The Journal of Experimental Biology **199**, 1791–1800 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0304

CARDIORESPIRATORY SYNCHRONY IN TURTLES

TOBIAS WANG* AND JAMES W. HICKS
Department of Ecology and Evolutionary Biology, University of California at Irvine, Irvine, CA 92717, USA

Accepted 29 April 1996

Summary

Many reptiles, particularly diving species, display characteristic cardiovascular changes associated with lung ventilation (cardiorespiratory synchrony). Previous studies on freshwater turtles show that heart rate and pulmonary blood flow rate ($\dot{Q}_{pul}$) increase two- to fourfold during ventilation compared with breath-holding, and some studies report concomitant decreases in systemic blood flow rate ($\dot{Q}_{sys}$). The primary aim of this study was to provide a detailed description of cardiorespiratory synchrony in free-diving and fully recovered turtles (*Trachemys scripta*). During breath-holds lasting longer than 5 min, $\dot{Q}_{pul}$ averaged 15 ml min^{-1} kg^{-1} and increased more than threefold to a maximum value of 50 ml min^{-1} kg^{-1} during ventilation. $\dot{Q}_{sys}$ also increased during ventilation compared with during breath-holds lasting longer than 5 min (from 44 to 73 ml min^{-1} kg^{-1} during ventilation). Neither $\dot{Q}_{pul}$ nor $\dot{Q}_{sys}$ was affected by the number of breaths in the ventilatory periods. Changes in $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ were accomplished entirely through a significant increase in heart rate during ventilation, while total stroke volume (systemic + pulmonary) remained constant. Irrespective of the ventilatory state, $\dot{Q}_{sys}$ exceeded $\dot{Q}_{pul}$ by 20–30 ml min^{-1} kg^{-1}. Nevertheless, because $\dot{Q}_{pul}$ increased relatively more than $\dot{Q}_{sys}$ during ventilation, $\dot{Q}_{pul}/\dot{Q}_{sys}$ increased from 0.29 during apnoea to 0.80 during lung ventilation. This study confirms cardiorespiratory synchrony in the turtle *Trachemys scripta* but, in contrast to earlier studies, a net right-to-left cardiac shunt prevailed regardless of ventilatory state.

Key words: reptile, turtle, *Trachemys scripta*, cardiovascular, intracardiac shunts, cardiorespiratory coupling.

Introduction

Ventilation in most ectothermic vertebrates is episodic and, particularly in aquatic species, the ventilatory pattern can be characterized as brief ventilatory bouts consisting of a variable number of breaths interspersed with breath-holds of variable duration (e.g. Glass *et al.* 1983; cf. Milsom, 1991). Commonly, the episodic breathing pattern is associated with characteristic changes in central vascular blood flow, an association often referred to as cardiorespiratory synchrony. For example, in vertebrate groups such as lungfish, anuran amphibians, turtles, crocodiles and snakes, pulmonary blood flow ($\dot{Q}_{pul}$) and heart rate increase several-fold during ventilation compared with during breath-holding (White and Ross, 1966; White, 1968; Johansen *et al.* 1970; Shelton, 1970; Lillywhite and Donald, 1989). These changes in central vascular blood flows result from both cholinergic and adrenergic mechanisms (see Hicks, 1994) and, at least in turtles, the increase in $\dot{Q}_{pul}$ during ventilation is mediated through a reduction in pulmonary vascular resistance (Burggren, 1977; Milsom *et al.* 1977).

Cardiorespiratory synchrony, including the attendant changes in cardiac shunt patterns, are particularly well described for freshwater turtles. With one exception (Heisler and Glass, 1985), all studies on resting animals indicate that the right-to-left (R–L) cardiac shunt increases during breath-holding relative to ventilation, and some studies showed large increases in the left-to-right (L–R) cardiac shunt during ventilation (White and Ross, 1966; Shelton and Burggren, 1976; Burggren and Shelton, 1979; White *et al.* 1989). These changes in cardiac shunt have important implications for arterial blood gas composition (e.g. Wood, 1982), but the functional role of cardiac shunts remains to be determined (e.g. Burggren, 1985; Hicks and Wang, 1996).

Previous studies on cardiorespiratory synchrony in turtles provide information on the changes in blood flows during ventilation and breath-holding (Burggren, 1975; Shelton and Burggren, 1976; West *et al.* 1992), but a detailed description regarding the changes in central vascular blood flows during short compared with long breath-holds is not available. Similarly, it is not known whether ventilatory periods consisting of many breaths, on average, are accompanied by larger cardiovascular changes than ventilatory periods consisting of few breaths. Such information is important in order to understand the integration of ventilatory and cardiovascular control and would allow us to predict cardiovascular changes following changes in breathing pattern.

*Present address: Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, Canada V6T 1Z4.

The primary objective of this study is to provide a detailed description of cardiorespiratory synchrony in free-diving and fully recovered turtles.

Materials and methods

Animals

Seven freshwater turtles *Trachemys scripta* Gray (1.2–2.0 kg) were obtained from Lemberger Inc. (Oshkosh, WI, USA) 2–6 weeks before experimentation. During this period, they were kept in large aquaria, under a 12 h:12 h L:D photoperiod, with free access to dry basking areas and infrared lamps to allow behavioural thermoregulation. The turtles were fed goldfish *ad libitum*, but food was withheld for 3 days before surgery.

Anaesthesia and surgery

Turtles were intubated by inserting a short piece of soft rubber through the glottis. Thereafter, the turtles were artificially ventilated at a rate of 8–15 breaths min^{-1} at a tidal volume of 10–20 ml kg^{-1} using a SAR-830 CWE Inc. ventilator (Ardmore, PA, USA) with a gas mixture consisting of 30 % O_2, 3 % CO_2 (balance N_2) prepared with a gas-mixing flowmeter (GF-3, Cameron Inst., TX, USA). This mixture passed through a Halothane vaporizer (Dräger, Lubeck, Germany) set at 3–4 % to induce anaesthesia. After 5–15 min, the turtles stopped responding to pinching of the legs and the Halothane level was reduced to 0.5–1 %, where it was maintained throughout surgery. When the surgery had been completed, artificial ventilation (with a gas mixture consisting of 30 % O_2, 3 % CO_2, balance N_2) was continued until the turtle regained consciousness and started breathing on its own. The turtle was then placed in a small holding tank. No experiments were conducted within 48 h after surgery. All experiments were conducted at 25 °C.

To implant blood flow probes, the heart and central blood vessels were exposed by removing a 4 cm×5 cm portion of the plastron with an electric bone saw. Attached muscles were gently removed from the plastron piece and bleeding (if any) was stopped by either cauterization or suturing the damaged vessel(s). Short sections (1–1.5 cm) of the central blood vessels were freed from connecting tissue to allow placement of four blood flow probes (for locations, see below). Acoustic coupling gel was infused around the probes to enhance the signal and, in some cases, flow probes were secured by sutures to surrounding connective tissue. In addition, a small blood vessel branching from the left subclavian artery was occlusively cannulated with PE 50 tubing filled with heparinized saline (100 i.u. ml^{-1}) to prevent blood coagulation. The cannula was moved forward into the subclavian artery before being secured in place with one or two sutures. Leads from the probes, and the catheter, were carried out ventrally and strapped to the carapace. Finally, the excised piece of plastron was glued in place with silicone and two-component epoxy glue. All turtles appeared healthy and all were used in subsequent experiments for up to 3 months following surgery. Ultimately, all turtles were killed by injections of Nembutal (according to University of California guidelines) in order to recover the blood flow probes.

Placement of flow probes and measurement of blood flow rates

Blood flows were measured using 2R transit-time ultrasonic blood flow probes (Transonic System, Inc., Ithaca, NY, USA) constructed for vessel diameters ranging from 1.8 to 2.5 mm. These flow probes were calibrated at the factory at 25 °C and we periodically verified the calibration by generating known flows through either excised vessels or polyurethane tubing.

The four blood flow probes were implanted around the left pulmonary artery (LPA), the left aortic arch (LAo), the right branch of the right aortic arch (RRAo) and, finally, one probe was placed around both the right subclavian (Rsub) and the right carotid artery (Rca). At the position of probe placement, the Rsub and Rca lie parallel and both blood vessels could be inserted into a single flow probe without distorting their normal positions. The use of a single probe for simultaneous measurement of blood flow in two vessels has been validated by Akagi *et al.* (1987) and has been successfully employed previously in anaesthetized turtles (Comeau and Hicks, 1994). Nevertheless, we determined the validity of this arrangement in a separate series of experiments. In these experiments, blood flow in the subclavian and carotid arteries was measured as described above. In addition, the distal portion of the subclavian artery was placed within a single flow probe and the distal carotid artery was also placed within a single flow probe and blood flows were altered pharmacologically by intravenous injection of acetylcholine (0.1 mg kg^{-1}) or isoproterenol (0.1–0.3 μg kg^{-1}). The sum of the independently measured subclavian ($\dot{Q}_{Rsub}$) and carotid blood ($\dot{Q}_{Rca}$) flows was compared with the blood flow measured when both arteries were placed within a single probe ($\dot{Q}_{tot}$). The combined results from two animals are shown in Fig. 1. In general, there was good agreement between the two methods of measuring blood flows, described by the equation $\dot{Q}_{Rsub} + \dot{Q}_{Rca} = 0.625 + 0.8\dot{Q}_{tot}$; $r^2=0.82$, $P<0.001$. However, at very low flows (less than 1.0 ml min^{-1} kg^{-1}), the use of a single probe around both vessels tended to underestimate blood flow. Such low flows did not occur in our experiments and since subclavian + carotid blood flows make up approximately 20–25 % of the total systemic blood flow ($\dot{Q}_{sys}$) (Comeau and Hicks, 1994), it is unlikely that our approach would lead to a significant underestimation of $\dot{Q}_{sys}$.

All four flow probes were connected to two dual-channel blood flowmeters (T201, Transonic System, Inc.) for simultaneous measurement of mean blood flows. The signals from the two flowmeters were continuously recorded at 15 Hz using the Acknowledge data acquisition system (Biopac Systems, Inc., CA, USA).

Calculation of $\dot{Q}_{sys}$, $\dot{Q}_{pul}$, *net shunt blood flow, heart rate and stroke volumes*

For both the subclavian and the carotid arteries, the right and

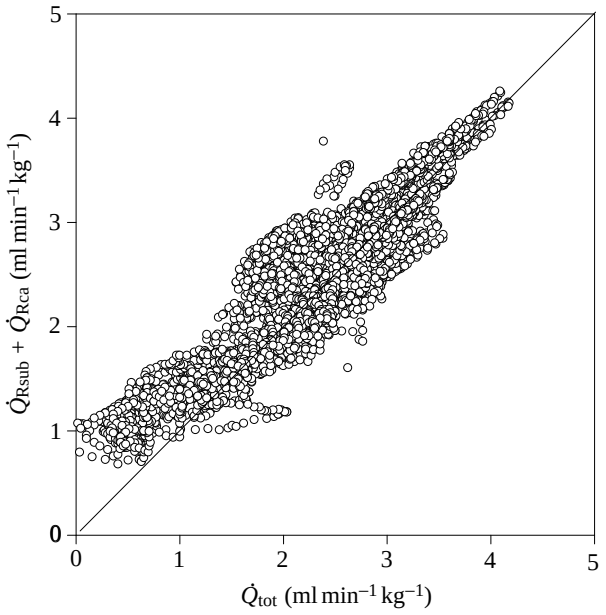

Fig. 1. The relationship between the sum of blood flow rates measured independently in the right subclavian ($\dot{Q}_{Rsub}$) and the right carotid artery ($\dot{Q}_{Rca}$) and the blood flow measured using a single flow probe on both vessels ($\dot{Q}_{tot}$). Blood flows were altered pharmacologically by intravenous injection of acetylcholine or isoproterenol in two turtles.

left vessels are of similar diameter and are perfused by virtually identical blood flows in anaesthetized turtles (Comeau and Hicks, 1994). Thus, assuming that this is true in non-anaesthetized turtles, total systemic blood flow ($\dot{Q}_{sys}$) was calculated as:

$$\dot{Q}_{sys} = \dot{Q}_{LAo} + \dot{Q}_{RRAo} + 2(\dot{Q}_{Rsub} + \dot{Q}_{Rca}) .$$

Similarly, assuming that blood flow in the right pulmonary artery equals that in the left pulmonary artery, pulmonary blood flow ($\dot{Q}_{pul}$) was calculated as $2\dot{Q}_{LPA}$. This assumption has been employed previously by Shelton and Burggren (1976) and has been verified in anaesthetized turtles (Shelton and Burggren, 1976; Comeau and Hicks, 1994). The net shunt flow ($\dot{Q}_{shunt}$) was calculated as the difference between $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ ($\dot{Q}_{shunt}=\dot{Q}_{pul} - \dot{Q}_{sys}$). Using this representation, a negative $\dot{Q}_{shunt}$ value indicates a *net* R–L shunt, whereas a positive $\dot{Q}_{shunt}$ value indicates a *net* L–R shunt.

Heart rate (f_H) was obtained from continuous recording of LAo blood flow, and total stroke volume (V_{Stot}) was calculated as total blood flow (systemic + pulmonary) divided by heart rate. Similarly, systemic and pulmonary stroke volumes (V_{Spul} and V_{Ssys}, respectively) were calculated as $\dot{Q}_{pul}/f_H$ and $\dot{Q}_{sys}/f_H$.

Measurement of ventilation rate

During experiments, the turtles were allowed to move freely within an experimental chamber (30 cm×30 cm×60 cm), which was covered in dark plastic to minimize visual disturbance. Ventilation rate was measured using an apparatus similar to that described by Glass *et al.* (1983). Briefly, the water surface was covered by a grid, except at a funnel-shaped breathing hole (7 cm in diameter). A pneumotachograph (0-5 LPM, Hans Rudolph, Inc, MO, USA) connected to a Validyne differential pressure transducer (DP45-14) was placed at the gas inlet to the breathing funnel and the air flow leaving the funnel was maintained constant at 500 ml min^{-1} by use of a Beckman pump. Because the air flow leaving the breathing funnel was constant, air flow through the pneumotachograph increased during inhalation and decreased during exhalation. The signal from the differential pressure transducer was recorded at 15 Hz using the Acknowledge data acquisition system for later analysis. The pneumotachograph was calibrated by manually simulating breaths using a syringe connected directly to the breathing funnel. In accordance with Funk *et al.* (1986), we found the integrated flow signal to be highly rate-dependent and a calibration at several frequencies was therefore necessary. The instantaneous breathing frequency of several turtles was analyzed in detail to provide the exact correction factor of the flow signal (1.83).

Data analyses and statistics

All recordings of blood flow and ventilation rates were analyzed using the Acknowledge data analysis system. For each turtle, blood flow and ventilation rates were recorded for a minimum of 4–6 h and a continuous period of 120–180 min was analyzed.

In the analysis of the relationship between ventilation and cardiovascular variables, we defined a non-ventilatory period (NVP) as any period in which there was no ventilatory flow across the nostrils, i.e. the flow signal from the pneumotachograph was stable and identical to the baseline value. Using this strict definition, a NVP could be as short as a few seconds, which approximates the duration of one breath. Similarly, a ventilatory period (VP) was defined as a continuous and uninterrupted series of breaths. Each NVP and VP was manually identified and analyzed for average blood flows and heart rate (for VPs, the integrated air flow was also recorded). Stroke volumes were calculated for all individual periods. For NVPs, the values obtained were subsequently grouped according to duration (0–30, 30–60, 60–120, 120–180, 180–240, 240–300 and >300 s), while VPs were grouped according to the number of breaths (1, 2, 3, 4 or >5 breaths). For each turtle, an average for each group was calculated for all physiological variables, and these individual averages were employed in the subsequent statistics (see below). The data represent means (± 1 S.E.M.) of these averages. It is important to emphasize that these mean values do not represent the temporal events occurring *within* a given NVP or VP.

The effects of ventilation on blood flow and heart rates were tested for statistical significance using a one-way analysis of variance (ANOVA) for repeated measures. Differences among means were subsequently assessed using a Student–Newman–Keuls test, applying a fiducial limit for significance of $P \leqslant 0.05$.

Results

The changes in central vascular blood flow and heart rates

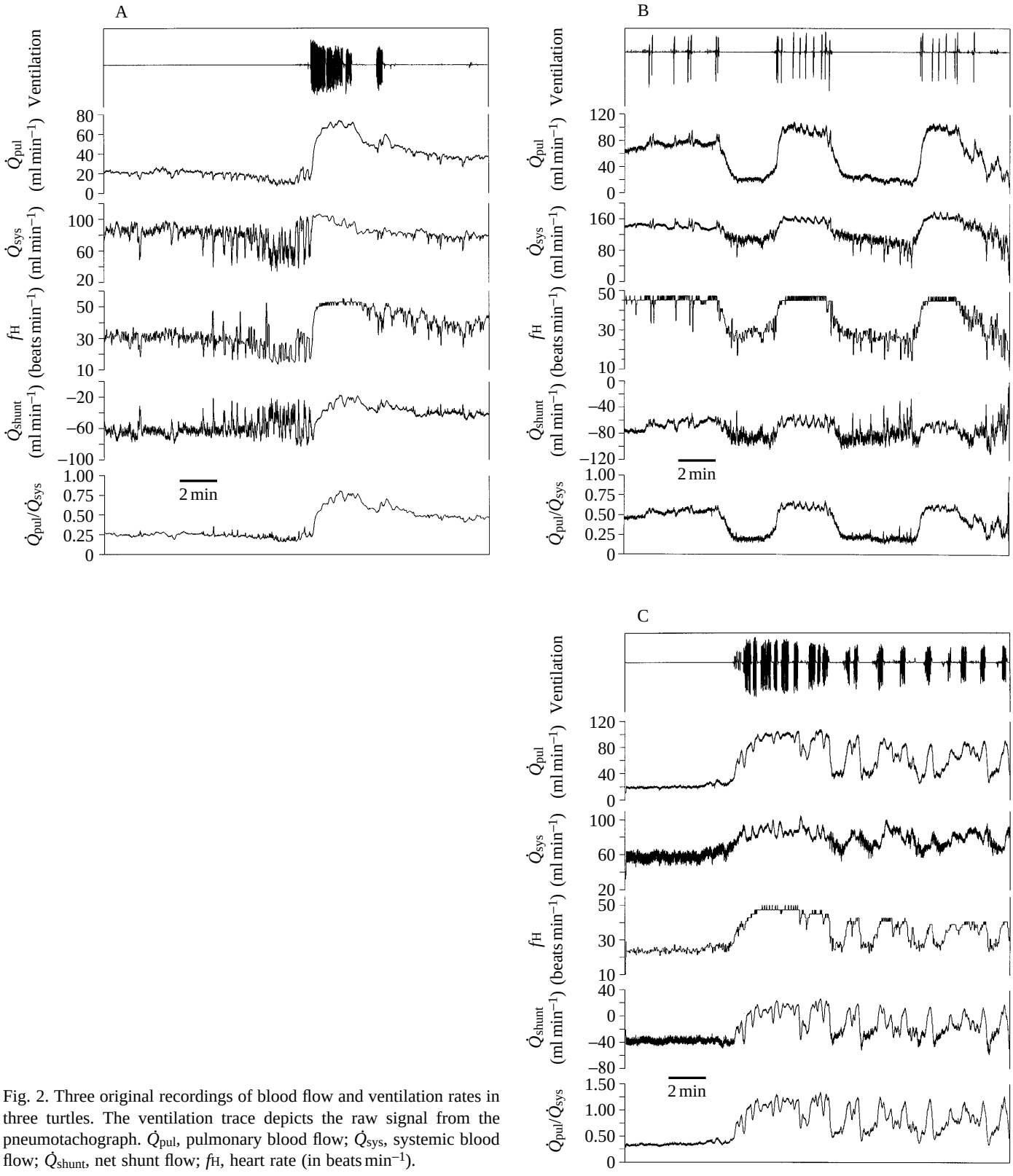

Fig. 2. Three original recordings of blood flow and ventilation rates in three turtles. The ventilation trace depicts the raw signal from the pneumotachograph. $\dot{Q}_{pul}$, pulmonary blood flow; $\dot{Q}_{sys}$, systemic blood flow; $\dot{Q}_{shunt}$, net shunt flow; f_H, heart rate (in beats min^{-1}).

(f_H) during lung ventilation in three turtles are presented in Fig. 2. These recordings show several similarities and differences among individuals. In all examples, both $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ as well as f_H increased abruptly at the onset of lung ventilation. At the end of a VP, f_H and blood flows decreased slowly (see Fig. 2A for an example), but this response varied greatly between and within individuals. In most turtles, blood flows and f_H remained high during short NVPs (see Fig. 2B).

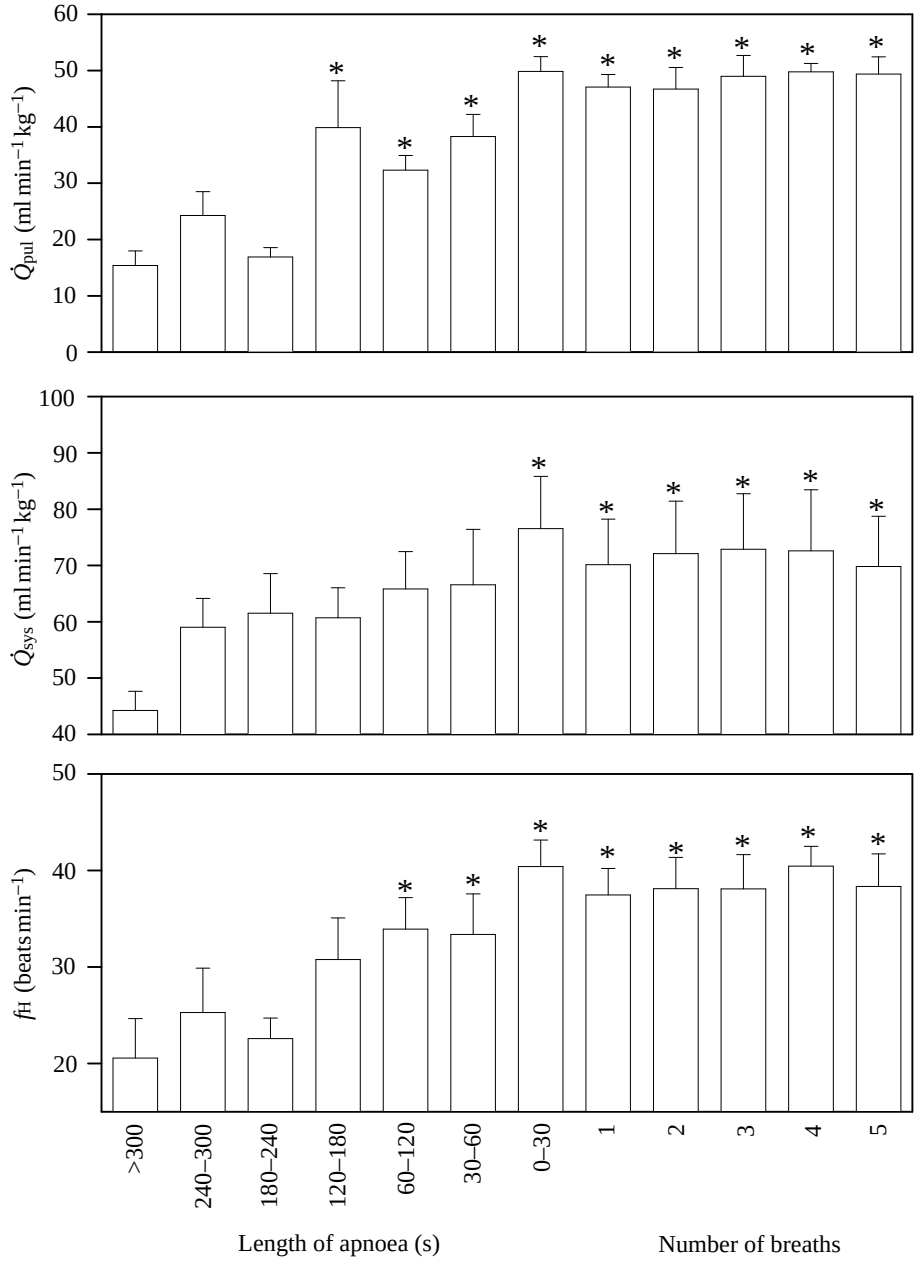

Fig. 3. Cardiorespiratory synchrony in turtles. Pulmonary blood flow rate ($\dot{Q}_{pul}$), systemic blood flow rate ($\dot{Q}_{sys}$) and heart rate (f_H) are depicted for non-ventilatory periods of different duration and for ventilatory periods consisting of 1, 2, 3, 4 or >5 breaths. Each value does not represent a consecutive temporal event, but rather an average of the given variable over a given non-ventilatory or ventilatory period. Values are mean + 1 S.E.M. (N=7). Systemic and pulmonary blood flow rates and heart rate changed significantly with ventilatory state ($P \leqslant 0.001$). Values significantly higher than the mean obtained during non-ventilatory periods longer than 180 s are marked with an asterisk.

However, in two of the seven turtles, a bradycardia and a reduction in blood flows developed rapidly (Fig. 2C). In all three examples $\dot{Q}_{shunt}$ decreased during ventilation, and in one of the examples (Fig. 2C) $\dot{Q}_{shunt}$ reversed. In all seven turtles, the relative increase in $\dot{Q}_{pul}$ was greater than the relative increase in $\dot{Q}_{sys}$. Thus, the ratio $\dot{Q}_{pul}/\dot{Q}_{sys}$ invariably increased during ventilation.

Average values for blood flows and f_H during NVPs of varying duration and VPs consisting of various numbers of breaths are presented in Fig. 3. Values in this and later figures do not represent consecutive temporal events, but rather the average of each physiological variable during a NVP of a given length or a VP consisting of a given number of breaths. During NVPs longer than 300 s, $\dot{Q}_{pul}$ averaged 15.4±2.6 ml min^{-1} kg^{-1} and increased more than threefold to a maximum value of 49.8±1.5 ml kg^{-1} min^{-1} during ventilation. $\dot{Q}_{pul}$ during NVPs lasting 0–30 s (49.9±2.6 ml min^{-1} kg^{-1}) was indistinguishable from that recorded during ventilation. Furthermore, $\dot{Q}_{pul}$ was independent of the number of breaths taken during a VP. $\dot{Q}_{sys}$ was also significantly elevated during VPs compared with NVPs lasting longer than 180 s. During NVPs lasting longer than 300 s, $\dot{Q}_{sys}$ was 44.2±3.4 ml min^{-1} kg^{-1} and increased to a maximum value of 72.9±9.9 ml min^{-1} kg^{-1} during ventilation. $\dot{Q}_{sys}$ was not affected by the number of breaths in the VPs.

The changes in $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ were accomplished entirely through a doubling of f_H during ventilation (20.6±2.3 beats min^{-1} during NVPs longer than 300 s to 38.5±1.0 beats min^{-1} during ventilation; Fig. 3), while total stroke volume did not change significantly (2.60± 0.13 ml kg^{-1} during NVPs longer than 300 s *versus*

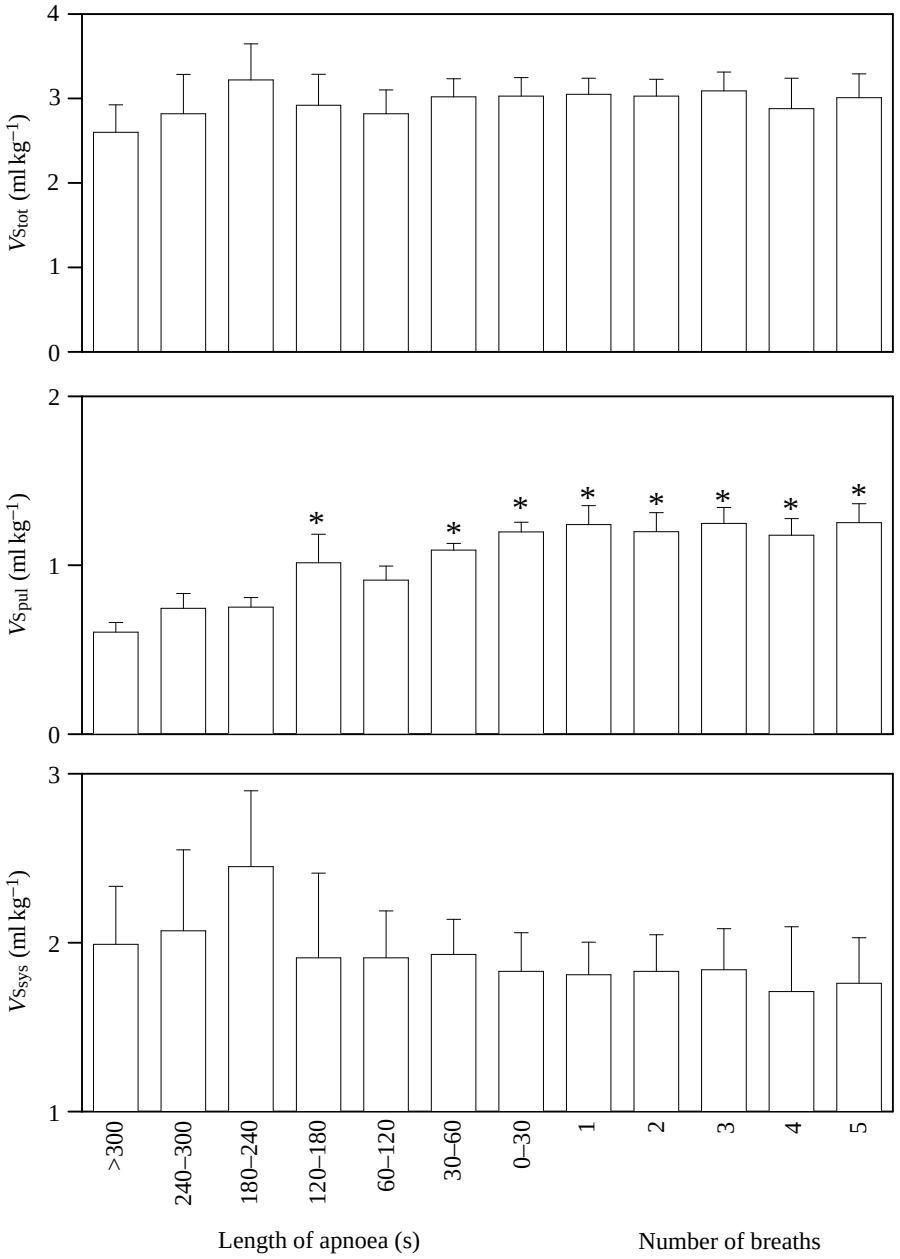

Fig. 4. Cardiorespiratory synchrony in turtles. Total stroke volume (V_{Stot}), pulmonary stroke volume (V_{Spul}) and systemic stroke volume (V_{Ssys}) are depicted for non-ventilatory periods of different duration and for ventilatory periods consisting of 1, 2, 3, 4 or >5 breaths. Values are mean + 1 S.E.M. (N=7). V_{Stot} did not change significantly with ventilatory state (P=0.27), while both V_{Spul} and V_{Ssys} changed significantly with ventilatory state ($P \leqslant 0.001$). Values significantly higher than the mean obtained during non-ventilatory periods longer than 180 s are marked with an asterisk.

3.09±0.09 ml kg^{-1} during ventilation; Fig. 4). However, pulmonary stroke volume (V_{Spul}) doubled from 0.60±0.02 ml kg^{-1} during NVPs longer than 300 s to a value of 1.25±0.04 ml kg^{-1} during ventilation (Fig. 4). Concomitantly, systemic stroke volume (V_{Ssys}) decreased from 1.99±0.14 ml kg^{-1} during NVPs longer than 300 s to 1.71±0.16 ml kg^{-1} during ventilation (Fig. 4).

Regardless of the ventilatory state, $\dot{Q}_{sys}$ exceeded $\dot{Q}_{pul}$, and average net shunt flow ($\dot{Q}_{shunt}$; $\dot{Q}_{pul} - \dot{Q}_{sys}$) was therefore always negative (Fig. 5). Furthermore, average net shunt flow was not affected by the ventilatory state and ranged between –20 and –30 ml min^{-1} kg^{-1} throughout the ventilatory cycles. In contrast, because $\dot{Q}_{pul}$ increased relatively more than $\dot{Q}_{sys}$ during ventilation, the $\dot{Q}_{pul}/\dot{Q}_{sys}$ increased significantly during ventilation (Fig. 5). In fact, this ratio almost tripled from 0.29±0.04 during NVPs of 180–240 s, to a value of 0.80±0.11 during ventilation.

Discussion

This study provides a detailed description of the interaction between ventilation and central vascular blood flows in free-diving turtles (*Trachemys scripta*). In general, our data agree with previous studies on turtles, with the notable exception that large net L–R shunts during ventilation were not apparent in our study (Figs 2, 5). For comparison, previous measurements of blood flows and heart rates are compiled in Table 1. Consistent with earlier studies (White and Ross, 1966; Burggren, 1975; Shelton and Burggren, 1976), ventilation was associated with large increases in $\dot{Q}_{pul}$ and f_H (Figs 2, 3). In

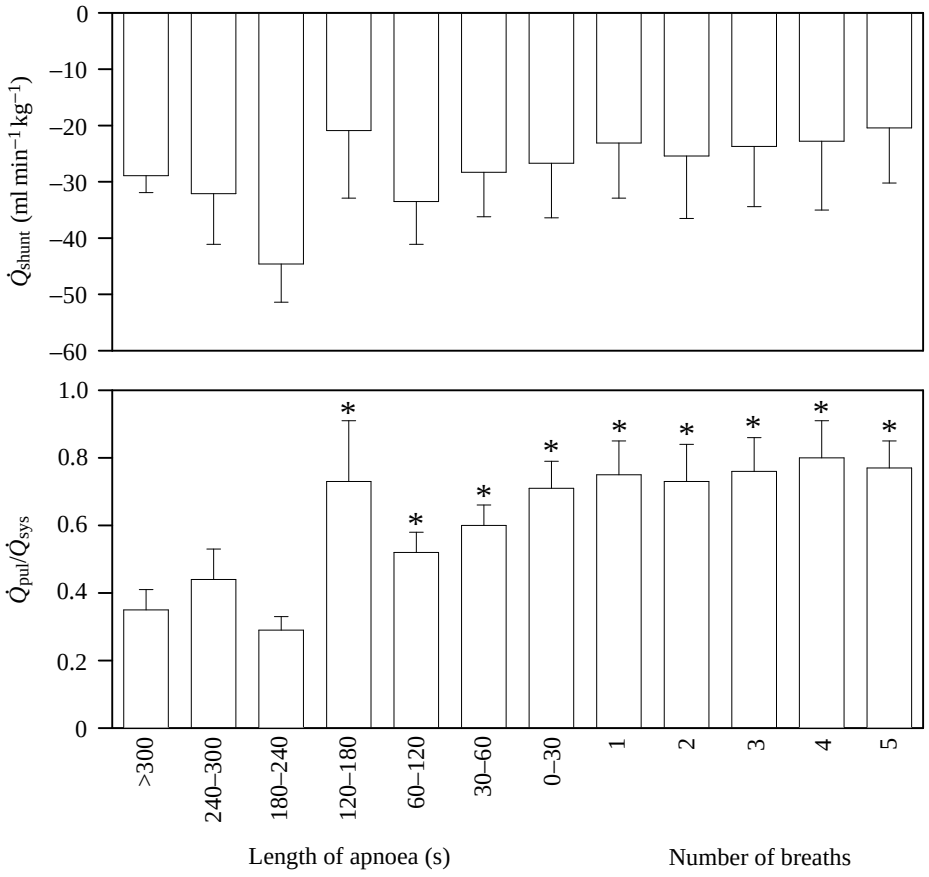

Fig. 5. Cardiorespiratory synchrony in turtles. Net shunt flow ($\dot{Q}_{shunt}$), calculated as pulmonary flow rate ($\dot{Q}_{pul}$) minus systemic flow rate ($\dot{Q}_{sys}$), and $\dot{Q}_{pul}/\dot{Q}_{sys}$ are depicted for non-ventilatory periods of different duration and for ventilatory periods consisting of 1, 2, 3, 4 or >5 breaths. Values are mean + 1 S.E.M. (N=7). $\dot{Q}_{shunt}$ changed significantly with ventilatory state (P=0.023), but multiple comparison (Student–Newman–Keuls test) failed to detect differences among means. $\dot{Q}_{pul}/\dot{Q}_{sys}$ changed significantly with ventilatory state ($P \leqslant 0.001$) and values significantly higher than the mean obtained during non-ventilatory periods longer than 180 s are marked with an asterisk.

Table 1. *Previous determinations of blood flow and heart rates during non-ventilatory periods and ventilatory periods in non-anaesthetized turtles (*Trachemys scripta*)*

Ventilatory state	$\dot{Q}_{pul}$ (ml min^{-1} kg^{-1})	$\dot{Q}_{sys}$ (ml min^{-1} kg^{-1})	$\dot{Q}_{shunt}$ (ml min^{-1} kg^{-1})	$\dot{Q}_{pul}/\dot{Q}_{sys}$	Heart rate (beats min^{-1})	Temperature (°C)	Reference
NVP	–	–	–	–	7.8	17	1
VP	–	–	–	–	17.9	17	1
NVP	14.8	18.5	–3.7	0.80	11	19	2
VP	46.2	25.1	21.1	1.84	23	19	2
–	42.7	58.6	–15.9	0.73	23.4	25	3
NVP	6.3	13.2	–6.9	0.48	–	15	4
VP	30.0	41.6	–11.6	0.72	–	15	4
NVP	61.6	65.0	–3.4	0.95	–	30	4
VP	80.7	85.7	5.0	0.94	–	30	4
NVP	18.9	54.9	–35.2	0.36	22.8	25	5
VP	50.1	71.5	–23.1	0.76	40.7	25	5

1, Burggren (1975); electrocardiogram. 2, Shelton and Burggren (1976); electromagnetic flow probes. 3, Kinney *et al.* (1977); Fick principle. 4, Heisler and Glass (1985); microsphere injection. 5, Present study (NVP longer than 180 s), flow probes.

$\dot{Q}_{pul}$, pulmonary blood flow; $\dot{Q}_{sys}$, systemic blood flow; $\dot{Q}_{shunt}$, net shunt flow; NVP, non-ventilatory period; VP, ventilatory period.

our study, $\dot{Q}_{sys}$ increased during ventilation (Figs 2, 3) and the absolute blood flows were similar to those from studies based on the Fick principle (Kinney *et al.* 1977) and on microsphere injection (Heisler and Glass, 1985). However, the $\dot{Q}_{sys}$ reported by Shelton and Burggren (1976) is 2–3 times lower than that obtained in our study (Table 1). In their study, $\dot{Q}_{sys}$ was estimated by multiplying right aortic arch blood flow by a factor of 2.75, while our only assumption was that the right and left subclavian and carotid blood flow rates were equal. Nevertheless, in anaesthetized turtles, Comeau and Hicks (1994) confirmed the validity of estimating systemic blood flow as a simple function of right aortic arch blood flow (by using a factor of approximately 2.80), and in this study we obtained a value of 2.86 (data not shown). We found this

Table 2. *Previous determinations of cardiac shunt and net shunt flows during non-ventilatory periods and ventilatory periods in non-anaesthetized turtles (*Trachemys scripta*)*

Ventilatory state	R–L shunt fraction	L–R shunt fraction	Temperature (°C)	Reference
NVP	68.0	11.0	18–20	1
VP	38.5	12.0	18–20	1
NVP	59.5	14.8	15	2
VP	41.0	18.3	15	2
NVP	27.0	22.4	30	2
VP	31.8	27.6	30	2
NVP	76.4	10.0	15	3
VP	25.1	18.6	15	3
NVP	79.0	30.5	15	4
VP	64.6	24.9	15	4

1, Burggren and Shelton (1979); P_{O_2}. 2, Heisler and Glass (1985); microsphere injection. 3, White *et al.* (1989); O_2 content. 4, White *et al.* (1989); microsphere injection.

L–R shunt fraction is defined as the L–R shunt flow relative to the pulmonary blood flow; R–L shunt fraction is defined as the R–L shunt flow relative to the systemic blood flow.

NVP, non-ventilatory period; VP, ventilatory period.

relationship to be independent of ventilatory state, and therefore it seems unlikely that the values of Shelton and Burggren (1976) differ from ours owing to incomplete measurements of blood flows in systemic vessels. Furthermore, the total stroke volume estimated by Shelton and Burggren (1976) is in excellent agreement with the value from our our study (3.1 and 3.0 ml kg^{-1}, respectively). It is possible that part of the discrepancy results from our study being conducted at a higher temperature (25 *versus* 19 °C).

In our study, total cardiac output ($\dot{Q}_{pul} + \dot{Q}_{sys}$) increased during ventilation. This increase was accomplished by changes in f_H (Fig. 3) while $V_{S_{tot}}$ remained constant (Fig. 4). Nevertheless, $V_{S_{pul}}$ and $V_{S_{sys}}$ changed in a reciprocal manner with ventilation (Fig. 4), consistent with previous studies (Shelton and Burggren, 1976; West *et al.* 1992) but in contrast to results observed in an *in situ* turtle heart preparation (Franklin, 1994). In this *in situ* study, all changes in cardiac output were ascribed to changes in total stroke volume, while f_H remained constant. As shown in our study and in previous studies on chelonians (White and Ross, 1966; Shelton and Burggren, 1976; Burggren, 1975; Butler *et al.* 1984; White *et al.* 1989; West *et al.* 1992), f_H is not normally constant *in vivo*. The dynamic changes in f_H and blood flow *in vivo* probably result from reciprocal interactions of the parasympathetic and sympathetic nervous systems (see Hicks, 1994). Thus, results from *in situ* turtle heart preparations in which f_H remains constant or where the influence of the autonomic nervous system is eliminated must be interpreted with caution.

The dynamics of blood flow and heart rate in turtles

All turtles in this study displayed rapid and large increases in $\dot{Q}_{pul}$ and f_H at the onset of ventilation. As in green sea turtles *Chelonis mydas* (West *et al.* 1992), these increases often occurred immediately before ventilation commenced, suggesting that afferent input from pulmonary stretch receptors alone cannot explain these changes, as proposed by Johansen *et al.* (1977) (see Wang *et al.* 1997 for a discussion on the role of pulmonary stretch receptors in the regulation of $\dot{Q}_{pul}$). In contrast to the rapid changes at the onset of ventilation, blood flows and f_H decrease slowly after a breathing bout (Fig. 2), which explains why blood flows and f_H during short NVPs resemble those during VPs (Figs 2–5).

On the basis of our results (Figs 2–5), cardiorespiratory synchrony in *Trachemys scripta* can be divided into three phases: (1) VPs, (2) NVPs of short duration (0–180 s) and (3) NVPs of long duration (>180 s). During VPs, f_H and blood flows were twice those measured during long-lasting NVPs, while during NVPs of shorter duration, f_H and blood flows were of intermediate values. Furthermore, our data show that cardiovascular changes during a VP were independent of the number of breaths. However, because our data (Figs 3–5) represent the average values during a VP regardless of the length of the preceding NVP, this presentation does not reveal the cardiovascular changes *within* a single VP. Previous studies on chelonians have shown that following a long NVP, blood flows and f_H progressively increase during the first few breaths of a VP (Burggren, 1975; West *et al.* 1992). Our recordings confirm this pattern (Fig. 2). For example, in Fig. 2A, following a long NVP (>10 min), $\dot{Q}_{pul}$, $\dot{Q}_{sys}$ and f_H progressively increased during the first few breaths of the initial VP. Throughout the remaining portion of the VP, and the three following VPs, the cardiovascular variables remained relatively stable at high levels.

Cardiac shunting in turtles

In our study, $\dot{Q}_{sys}$ exceeded $\dot{Q}_{pul}$ throughout the ventilatory cycle, revealing that a *net* R–L cardiac shunt occurred during both ventilation and apnoea (Figs 2, 5). Previous studies report large net R–L shunts during breath-holding, but also the development of a net L–R shunt during ventilation (Shelton and Burggren, 1976; White and Ross, 1966). Nevertheless, it should not be concluded that our results contradict these studies. In turtles and squamate reptiles, quantification of cardiac shunts is complicated by the potential for bidirectional shunting, where L–R and R–L shunts occur simultaneously. Under this condition, measurements of $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ alone do not fully describe shunt patterns (Hicks, 1994). For example, if R–L and L–R shunts are of similar magnitude, the net shunt flow is zero. Thus, a net R–L shunt only implies that the R–L shunt is larger than the concurrent L–R shunt, and a more detailed description of cardiac shunts requires simultaneous measurements of blood flows and blood oxygen levels from several cardiac and central vascular sites (see, for example, Ishimatsu *et al.* 1988). In turtles, the existence of bidirectional shunting has been documented in several studies (Burggren and Shelton, 1979; Heisler and Glass, 1985; White *et al.* 1989), and the results of these studies are listed in Table 2, in which the shunt flows are expressed relative to $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ (i.e.

shunt fractions; $\dot{Q}_{L-R}/\dot{Q}_{pul}$ and $\dot{Q}_{R-L}/\dot{Q}_{sys}$). In spite of the considerable variation between these studies (see White *et al.* 1989 for a critical discussion of microsphere use to study cardiac shunts), these results support the view that the R–L shunt fraction increases during non-ventilatory periods and that the L–R shunt fraction increases during ventilation. It is of interest to note that, in all the studies, the L–R shunt fraction never exceeds the R–L shunt fraction (Table 2). This pattern supports the finding of our study, that large net L–R shunts do not normally develop during ventilation.

As an alternative to shunt fractions, cardiac shunts can be evaluated on the basis of $\dot{Q}_{pul}/\dot{Q}_{sys}$ which, in essence, expresses the net shunt flow relative to the absolute blood flows. This ratio may be a better indicator of the effects of cardiac shunting on arterial blood gas composition than net shunt flow *per se*. In anaesthetized turtles, an increase in $\dot{Q}_{pul}/\dot{Q}_{sys}$ leads to an increase in both systemic and pulmonary arterial O_2 contents, attesting to a reduction in the R–L shunt and a simultaneous increase in the L–R shunt (A. Ishimatsu, J. W. Hicks and N. Heisler, unpublished data). In this context, it is interesting that all existing studies report an increase in $\dot{Q}_{pul}/\dot{Q}_{sys}$ during ventilation, while the changes in net shunt are much less uniform (Figs 2, 3, 5; Tables 1, 2).

In conclusion, the present description of cardiorespiratory synchrony in turtles is, in general, consistent with previous studies. Both $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ increased significantly during ventilation, but $\dot{Q}_{sys}$ exceeded $\dot{Q}_{pul}$ by 20–30 ml min^{-1} kg^{-1} regardless of the ventilatory state. Nevertheless, because $\dot{Q}_{pul}$ increased proportionally more than $\dot{Q}_{sys}$ during ventilation, $\dot{Q}_{pul}/\dot{Q}_{sys}$ increased almost threefold (0.29–0.80), indicating a reduction in the R–L shunt fraction during ventilation compared with breath-holding. These changes in cardiac shunts have important effects on levels of arterial blood gases, but the physiological function of cardiac shunts remains unknown (see Burggren, 1987; Hicks and Wang, 1996).

This study was financially supported by an NSF grant (IBN-9218936) to J.W.H., and T.W. was in receipt of a Postdoctoral Fellowship from the Carlsberg Foundation (Denmark). We are delighted to acknowledge the useful comments provided by Dr Egle Krosniunas, Dr Gillian Muir and Dr Stephen J. Warburton and the anonymous referees on an earlier version of this manuscript.

References

AKAGI, K., ENDO, C., SAITO, J., ONODERA, M., KUZO, M., TANIGAWARA, SH., OKAMURA, K., YAJIMA, A. AND SATO, A. (1987). Ultrasonic transit-time measurement of blood flow in the animal chronic preparation model. *Jap. J. med. Ultrasonics* **14**, 104–110.

BURGGREN, W. W. (1975). A quantitative analysis of ventilation tachycardia and its control in two chelonians, *Chrysemys scripta* and *Testudo graeca*. *J. exp. Biol.* **63**, 367–380.

BURGGREN, W. W. (1977). The pulmonary circulation of the chelonian reptile: morphology, haemodynamics and pharmacology. *J. comp. Physiol.* **116**, 303–323.

BURGGREN, W. W. (1985). Hemodynamics and regulation of central cardiovascular shunts in reptiles. In *Cardiovascular Shunts: Phylogenetic, Ontogenetic and Clinical Aspects* (ed. K. Johansen and W. W. Burggren), pp. 121–142. Copenhagen: Munksgaard.

BURGGREN, W. W. (1987). Form and function in reptilian circulation. *Am. Zool.* **27**, 5–20.

BURGGREN, W. W. AND SHELTON, G. (1979). Gas exchange and transport during intermittent breathing in chelonian reptiles. *J. exp. Biol.* **82**, 75–92.

BUTLER, P. J., MILSOM, W. K. AND WOAKES, A. J. (1984). Respiratory cardiovascular and metabolic adjustments during steady state swimming in the green turtle, *Chelonia mydas*. *J. Comp. Physiol.* B **154**, 167–174.

COMEAU, S. AND HICKS, J. W. (1994). Regulation of central vascular blood flow in the turtle. *Am. J. Physiol.* **267**, R569–R578.

FRANKLIN, C. E. (1994). Intrinsic properties of an *in situ* turtle heart (*Emydura signata*) preparation perfused *via* both atria. *Comp. Biochem. Physiol.* **107**A, 501–507.

FUNK, G. D., WEBB, C. L. AND MILSOM, W. K. (1986). Non-invasive measurement of respiratory tidal volume in aquatic, air-breathing animals. *J. exp. Biol.* **126**, 519–523.

GLASS, M. L., BOUTILIER, R. G. AND HEISLER, N. (1983). Ventilatory control of arterial P_{O_2} in the turtle *Chrysemys picta bellii*. Effects of temperature and hypoxia. *J. comp. Physiol.* **151**, 145–153.

HEISLER, N. AND GLASS, M. L. (1985). Mechanisms and regulation of central vascular shunts in reptiles. In *Cardiovascular Shunts: Phylogenetic, Ontogenetic and Clinical Aspects* (ed. K. Johansen and W. W. Burggren), pp. 334–353. Copenhagen: Munksgaard.

HICKS, J. W. (1994). Adrenergic and cholinergic regulation of intracardiac shunting. *Physiol. Zool.* **67**, 1325–1346.

HICKS, J. W. AND COMEAU, S. G. (1994). Vagal regulation of intracardiac shunting in turtles. *J. exp. Biol.* **186**, 109–126.

HICKS, J. W. AND WANG, T. (1996). Functional role of cardiac shunts in reptiles. *J. exp. Zool.* (in press).

ISHIMATSU, A., HICKS, J. W. AND HEISLER, N. (1988). Analysis of intracardiac shunting in the lizard, *Varanus niloticus*: A new model based on blood oxygen levels and microsphere distribution. *Respir. Physiol.* **71**, 83–100.

JOHANSEN, K., BURGGREN, W. W. AND GLASS, M. L. (1977). Pulmonary stretch receptors regulate heart rate and pulmonary blood flow in the turtle, *Chrysemys scripta*. *Comp. Biochem. Physiol.* **58**A, 185–191.

JOHANSEN, K., LENFANT, C. AND HANSON, D. (1970). Phylogenetic development of pulmonary circulation. *Fedn Proc. Fedn Am. Socs exp. Biol.* **29**, 1135–1140.

KINNEY, J. L., MATSUURA, D. T. AND WHITE, F. N. (1977). Cardiorespiratory effects of temperature in the turtle. *Respir. Physiol.* **31**, 309–325.

LILLYWHITE, H. B. AND DONALD, J. A. (1989). Pulmonary blood flow regulation in an aquatic snake. *Science* **245**, 293–295.

MILSOM, W. K. (1991). Intermittent breathing in vertebrates. *A. Rev. Physiol.* **53**, 87–105.

MILSOM, W. K., LANGILLE, B. L. AND JONES, D. R. (1977). Vagal control of pulmonary vascular resistance in the turtle *Chrysemys scripta*. *Can. J. Zool.* **55**, 359–367.

SHELTON, G. (1970). The effect of lung ventilation on blood flow to the lungs and body of the amphibian, *Xenopus laevis*. *Respir. Physiol.* **9**, 183–196.

SHELTON, G. AND BURGGREN, W. W. (1976). Cardiovascular dynamics of the chelonia during apnoea and lung ventilation. *J. exp. Biol.* **64**, 323–343.

WANG, T., KROSNIUNAS, E. H. AND HICKS, J. W. (1997). Regulation of cardiac shunts in turtles. *Am. Zool.* (in press).

WEST, N., BUTLER, P. J. AND BEVAN, R. M. (1992). Pulmonary blood flow at rest and during swimming in the green turtle, *Chelonia mydas*. *Physiol. Zool*. **65**, 287–310.

WHITE, F. N. (1968). Functional anatomy of the heart of reptiles. *Am. Zool*. **8**, 211–219.

WHITE, F. N., HICKS, J. W. AND ISHIMATSU, A. (1989). Relationship between respiratory state and intracardiac shunts in turtles. *Am. J. Physiol*. **256**, R240–R247.

WHITE, F. N. AND ROSS, G. (1966). Circulatory changes during experimental diving in the turtle. *Am. J. Physiol*. **212**, 15–18.

WOOD, S. C. (1982). Effect of O_2 affinity on arterial P_{O_2} in animals with central vascular shunts. *J. appl. Physiol*. **53**, 1360–1364.