Breathing frequencies of northern elephant seals at sea and on land revealed by heart rate spectral analysis

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Abstract

Elephant seals breathe episodically at sea and on land and surprisingly long apnoeas occur in both situations. An important difference is that recovery from apnoeic periods is much quicker at sea, which might be due, in part, to differences in the ventilatory response. Respiratory frequencies of juvenile northern elephant seals diving at sea and resting on land were estimated from time-frequency maps of the Wigner distribution of heart rate variability. Simultaneous direct measurement of respiration and estimation of respiratory frequency (f_R) in the laboratory demonstrated that the error of estimation was small (mean $\pm$ S.D. $= 1.05 \pm 1.23\%$) and was independent of the magnitude of f_R. Eupnoeic f_R at sea was 2.4 times higher than on land (22.0 ± 2.0 vs. 9.2 ± 1.3 breaths min^{-1}, respectively), facilitating quick recovery from the preceding dive and allowing a 34% increase in time spent apnoeic at sea versus on land. The overall f_R (no. of breaths in a eupnoea divided by the total time of the apnoea + eupnoea cycle) of 2.3 ± 0.6 breaths min^{-1} at sea was no different from the rate on land and was inversely related to the preceding dive duration, suggesting that metabolism on longer dives may be reduced. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

It has been suggested that the ability to maximise the percentage of time spent submerged is just as important to underwater foragers as their capacity for making long dives (Fedak et al., 1988). The northern elephant seal (*Mirounga angustirostris*) is a species that possesses extreme capabilities in both categories. Mean dive durations of juveniles and adults range from 15 to 20 min, with the maximum observed duration being 119 min and yet dives are almost always followed by short surface intervals of ≈ 2 min (Le Boeuf et al., 1988, 1996; Stewart and DeLong, 1995). Ele-

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phant seals are, therefore, very efficient divers, spending 80–95% of their time at sea underwater.

This exceptional ability must be related to high rates of gas exchange at the surface, but little is known about the physiology of recovery from diving in this species. Given that elephant seals are more accurately described as 'surfacers' than divers, their 'surface physiology' deserves as much attention as the more commonly studied 'diving physiology'. Heart rate between dives is 174% greater than during dives and 64% higher than the rate during eupnoeic periods on land (Andrews et al., 1997). Such high heart rates must facilitate rapid gas transport between tissue and lung and are presumably accompanied by high breathing rates to ensure similarly rapid exchange between lung and environment. Ventilation is positively related to the duration of the preceding dive in Weddell seals (*Leptonychotes weddellii*), plateauing at about six times the resting minute volume after dives of 15 min or longer. This hyperventilation is due to a 1.5–2 times increase in tidal volume and a 3-fold increase in respiratory frequency (Kooyman et al., 1971). Unfortunately, Weddell seals are the only pinniped species for which it has been possible to quantify the ventilatory response to voluntary diving in nature.

In preliminary experiments conducted as part of an earlier study (Andrews et al., 1997), attempts to record respiration from freely diving northern elephant seals using nasal thermistors were unsuccessful. The seals always managed to remove the thermistors before being released at sea. We were able, however, to obtain R-R interval time series at sea and on land for four seals equipped with electrocardiogram (ECG) recording Holter monitors. Northern elephant seals display a pronounced respiratory sinus arrhythmia (RSA) when on land, with heart rate increasing on inspiration and falling on expiration (Bartholomew, 1954; Castellini et al., 1994a,b). RSA also occurs during the eupnoeic periods between short dives in laboratory tanks, albeit with a reduced amplitude (personal observations) and rhythmic heart rate oscillations that appear to be due to RSA were also seen in sea-going northern elephant seals when at the surface (Andrews et al., 1997). We therefore sought to esti-

mate the respiratory frequency (f_R) of freely diving elephant seals through the analysis of these rhythmic fluctuations in heart rate.

We required an objective method that could be automated for analysis of large amounts of data and initially attempted to use the Womack (1971) method for the estimation of the time of occurrence of individual breaths. In this method, a low-pass filter is applied to the heart rate time series, deriving a smoothed function which is then compared with the unfiltered time series in order to detect inspiration-induced peaks that rise above the local average. Despite trying many different low-cut-off frequencies and various other filtering algorithms, we were not able to derive a satisfactory method for application to the at sea data because the RSA amplitude was very low. Instead of attempting to identify the exact time of each individual breath, we then decided to estimate the mean frequency of heart rate oscillations over a breathing period by using spectral analysis. The Fourier transform is often used for heart rate spectral analysis, but the breathing periods at sea are usually very short, while on land the breath to breath intervals can vary considerably over a short time period. A more appropriate method for short, nonstationary data is the discrete Wigner distribution (DWD), a form of time frequency mapping which provides a two-dimensional function of time and frequency (Wigner, 1932; Claasen and Mecklenbrauker, 1980). The DWD has been shown to correctly map the instantaneous changes in R-R interval spectral content even during continuous slowing of respiration in humans (Novak et al., 1993). Novak et al. (1993) showed that the dominant frequency of the R-R interval fluctuations closely followed the changing respiratory frequency over a large range, from 28 to 3 breaths min^{-1}. In this paper we present data to show that the DWD of heart rate variability provides a good estimate of f_R in northern elephant seals. We then compare our estimates of f_R at sea and on land to determine whether an increase in f_R during at sea breathing intervals is an important element of the elephant seals ability to minimise the time spent at the surface.

2. Materials and methods

2.1. Laboratory validation of f_R estimation method

Four juvenile northern elephant seals (Table 1) were captured at Año Nuevo State Reserve, CA and held in captivity at the Long Marine Laboratory, Santa Cruz, CA for up to 5 days. Details of the immobilisation, capture and transport methods are presented in Andrews et al. (1997). The surface ECG was recorded from two of these seals (L1 and L2) onto channel 1 of a 2-channel analogue Holter monitor (Model 90205, Space Labs Inc., Redmond, WA) attached to the fur in the mid-dorsal region (Andrews et al., 1997). Respiration was monitored in these two seals by recording, on channel 2 of the Holter monitor, the signal from a thermistor glued to the fur just above one of the nares. Respiratory and electrocardiographic signals were recorded from seals L1 and L2 for at least 4 h during daylight hours, while they rested in a fenced, outdoor pen. The Holter monitor tapes were scanned and digitised at 200 Hz using an FT2000A Medical Workstation computer system (Space Labs, Inc.).

The ECG of seals L3 and L4 was monitored by using needle electrodes attached subdermally along the dorsal midline. Respiratory movements of these seals were monitored using two additional needle electrodes placed subdermally on each side of the thorax at the level of the posterior end of the foreflippers. These electrodes were attached via long leads to an impedance respirometer. The ECG and respiration signals were acquired and stored on a personal computer equipped with an analogue-to-digital converter card. The ECG was sampled at 200 Hz, while respiration was sampled at 2 Hz. Seals L3 and L4 were held in a foam-padded metal cage for ≈ 4 h of daytime monitoring, after which their electrodes were removed and they were returned to an outdoor pen.

Sections of the records from all four seals were chosen for analysis by selecting time periods during which the seals were apparently sleeping or resting quietly. Only complete apnoea cycles (an apnoea and subsequent eupnoea) that were followed by another apnoea were analysed. To obtain R-R interval time series, the QRS complexes of the ECG were detected and the R-R intervals were calculated. Data segments from at least five apnoea cycles were chosen for each seal. Each segment consisted of the data 30 sec before the start of an eupnoea and continued until the end of the eupnoea. The time series were linearly interpolated at 4 Hz and the low-frequency baseline trend was removed by applying a moving fourth-order polynomial function. Time-frequency maps of the R-R interval variability for each data segment were constructed using the discrete Wigner distribution, with the parameter settings suggested by Novak et al. (1993). Each map consisted of spectra calculated at 0.5-sec intervals (Fig. 1). For each of these spectra, the frequency at which the maximum power occurred (peak power frequency) was determined. The frequency resolution of these individual spectra was 0.0156 Hz, or 0.94 cycles min^{-1}. Although the peak power frequency is a function of the time and frequency smoothing of the Wigner distribution, we used it to provide an estimation of the f_R for that time period. The individual spectra of the time-frequency maps were only used to estimate f_R if the spectrum was dominated by a single peak. Spectra that contained multiple peaks at similar power levels were discarded. This sometimes occurred at late stages in eupnoeas when the breathing intervals could become especially irregular. If $> 10\%$ of the spectra from a time-frequency map for an eupnoeic segment were discarded, then that segment was not included in the analysis.

In order to calculate the error involved in estimating f_R from the spectral analysis of R-R interval variability, f_R was directly measured by counting the number of breaths (including fractions of a breath cycle) that occurred in the period 15–60 sec after the start of an eupnoea. This count was then divided by the duration of the period (45 sec) to obtain the mean measured f_R for individual eupnoeas. Over the same period, the mean estimated f_R was determined by calculating the mean of the peak power frequencies from each of the time-frequency map spectra for that period. These 45 sec periods were chosen for analysis to be consistent with the analyses of

Table 1
Summary of the seals in the laboratory, their apnoea/eupnoea durations, and the error in the estimation of eupnoeic respiratory frequency from the spectral analysis of heart rate variability[a]

Seal no.	Age (months)	Mass (kg)	n	Mean apnoea duration (min)	Mean eupnoea duration (min)	Mean measured fR (breaths min^{-1})	Mean estimated fR (breaths min^{-1})	Mean % Δ	Mean absolute value of % Δ
L1	16	145	11	5.8 ± 1.2	5.6 ± 2.0	14.1 ± 0.8	14.2 ± 0.8	1.12 ± 0.8	2.29 ± 2.04
L2	16	198	10	7.3 ± 1.2	5.3 ± 1.6	8.2 ± 1.7	8.4 ± 1.8	1.88 ± 4.38	3.39 ± 3.22
L3	21	154	9	8.3 ± 3.9	4.9 ± 1.9	7.1 ± 1.2	7.0 ± 1.3	−0.71 ± 2.92	2.50 ± 1.44
L4	21	153	5	8.4 ± 1.8	3.8 ± 0.3	7.1 ± 1.1	7.2 ± 1.3	1.91 ± 4.49	4.15 ± 1.80
Grand mean				7.4 ± 1.2	4.9 ± 0.8	9.1 ± 3.4	9.2 ± 3.4	1.05 ± 1.23	3.08 ± 0.85

[a] n is the number of apnoea/eupnoea cycles. Mean values are presented as mean ± S.D.; a grand mean is the mean of the individual seal means. Measured fR, respiratory frequency measured by counting breaths in the periods 15–60 sec after the start of a eupnoea. Estimated fR, respiratory frequency estimation based on the mean peak power frequency from the time frequency map of the R-R variability for the period 15–60 sec after the start of a eupnoea. %Δ, the difference between the measured and estimated fR expressed as a percentage.
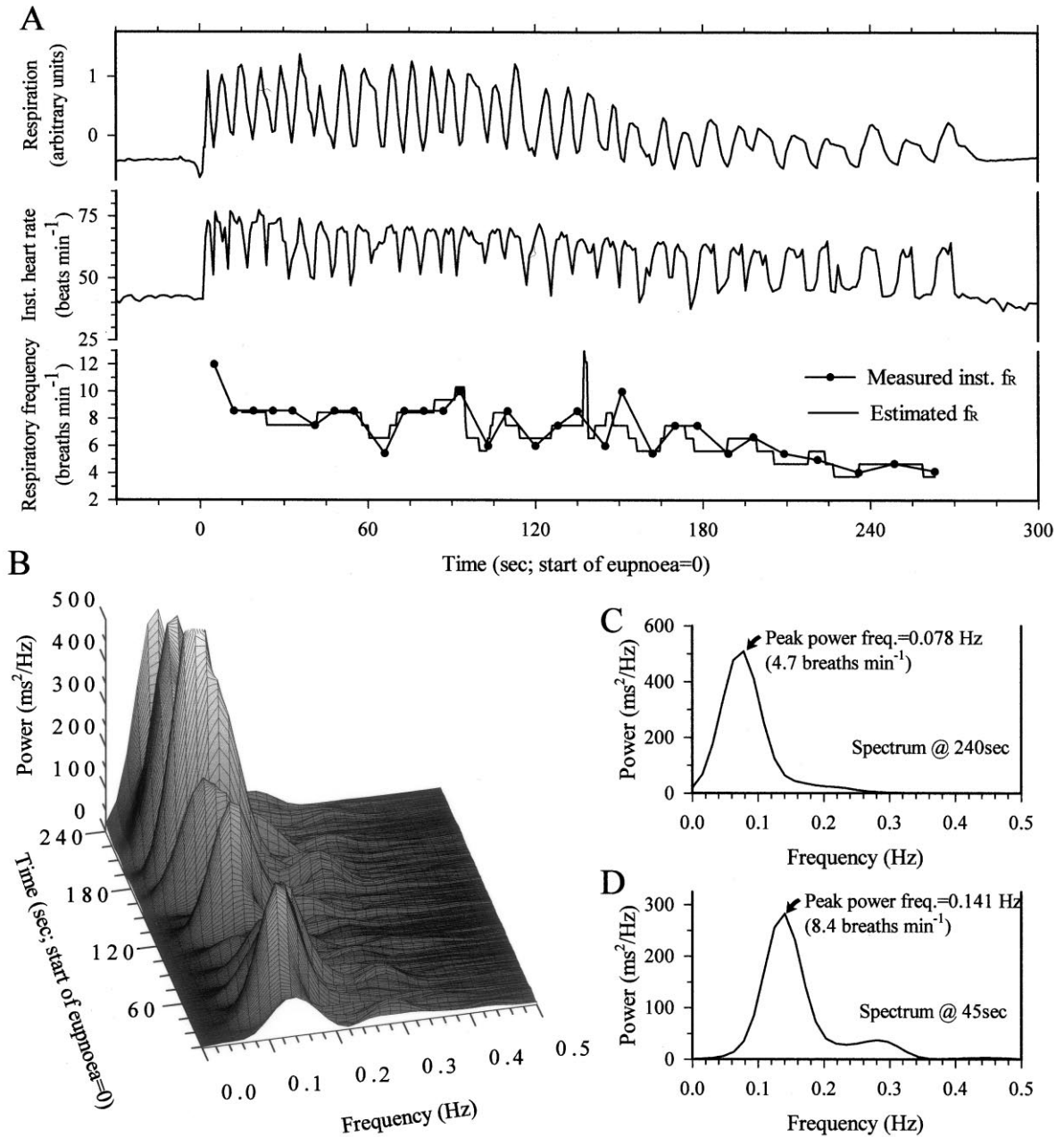
Fig. 1. Comparison of directly measured respiratory frequency (fR) and estimation of fR from the R-R interval time-frequency map. (A) Time course of the respiration signal, instantaneous heart rate (reciprocal of the R-R interval), and fR, both the measured instantaneous fR (reciprocal of the interbreath interval) and the estimated fR. (B) Time-frequency map of the R-R interval time series from the eupnoeic period in A. (C,D) The spectra from the time-frequency map at 45 sec (D) and 240 sec (C). The peak power frequency of each spectrum is indicated, which also provides an estimate of the fR at that time instant. Over the period from 15 to 263 sec, the mean measured fR = 6.64 breaths min^{-1}, while the mean estimated fR = 6.66 breaths min^{-1}.

at-sea eupnoeas. At-sea eupnoeas lasted on average for only 74 sec and the first 15 sec were ignored because of the imprecision in determining the start time of eupnoeas at sea.

2.2. Estimation of f_R for seals at sea and on the beach

The dive behaviour and ECG were recorded from four translocated seals (three males and one female; age: 16–34 months; mass: 150–245 kg, mean mass: 201 kg) as reported in Andrews et al. (1997). These seals were captured at Año Nuevo State Reserve, instrumented at the Long Marine Laboratory, transported either out to sea or to the opposite side of Monterey Bay and then released. The ECG was recorded as the seals dove continuously on their return to Año Nuevo and for 12–18 h after they reached land. Only eupnoeic periods longer than 32 sec, in which the ECG was completely noise-free and did not contain any ectopic or missing beats, were chosen for analysis. The start and end of eupnoeas at sea could be determined with an accuracy of $\approx \pm 5$ sec, because of the 5 sec sampling interval of the time-depth recorder (TDR). On land, the start of eupnoeas and apnoeas was determined from the marked changes in heart rate that accompany these transitions (Andrews et al., 1997).

The R-R interval time series were treated as described above for the seals in the laboratory and time-frequency maps were created for each eupnoea. For eupnoeas at sea, an 'estimated eupnoeic $f_{R_{1st\ min}}$, was calculated for the period from 15–60 sec (for the few eupnoeas < 60 sec, f_R was estimated for the period from 15 sec to the end of eupnoea). The mean eupnoea duration at sea was only 74 sec, so the estimated eupnoeic $f_{R_{1st\ min}}$ was multiplied by the total duration of the eupnoea in order to estimate the number of breaths in each eupnoea. The 'estimated overall f_R' was defined as the quotient of the number of breaths in an episode of eupnoea and the total duration of the apnoea cycle (apnoea duration plus eupnoea duration).

For eupnoeas on land, an 'estimated eupnoeic $f_{R_{1st\ min}}$' was calculated as above for the first minute, and for each subsequent minute of the

eupnoea an estimate was derived for the entire minute (e.g. 'estimated eupnoeic $f_{R_{2nd\ min}}$' covers the range from 60–120 sec). For the last fraction of a minute in a eupnoea, an estimate was derived for the total remainder of time (e.g. for a 3.6 min eupnoea, the 'estimated eupnoeic $f_{R_{4th\ min}}$, covers the range from 180–216 sec). The total number of breaths in a eupnoea on land were calculated iteratively, by multiplying the fraction of time spent in each 1 min bin by the estimated f_R for that bin and then calculating the sum. In some of the data segments for the later periods of eupnoeas (e.g. in the fourth and fifth minute) heart rate became quite arrhythmic and $> 10\%$ of the spectra did not contain a single dominant peak. In those cases, the fraction of time spent in a 1 min bin was multiplied by that seal's mean value for estimated f_R for that bin. For example, if the period from 240–300 sec of a 5.0 min eupnoea had to be discarded, then the number of breaths for that 1 min period were estimated by multiplying the mean estimated eupnoeic $f_{R_{5th\ min}}$ value from that seal's other eupnoeas by 1 (because the eupnoea lasted 5 min the fraction of time spent in the 5th minute bin was 1).

Student's paired t-tests were used to compare apnoea and eupnoea durations and respiratory frequencies at sea and on land, except where it was appropriate to perform a one-way repeated measures ANOVA. Significance was accepted at the level of $P < 0.05$ except when it was necessary to use the sequential Bonferroni method to minimise type-I errors (Rice, 1989). Relationships were examined using least-squares linear regression.

3. Results

3.1. Laboratory validation

During periods of simultaneous monitoring of respiration and heart rate, the estimated f_R from the 0.5 sec spectra of the DWD time-frequency map matched the measured instantaneous f_R closely, even when the breathing intervals were quite variable over time (Fig. 1). Therefore, the mean estimated f_R was usually within a few per-
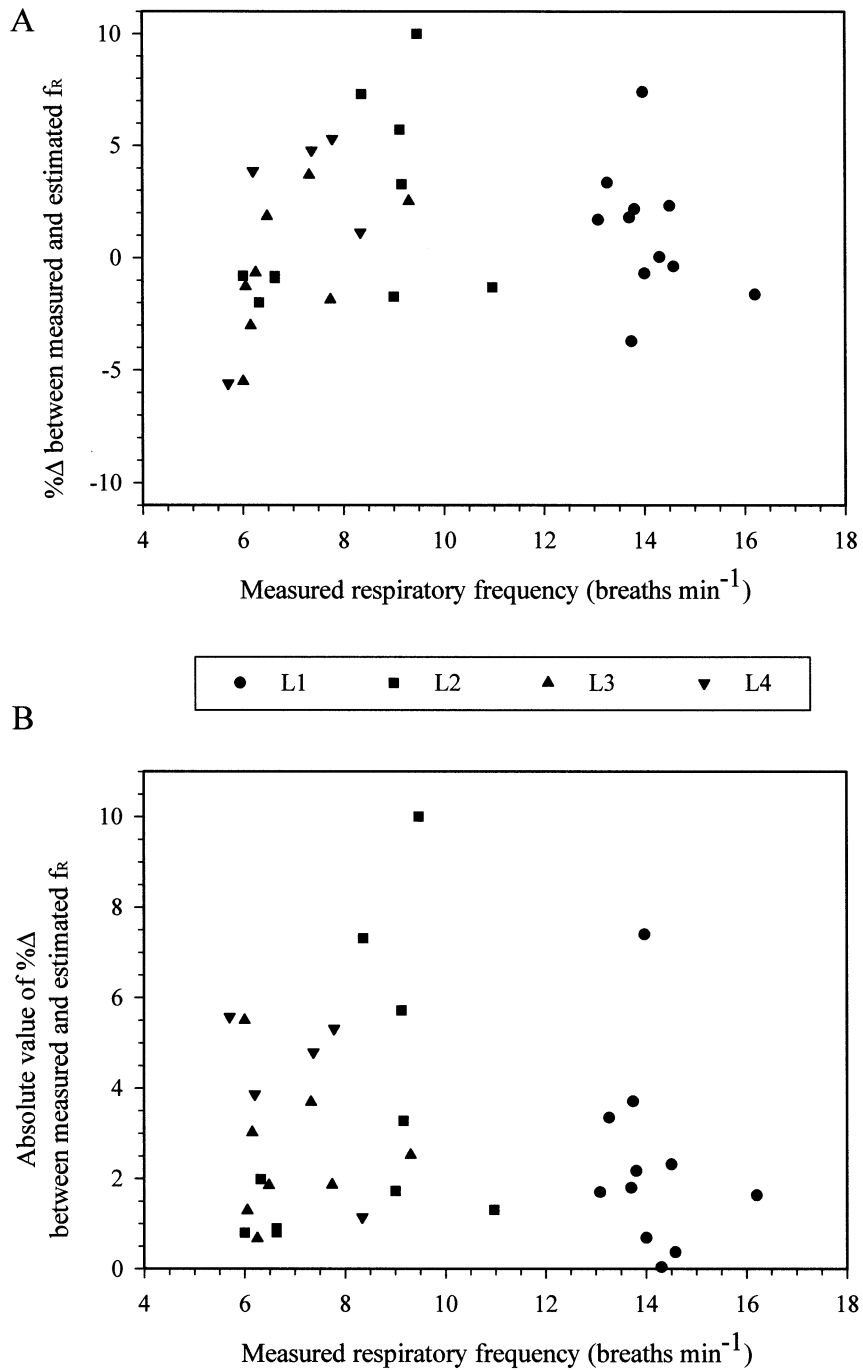
Fig. 2. The algebraic (A) and absolute value (B) of % Δ, the difference between measured and estimated f_R, plotted against the measured f_R for individual eupnoeic segments from all four seals (L1–L4).
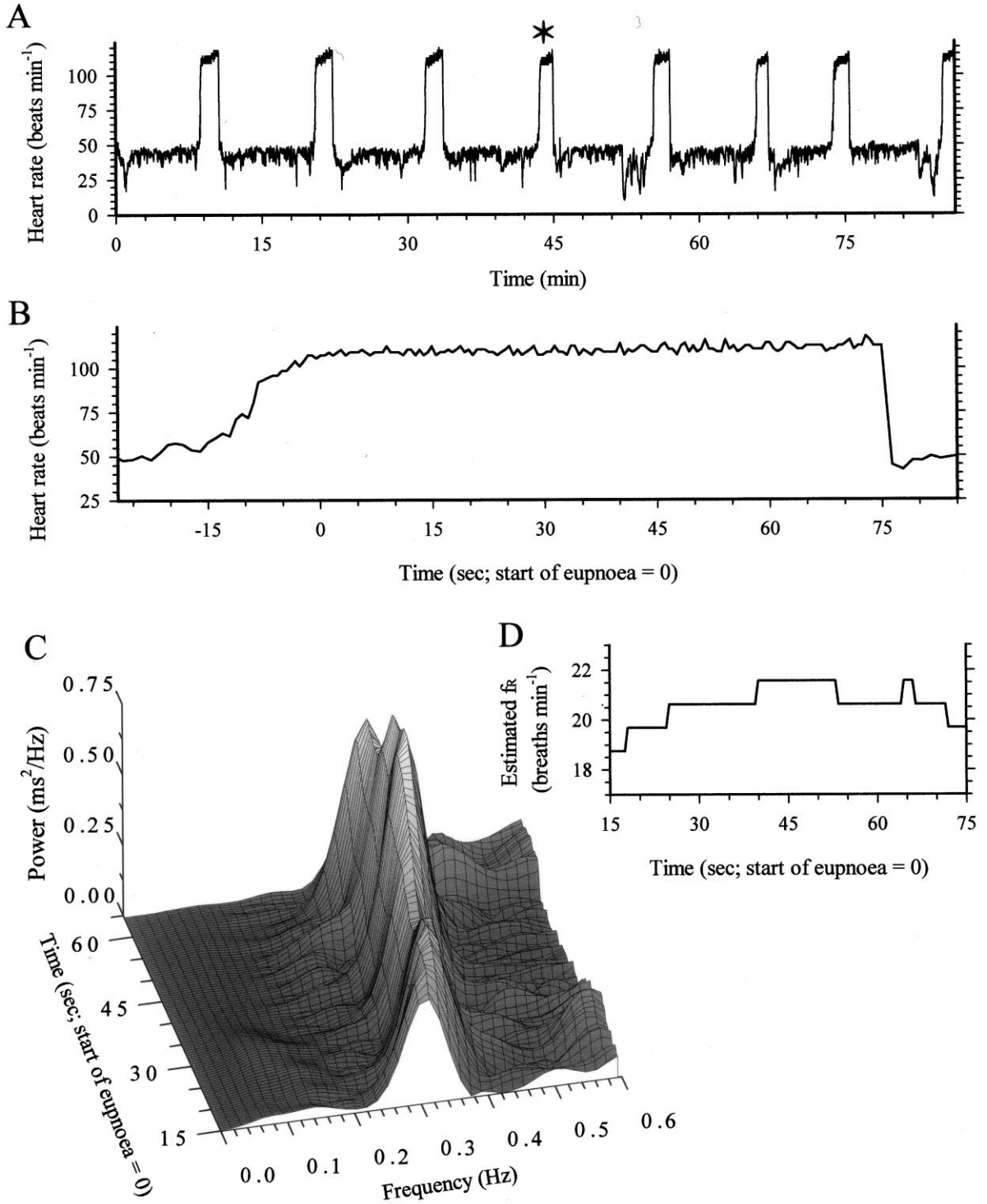
Fig. 3. Calculation of 'estimated eupnoeic $fR_{1st\ min}$, from the heart rate for a 1.25 min surface interval marked (*) in (A) and shown in an expanded view in (B). (C) The DWD time-frequency map of the R-R variability. (D) Time series of the estimated fR based on the peak power frequency of each of the spectra taken at 0.5 sec intervals from the time-frequency map. The estimated eupnoeic $fR_{1st\ min}$ was 20.6 breaths min^{-1}.
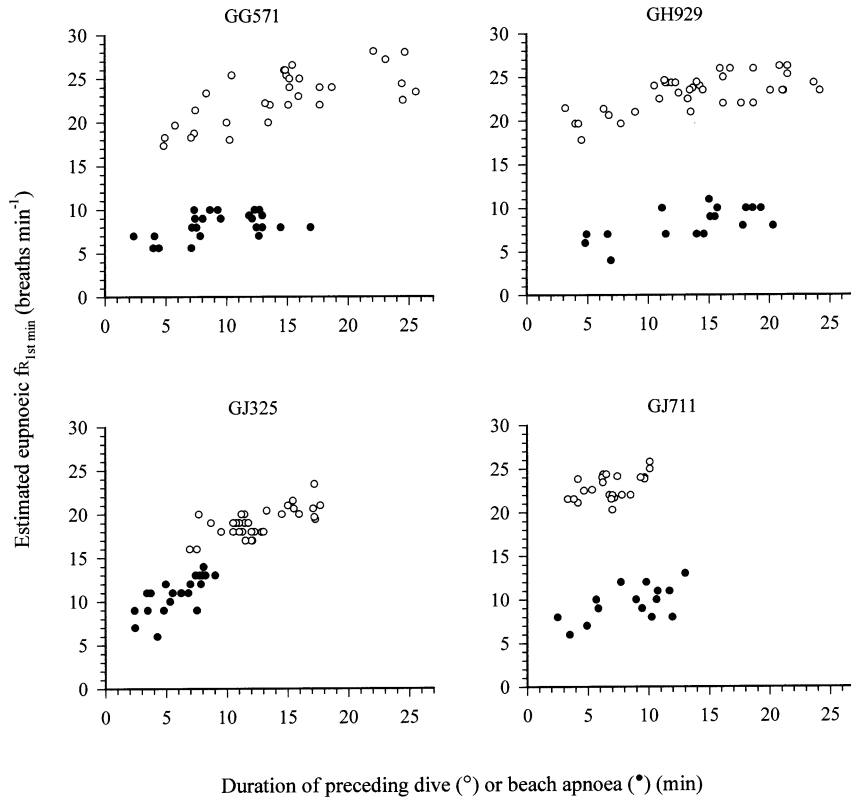
Fig. 4. Estimated eupnoeic $fR_{1st\ min}$ plotted against the duration of the preceding dive or beach apnoea for each of the translocated seals.

cent (range -5.6 to $+10.0\%$) of the mean measured fR for individual eupnoeic segments (Fig. 2). The mean algebraic error in the estimation of fR for all seals was only $1.05 \pm 1.23\%$ and the mean absolute value of the error was $3.08 \pm 0.85\%$ (Table 1). There was no relationship between the measured fR and the error in its estimation (Fig. 2).

3.2. Respiration at sea and on the beach

The DWD time-frequency maps of heart rate variability for eupnoeic segments at sea were usually dominated by a peak at the presumed respiratory frequency, although there was often considerable spectral power at other frequencies as well (Fig. 3C). The estimated eupnoeic $fR_{1st\ min}$ for individual surface intervals tended to increase with the duration of the preceding dive (Fig. 4).

In the two seals that made many dives in excess of 15 min, the estimated eupnoeic $fR_{1st\ min}$ appeared to plateau at ≈ 25 breaths min^{-1} (Fig. 4). The mean estimated eupnoeic $fR_{1st\ min}$ for individual seals ranged from 19.0 ± 1.6 to 23.3 ± 2.1 breaths min^{-1} (Table 2). There was a significantly positive linear relationship for each seal ($r^2 = 0.20 - 0.67$) between the estimated number of breaths per episode and the duration of the preceding dive. However, there was a negative relationship between the estimated overall fR and preceding dive duration (significant for three seals, $r^2 = 0.23 - 0.43$). Overall fR was even more strongly related in an inverse fashion to the percent time submerged (significant for all four seals, $r^2 = 0.28 - 0.96$).

Time-frequency maps of heart rate variability during eupnoeas on the beach were very similar to those for the seals in the laboratory. There was a

Table 2
Durations of apnoea and eupnoea and respiratory frequencies for translocated seals at sea and on land[a]

Seal tag no.	n	Mean apnoea duration (min)	Mean eupnoea duration (min)	Mean % time apnoeic	Mean estimated eupnoeic $fR_{1st min}$ (breaths min^{-1})	Mean estimated overall fR (breaths min^{-1})	Mean estimated no. of breaths per episode
At sea:							
GG571	31	14.6 ± 6.1	1.21 ± 0.34	91.6	22.9 ± 3.0	1.9 ± 0.5	28.3 ± 10.4
GH929	38	13.9 ± 5.7	1.10 ± 0.26	91.6	23.2 ± 2.1	1.9 ± 0.8	25.6 ± 7.1
GJ325	34	12.5 ± 2.9	1.59 ± 0.34	88.3	19.0 ± 1.6	2.2 ± 0.5	30.4 ± 7.1
GJ711	23	6.9 ± 2.0	1.06 ± 0.30	86.2	22.9 ± 1.4	3.2 ± 0.6	24.6 ± 8.1
Grand mean:		12.0 ± 3.5	1.24 ± 0.24	89.4 ± 2.6	22.0 ± 2.0	2.3 ± 0.6	27.2 ± 2.6
On land:							
GG571	24	9.4 ± 3.7	5.1 ± 1.5	62.9	8.2 ± 1.4	2.8 ± 0.8	38.6 ± 10.3
GH929	17	13.5 ± 5.1	4.4 ± 1.6	74.2	8.2 ± 1.8	2.0 ± 0.6	33.9 ± 11.8
GJ325	23	6.0 ± 2.0	4.3 ± 2.1	58.0	11.0 ± 2.1	3.5 ± 0.7	36.0 ± 15.4
GJ711	15	8.4 ± 3.2	3.2 ± 1.3	71.6	9.6 ± 2.0	2.3 ± 0.4	26.4 ± 9.6
Grand mean:		9.3 ± 3.1	4.2 ± 0.78*	66.7 ± 7.5*	9.2 ± 1.3*	2.6 ± 0.6	33.7 ± 5.2

[a] Mean values are presented as mean ± S.D.; a grand mean is the mean of the individual seal means. Eupnoeic $fR_{1st min}$, the estimated fR for the period 15–60 sec after the start of a eupnoea. Overall fR, the estimated number of breaths of a eupnoea divided by the duration of the complete apnoea cycle. Sequential Bonferroni procedure was used to minimise Type 1 errors in the multiple (6) paired *t*-tests.

* Significant difference between the 'at sea' and 'on land' value.

trend for the estimated eupnoeic $fR_{1st\ min}$ for individual eupnoeas on the beach to be directly related to the duration of the preceding apnoea, at least for apnoeas up to 10 min long (Fig. 4). Mean estimated eupnoeic $fR_{1st\ min}$ for all seals was 9.2 ± 1.3 breaths min^{-1}, which was 58% lower than the estimated eupnoeic $fR_{1st\ min}$ at sea (Table 2). The mean estimated fR for each subsequent minute of eupnoea tended to decrease slightly. There were adequate sample sizes from all four seals to allow comparison of $fR_{1st\ min}$, $fR_{2nd\ min}$, $fR_{3rd\ min}$ and $fR_{4th\ min}$, with a repeated measures ANOVA (multiple pairwise comparisons done with the Student-Newman–Keuls test). Only $fR_{3rd\ min}$ and $fR_{4th\ min}$ was significantly lower than $fR_{1st\ min}$ and the mean $fR_{4th\ min}$ was 7.7 ± 1.0 breaths min^{-1}. The mean estimated number of breaths per episode on land was 34 ± 5, which was not significantly different from the mean value of 27 ± 3 breaths per episode at sea (Table 2). The mean estimated overall fR was 2.6 ± 0.6 breaths min^{-1} on land and 2.3 ± 0.6 breaths min^{-1} at sea (Table 2). Because the number of breaths for some long eupnoeas on land had to be calculated with mean fR values for individual 1 min bins instead of with the actual estimated fR for that min of the eupnoea, we did not examine relationships between the duration of the preceding apnoea and either the number of breaths per episode or the overall fR.

4. Discussion

The estimation of respiratory frequency from the spectral analysis of heart rate using the discrete Wigner distribution appears to be a satisfactory method for situations in which it is possible to record the ECG but not respiration. Although we were not able to validate the method by simultaneous recording of respiration and estimation of fR in seals at sea, we are confident nonetheless that our estimates of fR for seals at sea are reasonable. In the laboratory we observed no correlation between measured fR and the error in estimating fR, even though one of the seals (L1) breathed at an unusually high rate (Table 1, Fig. 2). The RSA amplitude was reduced in seal L1

compared with the others, and the magnitude of the peak R-R interval spectral power was diminished, but the error in estimating fR was still very low. RSA amplitude in the seals breathing at sea was even lower, so the level of spectral power at the peak power frequency (the presumed fR) was very low (compare Figs. 1 and 3). Overall spectral power, however, was also reduced and so there was still a single, obviously dominant, peak in most spectra.

A reduction in R-R interval spectral power at the respiratory frequency and at all other frequencies is also seen at high respiratory rates during exercise in humans (Arai et al., 1989). RSA is dependent upon both fR and tidal volume (V_T), and while the phase and amplitude may change, there is usually a 1:1 relationship between the respiratory movements and RSA heart rate fluctuations (Angelone and Coulter, 1964; Hirsch and Bishop, 1981). The coherence between the R-R interval power spectrum and the respiratory signal power spectrum is usually near one and is independent of fR in humans (Patwardhan et al., 1995). This would appear to be the case for elephant seals as well, at least for seals on the beach breathing at a limited range of frequencies. Our estimates of fR for seals at sea are also supported by recent, serendipitous measurements of fR from the recording of breath sounds of translocated northern elephant seals carrying acoustic monitoring devices (Fletcher et al., 1996). The mean eupnoeic fR, between dives that averaged 14.7 min, ranged from 22.0 ± 1.0 to 24.6 ± 1.6 breaths min^{-1} for three seals. These values are very similar to the values of fR that we estimated in this study.

The mean post-dive eupnoeic $fR_{1st\ min}$ was 2.4 times greater than the mean post-apnoea eupnoeic $fR_{1st\ min}$ on land (Table 2). Eupnoeic $fR_{1st\ min}$ at sea also increased in direct relation to the duration of the preceding dive, at least for dives up to 15 min in length (Fig. 4). These high breathing rates permit elephant seals to spend only short periods at the surface for gas exchange, resulting in a high percentage of time spent submerged, or apnoeic. The mean duration of apnoea at sea was not significantly different from that on land, and the number of breaths per episode was also not differ-

ent. The much higher fR at sea, however, resulted in 34% increase in the time spent apnoeic at sea compared with apnoeic time on land.

This difference in fR between diving seals and seals resting on land contrasts with a study of northern elephant seal pups. In the laboratory, pups performed equivalent length breath-holds whether sleeping underwater in a shallow tank or sleeping in dry conditions, and there was no difference in the eupnoeic fR between wet and dry conditions (Castellini et al., 1994a). It is possible that the increased cardiac and respiratory rates of juveniles during recovery from dives compared with recovery from sleep apnoeas are due to more extreme changes in blood gas levels and therefore, greater levels of respiratory drive during recovery from diving. When northern elephant seal pups that are awake and breathing regularly, without extended apnoeas, are exposed to hypercapnic breathing gas, fR approximately doubles (Milsom et al., 1996). Exposure to hypercapnia during bouts of sleep apnoea, however, causes only a small increase in the instantaneous breathing rate for the first five breaths after an apnoea, and moderate hypoxia has no effect on fR whether the pups are asleep or awake. It is therefore not clear whether increased Pa_{CO_2} and decreased Pa_{O_2} would be expected to cause as large an increase in fR as the one we saw in recovery from dives compared with recovery from apnoeas on land in elephant seals.

It is not even clear whether blood gas levels will differ at the end of dives and beach apnoeas of similar duration. Furthermore, the direction of any difference may not be what one might expect, despite that during a dive seals are swimming and during most apnoeas on land seals are simply sleeping. For instance, during one episode of sleep apnoea in a Weddell seal lying on ice, Pa_{O_2} fell to 25 mmHg and Pa_{CO_2} rose to 55 mmHg after only 4 min (Kooyman et al., 1980). Such a low Pa_{O_2} was not usually seen in freely diving seals until after at least 15 min of submergence and Pa_{CO_2} never rose above 53 mmHg, even in dives as long as 27 min (Qvist et al., 1986).

Although simultaneous measurements of fR, VT and alveolar and blood gases in elephant seals during both diving and sleep apnoea are needed before an attempt to resolve the issue can be made, it seems safe to propose that there are important differences in the control of the cardiorespiratory responses to diving and sleep apnoea. Perhaps during diving there is a change in the set point and gain of the ventilatory CO_2 response. Rather than being an automatic response to respiratory drive, irrespective of situation, it appears that the high heart rates and breathing rates after a dive uniquely serve to ensure rapid, as opposed to adequate, gas exchange, thereby decreasing the amount of time spent at the surface.

Although the elephant seal's mean eupnoeic breathing rate at sea of 22 breaths min^{-1} is 2.4 times higher than on land, terrestrial mammals are often capable of increasing fR by four to eight times during strenuous exercise. If elephant seals are under pressure to decrease the fraction of time they spend at the surface, one might ask why they do not breathe even faster between dives. Even sedentary calves (mass ≈ 180 kg) are able to increase fR up to 65 breaths min^{-1} during exercise (Kuhlmann et al., 1985), and the fR of ponies (mass ≈ 150 kg) exercising near their maximal work load reaches 95 breaths min^{-1}, an increase of five times over resting fR (Bisgard et al., 1978). Of course, respiration in elephant seals floating motionless at the surface cannot be assisted by coupling between respiratory and locomotor movements, as may be the case in running mammals.

Nevertheless, if elephant seals breathe with very large tidal volumes after dives, like Weddell and grey (*Halichoerus grypus*) seals (Kooyman et al., 1971; Reed et al., 1994), reasonably high levels of ventilation may still be achieved. Based on the relationship between body mass and lung volume derived from other marine mammal species (Kooyman, 1989), the predicted total lung capacity of a 201-kg elephant seal is 16.2 L. The maximal VT during recovery from voluntary dives in Weddell and grey seals is between 46 and 49% of total lung capacity (TLC) (Kooyman et al., 1971; Reed et al., 1994). If we assume that VT in elephant seals is 50% of TLC, or 8.1 L, then with a fR of 22 breaths min^{-1} they would have an expired ventilation of 178 L min^{-1}. At a maximal

breathing rate of 27 breaths min^{-1}, ventilation would reach 219 L min^{-1}. Similarly sized calves and ponies reach ventilation levels of 251 and 435 L min^{-1}, respectively, but a comparison of alveolar ventilation may be more appropriate because the dead space (V_D) to V_T ratio tends to be very small in phocid seals. (Kooyman et al., 1971; Craig and Påsche, 1980). With a V_D/V_T of 0.15, at the maximal f_R of 27 breaths min^{-1}, elephant seal alveolar ventilation might reach 186 L min^{-1}, exceeding the alveolar ventilation reached by calves (160 L min^{-1}), but still much less than that observed in the highly athletic ponies (234 L min^{-1}).

A factor that may be limiting total ventilation in elephant seals is the extra work required to breathe while immersed in water. When juvenile elephant seals reach the surface and begin to breathe they are still immersed up to the level of the base of the skull, and the midpoint of the lung is subject to a hydrostatic pressure of ≈ 40 cm of seawater (4.0 kPa). The work of breathing increases by 60% when humans are immersed up to the neck, and this is partly due to an increase in airway resistance because of compression of the extrathoracic airways (Agostoni et al., 1966; Hong et al., 1969). The trachea, bronchi and terminal airways of pinnipeds, however, have an unusual amount of muscular and cartilaginous reinforcement (Denison and Kooyman, 1973; Tarasoff and Kooyman, 1973). The function of this reinforcement may be to limit nitrogen absorption during deep dives, by allowing orderly collapse of the lung (Scholander, 1940), or to allow high expiratory flow at low lung volumes (Denison and Kooyman, 1973; Kerem et al., 1975). On the other hand, the strengthened airways may also serve to resist hydrostatic compression during immersed breathing. Nonetheless, Kerem et al. (1975) observed that inspiratory flow rates of immersed California sea lions (*Zalophus californianus*) were much lower than expiratory rates and although they thought this result was puzzling, it is likely that while pinnipeds can expire large volumes very quickly, inspiration is limited by the difficulty of breathing against a large positive pressure.

A potential trade-off between oxygen transport and oxygen storage capacity may also limit the maximum ventilation rate that elephant seals can, or should, achieve. The resting hematocrit (Hct) of northern elephant seals is quite high (range ≈ 50–67%), and it can increase both during apnoeas on land and during diving in the laboratory (Castellini et al., 1986; Hedrick et al., 1986; Wickham et al., 1990; Hedrick and Duffield, 1991; Thorson, 1993; Thorson and Le Boeuf, 1994). Although such a high Hct is an important component of the northern elephant seal's exceptional oxygen storage capacity, the concomitant exponential increase in blood viscosity may severely limit maximal oxygen transport (Hedrick et al. 1986; Hedrick and Duffield, 1991). There is little difference in surface interval heart rate between short and long dives at sea (Andrews et al., 1997), suggesting that a heart rate of ≈ 110–120 beats min^{-1} is the highest rate possible in juvenile elephant seals. If during recovery from dives cardiac output reaches a limit, then further increases in ventilation would contribute very little to gas exchange at the expense of increases in the work of breathing. Extremely high Hct may increase oxygen storage, enabling longer maximal dive times, but would also decrease oxygen transport, extending the time needed for recovery. Consequently, there may be a trade-off between the ability to make very long dives and the ability to reduce the surface interval between dives. With dive durations of ≈ 20 min and 90% of time at sea spent submerged, elephant seals may have reached the limit.

Elephant seals do, however, sometimes make exceptionally long dives that are nonetheless followed by short surface intervals, which suggests that metabolism must be reduced in such dives so that an extended recovery period at the surface is not necessary. Although metabolic rate reductions during diving have been proposed frequently (for reviews see Boyd and Croxall (1996), Butler and Jones (1997) and Kooyman and Ponganis (1998)), there has never been a direct measurement of metabolic rate during a dive for any marine mammal. In translocated juvenile elephant seals, there is an inverse relationship between diving heart rate and dive duration, suggesting that metabolic rate may be adjusted in a similar fashion (Andrews et al., 1997). In this study we reported that the overall f_R (number of breaths per dive cycle divided by the

dive plus surface interval duration) is also inversely related to dive duration, providing further support that metabolic rate is reduced on longer dives.

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