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THE ROLE OF THE GILLS AND BRANCHIOSTEGITES IN GAS EXCHANGE IN A BIMODALLY BREATHING CRAB, *HOLTHUISANA TRANSVERSA*: EVIDENCE FOR A FACULTATIVE CHANGE IN THE DISTRIBUTION OF THE RESPIRATORY CIRCULATION

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SUMMARY

The respiratory circulation was investigated in air-breathing and water-breathing *Holthuisana transversa* von Martens by analysis of the distribution of radioactive microspheres injected into the haemocoel at seven locations. The gills and putative lungs (branchiostegites and membraneous thoracic walls) both trap approximately 90 % of the microspheres entrained in their afferent circulations. The main blood supply to the branchiostegites is from the venous sinuses and constitutes a substantial fraction of the total venous return, which is consistent with earlier inferences, based on morphological information, of their possible involvement in gas exchange. In air-breathing crabs, a mechanism exists which directs a greater proportion of the total venous return *via* the lungs. From the sinus at the base of walking leg 2, the ratio lung: gill flow was estimated as 86·9: 13·1 ± 5·7 % in hydrated crabs that had been air-breathing for more than 1 day, and 19·5: 80·5 ± 7·12 % in water-breathers. A factor in this circulatory switch may be an increase in branchial resistance in air caused by surface tension of water adherent to the gill lamellae. The direct arterial circulation to the gills represents about 3 % of cardiac output and is therefore an insignificant component of the total respiratory circulation. Patterns of microsphere distribution among different gills and different regions of the lung provide information on flow patterns within the thoracic sinus. Neither the thoracic sinus as a whole nor the infrabranchial sinuses can be considered as reservoirs of truly mixed venous blood in *H. transversa*.

INTRODUCTION

The crab *Holthuisana transversa* (Parathelphusoidea, Sundathelphusidae) may have to depend for extended periods on either aquatic or aerial respiration (Greenaway & MacMillen, 1978; MacMillen & Greenaway, 1978). Its mechanisms of gas

Key words: Land crab, gas exchange, respiratory circulation.

exchange appear to be truly bimodal. For example, the rates of oxygen consumption measured at rest and immediately after exercise are comparable in water-breathing and in air-breathing crabs (Greenaway, Bonaventura & Taylor, 1983*a*; Greenaway, Taylor & Bonaventura, 1983*b*). The mechanism of aquatic gas exchange is essentially similar to that of aquatic decapods (e.g. *Carcinus*; Hughes, Knights & Scammel, 1969), the scaphognathite being employed for ventilation of the gills. However, it has been suggested (Greenaway & Taylor, 1976; Taylor & Greenaway, 1979) that, in the aerial mode, gas exchange may take place in 'lungs' formed from the expanded prebranchial and epibranchial regions of the branchial chambers. This suggestion is supported by the following observations. Firstly, in air, the gills normally retain moisture between their lamellae and coalesce together against the postero-ventral walls of the chamber, presenting a relatively small area for gas exchange. Secondly, the anterior chamber is ventilated tidally by lateral oscillations of membraneous regions of the thoracic walls so that air is unlikely to move between the gills. Thirdly, the branchiostegites, or gill covers, which delimit this chamber externally appear to be highly vascular. Finally, the internal cuticle and epidermis of the branchiostegites are extremely attenuated, the blood/medium diffusion barrier being only 250–300 nm, less than one-twentieth of that in the gills.

A role in aerial gas exchange for the branchiostegites of amphibious and terrestrial crabs has been inferred by other authors (von Raben, 1934; Bliss, 1968; Storch & Welsch, 1975; Diaz & Rodriguez, 1977; Wood & Randall, 1981) and various structural adaptations, including apparently increased vascularity of this region, have been described. In a morphological study of crabs from several families, von Raben (1934) concluded that the branchiostegites were supplied with deoxygenated systemic blood from the thoracic sinuses and returned oxygenated blood to the pericardium. However, the relative quantitative importance of the branchiostegal and the usual branchial routes for venous return was not considered. Indeed, despite considerable interest recently in the physiological adjustments associated with aerial exposure in decapods (see Taylor, 1982 for review), the actual site of aerial gas exchange has received scant attention.

In an attempt to clarify the situation for *Holthuisana* we addressed three main questions.

(1) Do the branchiostegites in fact receive systemic blood from the thoracic sinus? The question is apposite since Vuillemin (1963) described a system of true arteries within the branchiostegites of another freshwater crab, *Bottia madagascariensis*.

(2) Is the venous perfusion of the branchiostegites of sufficient magnitude in relation to the total venous return for them to be important in overall gas exchange?

(3) Is there evidence for a change in distribution of venous return between appropriate gas exchange surfaces associated with breathing mode? Appreciable shunting of blood *via* an ineffective gas exchange route would be expected to affect adversely the overall gas exchange and transport.

The approach taken was to examine the fate of radioactive microspheres injected into the haemocoel at various locations. This technique has been applied to numerous circulatory studies in vertebrates (e.g. Wagner, Rhodes, Sasaki & Ryan, 1969; Hales, 1974). Quantification of the method is less satisfactory in the case of crustaceans. This is particularly so when applied to the venous system which is composed not of discre

vessels but of a complicated system of interconnected sinuses containing relatively slowly moving blood and with multiple afferent and efferent pathways. Nevertheless, unequivocal, affirmative answers to the above questions were obtained together with other semi-quantitative information on the functioning of the open haemocoelic blood system of a crab.

MATERIALS AND METHODS

Fifty specimens of *H. transversa*, 20–30 g, were collected from Bourke or Gulargambone, New South Wales and maintained in the laboratory in shallow water as described previously (Greenaway & MacMillen, 1978). Both sexes were included and experiments were carried out at 20–25 °C.

Radioactive microspheres labelled with ^{57}Co, ^{46}Sc or ^{113}Sn (New England Nuclear, 20 KBq, nominally 15 μm diameter, approximately 250 000 suspended in 10 μl saline containing 0·01 % Tween) were injected into the haemocoel at one of seven locations as follows. (A) Into the pericardial cavity *via* a small medial hole pre-drilled in the posterior cardiac region of the carapace. (B–F) Through the arthrodial membrane between the coxopodite and basipodite of a cheliped or one of the four walking legs on one side only. The tip of the syringe was positioned within a sinus communicating with the leg sinus and located ventrally between the endosternites of the adjacent somite. (G) Into venous sinuses associated with the digestive gland *via* a small hole pre-drilled in the protogastric region of the dorsal carapace.

Crabs referred to as water-breathing were removed from the essentially aquatic culture conditions and totally submerged in aerated water for at least 1 day. All air was expelled from the branchial chambers initially by holding the crabs with their exhalant apertures upwards for a short time. Bilateral activity of the scaphognathites was confirmed visually just before injection of microspheres into the crabs which were held in a clamp under water. Air-breathing crabs were removed from water at varying times prior to injection, blotted dry externally and maintained in humid conditions to prevent dehydration. Tidal ventilation was confirmed by visual inspection in crabs supported in air just before injection. In both air- and water-breathers the legs and body were gently stimulated to promote a moderate level of activity during and after the injection. In order to minimize sedimentation in the syringe, a relatively wide-bore gas-tight syringe was used (total capacity 100 μl) and this was rotated continuously prior to the injection. After injection 1–2 μl of haemolymph were aspirated into the syringe and re-injected to improve mixing. After allowing 5–10 min for entrapment in vascular beds the distribution of injected microspheres was determined by gamma scintillation counting. Longer circulation times (35 min) did not result in any obvious change in distribution. Samples of haemolymph (300–500 μl) taken from the pericardium and ventral sinus 2–3 min after injection contained no detectable microspheres. The radioactivity of the suspending medium was negligible.

Each animal was rapidly dissected into more than 30 tissue samples which were placed in separate gamma counting vials. Each of the fourteen gills was counted individually. The branchiostegites were cut off along their medial junction with the thoracic carapace and then divided into dorsal and ventral halves along the ridge at their lateral border and each further divided into anterior and posterior sections. The

thin flexible regions of the thoracic integument within the branchial chambers which are involved in tidal ventilation (Taylor & Greenaway, 1979) were carefully stripped off after separation from attached muscles and also considered as lung samples.

The chelipeds were counted individually. Usually the four walking legs on each side were pooled, but in some individuals single limbs were counted. Digestive gland, gonads and foregut were counted separately. In many cases blood samples were taken before dissection as noted above, and in some animals individual somites of the endophragmal skeleton including, and adjacent to, the injection site, were taken in order to assess the efficiency of entrainment of microspheres in the venous flow. In each case the entire animal was counted, including the paper tissue on which the animal was dissected, in order to determine the total injected load.

Counting was performed using a Packard automatic gamma counter or manually using an ORTEC well counting system. Counting statistics allowed a precision of $<0{\cdot}1$ % of total injected load for all samples and a detection limit of $<0{\cdot}001$ % of total injected load in the lowest activity samples. Errors due to sample geometry and self absorption were negligible.

The anatomy of the venous system was investigated in crabs injected with the latex compound, Microfil (Canton Biomedical Products, Inc.) injected at a limb base and cleared in glycerol.

Terminology referring to the areas of the carapace is taken from Chace & Hobbs (1969) and other anatomical terms are generally after Pearson (1908).

RESULTS

General organization of the circulation in Holthuisana

Although a full description of the circulation of *H. transversa* is beyond the scope of this paper, it is briefly summarized here as there are important differences from the scheme reported for other crabs.

The main circulatory routes within *H. transversa* are illustrated schematically in Fig. 1. The circulation common to all aquatic decapods (e.g. Pearson, 1908; Pike, 1947; Maynard, 1960; Blatchford, 1971) is shown on the right. After leaving the heart, blood enters the arterial system which supplies the musculature and all of the principal organs. The finest arterial branches of crabs are of the order of 2–10 μm in diameter (Sandeman, 1967, *Carcinus*; Taylor & Greenaway, 1979, *Holthuisana*) and would thus be expected readily to trap the 15 μm diameter microspheres used in this study. Blood then passes through a series of narrow capillaries intimately associated with the individual tissues, and these lead into larger haemocoelic lacunae. Deoxygenated blood then drains into a system of sinuses within the thorax.

The intercommunication of the whole thoracic sinus system was observed in the Microfil-injected crabs. These preparations also demonstrated the infrabranchial sinuses which run along each side of the thorax at the base of the gills and interconnect the afferent branchial vessels. In *H. transversa* these sinuses are not large, the connections quite narrow and, as discussed below, antero-posterior mixing within them is probably incomplete.

In another brachyuran crab, *Cancer pagurus*, Pearson (1908) described a set of 'branchial sinuses' which run down the pleural muscle chambers in each thorac

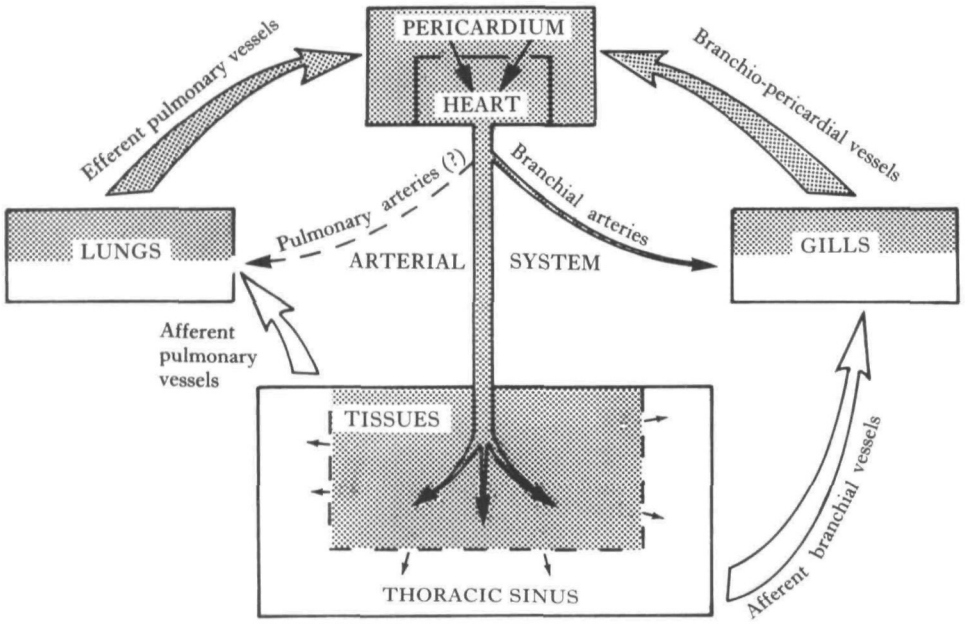


Fig. 1. Schematic diagram of circulatory routes in the bimodally breathing crab, *Holthuisana transversa*. Stippled regions contain oxygenated blood. See text for description.

somite and connect the main sternal sinus with the infrabranchial sinus on each side. In *H. transversa* such connections probably exist also but, in addition, there are sinuses which lie close to the ventral surface in each somite and lead upwards into the infrabranchial sinus as described by Pike (1947) for *Galathea*. These also communicate with the leg sinuses and it is into these channels that injections were made in the present study.

The left-hand circuit of Fig. 1 represents an alternative route for venous return through the branchiostegites which is also a potential respiratory pathway in *Holthuisana*. The afferent venous circulation to the branchiostegites as seen in Microfil-injected crabs consists of a series of more or less parallel channels extending directly from dorsal thoracic sinuses into the anterodorsal (epibranchial and hepatic) areas of the branchiostegite and a series of roughly radial channels extending into the antero-ventral (pterygostomial) region of the branchiostegites from a sinus below the orbit (Fig. 2). A distinct afferent pulmonary vessel (Kiemendeckelarterie), as described in several other families of terrestrial crabs by von Raben (1934), was not observed in *Holthuisana*. When viewed from the internal surface of the branchiostegite, each of the main channels is seen to be the trunk of a delicate arborization close to the internal integument. A similar set of efferent channels, in the postero-ventral half of each branchiostegite, collects into a clearly defined efferent pulmonary vessel (Kiemendeckelvene) which runs around the posterior margin of the branchiostegite on each side and leads directly into the pericardial sinus.

In addition to the afferent branchial sinuses which supply the gills with venous blood, each gill receives blood directly from the heart *via* a pair of true arteries (hypobranchial and epibranchial) which arborize within each lamella (Taylor &

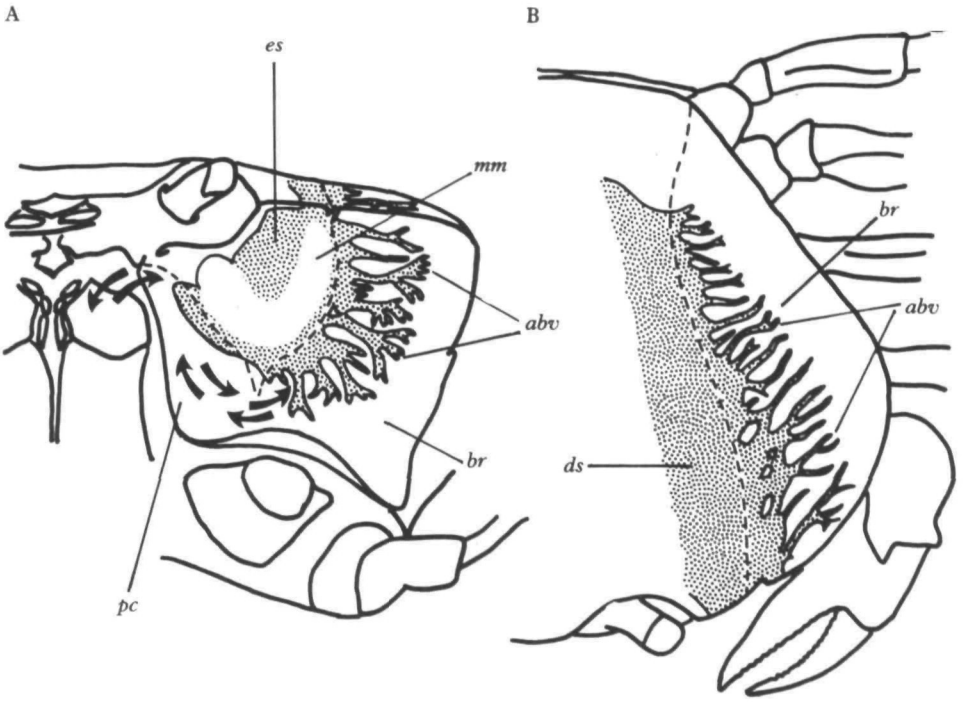


Fig. 2. (A), Antero-ventral and (B) dorsal aspects of *Holthuisana transversa*. L.H. branchiostegite (*br*) is attached to thoracic wall along dashed line which also delimits the inner boundary of the air- or water-filled branchial chamber. Along this line, Microfil (stippled), initially injected at the base of walking leg 2, passes out of the dorsal sinuses (*es*, *ds*) (complex deeper structure not shown) into afferent branchiostegal vessels (*abv*) interposed between the inner and outer integuments of the branchiostegite. Radioactive microspheres injected at the same point were also concentrated in the same regions, suggesting that the branchiostegites are supplied with deoxygenated, systemic haemolymph. Clear zone (*mm*) in eye sinus (*es*) is area of attachment to cuticle of mandibular muscles. Arrows indicate direction of tidal air flow and exhalant water flow through prebranchial chamber (*pc*) which encloses the scaphognathite.

Greenaway, 1979). Although not generally mentioned, a similar system is certainly present in some other crabs (see Taylor & Greenaway, 1979 for references). Thus its relative contribution to the respiratory circulation must be considered. A direct arterial supply to the branchiostegites may also be present. Vuillemin (1963) described a system of true pulmonary arteries in the potomid crab *Bottia madagascariensis*. As yet we have not verified its existence in *Holthuisana* anatomically although our data below indicate that, if present, it is not an important component of the respiratory circulation.

Preliminary considerations of microsphere distribution

In Table 1 are shown the detailed final distributions of radioactive microspheres initially injected into the infrabranchial sinus at the base of the walking leg 2 for six water-breathing (>2 weeks) and seven air-breathing (>5 h) crabs. Radioactivities of individual tissues are expressed as percentages of the total injected load.

The largest proportion of microspheres is found in the compartment designated 'carcase', comprising the internal and external skeleton of the cephalothorax and all

Table 1. *Distribution of radioactive microspheres in various tissues (percentage of injected load)*

TISSUE	Injection into venous sinus at base second R.H. walking leg		Pericardial injection
	Water-breathing ($N = 6$)	Air-breathing ($N = 7$)	($N = 8$; 4 air + 4 water)
	$\bar{x}$ S.E.	$\bar{x}$ S.E.	$\bar{x}$ S.E.
Gills (R.H.) 1	0·42 ± 0·22	0·53 ± 0·22	0·03 ± 0·01
2	4·18 ± 1·37	1·44 ± 0·81	0·23 ± 0·09
3	2·53 ± 0·94	2·83 ± 1·31	0·31 ± 0·10
4	7·52 ± 2·90	1·04 ± 0·42	0·34 ± 0·11
5	6·70 ± 1·80	0·59 ± 0·21	0·35 ± 0·15
6	4·83 ± 1·58	0·42 ± 0·19	0·40 ± 0·16
7	0·15 ± 0·08	0·05 ± 0·04	0·16 ± 0·07
(L.H.) 1	0·06 ± 0·04	0·00 ± 0·00	0·05 ± 0·02
2	0·30 ± 0·21	0·02 ± 0·00	0·10 ± 0·05
3	0·05 ± 0·02	0·01 ± 0·00	0·21 ± 0·09
4	0·03 ± 0·01	0·01 ± 0·00	0·26 ± 0·11
5	0·02 ± 0·00	0·01 ± 0·00	0·34 ± 0·19
6	0·01 ± 0·01	0·01 ± 0·00	0·35 ± 0·20
7	0·01 ± 0·00	0·01 ± 0·00	0·21 ± 0·11
TOTAL GILLS	26·80 %	6·95 %	3·38 %
Branchiostegite (R.H.) AD	1·84 ± 1·61	2·69 ± 0·77	0·14 ± 0·04
AV	3·62 ± 1·67	6·27 ± 1·69	0·57 ± 0·23
PD	0·07 ± 0·02	0·28 ± 0·08	0·30 ± 0·18
PV	0·04 ± 0·02	0·05 ± 0·02	0·39 ± 0·09
(L.H.) AD	0·15 ± 0·08	2·63 ± 0·96	0·20 ± 0·07
AV	0·60 ± 0·32	3·55 ± 1·29	0·44 ± 0·13
PD	0·03 ± 0·01	1·32 ± 0·86	0·32 ± 0·13
PV	0·03 ± 0·01	0·04 ± 0·02	0·31 ± 0·09
Thoracic wall	0·29 ± 0·10	1·47 ± 0·37	0·47 ± 0·16
TOTAL LUNGS	6·67 %	18·29 %	3·14 %
Cheliped (R.H.)	0·20 ± 0·05	0·08 ± 0·01	5·31 ± 1·29
(L.H.)	0·25 ± 0·08	0·07 ± 0·02	6·97 ± 2·31
Walking legs (R.H.)	0·35 ± 0·15	0·19 ± 0·10	5·64 ± 1·17
(L.H.)	0·27 ± 0·08	0·09 ± 0·02	6·27 ± 1·23
TOTAL LIMBS	1·06 %	0.41 %	24·18 %
Abdomen	0·18 ± 0·04	0·13 ± 0·04	3·13 ± 0·59
Digestive gland	1·03 ± 0·43	3·11 ± 1·56	8·05 ± 3·31
Foregut	0·14 ± 0·06	1·05 ± 0·78	1·18 ± 0·25
Carcase	64·09 ± 5·18	70·98 ± 3·16	57·05 ± 6·72
TOTAL BODY	65·44 %	75·27 %	69·42 %
GRAND TOTAL	99·98 %	100·92 %	100·13 %

the internal musculature which remained after removal of the other tissues and appendages. In some crabs this compartment was further divided for counting and it was shown that these microspheres were not widely distributed but almost entirely resided within the musculature and endophragmal skeleton within a few millimetres of the injection site. It was concluded that these microspheres were not effectively entrained the venous flow and settled out more or less immediately. Attempts to improve the

proportion entrained by including dextrose in the suspension medium and by more expeditious injection technique were unsuccessful. This was no doubt partly an unavoidable consequence of the injection of a small volume of a suspension into a small irregular sinus containing slowly moving blood. However, these difficulties may also be related to particular features of the venous circulation of *Holthuisana*, e.g. the proposed requirement for a proportion of the venous circulation to move dorsally through the thoracic sinus system to the branchiostegites. It is noteworthy that much higher entrainments (only 2–20% remaining in the carcase) were obtained using essentially the same technique in the crab, *Hemigrapsus crenulatus* and the crayfish, *Paranephrops zealandicus*, two species in which the branchiostegal circulation appears to be less important in gas exchange (H. H. Taylor, unpublished observations).

Nevertheless, Table 1 shows that about one-quarter to one-third of the microspheres injected at the base of walking leg 2 were entrained into the venous circulation and passed to the gills (mainly on the injection side) and also to the branchiostegites (both sides). This confirms that both the gills and the putative lungs (including also the membraneous thoracic walls) receive deoxygenated systemic blood and could potentially function in gas exchange.

The distribution of microspheres within the respiratory organs should also reflect the relative venous blood flow to these regions from this particular injection site. The reciprocal differences in gill/lung microsphere distributions observed in comparisons of water-breathing with air-breathing crabs in Table 1 suggest the existence of mechanisms which increased the relative venous blood supply to gills in water and increased the relative lung supply in air.

These conclusions are valid providing that both lungs and gills retain a large fraction of the microspheres which pass to them on the first circuit. Table 1 also shows the proportions of microspheres which are trapped in the same regions of the body after injection into the pericardium of eight crabs. Data from air-breathing and water-breathing crabs were pooled. In this case, large numbers did not remain at the injection site and the high percentage in the carcase results from their widespread distribution. This distribution should be proportional to the partitioning of cardiac output. Thus the mean proportion of the cardiac output directed to the limbs was 24·18%. This estimate seems reasonable in view of their relatively high muscle content and the high activity levels which were maintained during and after injection.

The microsphere count in the limbs after venous injection may be used to assess the trapping efficiency of the respiratory organs since microspheres which are not trapped by the gills or lungs enter the pericardium and should be distributed identically to those injected directly into the pericardium. Thus the fraction retained by the respiratory organs

$$F = \frac{\text{total trapped by respiratory organs}}{\text{total entrained microspheres}} = \frac{G + P}{G + P + Lv/La}$$

where G, P and Lv are the numbers (or % load) found in the gills, lungs and limbs respectively after venous injection and La is the fraction trapped within the limbs after pericardial injection. Using the data in Table 1, F has a mean value of 0·884 for water-breathing and 0·937 for air-breathing crabs injected at walking leg 2. The overall mean value of F for all crabs injected into the venous system was 0·879 ± 0·016 (S.E.

(n = 44). There was no evidence that the trapping efficiencies of the gills and lungs were substantially different (determined from a comparison of F values in all crabs in which more than 50 % of the total microspheres trapped in the respiratory organs were trapped in the lungs with those in which more were found in the gills). In fact for a majority of crabs, F was >0·95 and the mean is biased by a small number which had very high limb counts. It is possible that such high counts were caused by refluxing of microspheres back through the venous system, since in cases where individual limbs were counted, such crabs usually showed higher count rates in limbs adjacent to the injection site.

After pericardial injection, about 3 % of the microspheres were found in the gills and a similar proportion in the lungs (Table 1). Therefore, after venous injection, the numbers of microspheres which settled in the respiratory organs after multiple circuits must have been negligible.

From the above considerations we conclude that an estimate of the proportion of the respiratory circulation which passes from a particular venous injection site through the lungs is provided by expressing the total microsphere count in all of the lung samples as a percentage of the overall total in all of the respiratory organs. This is the form in which the data are presented in Tables 2 and 3. The remaining percentage, of course, represents the branchial component of the respiratory flow from this site.

Another potential source of error in these estimates is rather more difficult to assess and quantify. This results from the possible settling out of microspheres on the walls of vessels or sinuses between the point of injection and the respiratory organs. Such effects would tend to underestimate lung flow relative to gill flow from a ventral injection site and to underestimate relative gill flow from a dorsal injection site. Possibly, this accounts for the higher numbers of microspheres observed in the carcase, digestive gland and foregut fractions of air-breathing crabs (Table 1). However, although such effects could introduce distortions of the calculated partitioning of the respiratory circulations that are presented below, the major conclusions, based on differences between air-breathing and water-breathing crabs, are still valid.

Arterial routes in the respiratory circulation

The recovery of microspheres, initially injected into the pericardium, from the gills and lungs (Table 1) implies a direct arterial supply to those regions. Such arteries have been described in the gills of *H. transversa* by Taylor & Greenaway (1979) but have not yet been observed in the lungs. It is also possible that some of the microspheres may have arrived in the respiratory organs *via* the venous route after escape from the arterial system. In any event, it is clear that in *H. transversa* the arteries of the gills and lungs do not contribute significantly to the respiratory flow, since at maximum they represent a total of 6·4 % of the cardiac output.

Relative venous flow to gills and lungs from a mid-ventral sinus during water and air breathing

In Table 2 the numbers of microspheres retained by the lungs following injection into a venous sinus at the base of the second walking leg are expressed as a percentage of the total recovered in the gills plus lungs. As discussed above, this should be

Table 2. *Numbers of microspheres trapped in lungs (as % total in gills plus lungs) after injection at base of second walking leg*

	% in lungs	Mean S.E.
Water-breathing (>2 weeks)	3·15	
(>2 weeks)	8·04	
(>2 weeks)	10·88	19·52 ± 7·12
(>2 weeks)	16·98	
(>2 weeks)	27·30	
(>2 weeks)	50·81	
Air-breathing (<3 min)	12·16	29·16
(<3 min)	44·41	
(5 h)	41·56	48·06
(5 h)	54·56	
(1–10 days)	72·48	
(1–10 days)	73·90	
(1–10 days)	92.95	86·93 ± 5·68
(1–10 days)	97·15	
(1–10 days)	98·17	

approximately equal to the proportion of venous blood returning *via* the lungs from this injection site. Data for the thirteen animals represented in Table 1 are shown individually. Two more animals are included which were injected as soon as possible after removal from water and visual inspection had confirmed that tidal aerial ventilation had begun.

It is evident that while crabs breathing either medium may simultaneously direct a proportion of the blood *from this leg-base via* both respiratory routes, crabs breathing water are capable of directing almost the entire flow *via* the gills and air breathers are capable of directing almost the entire flow *from this site via* the lungs. The difference in mean percentage lung flow between crabs breathing water (19·52) and crabs breathing air (86·93) for more than one day is highly significant ($P < 0{\cdot}001$, t-test using arcsine transformation, Sokal & Rohlf, 1969). Crabs air-breathing for a few minutes or hours showed intermediate patterns, indicating that the circulatory switch is not an immediate response to emersion. In addition, as all air-breathers were observed to be ventilating tidally and scaphognathite activity was observed in all water-breathers, it is obvious that the circulatory pattern cannot be linked specifically to the ventilation mode.

Venous return from other locations in the thoracic sinus system

Table 3 shows the results of similar comparisons between water-breathing and air-breathing (>1 day) crabs after venous injections at the base of each of the five limbs and through the carapace. The patterns of venous return from different locations are obviously rather different. Blood returning from the neighbourhood of the cheliped-base had a similar distribution to that from the second walking leg. However, most of that from walking leg 1 passed to the gills in both breathing modes and most from walking leg 4 and the dorsal site passed to the lungs in both modes. Importantly, it can be seen that in each of the six comparisons the estimated mean lung flow is higher in the air-breathing crabs than in the water-breathers. Without regard to the magnitude of th

Table 3. *Numbers of microspheres trapped in lungs (as % total in gills plus lungs) after injection into venous sinus at various points in crabs breathing water or air*

Injection site	Breathing mode*	% in lungs		
		Mean	S.E.	*N*
Cheliped	W	24·08	9·91	3
	A	89·77	5·31	3
Walking leg 1	W	6·97	4·73	3
	A	26·30	2·85	3
Walking leg 2	W	19·52	7·12	6
	A	86·93	5·68	5
Walking leg 3	W	40·16	–	1
	A	68·15	–	2
Walking leg 4	W	73·31	19·32	3
	A	73·43	11·48	3
Dorsal	W	98·38	0·89	3
	A	99·44	0·42	3

* W, water-breathing, >2 weeks; A, air-breathing, >1 day (hydrated animals).

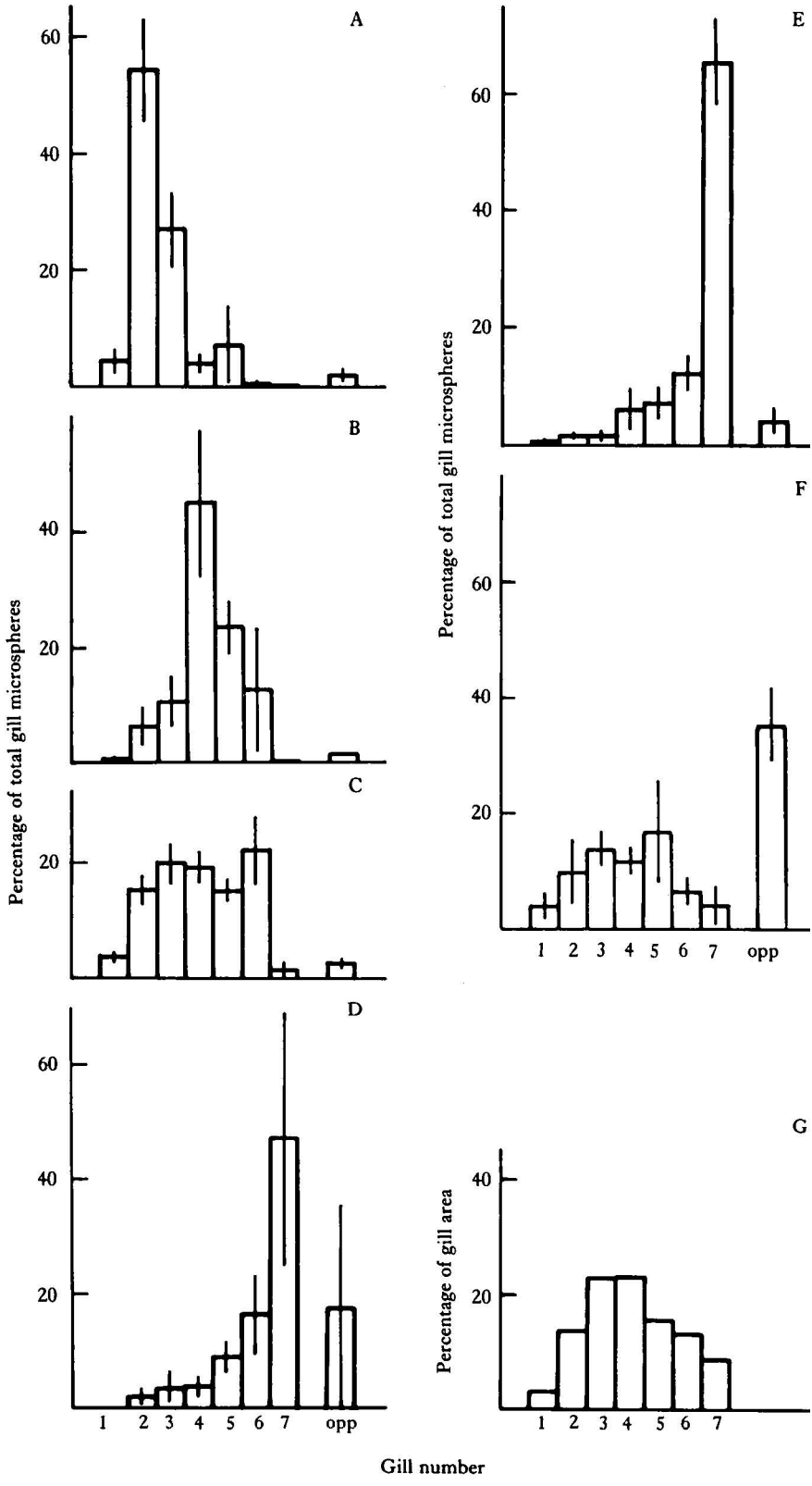
individual changes, such a result is statistically significant ($P = 0{\cdot}016$, one-tailed Wilcoxon's signed ranks test, Sokal & Rohlf, 1969) and suggests a general increase in the relative blood flow towards the lungs from most regions of the thoracic sinus in air-breathing crabs.

There is no accurate way in which the information from such separate measurements can be weighted or integrated in order to estimate the distribution of total venous return between the alternative routes since it is not known what proportion of the total flow each injection locus samples. Also, it is quite possible that this proportion is different between the two breathing modes. *A priori*, one would expect that the mainstream of venous return would flow in the neighbourhood of the anterior limb bases. This region is central, drains blood from the largest muscle masses and, as shown below, supplies blood to the largest gills. Thus, the large changes in distribution of respiratory microspheres initially injected at the base of the cheliped and the first and second walking legs probably reflect quite large redistributions of the total venous return.

Although almost the entire venous return from the dorsal injection site appears to be routed *via* the lungs it should be realized that in terms of the relative gill flow the values in Table 3 suggest a three-fold increase in the water-breathing animals. Also it is likely that this flow is somewhat underestimated because of the longer and more tortuous route to the gills.

Distribution of venous return among individual gills and regions of the lung from different locations in the thoracic sinus

In Fig. 3 the mean microsphere count in individual gills, expressed as a percentage of the total gill microspheres, is shown for each group of crabs injected at a particular venous location. It is probable that this reflects the partitioning of venous return from


A
B
C
D
E
F
G
60
40
20
Percentage of total gill microspheres
Percentage of gill area
1 2 3 4 5 6 7 opp
Gill number

ch location among the different gills. This graph also shows the percentage contributions of each gill pair to total gill area (from Greenaway, 1984), which, on general grounds one would expect to be approximately equal to the percentage of total branchial flow within each gill pair. The results for air-breathing and water-breathing crabs were pooled, because their patterns were essentially similar despite the differences in total branchial flow inferred above.

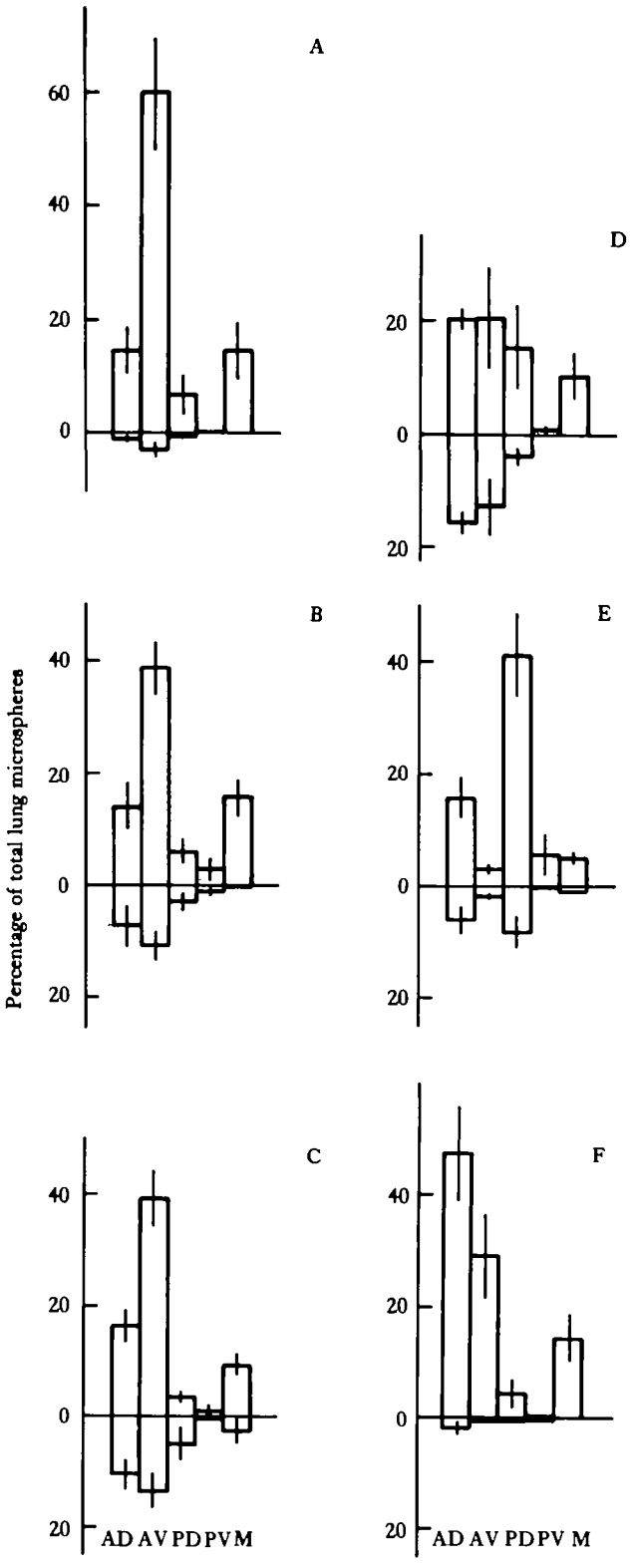
The individual patterns from the six locations are all different and do not accurately reflect gill area. In general, microspheres injected ventrally were found in one or two gills close to the injection site on the same side, although small numbers were found in all gills on this side and sometimes on the opposite side. Thus, almost all of the microspheres injected at the base of the cheliped were recovered in gills two and three. The relatively small fraction of these microspheres recovered in the first gill is not surprising in view of the small size of this demibranch pair (about 3 % total area). However, it is interesting that gill seven, which represents only 8·7 % of the total area on each side, apparently receives about 47 % of the branchial flow from the neighbourhood of the base of the third walking leg and about 65 % from the fourth. In the case of walking leg 2, averaging has tended to obscure the highly localized distribution seen in individuals. In all 24 of these animals, 53–96 % of all branchial microspheres were located within only two gills, although the peak count was observed in gills numbered two to six in different crabs.

Microspheres from the dorsal injection site were trapped in all seven pairs of gills, their proportions roughly reflecting gill areas (although as noted earlier the total branchial flow in air and water is very low from this site). Compared with the ventral injections, a much larger proportion of gill microspheres (mean 36 %) was found on the contralateral side, confirming a functional continuity laterally in the thoracic sinus.

Fig. 4 shows the patterns of distribution of microspheres among the five regions of the lung on each side, in the same six groups of crabs. The quadrants of each branchiostegite and the sections of thoracic wall were all roughly similar in area (although the latter are stretched to about double this area during the expiratory phase of aerial ventilation). In most cases the largest numbers of microspheres were detected in the anterior regions of the branchiostegites, particularly ventrally. This is in accord with the observations on the afferent pathways to the lungs in Microfil-injected crabs (Fig. 2). It is interesting that microspheres injected at the base of the most posterior walking leg were directed in largest numbers towards the postero-dorsal quadrant of the branchiostegite, suggesting that blood from this neighbourhood may take a different route through the thoracic sinus system. In this context, it should be recalled that partitioning of venous return from this site appears to be strongly in favour of the lungs in both air- and water-breathers (Table 3).

Fig. 4 also demonstrates that microspheres injected laterally at the base of a limb are often detected in large numbers in the lungs on both sides. This was most

Fig. 3. (A–F) Patterns of distribution of microspheres among different gills after initial injection into the thoracic sinus at various locations. Expressed as percentage of total gill microspheres. Air- and water-breathers pooled. 1–7, gill number from anterior on injection side; opp, all gills on opposite side. Injection at: (A) base cheliped; (B) base walking leg 1; (C) base walking leg 2; (D) base walking leg 3; (E) base walking leg 4; (F) dorsal carapace; (G) percentage of total gill area of each gill pair from Greenaway (1984).


A
B
C
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60
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0
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0
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40
20
0
20
40
20
0
20
Percentage of total lung microspheres
AD AV PD PV M
AD AV PD PV M
Region of lung

frequently observed in crabs injected *via* walking legs 1 to 3 and indeed in four of these individuals the microsphere count was higher on the contralateral side than on the injection side. Interestingly, the pattern of distribution on the contralateral side mirrored that on the injection side, even in the case of walking leg 4.

In the case of injections at the dorsal site and also at the base of the cheliped, very few microspheres were detected in the contralateral lung.

Depending on the site of injection, 6–16 % of the pulmonary microspheres were located within the extensible thoracic wall of the branchial chamber.

DISCUSSION

The experiments reported here demonstrate that the bimodally breathing crab, *Holthuisana transversa*, possesses dual routes for the return of venous blood to the heart. Thus, deoxygenated haemolymph within the thoracic sinus system may return either *via* the gills, as in primarily aquatic crabs, or *via* vascular beds within the branchiostegites. Both sites must therefore be considered as capable of contributing significantly towards the overall gas exchange in the animal. Although the total area of the branchiostegites is only about 10 % of the lamellar area of the gills (Greenaway, 1984), in terms of diffusive conductance this would be more than compensated by the extremely thin internal cuticle and epidermis of the branchiostegites (total thickness 250–300 nm, compared with 5–8 μm for the gills; Taylor & Greenaway, 1979). Greenaway's (1984) estimates of lung area did not include the membranous regions of the medial walls of the branchial chambers interposed between the epimera and the branchiostegites. These extensible membranes are provided with muscle attachments internally and by alternate expansion into and retraction from the epibranchial spaces, they are responsible for tidal aerial ventilation (Greenaway & Taylor, 1976). As 6–16 % of the lung microspheres recovered after venous injection were located within these membranes and, depending on the phase of the ventilation cycle, they may have an area up to one-half that of the branchiostegites, it is possible that they also contribute to aerial gas exchange. However, further morphological information on vasculature and gas diffusion distances is required to clarify this.

It has also been shown that the relative venous flow to the lungs and gills changes, depending upon whether the animal is breathing air or water. To date, studies of crustacean circulatory control (reviewed by Taylor, 1982) have mainly concentrated on the regulation of heart rate and cardiac output. Thus this appears to be the first demonstration in any crustacean of an apparent regulation of the distribution of the peripheral circulation either arterial or venous.

It is clear that in most of the animals the switchover was not complete, some blood passing through both routes in both media and with certain venous sinuses supplying

Fig. 4. Patterns of distribution of microspheres among different regions of the lung after initial injection into the thoracic sinus at various locations. Expressed as percentage total in all regions of lung. Air- and water-breathers pooled. Upper half of graphs, regions of lung on injection side; lower, opposite side. AD, antero-dorsal quadrant of branchiostegite; AV, antero-ventral; PD, postero-dorsal; PV, postero-ventral; M, membraneous regions of thoracic wall in branchial chambers. Injection at: (A) base cheliped; (B) base walking leg 1; (C) base walking leg 2; (D) base walking leg 3; (E) base walking leg 4; (F) dorsal carapace.

blood preferentially to the lungs or to the gills irrespective of breathing mode. This is consistent with the expectation that both gills and lungs would retain at least a limited capacity for gas exchange in either medium.

During aquatic ventilation, water entering the branchial chambers at the leg bases passes first over the gills, and these are therefore likely to be the main sites of aquatic gas exchange. However, water leaving the gill then passes through the epibranchial chambers and it is likely that the thin inner integument of the branchiostegites and medial walls would also function in aquatic gas exchange. Despite relatively reduced gill area (Greenaway, 1984), *H. transversa* is certainly competent in aquatic gas exchange and has a mean oxygen extraction efficiency of 46% in normoxic water (Greenaway *et al.* 1983*a*). Just as the lungs may contribute towards aquatic gas exchange, it is also possible that the gills may provide a component of aerial gas exchange. However, the gills are not structurally adapted for aerial gas exchange and trap water between their lamellae on emergence from water. Thus aerial gas exchange is effectively limited to the relatively small area of the exposed afferent branchial vessels and marginal canals of the lamellae. As the integumental diffusion barrier in the gills is more than an order of magnitude greater than that of the branchiostegites, the total diffusional conductance of the gills in air must be very much less than that of the branchiostegites. The reduction in relative blood flow through the gills observed in air-breathing crabs would be advantageous since venous return through an ineffective gas exchanger would reduce both the oxygen content of arterial blood and the total oxygen store and also waste energy in unnecessary cardiac output.

On exposure of aquatic crabs to air, the activity of the scaphognathites continues in an intermittent fashion for a minute or so until the branchial chambers are cleared of water. Tidal ventilatory movements commence almost immediately and assist in the draining of the chambers. It is not known what neural mechanisms are responsible for switching these quite separate oscillatory activities. Conceivably, stimulation by water of mechanoreceptors on the scaphognathite, gills or branchial walls could reflexly promote aquatic ventilation and inhibit aerial ventilation. However it is also possible that a distinct pattern of blood gas tensions is important in determining whether the animal maintains aquatic or aerial ventilation. In this respect it should be noted that in air Pa_{O_2} and Pa_{CO_2} are normally both high, whereas in water they are low (Greenaway *et al.* 1983*a*,*b*). Support for a chemical component in these switches is provided by the observation that when air-breathing animals were exposed to extreme hypoxia by gassing with pure N_2, the normal inhibition of aquatic ventilation appeared to be removed and scaphognathite activity reappeared simultaneously with tidal ventilation (Greenaway *et al.* 1983*b*). Whatever the basis for these switches in ventilatory mode, it appears that separate rate-controlling mechanisms exist for the two oscillatory activities. Thus aquatic ventilation is primarily O_2-sensitive, whereas aerial ventilation is primarily CO_2-sensitive (Greenaway *et al.* 1983*a*,*b*).

The circulatory changes reported here do not appear to be specifically linked to either of the ventilatory mechanisms, as the time course for the full development of the air-breathing pattern is of the order of many hours rather than minutes. Without ruling out the possibility of more complex control mechanisms, a simple physical explanation for the redistribution of venous return is that the surface tension of water adhering to the gills after emersion slowly compresses the gills as they drain and d

and this increases the overall branchial resistance, diverting more blood *via* the pulmonary route. In other crabs, branchial resistance is certainly sensitive to small changes in branchial pressure caused by ventilatory reversals (Blatchford, 1971; Taylor, 1982). Indeed, similar increases in general branchial pressure probably accompany cessation of scaphognathite activity in air in *H. transversa*. However, as already mentioned, the time course of this latter change is such that it cannot be primarily responsible for the changes in branchial perfusion. In addition it might be expected that similar changes in resistance would be produced in the pulmonary route. Temporal considerations also mean that alternating pressures within the thoracic haemocoel, associated with tidal breathing, cannot be primarily responsible for the circulatory redistribution, although their possible role in potentiating the upward movement of blood through the complex thoracic sinus system cannot be ignored.

The branchiostegites of terrestrial and semiterrestrial crabs from several other families are also highly vascularized and these observations, together with the morphological specializations of the inner surface which appear adapted to facilitate gas exchange, have prompted several authors to suggest that these structures may function as lungs (Harms, 1932; von Raben, 1934; Bliss, 1968; Storch & Welsch, 1975; Diaz & Rodriguez, 1977; Wood & Randall, 1981). The anomuran, *Birgus*, is able to maintain gas exchange in air even when the gills are removed (Harms, 1932), which strongly implies a respiratory role for the highly modified walls of the branchial cavity. For other air-breathing brachyurans, the relative importance of the putative lungs and the gills is difficult to estimate. An important role for the gills of most other terrestrial crabs is implied by the continued activity of the scaphognathite in moving air across the gills and movements of the flabellae which mix the water surrounding the gills in *Cardisoma carnifex* (Wood & Randall, 1981). However, the crucial information on branchiostegal blood flow is completely lacking.

The branchiostegal route for venous return from the thoracic sinus may be present to some degree in all decapods. Bouvier (1890) regarded this circulation as ontogenetically and phylogenetically the earlier circulation. It is the principal respiratory circulation in the larval stages of aquatic decapods, the gills developing secondarily in the adult. Retention of this route, even as a minor component of venous return, could provide an emergency gas-exchange mechanism if the branchial chambers accidentally became air-filled and the gills waterlogged. The existence of such a device would of course pre-adapt decapods for the kind of evolutionary development which appears to have occurred in *Holthuisana*.

It is clear that there are still very large gaps in our understanding of the anatomy and of the operation of the circulatory system in *H. transversa* and other crabs. In particular, we are still largely ignorant of blood flow patterns within the thoracic sinus system. Potentially it forms a single vascular compartment with multiple inputs of systemic blood and numerous exits to the respiratory structures. However, no doubt certain dominant flow pathways exist. In *Holthuisana* some of these pathways probably differ both in direction and magnitude of flow between air- and water-breathers. Whether any mechanism of control of these flows exists within the thorax itself remains to be determined. A number of authors have implied that the infrabranchial sinuses in crabs serve as reservoirs of mixed venous blood receiving blood from the

general thoracic sinus system and distributing it to the gills (Pearson, 1908; Blatford, 1971). In *Holthuisana* at least, the degree of antero-posterior mixing which occurs in this sinus appears to be quite limited, even during active leg movements, blood apparently streaming from certain limbs and regions of the thorax directly towards certain gills or particular regions of the lung. Although this does not necessarily imply any significant local variation in oxygen content of the haemolymph within the sinus, these observations reinforce Taylor's (1982) caution concerning the assumption that infrabranchial haemolymph samples are mixed venous samples in estimates of cardiac output based on the Fick principle.

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