

## Acoustic measurements of post-dive cardiac responses in southern elephant seals (*Mirounga leonina*) during surfacing at sea

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## Summary statement

This paper demonstrates the reliability of an acoustic method to extract and analyse the cardiac function of free ranging southern elephant seals in order to study post-dive recovery.

## Abstract

Measuring physiological data in free-ranging marine mammals remains challenging, owing to their far-ranging foraging habitat. Yet, it is important to understand how these divers recover from effort expended underwater, as marine mammals can perform deep and recurrent dives. Among them, southern elephant seals (*Mirounga leonina*) are one of the most extreme divers, diving continuously at great depth and for long duration while travelling over large distances within the Southern Ocean. To determine how they manage post-dive recovery, we deployed hydrophones on four post-breeding female southern elephant seals. Cardiac data were extracted from sound recordings when the animal was at the surface breathing. Mean heart rate at the surface was  $102.4 \pm 4.9$  beats.min<sup>-1</sup> and seals spent on average  $121 \pm 20$  s breathing. During these surface intervals, the instantaneous heart rate is increasing with time. Elephant seals are supposed to drastically slow their heart rate (bradycardia) while they are deep underwater, and increase it (tachycardia) during the ascent towards the surface. Our finding suggests that tachycardia continues while the animal stays breathing at the surface. Also, the measured mean heart rate at the surface was unrelated to the duration and swimming effort of the dive prior to the surface interval. Recovery (at the surface) after physical effort (underwater) appears to be related to the overall number of heart beats performed at the surface, and therefore total surface duration. Southern elephant seals recover from dives by adjusting the time spent at the surface rather than their heart rate.

## Introduction

Diving marine mammals face a strong dilemma: their food resources are located at depth while they need to restore oxygen supply at the surface. This specificity makes them particular because their breath hold capability limits the time spent foraging. Hence, surfacing is essential to reconstitute oxygen stores by breathing and restoring oxygen levels in muscles and organs. Oxygen stores are higher for diving species than non-diving ones (Butler and Jones, 1997) and are located in the blood and muscles (Hassrick et al., 2010; Kooyman et al., 1983). Bradycardia is the common response to diving in marine mammals and diving seabirds (Ponganis, 2015), with, for instance, northern elephant seal reducing its heart rate by 64% (Andrews et al., 1997). Regulation of heart rate, cardiac output, and the degree of vasoconstriction and blood circulation shutdown is critical to the management and utilization of oxygen stores.

Measures of physiological data are essential to understand diving mammal metabolism (Butler and Jones, 1997). Ideally, physiological parameters should be recorded on free-ranging animals diving voluntarily (Webb et al., 1998). However, accessing physiological data *in situ*, like cardiac response at the surface, on free-ranging animals in the open ocean remains difficult. The pinnipeds share their time both at sea and on land or ice (Harrison and Kooyman, 1968). This bimodal cycle coupled to their large size makes them a unique system to study their physiological adaptations to deep dives, because the deployment and recovery of loggers are eased on land (Costa et al., 2004). Kooyman et al. (1968; 1971; 1973) were the first to access physiological data and calculate metabolic rate using a respiratory chamber on free-ranging Weddell seals, *Leptonychotes weddellii*, with the man-made ice-hole experiments under semi-natural conditions.

Due to difficulties to employ this technique *in situ* on other marine mammals species, methods based on heart rate were used as a reliable indicator of field metabolic costs (Butler et al., 2004; McPhee et al., 2003; Ropert-Coudert et al., 2012). For instance, Weimerskirch et al. (2000) successfully used heart rate as a proxy of energy expenditure and instantaneous effort in flying wandering albatrosses, with the highest heart frequencies observed while albatrosses were walking on land and taking off. This method also provides advantages over doubly labelled water (DLW) in pinnipeds, since it provides an estimation of the metabolic rate of specific activities, such as those occurring during a dive cycle (Butler et al., 2004). The electric approach, which measures the electrical signal of the heart, is the most common way to record heart rate in free-ranging diving mammals (Ropert-Coudert et al., 2012; Webb et al., 1998).

Heart rate studies on elephant seals with an electric method showed that during diving, they exhibited bradycardia. Heart rate rapidly decreased by 50-80% at the beginning of the dive and remained low while the seal was submerged (Andrews et al., 1997). Hindell and Lea (1998) recorded extreme bradycardia with heart rate reaching 2 beats.min<sup>-1</sup> in 23 dives. Heart rate then increased gradually as the seal rose to the surface (Andrews et al., 1997). Bradycardia, apnoea and vasoconstriction of the peripheral system constitutes the dive response in pinnipeds (Harrison and Kooyman, 1968). However, the electrical method requires the fixation of an electrode into the body which can cause complications in the field (Ropert-Coudert et al., 2012).

Fletcher et al., 1996, using an acoustic approach, provided the first record of a respiratory rate at the surface of translocated northern elephant seals (*Mirounga angustirostris*). Between breaths, putative heart beats were distinguished and cardiac frequency extracted. Several acoustic studies confirmed that this method could provide physiological data such as breath frequency or heart rate at the surface (Burgess et al., 1998; Génin et al., 2015; Le Boeuf et al., 2000).

The aim of this study is to investigate the cardiac response at the surface to active dives in free-ranging southern elephant seals (SES here after). SESs are a major predator in the Southern Ocean. At sea, they dive repeatedly to around 500 m during 20-30 minutes with surface intervals lasting on average 2 minutes but extreme depth records reached over 1,800 m (Hindell et al., 1991; McConnell et al., 1992). They come back on land twice a year for mating during the southern spring, and moulting in the summer, with a high-site fidelity (Fabiani et al., 2006).

Génin et al (2015) have shown that the number of breaths is tightly related to surfacing time and mainly explained by dive duration and swimming effort made by SESs. Yet, in terms of recovery, the cardiac function might play a major role. In this study, we intend to explore the recovery behaviour of SES through examining variation in heart frequency. First, we investigate how the instantaneous cardiac frequency evolves at the surface. Second, we study the relation between the mean cardiac frequency during the surface interval, and the dive duration and foraging effort performed by SESs during the previous dive.

## Materials and methods

### Ethics statement

All animals in this study were treated in accordance with the French Polar Institute (IPEV) ethical and Polar Environment Committees guidelines. All scientific procedures conducted on SES had been validated beforehand.

### Deployment of devices and data collection

This study is based on data collected on four post-breeding SES females (Table 1): two equipped in October 2011 and another two in October/November 2012 on Kerguelen Islands (49°20'S, 70°20'E). Individuals were captured, then anaesthetized using 1:1 combination of tiletamin and zolazepam (Zoletil 100), which was injected intravenously (0.8 mg/100kg; McMahon et al., 2000). They were then equipped with two devices glued on the head or the back of the individual, using quick-setting epoxy (Araldite AW 2101, Ciba) after cleaning the fur with acetone. First, an Argos-GPS satellite tag (Splash 10-F, Wildlife Computer, USA) was glued to the head of the seals. It provided real-time position of the seals through the Argos system and also collected GPS location data. Second, an autonomous acoustic/accelerometer/magnetometer and pressure logger named Acousonde™, model 3A (Acoustimetrics, Greeneridge Sciences, Inc, USA) (Burgess, 2000; Burgess et al., 1998) was fixed on the dorsal fur on the longitudinal axis, 10 cm behind the scapula. Acousondes™ recorded at a sampling frequency of 6.3 kHz in 2011 and 12.2 kHz in 2012, with both an acoustic sampling resolution of 16 bits. This difference in sampling rates does not interfere in our study as cardiac and respiratory events occur in a frequency range inferior to 1 kHz. To save battery power and storage space, it was programmed to record sound 3 h every 12 h in 2011 and 4 h every 24 h in 2012. All devices provided measurements of time, location and depth at 1 Hz, as well as the three-dimensional magnetic field strength and acceleration at a 5 Hz frequency. The instruments sampled acoustic data until battery exhaustion, which occurred between 10 and 20 days after deployment. All devices were retrieved once individuals returned ashore to moult after their foraging trip in January/February following deployments. Seals were located on land using their Argos position.

## Acoustic data processing

### *Cardiac occurrences detection*

When the animal is surfacing, the water flow noise produced by swimming ceases and most of the sound is due to breathing. Respiratory signals are contained between frequencies within the 0 and 700 Hz range. Between two respirations, spectrograms (time-frequency representation) showed putative cardiac occurrences (Fletcher et al., 1996; Le Boeuf et al., 2000). Heart sounds are expected to be dual due to the closure of mitral and the aortic valves (Burgess et al., 1998). The two sounds are undistinguishable, as they occur too close together in time. Hence, cardiac occurrences (a combination of the two valves' sounds) are brief and regular temporal impulsions at frequencies from 0 to 150 Hz (Burgess et al., 1998).

Acoustic recordings of surface intervals were visualized and analysed using the software Raven (The Cornell Lab of Ornithology – Bioacoustics Research Program) to generate a spectrogram for each surface interval. The same parameters were used for the computation of all spectrograms: a window of Hann type and a size of 512 samples, an overlap of 50 % and a Discrete Fourier Transform (DFT) calculated with 512 samples.

Each cardiac beat was determined using visual and auditory cues (Fig. 1). Hence, each occurrence is characterised by its temporal abscissa. When two beats are consecutive, an instantaneous cardiac frequency (beats.min<sup>-1</sup>) is calculated using the temporal difference between the two occurrences (Eqn. 1).

**Equation 1:** Instantaneous cardiac frequency ( $f_{Hinst}$ ) =  $\frac{60}{x_{t+1} - x_t}$

Some surface intervals contained high-amplitude noise from external sources (water splashes possibly due to rough weather conditions or due to certain animal behaviours such as grooming). A noisy acoustic environment prevents access to heart data. As such, a surface interval was only kept for further analyses when a minimum of five cardiac beats were measured, so that the cardiac frequency could be estimated with confidence. Cardiac frequency for a surface interval  $f_H$  was computed as the average of instantaneous heart frequency  $f_{Hinst}$  (Eqn. 1) during each surface interval.

### *Surface interval duration*

The beginning, end and duration of each surface interval, to the nearest second, were determined using visual and auditory cues. A surface interval begins with the first respiration and ends with the last one.

## Dive cycle

A dive cycle is composed of a dive followed by a surface interval. SESs were considered to be diving when they reached depths greater than 15 m, to avoid considering subsurface movements as dives. Each dive was decomposed into three phases: descent, bottom and ascent. Each phase was determined using a vertical speed criterion. Vertical speed was modelled with an order four polynomial function adjusted on instantaneous vertical speed using a custom-written Matlab code (Matlab software 8.1, The MathWorks, Natick, MA, USA). Ascent and descent phases were identified as periods before or after surfacing where the modelled vertical speed exceeded  $0.75 \text{ m}\cdot\text{s}^{-1}$ . Bottom phases were identified as periods between ascent and descent phases where the modelled vertical speed remained below  $0.75 \text{ m}\cdot\text{s}^{-1}$  (Jouma'a et al., 2016; Vacquié-Garcia et al., 2015).

## Dive parameters

For each surface interval where  $f_H$  ( $\text{beats}\cdot\text{min}^{-1}$ ) was measured, data from the previous dive were extracted from the Acousonde<sup>TM</sup> at a 5 Hz resolution. Statistics on each dive were then calculated to give: maximum depth reached (m), dive duration (min), descent, bottom and ascent duration (min) and the location (latitude and longitude) when the SES reached the surface. Elephant seals perform dives where they passively descend through the water column over a large proportion of time (Crocker et al., 1997). These passive 'drift' dives were identified based on the method designed by Dragon et al., (2012) using the package "rbl" (Le Bras, 2016) in R. A dive was considered as a passive drift dive when passive phases were detected with the following parameters: a minimum duration of 50 s, an absolute roll superior to  $90^\circ$  and a drift rate (i.e. an absolute vertical speed) ranging between  $-0.4$  and  $0.6 \text{ m}\cdot\text{s}^{-1}$ .

Another important dive parameter is total acceleration. It can be decomposed into two types: static and dynamic. Static acceleration is caused by earth's gravitational pull whereas dynamic acceleration results from the animal's movements (body waves, tail strokes, head motions). The static component corresponds to low frequencies and the dynamic one, to higher frequencies (Génin et al., 2015; Richard et al., 2014).

### *Mean swimming effort index*

Data provided by accelerometers were used to calculate absolute and mean swimming effort index for each dive phase and for the entire dive (Eqn. 2). The lateral axis of the accelerometer contains information on putative turning and rolling movements (static acceleration) and on flipper stroke (dynamic acceleration) (Richard et al., 2014). Swimming effort was obtained by summing the absolute values of the local extrema of the lateral axis of the acceleration filtered with a band-pass

filter which cuts frequencies below 0.44 Hz and above 1 Hz (Jouma'a et al., 2016; Richard et al., 2014). Mean swimming effort index of the dive was then calculated by dividing the absolute swimming effort by the duration ( $\Delta t$ ) spent underwater.

**Equation 2:**  $Mean\ Swimming\ Effort\ index = \frac{\sum|peaks|}{\Delta t}$

#### *Putative capture rate*

Putative capture rate ( $s^{-1}$ ) is the number of Prey Encounter Events (PEE) divided by the bottom duration of a dive. PEE were extracted from the acceleration signal which contained head movements by adapting the method developed by Viviant et al. (2009): on each axis, a high-pass filter with a cut-off frequency of 0.33 Hz was applied. Standard deviation was then calculated with a 1 second-fixed-window and then, a 5 second-moving-window. Significant peaks in this filtered signal were considered as PEE when they were detected simultaneously on all three axes (for details see Vacquié-Garcia et al., 2015).

#### **Data design and statistical analyses**

Analyses were conducted at two scales: surface interval level and dive cycle level. At the surface interval scale, the statistical unit is a measure of  $f_{Hinst}$  associated with temporal abscissa in seconds (0 corresponding to the beginning of the surface interval). At the dive cycle scale, the statistical unit is a dive cycle. We aimed to explain the mean heart frequency measured at the surface ( $f_H$ ) using dive and surface interval parameters.

#### *Instantaneous heart frequency at the surface*

This part intended to study the evolution of  $f_{Hinst}$  within a surface interval. The effect of the time on  $f_{Hinst}$  was examined using linear regression (lm function in "stats" R package; R Development Core Team, 2015). The regressions were conducted for each surface interval of each individual. First order effects were selected over second order effects based on an Akaike Information Criterion (AIC) selection (Zuur, 2009). Estimated slopes were used to determine the nature of  $f_{Hinst}$  variations. Then, a linear mixed model computed with the 'nlme' package (Pinheiro et al., 2015) was run on all the surface intervals of the four individuals at the same time to explore the importance of the time on the  $f_{Hinst}$  (standardized values;  $n = 2978$ ). Individual was set as a random factor. To take into account temporal correlation, an auto-correlation structure was included in our models, using an auto-regressive correlation structure of order 1 (with the function corAR1 from the package 'nlme'; Pinheiro et al., 2015). The most relevant model, between random effects model, random intercept model or without random effect model, was selected based on AIC (Zuur, 2009).

### *Mean heart rate and number of beats at the surface*

To investigate the contribution of previous diving behaviours on mean heart rate at the surface and on the number of cardiac beats at the surface, we used linear mixed-effects models, also from the package 'nlme'. Values outside the 1.5 interquartile range were removed from the data. All explanatory variables were centred and standardised at the population scale to keep individual differences and allow comparisons between slope estimates. Time (in days) was included in the model as we assumed a possible impact on the mean heart rate. To test linear and quadratic effects of the time, both variables were included in the model. Individual was set as a random factor, and an auto-regressive correlation structure was included (corAR1). As previously, the most parsimonious model, between a random effect model, random intercept model or without random effect model, was selected based on AIC (Zuur, 2009). Same models and same protocol were used to explain the number of beats at the surface.

All statistical analyses were conducted using the software R (R Development Core Team, 2015). For each model, normal distributions of the explained variable and of the residuals, and residuals' homogeneity were checked up. All results are expressed as mean  $\pm$  standard deviation for single parameters. The significance level was set at  $p = 0.05$ .

## Results

### Foraging trips and overall diving behaviour

Each SES travelled eastward of Kerguelen Islands. Acousondes™ provided data for the first days of foraging trips. We obtained 296 hours of sound recorded in 84 files of which 15 (53 hours) were immediately put aside because the animal was still on land or data were too bad to be exploited. Of the 243 hours left, there were 688 dive cycles and we kept the cycles that counted more than 5 heart beats. 284 dive cycles were kept for this study. On average, seals dived for  $18.4 \pm 3.7$  min with a mean depth of  $546 \pm 159$  m. Time spent at the surface recovering averaged  $121 \pm 19$  s (*i.e.* 2 min 1 s; Table 2) with a maximum of 208 s (*i.e.* 3 min 28 s). Consequently, seals were submerged on average 90.1 % of the time, ranging from 89.3 % for ind. 3 to 91.1 % for ind. 4. The four individuals showed differences in their diving strategies. Ind. 1 performed deep long dives whereas ind. 2 performed shallower and shorter dives. Ind. 3 had a greater number of prey event encounters and higher putative capture rate compared to the three others (Table 3). The longest (33 min 12 s) and the deepest (938.2 m) dives were both performed by ind. 1.

There was a strong negative relationship between dive duration and mean swimming effort index per dive across the four individuals (Pearson's correlation coefficient = -0.74,  $p \leq 0.001$ ; Fig. 2).

### Instantaneous cardiac frequency during surface intervals

There was a positive relationship between  $f_{\text{Hinst}}$  and time in more than 90 % of the 284 dive. Mean R squared (meaning the percentage of the variance explained by models) was  $0.32 \pm 0.26$  with a minimum of  $6.10^{-5}$  and a maximum of 0.97. Linear models showed that  $f_{\text{Hinst}}$  increased significantly with the time spent at the surface (Fig. 3, estimate =  $0.009 \pm 0.001$ ,  $t = 7.41$ ,  $p \leq 0.001$ ) with no individual effect.

### Mean heart rate at the surface and underwater

At the surface, mean heart rate was  $102.4 \pm 4.9$  beats.min<sup>-1</sup> with significant differences between seals (Kruskal-Wallis test:  $\chi^2_3 = 42.8$ ,  $p \leq 0.001$ ; Table 2). The mean heart rates for individual seals ranged from  $99.0 \pm 4.7$  (ind. 4) to  $105.7 \pm 5.2$  (ind. 3) beats.min<sup>-1</sup> while they were breathing at the surface.

In most dives, flow noise generated by seal movements prevented the detection of heart beats. However quiet recording conditions observed during the passive drift phases allowed to detect beats

(e.g. short impulse signals), in the frequency range between 0 and 40 Hz, which is likely be attributed to heart beats. Drift dives were mainly observed in ind. 1. The low-frequency pattern appeared during the whole passive drift event. A simple calculation of the frequency of occurrence based on drift events exhibited a mean of  $20.2 \pm 5.1$  beats.min<sup>-1</sup>, which represents an 80.3% reduction in heart rate compared to surface measurements for that individual.

### **Mean surface heart rate in relation to dive parameters**

The most appropriate model in order to explain mean heart rate at the surface with dive parameters and time was the one without individual effect. The mean surface heart rate was found to be positively correlated with both the number of days elapsed since the departure from Kerguelen Island and the putative capture rate (Table 4). The quadratic time term significantly contributed to changes in the relationship. Its estimated coefficient was negative which means that the relation between heart rate and time directed towards a concave shape. Therefore, heart rate increased with time spent at sea, followed by a “plateau” effect (Fig. 4). Dive duration and swimming effort did not influence heart rate at the surface for the seals studied (Table 4).

### **Total number of heart beats at the surface in relation to dive parameters**

To explore the variations of the number of beats counted in surface intervals, we used the same explanatory variables as above. As expected, a strong correlation between the number of heart beats and surface duration was found (Pearson's coefficient = 0.96,  $p \leq 0.001$ ). In this case, there were random effects (on the slope and the intercept) across individuals. The total number of heart beats during the surface interval was positively related to both the mean swimming effort index and dive duration. However the total number of cardiac beats at the surface was unrelated to putative prey capture rate and time (Table 4).

## Discussion

### Measuring heart rate through acoustic records

This study provides one of the very few datasets of heart rate, simultaneously with breathing rate (Génin et al., 2015) for free ranging post breeding female SESs. Previous studies carried out on heart rate used mainly captive or translocated animals (Andrews et al., 1997; Burgess et al., 1998; Fletcher et al., 1996; 1998; Le Boeuf et al., 2000). Acoustic records offer the possibility to access free-ranging SES heart rates during post-dive surface intervals, although records could contain heart sounds under water only when the flow noise stops (Burgess et al., 1998). This condition of quiet soundscape is satisfied when SESs are passively drifting through the water column. Therefore, heart rate could not be quantified while the seals were actively swimming or gliding underwater due to associated flow noise. In our study, mean heart rate at the surface measured in the four voluntary diving post-breeding females was  $102.4 \pm 4.9$  beats.min<sup>-1</sup>. With northern elephant seals, previous acoustic studies recorded a mean heart rate at the surface of 86 beats.min<sup>-1</sup> for adult males (Le Boeuf et al., 2000) and ranging from 106 to 121 beats.min<sup>-1</sup> for juveniles (Andrews et al., 1997; Burgess et al., 1998; Fletcher et al., 1996; Le Boeuf et al., 2000). All these studies demonstrate the reliability of the acoustic method to analyse the cardiovascular system. In our data, heart rate is detected only at the surface. Hindell and Lea (1998), using an electrical approach, extracted heart rate at the surface of one post-breeding SES female over a 50 days period, and found a heart frequency ranging between 65 and 95 beats.min<sup>-1</sup> while at the surface breathing. They estimated that this number was underestimated by 10-15 % due to the sampling biases. Hence, both measures are in the same order of magnitude.

The electrical method also allowed the detection of heart beats while the SES was swimming underwater. During a dive, an elephant seal exhibits pronounced bradycardia (Elsner et al., 1966), a finding confirmed in free-ranging seals (Burgess et al., 1998). Heart rate during diving of free-ranging SESs decreases from 40 beats.min<sup>-1</sup> for dives less than 13 min to 14 beats.min<sup>-1</sup> for dives lasting between 13 min and 37 min (Hindell and Lea, 1998). In this study, we estimated heart rate during one drift dive and found  $20.2 \pm 5.1$  beats.min<sup>-1</sup>, which is consistent with the values found by Hindell and Lea, (1998) in their Fig. 3 and represent an 80% reduction of heart rate compared to surface value, which tends to be more important compared to the mean 64% reduction found in northern elephant seals for all dives combined (Andrews et al., 1997). This higher reduction might be related either to drift dive which is supposed to represent a recovery behaviour (Crocker et al. 1997) or possibly to the longest duration of drift dive which are on average 25%

longer than foraging/travelling dives (Guinet unpublished data). The high standard deviation calculated here indicates a high variation between inter-beat intervals suggesting that bradycardia might be unstable at depth. This is consistent with the hypothesis of Williams et al. (2015b) who found that both depth and exertion garble bradycardia in Weddell seals (*Leptonychotes weddellii*) and bottlenose dolphins (*Tursiops truncatus*). However, this diving heart rate obtained by acoustics has to be validated in laboratory condition, using electrocardiogram methods for example.

### **Post-dive recovery and instantaneous heart rate**

During surface interval periods, the instantaneous heart frequency increases with time: it is significantly higher at the end of the surface interval than at the beginning. Additionally, all seals exhibited the same pattern. This result probably represents a part of the dive response of the SES. Indeed, after reaching very low values when the seal chases at the bottom of its dive, heart frequency increases gradually while the seal ascends toward the surface (Harrison and Kooyman, 1968). Andrews et al. (1997) found that the rate of increase of tachycardia was most marked just prior to surfacing, approximately during the last 15 s of ascent. Therefore, increasing heart rate observed through the surface interval in this study is likely to correspond to the decelerating phase of the tachycardia which reaches its maximum value at the end of the surface interval, prior to diving. Periods of tachycardia enable rapid oxygen loading at the surface, in both blood and muscle stores, and eliminating carbon dioxide accumulated during the previous dive (Reed et al., 1994). Associated with high breathing frequency, high heart rate eases quick gas exchange at the surface and a more efficient recovery (Fedak et al., 1988; Le Boeuf et al., 2000). Hence, surface duration is minimised and submergence times are maximized. Indeed, the four seals studied here spent about 90 % of time under the water, enabling this central place predator to take full advantage of its underwater preys.

### **Post-dive recovery and physical effort exerted by the SES**

The costs associated with diving are a central component of a marine mammal's energy budget (Maresh et al., 2015). This budget can be decomposed into oxygen-consuming additive elements: basal metabolic costs, locomotor costs, feeding costs and thermoregulatory costs (Costa and Williams, 1999). In this study, exertion levels during diving have been evaluated via three parameters: dive duration, mean swimming effort index and putative capture rate. Contrary to our expectation that, like terrestrial mammals, heart rate should increase with increasing foraging effort no relationship was found between mean heart rate at the surface and dive duration or swimming effort. However a positive relationship was found with both putative prey capture rate and the number of days elapsed since Kerguelen Island departure. Dive duration may not reflect the

exertion level during a dive as it could be biased as SESs reduce their relative mean swimming effort with increasing dive duration (Fig. 2). Swimming effort *i.e.* movements of the hind flippers, appear to be a reliable indicator of costs due to the locomotion (Williams et al., 2004; Wilson et al., 2006). Putative capture rate can easily be linked to foraging costs during a dive. Feeding events are responsible for an increase of 44.7 % of the energetic budget in Weddell seals (Williams et al., 2004). Nevertheless, interpretations should be presented with precautions as putative capture rate is calculated from prey catch attempts and not effective catches. Indeed, around 90 % of the dive cycles analysed in this study had at least one capture attempt.

Our results indicate that SESs manage their recovery by increasing the duration of the post-dive interval and therefore by increasing the total number of cardiac beats (*i.e.* the duration of the tachycardia) rather than acting on heart rate while at the surface. Indeed, the total number of heart beats was highly correlated with the time spent at the surface breathing. Dive duration and mean swimming effort were the two parameters that positively influenced time spent at the surface. Hence, a long dive and/or a dive where the SES gave a high quantity of large tail movements implies a long surface duration. This is in accordance with previous results obtained by Génin et al. (2015) with SES. In Weddell seals, *Leptonychotes weddellii*, the energy expenditure approximated by the number of flipper strokes taken is highly correlated with the oxygen consumption (Williams et al., 2004). Maresh et al. (2014) showed that with an artificially increased cost of locomotion, northern elephant seals, *Mirounga angustirostris*, spent more time breathing and thus recovering. The relationship between dive duration and surface interval duration had already been demonstrated in several marine mammal species such as northern elephant seals (Andrews et al., 1997; Le Boeuf et al., 2000), grey seals, *Halichoerus grypus* (Thompson and Fedak, 1993) alongside diving birds including thick-billed murre, *Uria lomiva* (Croll et al., 1992).

Putative capture rate positively influenced mean heart rate at the surface. This result might indicate that seals recover from a foraging dive with a higher heart rate. Hence, the increase of surface mean heart rate after putative prey capture attempts could be explained by factors unrelated to swimming effort, such as the added energy required for prey warming and digestion. This hypothesis is consistent as prey assimilation affects both resting and diving metabolic rates (Williams et al., 2004).

This study strongly supports that the time spent at the surface and therefore the total number of breaths and heart beats, rather than heart beat frequency, appears to be the main driver of the post-dive recovery behaviour in SES. As such, the cardio-respiratory system as a whole needs to be considered to understand SES recovery strategy. Nonetheless, it is critical to bear in mind the complexity of the cardiac responses observed: cardiac regulation is controlled by neural drivers

which themselves react to multiple factors such as the environment or a change in the behaviour (Williams et al., 2015b).

This study also revealed that the mean heart rate at the surface of the four seals varied with the time spent at sea with a non-linear relationship (Fig. 4). In the first 5-10 days of trip, the mean heart rate at the surface increased with time. Then, mean heart rate might decrease (ind. 3) or remain stable (ind. 1, 2 and 4). Females left Kerguelen Island after the breeding season in poor body condition as they lost 25-50 % of their original weight (McCann et al., 1989). Increasing mean heart rate could then reflect the adaptation, or a response to cardio-vascular training during the first few days spent at sea after one month spent on land. By assuming that the higher the heart rate is during surface breathing, the faster the gas exchanges should be, and so, recovery would be more efficient, we suppose that an “optimal” heart rate exists (linked with individual characteristics as body mass or composition) to maximise gas exchange at the surface. Therefore, future analyses with access to larger datasets and more individuals observed over a longer time period should be able to investigate this hypothesis.

## Conclusion

This study indicates that SESs manage their post dive recovery by modulating their post dive surface duration, and therefore the number of breaths and heart beats, rather than acting on their breathing rate (Genin et al. 2015) or their heart rate as we found in this study.

Sound recording can be a powerful tool as it provides the simultaneous detection of breathing and heart frequencies, allowing the investigation of the cardio-respiratory system in its entirety (Génin et al., 2015; Le Boeuf et al., 2000). Both physiological data are essential to study post-dive recovery of marine mammals and seabirds. However, the main limitation is that we access the heart rate only when SESs are breathing at the surface. A study on harbour seals (*Phoca vitulina*) suggested that the mean heart rate of the complete dive cycle (*i.e.* dive and surface) could be easily explained by the percent of dive time and link to oxygen consumption (Fedak et al., 1988). An improvement in data collection is essential to fully exploit the possibilities of the acoustic method. A major breakthrough would be to trigger audio recording based on external events of interest (e.g. using acceleration data). This would save battery and allow long-term dataset. Examples of interest include recording acoustic data when the animal is at the surface (to study surface heart rate) and/or during drift dive (to study underwater heart rate).

In addition, sound records can also be used to explore other aspects of the elephant seal behaviour and environment. They allow the collection of abiotic sounds, such as those generated by wind and rain, which represent a great interest for oceanographers as the Southern Ocean is difficult to observe. Acoustics offer many possibilities, and non-invasive bio-logging data collection could easily be improved by concertation between the users and the research teams in the future.

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## Competing interests

No competing interests declared.

## Author contribution

Conceptualization: C.G.; Methodology: L.D., J.J. and J.B.; Validation: J.B., J.J.; Formal analysis and investigation: L.D. and J.J.; Writing - original draft preparation: L.D.; Writing - review and editing: L.D., J.J., C.G. and J.B.; Resources: C.G. and J.B.; Supervision: C.G. and J.B.; Funding acquisition: C.G.

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## Data availability

Data are available under request.

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## Tables

**Table 1: Descriptive information about the four post-breeding female SESs at deployment**

Seal ID	Acousonde ID	Body mass (kg)	Size (cm)	Departure day
1	A031	255	254	10 Oct 2011
2	A032	245	238	28 Oct 2011
3	626019	230	232	28 Oct 2012
4	626040	292	225	01 Nov 2012

**Table 2: Surface interval parameters (mean  $\pm$  sd)**

<b>Individual</b>	<b>Number of dives cycle</b>	<b>Surface interval (s)</b>	<b>Heart rate (beats.min<sup>-1</sup>)</b>
1	102	132 $\pm$ 19	102.4 $\pm$ 4.6
2	85	107 $\pm$ 13	101.0 $\pm$ 3.7
3	70	122 $\pm$ 13	105.7 $\pm$ 5.2
4	28	121 $\pm$ 18	99.0 $\pm$ 4.7

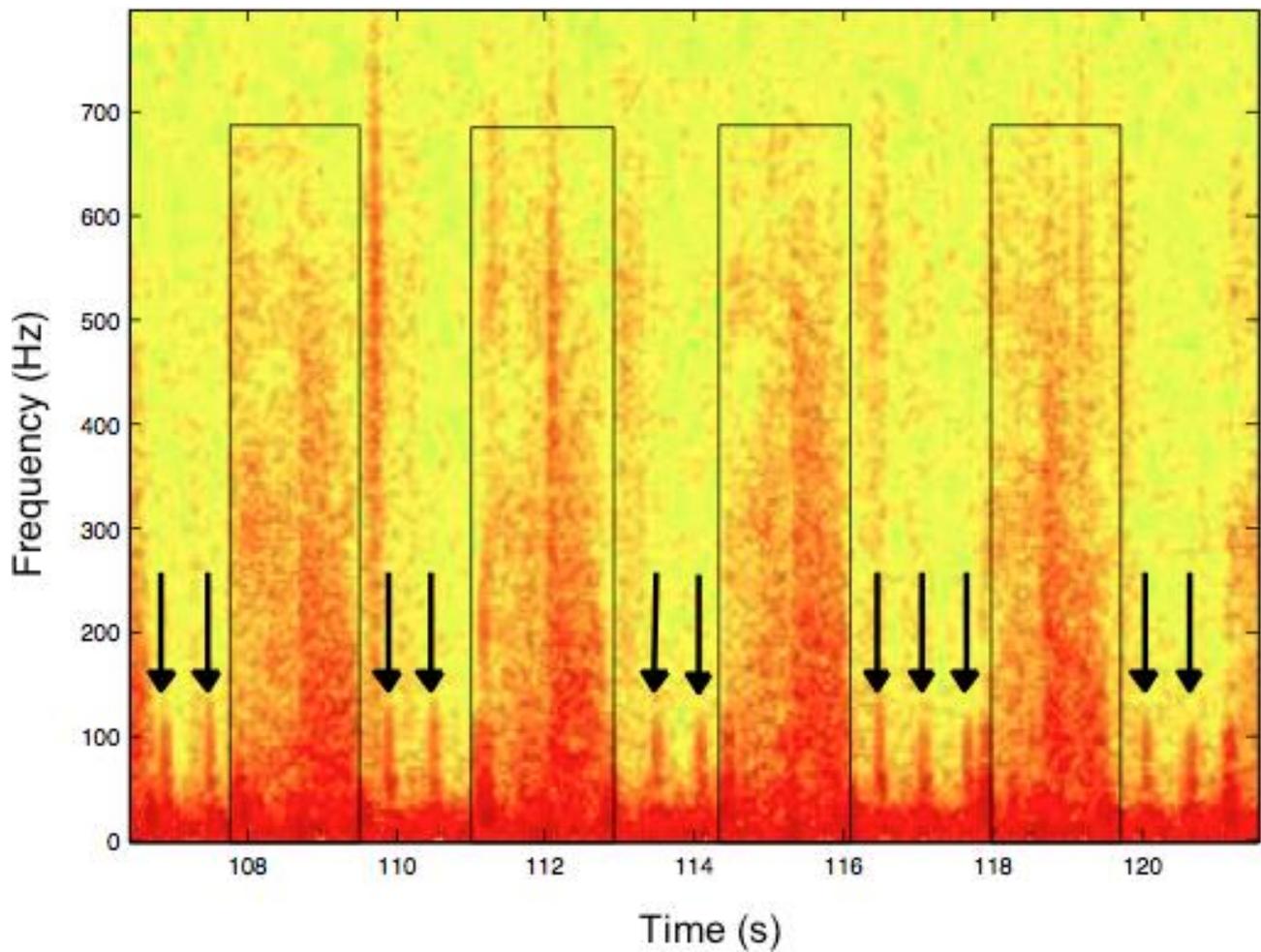
**Table 3: Dive parameters of the four female SESs (mean  $\pm$  sd)**

<b>Ind.</b>	<b>Maximum depth (m)</b>	<b>Dive duration (min)</b>	<b>Number of PEE</b>	<b>Putative capture rate (s<sup>-1</sup>)</b>	<b>Mean swimming effort index (m.s<sup>-2</sup>)</b>
1	611.5 $\pm$ 164.4	21.2 $\pm$ 3.1	4.9 $\pm$ 3.4	0.015 $\pm$ 0.023	3.3 $\pm$ 0.6
2	434.9 $\pm$ 114.9	15.5 $\pm$ 2.5	6.4 $\pm$ 5.0	0.014 $\pm$ 0.011	5.2 $\pm$ 0.5
3	575.3 $\pm$ 138.5	17.0 $\pm$ 2.4	8.9 $\pm$ 5.4	0.026 $\pm$ 0.020	4.6 $\pm$ 0.4
4	579.7 $\pm$ 128.2	20.5 $\pm$ 2.8	6.6 $\pm$ 5.7	0.015 $\pm$ 0.015	3.7 $\pm$ 0.7

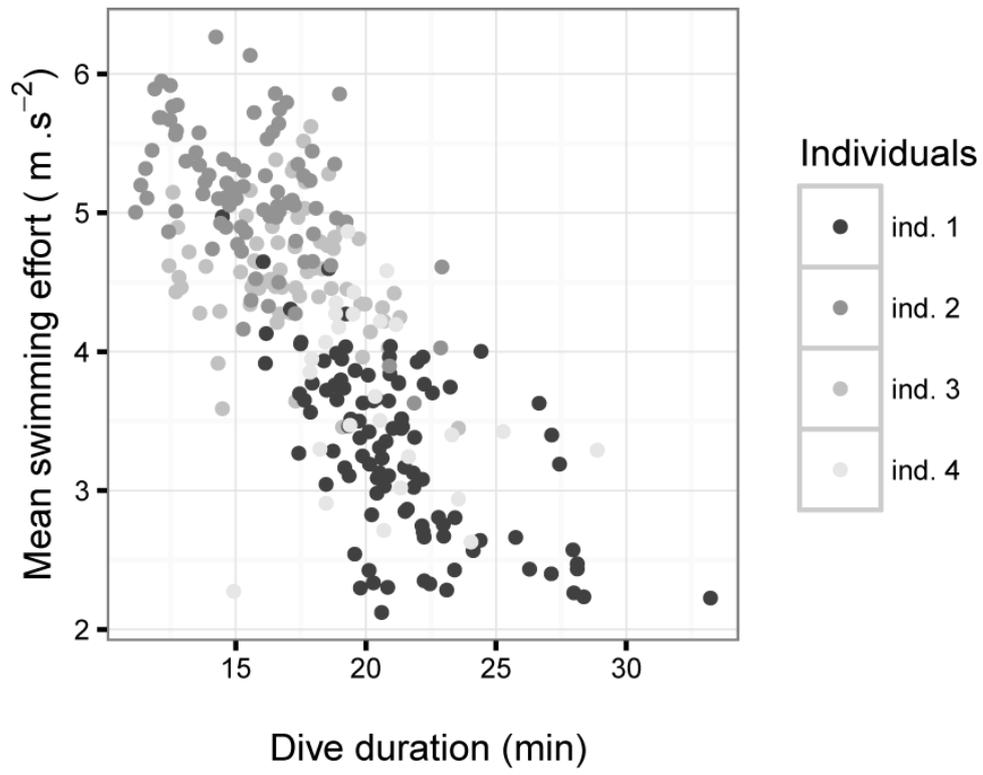
**Table 2: Results of linear models looking at mean surface heart rate or at the number of beats in relation to dive parameters and time spent at sea (NS: no statistically significant)**

Model	Parameter	Coefficient estimate	p-value
$f_H \sim$ <i>gls model</i>	Intercept	$94.2 \pm 1.5$	$\leq 0.001$
	Mean swimming effort (m.s <sup>-2</sup> )		NS
	Dive duration (min)		NS
$corr \sim time (s) / ind$	Putative capture rate (s <sup>-1</sup> )	$0.3 \pm 0.1$	0.02
	Time (days)	$1.5 \pm 0.4$	$\leq 0.001$
	Time <sup>2</sup>	$-0.05 \pm 0.02$	0.02
<b>Number of beats</b> ~ <i>lme model</i>	Intercept	$225.2 \pm 22.5$	$\leq 0.001$
	Mean swimming effort (m.s <sup>-2</sup> )	$20.4 \pm 8.4$	0.01
	Dive duration (min)	$14.9 \pm 7.5$	0.04
$random \sim slope / ind$	Putative capture rate (s <sup>-1</sup> )		NS
$corr \sim time (s) / ind$	Time (days)		NS
	Time <sup>2</sup>		NS

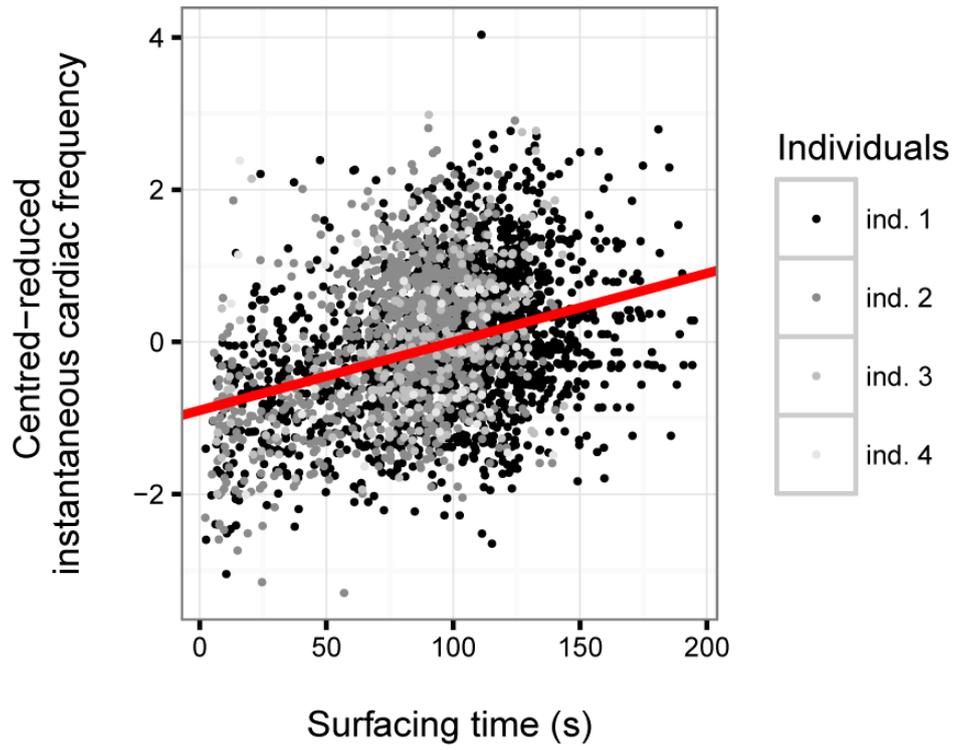
## Figures



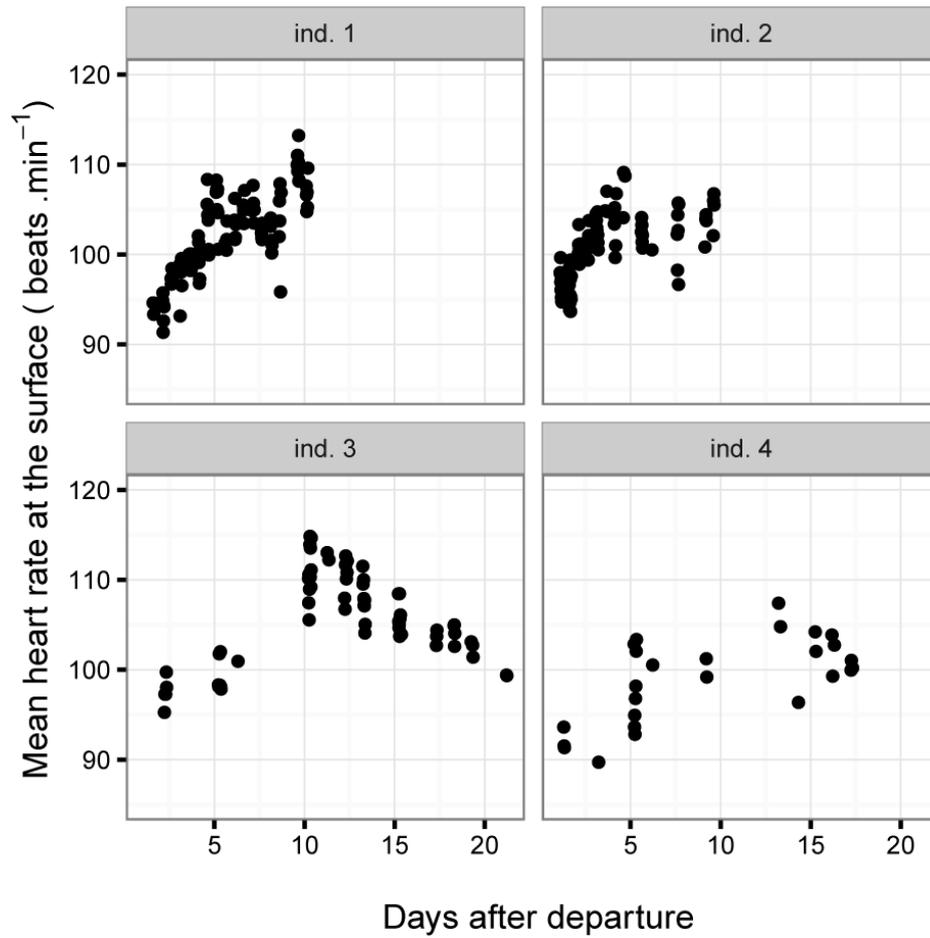
**Figure 1:** spectrogram of recorded sound performed by ind. 1 during a surface interval showing five respiratory cycles (rectangles in the 0-700 Hz range) with cardiac occurrences between breaths (black arrows in the 0-150 Hz range)



**Figure 2:** relationship between the dive duration and the mean swimming effort for the four SES studied



**Figure 3:** linear relationship between time and  $f_{Hinst}$  of the four seals:  $f_{Hinst} = -0.9 + 0.009 \cdot \text{Surfacing time}$



**Figure 4:** relationship between time spent at sea after breeding season on Kerguelen Island and the mean heart rate measuring during post-dive interval surface for the four female elephant seals