

Stroke volume and cardiac output in juvenile elephant seals during forced dives

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Summary

The aim of this study was to examine the effect of forced diving on cardiac dynamics in a diving mammal by evaluating cardiac output and heart rate. We used MR Imaging and phase contrast flow analysis to obtain accurate flow measurements from the base of the aorta. Heart rate (f_H) and cardiac output (\dot{Q}) were measured before, during and after dives in four restrained juvenile northern elephant seals, *Mirounga angustirostris*, and stroke volume (V_S) was calculated ($V_S = \dot{Q}/f_H$). Mean \dot{Q} during diving ($4011 \pm 387 \text{ ml min}^{-1}$) and resting ($6530 \pm 1018 \text{ ml min}^{-1}$) was not significantly different (paired t -test; $P < 0.055$). Diving was accompanied by a 20% increase in V_S over the pre-dive level. Pre-dive, post-

dive or diving f_H was not significantly correlated with V_S during any state. Diving V_S correlated negatively with the bradycardic ratio (diving f_H to pre-dive f_H). In this study, the degree of bradycardia during diving was less than in previous pinniped studies, suggesting that the reduction in vagal input may contribute to the observed increase in V_S .

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Key words: diving, elephant seal, *Mirounga angustirostris*, cardiac output, stroke volume, magnetic resonance imaging.

Introduction

Monitoring the interplay of heart rate (f_H), stroke volume (V_S) and cardiac output (\dot{Q}) is vital for understanding the complex interactions that dictate blood flow and oxygen distribution in diving seals. Simultaneous measurement of these variables has proven elusive. Most studies have simply employed electrocardiograph (ECG) measurements in the laboratory or measurement of f_H in instrumented free-ranging pinnipeds to provide information on the frequency of cardiac contraction under different conditions. Typically, V_S in marine mammals is not measured directly but rather calculated from simultaneous f_H measurements and \dot{Q} ($V_S = \dot{Q}/f_H$). In pinnipeds, a number of methods have been used to evaluate \dot{Q} : Fick principle, dye dilution, thermodilution, and Doppler or transducer flow evaluation (Elsner et al., 1964; Murdaugh et al., 1966; Blix et al., 1976, 1983; Sinnett et al., 1978; Zapol et al., 1979; Ponganis et al., 1990). These procedures are difficult to employ and invasive, whether conducted in the field or under controlled laboratory conditions. All seven of these studies of four species of pinnipeds report a \dot{Q} associated with dive-induced bradycardia. Stroke volume, by contrast, remained constant during diving in some experiments and declined in others.

In light of the reported variation in \dot{Q} measurements and V_S calculations in diving pinnipeds, as well as differences in interpretation, our objective was to elucidate the effect of diving on cardiac dynamics using MR Imaging and phase contrast flow analysis. Using this approach, \dot{Q} was measured before, during and after forced dives in restrained juvenile northern elephant seals, *Mirounga angustirostris* (L).

Elephant seals are ideal subjects for a study of cardiac function because they stand out among diving mammals for their deep, long-duration, continuous diving (Le Boeuf et al., 1988, 1993, 2000a). The diving pattern of juveniles, although reduced in scale, is similar to that of adults. By one year of age, after having spent five months at sea foraging, they are accomplished divers with oxygen stores equivalent to 84% of adult female storage ability (Thorson and Le Boeuf, 1994). The dives of yearlings average 15 min duration, with only 2 min at the surface between dives; 88% percent of the time at sea is spent underwater (Le Boeuf et al., 1996). Heart rate of free-ranging juveniles decreases from a mean 107 beats min^{-1} at the surface (between dives) to a mean of 35 beats min^{-1} during dives, with maximum decreases observed as low as

3 beats min^{-1} (Andrews, 1997; Webb et al., 1998; Andrews et al., 2000; Le Boeuf et al., 2000b). Juvenile elephant seals are also excellent subjects for MR Imaging, as they are amenable to restraint, transport and handling.

Materials and methods

Animals

Four 10-month-old juvenile northern elephant seals were collected on the rookery at Año Nuevo, CA, USA in October, as they returned from a five-month foraging trip (National Marine Fisheries Service permit #938; UCSC Chancellor's Animal Research Committee protocol #COST97.10-2; Stanford University's Institutional Animal Care and Use Committee protocol #5091). At this time, the animals are in a fasted state and rest on the beach until their return to the sea in December. The two males and two females, weighing a mean (\pm S.D.) of 102.2 ± 3.6 kg, were transported by truck to the Long Marine Laboratory at the University of California in Santa Cruz, where they were housed in cages with sand substrates until used as experimental subjects. On the day of an experiment, a seal was transported by truck to the Lucas Center for Advanced MR Technology at Stanford University (transport time ~ 2 h).

Prior to imaging, animals were fitted with a diving helmet manufactured from a 35 cm-diameter Plexiglas tube, an inner neoprene seal and a secondary outer latex neck seal. The seal was allowed to acclimate for approximately 30 min, during which time a vacuum hose was attached to the helmet to ensure sufficient airflow through the open valves. At the initiation of a diving experiment, the vacuum hose was removed and the helmet was filled with cold water. Timing of the forced apnea or 'dive' commenced when the animal's nostrils were completely submerged and continued until the helmet was drained and the first inspiration occurred. Each animal was subjected to 2–5 forced dives with a mean duration of 6.28 ± 1.07 min (\pm S.D.). These forced dives were approximately 63% of the mean duration of natural dives of similar aged juveniles at sea (Le Boeuf, 1994) and slightly less duration than sleep apneas of juveniles sleeping on the beach (Blackwell and Le Boeuf, 1993). Minimum time between dives was 13 min to allow for full recovery. Cardiac measurements were collected during the pre-dive, dive and post-dive periods. Values referred to as 'resting' were obtained during the quiescent 5 min period immediately preceding a dive.

Heart rate

At the MR unit, the seal was physically restrained while four ECG electrodes were glued to the ventral surface of the animal using cyanoacrylate adhesive. The seal was then placed in a custom-made conical nylon jacket and strapped to a restraining board in a prone position. The board was placed in a PVC cradle that served as a fluid containment unit to prevent water damage to the magnet. Once in position on the magnet bed, the ECG leads were connected and the strap of the respiratory

bellows was threaded under the animal at the level of the diaphragm. Heart rate data were collected throughout the experimental protocol on a Macintosh IISI with an 8-channel Powerlab™ (ADInstruments, Colorado Springs, CO, USA) interface and an ML 132 BioAmp. Pre-dive heart rates were collected during the 5 min acclimation period immediately preceding the first dive. Heart rate was acquired by recording interbeat intervals using Chart™ (ADInstruments).

MR Imaging

All images were collected using a high-performance 1.5 T system (Signal Horizon Echo Speed, GE Medical Systems, Milwaukee, WI, USA). Localizer images were acquired in the axial plane at the base of the aortic bulb (Fig. 1; Movie 1 in supplementary material). Phase contrast (PC) MRI was used to acquire through-plane velocity data using a conventional 2-D cine PC sequence and an experimental cine-spiral PC sequence (Liao et al., 1995). Scan parameters were TR=55, TE=6.2, NSA=1, FOV=48×48 cm^2 and slice thickness=7 mm. The spiral sequence used 12–16 interleaves, allowing much faster data acquisition than the conventional sequence.

The PC scans provided values of the through-plane velocity in each pixel of the image (Pelc et al., 1991). Since cine MR data are acquired over a large number of cardiac cycles (128–256 for the conventional sequence and 12–16 for the spiral sequence), the PC measured velocities represent the average physiology over a number of cardiac cycles. Velocity data were analyzed using software written at Stanford. For each image in the cine sequence, integrating the velocity across the lumen of a blood vessel provides an estimate of the flow rate (in ml s^{-1}) at that point in the cycle (Pelc et al., 1994). When this is done at the aortic root, the average over the cardiac cycle provides an estimate of \dot{Q} . Stroke volume is computed by dividing \dot{Q} by f_H . Regions of interest for flow analysis were defined for each frame in the cine sequence using a semiautomated algorithm based on thresholding the MR magnitude images (Pelc et al., 1994). Baseline velocity offsets were estimated using a linear fit to static regions and subtracted from the region of interest.

Statistical analysis

Statistical analysis of physiological parameters was conducted using JMP 3.2.1; reported values are means \pm S.E.M. unless otherwise indicated.

Results

Heart rate

Mean pre-dive heart rate (f_H) was 61.4 ± 6.1 beats min^{-1} . Mean diving f_H (from 1 to 5 min) was 31.8 ± 3.6 beats min^{-1} . Mean dive f_H at 5 min into a dive was even lower, 29.2 ± 4.9 beats min^{-1} . Mean diving f_H was not significantly different from diving f_H at any given minute during the dive (ANOVA; from 1 to 5 min, $F_{5,18}=0.61$, $P=0.69$). Pre-dive, post-dive and diving f_H s did not correlate significantly with V_S during any state.

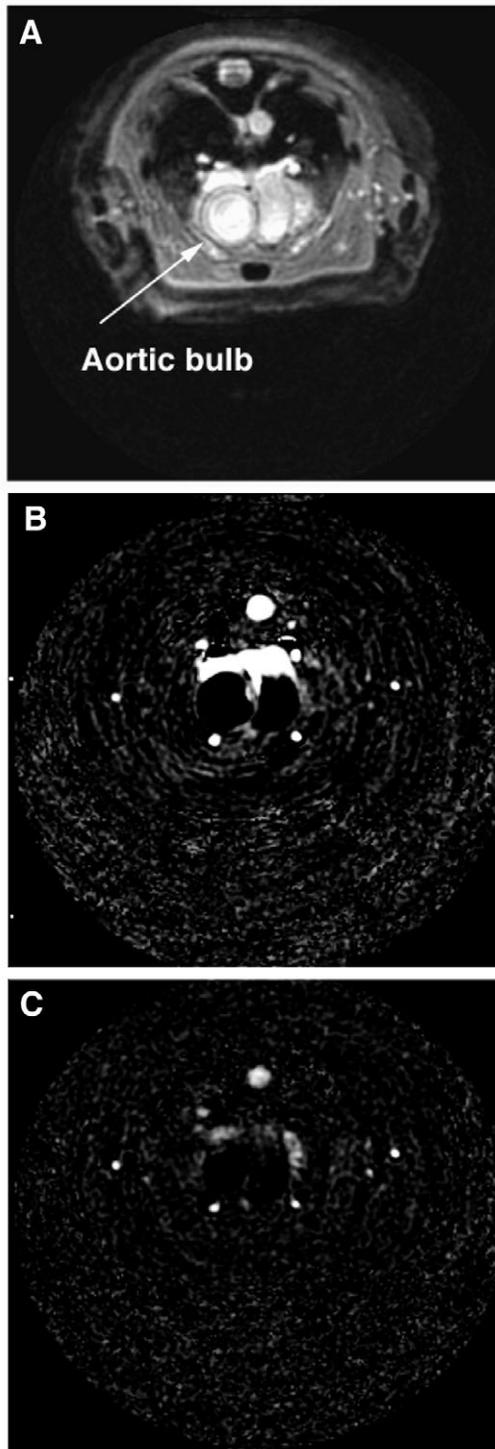


Fig. 1. MR axial images of a northern elephant seal pup during cardiac phase-contrast data acquisition. Localizer image (A) shows the location selected for flow analysis and the position of the aorta. In phase-contrast images A and B, image intensity is proportional to the velocity component of the blood along the superior/inferior direction. Image B was obtained during systole (frame 4 of 36), and image C is near the end of diastole (frame 27 of 36). Black indicates superior (cranial) flow and white represents flow in the inferior (caudal) direction.

Cardiac output

Mean cardiac output (\dot{Q}) during diving ($4011 \pm 387 \text{ ml min}^{-1}$) and resting ($6530 \pm 1018 \text{ ml min}^{-1}$) was not significantly different (paired t -test; $P < 0.055$, $N = 4$).

Stroke volume

Stroke volume (V_s) increased significantly during forced dives from a mean resting level of $104.9 \pm 4.1 \text{ ml}$ to a mean value of $126.1 \pm 3.9 \text{ ml}$ (paired t -test; $P < 0.008$, $N = 4$). f_H ratio (mean diving f_H /pre-dive f_H) represents the diving bradycardic response (Fig. 2) and is negatively correlated with stroke volume ($r^2 = 0.98$, $P = 0.01$).

Cardiac data from one animal was acquired rapidly enough to permit flow pattern evaluation during individual cardiac cycles (Fig. 3). Blood flow (ml) over a cardiac cycle during the dive exhibited a distinct peak in the first quarter of the cardiac cycle then decreased momentarily before leveling off. In the second half of the cardiac cycle (frames 17–32), flow did not fall to zero at any point (mean flow per frame = $2.23 \pm 0.08 \text{ ml}$). Post-dive flow data exhibited a diminished peak and did not show a distinct post-peak reduction.

Discussion

The most striking feature of this study was the 20% elevation of V_s during transition from resting to forced diving. Animals that exhibited a more profound bradycardia had a higher V_s than individuals expressing a less profound reduction in f_H . This finding stands in contrast to other pinniped cardiac output studies, reporting either no change or a reduction in diving V_s over resting levels. Why is our finding different from that of other studies? Might this difference be due to the experimental procedure used, the species under observation,

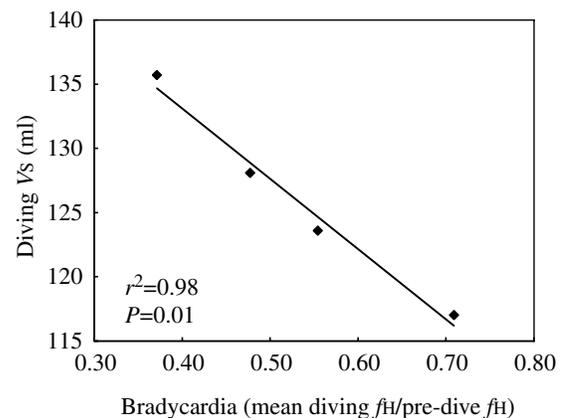


Fig. 2. Diving stroke volume (V_s) correlates with the degree of bradycardia expressed during the dive [ratio of mean diving heart rate (f_H) to mean pre-dive f_H ($P = 0.01$, $r^2 = 0.98$; $y = -47.83x + 150.56$)]. Although the degree of bradycardia is correlated with V_s , the absolute f_H during the dive is not, suggesting that it is the degree of cardiac response to diving that is driving the increase in V_s rather than the absolute rate of contraction.

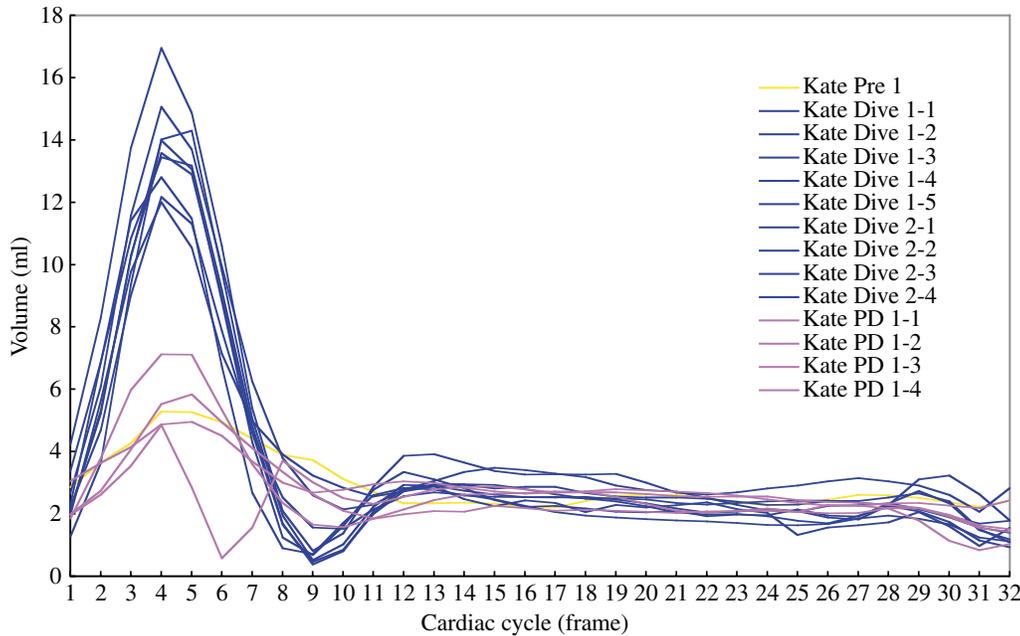


Fig. 3. Stroke volume data from an individual northern elephant seal during the pre-dive, dive and post-dive (PD) periods. Blood flow (ml) over a cardiac cycle during the dive exhibited a distinct peak in the first quarter of the cardiac cycle, then decreased momentarily before leveling off. In the second half of the cardiac cycle (frames 17–32), flow did not fall to zero at any point [mean flow (\pm S.E.M.) per frame = 2.23 ± 0.08 ml].

the development stage of the subjects, the forced dive pattern employed or a combination of these variables?

Experimental procedure

Velocity-encoded cine MR imaging, or MR flow velocity mapping, is increasingly used clinically for the quantification of arterial and transvalvular blood flow. Velocity and flow rate measurements with PC MRI have been repeatedly validated (e.g. Evans et al., 1993; Pelc et al., 1992). The principal source of error on the measured velocity is eddy currents induced by switched gradients, which can introduce a non-zero baseline. This is generally well controlled using corrections based on the apparent velocity of tissues known to be static. Flow measurements require integration over the vessel lumen, and errors can be introduced by imperfect definition of this region. This is more serious for small vessels than for channels as large as the aortic root. For more details on these effects, see Pelc et al. (1994).

Almost all cardiac MRI techniques employ a data acquisition protocol that is gated by ECG, which serves to eliminate motion artifacts due to the contractile motion of the heart. However, gated cardiac studies are reliant on the ability of the MR system to accurately assess each point in the cardiac cycle and require a significant amount of time to acquire a series of images. For the most part, the animals used in this study maintained a steady f_H during the dive except during periods of struggling, where f_H would increase during movement, then drop for a period of 5–10 s in the post-movement phase before leveling. We used a spiral sequence that employs 12–16 interleaves, allowing for much faster data acquisition than the conventional sequence. Application of this technique to 2-D slice data allows for computation of flow over the entire cardiac cycle by interleaving data from different beats. When the acquisition of a clear ECG signal was

occasionally impaired by animal movement or lead displacement, the gating sequence would ‘stall’ until a suitable ECG signal was obtained. The reconstructed cardiac cycle would then consist of images obtained over a period of 1–2 min. As the average f_H for any given minute of the dive was not significantly different from the mean diving f_H , flow data based on reconstructed cardiac cycles was accepted as an accurate representation of blood flow during the dive.

Diving ability

A key variable for explaining the results of forced dive studies on cardiac function is the diving schedule imposed on the seal and the diving capabilities of the subjects. Juvenile elephant seals have the capacity to dive longer than adult California sea lions (*Zalophus californianus*), grey seals (*Haliocoerus grypus*), harbor seals (*Phoca vitulina*) and Weddell seals (*Leptonychotes weddelli*), the subjects of other similar studies. The diving schedule we used, dives of 6 min followed by a surface rest of at least 13 min for complete recovery, is shorter in duration than dives performed in nature. On their first trip to sea, juvenile elephant seals exhibit a continuous diving pattern similar to adults and are submerged 85% of the time. The mean dive duration is about half that of adults, with a maximum of 22.3 min. Dives at sea are normally undertaken as part of a continuous diving cycle, or ‘bout’, with brief surface intervals between 1.4 and 1.8 min. This results in at-sea pre-dive heart rates that are strongly influenced by the previous dive and are tachycardic when compared with resting rates. The short surface interval could not be emulated under our experimental conditions, as the animal was removed from the magnet between dives to assess flipper temperature.

The selection of a 6 min experimental dive time was based on previous experience with juvenile elephant seals. In general, animal movement increases after 6 min of forced

submergence, which would have negative effects on cardiac gating. Although f_H has been observed to decrease slightly as a dive progresses in natural dives, no significant differences between Min 1 f_H and Min 5 f_H were observed in these experiments. This allowed us to use cardiac assessments gathered at different points within a dive.

Bradycardia

Assuming that the animals adapted to the experimental paradigm, as evidenced by reduced signs of stress, the animals never had to 'defend against asphyxia' (Elsner and Gooden, 1983) by exhibiting extreme bradycardia. That is, diving f_H did not approach the 3 beats min^{-1} of which they are capable. The response of juvenile seals in this study was much like that observed in elephant seals sleeping on the beach in the hot sun with their heads in a pool of water; a 'diving response' is observed but the decrease in heart rate from eupnea to apnea is less extreme than during dives at sea, where the tachycardic interdiver heart rate results in a greater degree of bradycardia during the dive.

Juvenile elephant seals are tractable and appear to be tolerant of forced diving protocols. The animals used in this experiment were also subjects in a concurrent study and had been previously exposed to the diving protocol. The profound bradycardia that normally accompanies forced diving protocols was not observed in this study. Forced diving f_H s are thought to be partly due to fear and the effects of restraint and handling; therefore, acclimation to the experimental protocol may have attenuated the bradycardic response.

Another possible contributing factor to the comparatively high forced diving f_H s (31.8 ± 3.6 beats min^{-1}) is thermoregulation. Animals are only subject to facial immersion and not whole body submergence, therefore heat retention may become a physiological challenge over the course of an experiment. During such experimental protocols, restraining devices may further restrict cutaneous heat loss from the animal. Temperature regulation in phocids is accomplished primarily through peripheral vasoregulation (McGinnis et al., 1971), therefore a decrease in total peripheral resistance due to heat dissipation may have resulted in a higher diving heart rate ($\dot{Q} = \text{mean arterial blood pressure} / \text{total peripheral resistance}$). However, experiments on harbor seals (Hammel et al., 1977) showed that diving-induced peripheral vasoconstriction overrides the increased cutaneous blood flow normally elicited in the presence of a thermal load but results in an increase in flipper vasodilation, as indicated by a 10°C rise in flipper temperature. In our study, the animals' fore and rear flippers were palpated before and after each dive (a commonly employed means of assessing thermal stress in pinnipeds). Flippers remained cool to the touch, indicating that animals were not dissipating excessive heat during the experiments.

Stroke volume

In this study, elephant seal V_s did not correlate with f_H during the pre-dive, dive or post-dive state. However, there

exists a negative correlation between V_s and the *ratio* of diving f_H to pre-dive f_H (the ratio is indicative of the degree of bradycardia expressed by the individual: mean diving f_H /pre-dive f_H). Animals that exhibit a more profound bradycardia have a higher V_s than individuals who express a less profound reduction in f_H (Fig. 2). Although the degree of bradycardia correlated negatively with V_s , the absolute f_H during the dive did not show a correlation. This finding suggests that it is the magnitude of the f_H response to diving that is driving the observed increase in V_s , rather than the absolute rate of contraction.

The extended diastolic filling time that accompanies bradycardia may partially account for the increase in V_s . An increased strength of myocardial contraction is normally thought to accompany bradycardia and compensate for the reduction in \dot{Q} caused by a decrease in cardiac frequency. This positive inotropic effect is achieved through the increased left ventricular filling time that is associated with extended diastole, causing increased myocardial preload and ventricular performance (Starling relationship). Right ventricular dilation is observed during diving, indicating a significant increase in preload (Hol et al., 1975; Blix and Hol, 1973). An increased ventricular volume also stimulates the ventricle to contract more rapidly, further augmenting the interbeat filling time (Frank–Starling Law). Contraction of the caval sphincter that occurs during diving in phocid seals may serve as a mechanism for controlling the level of preload. Hepatic sinus filling accounts for the increased volume of blood resulting from splenic contraction (Thornton et al., 2001), but may not attenuate the increased preload resulting from peripheral vasoconstriction. Thus, during diving, some increase in ventricular pressure would be anticipated.

In one animal, the combination of a clear ECG signal and lack of movement during the dive resulted in rapid acquisition of flow measurements (Fig. 3). Aortic blood flow during the dive peaked following ventricular contraction and aortic valve opening. Peak flow was followed by a reduction of flow approaching zero, which corresponds to closure of the aortic valve and phase lag effects (Nichols and O'Rourke, 1990). Flow was then maintained at a relatively constant rate throughout diastole, as evidenced by the latter half of the flow trace. The maintenance of flow during diastole is largely due to the windkessel effect of the aortic bulb, which continues to deliver blood *via* the elastic recoil of the stretched arterial walls (Jones, 1992).

Cardiac output

Previous studies of pinniped \dot{Q}_s all report significantly higher values than those predicted by allometric formulas (Table 1; Stahl, 1967). Although it was originally proposed that the metabolic rates of marine mammals scale differently in relation to body mass than terrestrial mammals (Platt and Silvert, 1981), this proposition was soundly disputed (Lavigne et al., 1985, 1986). Eupneic f_H s in phocids are reportedly higher than the predicted relationship ($M_b^{-0.25}$, where M_b is body mass; Castellini and Zenteno-Savin, 1997); however, the

Table 1. Comparison of literature values documenting pinniped cardiac output (\dot{Q}) and stroke volume (V_s) during pre-dive and diving state

Species	Mass (kg)	V_s , rest (ml)	\dot{Q} , rest (l min ⁻¹)	Stahl's \dot{Q} prediction* (ml min ⁻¹)	V_s , dive (ml)	\dot{Q} , dive (l min ⁻¹)	Method	Reference
Sea lion	~30	–	–	2.9	No change	75% decline	Doppler flowmeter	Elsner et al. (1964)
Harbor seal	15–27	31.2±16.0	5.18±2.33	1.7–2.7	39.61±27.2	0.62±0.35	Dye dilution	Murdaugh et al. (1966)
Grey seal	~39	59.85	7.9	3.6	60	0.6	Microspheres	Blix et al. (1976)
Harbor seal	35–52	128	11.5	4.14	83	~2	Thermodilution	Sinnett et al. (1978)
Weddell seal	334–496	826.21±317.64	39.79±10.21	20.7–28.5	365.87±178.38	5.62±3.42	Thermodilution, microspheres	Zapol et al. (1979)
Harbor/grey seal	30–40	70	9.7	2.9–3.7	40–70	0.6–0.8	Microspheres	Blix et al. (1983)
Harbor seal	28–39	100.2±15.2	9.14±2.17	2.8–3.6	30–50	2.5–5.6	Thermodilution	Ponganis et al. (1990)
Elephant seal	99–105	104.9±6.43	6.53±1.02	7.7–8.1	126.7±6.97	4.01±0.39	MRI phase contrast	Present study

*Stahl's power law prediction equation: 'resting' $\dot{Q}=187(\text{body mass})^{0.81}$ (Stahl, 1967).

increase in f_H is not enough to account for the elevated \dot{Q} values. An increase in V_s over predicted values is required in order to produce the resting \dot{Q} values reported for pinnipeds. As seal hearts scale isometrically with M_b , it is unlikely that resting V_s s would be 2–3 times higher than predicted values. One explanation for the discrepancy in measured v_s predicted values is that the allometric equation is based on resting measurements from a myriad of species. The definition of the phocid 'resting' condition is elusive, as many species spend a considerable amount of time in the submerged, apneic and bradycardic condition (Castellini and Zenteno-Savin, 1997). In general, most studies (including this one) loosely define a quiescent eupneic period as resting, introducing considerable variability into this evaluation. It is plausible that the \dot{Q} measurements from previous pinniped studies were obtained during a time when the animal was not in a resting state, thus creating a disparity between the predicted \dot{Q} and the measured value.

In most systems, comparison of \dot{Q} between a control and experimental condition will illustrate differences in systemic oxygen distribution and availability. These comparisons are based on the reasonable assumption that the oxygen content (Ca_{O_2}) of arterial blood leaving the heart remains relatively constant. However, when examining variations in phocid cardiac output, one must take into account the effect of the increased haematocrit and continued hemoglobin desaturation that accompanies diving. The blood that is expelled from the heart in the pre-dive state does not carry as much oxygen as that expelled in the first few minutes of diving. With respect to oxygen distribution, V_s and \dot{Q} are not directly comparable between control and experimental conditions. This is further complicated by the fact that, after an initial rise in Ca_{O_2} caused by the increase in haematocrit, the blood oxygen content in the latter part of the dive continuously decreases as hemoglobin desaturation occurs (Qvist et al., 1986). Although the effect of decreased Ca_{O_2} on cardiac contractile force in seals is not known, any chemoreceptor effect elicited by a reduced oxygen content would likely be overridden by the vagal input.

Conclusions

During diving, a reduction in cardiac frequency occurs and is accompanied by an increase in total peripheral resistance. In these experiments, the degree of bradycardia is considerably less than that reported in the seven previous studies reporting \dot{Q} measurements for diving pinnipeds and may be responsible for the observed disparity in V_s results. As the diving f_H values recorded in this study are close to those observed in freely diving animals, the data presented suggest that V_s may increase during free dives when bradycardia is less profound, rather than decrease as suggested by previous studies. These findings indicate that the effect of diving on V_s is correlated to the degree of bradycardia, suggesting that f_H alone may not be an accurate indicator of oxygen distribution in a diving animal.

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