



# Cardiorespiratory patterns of male South American sea lions (*Otaria flavescens*) resting on land

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## Abstract

The goal of this study was to characterize the cardiorespiratory patterns of male South American sea lions (SASLs, *Otaria flavescens*) resting on land. We recorded respiratory and heart rate ( $n = 360$  individuals studied) by observing the nostrils, chest movements and the impact of the heart on the thoracic wall. The sea lions breathe apneustically with a pause on inspiration, representing 74% of the respiratory cycle. The mean breathing frequency was  $3.2 \pm 1.0$  breaths  $\text{min}^{-1}$ , with a breathing cycle presenting periods of bradypneas, tachypneas, and long-term post-inspiratory pauses. The normal heart rate (nHR) was  $73.4 \pm 14.5$  beats  $\text{min}^{-1}$  and no significant differences were observed between age classes. All animals showed variability in HR in relation to respiratory phases (Inspiration:  $101.2 \pm 18.4$  beats  $\text{min}^{-1}$ ; post-inspiratory pause:  $73.4 \pm 14.5$  beats  $\text{min}^{-1}$ ; expiration:  $64.6 \pm 17.7$  beats  $\text{min}^{-1}$ ), consistent with respiratory sinus arrhythmia (RSA). The mean HR (measured during all respiratory phases) was  $79.9 \pm 22.7$  beats  $\text{min}^{-1}$ , and was significantly different between age classes. The total duration of respiratory cycle, and duration of both inspiration and expiration, decreased with an increment in ambient temperature, with no variation in the pause duration. Heart rate during pause and expiration was significantly higher during high temperatures. Similar changes in cardiorespiratory patterns have been reported in other pinnipeds. Our results showed ontogenetic differences in development and typical variations with environmental and behavioral variables.

**Keywords** Cardiorespiratory patterns · Respiratory sinus arrhythmia · South American sea lion · Otariidae · Pinnipeds

## Introduction

Marine mammals have evolved physiological, morphological, and behavioral adaptations that maximize aerobic dive duration, including modifications on the cardiorespiratory system (Kooyman 1989; Ponganis 2015; Wartzok 2002; Mortola and Limoges 2006; Mortola and Seguin 2009; Davis 2019). In most of the species studied so far, these changes include increased relative lung size (Mortola and Limoges 2006), increased tidal volume (relative to total lung volume; Kooyman 1973; Mortola and Seguin 2009),

cartilaginous enhancement of the upper airways (Kooyman 1973), increased oxygen stores in the blood (hemoglobin) and muscle (myoglobin; Wartzok 2002; Davis 2019), increased ability to control ventilation (Mortola and Limoges 2006; Mortola and Seguin 2009), development of mechanisms to reduce water loss in ventilation, and changes in respiratory and cardiac behavior (Davis 2014, 2019; Ponganis 2015).

In pinnipeds, these behavioral modifications affect not only the cardiorespiratory pattern during diving but also while resting on land (Castellini 1996; Andrews et al. 1997; Falabella et al. 1999). Analogous ontogenetic aspects of the development of terrestrial apnea and diving capacity suggest that these traits share a common physiological foundation (Blackwell and Le Boeuf 1993; Castellini 1996; Andrews et al. 1997; Boily and Lavigne 1997; Falabella et al. 1999). They are thought to represent a dual adaptation for enabling protracted periods of submersion while foraging at sea, as well as for conserving water and energy during fasting life-history phases on land (Blackwell and Le Boeuf 1993; Motishaw et al. 1999; Lester and Costa 2006). For example,

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during open sea diving, successive dives are alternated by short or long surface periods, characterized by hyperventilation that removes the carbon dioxide excess accumulated during breath holding (Andrews et al. 2000; Wartzok 2002). Similarly, several behavioral strategies occur while resting on land, involving periods of apnea, associated with bradycardia and tachycardia (Andrews et al. 1997, 2000; Wartzok 2002; Ponganis 2015). Although land-based cardiorespiratory patterns may be associated with thermoregulatory processes (Riedman 1990; Castellini 2002a; Randall et al. 2002; Ponganis 2015), they may also reflect adaptations for breath-hold diving (Castellini 1994).

Most studies of cardiorespiratory patterns in marine mammals involving animal handling and instrumentation have focused on the analysis of apneic and eupneic breathing, giving less importance to the detailed study of the respiratory phases associated with eupnea (Kastelein and Meijler 1989; Blackwell and Le Boeuf 1993; Castellini et al. 1994; Andrews et al. 1997, 2000; Ponganis et al. 1997; Boyd et al. 1999; Ponganis and Kooyman 1999; Cummings et al. 2015). In pinnipeds, the cardiorespiratory behavior at sea or resting on land has been studied through the attachment of different recording devices (Pasche and Krog 1980; Boyd et al. 1999; Falabella et al. 1999), but only a few have focused on the detailed study of the cardiorespiratory cycle, recording variations in respiratory phases and their coupling with the cardiac cycle (Lin et al. 1972; Lyamin et al. 2002; Deacon and Arnould 2009; Fahlman et al. 2017). The study of cardiorespiratory function on unrestrained pinnipeds using non-invasive techniques has previously been performed only on elephant seals (*Mirounga angustirostris* and *M. leonina*), Antarctic fur seals (*Arctocephalus gazella*), and Weddell seals (*Leptonychotes weddellii*) (Bartholomew 1954; Blackwell and Le Boeuf 1993; Salwicka and Stonehouse 2000). To date, several variables during sleep and wakefulness have been studied in the South American sea lion (SASL; *Otaria flavescens*; Lyamin et al. 2002), and it is also known about submerged swimming and resting in water metabolic rates (Dassis et al. 2012), and resting in land metabolic rates (Dassis et al. 2012; Fahlman and Madigan 2016). However, there are no data on cardiorespiratory patterns on land in non-disturbed and non-instrumented animals. We characterized cardiorespiratory variables (heart rate, breathing frequency, and durations of the respiratory cycle and phases) of SASL males while resting and examined whether they were affected by age, temperature, time of day, or state of sleep using visual non-invasive techniques.

Results obtained here have provided new insights on the coupling between respiration and cardiac function for this species resting on land, with details of the different respiratory phases and their relation to the heartbeat, providing detailed information on the cardiorespiratory pattern. Also, our results are discussed in terms of their potential

relationship with the cardiorespiratory cycle during immersion-surface periods at sea. As was suggested for seals that usually dive by exhaling air from their lungs (Castellini 1994; Falabella et al. 1999), we hypothesize that the close relation between cardiorespiratory pattern on land and at sea would also occur in otariids characterized as inhaler divers (Kooyman 1973, 1985), such as the SASL, and we provided baseline data for further investigation of this hypothesis in the future.

## Methods

### Study site and data recording

This study was carried out at Puerto Mar del Plata (38° 02' 30" S, 57° 32' W), in a permanent and non-reproductive harbor haul-out of South American sea lions (SASLs), mainly composed of juvenile and subadult males, with lower concentrations of annuals and adults (Giardino et al. 2014). The observation period extended from March to July 2014. We studied resting and deemed healthy animals randomly selected; a total of 360 male SASLs from different age classes were registered for the study. The resting behavior involves quiet animals in two different positions: sitting upright or lying down (Gana 2016). The latter includes both recumbency with the back completely on the floor or only partially with the body slightly on its side, as well as ventral recumbency. All animals studied were considered healthy based on general behavior, the absence of evident injuries, and a visually appreciable good body condition. The age classification was defined as juvenile, subadult, and adult (Crespo 1988; Rodríguez 1996). For analysis, only animals that had not moved or interacted with other sea lions in the preceding ten minutes were included.

The observations were made alternatively at two different times of day, morning (9:00 a.m.–12:00 p.m.) and afternoon (3:00 p.m.–6:00 p.m.) for different states of sleep (“presumably asleep” or “awake”) and at different ambient temperatures. We considered that the animal was presumably asleep when it was resting with eyes closed for at least two minutes (Blackwell and Le Boeuf 1993). Data for ambient temperature (°C) were obtained from the National Meteorological Service of Argentina archives at three hourly sampling intervals.

### Respiratory cycle observation

Respiratory frequency ( $f$ , breaths  $\text{min}^{-1}$ ) was based on visual observations of open nostrils and chest movements resulting from inflation and deflation of the lungs during the breathing cycle (online resources 1 and 2). Each breath was divided into distinct phase: inspiration, pause and expiration.

Inspiration is recognized by a total opening of the nostrils, as opposed to expiration where the nostrils open partially. During the pause, the nostrils remain completely closed. Ten breaths were counted and the elapsed time was recorded, and then referred to 1 min as follows:  $[60 \text{ s} \cdot 10 / \text{elapsed time for those 10 breaths (in s)}]$ . A total of three  $f$  measurements were recorded for each animal, taken consecutively within approximately 10 min. Subsequently, the average value of the three measurements of each animal was selected. Using a stopwatch, we also measured the duration of each of the phases of the respiratory cycle.

In order to record information on the respiratory cycle over time and follow successive breathing cycles in the same animal, a subtotal of 50 animals were randomly selected to individual filming for a period of 20 min. Circa 17 filming hours were recorded, and the duration of each respiratory phase was also recorded with a stopwatch. To assess the efficacy of the respiratory data estimation, we randomly selected 10 subjects who were monitored by both direct observation and video recordings, and we statistically compared the results obtained from both methods.

### Cardiac cycle observation

We recorded heart rate (HR,  $\text{beats min}^{-1}$ ) visually based on precordial pulsations originating from the heart, which were visible on the anterior chest wall (Salwicka and Stonehouse 2000). We counted 10 consecutive heart contractions and then referred them to 1 min as follows:  $[60 \text{ s} \cdot 10 / \text{elapsed time for those 10 beats (in s)}]$ . A total of three consecutive HR measurements were recorded during the longest and therefore most representative phase of the breathing cycle (pause) within a 10 min period for each animal and then averaged. This value was henceforth called normal HR (nHR) and refers to the HR value typical or representative of each animal, recorded only during the respiratory pause.

Additionally, to further investigate cardiac and breathing relationship, HR was also recorded separately on each respiratory phase (inspiration/pause/expiration). For inspiratory and expiratory phases, only three precordial pulsations could be counted because of their short duration. A mean heart rate value from all respiratory phases pooled, was also calculated and used to compare with most of previous studies.

### Validation of the HR estimation method

To evaluate the effectiveness of the heart rate estimation method, we worked with two SASLs housed in professional care and trained for different veterinary examination routines at Mundo Marino Oceanarium (San Clemente del Tuyú, Argentina). One adult male and one adult female were selected and trained by operant conditioning to remain in a resting state during examination. Heartbeats

were simultaneously determined by both, auscultation and direct observation of the thorax by two observers. A Littmann stethoscope was used for auscultation while a second assistant directly observed the chest movement. In both cases, the heartbeats were counted and the elapsed time was recorded with a stopwatch.

### Data analysis and statistics

Using exploratory graphical techniques, we identified outliers in the continuous response variables (total duration of the respiratory cycle, duration of each respiratory phase,  $f$  and HR). These outliers corresponded to measurement errors that yielded values of apnea duration or HR that are not physiologically possible, and were discarded. We also assessed collinearity-correlation among explanatory variables [age class, time of day (morning/afternoon), animal identity, ambient temperature and state of sleep or wakefulness (presumably asleep/awake)] with multiple pair-wise scatterplots (pair plots; Zuur et al. 2009, 2010). We examined the response variables for normality visually using a histogram, and any factor explanatory variables were tested for equal variances across the response variable (Bartlett's test).

Animals were grouped into age classes to evaluate if age had a significant influence on durations of respiratory phases and/or total duration of the respiratory cycle using Kruskal–Wallis test and the respective post hoc test.

We employed generalized linear mixed models (GLMMs) to assess sources of variation in durations ( $y$ ) across distinct respiratory phases and the total respiratory cycle duration. For this, the negative binomial distribution with a log link function was utilized, and random effects encompassed animal identities. Age class was considered a categorical variable (juvenile, subadult, adult). Additionally, temperature, examined within 5 °C ranges, corresponded to six categories (0–5 °C; 6–10 °C; 11–15 °C; 16–20 °C; 21–25 °C; 26–30 °C). The categorical variables of state of sleep (presumably asleep/awake) and time of day (morning/afternoon) were also incorporated. Another GLMM, utilizing a gamma distribution with an inverse link function, was employed to ascertain the prime sources of variation influencing changes in  $f$ . Age class, temperature, state of sleep, and time of day were regarded as predictor variables in this analysis. We used a generalized linear model (GLM) to evaluate HR in each respiratory phase. The error distribution was Gaussian with an identity link function. The predictor variables were age class, temperature, state of sleep and time of day.

We tested for significant differences in categorical variables across levels using a post hoc general linear hypotheses and multiple comparisons test using the Tukey method with the function `glht` from the R package `multcomp` (Hothorn et al. 2013). The statistical analyses were carried out using

R software version 3.4.4 (R Core Development Team 2018) and the contributed package lme4 (Bates et al. 2014). All means are expressed  $\pm$  SD.

## Results

### Respiratory cycle characterization

Respiratory data were collected from 303 male SASLs from different age classes [juveniles (N=47), subadults (N=97), and adults (N=159)] while resting on land. Respiratory cycle was composed of three well-defined phases: inspiration, post-inspiratory pause and expiration. The pause was defined as the period between the end of inspiration to the beginning of the next expiration, and characterized as “full lung apnea”. There was no expiratory pause.

The durations of the respiratory phases were significantly different (Kruskal Wallis Test:  $H = 441.75$ ,  $p < 0.0001$ ,  $N = 228$ ), with post-inspiratory pause > expiration > inspiration (Table 1). This pattern was observed for all age classes (*Juveniles*: Kruskal Wallis Test:  $H = 48.42$ ,  $p < 0.0001$ ,  $N = 24$ ; *Subadults*: Kruskal Wallis Test:  $H = 145.99$ ,  $p < 0.0001$ ,  $N = 74$ ; *Adults*: Kruskal Wallis Test:  $H = 250.66$ ,  $p < 0.0001$ ,  $N = 130$ ; Table 1). Although

there were slight variations in the mean duration of the phases, the post-inspiratory pause was always the longest, representing approximately a 74% of the total respiratory cycle, followed by the expiration and the inspiration in all animals pooled (Table 1).

The mean  $f$  was  $3.2 \pm 1.0$  breaths  $\text{min}^{-1}$ , with a minimum of 0.5 breaths  $\text{min}^{-1}$  and a maximum of 8.1 breaths  $\text{min}^{-1}$  (N = 303). No significant differences were observed among different age classes (Kruskal Wallis Test:  $H = 2$ ,  $p > 0.99$ ; Table 1).

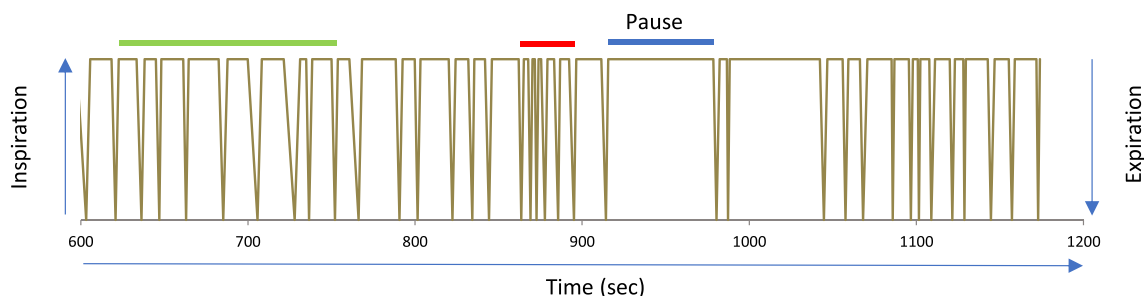
The subset of animals that were filmed showed that the breathing cycle was not constant over time, presenting periods of bradypnea and tachypnea, that is, periods in which the respiratory rate decreased and increased, respectively (Fig. 1). Long-term pauses were also identified, reaching a maximum of 78 s. (Fig. 1).

Regarding the two methodological approaches used (direct observations and videos), no significant differences were observed in either the breathing frequency (Paired t-test;  $t = 1.72$ ;  $df = 32$ ;  $p = 0.095$ ) or the durations of the respiratory phases (*Inspiration*: Paired t-test;  $t = 0.50$ ;  $df = 49$ ;  $p = 0.62$ ; *Post-inspiratory pause*: Paired t-test;  $t = 1.91$ ;  $df = 49$ ;  $p = 0.062$ ; *Expiration*: Friedman test;  $\chi^2 = 0.02$ ;  $df = 1$ ;  $p = 0.89$ ) between both methods.

**Table 1** Mean  $\pm$  SD and range of the respiratory phases duration (seconds), mean and ranges of the breathing frequency (breaths  $\text{min}^{-1}$ ) and normal heart rate (beats  $\text{min}^{-1}$ ) of male South American sea lions from different age classes resting on land

	Juveniles (N=24)		Subadults (N=74)		Adults (N=130)		Pooled (N=228)		nHeart rate (N=216)
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	
Inspiration	$1.74 \pm 0.54^b$	0.93–2.83	$2.28 \pm 0.86^b$	0.56–4.82	$2.61 \pm 1.16^c$	0.98–11.58	$2.41 \pm 1.06^c$	0.56–11.58	
Post-inspiratory pause	$14.95 \pm 9.01^a$	5.47–48.21	$18.48 \pm 9.21^a$	6.22–45.90	$15.22 \pm 7.16^a$	3.32–41.02	$16.25 \pm 8.19^a$	3.32–48.21	$73.4 \pm 14.5$
Expiration	$2.50 \pm 1.43^b$	0.64–6.25	$2.92 \pm 2.04^b$	0.39–12.52	$3.60 \pm 2.20^b$	0.72–13.40	$3.26 \pm 2.11^b$	0.39–13.40	
Breathing frequency	$3.5 \pm 0.9^*$	2.1–7.0	$3.2 \pm 1.1^*$	1.3–8.1	$3.1 \pm 1.0^*$	0.5–7.3	$3.2 \pm 1.0$	0.5–8.1	

Means with a common letter are not significantly different between respiratory phases (all post hoc comparisons:  $p > 0.05$ ). Means with a common asterisk are not significantly different between age classes (all post hoc comparisons:  $p > 0.05$ )



**Fig. 1** Selected respiratory cycle of a male South American sea lion (*Otaria flavescens*) resting on land. Bradypnea (green line) and tachypnea (red line); long-term pause is highlighted with a blue line

## Cardiac cycle characterization

Regarding the validation of the HR estimation method, no significant differences were found between the two techniques used, direct observation and auscultation, for either of the two specimens studied (t-test;  $t = 0.25$ ;  $df = 12$ ;  $p = 0.81$ ; t-test;  $t = -0.09$ ;  $df = 12$ ,  $p = 0.93$ ).

The normal heart rate (nHR) was recorded for 216 male SASLs ranging in age from juveniles to adults. The nHR for all animals pooled was  $73.4 \pm 14.5$  beats  $\text{min}^{-1}$  (range 46–120 beats  $\text{min}^{-1}$ ; Table 1), and there were no significant differences among age classes (Kruskal Wallis Test;  $H = 0.63$ ;  $p = 0.72$ ). Mean HR during all respiratory phases pooled ( $79.9 \pm 22.7$  beats  $\text{min}^{-1}$ ,

range = 30–180 beats  $\text{min}^{-1}$ ) was significantly different between age classes (Kruskal Wallis Test;  $H = 6.22$ ;  $p = 0.04$ ; *juveniles* =  $89.9 \pm 29.4$ , *subadults* =  $81.4 \pm 24$  and *adults* =  $77.9 \pm 20.8$  beats  $\text{min}^{-1}$ ), with adults significantly lower than juveniles ( $p < 0.05$ ). No differences were observed between juveniles and subadults.

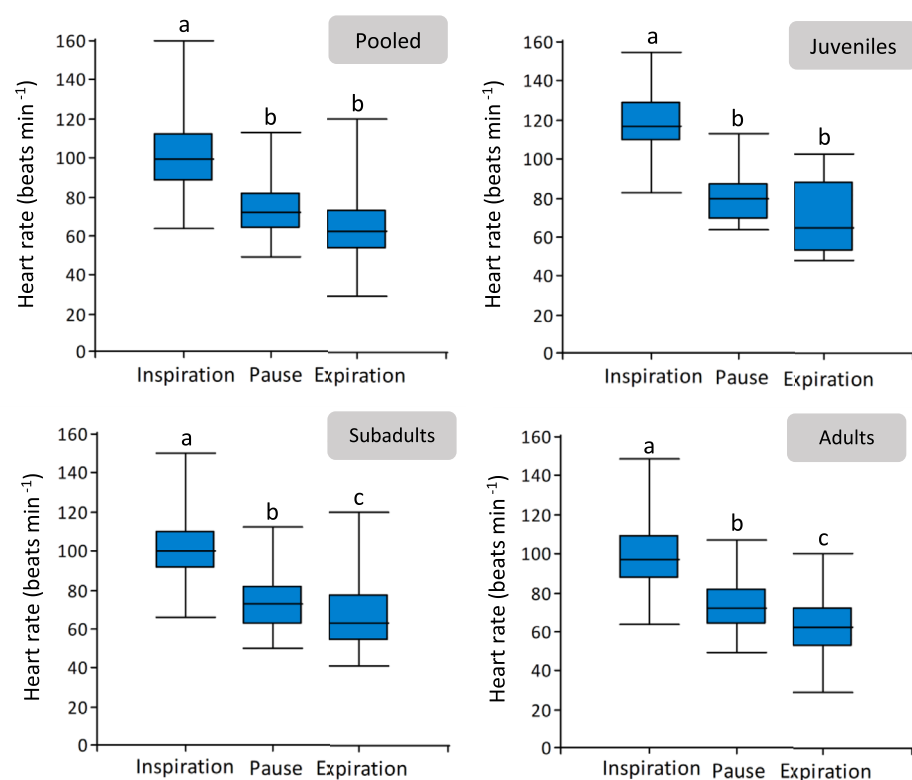
As expectable according to the RSA, all animals showed variability in HR in relation to respiratory phases, (Kruskal Wallis Test;  $H = 254.91$ ;  $p < 0.001$ ;  $N = 167$ ), with a high HR during the inspiration ( $101.2 \pm 18.4$  beats  $\text{min}^{-1}$ ) and a low HR during the expiration ( $64.6 \pm 17.7$  beats  $\text{min}^{-1}$ ). The pause period had an intermediate HR between inspiration and expiration (Table 2; Fig. 2). This pattern was similar between age classes (Table 2; Fig. 2).

**Table 2** Mean heart rate  $\pm$  SD during respiratory phases for male South American sea lions from different age classes resting on land

Age class	Heart rate (beats $\text{min}^{-1}$ )		
	Inspiration	Pause	Expiration
<i>Juveniles</i>	$120.7 \pm 24.6$ ( $n = 14$ ) <sup>a</sup>	$80.6 \pm 13.9$ ( $n = 14$ ) <sup>c</sup>	$67.6 \pm 17.7$ ( $n = 14$ ) <sup>d</sup>
<i>Subadults</i>	$101.7 \pm 18.9$ ( $n = 46$ ) <sup>b</sup>	$73.2 \pm 12.9$ ( $n = 46$ ) <sup>c</sup>	$68.2 \pm 24.4$ ( $n = 46$ ) <sup>d</sup>
<i>Adults</i>	$98.5 \pm 15.8$ ( $n = 107$ ) <sup>b</sup>	$72.4 \pm 12.8$ ( $n = 107$ ) <sup>c</sup>	$61.9 \pm 14.0$ ( $n = 107$ ) <sup>d</sup>
<i>Pooled</i>	$101.2 \pm 18.4$ ( $n = 167$ ) <sup>*</sup>	$73.4 \pm 14.5$ ( $n = 167$ ) <sup>**</sup>	$64.6 \pm 17.7$ ( $n = 167$ ) <sup>***</sup>

Means with a common letter are not significantly different between age classes ( $p > 0.05$ –LSD Fisher). Means with varying numbers of asterisks (\*, \*\*, \*\*\*) are significantly different between respiratory phases (all post hoc comparisons:  $p > 0.05$ ). HR increases during inspiration and decreases during expiration, with intermediate HR during the pause, consistent with the RSA

**Fig. 2** Median, interquartile range (box) and range (bars) of heart rate (beats  $\text{min}^{-1}$ ) during respiratory phases of male South American sea lions resting on land, showing the occurrence of the respiratory sinus arrhythmia in different age classes (juveniles, subadults and adults) and for all animals pooled. Boxes with a common letter (within each age class and for all animals pooled) are not significantly different (all post hoc comparisons:  $p > 0.05$ )





When we applied a GLM of HR in each respiratory phase, we found that HR during inspiration is only affected by age class ( $F=9.54$ ;  $p=0.0001$ ), with no significant effect of other variables. Mean HR of the inspiration in juveniles was significantly higher than subadults and adults (Table 2). In the models for the pause and the expiration, age class was not significant (Table 2), but ambient temperature was (see section “Effect of ambient temperature on HR”).

### Breathing rate and averaged heart rate relation

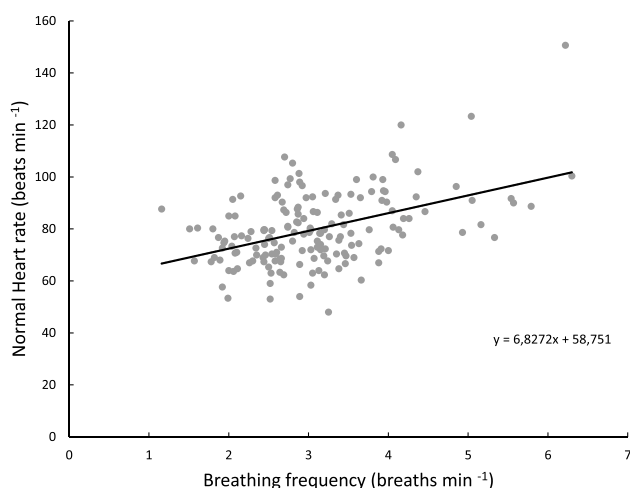
Mean  $f$  and mean nHR were weakly but significantly positively correlated (Spearman correlation,  $r^2=0.37$ ,  $n=167$ ,  $p<0.01$ ; Fig. 3).

### Cardiorespiratory variations

GLMMs of total duration of the respiratory cycle indicated that ambient temperature ( $p<0.05$ ) was the only significant variable, with no effect of age class, state of sleep and time of day. GLMMs of their respective phases' duration also showed that ambient temperature ( $p<0.05$ ) was the only significant variable for expiration and inspiration. The pause was not affected by any of the variables.

### Effect of ambient temperature on respiration

There were some significant differences in the total duration (TD) of the respiratory cycle in relation to ambient temperature. The TD from temperatures ranging from 21 to 25 °C was  $14.42 \pm 7.60$  s, which was significantly lower than both the TD at temperatures from 11 to 15 °C ( $20.62 \pm 9.0$  s; post hoc comparisons:  $Z=-3.15$ ,  $p=0.008$ ; Table 2) and



**Fig. 3** Relationship between the normal heart rate (beats  $\text{min}^{-1}$ ) and breathing frequency ( $f$ —breaths  $\text{min}^{-1}$ ). The positive relationship indicates that tachycardia occurs when the respiratory rate increases

at temperatures from 6 to 10 °C ( $20.52 \pm 8.52$  s; post hoc comparisons:  $Z=-2.60$ ,  $p=0.044$ ; Fig. 4). The remaining post hoc comparisons were not significant (all post hoc comparisons:  $p>0.05$ ).

There was a significant reduction in phase duration with ambient temperature in both inspiration ( $\chi^2=11.81$ ,  $p=0.008$ ) and expiration ( $\chi^2=12.28$ ,  $p=0.07$ ), with no significant effect on pause duration ( $\chi^2=8.05$ ,  $p=0.05$ ). The duration of inspiration at temperatures ranging from 21 to 25 °C was significantly lower than the duration at temperatures from 11 to 15 °C (post hoc comparisons:  $Z=-3.6$ ,  $p=0.002$ ; Fig. 4). There were no significant differences in the duration of inspiration among the other temperature ranges (Fig. 4). The duration of the expiration at temperatures ranging from 21 to 25 °C was significantly lower than the duration at temperatures from 11 to 15 °C (post hoc comparisons:  $Z=-3.7$ ,  $p=0.001$ ; Fig. 4) and at temperatures from 6 to 10 °C (post hoc comparisons:  $Z=-3.04$ ,  $p=0.01$ ; Fig. 4). There were no significant differences in the duration of the expiration between temperatures from 6 to 10 °C and from 11 to 15 °C (Fig. 4). No data on TD and respiratory phases' durations during temperatures between 0 and 5 °C were recorded.

### Effect of state of sleep on $f$

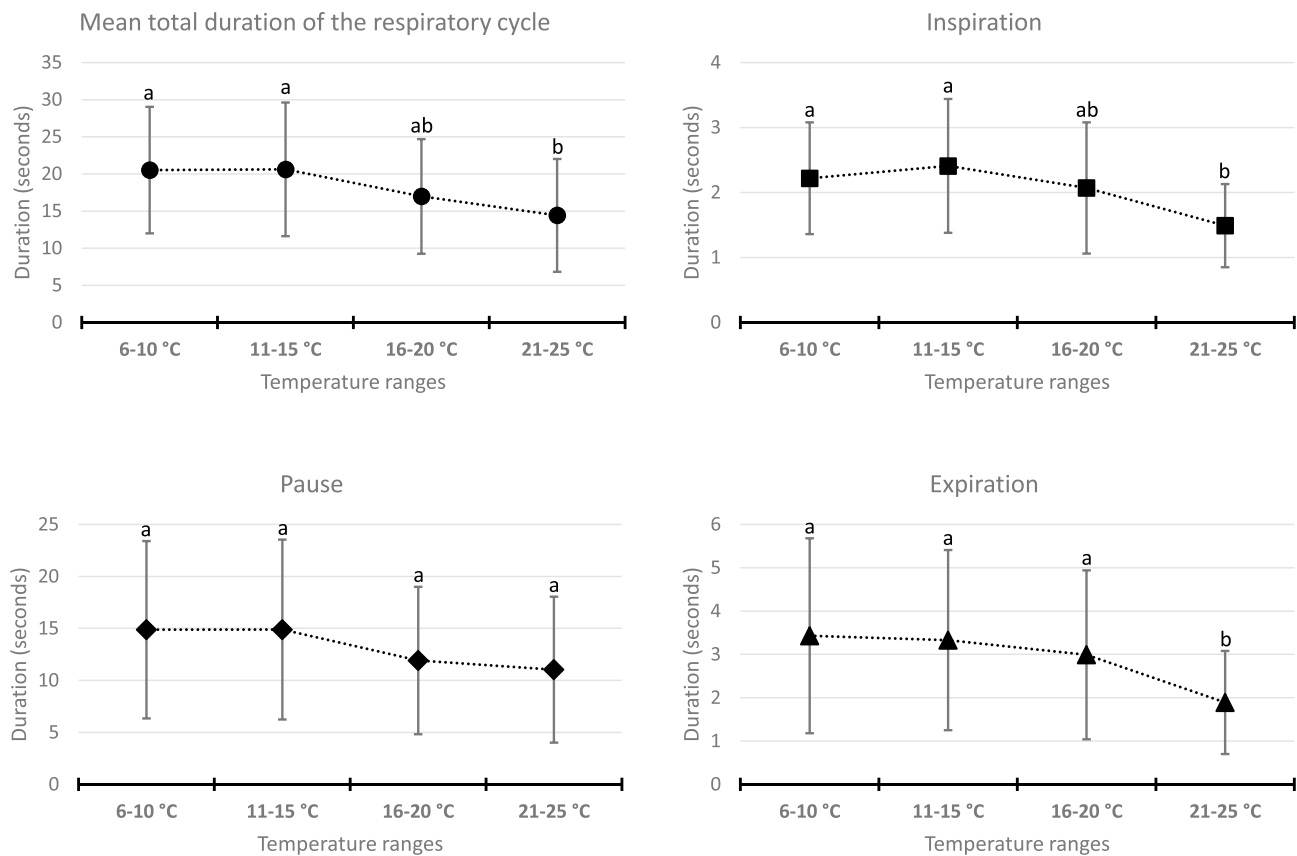
A GLMM of  $f$  showed that only the state of sleep ( $t=11.87$ ,  $p<2e^{-16}$ ) was significant, with no effect of age class, ambient temperature, and time of day. The  $f$  was significantly lower when the animals were presumably asleep ( $3.1 \pm 0.9$  breaths  $\text{min}^{-1}$ ), compared to animals that were awake ( $3.3 \pm 1.1$  breaths  $\text{min}^{-1}$ ).

### Effect of ambient temperature on HR

A GLMM of HR during both the pause ( $F=4.99$ ,  $p=0.0008$ ) and the expiration ( $F=14.71$ ,  $p<0.0001$ ) showed that temperature had a significant effect. The HR during respiratory pause was significantly higher for temperatures of 11–15 °C, 16–20 °C, and 21–25 °C than during temperatures of 0–5 °C and 6–10 °C (Fig. 5). The HR during expiration was significantly higher during temperatures of 26–30 °C than during temperatures of 16–20 °C. Likewise, HR during expiration was also significantly higher for temperatures of 16–20 °C and 21–25 °C than during temperatures of 0–5 °C, 6–10 °C, and 11–15 °C (Fig. 5).

## Discussion

This study has described the cardiorespiratory parameters of wild male SASLs resting on land, providing novel information on the coupling between respiratory and cardiac cycles.



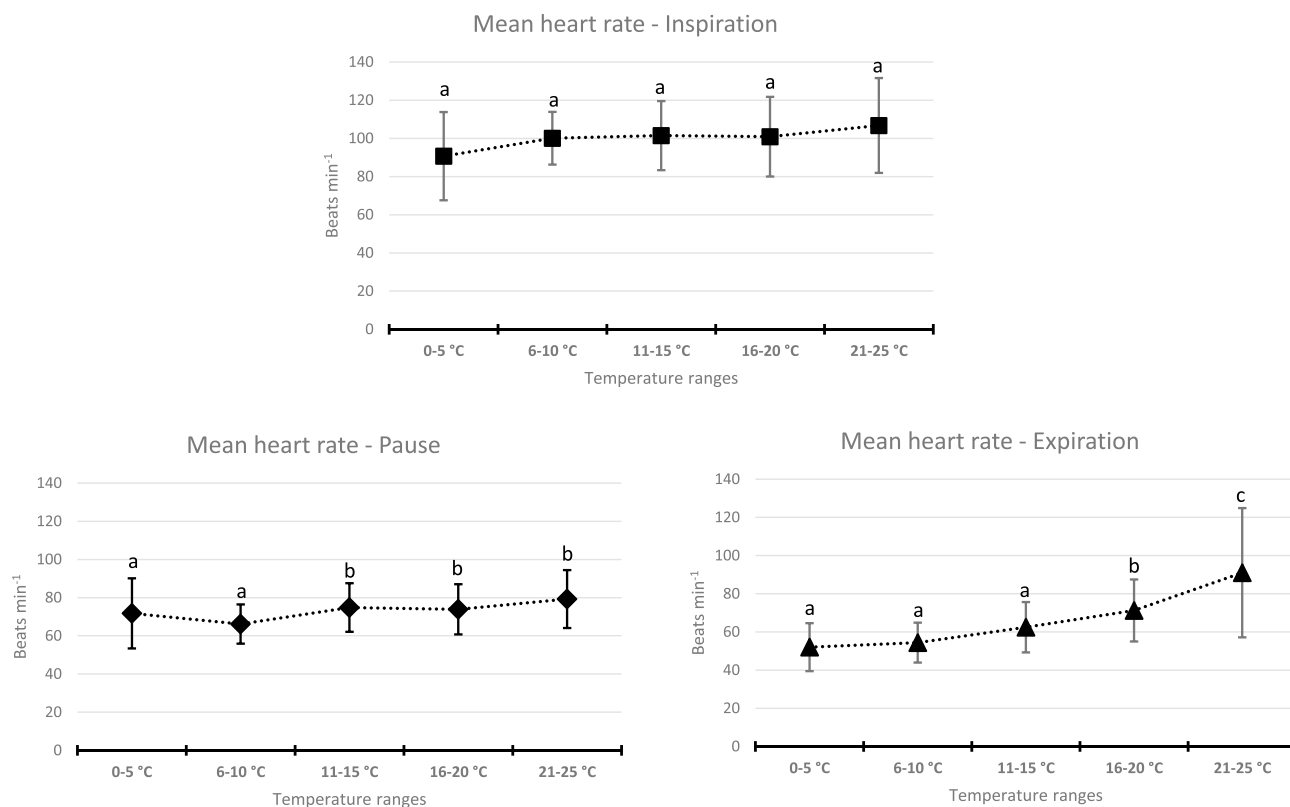
**Fig. 4** Mean duration (seconds)  $\pm$  SD of respiratory cycle and their phases for male South American sea lions resting on land from different temperature ranges. Means with a common letter (from each duration) are not significantly different (all post hoc comparisons:  $p > 0.05$ )

We have reported for the first time for SASL the resting HR at different respiratory phases, and characterized variations in respiratory phase duration and HR as a function of age, temperature, time of day, and state of sleep. Our study was carried out only by visual methods, which allowed us to study undisturbed animals resting in their natural habitat. Our results for respiration and HR were comparable with those obtained using electrocardiogram and respiratory impedance electrodes in other pinnipeds (Lin et al. 1972; Deacon and Arnould 2009), and were also coincident with those recorded for SASL housed in professional care (Fahlman and Madigan 2016) or captured in their natural colonies (Lyamin et al. 2002). Therefore, despite limitations such as the need for a suitable observation point to accurately view cardiorespiratory movements, this non-invasive method allows a similar quality of information to that obtained by instrumentation, but without the stress and cost associated with animal handling.

In agreement with other species of aquatic mammals studied so far, the respiratory cycle consisted of three phases (inspiration, post-inspiratory pause, and expiration). The post-inspiratory pause, or the full lung apnea, was always the longest respiratory phase in all age classes. The later has

already been observed in *Otaria flavescens* (Lyamin et al. 2002; Fahlman and Madigan 2016) and *Zalophus californianus*, with a long post-inspiratory pause representing 84% of the total respiratory cycle (Lin et al. 1972). Similarly, we observed that the post-inspiratory pause duration accounted for 74% of the total respiratory cycle duration. The post-inspiratory pause has been reported for a variety of marine mammals (Scholander 1940; Spencer et al. 1967; Olsen et al. 1968; Lin et al. 1972; Kooyman 1973; Castellini et al. 1986; Gallivan et al. 1986; Mortola and Lanthier 1989; Mortola and Seguin 2009). The fact that the lungs remain full above the passive volume of the respiratory system (amount of air remaining in the lungs after a normal expiration) has been considered an adaptation to aquatic life (Scholander 1940; Spencer et al. 1967; Olsen et al. 1968; Kooyman 1973; Castellini et al. 1986; Gallivan et al. 1986; Mortola and Lanthier 1989; Mortola and Seguin 2009) since this would provide more time for oxygen in the air within the lungs to be transferred to the blood.

The breathing frequency of male South American sea lions resting on land ( $3.2 \text{ breaths min}^{-1}$ ) was similar to those already reported in the SASL (Fahlman and Madigan 2016), and also in other aquatic mammals resting on land,



**Fig. 5** Mean heart rate (beats min<sup>-1</sup>) ± SD during respiratory phases for male South American sea lions resting on land at different temperature ranges. Means with a common letter (from each duration) are not significantly different (all post hoc comparisons:  $p > 0.05$ )

where  $f$  was in the range of 1–7 breaths per minute (Scholander and Irving 1941; Andersen 1966; Gallivan 1980; Mann and Smuts 1999; Andrews et al. 2000; Le Boeuf et al. 2000; Mortola and Limoges 2006). It is well known that breathing frequencies of mammals at rest decrease with the increase in body weight (Guyton 1947; Stahl 1967; Mortola and Seguin 2009; Davis 2019), and those changes can be attributed in part to an increase in total body oxygen stores that occurs with increasing size and age (Kodama et al. 1977; Ponganis et al. 1993; Thorson and Le Boeuf 1994; Burns et al. 1999; Clark 2004). According to this negative allometry (Mortola and Limoges 2006; Davis 2019), we might expect to observe a decrease in  $f$  in our adult specimens, that are expected to be heavier than juveniles (Winship et al. 2001; Grandi et al. 2010). However, no significant differences were found in  $f$  of animals of different age classes. We only observed a slight tendency of lower  $f$  values in older specimens, highlighting the need to clarify this through the study of respiratory frequency in animals of known weight.

The respiratory cycle was not constant over time and there were variations in the respiratory frequency within individuals. In the same animal, periods of bradypnea and tachypnea were recognized. Likewise, the presence of long pauses between the periods of bradypnea and tachypnea

revealed the high variability of the respiratory cycle of this species. In some phocids studied during sleep states, post-inspiratory pauses alternating with periods of tachypnea have been recorded (Mukhametov et al. 1984; Lyamin 1993; Lyamin et al. 1993, 2002; Castellini et al. 1994; Castellini 2002b). This has also been studied in other pinnipeds (*Callorhinus ursinus*; Mukhametov et al. 1985; Lyamin and Mukhametov 1998), showing that the respiratory cycle of South American sea lion is in agreement with other species within this taxon. Reason behind these cardiorespiratory variations during resting might be related to several factors, such as maintenance of normal levels of alveolar and arterial  $PO_2$  and  $PCO_2$  (Mortola and Seguin 2009; Davis 2019), thermoregulation (Castellini and Mellish 2015; Davis 2019), and also the potential correlation between cardiorespiratory behavior during dispersal at sea.

The study of the cardiorespiratory behavior of some pinniped species during their resting phase on land has shown some correlation with the cardiorespiratory behavior during diving. It has been shown, for example, that adult elephant seals resting on land experience periods of post-expiratory apnea of similar duration to the post-expiratory apneas they perform during their typical dives (Kenny 1979; Blackwell and Le Boeuf 1993). The pattern of relative duration



of apnea-eupnea phases when they remain on land is similar to the pattern imposed by sea diving, which consists of successive dives (post-expiratory apneas) interspersed by eupneic periods of surface breathing (Bartholomew 1954; Hubbard 1968; Blackwell and Le Boeuf 1993; Andrews et al. 1997, 2000; Wartzok 2002). The recorded duration of post-expiratory apnea on land and the duration of typical dives in elephant seals suggest the existence of a similar physiological basis (Blackwell and Le Boeuf 1993), and therefore, land and dive apneas could be governed by the same control mechanisms (Castellini et al. 1994). Some of the physiological changes that are recorded during diving (bradycardia, peripheral vasoconstriction and reduction in metabolic rate), are likely to be observed also during post-expiratory apneas on land (Scholander 1940; Elsner et al. 1964; Jones et al. 1973). In fact, it has been observed that changes in duration of terrestrial apnea would coincide with changes in the diving capacity of individuals (Blackwell and Le Boeuf 1993), so their study has been used to complement the study of the diving physiology of these animals (Bartholomew 1954; Blackwell and Le Boeuf 1993).

Unlike the aforementioned phocids, commonly known as exhaler divers, the sea lions undergo typical dives by performing post-inspiratory apneas (Bartholomew 1954; Blackwell and Le Boeuf 1993; Lyamin et al. 2002), and are therefore often referred to as inhaler divers (Favilla and Costa 2020). Almost all dives (99% of the routine dives) of SASL females instrumented with TDRs reported by Rodríguez et al. (2013) were shorter than 2 min, with the most frequent duration at about 1–1.5 min. The latter is comparable with the terrestrial maximum duration of the post-inspiratory pauses reported in the present study, which is approximately 80 s. Therefore, and comparable to what was suggested for phocids, it is highly probable that on land cardiorespiratory patterns for this species are closely related and share a physiological basis with the cardiorespiratory patterns at sea.

The nHR (73.4 beats  $\text{min}^{-1}$ ) was similar to the 75 beats  $\text{min}^{-1}$  we measured previously in a non-sedated SASL using echocardiography (Castro et al. 2018). These results were also similar to that reported in resting Californian sea lions, with an average value of 80–84 beats  $\text{min}^{-1}$  (Lin et al. 1972; Ponganis et al. 1997). Although no significant differences were observed in nHR among age classes, mean HR from all respiratory phases pooled (during complete breathing cycle) were significantly different among age classes. Several authors have studied ontogenetic variations in mean HR of the eupneic cycle, considering HR during all respiratory phases grouped, and have reported similar ontogenetic differences in pinnipeds (Lin et al. 1972; Castellini et al. 1994), where juveniles have a higher HR than adults. Ontogenetic differences in mean HR but not in nHR are probably due to the fact that significant variations in HR with age were recorded only during the inspiration

(Table 2), thus producing differences only in mean HR values, and no in the nHR that only considers HR during the pause. These results (significant HR differences with age only during inspiration) highlight the need to further investigate ontogenetic variations in HR in the different respiratory phases of pinnipeds. The latter has not been studied in depth in other species, and raises new questions such as whether HR decrease with age occurs in all respiratory phases, or is mostly a reflection of a lower HR (in larger animals) during inspiration only.

Since variations in HR among respiratory phases were statistically significant, we consider that respiratory sinus arrhythmia (RSA) in SASL can certainly be assumed. RSA consists of a decrease in HR during the expiratory phase and an increase in HR during inspiration (Grossman and Kollai 1993). The HR of sea lions ranged around a mean value of 73 beats  $\text{min}^{-1}$  during the post-inspiratory pause, with inspiratory tachycardia averaging 101 beats  $\text{min}^{-1}$  and expiratory bradycardia averaging 65 beats  $\text{min}^{-1}$ . As observed in almost every terrestrial mammal, RSA has been reported in marine mammals including harbor porpoise (*Phocoena phocoena*; Kastelein and Meijler 1989), Antarctic fur seal (*Arctocephalus gazella*; Boyd et al. 1999), California sea lion (*Zalophus californianus*; Lin et al. 1972) and the northern (*Mirounga angustirostris*) and southern elephant seals (*Mirounga leonina*; Bartholomew 1954; Blackwell and Le Boeuf 1993; Castellini et al. 1994; Andrews et al. 1997; Falabella et al. 1999; Andrews et al. 2000).

The physiological role of the RSA has been widely discussed. Hayano and Yasuma (2003) proposed that during rest, as the oxygen demand is decreasing, RSA may represent energy savings by reducing unnecessary heartbeats and wasted ventilation without compromising respiratory gas exchange performance. Also, Kanwisher and Ridgway (1983) suggest that acceleration of HR during inspiration allows acceleration of oxygen uptake into the blood. Such a high HR may facilitate rapid gas transport between the tissue and the lung, and is presumably accompanied by high  $f$  to ensure a similar rapid exchange between the lungs and the environment (Kooyman et al. 1971; Andrews et al. 2000). More recently, Ben-Tal et al. (2012) proposed a new hypothesis, using theoretical models, where they argue that RSA minimizes the work done by the heart while maintaining a desired average partial pressure of  $\text{CO}_2$ . The positive relationship between mean  $f$  and nHR shown in Fig. 3, also confirmed the RSA. As is already known, RSA is an oscillation of the heart period in synchrony with respiration. This is, when  $f$  increases, so does HR. These results are consistent with those reported in different species of cetaceans, where  $f$  was positively related to both mean and minimum HR (Fahlman et al. 2020; Blawas et al. 2021). Several authors suggested that cardiorespiratory coupling help cetaceans maximize gas

exchange during short surfacing intervals by producing a large cardiac response (Blawas et al. 2021). In addition, RSA could represent a mechanism to mitigate the disparity between the intermittent flow of air and the continuous flow of blood by varying the distribution of heart beats within a breath (Hayano and Yasuma 2003; Yasuma and Hayano 2004).

The cardiorespiratory cycle can undergo spontaneous variations in both  $f$  and HR, which is characteristic of many aquatic mammals (Parker 1922; Swindle 1926; Scholander and Irving 1941; Gunther 1949; Kenny 1979; Blackwell and Le Boeuf 1993). HR and  $f$  may vary among individuals, depending on their characteristics, and would be influenced by activity level (Stephenson et al. 1988; Williams et al. 1991). For example, oxygen uptake and HR in sea lions vary with swimming speed (Williams et al. 1991; Butler et al. 1992; Boyd et al. 1995, 1999). Also, during exposure to high ambient temperatures, the  $f$  may increase slightly (Whittow et al. 1972). Although we did not find statistical differences in  $f$  with temperature, this would be as well reflected in our results, which showed a decrease in the total respiratory cycle duration, as well as the duration of inspiration and expiration, during temperatures around 21–25 °C. This is, a shorter duration of expiratory/inspiratory events would suggest an increased  $f$  (breaths per minute) (Hill et al. 2004; Van Diest et al. 2014). Changes in  $f$  can be observed as the ambient temperature gradually increases, and has been described that it becomes much more evident when temperatures exceed 30 °C (Lin et al. 1972; Whittow et al. 1972; Matsuura and Whittow 1973). However, our respiratory duration results showed significant differences at 20 °C and above. Our data indicate a stepwise variation, rather than a continuous variation. Additionally, it would also be interesting to monitor the body temperature of the animals to determine the range of ambient temperature that affects body temperature (Lin et al. 1972; Whittow et al. 1972), when changes in  $f$  and phase durations could be expected.

The HR during both pause and expiration was significantly higher during temperatures above 16 °C. Our results are in agreement with those reported by several authors who have analyzed the effect of ambient temperature on different cardiorespiratory parameters in pinnipeds (Lin et al. 1972; Whittow et al. 1972; Matsuura and Whittow 1973). As ambient temperature increases, HR also increases when animals become hyperthermic, and clear effects on  $f$  can be observed (Seath and Miller 1946; Shrode et al. 1960; Lin et al. 1972; Whittow et al. 1972; Matsuura and Whittow 1973; Legates et al. 1991), demonstrating the cardiorespiratory adjustments that these mammals display in reaction to changes in environmental conditions. It is noteworthy that significant differences were only observed in the pause and expiration phases but not in the inspiratory phase, which is the most tachycardic phase of the cycle.

Breathing frequency was significantly lower when the animals were presumably asleep. The variation in the breathing rhythm across the sleep–waking cycle was similar to that described electrophysiologically in SASL and other pinnipeds (Lyamin et al. 2002). In addition, oxygen uptake is influenced by the state of sleep or wakefulness, with oxygen consumption being higher in awake animals (Matsuura and Whittow 1973). There are also studies that assess changes in  $f$  across various sleep states, with an observed increase accompanied by evident tachycardia during the REM sleep phase (rapid eye movement) (Lin et al. 1972; Ridgway et al. 1975).

In summary, our study showed the interplay between the respiratory and cardiac cycles in male SASLs while resting on land, providing a detailed examination of respiratory phases, their coupling with HR and the RSA. Furthermore, the results of the cardiorespiratory pattern during resting on land provide the basis for discussing its potential connection with the cardiorespiratory cycle present during their dispersal at sea. The investigation of terrestrial post-inspiratory pauses and the pattern of respiratory cycle variations on land offers an opportunity to increase our understanding of specific aspects of the physiology of inhaler divers, which can be challenging to explore during diving. Future research should include females and incorporate additional information about the behavioral and physiological mechanisms that influence the cardiorespiratory cycle.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Competing interest** The authors have no relevant financial or non-financial interests to disclose.

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