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Heterogeneous perfusion of the paired gills of the abalone *Haliotis iris* Martyn 1784: an unusual mechanism for respiratory control

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Summary

The abalone *Haliotis iris* retains the ancestral gastropod arrangement of a pair of bipectinate gills (ctenidia). The gills share a single branchial chamber, are supplied from a common haemolymph sinus and effectively support the whole of oxygen uptake by the animal. Using chronic indwelling cannulae and pulsed Doppler probes, post-branchial haemolymph oxygen partial pressures ($P_{a_{O_2}}$) and haemolymph flow rates were measured in the left and right efferent ctenidial veins. During periods of internal hypoxia following emersion and handling, total branchial haemolymph flow (24.4±3.6 ml kg^{-1} min^{-1}) was partitioned nearly equally between the left and right gills (13.3±2.6 and 10.8±1.4 ml kg^{-1} min^{-1}, respectively) and their $P_{a_{O_2}}$ values were similar (81.9±6.1 and 87.3±4.7 mmHg, respectively). In animals settled for >24 h, branchial haemolymph flow decreased to 9.1±2.1 ml kg^{-1} min^{-1}, primarily resulting from a virtual shutdown of the left gill flow to only 4.6% of total flow (left, 0.41±0.34 ml kg^{-1} min^{-1}; right, 8.6±2.0 ml kg^{-1} min^{-1}). At rest, right gill $P_{a_{O_2}}$ (85.5±6.8 mmHg) was essentially unchanged while $P_{a_{O_2}}$ of the slowly perfused left gill rose to 105.3±10.2 mmHg, close to the P_{O_2} of the exhalant seawater (104.5±3.1 mmHg). The aerobic metabolic scope of *H. iris* therefore appears to be met primarily by circulatory adjustments at the left gill, which at rest is highly perfusion limited (left L_{diff}, 0.14±0.07; right L_{diff}, 0.44±0.08).

Key words: abalone, bipectinate gill, haemolymph, oxygen uptake, branchial perfusion, cardiac output.

Introduction

In the course of evolution, gastropod molluscs have tended to abandon the bilaterally symmetrical body plan and paired organs present in ancestral forms (Fretter and Graham, 1994). Amongst gastropods, the need to accommodate the visceral organs within a coiled shell has led to a characteristic reduction or loss of the ancestral right-side organs (e.g. Voltzow, 1994). However, a small number of extant gastropod families, including the Haliotidae or abalone, have retained functional right-side organs. Haliotids are not perfectly bilaterally symmetrical; the visceral organs are displaced to the left by the large right shell adductor muscle (Yonge, 1947) and, although both right and left kidneys are present, they are morphologically and functionally disparate (Harrison, 1962) and have lost specific association with the right or left side vasculature (Crofts, 1929; Bourne and Redmond, 1977; Russell and Evans, 1989). However, the bipectinate right and left gills (ctenidia) are morphologically similar (Crofts, 1929), are supplied from a common haemolymph sinus and deliver haemolymph into the left and right auricles of the heart, respectively. Thus, the gills and associated vasculature are believed to closely resemble the ancestral condition.

Recent investigations of gas exchange in *Haliotis iris* have demonstrated that the gills are responsible for essentially all oxygen uptake under both normoxia and hypoxia and are capable of high oxygen extraction efficiencies (Taylor and Ragg, 2005). The authors have also shown that ventilation, perfusion and diffusion are well matched in the right gill of *H. iris* (Ragg and Taylor, in press). The current study extends these observations by considering the overall efficiency of the two gills and, in particular, the capacity of the gill system to accommodate increased oxygen demand by adjustments to branchial haemolymph flow following a period of internal hypoxic stress. Changes in left and right post-branchial oxygen pressures were measured by sampling from chronic indwelling cannulae, and haemolymph flows in the left and right efferent ctenidial veins were recorded by means of pulsed Doppler flow probes. It is demonstrated that the circulatory responses of the left and right gills are, in fact, quite different.

Materials and methods

Collection and holding systems

Adult *Haliotis iris* (250–615 g, mean 367.8 g) were collected from South Bay, Kaikoura (New Zealand) and

acclimated to a 15°C recirculating seawater system for 2 months prior to experimentation. The animals received a diet of Adam & Amos™ AAFD pellets (Mount Barker, South Australia) and *Gracilaria* spp. fragments fed *ad libitum*.

Experimental design

Measurements were made on animals in three states, defined as follows.

(1) *Stressed*. The condition of the abalone in the period up to 2 h after immersion in the experimental system having just endured approximately 1 h of emersion (environmental hypoxia), desiccation and physical disturbance associated with the insertion of cannulae and flow probes.

(2) *Recovering*. 12–24 h after cannulation, abalone generally clamped, with cephalic and epipodal tentacles withdrawn ('quiescent' in the system of Donovan and Carefoot, 1998).

(3) *Resting*. Undisturbed for at least 24 h following cannulation, alert with all tentacles extended ('alert', using the definitions of Donovan and Carefoot, 1998).

Sets of measurements of oxygen partial pressure in haemolymph samples from the left and right efferent ctenidial veins ($P\mathrm{a}_{\mathrm{O}_2}$), and in the exhalant water, were obtained in triplicate for each animal in each of the above states. Continuous records of haemolymph flow in each vessel and heart rate were obtained throughout the period of haemolymph sampling (2–3 min per sample). On several occasions, animals exhibited avoidance behaviour, either clamping to the chamber floor or raising and twisting the shell. Measurements were also made under these activity conditions.

Animal preparation

Abalone were starved for 24 h prior to manipulations, which took place in moist air at 4–6°C. A high-speed diamond grinding wheel (Dremel™; Emerson Electric Co., St Louis, MO, USA) was used to cut two openings in the shell. One opening (20×10 mm) exposed the right efferent ctenidial vein posterior to the shell apertures. A second (40×10 mm), cut ventrally to the first, exposed the left mantle surface. Two 1.5 mm holes were drilled through the shell either side of the pericardial region, and impedance leads (0.2 mm insulated copper wire, coiled bared ends contacting the mantle epidermis) for recording of cardiac activity were inserted and secured with cyanoacrylate glue. The animals were given a minimum of 2 days to recover in the holding system.

Once recovered, i.e. alert and feeding and showing no sign of tissue damage or haemorrhage, each abalone was cannulated, and customised Doppler probes were inserted to measure blood velocity. A 1 mm^2 crystal sub-assembly (Iowa Doppler Products, Iowa City, IA, USA) was encased in epoxy resin and attached to a 5 mm section of 0.86 mm (i.d.) polyethylene tubing. PVC wings were attached to the tubing to prevent rotation once in position against the vessel wall. The cannula, a 20 cm length of 0.6 mm (i.d.) × 0.8 mm (o.d.) PE tubing with a tapered end was threaded through the sleeve of the pulsed-Doppler assembly. A 23-gauge needle was used to puncture the right efferent ctenidial vein approximately 10 mm anterior to the pericardium, and 5 mm of cannula was inserted retrograde to haemolymph flow. The cannula was tested for patency and secured to the shell with cyanoacrylate glue. The Doppler crystal assembly was then manoeuvred onto the mantle surface immediately posterior to the cannula insertion point, and its leads secured to the shell by means of a friction mount, permitting subsequent minor adjustments in crystal position. The left efferent ctenidial vein, located by displacing the mantle roll dorsally, was prepared in a similar way, using a U-shaped cannula.

The cannulae were stoppered, the impedance and Doppler leads connected and the animal placed into an experimental chamber. Each chamber consisted of a 1.0-litre circular polycarbonate bowl supplied with 15°C seawater from a recirculating reservoir. The cardiac leads were connected to an impedance coupler (A100; Strathkelvin Instruments, Glasgow Strathclyde, UK), and Doppler signals were processed using a directional pulsed-Doppler flowmeter (20 MHz; 545C-4; Bioengineering, Iowa City, IA, USA). Output was digitally recorded using PowerLab™ 4/20 (ADInstruments, Bella Vista, NSW, Australia) data acquisition hardware and Chart™ 4.1.2 (ADInstruments) software.

Haemolymph flow measurement

Signals from the left and right efferent ctenidial probes were acquired simultaneously. Minor adjustments were made to the crystal positions and range settings until a maximal signal was obtained. The mean Doppler output was calculated by integration over the whole sampling period (~5 min).

Calibration of the Doppler signal in terms of volumetric flow was achieved by two methods. *In situ* calibration was attempted for each crystal placement. At the end of the experiment the abalone was decapitated and the ventricle cut to create a low-resistance outflow path. A suspension of zeolite particles (<80 μm, filtered barbecue deodorizer) in seawater was passed through each cannula at a range of preset flows *via* a peristaltic pump, and the mean Doppler signals were recorded and used to create the calibration curve.

In the second calibration technique, geometric assumptions were used to calculate the flow velocity using the 'Doppler equation' (University of Iowa Bioengineering, 1986; Levick, 1991):

$$V = F_{\mathrm{d}}C / 2F_{\mathrm{o}}\cos A\ , \qquad (1)$$

where V is the mean velocity of blood across the vessel diameter ($\mathrm{mm\ s^{-1}}$), F_{d} is the Doppler shift frequency (in this case 0.5 V≡1 kHz shift), C is the velocity of sound in blood (1 565 000 $\mathrm{mm\ s^{-1}}$), F_{o} is the transmitter frequency (20 000 kHz) and A is the angle between the beam and the blood velocity vector (assumed to be 45°).

Vessel cross-sectional area was determined by low-pressure injection of amaranth-stained gelatin dissolved in seawater (1:15 w/w). Transverse sections of the fixed tissues (70% ethanol) were photographed, and internal cross-sectional area (Fig. 1A) was measured using image analysis software (Scion Image™ beta 4.0.2; www.scioncorp.com). The product of

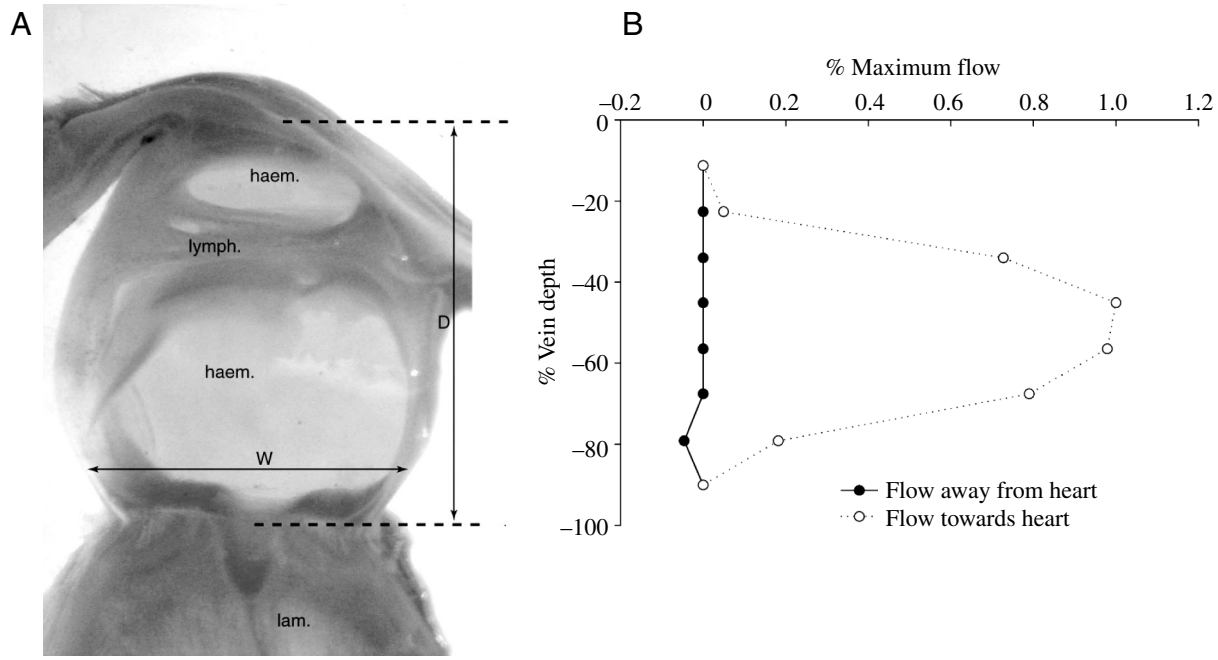

Fig. 1. (A) Transverse section of the right efferent ctenidial vein of *Haliotis iris* filled with amaranth-stained gelatin. Haemocoelic spaces (haem.) and 'lymphoid' tissue (lymph.) are marked, along with the depth (D) and width (W) dimensions. (B) Velocity flow profile with respect to probe depth range from a representative animal, showing a parabolic distribution. Points are mean flow velocities expressed as a fraction of the peak measured velocity.

vessel cross section and mean velocity thus gave an estimate of mean flow.

The Doppler equation assumes that the vessel is circular in section and that blood flow is laminar with a parabolic profile. In such cases, the maximum midstream flow is twice the mean flow velocity (University of Iowa Bioengineering, 1986; Vogel, 1994). The efferent ctenidial veins of abalone clearly are not perfectly circular due to the presence of a thick band of connective and 'lymphoid' tissue transecting the lumen (Fig. 1A; Crofts, 1929). The assumption of parabolic flow was therefore tested by plotting the Doppler signal strength against focal depth, which was increased in 50 mV (0.5 mm penetration) increments.

Geometric flow estimates were more variable and lower (74.5±11.7%, N=13) than those determined by known-flow calibration. Geometrically calibrated flows were accordingly corrected using this factor to provide the best estimate of flow and only used when known-flow calibration was unavailable.

Oxygen partial pressure and content

A syringe (Hamilton Gastight™; 25-gauge) was used to withdraw 100 μl of haemolymph from the cannula line to clear dead-space and 'prime' the oxygen electrode (MI730; Microelectrodes Inc., Bedford, NH, USA; Cameron water jacket at 15°C; Cameron Instruments, Guelph, ON, Canada; 781b oxygen meter; Strathkelvin Instruments). A further 100 μl were then withdrawn and injected into the electrode chamber, and P_{O_2} was recorded after a 2 min stabilizing period. As left and right efferent ctenidial samples could not be taken simultaneously, they were taken in random order, within 5 min of each other. Seawater P_{O_2} was also measured in conjunction with each set of haemolymph samples. Circulating water in the experimental container was taken to represent inhalant P_{O_2}. Branchial chamber water was sampled *via* a PVC cannula briefly inserted ~2 mm through the second oldest patent shell hole; this was assumed to represent exhalant water (after Volzow, 1983).

Oxygen contents (C_{O_2}; mmol l^{-1}) of haemolymph samples were calculated from P_{O_2} values (mmHg; 1 mmHg≡133 Pa) using oxygen binding curves determined for the same population of *H. iris*. As haemolymph P_{O_2} values observed here (80–105 mmHg) lay well above the P_{50} for *H. iris* haemocyanin (4–12 mmHg; Behrens et al., 2002), a linear relationship provided a satisfactory fit (slope 0.00199±0.00033 mmol l^{-1} $mmHg^{-1}$; intercept 0.178±0.022 mmol l^{-1}; N=139). Oxygen convection in the efferent ctenidial flow was therefore calculated as the product of C_{O_2} and flow rate.

Data are expressed as means ± standard error of the mean (s.e.m.). Statistical analyses were carried out using Statistica™ 6.0 software (StatSoft, Inc., Tulsa, OK, USA), using model I analysis of variance (ANOVA) with replicate samples nested within individual animal, followed by Fisher's least significant difference pairwise comparison of means. Statistical significance was accepted at P<0.05.

Results

A total of 29 abalone yielded data in this experiment. Records from six were subsequently discarded due to tissue damage, cannula leakage or poor physiological condition. All

parameters examined showed significant inter-individual variability.

Doppler calibration

A transverse flow profile was constructed for the right efferent ctenidial vein of 12 individuals. In all cases, a reasonable approximation of parabolic flow was observed (Fig. 1B).

Haemolymph flow

In stressed animals immediately following handling, total haemolymph flow through both gills was 24.4±3.6 ml kg^{-1} min^{-1} (N=13). This increased non-significantly to 25.8±5.7 ml kg^{-1} min^{-1} (N=8) in recovering animals and declined highly significantly to 9.1±2.1 ml kg^{-1} min^{-1} at rest (N=5, P<0.001).

There were marked differences in the relative perfusion of the left and right gills dependent upon animal state (Fig. 2). In stressed animals, mean right gill haemolymph flow was 10.77±1.37 ml kg^{-1} min^{-1} (N=14). During the recovery period, mean right gill flow was 5.86±1.59 ml kg^{-1} min^{-1} (N=9) and in settled animals it was 8.56±1.95 ml kg^{-1} min^{-1} (N=6). These values were not significantly different. By contrast, mean left gill flow was 13.32±2.63 ml kg^{-1} min^{-1} (N=14) in stressed animals, this was elevated (non-significantly) to 18.20±5.75 ml kg^{-1} min^{-1} (N=9) during recovery but decreased highly significantly by a factor of more than 30 to 0.41±0.34 ml kg^{-1} min^{-1} (N=6) in settled animals (P<0.001). That is, at rest, more than 95% of the respiratory haemolymph flow was carried by the right gill.

Haemolymph oxygenation

The mean P_{O_2} of haemolymph leaving the gills exhibited an inverse relationship with flow (Figs 2, 3). Right gill efferent P_{O_2} did not change significantly between the stressed, recovering or resting states (87.3±4.7 mmHg, N=11; 95.5±3.8 mmHg, N=10; 85.5±6.8 mmHg, N=7, respectively). Statistically similar values were also recorded in the left gill efferent haemolymph in stressed and recovering animals (81.9±6.1 mmHg and 88.2±3.9 mmHg, respectively). By contrast, the left efferent haemolymph P_{O_2} of resting animals (105.3±10.2 mmHg) was significantly higher than that of the right efferent (P<0.001) and similar to that of the exhalant seawater (104.5±3.1 mmHg). Exhalant P_{O_2} did not change significantly under stress (103.5±4.2 mmHg) or recovery (99.1±2.4 mmHg). Mean inhalant seawater P_{O_2} was 148.7±0.9 mmHg.

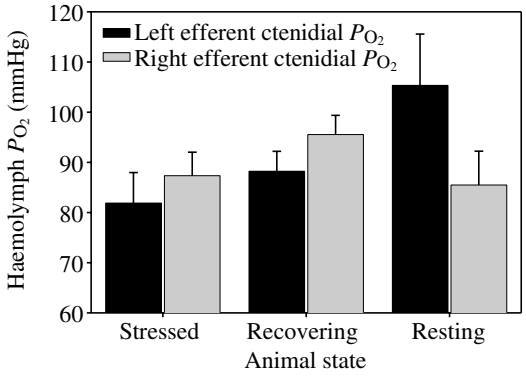

Fig. 3. Mean oxygen partial pressure (P_{O_2}) measured in the post-branchial haemolymph of the left and right gills of stressed, recovering and resting *Haliotis iris*. Values are means ± s.e.m.

Haemolymph P_{O_2} values were used to calculate oxygen contents from oxygen binding data (see Materials and methods) and were combined with efferent ctenidial flow rates to estimate the total output of oxygen from the gills (Fig. 4). In stressed abalone, the total post-branchial oxygen convection was 0.0075±0.0015 μmol g^{-1} min^{-1} falling significantly to 0.0027±0.0006 μmol g^{-1} min^{-1} in settled animals. Despite the higher post-branchial haemolymph P_{O_2} recorded in the left gill of settled animals, its contribution to total oxygen uptake was minimal because of the very low haemolymph flow.

Heart rate

No significant change in heart rate was associated with the greatly decreased gill perfusion in resting animals compared

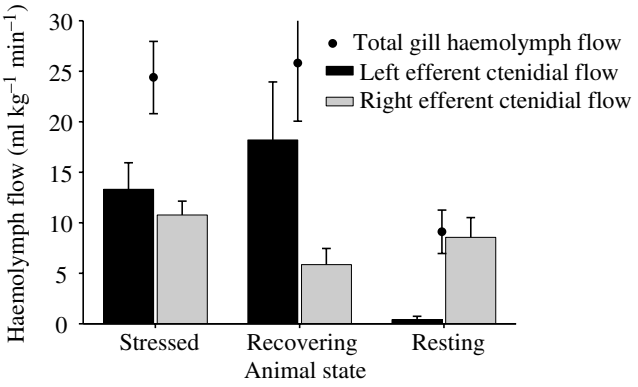

Fig. 2. Live mass-standardised haemolymph flow rates leaving the left and right gills of stressed, recovering and resting *Haliotis iris*. Values are means ± s.e.m.

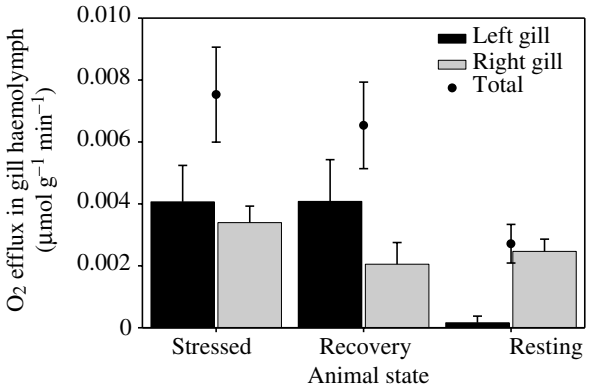

Fig. 4. Estimated oxygen content of post-branchial haemolymph leaving the left and right gills of *Haliotis iris* during conditions of stress, recovery and at rest. Values are means ± s.e.m.

with stressed animals (28.5±0.9 min^{-1} and 28.2±1.1 min^{-1}, respectively). A small but significant increase in heart rate was observed during the recovery period (31.8±0.8 min^{-1}; P=0.009).

The effects of activity

In 12 individuals, spontaneous activity was displayed during a period of data recording. Responses varied but some general patterns were apparent. At the onset of clamping (Fig. 5) or twisting (Fig. 6), the heartbeat became erratic or was arrested. During twisting (Fig. 6), the impedance signal was disrupted by the large body movements, but efferent ctenidial flow clearly fell to zero in the left gill and became intermittent in the right gill. A normal impedance signal was rapidly re-acquired after cessation of twisting (<20 s). Resumption of the regular heartbeat was followed by flow through the right and then the left gill, typically after an interval of 5–15 cardiac cycles. In animals that clamped for more than ~60 s, this recovery pattern ensued regardless of whether the animal released its clamp or not (Fig. 5).

Discussion

Regulation of oxygen uptake

Considering the paired gills of *Haliotis iris* as a single gas exchanger, respiratory haemolymph flow was increased nearly threefold relative to resting flow rate following the placement of cannulae and flow probes and during a subsequent recovery period lasting many hours. These abalone had experienced an extended period of combined environmental and functional asphyxia resulting from collapse of the gills during emersion and muscular activity in response to handling. As shown previously, these conditions produce a state of oxygen debt associated with the intracellular and extracellular accumulation of anaerobic metabolites such as tauropine and D-lactate (Gäde, 1988; Baldwin et al., 1992; Wells and Baldwin, 1995; Behrens et al., 2002).

The increased branchial blood flow would tend to promote increased oxygen uptake from the seawater and may be interpreted as one component of a suite of responses that contribute to the metabolic scope of abalone. Oxygen consumption was not measured in the present experiments so it remains unclear whether this was actually enhanced in the recovering animals. As discussed elsewhere (Taylor and Ragg, 2005), external water currents are required to augment the endogenous ciliary ventilation for maximum aerobic scope in *H. iris*. Nevertheless, increases in oxygen uptake by factors of 2.5–3.5 have been observed in crawling *H. kamschatkana* (Donovan and Carefoot, 1997, 1998) and by a factor of 1.9 by *H. laevigata* in response to elevated environmental ammonia (Harris et al., 1998).

In the absence of external water currents, the mean rate of oxygen uptake from seawater was previously reported as 0.47 μmol g^{-1} h^{-1} for intact settled *H. iris* (Taylor and Ragg,

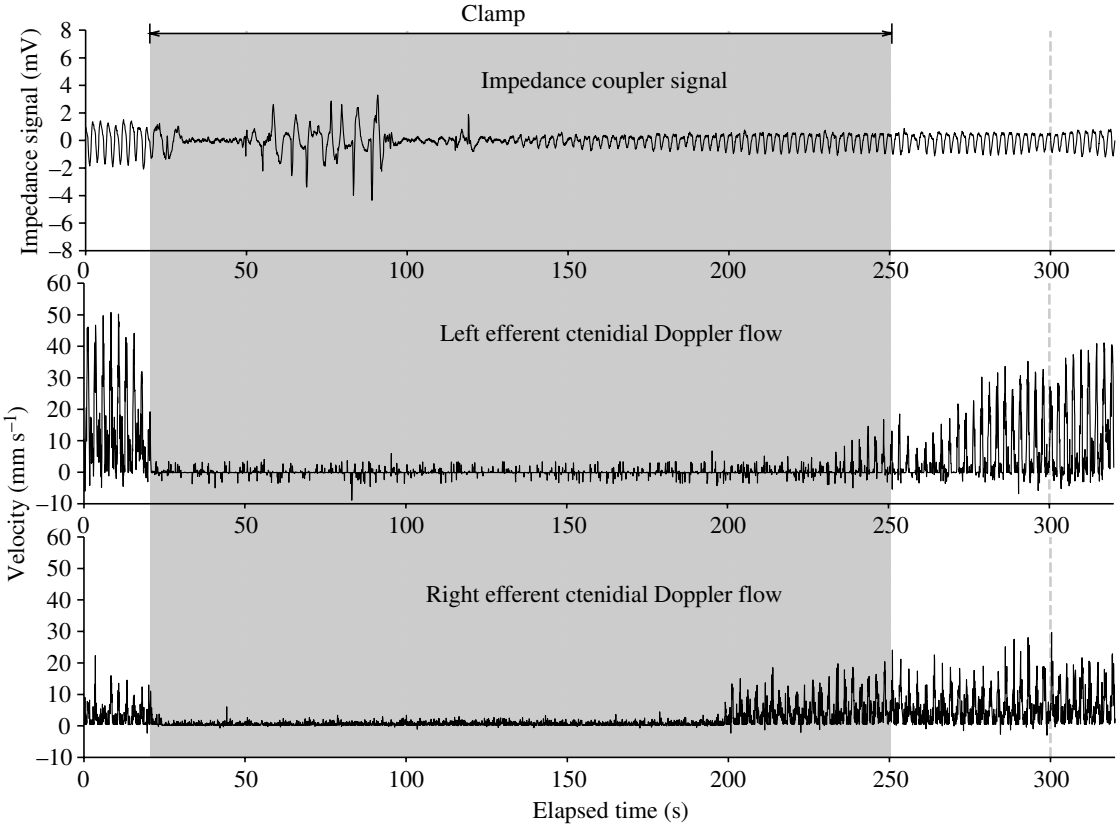

Fig. 5. Record showing the effect of clamping to the substratum on heart rate and post-branchial haemolymph flow in *Haliotis iris*. The shaded region represents a period of sustained spontaneous clamping.

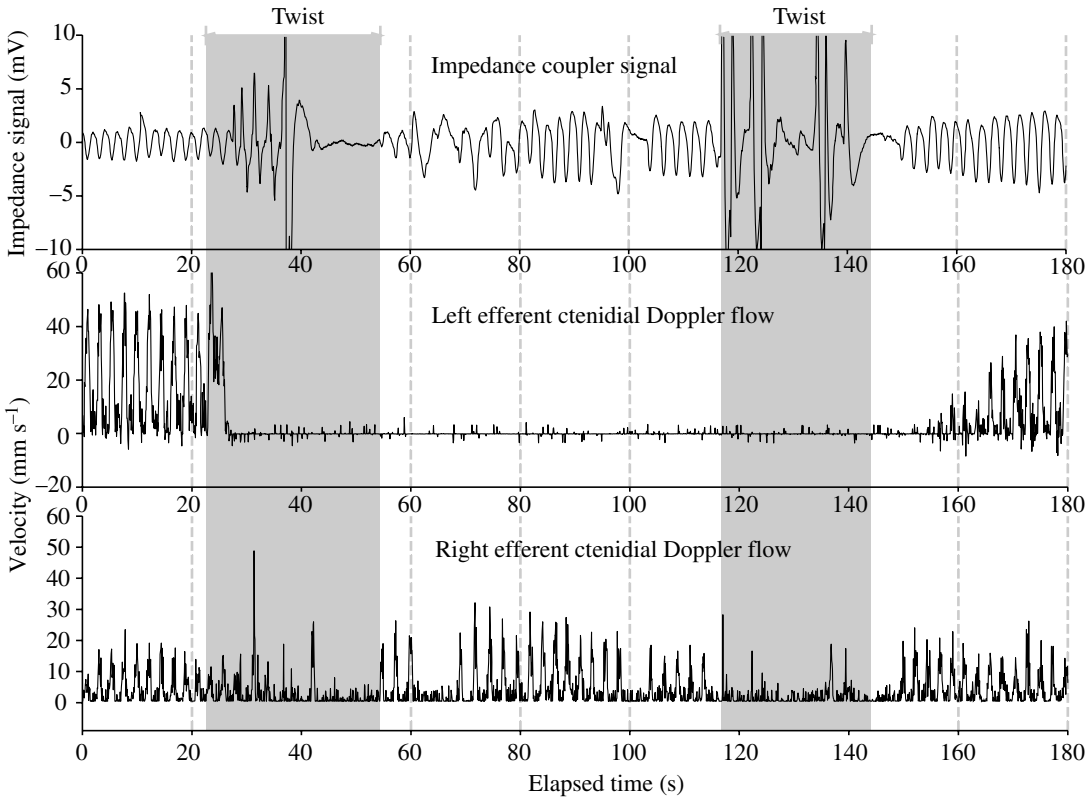

Fig. 6. Record showing the effect of twisting on heart rate and post-branchial haemolymph flow in *Haliotis iris*. The shaded regions represent bouts of spontaneous twisting activity.

2005) and slightly higher in recently cannulated abalone (0.54 μmol g^{-1} h^{-1}; Ragg and Taylor, in press). Based on the flow rates and estimated oxygen contents of haemolymph in the efferent ctenidial veins, the total convection of oxygen (bound and dissolved) in the post-branchial haemolymph was only 0.16 μmol g^{-1} h^{-1} in settled abalone and 0.45 μmol g^{-1} h^{-1} in stressed animals (Fig. 4). Care was taken to standardize conditions with those of previous respirometry trials: the possibility remains, however, that animals in the current experiment displayed unusually low oxygen uptake rate ($\dot{M}_{O_2}$). The authors suggest, however, that given that a substantial venous reserve is usually present (Ragg and Taylor, in press), these data imply that a significant proportion of oxygen uptake may not enter the post-branchial haemolymph. As virtually the whole of oxygen uptake is known to be abstracted from the branchial water flow (Taylor and Ragg, 2005), the possibility of direct uptake by metabolically active integumental surfaces within the branchial chamber (e.g. ciliary epithelia, mucous glands), or adjacent organs, requires further investigation.

Apart from a minor vascular route through the right mantle, the whole of the systemic venous return to the heart passes through the left and right gills (Crofts, 1929; Ragg, 2003; Taylor and Ragg, 2005). Thus, total gill flow, which increased 2.7 times from 9.1 ml kg^{-1} min^{-1} at rest to 24.4 ml kg^{-1} min^{-1} in stressed abalone, would have corresponded closely to cardiac output under these conditions. However, cardiac frequency was constant at about 28 min^{-1} confirming previous observations (Taylor and Ragg, 2005) of its insensitivity to disturbance and activity status. It is therefore deduced that cardiac compensation in *H. iris* resulted solely from adjustments to stroke volume, i.e. increasing from 0.37±0.15 ml kg^{-1} live mass at rest to 0.99±0.21 ml kg^{-1} in disturbed abalone.

Heart rates recorded for *H. iris* are slightly higher than those of the similar sized abalone *H. rubra* (25 min^{-1}; Russell and Evans, 1989) and *H. corrugata* (21 min^{-1}; Bourne and Redmond, 1977). Heart rate more closely resembles that of the smaller *H. discus hannai* (32 min^{-1} at 15°C; Fujino et al., 1984). The smaller (~50 g) and relatively active gastropod *Hemifusus tuba* showed a slower heart rate than *H. iris* (~18 min^{-1} at 15°C; Depledge and Phillips, 1986) but a larger stroke volume, resulting in a cardiac output of approximately 50 ml kg^{-1} min^{-1}. Resting decapod crustaceans of a corresponding size to *H. iris* typically exhibit rather higher rates of 30–50 min^{-1}; however, as with *H. iris*, increased stress or activity have minimal effect on heart rate (McMahon and Wilkens, 1983).

Heterogeneity in right and left gill perfusion

An important conclusion from the present study is that adjustments to cardiac output and respiratory blood flow were

accommodated almost entirely by changes in the perfusion of the left gill – from more than 75% of total flow in recovering abalone to less than 5% at rest. Heterogeneous perfusion, and circulatory switches between different gas exchangers, occur in animals in which there are clear differences in exchanger morphology, e.g. in relation to bimodal respiration in crustaceans (Taylor and Greenaway, 1984; Taylor and Taylor, 1992) and vertebrates (Johansen, 1982) but this appears to be the first report with respect to paired organs.

These changes in respiratory blood flow probably were not accompanied by corresponding changes in ventilation, as endogenous ciliary ventilation was similar in recently handled abalone and after settling overnight (Taylor and Ragg, 2005). In fishes, regulation of gas exchange does not involve shutdown of whole gills, but at rest the number of perfused lamellae may be reduced by as much as 60% (Jones and Randall, 1978; Randall and Daxboeck, 1984; Farrell and Jones, 1992). As in abalone, the under-perfused lamellae are still ventilated. Similarly, amphibians reliant upon cutaneous gas exchange may recruit additional surface capillaries during increased O_2 demand, in the absence of obvious augmentation to ventilation (Feder, 1995).

In decapod crustaceans, a superficially analogous shutdown of gas exchange in one branchial chamber occurs during bouts of unilateral scaphognathite ventilation, although no left or right bias has been noted (Mangum, 1983; McMahon and Wilkens, 1983). In decapods, preferential perfusion of the ventilated gills may be effected by the associated changes in branchial transmural pressures (Taylor, 1990) but it is unlikely that flow ceases on the non-ventilated side. Non-perfusion of a gas-exchanger module is probably not a viable strategy for crustaceans and other higher taxa because of the attendant risk of thrombosis. By contrast, gastropod haemolymph lacks clotting factors (Armstrong et al., 1971; Taylor et al., 1994).

The gills of *H. iris* are supplied from a common venous compartment, the basibranchial sinus (Crofts, 1929; Ragg, 2003). As the constant volume model for the operation of the molluscan heart (Ramsay, 1952; Fretter and Graham, 1994) precludes any obvious mechanism for differential aspiration by the left and right auricles, large changes in the relative flow through the left and right gills must be associated with changes in the relative resistance of the perfusion paths. The contractility and vasoactivity of molluscan vessels in response to neuropeptides and bioamines is well established (e.g. *Aplysia*, Brownell and Ligman, 1992; *H. kamtschatkana*, Krajniak and Bourne, 1987, 1989). Varicose nerve fibres showing 5-hydroxytryptamine immunoreactivity are present in the walls of blood vessels of *H. rubra*, including the afferent ctenidial veins (Russell and Evans, 1989). However, a marked pulsatility observed in the reduced left gill flow suggests that resistance changes occurred in the gill itself rather than downstream in the efferent ctenidial veins. The vessels associated with the gills of *H. iris* are certainly muscular and are capable of lumen adjustment (N.L.C.R., unpublished observation). Longitudinal muscle blocks associated with cartilage on either side of the efferent ctenidial veins could obliquely distort the ctenidia and affect efferent drainage and therefore are also potential sites of flow control (Ragg, 2003). Clearly, the relative responsiveness of abalone ctenidia to vasoactive agents deserves further investigation.

In the constant volume model (Ramsay, 1952; Fretter and Graham, 1994), ventricular systole causes passive expansion of the two approximately equal-sized auricles by transmission of negative pressure through the pericardial fluid. Thus, reduced flow in the left efferent ctenidial veins could potentially deprive the left auricle of haemolymph. However, this is probably not the case. The left auricle is connected to the right efferent ctenidial vein by a valved vascular route passing through the left kidney (Crofts, 1929; Ragg, 2003). It therefore appears that when the left branchial resistance is increased, haemolymph is partially diverted from the right efferent ctenidial vein to the left auricle. In support of this suggestion is the observation that casting resin perfused at low pressure into the right efferent ctenidial vein preferentially passes through the left kidney and fills the left auricle before the right.

Gill perfusion during activity

Bourne and Redmond (1977) noted that the onset of activity in *H. corrugata* caused a transient hydrostatic pressure spike throughout the vascular system. As no pressure gradient was developed, they predicted that haemolymph flow would not be assisted by these muscular contractions. Direct flow recordings taken from the gills of *H. iris* during twisting and clamping corroborate this suggestion, and in fact branchial haemolymph flow was reduced due to cardiac arrest (Figs 5, 6). Aortic pressure traces from *H. midae* also show evidence of cardiac arrest with the onset of clamping (Trueman and Brown, 1985). Cardiac arrest may help prevent damage to the heart by pressure surges, as proposed for the tarantula *Eurypelma californicum* during hydraulic leg extension (Paul, 1986). Interestingly, when avoidance activity was sustained in *H. iris*, normal heart rate resumed, followed by renewed flow through the right gill. However, the left gill typically remained unperfused until activity ceased (Figs 5, 6).

Diffusion limitation in right and left gills

In an earlier study (Ragg and Taylor, in press), the performance of the right gill was analyzed in terms of oxygen extraction, ventilation perfusion ratio, diffusive conductance and the diffusion limitation index, L_{diff} (Piiper, 1982). Based on these criteria, it was concluded that the right gill operated efficiently and near optimally. Right gill L_{diff} values of ~0.5 were obtained for settled abalone, indicating that neither perfusion nor the diffusive conductance of the gill prevailed to limit the rate of oxygen transfer. Prebranchial oxygen partial pressure measurements were not obtained in the present study but, using the earlier values for resting animals (37.3±3.6 mmHg) and values obtained from a separate subset of abalone subjected to emersion and exercise (32.2±3.8 mmHg, N=12; N.L.C.R., unpublished observations), the L_{diff} of the right gill was estimated to be 0.40±0.6 and

0.44±0.8 for resting and stressed animals, respectively. By contrast, the left gill was highly perfusion limited at rest, with an estimated L_{diff} of 0.14±0.07. This rose to 0.46±0.07 in stressed animals. Adjustments to perfusion of the left gill therefore play an important role in the control of oxygen delivery in this animal.

Conclusions

An unusual gas exchange strategy has been revealed in the abalone *Haliotis iris*. The right gill appears to be perfused at a fairly constant rate, regardless of demand. The left gill, however, is chronically under-perfused in resting *H. iris*, to the extent that haemolymph flow almost ceases. Thus, oxygen taken up by the right gill, plus direct oxygen diffusion into peripheral tissues, effectively supports the routine metabolism of the abalone. During periods of increased oxygen demand, perfusion rates in the left gill increase approximately 30-fold, matching oxygen uptake of the right gill. Changes in oxygen uptake at the left gill therefore effectively support the metabolic scope of the abalone. The mechanisms determining differential gill resistance warrant further investigation, in particular the role of neurohormones. The greater flexibility in blood flow exhibited by the left gill is of interest in relation to the evolutionary abandonment of the paired gill design, i.e. the loss of the right gill by higher gastropods. Comparative studies examining the strategies utilised by pectinibranch snails to regulate oxygen uptake would therefore be of considerable interest.

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