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Ventilation and Oxygen Consumption in the Dungeness Crab, *Cancer magister*

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ABSTRACT The respiratory system of the crab *Cancer magister* has been investigated in unrestrained specimens fitted with electrodes for monitoring heart rate, branchial pressure catheters for monitoring scaphognathite rate, a ventilation mask containing an electromagnetic flow probe for measuring instantaneous branchial water flow and water sampling catheters for determining gill oxygen extraction and oxygen consumption. In animals recently stressed by physical restraint and air exposure, heart rate, branchial water flow and oxygen consumption were elevated and both scaphognathites pumped continuously in a predominantly forward direction, whereas in well-acclimated animals ventilation rate and oxygen consumption were low, gill oxygen extraction was elevated, unilateral ventilation predominated and both ventilatory pauses and periodic reversals of scaphognathite beating were common. Analysis of the relationship between branchial water flow and scaphognathite rate reveals that the scaphognathite operated at a constant stroke volume for forward pumping but not for reverse pumping. Furthermore, the stroke volume was significantly lower for the latter over the normal range of scaphognathite rates in this mode. Reverse pumping and ventilatory pauses are behaviors that often occurred in the apparent absence of extrinsic stimuli in this species, thus their possible roles in gas exchange have been analyzed and are discussed.

Since the major review by Wolvekamp and Waterman ('60) there has been a considerable increase in our understanding of the nature and performance of the scaphognathite system of the decapod crustaceans. The neuromuscular elements responsible for scaphognathite movement have now been described in detail (Wilkens et al., '74; Young, '75). The interrelationships between scaphognathite beating, branchial hydrostatic pressures and branchial water flow have been examined in a number of species (Hughes et al., '69; McDonald et al., '77; Cumberlidge and Uglow, '77; Batterton and Cameron, '78). Detailed respiratory studies (e.g. Johansen et al., '70; Batterton and Cameron, '78; McMahon et al., '79) have shown that decapod ventilatory systems are capable of producing flows and oxygen extraction efficiencies that are comparable to those of water-breathing vertebrates.

Paralleling these studies have been investigations showing that the scaphognathites also exhibit a variety of activities which, superficially at least, do not appear to be strictly involved in the maintenance of adequate oxygen delivery to the gills. These include periodic reversals of the direction of water flow over the gills (Borradaile, '22; Arudpragasam and Naylor, '64a), ventilatory pauses, (McMahon and Wilkens, '72, '77) and unilateral scaphognathite pumping (Cameron, '75; McDonald et al., '77; Batterton and Cameron, '78). Pauses constitute an abrupt bilateral cessation of scaphognathite beating accompanied by a pronounced slowing of heart rate, while unilateral pumping comprises prolonged periods during which only a single branchial cavity is ventilated. In a previous paper (McDonald et al., '77) we described the patterns of these activities in the subtidal brachyuran decapod *Cancer magister.* In recently disturbed animals, which exhibit high ventilatory rates, forward bilateral scaphognathite pumping predominates, with reversals and pauses being virtually absent, whereas at the low ventilatory rates characteristic of well-acclimated animals, pauses and reversals occur frequently and unilateral scaphognathite pumping is the predominant mode of gill ven-

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tilation. While extrinsic stimuli for some of these activities have been demonstrated, and non-respiratory roles proposed (McMahon and Wilkens, '72; Berlind, '77), they often occur in the apparent absence of any extrinsic stimuli (McMahon and Wilkens, '77; McDonald et al., '77). This latter observation raises the possibility that they may be intrinsically generated to play some role in respiration. For example, Aradpragasam and Naylor ('64a) have suggested that reversals serve to ventilate portions of the branchial cavities not normally ventilated during forward ventilation.

The purpose of the present investigation was, therefore, to examine the patterns of ventilatory flow, gill oxygen extraction and oxygen consumption that correlate with these patterns of scaphognathite activity in *Cancer magister* and to determine what (if any) role, pauses, reversals and unilateral ventilation play in normal respiration. In this study we have employed a sensitive electromagnetic technique to measure instantaneous branchial water flow (after Johansen et al., '70), and pressure monitoring catheters implanted in the branchial cavities (after Hughes et al., '69) to determine the rate and direction of scaphognathite pumping. These techniques, together with simultaneous measurements of oxygen levels in inspired and expired water, permitted essentially instantaneous determinations of ventilatory volume and Fick estimates of oxygen consumption in relatively unrestrained and undisturbed animals exhibiting normal patterns of respiratory activity. These techniques also allowed a detailed analysis of the relationship between scaphognathite rate and branchial water flow for both forward and reverse pumping.

MATERIALS AND METHODS

Experimental animals

Adult *Cancer magister* (262–960 gm) in intermolt stage C (Drach and Tchernigovtzeff, '67) were collected in the waters off San Juan Island, Washington, or obtained from either the Vancouver Public Aquarium or a commercial supplier (Whitney-Fidalgo Sea Foods Inc., Anacortes, Washington). Prior to experimentation crabs were held in large aquaria filled to a depth of at least 8 cm with fine beach sand and continuously supplied with well aerated seawater at a temperature of 8 ± 1°C and salinity of 27 ± 1ppt. Crabs were fed twice weekly with chopped fish and kept on an artificial 12-hour day/night cycle.

Experimental protocol

Crabs were physically restrained and air exposed for 30–45 minutes to enable installation of water sampling and pressure monitoring catheters, electrodes and a ventilation mask. Following this procedure crabs were placed in a shielded, sandy-bottomed, 10 liter aquarium continuously supplied with well-aerated seawater (temperature and salinity as above). Recording of heart and scaphognathite activity, branchial water flow and expired oxygen tensions began immediately.

The techniques for recording heart and scaphognathite activity are described in detail in McDonald et al., ('77). In brief, heart rate (f_H) was obtained from fluctuations in impedance between two fine (0.13 mm o.d.) insulated stainless-steel wires implanted above the pericardial cavity on either side of the heart and connected to an impedance converter (Biocom Inc.). Scaphognathite rate (f_{SG}) was obtained from fluctuations in hydrostatic pressure in the branchial cavities caused by movement of the scaphognathites. Water-filled polyethylene cannulae (PE 160) connected to pressure transducers (Hewlett Packard 267 BC) were tightly fitted into both epibranchial cavities through holes drilled in the posterior margin of the carapace overlying gills 7 and 8.

Instantaneous branchial water flow (ventilation volume, $\dot{V}w$) was determined by a direct method similar to that of Johansen et al. ('70). Crabs were fitted with a lightweight mask constructed of heat-molded Butyrex plastic (< 1 mm thickness) which, when fitted, isolated the animals' exhalant apertures. The mask was sealed to the carapace with strips of latex rubber sheet (dental dam) and cyanoacrylate cement. To obtain a good seal it was necessary to dry the carapace and scrape away the fringe hairs that cover the ventral surface. Although the mask was designed to fit closely to the animal and thereby reduce its dead volume it still allowed free movement of the oral appendages. An electromagnetic flow probe (Biotronix 2100, 10 mm i.d. cannulation type) was fitted to the mask through a small aperture in a latex rubber septum glued over the anterior mask opening. The fitting was leak-proof, yet the probe could easily be installed or removed without removing the mask or seriously distrubing the animal. In situ, the design of the mask oriented the probe in the normal excurrent water stream, 1–2 cm from the exhalant openings. The large size of probe chosen offered no appreciable resistance (measured as pressure difference) to flow. The probe was connected to a Biotronix model 610 electromagnetic flowmeter, set to mean flow. The output of the flowmeter was displayed, together with branchial pressure and heart impedance signals, on a rectilinear writing chart recorder (Gilson, M8PM). The system was calibrated by timed collections of seawater passed through the probe. The periodic occurrence of ventilatory pauses provided a natural and non-disturbing means of determining zero flow (e.g. Figs. 1 and 2). For periods when animals were not exhibiting ventilatory pauses, flow zero was periodically determined by momentary finger occlusion of the flow probe.

Inspired and expired water samples were collected from catheters positioned in five different sites. Two catheters, constructed of PE 160 polyethylene tubing and short sections of 90° bent 18-gauge stainless-steel tubing, were positioned to lie directly within the exhalant openings about 1 cm anterior to the forward edge of the scaphognathites. Once positioned these catheters were sealed to the carapace to prevent any subsequent movement. Two similar catheters were positioned and sealed in place about 0.5 cm above the inhalant (Milne-Edwards) openings located at the bases of the chelipeds. A fifth catheter was inserted through a rubber ring fitted over the anterior end of the flow probe with the catheter tip positioned to lie in the centre of the probe opening.

Preliminary experiments established that the catheter positioned anterior to the flow probe permitted sampling of mixed expired water during forward pumping. This mixed condition was confirmed by measurements of oxygen tension on water samples collected simultaneously from the catheters positioned anterior to the flow probe and within the left and right exhalant channels. Preliminary experiments also established that the catheters positioned above the Milne-Edwards openings permitted representative sampling of water expired during reverse pumping. This was confirmed by dye injection experiments which showed that > 90% the water expired during reverse pumping exited from these openings. Appearance of dye at openings above the other limb bases was negligible.

Oxygen tensions in the water were usually measured by continuous siphoning (flow rate, 1 ml/min) from the catheters past a Radiometer oxygen electrode thermostatted to the experimental temperature. The electrode was calibrated at the same flow rate to correct for elevation of electrode output (5–10%) caused by flow past the measuring surface. This method permitted continuous monitoring of PO_2 and avoided possible visual and vibrational disturbances to the crab associated with direct samples (see below). A drawback was that expired PO_2s monitored from exhalant catheters during forward pumping were artificially elevated for 1–2 minutes following a period of reverse scaphognathite pumping. This period reflected the oxygen electrode response time and the clearance time of the dead volume of the ventilation mask and sampling catheter. Thus direct samples were occasionally drawn to confirm the accuracy of siphon samples and were routinely drawn whenever reversal incidence exceeded 5/10 min. These samples had to be drawn with care, as rapid withdrawal tended to stimulate either reversals or pausing (when samples drawn during forward pumping) or cause premature termination of reversals (when samples drawn during reverse pumping). During experimental recording PO_2s were normally simultaneously monitored at two different sites, with the site selection dependent on the respiratory patterns of the crab being examined. For example, during unilateral ventilation, oxygen tensions were monitored from the catheters located in the exhalant and inhalant openings of the ventilated branchial cavity.

Calculations

1. Percent oxygen extraction (% Ext).

$$\% \text{ Ext} = \frac{(P_IO_2 - P_EO_2)}{P_IO_2} \times 100$$

where P_IO_2 is the inspired oxygen tension (torr) and P_EO_2 the mixed expired oxygen tension (torr). P_IO_2 normally exceeded 130 torr.

2. Oxygen consumption ($\dot{M}O_2$)

$$\dot{M}O_2 = (P_IO_2 - P_EO_2) \times \dot{V}w \times \alpha_wO_2$$

where $\dot{V}w$ is the branchial flow rate in mls H_2O/min and α_wO_2 is the oxygen solubility coefficient in water in μmoles O_2/ml H_2O/torr at the appropriate temperature and salinity (Carpenter, '66).

RESULTS

Heart and scaphognathite activities, branchial water flow, and inspired (P_IO_2) and expired (P_EO_2) oxygen tensions were monitored

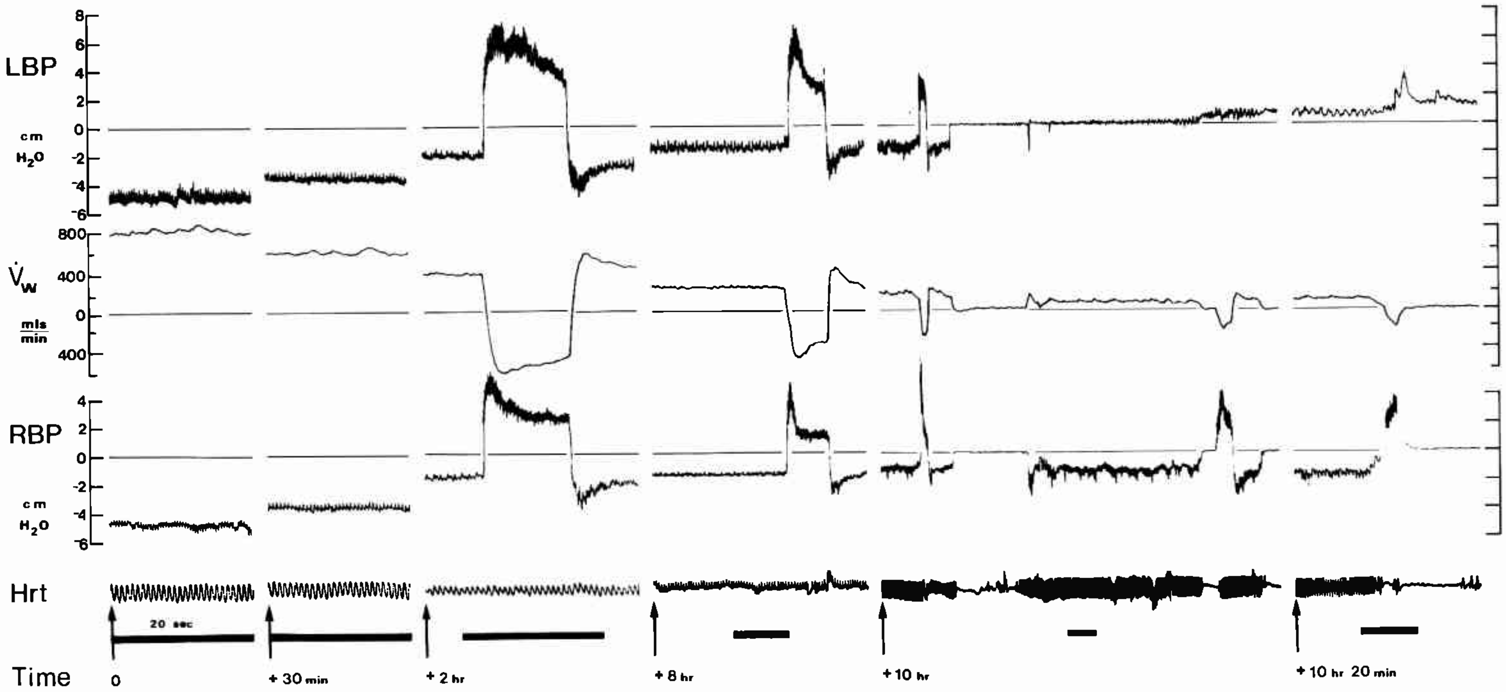

Fig. 1. Six excerpts (Time 0, +30 min, +2 hr, +8 hr, +10 hr and +10 hr 20 min) from a continuous record of branchial water flow ($\dot{V}w$), hydrostatic pressure in the left and right branchial cavities (LBP and RBP), and heart activity (Hrt) on an 864 g *Cancer magister* (T = 8°C, salinity = 27%, P_IO_2 = 134 torr). Each horizontal bar indicates a 20 sec period, recording speed varied between excerpts. Recording was begun immediately following the installation of the monitoring equipment. Pressure fluctuations below ambient indicate forward scaphognathite pumping and above ambient, reverse pumping. Ventilatory pauses are indicated by an abrupt fall in $\dot{V}w$ to zero, by the absence of pressure fluctuations in the branchial cavities and by a pronounced slowing in heart rate (+10 hr excerpt). The onset of unilateral ventilation (+10 hr excerpt) is indicated by a reduction in $\dot{V}w$ and the initial absence of pressure fluctuations in the left branchial cavity. Note the appearance of anomolous pressure fluctuations (as described in McDonald et al., '77) in the left (unventilated) branchial cavity in the latter part of this excerpt and that these fluctuations do not result in water flow (+10 hr, 20 min excerpt).

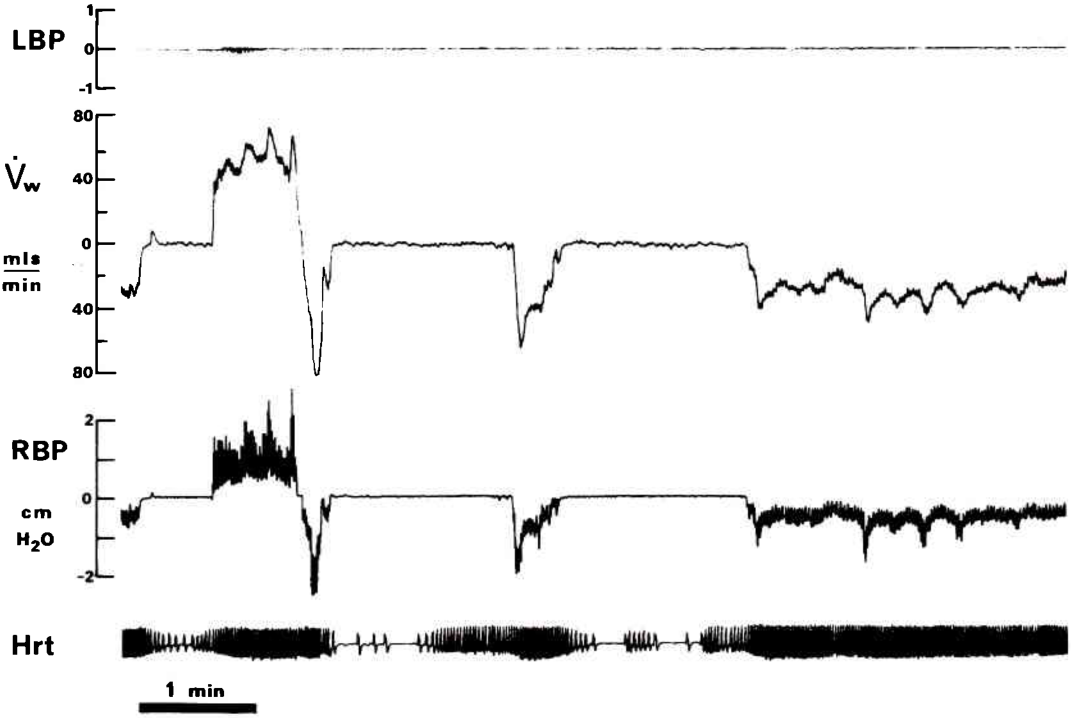

Fig. 2. Unilateral ventilation in a 262 gram *Cancer magister*. Axes labeled as in Figure 1. Note the absence of pressure fluctuations in the left (unventilated) branchial cavity, the pronounced slowing of heart rate during pauses by the right scaphognathite and the close correspondence between RBP and $\dot{V}w$.

on each animal under daylight conditions for an average of 8 hours/day over a 2–3-day period. All animals were usually inactive throughout this recording period except at the beginning when most exhibited brief, sporadic attempts to dislodge the ventilation mask. Over this initial period crabs maintained an alert posture on the top of the sand. Subsequently they burrowed into the sand and remained in this position for the remainder of the day. Crabs would become active in the dark and move about the experimental chamber, but no attempt was made to monitor respiratory parameters during this time. In the subsequent analysis of the data all periods of overt locomotory activity have been excluded.

The patterns of heart and scaphognathite activity observed in crabs with ventilation masks were similar to those we have previously described in detail for individuals of this species where ventilation masks were absent (McDonald et al., '77). That is, as the masked crabs acclimated to the experimental conditions the forward scaphognathite rate gradually declined and the incidence of reverse scaphognathite pumping and pausing increased until eventually, in all animals examined, one scaphognathite ceased pumping for prolonged periods (unilateral ventilation). Typical records illustrating the major patterns observed are shown in Figures 1 and 2.

The time elapsed from the installation of monitoring equipment to the initial onset of unilateral pumping was highly variable among individuals but in general was longer in crabs with ventilation masks than in those without (8 hr to 2 days, compared to 4–9 hr, McDonald et al., '77). This may be due to irritant properties of the mask and to the extended period of air exposure required for its installation (30–45 minutes, compared to 20–30 minutes, McDonald et al., '77).

Patterns of ventilation, oxygen consumption and oxygen extraction

Forward pumping

In all crabs branchial water flow ($\dot{V}w$) and oxygen consumption ($\dot{M}O_2$), decreased with

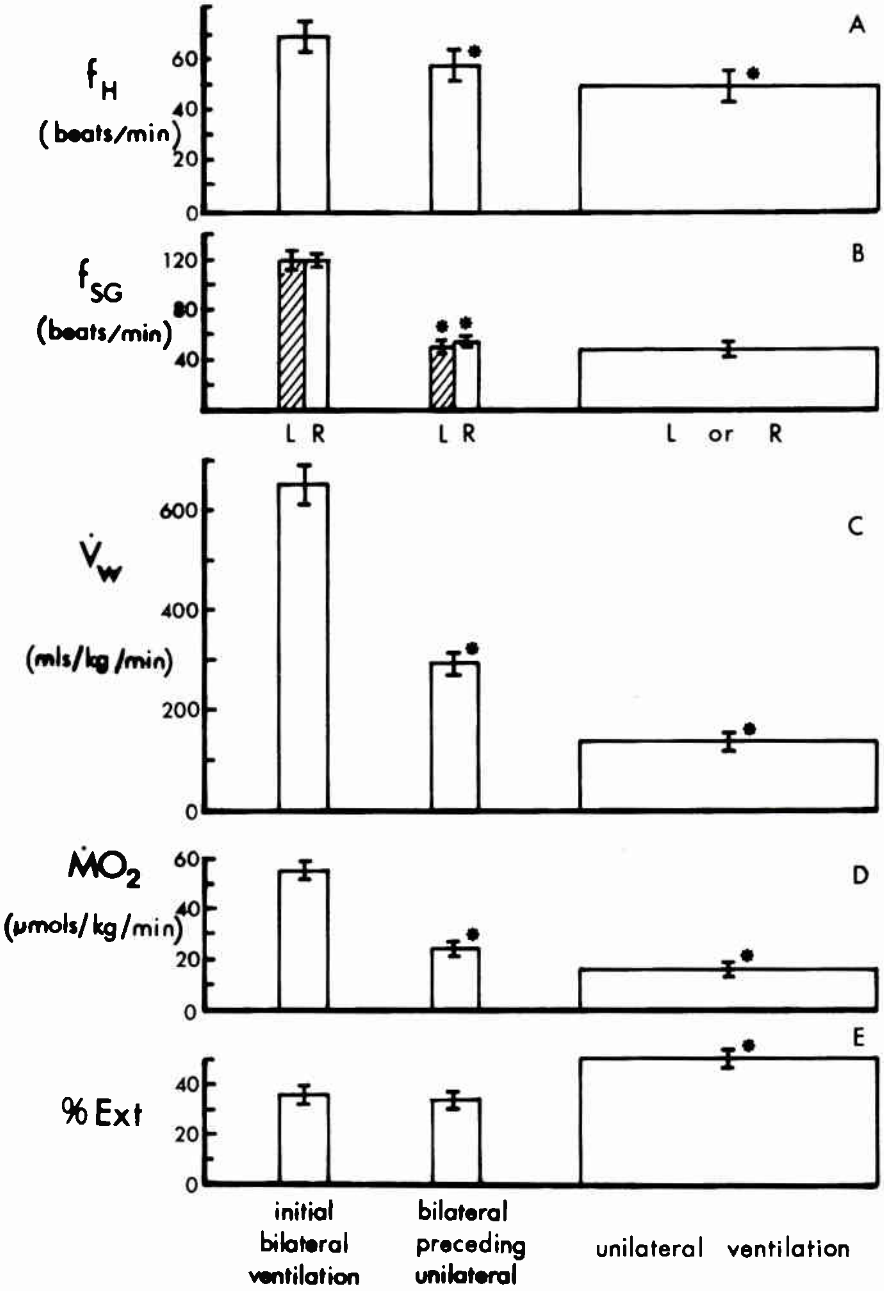

Fig. 3. Histogram summarizing respiratory performance during forward ventilation in *Cancer magister*. (T = 8°C, salinity = 27ppt. $P_IO_2 > 130$ torr). A. heart rate (f_H). B. scaphognathite rate (f_{SG}); L = left, R = right. C. branchial water flow ($\dot{V}w$). D. oxygen consumption ($\dot{M}O_2$). E. percent oxygen extraction (% Ext). Data are means (± one SEM, N = 11 crabs with ≥ 5 measurements/crab) for three time periods: initial 20 minutes of bilateral ventilation, 20 minutes of bilateral ventilation preceding the onset of unilateral ventilation and 2 hours of unilateral ventilation. Data from each animal were selected not only to span the 20-min or 2-hr period but also to avoid possible disturbances to % Ext and $\dot{M}O_2$ estimates caused by periodic reversals and pauses (see Tables 1 and 2). Asterisks indicate means significantly different ($P < .01$, paired "t" test) from mean of preceding time period. Means and statistical tests on % Ext values were computed after arcsin$\sqrt{x}$ transformation of the data.

time concomitant with the gradual decrease in forward scaphognathite rate. Since the rates of decline varied widely among individuals the respiratory data have been analyzed over three periods: the first 20 minutes of recording, the 20-minute period of bilateral pumping immediately preceding the onset of unilateral pumping, and the first 2 hours of unilateral pumping (Fig. 3). Within each time block the data presented are averages for measurements made during forward scaphognathite pumping only (i.e. periods of reverse pumping and pausing excluded).

Over the bilateral pumping period the mean heart rate ($\bar{f}_H$) fell slightly but significantly (65 ± 3 to 58 ± 3 beats/min; $\bar{x}$ ± SEM, N = 11) while the mean forward scaphognathite rate ($\bar{f}_{SG}$) fell markedly (121 ± 6 to 52 ± 5 beats/min, a 57% decrease). Since the scaphognathite, in the forward mode, operates as a fixed stroke volume pump (see below) the decrease in $\bar{f}_{SG}$ resulted in a nearly identical decrease (56%) in mean $\dot{V}w$ (650 ± 36 to 288 ± 22 ml/kg/min). Percent oxygen extraction (% Ext), however, remained essentially constant over the bilateral pumping period (36 ± 3% to 34 ± 2%). Bilateral oxygen consumption ($\dot{M}O_2$) thus was a simple function of $\dot{V}w$; decreasing by an equivalent amount (56%; 55.5 ± 2.6 to 24.5 ± 2.3 μmoles O_2/kg/min).

With the onset of unilateral ventilation there was again a slight but significant decrease in $\bar{f}_H$ (58 ± 3 to 50 ± 3 beats/min), whereas the active scaphognathite remained pumping at virtually the same rate as during the preceding bilateral phase (52 ± 5 to 49 ± 5 beats/min). Since f_{SG} remained constant, $\dot{V}w$ of the single ventilated branchial cavity similarly remained constant, and the total branchial water flow was effectively halved (288 ± 22 to 135 ± 13 ml/kg/min). $\dot{M}O_2$ was not halved, however, since % Ext (now measured directly on the single ventilated branchial cavity, see Methods) increased significantly (34 ± 2% to 50 ± 2%). Thus $\dot{M}O_2$ decreased, on average, by only 32 ± 5%.

The above mean extraction values indicate that, overall, there was no apparent correlation between % Ext and $\dot{V}w$, despite a more than twofold variation in the latter. However, momentary increases in f_{SG} and thus $\dot{V}w$ were invariably accompanied by a decrease in P_EO_2 whenever such changes could be reliably measured (see Methods). This was particularly evident during unilateral pumping. Under these circumstances expired water was directly sampled from the excurrent water stream of the ventilated branchial cavity, and thus its PO_2 could not be affected by mixing in the dead space of the ventilation mask. Similarly, during bilateral pumping, an inverse correlation between P_EO_2 on each side and $\dot{V}w$ on each side was apparent whenever the two scaphognathites beat at substantially different rates. As pointed out previously (see Table 2 of McDonald et al., '77) the two scaphognathite rates, though normally closely approximating one another (Fig. 3B), were rarely equal at any point in time. When these differences were marked (~ 20 beats/min) the P_EO_2 was routinely 8–10 torr lower (i.e. % Ext higher) in the excurrent stream from that branchial cavity ventilated at the lower rate.

Reverse pumping

Reversals are periods during which the scaphognathite pump reverses so as to generate water flow in a direction opposite to forward pumping, i.e. water is drawn in at the exhalant openings, passes over the gill lamellae in a direction co-current with hemolymph flow (Hughes et al., '69) and is expelled as the limb bases primarily via the Milne-Edwards openings. Reversals were usually brief in duration (10–15 seconds) and were usually characterized by substantially higher scaphognathite rates (> 150 beats/min) and consequently higher flows than adjacent periods of forward pumping (e.g. Figs. 1 and 2). Expired water samples collected from the Milne-Edwards openings during reverse pumping routinely had PO_2s at least 20 torr higher than those measured during forward pumping.

Most animals maintained reversals of this essential character throughout (e.g. Figs. 1 and 2). More rarely, a few animals exhibited reversals of longer duration (> 1 minute). During these reversals the scaphognathite rate steadily declined over the first 1–2 minutes and then stabilized at levels 10–50 beats/min above the preceding forward rate. P_EO_2 (at the Milne-Edwards opening) similarly declined but remained at least 10 torr higher than that measured at the normal exhalant opening during forward flow.

When these longer duration reversals occurred during unilateral ventilation it became possible to estimate $\dot{M}O_2$ from simultaneous measurements of P_IO_2 (at the normal exhalant opening), P_EO_2 (at the Milne-Edwards opening and $\dot{V}w$. Three crabs exhibited reversals of sufficient duration (2–5 minutes) during unilateral ventilation to allow $\dot{V}w$ and P_EO_2 to reach stable values and thus permit this anal-

TABLE 1. Mean values (± one SEM) for % Ext, $\dot{V}w$ (ml/kg/min), $\dot{M}O_2$ (μmoles/kg/min) and $\dot{V}w/\dot{M}O_2$ (ml H_2O/μmoleO_2) for long-duration (2–5 min) reversals occurring during unilateral ventilation compared with mean values for adjacent periods of forward ventilation in three crabs

		Forward pumping					Reverse pumping				
Crab	Weight (grams)	n	% Ext	$\dot{V}w$	$\dot{M}O_2$	$\dot{V}w/\dot{M}O_2$	n	% Ext	$\dot{V}w$	$\dot{M}O_2$	$\dot{V}w/\dot{M}O_2$
D	392	6	40.6 ± 0.8	195 ± 14	20.5 ± 1.4	9.5 ± 0.2	5	22.9 ± 0.3	333 ± 10	19.8 ± 0.6	16.8 ± 0.2
E	424	5	53.8 ± 5.4	154 ± 26	19.1 ± 1.7	8.0 ± 1.1	6	37.1 ± 4.7	94 ± 7	7.8 ± 1.0	12.5 ± 1.1
F	536	13	43.8 ± 1.7	125 ± 21	16.8 ± 5.0	9.9 ± 0.3	10	33.0 ± 2.4	120 ± 14	8.8 ± 0.7	13.7 ± 1.1
Overall means (n = 3)			46.0	158	18.8	9.1		31.0	182	12.1	14.3

TABLE 2. Values for % Ext, $\dot{V}w$ (ml/kg/min), $\dot{M}O_2$ (μmoles/kg/min) and $\dot{V}w/\dot{M}O_2$ (ml H_2O/μmole O_2) obtained prior to and following long duration pauses occurring during bilateral ventilation

			Before pause				Pause Duration	After pause			
Crab	Weight (grams)	n	% Ext	$\dot{V}w$	$\dot{M}O_2$	$\dot{V}w/\dot{M}O_2$	(min)	% Ext	$\dot{V}w$	$\dot{M}O_2$	$\dot{V}w/\dot{M}O_2$
G	522	1	32	159	13.4	11.9	7.5	51	159	21.5	7.4
I	864	4	22 ± 2	311 ± 15	17.3 ± 0.5	18.0 ± 1.0	7.6 ± 1.2	37 ± 5	302 ± 34	29.2 ± 3.7	10.6 ± 1.3
K	960	1	31	288	16.4	17.6	15.	38	345	35.9	9.6
Overall means (n = 3)			28	253	15.7	15.8	10.0	42	269	28.9	9.2

ysis (Table 1). Branchial water flows during these reversal periods were, on average, either lower (crab D), higher (crab E) or nearly the same (crab F) as flows during adjacent periods of forward pumping (Table 1). Nevertheless % Exts were significantly lower and the ventilation volume required per unit oxygen consumption (i.e., the convection requirement, $\dot{V}w/\dot{M}O_2$) significantly higher in all three animals when compared with adjacent periods of forward pumping.

Ventilatory pauses

Ventilatory pauses were characterized by an abrupt cessation of scaphognathite beating accompanied by a pronounced slowing of the heart rate (e.g. Figs. 1 and 2). Pauses normally did not exceed about 30 seconds, but occasionally pauses of substantially longer duration (6–10 minutes) were observed. Following short pauses there was usually a reduction in P_EO_2, but this reduction was very short-lived and it is uncertain whether this reflected an increase in true % Ext or simply the washout from the branchial cavities of water that had been deoxygenated during the pause period. Following long pauses the situation is somewhat clearer. Respiratory values obtained preceding and following these pauses are summarized in Table 2. For this analysis measurements of $\dot{V}w$ and P_EO_2 were made within 5 minutes preceding the pause and 2–3 minutes following the pause. At the ventilatory flow rates prevailing after the pauses (~ 270 ml/kg/min, Table 2) and on the basis of branchial cavity volume estimates of 50 ml/kg (B.R. McMahon and D.G. McDonald, unpublished results) the latter time was judged as being sufficient to ensure complete clearance of pause-deoxygenated water from the branchial cavities with the resumption of scaphognathite pumping. Although the data are limited in comparison to those obtained for reverse pumping (Table 1) % Exts were uniformly higher and water convection requirements uniformly lower than values at comparable $\dot{V}w$s obtained preceding the pause. Furthermore, while these differences usually diminished with time, they usually persisted for up to 8 minutes following the pause.

Scaphognathite rate and ventilatory flow

Least squares regression analyses were performed on the relationship between f_{SG} and $\dot{V}w$ in each animal for forward and, where possible, reverse pumping. For periods of bilateral pumping the total measured flow was halved and this value, together with the mean of the two scaphognathite rates was used for these analyses. Data from periods during

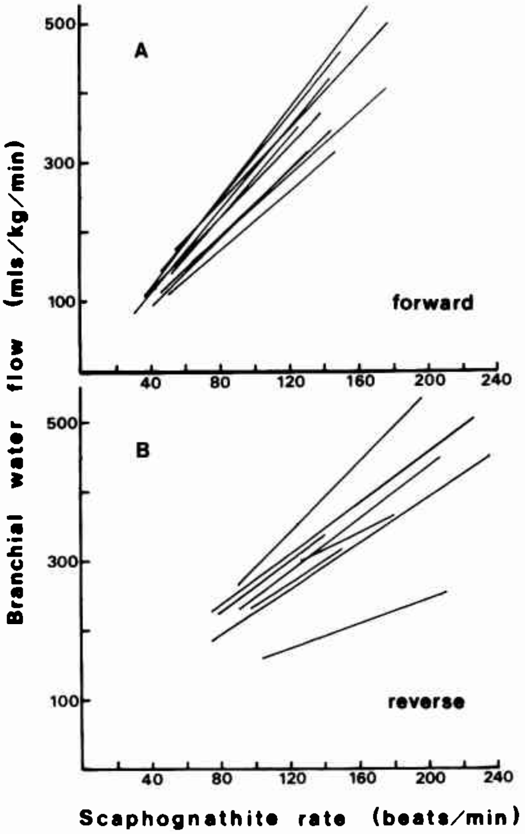

Fig. 4. Least-squares regression lines relating water flow through one branchial cavity (i.e. $\dot{V}w/2$ for bilateral pumping, $\dot{V}w$ for unilateral pumping) and scaphognathite rate in *Cancer magister* (262–960 g) during A, forward pumping, and B, reverse pumping. Each regression line is drawn from the maximum to the minimum scaphognathite rate recorded for each animal. All regressions significant ($P < 0.005$). Data points for individual animals have been omitted for the sake of clarity. See text for further details.

which the differences between the two scaphognathite rates exceeded 20 beats/min were not included. During unilateral pumping the absolute range in f_{SG} rarely exceeded 30 beats/min so it was generally not possible to perform separate regression analyses on f_{SG} versus $\dot{V}w$ for unilateral pumping. Instead, flow and rate data for unilateral pumping were included in the overall analysis. In no case did their inclusion significantly alter the slope or intercept of the resulting regression equation.

In all animals, with one exception excluded from Figures 4 and 5, there was a significant linear relationship ($P < .005$) between $\dot{V}w$ and forward scaphognathite rate over the whole range of rates exhibited (47 ± 4 to 147 ± 5 beats/min, N = 10). Furthermore the intercepts of the regression lines relating $\dot{V}w$ to $\overline{f_{SG}}$ (Fig. 4A) were not significantly different from zero thus indicating that the scaphognathite stroke volume ($\frac{1}{2}\dot{V}w \div \overline{f_{SG}}$ for bilateral pumping, $\dot{V}w \div f_{SG}$ for unilateral pumping) was constant at all scaphognathite rates. These stroke volumes increase linearly with increasing boby weight (Fig. 5) and thus allow indirect assessments of ventilation volume for *Cancer magister* from measurements of body weight and $\overline{f_{SG}}$.

For reverse pumping there was similarly a significant linear relationship ($P < .005$) between $\dot{V}w$ and reverse scaphognathite rate (Fig. 4B) over the range of rates exhibited (82 ± 7 to 198 ± 20 beats/min, N = 8) although two animals did not exhibit a sufficient range in rates to permit analysis. In contrast to forward pumping, the regression lines relating reverse $\overline{f_{SG}}$ to $\dot{V}w$ (Fig. 4B) had large positive intercepts, thus indicating that stroke volume decreases with increasing rate. These intercepts were significantly greater than zero in 4 of the 8 animals examined. For the remainder, the positive intercepts escaped significance because of variability in the rate vs. flow data. This variability was primarily due to the short duration and high rate of change of pumping rate during reversals (e.g. Figs. 1 and 2), which made it difficult to assess the precise association between the two parameters. To correct for any possible effect of rate on stroke volume, average stroke volumes for

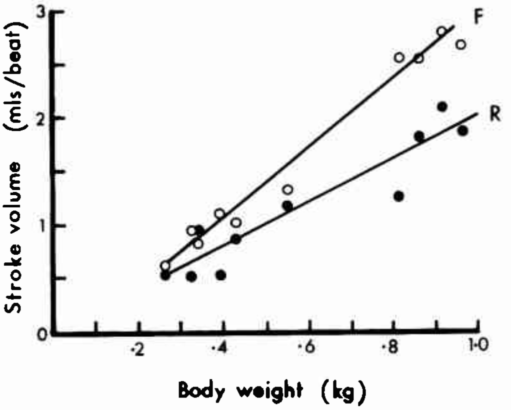

Fig. 5. Relationship between body weight and scaphognathite stroke volume ($\dot{V}w/2\overline{f_{SG}}$) for forward (F) and reverse (R) pumping. Mean stroke volumes (S.V.) for forward pumping calculated for whole range of $\overline{f_{SG}}$; means for reverse pumping calculated for $\overline{f_{SG}}$s of 140–170 beats/min only (see text). Regression equations: S.V. (F) = 3.22. Weight(kg)—0.24 ($r = .988$), S.V. (R) = 2.05. Weight(kg)—0.26 ($r = .929$).

each animal were computed over the range of 140–170 beats/min only (this represents a mid-range for the scaphognathite pumping in the reversed mode). These stroke volumes similarly increase linearly with increasing body weight (Fig. 5) but with a significantly lower slope than that for forward pumping.

DISCUSSION

Patterns of ventilation, oxygen consumption and oxygen extraction

For all animals examined, ventilation and oxygen consumption were at their highest levels (650 ± 36 ml/kg/min and 55.5 ± 2.6 μmoles/kg/min, respectively) immediately following physical restraint in air. These values closely approximate those seen following exhaustive exercise in this species (McMahon et al., '79), thus indicating that the crabs were initially respiring maximally despite the virtual absence of locomotor activity. This elevation of metabolism presumably reflects the repayment of an oxygen debt incurred as the result of the procedures required for the installation of monitoring equipment. Indeed, these procedures led to an elevation of hemolymph lactate to levels only slightly lower than those seen following maximal enforced exercise (5.2 ± 1.0 mEq/L, McDonald, '77 compared to 8.8 ± 0.6 mEq/L, McDonald et al., '79). The lactate determinations were made on animals additionally prepared for hemolymph sampling (McDonald, '77); nevertheless the experimental preparation was similar in both cases.

Subsequently $\dot{V}w$ and $\dot{M}O_2$ gradually declined in all animals until stabilizing at levels about 25% of initial values (Fig. 3). At these low levels unilateral ventilation predominated. Animals would normally return to bilateral ventilation only if disturbed or if they became active (e.g. during the dark), circumstances which would both probably lead to an increase in $\dot{M}O_2$. Thus it is apparent that *Cancer magister,* at 8°C at least, chooses to meet its minimum metabolic requirements via unilateral ventilation rather than by low-level bilateral ventilation. To explain this strategy it is possible, as we speculated elsewhere (McDonald et al., '77), that there may be a minimum rate below which the scaphognathite is unable to generate flow. This conclusion is supported by the observation that the scaphognathite rate rarely fell below 40 beats/min (Fig. 4A). If this represents a functional minimum rate, then bilateral ventilation at this rate would result in flows that are probably well in excess of those required to meet metabolic requirements at rest. As a strategy to reduce branchial water flow, unilateral ventilation may convey at least one major advantage to the animal. Since the onset of unilateral ventilation resulted in an increase in % Ext (Fig. 3E), there was a consequent significant reduction in the ventilation volume required per unit oxygen consumption (i.e., the water convection requirements, $\dot{V}w/\dot{M}O_2$) as compared to bilateral ventilation (see below). Any reduction in the water convection requirement would, of course, reduce the proportion of metabolism required to power ventilation (i.e. the metabolic cost of ventilation).

The increased % Ext accompanying the onset of unilateral ventilation must be related to an improvement in oxygen transfer conditions across the epithelium of the ventilated gills, since the flow rate through the ventilated branchial cavity remained the same when ventilation of the contralateral cavity ceased. The underlying reason for this improvement will be discussed more fully in a subsequent paper (McDonald et al., in preparation), but basically it appears to be due to a significant reduction in oxygen levels in prebranchial (i.e. venous) hemolymph and therefore an elevation of the diffusion gradient for loading oxygen into the hemolymph.

A similar increase in % Ext and reduction in water convection requirement occurs following pausing behaviors (Table 2). During pauses the animal must rely on hemolymph oxygen stores to support tissue oxygen demand, and this results in a reduction in hemolymph oxygen levels (McMahon and Wilkens. '77; McDonald et al., in preparation). Lower venous O_2 saturation (S_vO_2) following the pause will cause a transitory improvement in % Ext until hemolymph oxygen stores are replenished. Thus, in addition to the proposed role of ventilatory pauses as startle responses in masking the presence of a crab from a predator (McMahon and Wilkens, '77), periodic pauses may be another mechanism for reducing the overall convection requirement and cost of ventilation. For example, in bilaterally ventilating animals, long pauses increased % Ext and reduced $\dot{V}w/\dot{M}O_2$ to levels approximating those seen in unilaterally ventilating animals (compare Tables 2 and 3). Overall, the energetic significance of pausing in *Cancer magister* is probably greater during unilateral than during bilateral pumping, as pauses are relatively rare during the latter

TABLE 3. Mean values (± one SEM) obtained for $\dot{V}w$, % Ext, $\dot{M}O_2$ and the water convection requirement for crustaceans and fish by other investigators compared with values obtained for Cancer magister *in the present study*[1]

Reference	Animal	T°C	Weight (grams)	n	$\dot{V}w$ (ml/kg/min)	% Ext	$\dot{M}O_2$ µmoles/kg/min	$\dot{V}w/\dot{M}O_2$ ml H_2O/µmole O_2
Arudpragasam and Naylor ('64b)	*Carcinus maenas*	16°C	21–70	8	1276 ± 108	16 ± 2	51.5 ± 6.3	26.9 ± 3.3
Hughes et al. ('69)	*Carcinus maenas*	10°C	42–80	10	671 ± 66	15 ± 2	28.1 ± 3.7	26.7 ± 3.8
Johansen et al. ('70)	*Cancer magister*	9°C	650–1150	12	624 ± 36	18 ± 1	27.2 ± 1.7	23.8 ± 1.7
Batterton and Cameron ('78)	*Callinectes sapidus*	25°C	165–297	11	459 ± 60	55 ± 4	43.4 ± 5.7	11.0 ± 1.2
Present study	*Cancer magister*	8°C	262–960	11	288 ± 22	34 ± 2	24.5 ± 2.3	12.2 ± 0.8 bilateral ventilation
					135 ± 13	50 ± 3	15.9 ± 1.3	8.5 ± 0.4 unilateral ventilation
Davis and Cameron ('71)	*Salmo gairdneri*	9°C	210 ± 2	18	176 ± 9	46 ± 2	28.7 ± 1.1	6.2 ± 0.3
Cech and Wohlschlag ('73)	*Mugil cephalus*	18°C	79–116	10	363 ± 45	66 ± 4	62.4 ± 7.6	5.9 ± 0.3
Eddy ('74)	*Tinca tinca*	13°C	150–400	4	94 ± 25	56 ± 4	18.4 ± 4.3	4.8 ± 0.6
Wood et al. ('79)	*Platichthys stellatus*	8°C	300–1200	8	109 ± 6	68 ± 3	20.4 ± 1.2	5.3 ± 0.2

[1] Where necessary published data have been recalculated to obtain means ± SEM.

(McDonald et al., '77). However, in the closely related *Cancer productus,* rhythmic pauses of up to 20 minutes duration occurred repetitively during bilateral pumping in most specimens examined (McMahon and Wilkens. '77).

This sensitivity of % Ext to variations in venous oxygenation may also serve to explain the observation that % Ext did not vary significantly during bilateral ventilation despite a more than two-fold decrease in $\dot{V}w$ (Fig. 3C) during acclimation following physical restraint in air. Following strenuous exercise in *Cancer magister,* a condition that produced a similar lactacidosis (see above), the associated reduction in hemolymph pH led to a major reduction in S_vO_2, due to the Bohr effect (McDonald, '77; McMahon et al., '79). Consequently % Ext remained at pre-exercise levels despite a 2.3-fold increase in $\dot{V}w$. As hemolymph lactacidosis is only slowly corrected in *Cancer magister* (McDonald et al., '79) it is likely that throughout the period during which $\dot{V}w$ gradually decreased in the present study (Fig. 3C) S_vO_2 was slowly being returned to resting values. These two processes would have opposite effects on % Ext and thus would mask any intrinsic relationship between $\dot{V}w$ and % Ext. In contrast, an overall inverse correlation between $\dot{V}w$ and % Ext has been reported in a number of other studies on crustaceans (McMahon et al., '74; Batterton and Cameron, '78; Butler et al., '78). Presumably in these studies variations in $\dot{V}w$ were not accompanied by marked variations in S_vO_2, and thus flow effects on % Ext became apparent.

Table 3 compares % Ext, $\dot{V}w$ and $\dot{M}O_2$ values for resting bilateral ventilation in *Cancer magister* with values from previous studies on crustaceans. In general the present resting $\dot{V}ws$ are lower and the present % Exts higher than previously published values. In part this may be due to higher experimental temperatures in the other studies (e.g. *Callinectes sapidus* at 25°C, Batterton and Cameron, '78), but at least two other factors are likely of greater importance. First, earlier studies (Arudpragasam and Naylor, '64b; Hughes et al., '69) used an overflow technique for measuring $\dot{V}w$. In this technique, periodic reversals will artificially elevate P_EO_2 and thereby depress % Ext, a problem that was avoided by the water sampling procedures of the present study. Furthermore Johansen et al. ('70) found that the overflow method gave consistently higher values for $\dot{V}w$ than the electromagnetic flow probe technique. Second, in earlier studies, animals may not have been adequately acclimated to the experimental conditions when respiratory parameters were measured. For example, the conditions which Johansen et al. ('70) defined as resting for *C. magister* included "periods of spontaneous activity" during the measurement of ventilation, a circumstance we noted only at the beginning of experimental recording. Indeed, the average $\dot{V}ws$ these authors report (Table 3) are close to the maximums, whereas their % Exts are lower than the minimums recorded in the present study (Fig. 3). A number of authors have now noted that, for crustaceans, acclimation to experimental conditions is a relatively slow process, not necessarily signaled by an absence of locomotor activity, with up to 72 hours being required before resting metabolic levels are achieved (McMahon et al., '74; Batterton and Cameron, '78; Butler et al., '78).

Even with these methodological differences among the crustacean studies the reported $\dot{M}O_2s$ are comparable to resting values reported for fish species (Table 3). On the other hand, bilaterally ventilating crustaceans exhibit convection requirements that are at least two-fold higher than those of fish. Unilateral ventilation significantly reduced the convection requirement in *C. magister* from 12.2 ± 0.8 to 8.5 ± 0.4 ml H_2O/μmole O_2, but the latter value is still higher than the relatively uniform value of ~ 6 ml H_2O/μmole O_2 seen in fish (Table 3). While a number of factors could contribute to this phylogenetic difference, the most likely are a higher gill oxygen diffusion resistance (due to the presence of chitin in gill epithelial tissue; Krogh, '41) and lower blood oxygen-carrying capacities in crustaceans than in fish (0.5 to 3.0 vols/% for decapods vs. 5–20 vols/% for teleosts; Prosser, '73).

Scaphognathite rate and branchial flow

In the forward mode the scaphognathite operates at a fixed stroke volume in *Cancer magister.* This result has now been reported for a number of crustacean species: *Cancer novae-zelandiae* (Pilkington and Simmers, '73), *Callinectes sapidus* (Batterton and Cameron, '78), *Carcinus maenas* (Cumberlidge and Uglow, '77) and the crayfish *Astacus fluviatilus* (Lindroth, '38). For reverse flow it is uncertain whether a similar relationship holds. Stroke volume vs. rate relationships during reversals have not been described in previous studies and in the present study were somewhat difficult to resolve because of measurement inaccuracies (see Results). Nevertheless it is clear that at normal pumping frequencies the stroke volume of the scaphognathite in the reverse pumping mode is significantly lower than in the forward mode, and this difference increases with increasing body weight (Fig. 5). This may be due to change in either the operation of the scaphognathite itself when it switches from the forward to the reverse mode or to an alteration in the overall resistance of the branchial cavities to water flow.

Since reverse pumping is achieved simply by an inversion in the recruitment sequence of the same muscles that are involved in forward pumping, and since these muscles appear to be activated to a similar extent in both pumping modes (Young, '75), the former explanation seems unlikely. Absolute epibranchial hydrostatic pressures, on the other hand, are substantially higher during reverse pumping than during forward pumping at comparable scaphognathite rates (Hughes et al., '69; McDonald et al., '77). Furthermore these pressures increase gradually and linearly from zero pressure as rate increases during forward pumping (McDonald et al., '77; Batterton and Cameron, '78), whereas they increase rapidly in an exponential fashion during reverse pumping (McDonald et al., '77). If these measurements are indices of the pressure differential across the branchial cavities, then the effective resistance offered by the branchial cavities is higher for reverse pumping and may increase with increasing rate, whereas that for forward pumping is lower and is unaffected by rate. This would then explain both the lower overall stroke volumes during reverse pumping and the observation that stroke volume decreases with increased reverse jumping rate. Higher resistances during reverse pumping could result from direction-dependent decreases in the dimensions of the branchial water flow channels formed by the compliant gill lamellae or from decreases in inhalant or exhalant aperture sizes associated with appendage movements.

There is still a great deal of uncertainty concerning the actual function of reverse pumping. Clearly the scaphognathites must be operated at higher rates to produce flows comparable to forward pumping. Furthermore, reverse pumping is a less effective means of gill ventilation (Table 1), presumably because of the disruption of the counter-current arrangement between hemolymph and water flows. Further substantiation of this reduction in effectiveness comes from measurements of significantly lower postbranchial hemolymph oxygen tensions for reverse pumping compared to forward pumping (McDonald et al., in preparation).

One function that has been widely ascribed to reversals (e.g. Borradaile, '22; Berlind, '77) is in flushing the branchial cavities of accumulated debris. Reversals, however, often occur in clean seawater where a gill-clearing function is unlikely. Indeed, in well-acclimated animals, they may be the predominant mode of gill ventilation (see Fig. 5 of McDonald et al., '77). The suggestion by Arudpragasam and Naylor ('64a) that they serve to ventilate normally unventilated portions of the branchial cavity would also seem unlikely, given that reversals result in lower gill oxygen extractions (Table 1). One is still left with the conclusion (McDonald et al., '77; McMahon and Wilkens, '77) that reversals are simply the result of a preprogrammed motor output of the CNS which is modulated by a variety of neural, metabolic and possible hormonal factors. For example, when oxygen demand is high, reversals would be inhibited so as to maximize the effectiveness of gill ventilation.

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