Journal of Experimental Marine Biology and Ecology 394 (2010) 141–148

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Dive response differs between shallow- and deep-diving Steller sea lions (*Eumetopias jubatus*)

Allyson G. Hindle [a,]*, Beth L. Young [a], David A.S. Rosen [a], Martin Haulena [b], Andrew W. Trites [a]

[a] Marine Mammal Research Unit, University of British Columbia, 2202 Main Mall, Vancouver, BC, Canada V6T 1Z4
[b] Vancouver Aquarium, PO Box 3232, Vancouver, BC, Canada V6B 3X8

ARTICLE INFO

Article history:
Received 7 July 2010
Received in revised form 1 August 2010
Accepted 3 August 2010

Keywords:
Flipper stroking
Heart rate
Inter-beat interval
Overall dynamic body acceleration
Pinniped

ABSTRACT

Muscle exercise correlates with oxygen use, tissue perfusion and heart rate (f_H) in terrestrial animals, but the relationship between these physiological processes is less clear in diving animals. We found the mean heart rate of Steller sea lions trained to voluntarily dive to depths up to 40 m dropped by 40% while diving, and noted that mean bradycardia was 9% greater during shallow (10 m) compared to deep (40 m) dives. Longer dives resulted in lower heart rates, but only when they were shallow; on the other hand, minimum instantaneous f_H decreased consistently with dive duration. In general, instantaneous f_H did not reflect activity over short timescales. Our data suggest that our sea lions invoked a different dive response depending on whether they dove to shallow or deep depths. During shallow (10 m) dives only, the correlation between activity and f_H was indicative of vascular compromise between diving and exercise. However, during deep dives (40 m), there was no such correlation, suggesting that locomotory activity was uncoupled from dive bradycardia, which was possibly mediated by an absence of blood flow to active muscle. For both diving scenarios, surface f_H correlated with dive activity, suggesting that some underwater locomotory costs were deferred to the post-dive surface interval. Ultimately, our data support the speculation that Steller sea lion locomotory muscles become hypoxic during diving, regardless of dive depth.

1. Introduction

The relationship between diving locomotory activity and heart rate (f_H) has been an area of particular research interest given the seemingly contrary cardiovascular responses of diving animals to eupneic exercise and apneic diving. Indeed, a cardiovascular response to exercise, which facilitates oxygen (O_2) transport and use in active tissues (Brooks and White, 1978) is fundamentally at odds with the dive response that is designed to regulate O_2 consumption (Castellini et al., 1985; Hochachka, 1986; Davis et al., 2004). For animals exercising on land (Jones et al., 1989) and at the water surface (Fish, 1982; Feldkamp, 1987; Rosen and Trites, 2002), muscle work is an important determinant of O_2 consumption rates. Further, circulatory adjustments manage blood flow to contracting muscles in proportion to exercise output (Armstrong et al., 1987; Delp and Laughlin, 1998) such that exercise metabolism is strongly related to f_H (Baudinette, 1978). Air-breathing divers swimming in a flume demonstrate a typical exercise response (i.e., tachycardia, increased O_2 consumption, peripheral vasodilation; Castellini et al., 1985; Butler, 1988; Davis et al., 1991; Ponganis et al., 1991). By contrast, a decline in f_H during diving (i.e., bradycardia) is a primary component of the Scholander–Irving dive response (Scholander, 1940; Irving et al., 1941; Scholander et al., 1942) and has been documented in a variety of diving animals. Along with increased peripheral resistance, the presumed aim of such vascular responses is to conserve finite on-board O_2 for critical organs since these O_2 stores fuel underwater excursions (Butler and Jones, 1997; Davis et al., 2004).

Heart rate is the most commonly measured variable of the dive response, as it is relatively easy to telemeter from a free-diving animal (Ponganis, 2007). As a result of this, and given the apparent relationship between heart rate and whole-body O_2 use in air, many researchers have made a case for using f_H as an indirect measure of free-ranging metabolic rate (e.g., Butler and Woakes, 1982; Butler, 1993; Boyd et al., 1999; Green et al., 2001; McPhee et al., 2003). However, the relationships between activity, metabolic expenditure, and heart rate during apneic exercise are likely complicated by the cardiovascular adjustments associated with the dive response. Studies exploring voluntary or trained diving reveal that the dive response can be modulated by many factors including dive characteristics (Hill et al., 1987), body temperature (Hindle et al., 2006) and activity level (Davis et al., 2004). It is likely that diving in free-ranging vertebrates occupies a physiological middle-ground between the classic dive response (observed during forced submersions) and eupneic exercise

---

* Corresponding author. Department of Cell and Developmental Biology, University of Colorado Denver, PO Box 6511, Mail Stop 8108, Aurora, CO, USA 80045. Tel.: +1 303 724 3455; fax: +1 303 724 3420.
E-mail addresses: allyson.hindle@gmail.com (A.G. Hindle), b.young@fisheries.ubc.ca (B.L. Young), rosen@zoology.ubc.ca (D.A.S. Rosen), martin.haulena@vanaqua.org (M. Haulena), a.trites@fisheries.ubc.ca (A.W. Trites).

0022-0981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2010.08.006

(Castellini et al., 1985). It is not clear, however, what the outcome of such a physiological compromise would be on the management of body O_2 stores. Heart rate is a mechanistic indicator of whole-body changes in blood flow and thereby tissue O_2 delivery. In particular, the regulation of blood flow to exercising muscle could illustrate the compromise between diving and exercise should it exist; however blood flow to muscle vascular beds has not been directly monitored in a free-ranging diving vertebrate to date.

There is mixed evidence from indirect measures (blood O_2 partial pressure measurements from free-ranging translocated seals) and direct measures from other experimental scenarios (trained diving, simulated diving and simulation modeling) which suggests that a range of perfusion levels to working muscle is possible (Zapol et al., 1979; Davis et al., 1983; Bevan and Butler, 1992; Davis and Kanatous, 1999; Wright and Davis, 2006; Meir et al., 2009; Meir and Ponganis, 2010) or that it may be entirely absent (Ponganis et al., 2003; Meir et al., 2008). If locomotory muscle remains at least partially perfused during diving, we anticipate a correlation between underwater locomotion and diving f_H. On the other hand, for species in which there is likely no blood flow to muscle during diving (e.g., emperor penguins, Ponganis et al., 2003; Meir et al., 2008), measures of exercise output (e.g., stroke frequency) and f_H should be independent over the dive period (Meir et al., 2008).

Our study objective was to determine the relationship between f_H and underwater locomotion in a diving mammal, the Steller sea lion (*Eumetopias jubatus*, Schreber). Specifically, we examined diving f_H and activity (tri-axial acceleration and flipper stroking) in trained sea lions foraging in the open ocean to different depths. We also compared the patterns of diving bradycardia over single dives (with complete surface recovery of body O_2 stores) and dive bouts (having incomplete inter-dive O_2 recovery).

2. Materials and methods

2.1. Animals

Heart rate and tri-axial acceleration were recorded from three female Steller sea lions diving to 10 m and 40 m during August and September, 2008. Animals were housed in a purpose-built floating seawater pen located in a coastal inlet (Port Moody, British Columbia, Canada; 49°17′25″N, 122°54′55″W; Hastie et al., 2006a). The animals had been previously trained to voluntarily dive to depths up to 40 m. Sea lions ranged in age from 8 years (F00BO: 145.3 ± 2.1 kg; mean ± SE for this individual over the course of the study) to 11 years (F97SI: 217.6 ± 1.8 kg; F97HA: 172.2 ± 0.2 kg). All animal handling and experimental procedures were conducted in accordance with regulations of the Canadian Council on Animal Care (University of British Columbia animal use permit #A04-0311), Department of Fisheries and Oceans Canada (MML 2007-001) and the Vancouver Aquarium.

2.2. Telemetry

Heart rate (R–R interval) was measured continuously via HRX transmitter and HTR data logger (Wildlife Computers, Redmond, WA, USA). Under isoflurane anesthesia (0–5%), animals were outfitted prior to each trial with two temporary subcutaneous f_H electrodes (sterile 30-Ga Teflon-coated silver wire) placed caudal to the front flippers at the level of the heart, approximately 25 cm lateral of the dorsal midline (see Young et al., in press for details). Electrode leads were affixed to the sea lion's fur using neoprene strips and superglue (Loctite, Westlake, OH, USA), and all transmitters and data loggers were hard-mounted to a harness worn by the animals. Transmission of f_H from the HRX to the HTR data logger was periodically verified using a Polar brand f_H receiver watch (Polar Electro Canada Inc., Lachine, QC, Canada).

The sea lion harnesses were additionally outfitted with Mk-10-V data loggers (Wildlife Computers) which recorded depth, temperature, light level, wet-dry state, velocity, and tri-axial acceleration. Each harness was fitted specifically to the individual animal to minimize drag and noise in the acceleration data associated with a loose-fitting harness. Acceleration data was used to identify fore-flipper stroking as well as to calculate an integrated 3-dimensional measure of body motion (overall dynamic body acceleration, ODBA). Body heave (sampled at 64 Hz) was used to detect individual fore-flipper strokes. ODBA (*g*; Wilson et al., 2006) was determined from the 3-channel sum of dynamic acceleration, calculated as the absolute value difference between static (data smoothed with a three second running mean, per Shepard et al., 2008) and total (unsmoothed) acceleration values at 16 Hz.

2.3. Trial protocol

Dive trials were conducted in the open ocean either immediately adjacent to the sea lion holding pens, or at a dive site in a nearby fjord (49º19′30″N, 122º55′30″W). Sea lions were transported to the dive site in a 6.7 m transport boat (see Hastie et al., 2006a for complete description of research area). Trials (or transport) began immediately following electrode insertion and complete recovery from anesthesia. Modified bilge-pump systems delivered fish (20 g herring pieces) through two separate PVC feeding tubes at a set depth (both to 10 or 40 m). Feeding tubes were affixed to a floating research platform able to be towed to research moorings in different water depths. These feeding tubes were spaced ~6 m (about 3 body lengths) apart to encourage active swimming at depth. Trials were randomized and repeated three times at each depth (10 m, 40 m) for each animal, with a maximum of one trial per animal per day ($n = 6$ trials per animal). Aside from set surface times at the start and end of a bout the Steller sea lions controlled their own dive and surface interval durations.

The sea lions were trained from an early age to perform basic behaviors under trainer control. These three individuals had also been working in the open water for three (F97HA) to five (F97SI & F00BO) years, which included familiarization with diving to feeding tubes at varied depths (see Hastie et al., 2006a for additional details). We provided study-specific training (i.e. mock trials) for approximately three weeks prior to data collection. Each trial consisted of a resting period at the surface followed by two dive types: a single dive with a complete post-dive surface recovery period (~6 min); then a bout consisting of four dives to the same depth followed by a similar recovery period. Previous studies have revealed that this post-dive recovery time is sufficient for O_2 consumption rates to return to pre-dive baseline (Hastie et al., 2006b). For 40 m dive trials only, sea lions performed an additional single dive of longer duration ($n = 8$ pairs; encouraged via increased fish delivery rates) with full recovery at the end of the trial. We were unable to analyze several trial portions due to poor quality f_H data. Sample sizes therefore differed between 10-m single dives ($n = 9$), 10-m bout dives ($n = 9$), 40-m single dives (short initial: $n = 9$, long final: $n = 8$) and 40-m bout dives ($n = 8$).

2.4. Data analyses and statistics

R–R intervals were filtered for motion artifact using a series of algorithms (described in detail in Young et al., in press). Mean f_H for different periods (pre-dive rest, diving, post-dive and dive-surface) was calculated as the average of instantaneous f_H across the relevant interval. Pre-dive resting rates were measured over the 2-min immediately prior to submergence, diving f_H was measured over the dive duration, and post-dive f_H was measured over the initial 30 s at the surface. In many cases, transient noise in the f_H dataset made it difficult to accurately determine when post-dive f_H reached pre-dive resting level. Our analysis of the cleaner traces revealed that f_H returned to this pre-dive baseline within 15–30 s of surfacing. We therefore adopted a 30-s period of measurement for all dives. Dive-surface f_H was measured over the entire dive plus 30-s surface recovery period. For dive bouts, f_H means were taken across all underwater intervals (diving), all inter-dive surface intervals plus the final 30-s post-surface recovery (post-dive) or across the entire dive bout including the surface recovery period (dive-surface). Data were tested for normality using the Shapiro-Wilk statistic and homogeneity of variance was confirmed using a modified Levene test. Transformations were employed when necessary to meet the assumptions of parametric tests. Data were analyzed using SPSS statistical software (V 11.5.1, Chicago, IL, USA). Significance was set at $\alpha = 0.05$ and means are presented ± 1 SE.

Heart rates (mean f_H as well as minimum and maximum instantaneous f_H) for the intervals described above were compared against dive parameters (log dive duration, depth and type). For dive bouts, we incorporated mean dive duration (rather than cumulative dive duration) into the analysis. These relationships, along with those between underwater activity and f_H were explored using linear mixed effect models. For the latter, the dependant variable f_H was compared against fore-flipper stroke rates and mean ODBA using dive type (single or bout) and dive depth (10 or 40 m) as fixed factors, and dive duration (log-transformed) as a covariate. 'Animal ID' was included as a random (repeated measures) factor. We compared portions of the same trials (single dive vs. bout, for example) using paired *t*-tests, and applied Bonferroni's correction factor for sequential and related tests (Rice, 1989) to *p*-values generated from groups of *t*-tests where parameters where not independent. The temporal development of bradycardia was also compared with instantaneous ODBA over the course of single dives in a representative animal. Finally, data were visualized using linear regression. Heart rate data were regressed against dive duration (log-transformed) and activity measures. When statistical analyses revealed significant differences associated with dive depth or type, regressions for these groups of data were considered separately.

3. Results

3.1. Influence of dive characteristics on bradycardia

The duration (grand mean ± SE) of all dives to 10 m was 1.1 ± 0.05 min ($n = 18$), and that for all dives to 40 m was 2.7 ± 0.3 min ($n = 25$). The grand mean for 40 m trials includes the additional single dive of longer duration (4.2 ± 0.5 min vs. 2.1 ± 0.1 min for the initial single dive) at the end of the trial. The resting f_H for Steller sea lions was 86.3 ± 4.0 beats min^{-1} at the water surface, and mean f_H was reduced during diving to 51.6 ± 2.0 beats min^{-1} (40% overall reduction, range: 27.2–75.4 beats min^{-1}, $t = 8.79$, df = 21, $p < 0.001$). We detected a greater reduction in mean f_H for 10 m dives compared to 40 m dives (difference of 9%; $F_{1,37} = 5.50$, $p = 0.03$). Dive type (single versus bout diving) was not a significant factor in describing diving f_H ($p = 0.86$), although f_H was slightly reduced in single dives compared to bout dives (4–7.5% reduction for all sea lions tested; Table 1). Over the dive-surface cycle (single dives and bouts combined), mean f_H was 64.0 ± 2.3 beats min^{-1} (range: 39.0–83.9 beats·min^{-1}). This incorporates f_H during the dive period described above, along with inter-dive surface periods for bouts, plus a 30-s post-dive surface recovery period (where f_H averaged 100.2 ± 2.3 beats min^{-1}). When the entire dive plus surface recovery interval was considered, dive type and depth no longer had a significant influence on mean f_H. During the 30-s interval immediately following diving, the maximum instantaneous f_H recorded was 200 beats min^{-1}. Neither maximum nor mean f_H during the post-dive surface interval were related significantly to either dive depth or type.

Table 1
Characteristics of diving bradycardia in three adult female Steller sea lions (*Eumetopias jubatus*).

	Single dives	Bout dives
Dive duration (min)		
Shallow	1.2 ± 0.1	1.0 ± 0.1
Deep	2.1 ± 0.1/4.2 ± 0.5	1.7 ± 0.1
Dive f_H (beats min^{-1})		
Shallow	48.8 ± 3.8	49.0 ± 4.7
Deep	52.4 ± 4.1/50.3 ± 4.0	57.5 ± 5.8
Minimum f_H (beats min^{-1})		
Shallow	14.1 ± 2.1	12.3 ± 2.1
Deep	12.4 ± 1.0/9.0 ± 1.4	8.3 ± 0.9

Dive durations, diving heart rate (f_H) and minimum instantaneous f_H are presented for single and bouts dives (4 dives in series) to shallow (10 m) and deep (40 m) feeding stations. Trials consisted of 10-m single dives ($n = 9$), 10-m bout dives ($n = 9$), 40-m single dives (short initial: $n = 9$, long final: $n = 8$), and 40-m bout dives ($n = 8$). Data are presented mean ± SE.

The minimum instantaneous f_H documented for diving Steller sea lions in our study was 4 beats min^{-1}. Despite the noted effect of dive depth on mean diving f_H, there was no overall effect of depth ($p = 0.88$) on minimum observed f_H for each dive (grand mean = 11.2 ± 0.8 beats min^{-1}, Table 1). Rather, for this variable we noted a significant influence of dive type ($F_{1,33} = 6.80$, $p = 0.01$). Specifically, a paired *t*-test revealed that bout dives at both depths contained lower minimum instantaneous f_H compared to the initial single dives undertaken in the same trials ($t = 3.47$, df = 14, $p = 0.008$, Table 1). For the $n = 8$ trials to 40 m in which a longer single dive was also recorded, minimum instantaneous f_H was also significantly reduced compared to the initial (shorter) single dives ($t = 3.45$, $p = 0.018$; Table 1).

We noted a negative correlation between dive duration (log-transformed) and mean diving f_H, but only under specific conditions. In 10 m (shallow) dives, dive duration explained at least a portion of the variation in mean f_H underwater ($r^2 = 0.42$, $p = 0.005$), and this relationship was similar in both single and bout dives (Fig. 1A). However, no significant correlations between dive f_H and dive duration were noted for 40 m (deep) dives of any type (Fig. 1A). Dive duration correlated negatively with minimum instantaneous f_H at both trial depths (Fig. 1B), but only in dive bouts ($r^2 = 0.37$, $p = 0.02$). While a similar trend is apparent for single dives, this relationship was not statistically significant ($r^2 = 0.14$, $p = 0.07$; Fig. 1B).

On the other hand, measures of post-dive tachycardia were, in general, positively correlated with dive duration (Fig. 2). The mean of post-dive f_H taken over the first 30 s after surfacing was significantly related to dive duration (log-transformed) for single dives ($r^2 = 0.28$, $p = 0.01$) but not in dive bouts ($p = 0.96$; Fig. 2A). The maximum instantaneous f_H recorded during the surface period increased with dive duration (log-transformed); a relationship which was similar across all dive types and depths ($r^2 = 0.12$, $p = 0.04$).

3.2. Temporal development of diving bradycardia

Following submergence, instantaneous f_H dropped an average of 64% from pre-dive baseline (86 beats min^{-1}) within the first few seconds of the 10 m dives. Over the same period only an average 14% reduction was noted in 40 m dives. Minimum instantaneous f_H occurred 28.9 ± 9.4 s post-submergence in 10 m dives, on average, and 44.5 ± 3.2 s post-submergence in 40 m dives. Heart rate remained consistently reduced in the shorter duration 10 m dives (Fig. 3A). In 40 m dives, transient f_H increases were observed periodically throughout submergence (Fig. 3B). Ascent tachycardia (a term describing the release of bradycardia which occurs prior to surfacing) occurred for both 10 and 40 m dives. Deeper dives had a longer period of ascent tachycardia (Fig. 3B) compared to shallow dives (Fig. 3A) culminating in a mean f_H of 84 beats min^{-1} immediately prior to surfacing.

The time course of diving activity as quantified by instantaneous ODBA did not track the temporal patterns observed in f_H. Activity was high in the first 2 s of diving, preceding a pronounced decline of ~85%

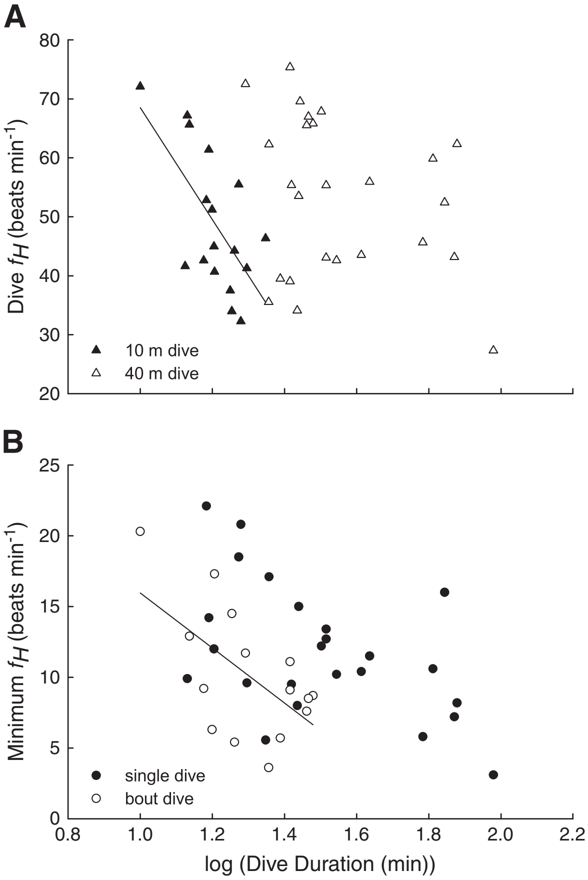


Fig. 1. Measures of dive bradycardia correlate with dive duration in three female Steller sea lions. Mean diving heart rate (f_H, panel A) had a significant negative linear relationship with dive duration (log-transformed) in 10 m dives (closed triangles, $r^2 = 0.42$, $p = 0.005$), but not for 40 m dives (open triangles). Minimum instantaneous f_H (panel B) also demonstrated a negative linear relationship versus dive duration (log-transformed), which described bout dives (open circles, $r^2 = 0.37$, $p = 0.02$) but not single dives (closed circles).

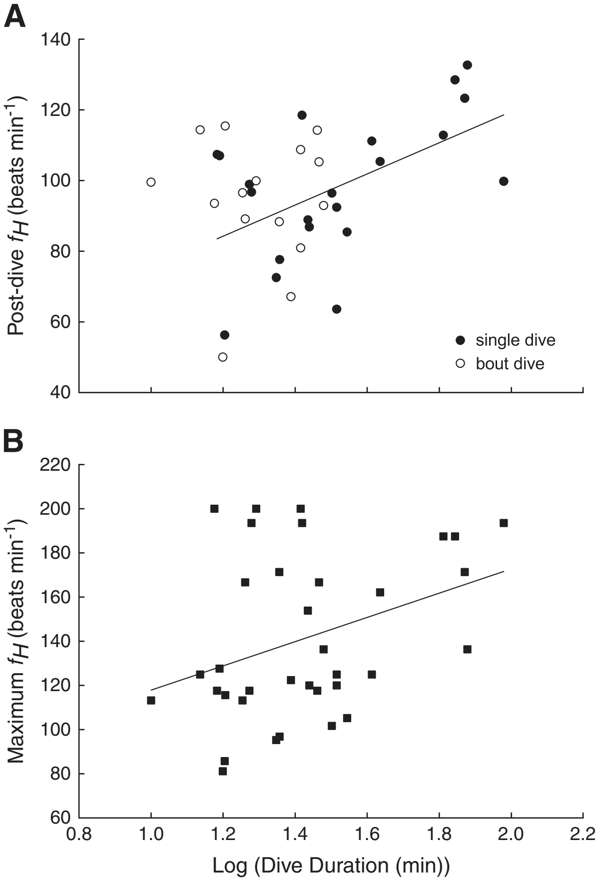


Fig. 2. Measures of post-dive tachycardia correlated with dive duration in three female Steller sea lions. The mean of post-dive heart rate (f_H) taken over the first 30 s after surfacing (panel A) was significantly related to dive duration (log-transformed) in bout dives (open circles, $r^2 = 0.37$, $p = 0.02$) but not for single dives (closed circles). Maximum instantaneous f_H (closed squares, panel B) also demonstrated a weakly positive linear relationship versus dive duration (log-transformed), which described the complete data set ($r^2 = 0.12$, $p = 0.04$).

for the representative dives presented at both depths (Fig. 3). This decline was prolonged in the 40 m dive, and was followed by a considerable rise ~20 s later corresponding to the dive's bottom-phase (Fig. 3B). Activity then fluctuated throughout the remaining underwater time, a pattern which was observed over the majority of the time course of 10 m dives. Contrary to the temporal pattern of f_H, and aside from a minor elevation in the final 3 s before surfacing, there was no clear elevation in ODBA during the ascent (Fig. 3).

3.3. Relationship between diving activity and heart rate

Mean ODBA was elevated by 18% in 10 m compared to 40 m dives ($F_{1,39} = 13.65$, $p = 0.001$); a difference which was not reflected in flipper stroke frequencies ($p = 0.10$). Neither variable was distributed by dive type. For the complete data set we noted a significant relationship between activity and f_H underwater for mean ODBA ($F_{1,36} = 4.46$, $p = 0.04$), but not for stroke frequency ($p = 0.91$; Fig. 4). This was driven by a significant positive linear relationship between ODBA and mean diving f_H ($r^2 = 0.45$, $p = 0.003$) at 10 m only (Fig. 4A). Mean ODBA during the dive period was also weakly correlated with minimum instantaneous f_H ($r^2 = 0.11$, $p = 0.04$; Fig. 4B) when all data were considered.

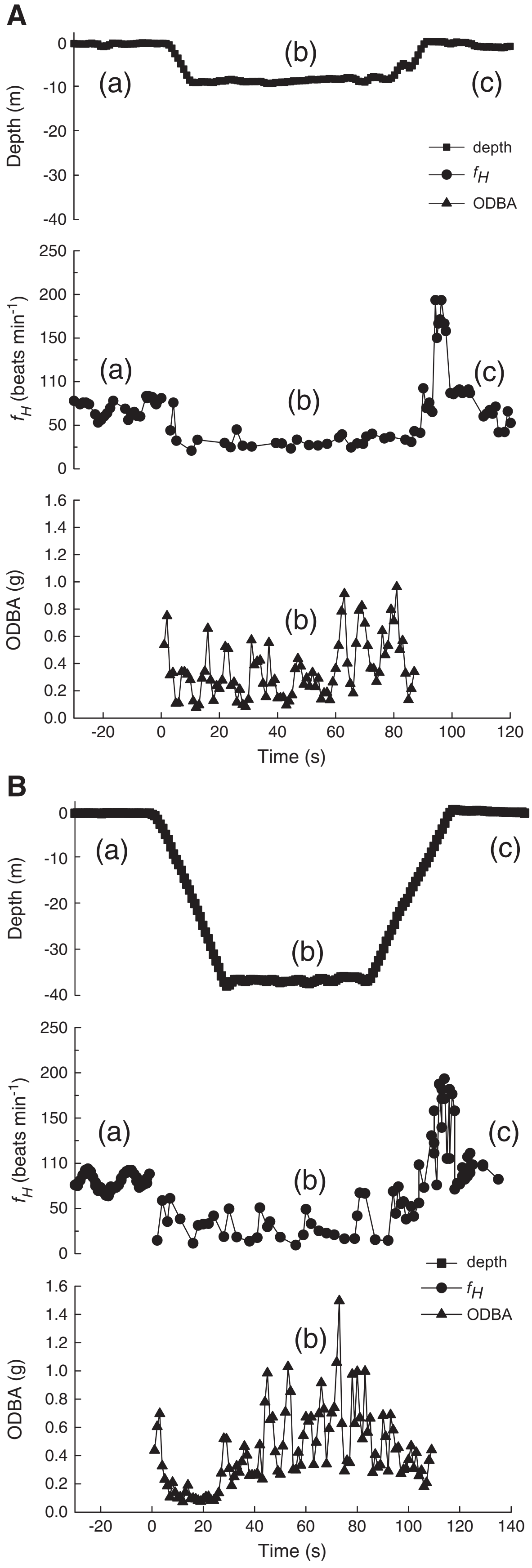
Underwater activity was correlated with f_H measures during the post-dive period. Mean f_H during the initial 30-s post-dive interval was significantly related to mean ODBA ($F_{1,27} = 4.58$, $p = 0.04$), resulting in a weak positive linear relationship ($r^2 = 0.19$, $p = 0.01$; Fig. 5A). Likewise, maximum instantaneous f_H recorded during this surface period was related to flipper stroke frequency ($F_{1,28} = 5.20$, $p = 0.03$), described by a similar positive linear relationship ($r^2 = 0.15$, $p = 0.02$; Fig. 5B).

4. Discussion

4.1. Diving bradycardia in Steller sea lions

Steller sea lions displayed bradycardia during all dive trials (overall 40% f_H reduction compared to resting levels). This degree of bradycardia over a complete, unrestrained dive was less than the 64% reduction recorded for a free-diving phocid, the northern elephant seal (*Mirounga angustirostris*; Andrews et al., 1997), but was greater than the 20% average dive bradycardia recorded for California sea lions (*Zalophus californianus;* Ponganis et al., 1991). Consistent with other diving animals (e.g., Thompson and Fedak, 1993; Andrews et al., 1997; Meir et al., 2008), the Steller sea lions also routinely displayed ascent tachycardia prior to surfacing.

Extended dive durations correlate with a greater bradycardia in other diving mammals and birds (Hill et al., 1987; Thompson and Fedak, 1993; Hindle et al., 2006; Meir et al., 2008). Steller sea lion dive durations correlated negatively with minimum instantaneous f_H, particularly in dive bouts (Fig. 1B), indicating that the dive response was strengthened over dive time. While mean diving f_H correlated negatively with dive duration, this was valid only for shallow (10 m) dives (Fig. 1A). Furthermore, we documented a 9% greater bradycardia



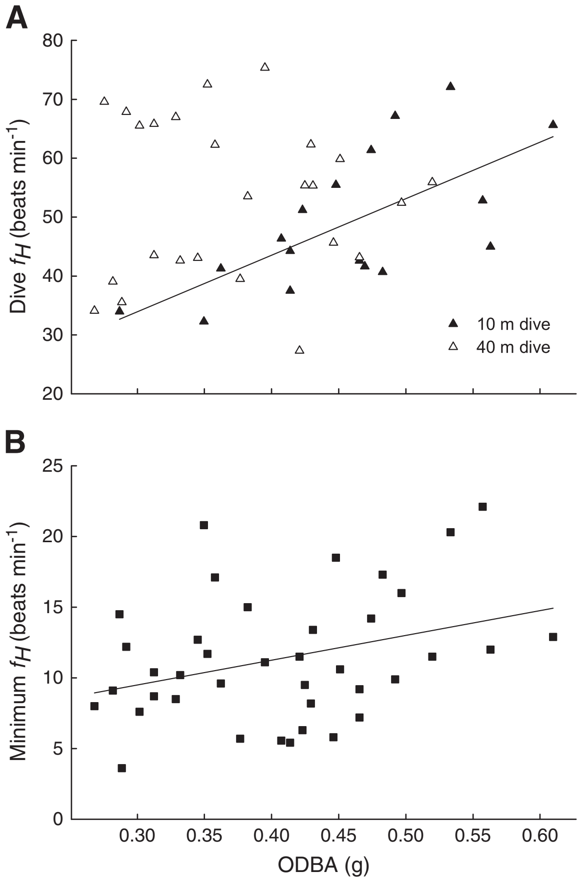


Fig. 4. Measures of dive bradycardia correlate with locomotory activity in three female Steller sea lions, under certain conditions. Mean diving heart rate (f_H, panel A) had a positive linear relationship with mean ODBA in 10 m dives (closed triangles, $r^2 = 0.45$, $p = 0.003$) but not 40 m dives (open triangles). Minimum instantaneous f_H (closed squares, panel B) also demonstrated a weakly positive linear relationship with mean ODBA which described the complete data set ($r^2 = 0.11$, $p = 0.04$).

during shallow (10 m) compared to deep (40 m) dives (Table 1), with no effect of dive depth on minimum recorded instantaneous f_H. This depth effect for mean diving f_H was counter to our expectations, since 40 m dives were generally longer.

Bradycardia was initiated upon submergence in all recorded dives regardless of depth. The absence of a dive duration effect in deep (40 m) dives may indicate a lack of bradycardia intensification via arterial chemoreceptor feedback as the dives progressed (Drummond and Jones, 1972; Elsner et al., 1977). This result is similar to that documented for double-crested cormorants (*Phalacrocorax auritus*) diving to 12 m (Enstipp et al., 2001), and could be mediated by the pressure effects of descent to depth. As explained for cormorants (Enstipp et al., 2001), the difference we noted in mean diving f_H could result from compression hyperoxia (Lanphier and Rahn, 1963; Qvist et al., 1986, 1993), in which elevated arterial O_2 pressure (PaO_2) impedes chemoreceptor influence on bradycardia. The predominantly gliding descents observed for Steller sea lions (Fig. 3B) will also reduce locomotor costs during this period, acting to keep PaO_2 high. Significantly lower minimum instantaneous f_H in the longer 40 m dives suggest that as dive length progresses and PaO_2 presumably falls, bradycardia does intensify (Drummond and Jones, 1972; Elsner

Fig. 3. The time courses of instantaneous heart rate (f_H) and instantaneous ODBA (calculated to the nearest 1 s) are presented for a representative 10 m (panel A, dive duration = 71 s) and 40 m (panel B, dive duration = 105 s) single dive. For comparative purposes, instantaneous f_H is also presented for the 30 s periods immediately prior to (labeled 'a') and following ('c') the dive period ('b'). Corresponding dive depth is presented in the top portion of each panel.

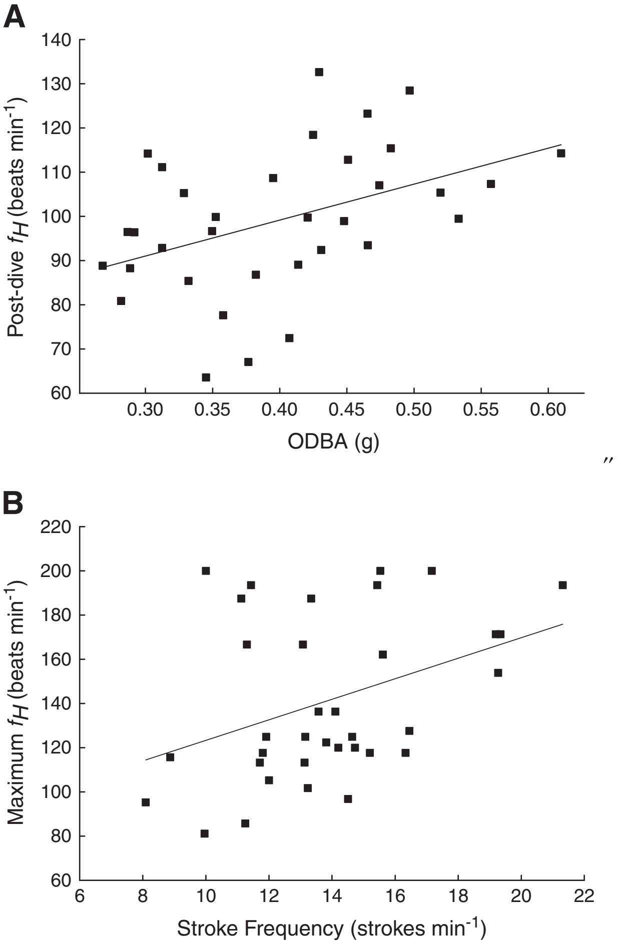


Fig. 5. Measures of post-dive tachycardia correlate weakly with locomotory activity in three female Steller sea lions. The mean of post-dive heart rate (f_H) taken over the first 30 s after surfacing (panel A) was related to mean ODBA ($r^2 = 0.19$, $p = 0.01$). Maximum instantaneous f_H (panel B) demonstrated a weakly positive linear relationship versus mean stroke frequency ($r^2 = 0.15$, $p = 0.02$).

et al., 1977). When averaged over the complete dive, however, early effects of compression could mask the response. Moreover, an extended period of ascent tachycardia in deep relative to shallow dives (Fig. 3) would also raise an average of f_H taken over the complete underwater period. It is possible that the 40 m dives recorded here were simply of insufficient length (i.e., lacked sufficient bottom time relative to the ascent and descent) to observe an effect of dive duration on bradycardia averaged over the entire dive.

No differences in mean diving f_H were observed for single dives compared to bout dives (Table 1). Further, the relationship between bradycardia and dive duration noted at 10 m (Fig. 1A) was similar for both single and bout dives. However, when single dives and bout dives within individual trials were considered as pairs, it was apparent that bout dives contained lower minimum instantaneous f_H (Table 1). As discussed above, this could reflect declining PaO_2 and the contribution of arterial chemoreceptor feedback to a strengthened dive response over time (Drummond and Jones, 1972; Elsner et al., 1977), which is detectable in a minimum parameter such as instantaneous f_H, but not when f_H was averaged across an entire dive. This line of reasoning is also supported by the fact the Steller sea lions develop a measurable O_2 debt over the course of dive bouts (Fahlman et al., 2008), making it likely that blood O_2 levels are generally lower in dive bouts compared to single dives.

Cardiac parameters were unaffected by dive characteristics (dive type, depth or duration) when the entire dive plus surface recovery interval was considered. This suggests any physiological impact incurred underwater related to these dive characteristics is accounted for during the immediate post-dive recovery period. Although Steller sea lion f_H returns quickly to baseline after diving is complete (~30 s), there is likely insufficient inter-dive surface time to permit much recovery within a dive bout (considered inter-dive O_2 consumption recovery; Fahlman et al., 2008). Positive correlations between measures of surface tachycardia (mean and maximum post-dive f_H; Fig. 2) and dive duration imply that an increasing physiological cost, such as an O_2 debt, is accrued as dive duration is extended.

4.2. Temporal development of diving bradycardia

In theory, the effects of a classic dive response and of eupneic exercise on the cardiovascular system are diametrically opposed. The result of diving is bradycardia coupled with peripheral vasoconstriction, which defends blood pressure as f_H falls (Scholander, 1940; Irving et al., 1941; Scholander et al., 1942). Conversely, eupneic exercise promotes elevated f_H and peripheral vasodilation to maximize perfusion of active locomotory tissues, even in divers (Castellini et al., 1985; Butler, 1988; Davis et al., 1991). If apneic exercise creates a compromised (yet present) peripheral perfusion in Steller sea lions driven by locomotory effort, we expect f_H and activity to track over short time scales.

Instantaneous ODBA and f_H in single dives (not part of dive bouts) appeared largely uncoupled when compared on a per-second time scale over the course of underwater time (Fig. 3). Both f_H and ODBA declined during the descent portion of dives; f_H as a result of dive bradycardia and ODBA due to a predominantly gliding descent (particularly in deep dives). The bottom (foraging) phase of the dives began when sea lions reached the feeding tubes at depth. This occurred almost immediately in 10 m dives and approximately 30 s after submergence in 40 m dives, and was associated with an elevation of ODBA due to an increased exercise output. By contrast, only a slight release of bradycardia was observed during this phase, suggesting that Steller sea lion dive f_H does not respond to underwater exercise output in a near-instantaneous manner. It has been suggested that limiting blood flow to muscle, as implied by the uncoupling of locomotory activity and f_H, allows sea lions to fuel this additional locomotory output by drawing down muscle O_2 stores (Davis et al., 2004). In other words, limited or absent muscle blood flow during apneic exercise could impose the hypoxic conditions necessary in muscle to extract the greatest durations of aerobic energy production via myoglobin (Davis et al., 2004). If this is indeed the case, it suggests that sea lions are either marshalling underwater time so as to surface without exhausting local aerobic reserves, or are shifting towards anaerobic energy production even in these short (<5 min) dives.

Heart rate and ODBA were clearly uncoupled during the dives' ascent phase, where bradycardia is released in anticipation of surfacing (Thompson and Fedak, 1993), and ODBA is relatively constant. This again suggests that the dive response of Steller sea lions (at least in dives of these durations and depths) is not affected by changes in activity level. The characteristic tachycardia in anticipation of surfacing (given that our animals had free access to the water surface, rather than the constraint of locating a breathing hole in fast ice, for example) would be especially beneficial if Steller sea lions do indeed develop a lactate load and an O_2 debt in swimming muscle. It would promote increased circulation of metabolic end-products and facilitate turnover during the surface interval, as well as deliver remaining blood O_2 to depleted tissues, thus maximizing the gradient for surface O_2 uptake (Thompson and Fedak, 1993).

There were several key differences noted between the f_H/ODBA time courses of 10 m compared to 40 m dives. Shallow (10 m) dives were dominated by the elevated (and variable) instantaneous ODBA indicative of feeding from the two stations at depth. This foraging activity was also predominant in deeper (40 m) dives, although it was delayed ~30 s, reflecting the time required for the sea lion to descend to the feeding stations. Initial bradycardia upon submergence was greater in 10 m (~64%) relative to 40 m dives (~14%), although a similar degree of bradycardia ultimately developed at both depths. This finding further supports an elevated PaO_2 during the initial phase of the deeper dives, resulting from the pressure effects of a 40 m descent (Lanphier and Rahn, 1963; Qvist et al., 1986, 1993) along with limited locomotory output (Fig. 3B). Ascent tachycardia was prolonged in 40 m dives, a time course which indicates that a release of bradycardia begins at the start of ascent, regardless of dive depth.

4.3. Diving activity and its relationship to mean heart rate over longer time scales

Mean ODBA was elevated by 18% in shallow compared to deep dives. This was partly due to the reduced ODBA noted early in deep dives (Fig. 3B), as negative buoyancy permits a predominantly gliding descent for Steller sea lions, and this descent interval comprised a greater proportion of deeper dives. In addition, 73% of an average shallow dive's duration was bottom time, compared to only 64% of deep dives. These differences could account for a reduced mean activity value over the entire trial.

On the other hand, stroke frequencies were not distributed by dive depth. Flipper stroking provides a good measure of activity and energetic output in some species (Williams et al., 2004), and is presumed to be a major component of ODBA. Our observation of relationships for ODBA but not for stroke frequencies is likely explained by the degree of stroke amplitude modulation and the translation of flipper strokes into surge possible for Steller sea lions (Hindle et al., 2010). Our center-mounted accelerometer had little ability to detect amplitude changes in flipper stroking, and we suspect such changes would be better measured by a device placed directly on the flipper. Neither ODBA nor stroke frequency were distributed by dive type, suggesting that any physiological differences noted between single or bout dives cannot be explained by changes in activity. However, it is noteworthy that a difference in minimum instantaneous f_H was the limit of the physiological differences we detected.

We noted a significant positive linear relationship between activity (mean ODBA) and mean dive f_H for 10 m dives only (Fig. 4A). Clearly, control of the dive response in Steller sea lions differs in shallow versus deep dives. The linear dive f_H–activity relationship documented for shallow dives implies that in this scenario, some vascular compromise between diving and exercise occurs in shallow dives (i.e., some muscle perfusion is maintained). A similar relationship was not observed between diving f_H and stroke frequency, presumably due to variability of stroke amplitude and the underlying muscle work (and muscle perfusion) not detected by our accelerometer. For deep dives, the lack of a linear f_H–activity relationship, considered alongside the disparate time course of f_H and activity suggests an uncoupling of these parameters in Steller sea lions. This is likely mediated by an absence of blood flow to active muscle, suggesting that perhaps more O_2 conservation is necessary in this scenario.

We also documented correlations between underwater activity and f_H measures during the post-dive period (Fig. 5), which indicates that at least a portion of underwater locomotory activity costs were deferred to the post-dive surface interval. Even for shallow dives in which blood flow to active muscle may occur as a compromise between dive-induced vasoconstriction, and exercise-induced vasodilation, any reduction in perfusion below that demanded for eupneic exercise would result in tissue hypoxia which must be recovered at the surface. This further supports the speculation that locomotory muscles of Steller sea lions become hypoxic during diving, regardless of dive depth. The likely mechanism for this occurrence is a limitation of perfusion to muscle vascular beds, presumably occurring to a lesser degree in shallow dives.

Acknowledgements

Funding was provided to the North Pacific Universities Marine Mammal Research Consortium by the North Pacific Marine Science Foundation and the National Oceanographic and Atmospheric Administration. We sincerely appreciate the assistance of the trainers, and technical and administrative staff at the Open Water Research Station and the Vancouver Aquarium. We are grateful to M. Horning for the loan of accelerometers. We also thank J. Meir for helpful comments and discussion on an earlier version of this manuscript. **[SS]**

References

Andrews, R.D., Jones, D.R., Williams, J.D., Thorson, P.H., Oliver, G.W., Costa, D.P., Le Beouf, B.J., 1997. Heart rates of northern elephant seals diving at sea and resting on the beach. J. Exp. Biol. 200, 2083–2095.

Armstrong, R.B., Delp, M.D., Goljan, E.J., Laughlin, M.H., 1987. Distribution of blood flow in muscles of miniature swine during exercise. J. Appl. Physiol. 62, 1285–1298.

Baudinette, R.V., 1978. Scaling of heart rate during locomotion in mammals. J. Comp. Physiol. 127B, 337–342.

Bevan, R.M., Butler, P.J., 1992. Cardiac output and blood flow distribution during swimming and voluntary diving of the tufted duck (*Aythya fuligula*). J. Exp. Biol. 168, 199–217.

Boyd, I.L., Bevan, R.M., Woakes, A.J., Butler, P.J., 1999. Heart rate and behavior of fur seals: implications for measurement of field energetics. Am. J. Physiol. 276, H844–H857.

Brooks, G.A., White, T.P., 1978. Determination of metabolic and heart rate responses of rats to treadmill exercise. J. Appl. Physiol. 45, 1009–1015.

Butler, P.J., 1988. The exercise response and the classical diving response during natural submersion in birds and mammals. Can. J. Zool. 66, 29–39.

Butler, P.J., 1993. To what extent can heart rate be used as an indicator of metabolic rate in free-living marine mammals? Symp. Zool. Soc. Lond. 66, 317–332.

Butler, P.J., Jones, D.R., 1997. Physiology of diving birds and mammals. Physiol. Rev. 77, 837–899.

Butler, P.J., Woakes, A.J., 1982. Telemetry of physiological variables from diving and flying birds. Symp. Zool. Soc. Lond. 49, 107–128.

Castellini, M.A., Murphy, B.J., Fedak, M., Ronald, K., Gofton, N., Hochachka, P.W., 1985. Potentially conflicting metabolic demands of diving and exercise in seals. J. Appl. Physiol. 58, 392–399.

Davis, R.W., Kanatous, S.B., 1999. Convective oxygen transport and tissue oxygen consumption in Weddell seals during aerobic dives. J. Exp. Biol. 202, 1091–1113.

Davis, R.W., Castellini, M.A., Kooyman, G.L., Maue, R., 1983. Renal glomerular filtration rate and hepatic blood flow during voluntary diving in Weddell seals. Am. J. Physiol. 245, R743–R748.

Davis, R.W., Castellini, M.A., Williams, T.M., Kooyman, G.L., 1991. Fuel homeostasis in the harbor seal during submerged swimming. J. Comp. Physiol. 160B, 627–635.

Davis, R.W., Polasek, L., Watson, R., Fuson, A., Williams, T.M., Kanatous, S.B., 2004. The diving paradox: new insights into the role of the dive response in air-breathing vertebrates. Comp. Biochem. Physiol. 138A, 263–268.

Delp, M.D., Laughlin, M.H., 1998. Regulation of skeletal muscle perfusion during exercise. Acta Physiol. Scand. 162, 411–419.

Drummond, P.C., Jones, D.R., 1972. Initiation of diving bradycardia in muskrats. J. Physiol. 222, 165–166.

Elsner, R., Angell-James, J.E., de Burgh Daly, M., 1977. Carotid body chemoreceptor reflexes and their interactions in the seal. Am. J. Physiol. 232, H517–H525.

Enstipp, M.R., Andrews, R.D., Jones, D.R., 2001. The effects of depth on the cardiac and behavioural responses of double-crested cormorants (*Phalacrocorax auritus*) during voluntary diving. J. Exp. Biol. 204, 4081–4092.

Fahlman, A., Svärd, C., Rosen, D.A.S., Jones, D.R., Trites, A.W., 2008. Metabolic costs of foraging and the management of O_2 and CO_2 stores in Steller sea lions. J. Exp. Biol. 211, 3573–3580.

Feldkamp, S.D., 1987. Swimming in the California sea lion: morphometrics, drag and energetics. J. Exp. Biol. 131, 117–135.

Fish, F.E., 1982. Aerobic energetics of surface swimming in the muskrat, *Ondatra zibethicus*. Physiol. Zool. 55, 180–189.

Green, J.A., Butler, P.J., Woakes, A.J., Boyd, I.L., Holder, R.L., 2001. Heart rate and oxygen consumption of exercising macaroni penguins. J. Exp. Biol. 204, 673–684.

Hastie, G.D., Rosen, D.A.S., Trites, A.W., 2006a. Studying diving energetic of trained sea lions in the open ocean. In: Trites, A.W., Atkinson, S., DeMaster, D.P., Fritz, L.W., Gelatt, T.S., Rea, L.D., Wynne, K. (Eds.), Sea lions of the world. Alaska Sea Grant College Program, University of Alaska, Fairbanks, pp. 193–204.

Hastie, G.D., Rosen, D.A.S., Trites, A.W., 2006b. The influence of depth on a breath-hold diver: predicting the diving metabolism of Steller sea lions (*Eumetopias jubatus*). J. Exp. Mar. Biol. Ecol. 336, 163–170.

Hill, R.D., Schneider, R.C., Liggins, G.C., Schuette, A.H., Elliott, R.L., Guppy, M., Hochachka, P.W., Qvist, J., Falke, K.J., Zapol, W.M., 1987. Heart rate and body temperature during free diving of Weddell seals. Am. J. Physiol. 253, R344–R351.

Hindle, A.G., Senkiw, R.W., MacArthur, R.A., 2006. Body cooling and the diving capabilities of muskrats (*Ondatra zibethicus*): a test of the adaptive hypothermia hypothesis. Comp. Biochem. Physiol. 144A, 232–241.

Hindle, A.G., Rosen, D.A.S., Trites, A.W., 2010. Swimming depth and ocean currents affect transit costs in Steller sea lions (*Eumetopias jubatus*). Aquat. Biol. 10, 139–148.

Hochachka, P.W., 1986. Balancing conflicting metabolic demands of exercise and diving. Fed. Proc. 45, 2948–2952.

Irving, L., Scholander, P.F., Grinnell, S.W., 1941. Significance of the heart rate to the diving ability of seals. J. Cell. Comp. Physiol. 18, 283–297.

Jones, J.H., Longworth, K.E., Lindholm, A., Conley, K.E., Karas, R.H., Kayar, S.R., Taylor, C.R., 1989. Oxygen transport during exercise in large mammals. I. Adaptive variation in oxygen demand. J. Appl. Physiol. 67, 862–870.

Lanphier, E.H., Rahn, H., 1963. Alveolar gas exchange during breathhold diving. J. Appl. Physiol. 18, 471–477.

McPhee, J.M., Rosen, D.A.S., Andrews, R.D., Trites, A.W., 2003. Predicting metabolic rate from heart rate in juvenile Steller sea lions *Eumetopias jubatus*. J. Exp. Biol. 206, 1941–1951.

Meir, J.U., Ponganis, P.J., 2010. Blood temperature profiles of diving elephant seals. Physiol. Biochem. Zool. 83, 531–540.

Meir, J.U., Stockard, T.K., Williams, C.L., Ponganis, K.V., Ponganis, P.J., 2008. Heart rate regulation and extreme bradycardia in diving emperor penguins. J. Exp. Biol. 211, 1169–1179.

Meir, J.U., Champagne, C.D., Costa, D.P., Williams, C.L., Ponganis, P.J., 2009. Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. Am. J. Physiol. 297, R927–R939.

Ponganis, P.J., 2007. Bio-logging of physiological parameters in higher marine vertebrates. Deep Sea Res. II 54, 183–192.

Ponganis, P.J., Kooyman, G.L., Zornow, M.H., 1991. Cardiac output in swimming California sea lions, *Zalophus californianus*. Physiol. Zool. 64, 1296–1306.

Ponganis, P.J., Van Dam, R.P., Levenson, D.H., Knower, T., Ponganis, K.V., Marshall, G., 2003. Regional heterothermy and conservation of core temperature in emperor penguins diving under sea ice. Comp. Biochem. Physiol. 135A, 477–487.

Qvist, J., Hill, R.D., Schneider, R.C., Falke, K.J., Liggins, G.C., Guppy, M., Elliott, R.L., Hochachka, P.W., Zapol, W.M., 1986. Hemoglobin concentrations and blood gas tensions of free-diving Weddell seals. J. Appl. Physiol. 61, 1560–1569.

Qvist, J., Hurford, W.E., Park, Y.S., Radermacher, P., Falke, K.J., Ahn, D.W., Guyton, G.P., Stanek, K.S., Hong, S.K., Weber, R.E., Zapol, W.M., 1993. Arterial blood gas tensions during breath-hold diving in the Korean ama. J. Appl. Physiol. 75, 285–293.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 45, 223–225.

Rosen, D.A.S., Trites, A.W., 2002. Cost of transport in Steller sea lions, *Eumetopias jubatus*. Mar. Mamm. Sci. 18, 513–524.

Scholander, P.F., 1940. Experimental investigations on the respiratory function in diving mammals and birds. Hvalrådets Skrifter 22, 1–131.

Scholander, P.F., Irving, L., Grinnell, S.W., 1942. Aerobic and anaerobic changes in seal muscle during diving. J. Biol. Chem. 142, 431–440.

Shepard, E.L.C., Wilson, R.P., Halsey, L.G., Quintana, F., Gómez Laich, A., Gleiss, A.C., Liebsch, N., Myers, A.E., Normans, B., 2008. Derivation of body motion via appropriate smoothing of acceleration data. Aquat. Biol. 4, 235–241.

Thompson, D., Fedak, M.A., 1993. Cardiac responses of grey seals during diving at sea. J. Exp. Biol. 174, 139–154.

Williams, T.M., Fuiman, L.A., Horning, M., Davis, R.W., 2004. The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. J. Exp. Biol. 207, 973–982.

Wilson, R., White, C., Quintana, F., Halsey, L., Liebsch, N., Martin, G., Butler, P.J., 2006. Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. J. Anim. Ecol. 75, 1081–1090.

Wright, T.J., Davis, R.W., 2006. The effect of myoglobin concentration on aerobic dive limit in a Weddell seal. J. Exp. Biol. 209, 2576–2585.

Young, B.L., Rosen, D.A.S., Haulena, M., Hindle, A.G., Trites, A.W., in press. Environment and digestion change the ability of heart rate to predict metabolism in resting Steller sea lions (*Eumetopias jubatus*). J. Comp. Physiol. B. doi:10.1007/s00360-010-0504-8. Published online 12-August-2010.

Zapol, W.M., Liggins, G.C., Schneider, R.C., Qvist, J., Snider, M.T., Creasy, R.K., Hochachka, P.W., 1979. Regional blood flow during simulated diving in the conscious Weddell seal. J. Appl. Physiol. 47, 968–973.

Glossary

f_H:	heart rate
ODBA:	overall dynamic body acceleration
PaO_2:	arterial oxygen partial pressure