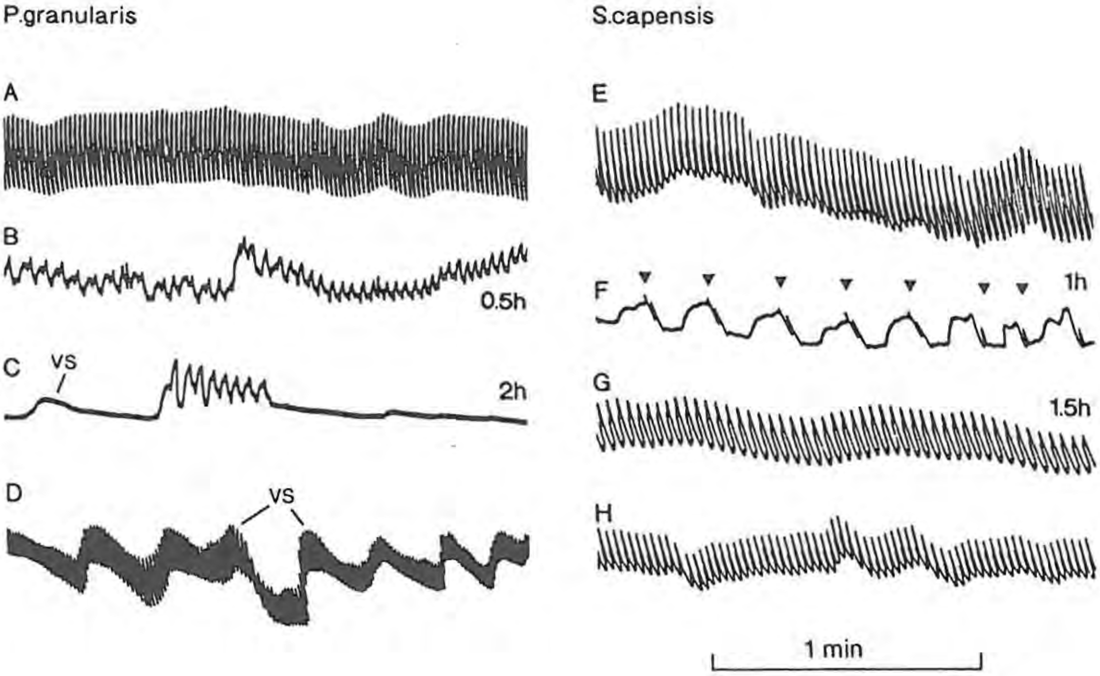

Fig. 7.4. Sequential cardiograms of an individual *Patella granularis* (A-D) and *Siphonaria capensis* (E-H) before (A,E), during (B,C,F,G), and 30 min after (D,H) hypoxic exposure. Durations of hypoxic exposure are indicated. Arrowheads show heart contractions. vs indicates ventilatory surge.

Table 7.1

Heart rate (beats/min) measured in air-saturated seawater (18°C; 37‰) before, and after hypoxic exposure (50 mm Hg) for the duration indicated. Fractional heart rate (recovery/initial) is also given.

Species	Duration	Heart rate (f_h)		
		initial f_h	recovery f_h	fractional f_h
P. granularis	3h	50.7 ± 2.5	71.7 ± 3.9	1.45 ± 0.11 (4) *
	6h	49.0 ± 3.5	71.3 ± 2.9	1.46 ± 0.12 (4) *
S. capensis	3h	37.6 ± 3.7	39.0 ± 4.3	1.04 ± 0.07 (6)
	6h	40.0 ± 6.2	39.5 ± 6.5	0.97 ± 0.06 (5)
	9h	43.0 ± 5.3	39.3 ± 3.1	0.95 ± 0.19 (3)

Values represent $\overline{X} \pm 1$ SD (n). Asterisk indicates a significant difference (*t*-test; $P < 0.05$) between initial and recovery heart rates.

There was variability in the heart beat response to hypoxia between individuals of *S. capensis*, thus making quantification of heart rate with progressive exposure difficult. After 1 h some individuals entered into extreme bradycardia (<10 beats/min), whereas others showed only a slight decline in heart rate. One limpet maintained a heart rate of 80% initial rate throughout the recording period (Fig. 7.3). An observation was made showing reversion of the characteristically low bradycardia (<10 beats/min), to a steady heart rate of 25 beats/min with increased exposure to hypoxia (Fig. 7.4 F,G). No shell lifting was noted by this limpet during hypoxia. Bradycardia was distinguishable from deflections of the recording trace resulting from ventilatory surging, by the often clearly definable cardiac contractions (Figs. 7.4 F and 7.5 F). On return to normoxia, heart rate returned to levels similar to those recorded initially in air-saturated water (that is, there was no overshoot of heart rate), irrespective of the duration of hypoxic exposure (Table 7.1; Fig. 7.4).

7.3.3 Effect of sand-inundation on heart beat

As during hypoxic exposure, gross cardiac adjustments were seen by *P. granularis* during sand-inundation. Within 1 h all four individuals, displayed long periods (minutes) of acardia, punctuated by bursts of cardiac activity (beat frequency was 15-23 beats/min), again not permitting meaningful quantification of rate. In one individual, acardia was observed after 5 min of sand- inundation, and ventilatory surging was associated with this (Fig. 7.5).

Heart rate of *S. capensis* (n = 4) declined slowly, being only slightly different from the initial rate after 1 h. All individuals showed bradycardia (<10 beats/min) after 2 h of sand-inundation (Fig. 7.5). On re-establishing sand-free conditions, *P.*

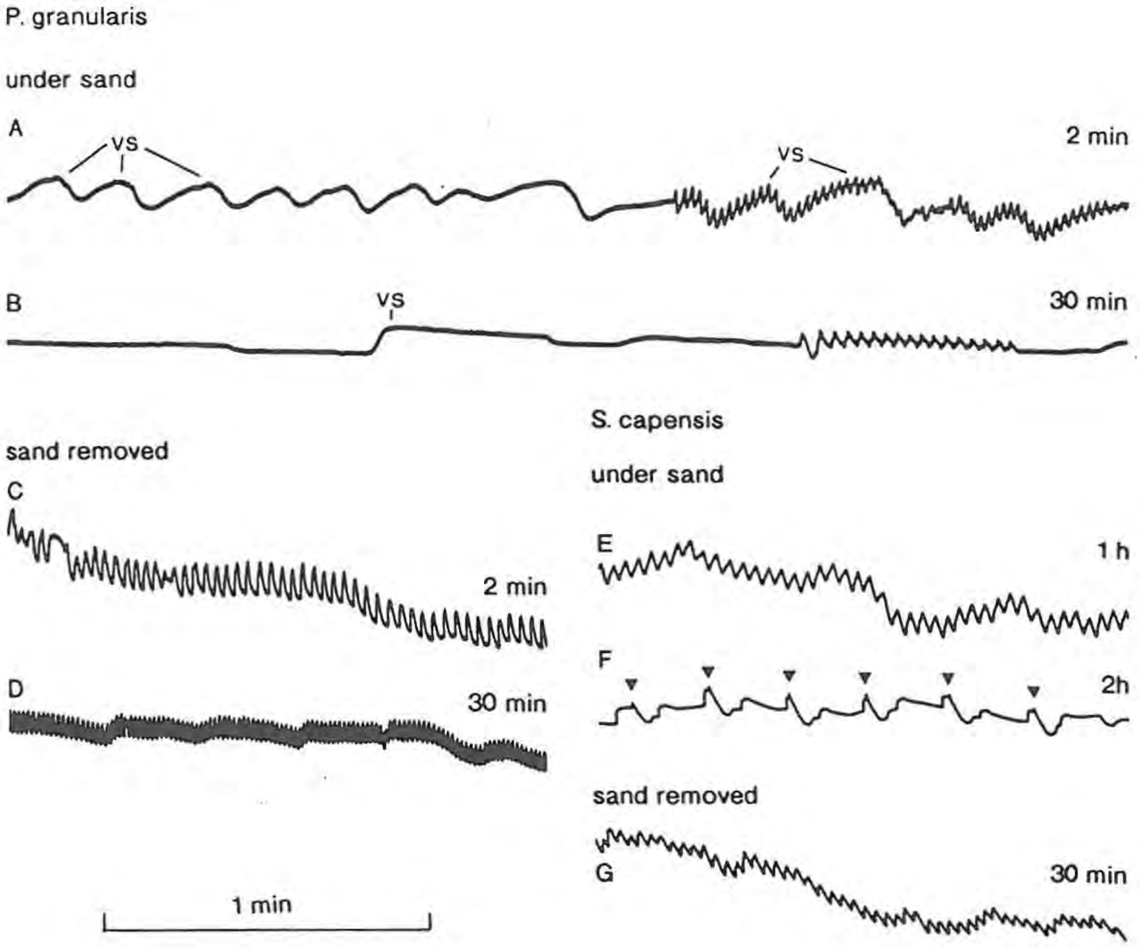

Fig. 7.5. Sequential cardiograms of an individual *Patella granularis* (A-D) and *Siphonaria capensis* (E-G) after sand coverage (A,B,E,F), and following the return to sand-free conditions (C,D,G). The duration of exposure to each condition is indicated. vs indicates ventilatory surge.

granularis, but not *S. capensis*, showed a significant (t-test; $P < 0.05$) overshoot of heart rate above the initial sand-free rate (Table 7.2; Fig. 7.5).

7.3.4 Effect of hyposalinity on heart beat

Mean heart rate (± 1 S D) of *P. granularis* declined from 45.5 ± 4.2 to 31.2 ± 7.7 (n = 7) within 3 h exposure to hyposaline seawater (20‰). During exposure, this species noticeably drew its shell firmly down onto the substratum, and on return to normal salinity showed excessive shell raising (and the associated undulations of the impedance recording trace). Although *P. granularis* displayed a significant post-hyposaline overshoot of heart rate (Table 7.3), this was not as marked as observed in the hypoxia and sand- inundation experiments.

All eight individuals of *S. capensis* underwent the typical extreme bradycardia within 3 h exposure to hyposalinity, and on return to normal salinity again showed no significant overshoot of heart rate (Table 7.3).

7.4 DISCUSSION

Many factors influence the respiratory responses of marine invertebrates to acutely declining oxygen tensions (see Herreid 1980, for review). For simplicity two extreme responses have been described: oxyregulation, the maintenance of a constant $\dot{V}O_2$ with declining PO_2, and oxyconformation, a proportional change in $\dot{V}O_2$ to change in ambient PO_2 (Mangum and van Winkle 1973). These responses represent opposite ends of a continuum, along which the distinction between them is often difficult (Mangum and van Winkle 1973; Taylor and Brand 1975; Herreid 1980). Various methods have however been proposed to determine the level of oxyregulation (Tang 1933; Mangum and van Winkle 1973; Herreid 1980; Yeager and Ultsch 1989), including the determination of: the oxygen tension (P_c) at which there is departure from regulation to conformation (good oxyregulators have lower P_c

Table 7.2

Heart rate (beats/min) measured in air-saturated seawater (18°C; 37‰) before, and after sand burial (3 h). Fractional heart rate (recovery/initial) is also given.

Species	Heart rate (f_h)		
	initial f_h	recovery f_h	fractional f_h
P. granularis	52.3 ± 5.4	69.6 ± 2.1	1.35 ± 0.11 (4) *
S. capensis	40.0 ± 6.5	41.0 ± 7.8	1.06 ± 0.17 (4)

Values represent $\overline{X} \pm 1$ SD (n). Asterisk indicates a significant difference (*t*-test; $P < 0.05$) between initial and recovery heart rates.

Table 7.3

Heart rate (beats/min) measured in air-saturated seawater (37‰) before, and after hyposaline (20‰) exposure.

Species	Heart rate (fh)		
	initial f_h	recovery f_h	fractional f_h
P. granularis	45.5 ± 4.2	51.9 ± 6.4	1.12 ± 0.07 (7) *
S. capensis	37.5 ± 5.6	41.1 ± 3.5	1.08 ± 0.19 (8)

Values represent $\overline{X} \pm 1$ SD (n). Asterisk indicates a significant difference (*t*-test; P <0.05) initial and recovery heart rates.

values); the B2 coefficient of a polynomial regression fitted to the relationship between $\dot{V}O_2$ and PO_2 (a more negative value indicates better independence). In the present study the B2 coefficient of individually fitted regressions proved to be of little use in characterizing the respective regulatory abilities, because of different initial (normal) oxygen consumption rates between the two species (Fig. 7.1). Standardized data, however, showed that under declining oxygen tension *Siphonaria capensis* displays greater respiratory independence than *Patella granularis* (Fig. 7.2) and appears to oxyregulate partially, to around 60 mm Hg.

Compensatory responses to increase oxygen supply in hypoxia include, increased ventilation of the gills, and increased removal of blood oxygen from the gills (or perfusion) (McMahon 1988; Wood 1991). Increased cardiac output would thus enhance oxygen uptake, however, an increase in heart rate is rarely observed in marine invertebrates in hypoxia. In some cases heart rate remains constant down to the critical tension, but it is usually lowered with a reduction in oxygen tension (deFur and Mangum 1979; Morris and Taylor 1985). Where extreme bradycardia (<10 beats/min) was not entered, the heart rate of *S. capensis* remained relatively higher for longer, than that of *P. granularis*. Whereas after 1 h hypoxia, all individuals of *P. granularis* displayed gross cardiac adjustments (acardia), the heart rate of one individual of *S. capensis* remained at 80% of the initial rate for 3 h hypoxia. Reversion of hypoxia-induced bradycardia was also observed in *S. capensis*. These cardiac responses by *S. capensis* may facilitate better oxygen uptake at low oxygen tension than by *P. granularis*.

Some authors, however, argue that fluid convection systems play only a minor compensatory role in low PO_2, with greater importance being placed on functioning of the blood pigment (Mangum 1980). Representatives of both *Patella* and *Siphonaria* possess heme- containing oxygen carriers (Bannister, Bannister and

Micallef 1968; Read 1968; Bonaventura and Bonaventura 1983), including haemocyanin, which has been identified in the blood of *S. zelandica* (Wells and Wong 1978). An investigation into the properties of the blood pigment, would allow better conclusions to be made concerning differences in oxygen uptake between the species.

In hypoxia or anoxia many intertidal invertebrates utilize environmental anaerobiosis (De Zwaan and Wijsman 1976; Gade 1983). End products of this generally accumulate in the tissues, and when normoxia is re-established are converted back into storage products, such as glycogen. Re-conversion results in elevation of aerobic rate relative to the pre-hypoxic rate. The so-called repayment of "oxygen debt" presumably does not occur in *S. capensis* on return to normoxia after hypoxic exposure, as no overshoot of heart rate was observed by this limpet (Table 7.1). An absence of oxygen debt is observed in other marine gastropods (McMahon and Russell-Hunter 1978; Brown and Wynberg 1987). However, this is not conclusive evidence for the absence of anaerobiosis, as anaerobic end products may be excreted (Ellington 1983). *Siphonaria capensis* has been shown to utilize anaerobic pathways common to other marine marine molluscs, evidenced by succinate accumulation in its tissues during prolonged anoxia (Marshall and McQuaid 1989), but whether this is utilized to any significant level in hypoxia is not known.

An alternative explanation for the absence of oxygen debt in *S. capensis*, is that it responds to lowered oxygen tension by lowering total energetic expenditure, rather than maintaining a constant metabolic rate by incorporating anaerobiosis. This is referred to as a *poikilometabolic response* (or type II oxyconformer) by Herreid (1980). This viewpoint is to some extent supported by observations of bradycardia in air by this limpet (pers. obs.) and *S. oculus* (Chapter 6), when anaerobic incorporation seems unlikely (see Discussion in Chapter 6).

Patella granularis exhibited an overshoot in heart rate of more than 140% of initial rate (Table 7.1), a magnitude suggested for repayment of oxygen debt during reconversion of anaerobic endproducts (50% elevation in aerobic rate; Curve IV; Herreid 1980). While other patellids, *P. caerulea* and *P. vulgata*, undergo facultative anaerobiosis (Brinkhoff, Stockmann and Grieshaber 1983; Lazou, Michaelidis and Beis 1989), *P. granularis* is intolerant of hypoxia, and accumulated no detectable succinate in anoxia (Marshall and McQuaid 1989). Oxygen debts occur for reasons other than reconversion of anaerobic endproducts, such as the replenishing of oxygen stores within the body fluids, and the regenerating of depleted phosphogen and ATP stores. These may explain the elevated heart rate by *P. granularis* on return to normoxia after hypoxic exposure.

To summarize then, although the limpet species exhibit different recovery responses after hypoxic exposure, these are difficult to interpret. Conclusive evidence for the utilization of anaerobic metabolism, clearly requires biochemical or calorimetric investigation. However, intolerance of hypoxia by *P. granularis* (Marshall and McQuaid 1989), and the capacity for aerobic rate depression by *Siphonaria* (Chapters 2, 3 and 6; Marshall and McQuaid 1991), suggest that neither may utilize anaerobiosis to any extent.

Sand burial is likely to induce respiratory stress for the following reasons: oxygen tension in the sediment interstitial water becomes lowered (pers. obs.; Andersen and Helder 1987); water flow through the sediment is reduced; the sand potentially forces the shell down, isolating the gills (an effect differing between patellid and siphonariid limpets; Marshall and McQuaid 1989). Therefore, as expected, response of heart beat to sand burial was in many respects similar to that during hypoxia (Figs. 7.4 and 7.5). Sand burial however, caused a more rapid change in the heart rate of *P. granularis*. This was presumably the result of smothering of its external pallial gills by sand particles. In contrast, heart rate of *S. capensis* became lowered over a longer period in sand, than in hypoxia. Because of the small pneumostomal opening to the mantle cavity, smothering of the gill of this limpet would seem unlikely.

Both species of limpet may clamp their shells down onto the substratum. This behaviour isolates their body tissues, in much the same way as in bivalves during valve closure, allowing them to escape potentially deleterious environmental conditions (e.g., extreme salinities). During the present experiments both species exhibited shell clamping in hyposalinity, and this response has been shown experimentally to enhance survival in low salinities of *Siphonaria*, including *S. capensis* (McAlister and Fisher 1968; Branch and Cherry 1985).

Cardiac and behavioural responses of *P. granularis* (lowering of heart rate, respiratory overshoot (Table 7.3), and shell raising on return to normal conditions) during and after exposure to hyposalinity, were indicative of respiratory stress. A respiratory stress would be expected to result from shell clamping in this limpet, as the pallial gills become isolated. In *S. capensis*, contact with the external environment can be maintained via the pnuemostome (siphon) (the shell is grooved to house this structure), even when the shell is clamped down. However, unfavorable salinities may induce isolation of the mantle cavity by closure of the pneumostome, and consequently, a bradycardia.

Isolation would be beneficial to both limpets in the short-term (e.g., during washing of the intertidal rocks by rainwater), but not in the long-term. Pool-dwelling *S. capensis* may be exposed to prolonged salinity reduction. In *S. pectinata, S. zelandica*, and *S. aspera*, a species sympatric with *S. capensis*, an adaptation to variable salinity exposure is suggested by their maintenance of hyperosmotic haemolymph in reduced salinities (Williams 1964; McAlister and Fisher 1968; Bedford 1969).

While ionic differences between haemolymph and environment may in part arise from an isolation response (McAlister and Fisher 1968; Branch and Cherry 1985), *S. zelandica* has been demonstrated to regulate levels of intracellular organic compounds (Bedford 1969), clearly an adaptation for long-term ionic perturbation.

In conclusion, the extension by *S. capensis* into potentially low PO_2 environments (tidal pools, sand-inundated rock), may in part be accountable for by its ability to oxyregulate, partially. Perhaps of greater importance for its survival in these temporally variable, unfavourable environments, is its capacity for metabolic rate depression (inferred from response of heart beat; Chapters 2, 3 and 6; Marshall and McQuaid 1991). Many terrestrial and freshwater snails withstand prolonged unfavourable conditions, because of their capacities for aerobic rate depression during dormancy/aestivation (Storey and Storey 1990). Characteristics of heart beat of *P. granularis* (e.g., erratic interspersion of often prolonged acardia, and respiratory overshoot) are suggestive of respiratory stress by this limpet in hypoxia, sand-inundation and hyposalinity.

7.5 SUMMARY

1. The B2 coefficient of the polynomial regression relating $\dot{V}O_2$ with PO_2 proved to be of little use in differentiating regulatory capacities of *Patella granularis* and *Siphonaria capensis*, because of differences in initial rates of oxygen consumption. Standardized rates (obtained by dividing by initial rate of oxygen consumption) showed that *S. capensis* was a better oxyregulator than *P. granularis.*

2. In both limpet species hypoxia caused major cardiac adjustments. *Patella granularis* showed bradycardia (above 15 beats/min) interspersed with periods of cardiac arrest, which were more prolonged after a period of hypoxic exposure. During hypoxia the heart rate of *S. capensis* usually fell progressively to an extreme

bradycardia (<10 beats/min). In one instance heart rate remained relatively constant throughout 3 h of hypoxia, and reversion of bradycardia during hypoxia was also observed. Whereas *P. granularis* showed a respiratory overshoot on return to normoxia, this was not so for *S. capensis.*

3. The cardiac responses by the limpets to sand inundation were similar to those during hypoxia, but were induced more rapidly following sand coverage in *P. granularis.*

4. Hyposalinity (20‰) induced a conservative lowering of heart rate in *P. granularis* (mean heart rate declined from 45.5 to 31.2 beats/min by 3 h), and the typical bradycardia was observed by *S. capensis.* Again, an overshoot in heart rate on return to normal salinity was observed only for *P. granularis.*

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CHAPTER 8

GENERAL DISCUSSION

Two phylogenetically distantly related families of intertidal limpet, the siphonariids and the patellids, occur along the coast of South Africa. They often show discrete local distributions. Patellids dominate in the low shore (Branch 1981) and in physico- chemically apparently more favourable intertidal habitats. Species of patellid may show intense intraspecific competition (Branch 1981), and findings by Creese and Underwood (1982) suggest that, through their feeding methods, they may gain competitive advantage over siphonariids. However, in physico-chemically extreme and variable intertidal habitats, siphonariids, to the exclusion of patellids, may occur in abundance. These limpets may be observed well into the upper-shore zone (open rock and tidal pools), in estuaries, on sand-inundated rock substrata and, in regions polluted by sewerage and industrial effluent (Chapter 1; Allanson 1958; Branch and Cherry 1985; Dower 1989; Marshall and McQuaid 1989; Coetzee 1991). Existence in such habitats is primarily influenced by adaptations facilitating tolerance of physico-chemical extremes, rather than biological factors such as feeding and competition. The present study therefore examined the physiological basis for discrepancies in local distribution of these limpet families. Specifically, differences in their vertical distribution, and distribution in tidal pools/sand-inundated regions were considered, by comparing the physiology of *Patella granularis*, *Siphonaria oculus*, and *S. capensis*.

Investigations of physiological adaptations for upper-shore existence by gastropods, have mainly examined tolerance of temperature and desiccation (Wolcott 1973; Underwood 1979; McMahon 1990) and respiratory/metabolic characteristics (Branch and Newell 1978; Newell and Branch 1980; McMahon 1988). Regarding respiratory/metabolic adaptations, there are two pertinent hypotheses. One being that upper-intertidal species should show efficient aerial respiration (McMahon 1988), and the other that they should be relatively good conservers of their energy resources (Branch and Newell 1978). While the ratio of aerial:aquatic respiration is poorly related to zonation for some gastropod groups (McMahon 1988), energy-conserving metabolic adaptations, such as a relatively low metabolic rate and lowered Q_{10} with increased temperature, have been shown for the upper-shore *P. granularis* (Branch and Newell 1978). Because *S. oculus* shows similar metabolic rate-temperature characteristics to *P. granularis* (Chapter 5), these may similarly facilitate its upper-shore distribution. *Siphonaria oculus*, however, differed in its metabolic response to long-term temperature change (Chapter 5). It showed absence of (or in some instances poor) thermal acclimation, and this may allow additional energy savings during the winter months.

However, when considering energy balance (i.e., energy input versus energy expenditure; Newell and Branch 1980), the vertically higher distribution of *S. oculus* is not entirely explained, as there is some evidence that *P. granularis* is a more gregarious feeder. When held in the laboratory, all individuals of *P. granularis* become active and commence radula movements immediately on reimmersion in water. This behaviour was not observed by all individuals of *S. oculus* as some remained inactive on reimmersion. Although energy expenditure of *P. granularis* may be relatively high during the winter months, such behaviour could increase energy input and so facilitate a higher distribution than predicted by a hypothesis considering energy expenditure alone.

However, energetic savings by *S. oculus* will be considerable if it utilizes, a further and the most remarkable metabolic characteristic observed by this limpet, depression of aerobic metabolism. Metabolic rate depression of siphonariids, characterized by extreme bradycardia (heart rate of <10 beats/min; Chapters 2,3,4,6 and 7; Marshall and McQuaid 1991) and relatively low levels of oxygen consumption ($\dot{V}O_2$ may fall to 18% of normal level; Chapter 6), was observed in laboratory experiments, when held continuously in air, and when exposed to reduced oxygen tension, sand coverage and hyposalinity (Chapters 6 and 7). Bradycardia was also recorded in the field when air exposed at around 30°C (Chapter 3). This metabolic characteristic of *S. oculus* may explain vertical separation of this limpet and *P. granularis* within an energetic framework. Recently, minimization of activity and aestivation have been suggested as adaptations by eulittoral fringe littorine species (McMahon 1990).

While the depletion of energetic resources (starvation) may influence vertical distribution of intertidal animals, another factor influencing this, is tolerance of continuous aerial exposure. *Patella granularis* is shown to have a lower tolerance of aerial exposure than *S. oculus* (Chapter 6). Important in enhancing survival in air is tolerance of, and/or resistance to evaporative water loss (Wolcott 1973; McMahon 1990). In addition, because aerial exposure potentially impedes oxygen uptake, the degree varying between species depending on respiratory organ morphology (see Chapter 6), tolerance of lowered tissue oxygen levels would also facilitate extended aerial exposure. Although *P. granularis* has a relatively low metabolic rate (Chapter 5; Branch and Newell 1978), in comparison to animals exhibiting metabolic rate depression, it depends on a relatively high minimal energetic requirement for cellular maintenance (metabolic homeostasis; Chapter 6). It is also vulnerable to impedance of oxygen uptake by the pallial gills during water loss and has a poor capacity for anaerobic metabolism (Marshall and McQuaid 1989). These effects suggest that metabolic stress (the falling of energetic level below the minimum cellular requirement) is the cause of mortality during exposure to air when neither the degree of desiccation nor temperature are at lethal levels. As mortality occurred after relatively little water loss, under conditions of low rate of desiccation metabolic stress is presumably induced before osmotic stress develops, unless this limpet has a relatively low tolerance of water loss (see Chapter 6 for comparison of LD_{50} values). By contrast, mortality of *S. oculus* held continuously in air is probably the result of osmotic stress (see LD_{50} and LT_{50} values in Chapter 6). This limpet, with its internal lung and capacity for metabolic rate depression, is not likely to experience significant respiratory stress (impedance of oxygen uptake) and could be expected to tolerate low tissue oxygen levels. Acid-base disturbance is another factor potentially influencing survival in air of both species, but little is known about this for gastropods in general (Burnett 1988).

Common to upper-shore pools and sand-inundated regions is a reduction in oxygen availability (Andersen 1987; Taylor 1988). *Siphonaria capensis* shows better tolerance of low ambient oxygen levels than *P. granularis* (Marshall and McQuaid 1989). The mechanism enhancing tolerance of low PO_2 by *S. capensis* may relate to depression of aerobic metabolism (inferred from heart rate; Chapter 7), as the capacity for this necessitates cellular maintenance at even low energetic levels, and thus low cellular oxygen levels. It is usually implied that aerobic rate depression in marine molluscs is accompanied by the incorporation of anaerobic metabolism, and this has been well demonstrated in some bivalves in hypoxia (Shick, Gnaiger, Widdows, Bayne and De Zwaan 1986). However, this may not be so for *Siphonaria*, as aerobic rate depression also occurs when oxygen is not limiting (Chapters 2,3, and 6). Furthermore, compensatory incorporation of anaerobiosis is unlikely as this limpet undergoes a low energy turnover (Chapter 6). *Siphonaria capensis* has, however, been shown to utilize anaerobiosis in anoxia (Marshall and

McQuaid 1989), the significance of which is unknown, and the determination, using direct calorimetry (Pamatmat 1878; Hammen 1980), of the apportionment of utilization of the respective pathways by this limpet should thus prove rewarding.

Upper-shore pools are variable in oxygen levels as well as in pH, temperature and salinity (Taylor 1988). Organisms inhabiting physico-chemically extreme and variable environments may tolerate exposure to, or limit interaction with (by isolating themselves), an adverse perturbation. Tolerance is usually only suffice for short-term exposure. Should the perturbation persist, the capacity for long-term isolation is necessary, and this is seen in many terrestrial and freshwater snails during aestivation. Because feeding is curtailed it is imperative to conserve energy resources during aestivation, and this is achieved by aerobic rate depression, which has been described in aestivating snails (Herreid and Rokitka 1976; Barnhart and McMahon 1987; 1988). Metabolic rate depression (inferred from heart rate; Chapter 7) is shown to be induced in *S. capensis* by conditions likely to be experienced in upper-shore pools and in sand-inundated habitats. Moreover, this limpet may show prolonged inactivity, a characteristic behaviour of aestivation. Individuals of *S. oculus* remained inactive for up to four months during continuous immersion in the laboratory. They were seen to remain on the same mark on perspex plates on daily observation. Immobility between observations was shown by the absence of radula or foot marks in an algal film which grew over the perspex plates. Active limpets made such marks.

It is suggested here that metabolic rate depression in *S. capensis*, which likely facilitates tolerance of hypoxia, also allows prolonged avoidance of other unfavourable physico-chemical variables by facilitating aestivation. This would allow their extension of distribution into upper-shore pools and sand-inundated habitats. By contrast, *P. granularis* is restricted in the range of intertidal habitats it can exploit because of its sensitivity to hypoxia, and its limited period of isolation due to lack of the ability for metabolic rate depression. Clearly, aestivation by *S. capensis* will allow it to survive in habitats were food intake is periodically restricted, be this a consequence of isolation, or a phenomenon of the environment, such as potential immobility imposed by sand burial.

The combination of the whole animal metabolic characteristics, non-acclimation and metabolic rate depression in *Siphonaria*, is interesting, as this is also seen in freshwater and terrestrial pulmonates (McMahon 1973; McMahon 1983; Bailey and Lazaridou- Dimitriadou 1991). Implicit in these characteristics is the capacity for sustained cellular functioning at lowered energetic levels. Inverse acclimation (similar to non-acclimation with regard to its ecological, energy-conserving significance) leads to declining cellular energetic levels during low winter temperatures, and has been suggested as forming part of the mechanism for dormancy in land snails (Bailey and Lazaridou-Dimitridou 1991). However, some teleost fish exhibit normal temperature acclimation in summer and metabolic rate depression during winter topor (Crawshaw, Lemons, Parmer and Messing 1982; Walsh, Foster and Moon 1983). Of further interest is the observation of non-acclimation in some upper-shore gastropods: the pulmonate, *Melampus bidentatus* (McMahon and Russell-Hunter 1981) and the prosobranch, *Littorina littorea* (Paul, Hatch, Jordan and Stein 1989). By interpreting aerial relative to aquatic oxygen consumption rate, it has been suggested that eulittoral fringe littorinids aestivate (McMahon 1990), however, detailed characterization of possible metabolic rate depression by these and other upper-shore species could prove worthwhile.

The successful invasion of terrestrial habitats (even arid habitats) by pulmonate snails can in part be ascribed to their capacity for dormancy and associated metabolic rate depression. While this metabolic feature may be ecologically determined, it may have a phylogenetic basis. The evolution of the pulmonates is a matter of controversy. An earlier theory (Morton 1955) suggests the origin of the Stylommatophora (land pulmonates) from marine Basommatophora (freshwater and marine pulmonates, including *Siphonaria*). More recently, it has been suggested that both orders originated independently, or that the Basommatophora are derivable from a group of Stylommatophora (Solem 1985). While this recent theory is based on fossil evidence showing an earlier appearance of the Stylommatophora in the Carboniferous (Solem 1985), *Siphonaria* has previously been reported as being the oldest extant pulmonate, dating back to the Devonian period (Morton 1955).

Siphonaria has mainly primitive morphological features (Morton 1955; Solem 1985), but has a secondary gill located internally in the mantle cavity. This may have been reacquired during a shift from intertidal conditions, to conditions of continuous submergence (species of *Siphonaria* live submerged in intertidal pools, and even subtidally), rather than representing readaptation from a terrestrial to an aquatic existence. A more convincing case for readaptation of an aquatic pulmonate from the terrestrial environment is provided by freshwater pulmonate limpets, which may occur in land-locked water bodies. Quite unlike the respiratory structures of *Siphonaria*, freshwater pulmonate limpets have lost the mantle cavity and bear neomorphic external gills (Russell- Hunter 1978).

Considering their earlier appearance in the fossil record, and their more primitive morphological characteristics, it is improbable that *Siphonaria* can be derived from the Stylommatophora, representing a readaptation to the marine environment. The capacity for aerobic rate depression among pulmonates, thus could have arisen in some common ancestral intertidal pulmonate from which both the Basommatophora and Stylommatophora originated.

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The influence of respiratory responses on the tolerance to sand inundation of the limpets *Patella granularis* L. (Prosobranchia) and *Siphonaria capensis* Q. et G. (Pulmonata)

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Abstract: Tolerance of sand inundation and reduced oxygen tensions by two phylogenetically distant limpet species, *Siphonaria capensis* and *Patella granularis*, were examined and related to their "within-shore" distributions. In the laboratory, the pulmonate *S. capensis* survives for considerably longer periods under sand and in water of reduced oxygen tensions than does the prosobranch *P. granularis*. Differences in mortality of the two limpet species can be explained by differential respiratory responses exhibited during exposure to seawater of reduced oxygen tensions. *S. capensis* responds to hypoxia by partial oxyregulation and in anoxic seawater exhibits anaerobic respiration, resulting in the accumulation of succinate in its tissues. By contrast, oxygen consumption of *P. granularis* is more dependent on external tension and succinate does not accumulate its tissues. These physiological differences can explain local distribution of the two species on rocky shores which are prone to sand inundation.

Key words: Anaerobiosis; Habitat segregation; Limpet; Respiratory response; Rocky shore; Sand-inundation

INTRODUCTION

Many rocky shores are prone to periodic inundation by sand. The frequency and severity of sand deposition vary between and within shores and influence species assemblages. Taylor & Littler (1982) demonstrated the importance of inundation of shores in maintaining the balance between psammophilic and psammophobic competitors for space. Frequently inundated or "mixed" shores constitute 31% of the coastline of South Africa (Bally et al., 1984) and are more common than rocky shores. Patellid and siphonariid limpets are abundant herbivores on this coast (Allanson, 1959; Branch, 1981) and, whereas species of both limpet families co-exist in sand-free rocky areas, only siphonariids extend their distribution to adjacent regions where sand deposition is a regular occurrence.

Apart from the normal stresses associated with intertidal life, sand inundation im-

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poses additional stresses, including a reduction in availability of dissolved gases (pers. obs.; Littler et al., 1983). The present study examines the hypothesis that patellid limpets, unlike siphonariids, are physiologically intolerant of sand coverage and that this is primarily due to their intolerance of reduced oxygen tensions.

Mechanisms employed by molluscs in order to tolerate hypoxia or anoxia are generally oxyregulation or anaerobic respiration (Newell, 1970). McMahon (1988) has suggested that the scope for either mechanism varies widely between species allowing them to fit physiologically into a particular ecological niche.

Respiratory studies on limpets have centered around oxygen consumption in relation to body weight, temperature and food intake (Branch & Newell, 1978; Branch, 1979; Innes et al., 1984; Branch et al., 1988) and comparisons between aerial and aquatic consumption rates (Bannister, 1974; Branch & Newell, 1978; Dye, 1987). However, the effect of reduced oxygen tension on consumption has not been examined. Similarly, although many investigations, mostly on bivalves, have shown the accumulation of anaerobic end products in mollusc tissue (De Zwaan, 1977; Wijsman et al., 1977; Livingstone & Bayne, 1977; Wieser, 1980), only a single study reports anaerobiosis in a patellid limpet (Brinkhoff et al., 1983) and there are no records of anaerobic respiration in siphonariid limpets.

Experiments were performed to compare these two limpet families for: (1) the degree of tolerance, measured as survival, of sand inundation and reduced water-oxygen tension; and (2) oxygen consumption at reduced tensions and the capacity for anaerobiosis. Two common species, *Patella granularis* L. and *Siphonaria capensis* Q. et G., were used as representatives of the families.

MATERIALS AND METHODS

Similarly sized individuals (15 mm shell length) of *P. granularis* and *S. capensis* were collected from a rocky shore at Kenton-on-Sea (33°41'S, 26°40'E), eastern Cape Province, South Africa. Specimens taken from a site of cohabitation in the balanoid zone (mid-shore) were maintained in the laboratory in well-oxygenated seawater. Experiments were started 24 h after collection and were run at 37‰ salinity and 18 °C (mean sea temperature for the area). To approximate conditions during inundation, experimental animals were starved and held inactive.

EFFECT OF SAND INUNDATION ON SURVIVAL

Animals were contained in plastic Petri dishes (five · dish^{-1}) through which holes had been drilled for ventilation. 15 animals of each species were subjected to experimental or control conditions. A 15-mm layer of sand was placed in Petri dishes over experimental animals while control animals, in the same aquarium, were not covered. Aquaria were kept well oxygenated. In a subsequent experiment, using only *P. granularis*, an additional treatment was introduced. A weight equivalent to that of a 15-mm sand layer was placed on top of the limpets. For both experiments, mortality was recorded daily. Each experiment was conducted three times and mean mortality rates were calculated.

EFFECT OF OXYGEN TENSION ON SURVIVAL

Seawater containing oxygen at three different concentrations (<0.8, 2.0–2.8 and 3.6–4.8 ml $\cdot$ l^{-1} O_2) was prepared by flushing with N gas for different periods of time. This was siphoned into 3.6-l experimental chambers containing 6 or 8 animals of either species. The chambers were then sealed. The relatively large chamber size and the small number of limpets $\cdot$ chamber^{-1} ensured that the oxygen tension (P_{O_2}) remained fairly stable throughout the experiment. Mortality and oxygen tension in each experimental chamber were recorded daily and, when necessary, tensions were readjusted. The experiment was repeated three times and mean mortality rates were calculated.

For all survival experiments, ANOVA was used to test for significant differences in mortality at the 1% probability level.

OXYGEN CONSUMPTION AT REDUCED OXYGEN TENSIONS

Marine invertebrates may modify the effect of oxygen tension on consumption depending on size (Bayne, 1971, 1973; Taylor & Brand, 1975; Murdoch & Shumway, 1980), acclimation and experimental temperatures (Bayne & Livingstone, 1977; Newell et al., 1978; McMahon & Russell-Hunter, 1981) or acclimation tensions (Kushins & Mangum, 1971; Taylor & Brand, 1975; McMahon & Russell-Hunter, 1978). Hence, animals of a narrow size range (mean dry weight: 270.0 ± 41.9 mg, $n = 12$) were used and subjected to identical conditions prior to experimentation.

Limpets were kept in well-oxygenated seawater at 18 °C and 37‰ salinity for at least 2 days before performing the experiment. Single animals that had clamped onto a thick polythene surface were placed in a Perspex respiratory chamber (similar to that illustrated by Crisp et al., 1978) containing 40 ml oxygen-saturated filtered seawater (GF/C Whatman filters). The chamber was immersed in a water bath over a magnetic stirrer. A YSI polargraphic electrode, coupled to an oxygen analyser (model 58) and pen recorder, was inserted in a tapered hole in the wall of the chamber. After allowing the animal 30 min to settle, the chamber door was closed and each animal held in the dark at 18 °C for 2 h. Animals were then separated from their shells and dried to a constant weight at 60 °C. Oxygen consumption was measured at 10-mm Hg intervals for oxygen tensions (P_{O_2}) between 10 and 110 mm Hg. This was done for six individuals of each species. Results are presented as weight-specific oxygen-consumption rates.

SUCCINATE ACCUMULATION

Limpets were maintained in anoxic seawater (0.01 ml $\cdot$ l^{-1} O_2) for 6-, 11- and 24-h periods. Only the 6- and 11-h treatments were used for *P. granularis* as no individuals survived for 24 h in oxygen-free water. Control animals were kept in oxygen-saturated seawater.

To prepare the substrate, the muscular foot of 10 animals was excised, washed, damp-dried and weighed. The tissue was minced, then homogenised for 3 min in 10 ml 6% perchloric acid with an Ultra-turrax homogeniser. Homogenisation was performed in centrifuge tubes and the homogeniser arm was washed with 5 ml 6% perchloric acid. The homogenate was then centrifuged at 12000 × *g* for 30 min. The supernatant was decanted off and neutralised with 3.9 ml 3 N KOH. The precipitate which formed ($KClO_4$) was removed by centrifugation at 5000 × *g* for 15 min. The clear supernatant was diluted to yield a volume of 25 ml. All operations were performed at ≈4 °C.

The concentration of succinate was determined for each treatment by an enzyme assay method (Boehringer/Mannheim test kit, Catalogue No. 176281). Concentrations were derived from absorbance values which were determined on a Shimadzu double-beam UV-spectrophotometer.

RESULTS

EFFECT OF SAND INUNDATION ON SURVIVAL

Sand coverage had a strong influence on the survival of *P. granularis* but less on the survival of *S. capensis*. In all replicates, no *P. granularis* individuals survived for >3 days under 15 cm of sand (Fig. 1). Mortality of *P. granularis* individuals weighed

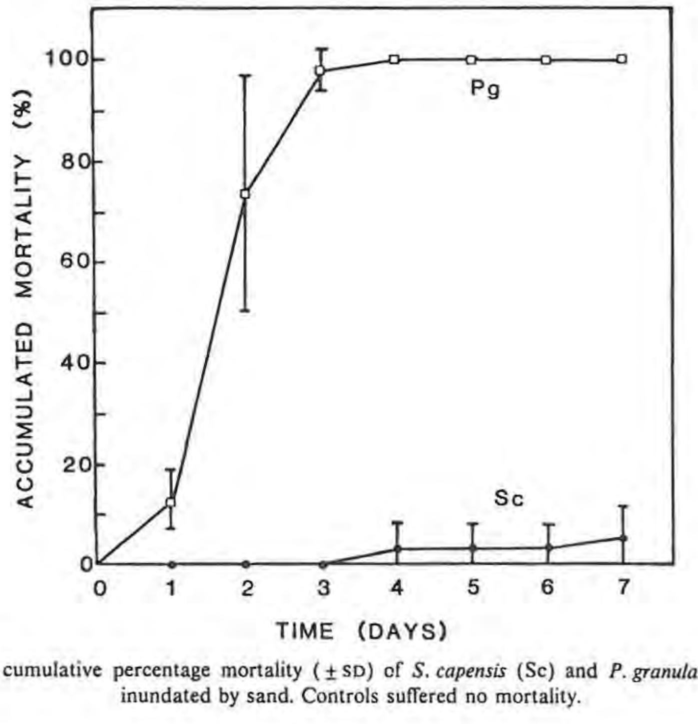

Fig. 1. Mean cumulative percentage mortality (± SD) of *S. capensis* (Sc) and *P. granularis* (Pg) when inundated by sand. Controls suffered no mortality.

down but not covered by sand was less than for sand-inundated individuals but significantly greater than for control animals (Fig. 2).

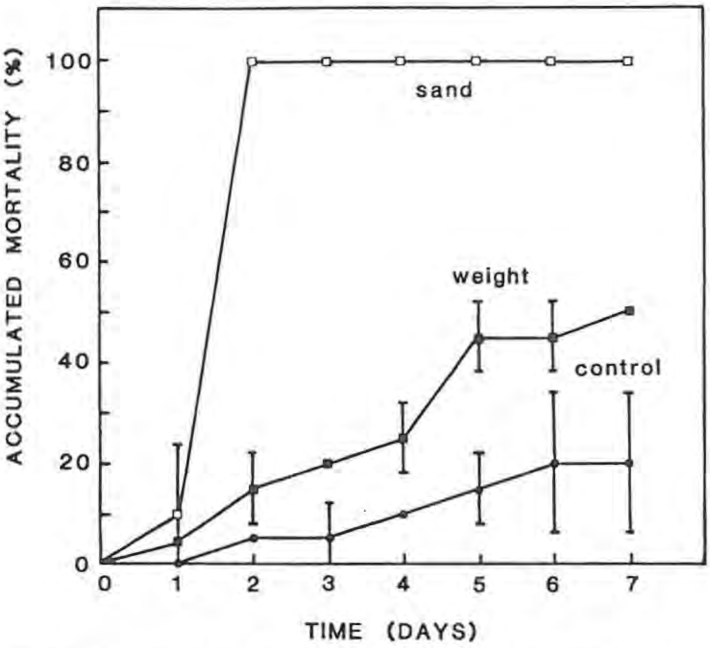

Fig. 2. Mean cumulative percentage mortality (± SD) of *P. granularis* when inundated by sand or depressed by an equivalent weight.

EFFECT OF OXYGEN TENSION ON SURVIVAL

Survival of both species decreased with a drop in oxygen tension but was significantly greater for *S. capensis* than *P. granularis* at all three oxygen tensions tested (Fig. 3). No individuals of *P. granularis* survived > 2 days at water-oxygen tensions of $<0.8\ \mathrm{ml \cdot l^{-1}}\ O_2$.

Seemingly stress-related behaviour was observed in *P. granularis* at low oxygen tensions. After an initial period of increased activity and waving of pallial tentacles, this limpet became inactive and mushrooming (excessive shell raising) was frequently observed. This presumably increased exposure of the mantle skirt, and hence the gills, to the surrounding water.

OXYGEN CONSUMPTION AT REDUCED OXYGEN TENSIONS

Polynomial regressions fitted to data relating Q_{O_2} to P_{O_2} for individual animals clearly differ between the two species (Fig. 4A). Q_{O_2} for *S. capensis* was more constant over the range of external oxygen tensions used. The B2 coefficient of a polynomial regression

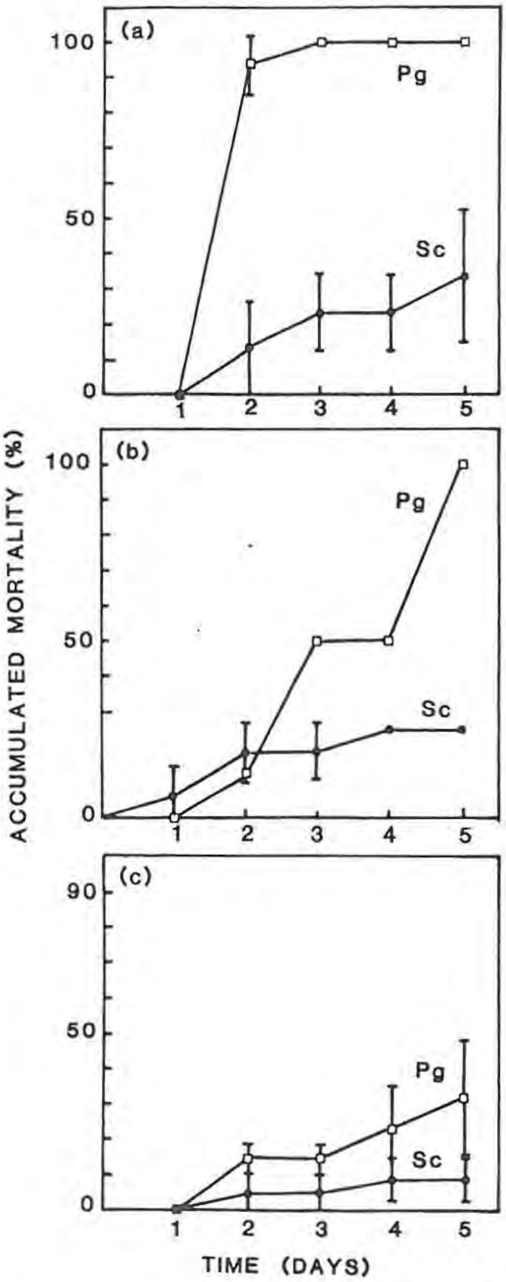

Fig. 3. Mean cumulative percentage mortality (± SD) of *S. capensis* (Sc) and *P. granularis* (Pg) at different water oxygen tensions: (a) <0.8, (b) 2.0–2.8 and (c) 3.6–4.8 $ml \cdot l^{-1}$ O_2.

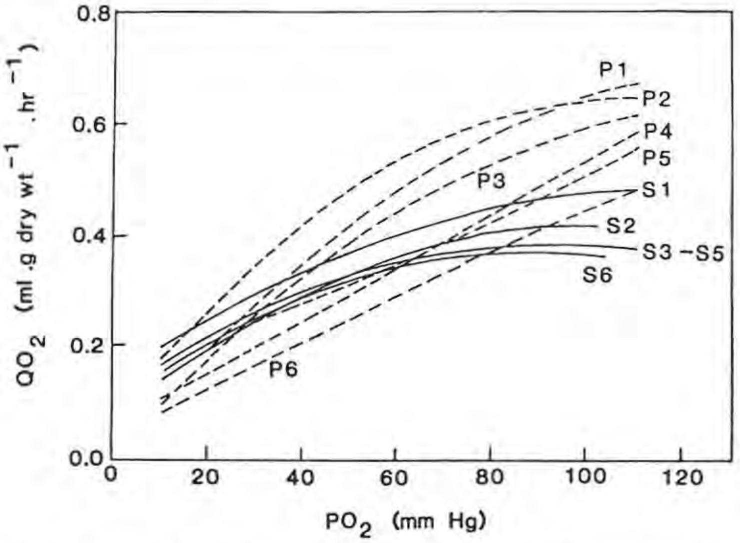

Fig. 4A. Weight-specific rate of oxygen consumption (Q_{O_2}) as a function of external oxygen tension (P_{O_2}) for six individuals of *P. granularis* (P1–6) and *S. capensis* (S1–6). Individual polynomial regressions are given below.

P1: $-0.0493 \times 10^{-3}x^2 + 0.012x + (-0.046)$; $r^2 = 0.99$ ($n = 10$)
P2: $-0.0491 \times 10^{-3}x^2 + 0.011x + 0.078$; $r^2 = 0.98$ ($n = 11$)
P3: $-0.0359 \times 10^{-3}x^2 + 0.009x + 0.012$; $r^2 = 0.98$ ($n = 11$)
P4: $-0.0027 \times 10^{-3}x^2 + 0.005x + 0.068$; $r^2 = 0.98$ ($n = 11$)
P5: $0.0099 \times 10^{-3}x^2 + 0.002x + 0.165$; $r^2 = 0.98$ ($n = 11$)
P6: $-0.0043 \times 10^{-3}x^2 + 0.004x + 0.037$; $r^2 = 0.99$ ($n = 10$)
S1: $-0.0265 \times 10^{-3}x^2 + 0.006x + 0.130$; $r^2 = 0.99$ ($n = 11$)
S2: $-0.0356 \times 10^{-3}x^2 + 0.007x + 0.084$; $r^2 = 0.94$ ($n = 10$)
S3: $-0.0372 \times 10^{-3}x^2 + 0.006x + 0.075$; $r^2 = 0.97$ ($n = 11$)
S4: $-0.0368 \times 10^{-3}x^2 + 0.006x + 0.111$; $r^2 = 0.92$ ($n = 11$)
S5: $-0.0339 \times 10^{-3}x^2 + 0.006x + 0.096$; $r^2 = 0.96$ ($n = 11$)
S6: $-0.0383 \times 10^{-3}x^2 + 0.007x + 0.103$; $r^2 = 0.90$ ($n = 10$).

has been used as an indicator of dependence of Q_{O_2} on P_{O_2}, with a more negative value indicating better independence (Mangum & Van Winkle, 1973). However, when, as in this case, initial oxygen-consumption rates (at 110 mm Hg) differ considerably, the B2 value is unreliable (see below).

Hence, the data for each animal were standardised by dividing Q_{O_2} values at each tension by the Q_{O_2} at 110 mm Hg [Q_{O_2} (initial)] and calculating the mean value for each species (Fig. 4B). The change in Q_{O_2}/Q_{O_2} (initial) between 110 and 50 mm Hg for *S. capensis* is ≈ 0.2 unit while that for *P. granularis* is > 0.4 unit, a difference of $> 100\%$. Between 50 and 10 mm Hg, the change in Q_{O_2}/Q_{O_2} (initial) for both limpet species is slightly > 0.4 unit. Although there is no obvious point of inflexion of the curve to indicate a change from regulation to conformation, this clearly demonstrates the superior regulatory ability of *S. capensis* down to ≈ 60 mm Hg.

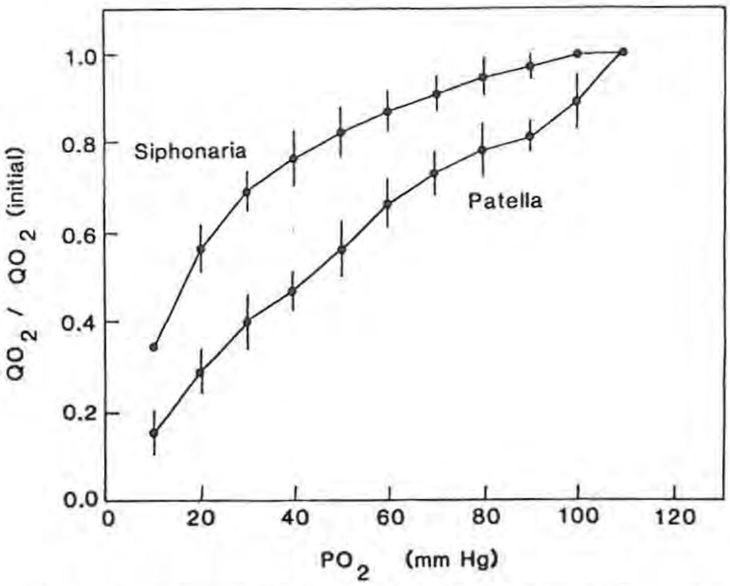

Fig. 4B. Mean Q_{O_2}/Q_{O_2} (initial) ± SD as a function of P_{O_2} for both limpets. For each species, $n = 6$.

SUCCINATE ACCUMULATION

While succinate was not detected in the tissues of *P. granularis* or control animals, it was detected in *S. capensis* after 11 h of anoxia and accumulated to 1.68 mg·g^{-1} tissue in <24 h (Table I).

TABLE I

Accumulation of succinate (mg·g^{-1} fresh weight) in foot of *S. capensis* and *P. granularis* when exposed to anoxic conditions (0.01 ml·l^{-1} O_2) or maintained in oxygen-saturated seawater.

Treatment	*S. capensis*	*P. granularis*
Saturated O_2	0.000	0.000
6 h: anoxia	0.000	0.000
11 h: anoxia	0.037	0.000
24 h: anoxia	1.680	–

DISCUSSION

Siphonariid and patellid limpets cohabit in sand-free intertidal rocky environments along the coast of South Africa but patellids are usually excluded from areas of the shore which are subject to frequent sand inundation. Siphonariid limpets may have a colonising advantage on sand-inundated shores as they cement a fertilized egg ribbon to the rock surface while patellids exhibit external fertilization. However, this habitat segregation may be explained by the greater physiological tolerance of sand coverage by *S. capensis* as demonstrated by laboratory experiments (Fig. 1).

During inundation, both limpets are exposed to: (1) reduced external water currents which will cause decreased irrigation of the gills; and (2) reduced oxygen tensions in the surrounding interstitial water (pers. obs.; Andersen & Helder, 1987). These effects will cause oxygen tensions at their respiratory surfaces to fall below normal.

In addition, morphological differences between the limpets, particularly the position of the gills, may cause oxygen tensions at their gill surfaces to differ. Patellid limpets have a ring of external pallial gills which are irrigated by a flow of water that passes to the mantle cavity between the shell margin and the foot. Depression of the shell presumably isolates the mantle cavity and, consequently, will cause a decline in oxgen tension of the water. Both inundation and a simple weight depressing the shell significantly increased mortality of *P. granularis* (Fig. 2).

The gills of siphonariids are located internally and are irrigated by water drawn in through an inhalent siphon. Water flow through the siphon originates internally due to ciliary action and is not likely to be inhibited by depression of the shell which is shaped to house this structure. Should these limpets utilise interstitial water during burial and maintain a flow through the mantle cavity, a degree of oxygen replenishment of the mantle water will occur. However, the siphon can be closed by means of a valve in which event the gills would be isolated from the external water and the oxygen tension in the mantle cavity would decline as suggested for *P. granularis*.

Because sand inundation generally reduces availability of oxygen, a physiological tolerance of hypoxia would increase the capacity to survive under sand. Mortality of *P. granularis* in water at reduced oxygen tensions is significantly higher than that of *S. capensis* (Fig. 3), thus *P. granularis*, which is likely to experience a more severe reduction in oxygen availability during inundation, is also less tolerant of hypoxia.

In molluscs, the relationship between oxygen consumption and external tension is particularly influenced by the intrinsic state of the animal. This varies with animal size, activity, ration and external conditions such as temperature. Whereas some studies have shown larger individuals within a species to be better regulators (Bayne, 1971; Taylor & Brand, 1975; Shumway, 1981), others showed the contrary (Brand & Morris, 1984). The siphonariid limpets which constitute sand-inundated populations tend to be smaller than those on exposed rocks. However, for these experiments, we selected the modal size of limpets living on noninundated rocks which was similar for both species and facilitated comparison.

Various methods of representing data for the relationship between oxygen consumption and tension have been proposed by Tang (1933), Mangum & Van Winkle (1973) and Herreid (1980). We fitted a second-degree polynomial curve (Mangum & Van Winkle, 1973) to data of individual responses and also represented the data as standardised mean values. The B2 coefficient of the second-degree polynomial proved to be of little use in characterising respective regulatory abilities because of difficulties when the difference in initial (normal) oxygen-consumption rates between species is large (in prep.). Standardised data, however, showed that in declining oxygen tensions *S. capensis* displays greater respiratory independence than *P. granularis* (Fig. 4B) and partially regulates to ≈60 mm Hg.

Apart from an ability partially to oxyregulate, *S. capensis* is also capable of anaerobic respiration when exposed to anoxia. This was demonstrated by the accumulation in the foot of succinate (Table I), one of several end products of environmental anaerobiosis in molluscs (Gade, 1983). By contrast, after 11 h under anoxia, succinate was not detected in the tissues of *P. granularis*. Although Ellington (1983) has reported the occasional excretion of succinate in bivalves and Brinkhoff et al. (1983) its accumulation in *Patella vulgata* held under anoxia, its total absence indicates that this anaerobic pathway is not utilised by *P. granularis*. The failure of even one individual to survive 24 h of anoxia indicates that possible alternative anaerobic pathways are of little ecological significance.

In conclusion, we propose that habitat segregation of these two limpets is caused by reduced oxygen availability during sand inundation. The ability of *S. capensis* to tolerate hypoxia through partial regulation of oxygen consumption and the use of an anaerobic pathway enables it to extend its distribution to environments which are regularly inundated with sand. The lower tolerance of hypoxia of *P. granularis* restricts its local distribution by confining it to sand-free environments.

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Metabolic rate depression in a marine pulmonate snail: pre-adaptation for a terrestrial existence?

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Summary. Terrestrial and freshwater pulmonate snails exhibit a marked depression of aerobic metabolism during estivation. This is an adaptation for existence in periodically harsh environments and, though marine gastropods may undergo anaerobic metabolism, they have not been shown to adaptively depress aerobic metabolic rate. We compared the metabolic response to progressive aerial exposure of two intertidal gastropod limpets, a prosobranch and a pulmonate. The prosobranch *Patella granularis* maintained a constant heart rate until shortly before death. In contrast, the pulmonate *Siphonaria oculus* underwent facultative depression of heart rate, accompanied by a decline in oxygen consumption. Both heart rate and oxygen consumption returned to normal levels on reimmersion in water. Metabolic rate depression is energy conserving, and may account for the ability of *S. oculus* to extend higher up the shore than *P. granularis*, into areas where food availability is low. *S. oculus* is a primitive, marine pulmonate, periodically subject to harsh conditions, and its capacity for metabolic rate depression may represent a pre-adaptation for life on land.

Key words: Limpets – Intertidal – *Siphonaria* – *Patella* – Metabolic rate depression

Many terrestrial and freshwater pulmonate snails survive unfavourable environmental conditions (food and water deprivation, low temperatures) by their remarkable capacity for depression of metabolic rate (McMahon 1983; Barnhart and McMahon 1987; Storey and Storey 1990). Although a few marine pulmonate species exist, there are no reports of depression of aerobic metabolism by marine gastropods (Storey and Storey 1990). We compared the metabolic response to continual aerial exposure, of two intertidal limpets, a prosobranch and a pulmonate, and report that, while the prosobranch showed no metabolic control, the pulmonate was capable of facultative metabolic rate depression. Comparisons were made between two upper-shore limpet species of distant phylogenetic relationship, *Patella granularis* L. (Gastropoda : Prosobranchia) and *Siphonaria oculus* Krauss (Gastropoda : Pulmonata). Conveniently, they have similar shell shapes and sizes, and thus water relation properties (Lowell 1984).

Methods

Limpets (mean dry weight ± SD: 161 ± 38 mg; $n = 37$) collected from Port Alfred (33°36′S; 26°53′E), South Africa, were held under constant laboratory conditions of temperature (25 ± 0.5° C; $\bar{n}$ ± SD; typical diurnal air temperature in summer), salinity (35‰) and subdued illumination, for 5 days prior to experimentation. A tidal cycle of 12 : 12 h air : water, was maintained. Metabolic rate response was inferred from recordings of heart rate.

Heart beat was monitored by impedance pneumography (Trueman 1967), by coupling a transducer (Z135 Bioscience) and strain gauge (FC 135, Bioscience), with a twin-channel George Washington oscillograph (MD 400). To prepare limpets for experiments, two holes were drilled through the shell near the heart, and a platinum electrode (0.3 mm diameter) with connecting lead, was inserted into each hole. Electrodes were set in position using dental wax and epoxy glue. After a settling period of at least 12 h in seawater, limpets were exposed to air (25 ± 0.5° C; 80 ± 5% R.H.; $\bar{x}$ ± SD) for 72 h, during which aerial heart rate (f_h) was recorded at 12 h intervals. Individual heart rates (beats/min) were determined from recording traces. In the event of variability, a mean was derived from fifteen 1-min intervals.

Results and discussion

Long-term aerial exposure induced different heart rate responses in the limpet species (Fig. 1). The mean heart rate of *P. granularis* remained relatively constant (50 60 beats/min) for the duration of the exposure period. High mortality of this limpet was observed after 48 h aerial exposure and only a single individual survived 72 h in air. In a few cases heart rate fell below 40 beats/min, but this only occurred in individuals which died within the next

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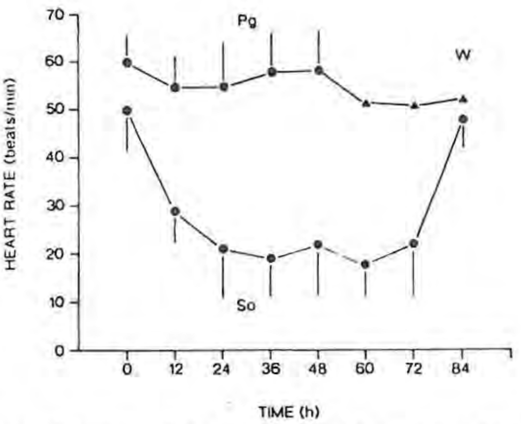

Fig. 1. Heart rate ($\bar{x} \pm 1$ SD) of *Patella granularis* (*Pg*) ($n = 8$–13, except for *triangles* where $n = 1$) and *Siphonaria oculus* (*So*) ($n = 12$), during continuous exposure in air (25 ± 0.5° C, 80 ± 5% R.H.), and following 12 h reimmersion in water (*W*)

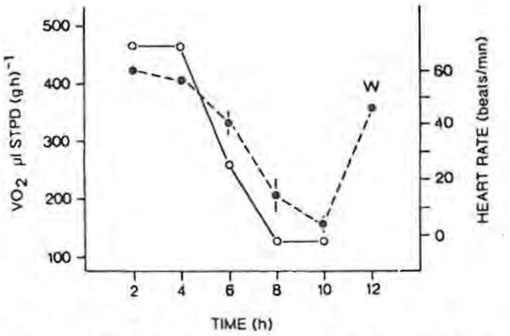

Fig. 2. Simultaneous measurements of heart rate *(closed circles)* and oxygen consumption ($\dot{V}O_2$ at 25° C; *open circles*) for an individual *S. oculus*, measured after intervals of exposure to a dry airstream (25° C), and following 2 h reimmersion in water (*W*). Heart rate is given as $\bar{x} \pm 1$ SD

12 h. Traces for *P. granularis* after long-term aerial exposure were characterized by beat to beat variability in amplitude and cardiac arrest (up to 1 min), characteristics indicative of stress. Periods of cardiac arrest and heart rates of dying limpets were not included in the analysis of the data. These results suggest that *P. granularis* depends on the maintenance of a relatively high metabolic rate. Lowered oxygen consumption rates following long-term aerial exposure (Marshall and McQuaid in prep.) must be a direct result of impairment of respiratory organ functioning, due to evaporative water loss.

Mean heart rate of *S. oculus* exposed to air declined rapidly (within 12 h) to 58% of the original rate, and then stabilized around 40% after 24 h. Heart rates of some individuals fell to below 10 beats/min (20% of original mean). No mortality was observed, and f_h returned to a level comparable to that recorded originally in air after 4 h of reimmersion. The relationship between aerial heart rate and oxygen consumption was confirmed by making simultaneous measurements (at 25° C) for four limpets exposed to a stream of silica-gel dried air. The oxygen consumption was measured with a Gilson Differential Respirometer (Gilson Medical Electronics). The respirometer flasks were modified to allow for limpet size and impedance connecting leads. Dry tissue weight-specific oxygen consumption ($\dot{V}O_2$) was standardised to STPD (Fig. 2).

In addition to the lowering of f_h and $\dot{V}O_2$, *S. oculus* exhibited behavioural responses (withdrawal of the mantle skirt, and closure of the pneumostome) during prolonged aerial exposure. Reversal of these behavioural responses and an increase of 50% in the lowered f_h resulted from physical disturbance or exposure to 100% humidity. We interpret these behavioural and physiological responses as representing a metabolic steady state well below "standard" metabolic rate. Environmental anaerobiosis by this limpet seems unlikely, as there is no evidence of the utilization of anaerobic pathways in intertidal gastropods during aerial exposure when oxygen availability is not reduced. Indeed, the absence of anaerobiosis in air-exposed gastropods has been demonstrated biochemically (Wieser 1980; Kooijman et al. 1982; Brinkhoff et al. 1983), and by direct calorimetry (Pamatmat 1978; Hammen 1980).

Species living in the upper intertidal zone are faced with physiological stress due to temperature variation and water loss. Inactivity during emersion reduces desiccation, but also limits the available time for feeding. Consequently, food shortages are often experienced, and energy conserving mechanisms are important (Branch et al. 1988). Energy of the uppermost South African patellid, *P. granularis*, is conserved by their low aerial metabolic rate and relative temperature independence (low Q_{10}), compared with the other *Patella* species (Branch and Newell 1978). Temperature relationships of *S. oculus* during normal metabolism are in many respects similar to those of *P. granularis*. However, *S. oculus* tolerates a higher level of desiccation and extends its distribution considerably higher up the shore than *P. granularis*. Temperature-independent metabolic rate depression during prolonged emersion will allow enhanced conservation of energy reserves by *S. oculus*.

Depression of aerobic metabolism by *S. oculus* in air can reach as low as 18% of the normal rate (Marshall and McQuaid in prep.) and is comparable to that of terrestrial and freshwater pulmonate snails deprived of food or water. These species depress their aerobic rate during estivation to 10–30% of that of resting, non-estivating snails (von Brand et al. 1957; Barnhart and McMahon 1987). This characteristic is important for the survival of pulmonate snails on land, particularly in dry regions, and for the ability of freshwater pulmonates to colonise temporary aquatic environments.

Although such physiological adjustments often have an ecological basis, the similarity of facultative aerobic depression in *S. oculus* to that seen in many families of freshwater and terrestrial pulmonates (Machin 1975; McMahon 1983; Riddle 1983), suggests that it is a phylogenetic character. *Siphonaria* is the oldest extant pulmonate genus, dating to the Devonian period (Morton 1955) and has mainly primitive features (Solem 1985). *Siphonaria* has therefore presumably evolved in the marine environment rather than re-adapted from a terrestrial or freshwater environment. Metabolic rate depression by this marine species may therefore indicate pre-adaptation for a terrestrial existence within primitive marine pulmonates.

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