EVALUATING CARDIAC FORM AND FUNCTION IN MARINE MAMMALS

by

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Abstract

The hearts of marine mammals frequently sustain marked changes in heart rate and vascular resistance when diving. However, it is not known how marine mammal hearts facilitate these changes. I examined cardiac function and electrical activity of marine mammal hearts to understand how they might differ from terrestrial mammals. I measured electrocardiographic parameters in 8 Steller sea lions, 5 northern fur seals, and 1 walrus—and echocardiographic function in all 8 Steller sea lions. I also compiled electrocardiographic parameters from 17 species of marine mammals (including my measurements) for comparison with 50 species of terrestrial mammals. I found that atrial and ventricular depolarization are slower in marine mammals after accounting for differences in body mass—and that the left ventricle of Steller sea lions contracts less than expected for a mammal of that size. These differences in cardiac timing and function may reflect specialized adaptations for diving. Electrocardiographic measurements of Steller sea lions, northern fur seals, and a walrus also varied between species and among the individuals of each species. For example, sinus arrhythmias occurred in 5 out of 8 individual Steller sea lions, but not in northern fur seals or the walrus. Mean electrical axes were also unique to each individual and varied greatly. Measurements ranged from -124° to 80°—with 3 of the Steller sea lions having extreme right axis deviation (-111° to -124°). Echocardiographic measurements showed that left ventricular form was similar between Steller sea lions and terrestrial mammals, except that Steller sea lions have larger aortic roots and larger left ventricular end-systolic dimensions than terrestrial mammals. Overall, my results show that marine mammals have functionally similar hearts to terrestrial mammals with a number of notable differences that likely support anatomical adaptations to diving.

Lay Summary

I compared the hearts of marine mammals with those of terrestrial mammals to identify the differences that allow marine mammals to dive for long durations. Overall, I found the functioning hearts of marine mammals were similar to those of terrestrial mammals, with two notable exceptions. One difference is the way in which their electrical conduction systems perform (which affects their heart rates); and the second is differences in the sizes of their left ventricles (which affects the extent of contractions and the volume of blood pumped out with each beat). These differences from terrestrial mammalian hearts are likely evolutionary modifications that enhance the diving abilities of marine mammals.

Preface

The research presented in this thesis is my original work. Under the guidance of Dr. Andrew Trites and Dr. David Rosen, I designed the experiments, organized procedures, assisted with data collection, compiled electrocardiographic data from the literature, performed the analyses, and summarized my findings in writing. Suggestions and feedback from my committee members, Dr. Trites, Dr. Rosen, Dr. Robert Shadwick, and Dr. Martin Haulena, were incorporated into the experimental design, data analysis, and final draft of my thesis. Before final submission, I also incorporated comments from my examiner, Dr. Bill Milsom. I formatted the data chapters (Ch. 2 and Ch. 3) as manuscripts for submission to peer-reviewed journals.

Conducting research with marine mammals was made possible with the help of the veterinary, training, and technical staff at the Vancouver Aquarium and Open Water Research Station. Dr. Marco Margiocco performed all of the echocardiograms and several of the electrocardiograms and made all of the cardiac measurements. I assisted with the echocardiograms and performed the remaining electrocardiograms. All research was conducted under the approval of the Animal Care Committees at the Vancouver Aquarium and University of British Columbia (Permit #A17-0092).

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List of Abbreviations

Ao	Aortic root or aortic root diameter
AV	Atrioventricular
AV Vmax	Peak systolic aortic blood velocity
BM	Body mass
CO	Cardiac output
ECG	Electrocardiogram
EG	Ecological group
FS	Fractional shortening
HR	Heart rate
IVS	Interventricular septum
IVSd	Interventricular septum thickness in diastole
IVSs	Interventricular septum thickness in systole
LA	Left atrium
LA:Ao	Left atrial to aortic root ratio
LAD	Left atrial diameter
LVIDd	Left-ventricular dimension in diastole
LVIDs	Left-ventricular dimension in systole
LVW	Left-ventricular free wall
LVWd	Left-ventricular free wall thickness in diastole
LVWs	Left-ventricular free wall thickness in systole
MAP	Mean arterial blood pressure
MEA	Mean electrical axis
M-mode	Motion-mode echocardiography
NFS	Northern fur seal
PM	Papillary muscle
PS	Physiological state
PV Vmax	Peak systolic pulmonary blood velocity
RV	Right ventricle
SA	Sinoatrial
SSL	Steller sea lion

SV	Stroke volume
TPR	Total peripheral resistance
2D	Two-dimensional echocardiography

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To my supervisor, Dr. Andrew Trites, thank you for allowing me to undertake a project adjacent to your area of expertise and trusting me to complete it. You taught me to be a better writer by teaching me to think of the whole story before beginning to write. To Dr. Dave Rosen, your mentorship through every stage of this thesis has helped me to develop into a confident researcher; thank you so much. To Dr. Martin Haulena, thank you for sharing your veterinary expertise on this project and allowing me to work with the animals under your care. To Dr. Robert Shadwick, thank you for inviting me into your lab, and thank you for your thoughtful questions and helpful suggestions on my thesis. To Dr. Bill Milsom, thank you for helping me to better understand my data and improve the final version of this thesis.

There is one person without whom my research would not have been possible. Dr. Marco Margiocco, thank you for the countless hours that you have volunteered to this project. You have taught me so much about echocardiography and electrocardiography and I am so grateful for your expertise. It was a pleasure looking at hearts with you.

Working with 200 kg Steller sea lions is not easy, but thanks to the trainers, vet staff, and technicians at the Vancouver Aquarium and Open Water Research Station, I never noticed. Although this list is not exhaustive, I would like to thank Billy Lasby, Nigel Waller, Malgosia Kaczmarska, Rebecca Barrick, Cesar Bertomeu, Troy Neale, Danielle Hyson, Sion Cahoon, Gabrielle Beer, Dr. Amy Gould, Dr. Megan Strobel, Rob Marshall and Brian Sheehan for their consistent involvement with this project. Your work has been critical to my success. I would also like to recognize all of the animals that we work with because without them this research would not be possible.

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Thank you to my friends outside of UBC for getting me away from the computer and out into nature. Having fun with you helped reenergize me to work on my thesis. I would especially like to thank Steffy Chwedoruk for her supportive ear throughout my degree, and Anderson Li for his daily encouragement and support during the final stages of this process, as well as his assistance with graphic design.

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Chapter 1: General introduction

Diving and the heart

Marine mammals are expert breath-holders that rely on their diving abilities for survival. Their underwater success is due in large part to significant anatomical and physiological adaptations that extend the duration of their dives. Their entire body is seemingly adapted to an aquatic life. However, the adaptations of one organ to an aquatic existence, the heart, remain unclear. Knowing how the hearts of marine mammals function can help to explain how they both facilitate and restrict the diving abilities of marine mammals.

The fact that marine mammal hearts are grossly anatomically similar to terrestrial mammal hearts suggests that they may function in similar ways. However, little research has been done on the functionality of freely diving and resting marine mammal hearts. At its most basic level, the heart is a pump that delivers blood to ensure adequate circulation of oxygen, carbon dioxide, nutrients, waste, heat, and hormones. Marine mammal hearts, like the hearts of all mammals, perform these same functions—but also must contend with circulatory changes that occur as part of adaptations for prolonged diving, namely the dive response.

The dive response is one of the most important contributions to a marine mammal’s ability to dive for long durations. It is found in all mammals, but is well-developed in marine mammals to support their impressive diving abilities. The dive response consists of breath-hold (apnea), constriction of blood vessels (vasoconstriction), and a decreased heart rate (bradycardia) (Kooyman and Ponganis, 1998; Scholander, 1940) that coupled with enhanced oxygen stores allows for the unrivalled diving abilities of marine mammals. During a dive, peripheral blood vessels constrict and heart rate decreases facilitating conservation of blood oxygen stores for the brain and heart, and use of muscle oxygen stores by the active muscles. Interestingly, the same response that is required for oxygen storage management while diving may challenge the normal functioning of the heart. It is unclear how marine mammal hearts accommodate such extensive circulatory changes that would likely have detrimental effects on the hearts of terrestrial mammals.

Mechanics of the dive response

Management of oxygen stores through changes to circulation are attained by peripheral vasoconstriction. The constriction of peripheral blood vessels conserves blood oxygen stores for organs that depend on aerobic respiration (i.e., the brain and heart) by restricting circulation to active muscles and inactive organs. A secondary consequence of peripheral vasoconstriction is that it creates a localized hypoxemic environment at the muscles that is conducive to offloading oxygen from myoglobin. This, in turn, makes the oxygen readily available for cellular respiration in active muscles (Davis et al., 2004). Increases in blood pressure that would normally occur while diving because of vasoconstriction must be prevented by adjustments to other aspects of the circulation.

The circulatory system maintains a relatively constant mean blood pressure via tight regulation of heart rate, stroke volume (the volume of blood ejected per heartbeat), blood vessel diameter (i.e., changing the resistance to blood flow via vasoconstriction or vasodilation), and peripheral vascular shunts (bypasses). This relationship is expressed by the mathematical equation:

$$MAP = HR \times SV \times TPR$$

where MAP is mean arterial blood pressure, HR is heart rate, SV is stroke volume, and TPR is the total resistance of the peripheral blood vessels (Boron and Boulpaep, 2017). In marine mammals, decreases in heart rate are expected to be matched by increases in total peripheral resistance of equal magnitude, while stroke volume does not change (e.g., Elsner et al., 1964; Ponganis et al., 2006, 1991). In this way, increased vasoconstriction and decreased heart rate appear to occur in tandem to prevent changes to blood pressure during a dive.

However, decreasing heart rate while maintaining stroke volume will result in decreased total blood flow through the circulatory system. Decreased blood flow due to bradycardia challenges the hearts of marine mammals because infrequent heartbeats can decrease oxygen delivery to the heart and brain. The flow of blood exiting the heart (cardiac output, CO) is a function of heart rate (HR) and stroke volume (SV), whereby:

$CO = HR \times SV$ (Boron and Boulpaep, 2017).

Cardiac output, that is blood flow, will decrease if heart rate decreases and stroke volume remains the same. This suggests that total circulation is reduced while marine mammals dive. However, selective vasoconstriction allows for the diversion of blood away from the peripheral muscles and inactive organs and helps maintain blood flow to the oxygen-sensitive heart and brain.

The aortic bulb is an adaptation in marine mammals that may also help maintain blood flow by what is known as the windkessel effect (Blix and Folkow, 1983). The aortic bulb is an elastic enlargement of the ascending aorta found in several species of marine mammals. It is thought to act like a blood-filled balloon that provides continuous blood flow by passively recoiling between heartbeats. It performs similarly to the human aorta, except that it is larger in size and more distensible which could potentially prolong blood flow for longer times in marine mammals.

Marine mammal hearts need to be able to rapidly change how fast they beat. During a dive, heart rate decreases, usually reaching its lowest rate as the animal reaches the maximum depth of its dive (e.g., Williams et al., 1991). When the animal approaches the surface, its heart rate increases to support replenishment of oxygen stores and removal of carbon dioxide and wastes from the blood. These changes in heart rate are mediated by input from the autonomic nervous system (Ponganis et al., 2017). Vagal stimulation to the heart's atrioventricular (AV) node signals a decrease in heart rate.

How marine mammal hearts can withstand the challenges imposed by the dive response is unclear. Understanding of the cardiovascular changes that occur in diving mammals would be best studied from direct, non-invasive measurements of blood pressure, blood flow, heart rate and stroke volume in freely diving marine mammals; but such measures are extremely logistically difficult to obtain. There is evidence suggesting that cardiac function in marine mammals is unique, but it is difficult to ascertain how cardiac function in marine mammals has been modified from terrestrial mammals. Determining how cardiac function in marine mammals differs from terrestrial mammals may reveal cardiac limitations to diving, and may help explain why marine mammals can dive for such long durations.

Current knowledge of cardiac function in diving marine mammals

Cardiovascular function in marine mammals was initially studied using invasive methods and forced dives which limit understanding of the natural cardiovascular responses of marine mammals. As more measurements from unrestrained animals were collected, it became apparent that the dive response was more variable and complex than what was initially reported. However, measurements from forced dives provided knowledge of the extremes of what marine mammals are capable of.

Fundamental studies of diving physiology have shaped how we think about cardiovascular responses to diving. Diving physiology was investigated by Scholander (1940) who described the bradycardia, vasoconstriction, and oxygen storage management now synonymous with the dive response. Using forced submersions of seals, Irving, Scholander, and Grinnel (1941) observed that blood pressure did not change during a forced dive, despite the decrease in heart rate, suggesting that heart rate decreases in synchrony with increasing vascular resistance. Using a similar protocol, Zapol (1979) showed the distribution of blood flow in Weddell seals when they are submerged. This study also showed that blood flow to the brain was maintained and blood flow to the heart decreased slightly, whereas blood flow to the muscles was greatly restricted. These results provided direct evidence of vasoconstriction and confirmed that oxygen-dependent tissues are supplied with blood during a dive. To this day, slowed heart rate, vasoconstriction, unchanged blood pressure, and redistribution of blood oxygen stores remain as the key cardiovascular components of the dive response.

The dive response elicited from forced dives produced an immediate marked bradycardia and vasoconstriction, which is not observed to the same extent in freely diving animals. Animals that perform short dives without exhausting their oxygen stores (within their aerobic dive limit) do not exhibit the classic dive response described by the early research (Butler, 1982). Their heart rates are also more variable and may correspond better to exercise than diving (Butler, 1982). However, the classic dive response is still observed during long-duration, deep dives.

More recently, variability in the dive response was further explored in freely diving animals. Minimally invasive biologging techniques have shown that cardiac arrhythmias occur at the bottom of Weddell seal and bottlenose dolphin dives, and that the dive response is not all or nothing, but instead a gradation that reflects cognitive control dive depth, dive duration, physical activity, and external stress (Elmegaard et al., 2016; Elsner et al., 1964; Hindle et al., 2010; Williams et al., 2017, 2015). There is debate over how the dive response and the exercise response interact in diving marine mammals as they have opposite effects on heart rate and vasoconstriction (Davis and Williams, 2012; Ponganis et al., 2017).

Throughout my thesis, I discuss the dive response in terms of all marine mammals. However, it is important to note that there is minimal data to show a similar response occurs in baleen whales. Understanding of the dive response mostly comes from studies on pinnipeds and dolphins because cardiovascular studies of large whales are extremely difficult to perform. The limited cardiac data from large whales comes from electrocardiographic recordings of a grey whale, two humpback whale and a fin whale (Meijler et al., 1992; Ponganis and Kooyman, 1999; Senft and Kanwisher, 1960). Due to this data deficit, large whales cannot be claimed to display the same dive response as observed in pinnipeds and dolphins. However, there is evidence from eupneic and apneic heart rates of a young grey whale to suggest that they similarly undergo diving bradycardia (Ponganis and Kooyman, 1999). In addition, the deep, long-duration dives performed by whales are suggestive of them having a pronounced dive response.

An additional complicating factor in documenting the dive response of large whales is that the majority of a large whale's daily activities take place underwater while they are breath hold diving and they only come to the surface to breathe. In studied marine mammals, the dive response appears to reflect the amount of time that a species spends diving compared to the time they spend hauled-out on land or at the surface. Generally speaking, empirical data has shown that species that spend more time diving than hauled out, such as phocids, have more pronounced dive responses than species that spend less time diving than time hauled out, such as otariids. It is unclear if this trend continues in large cetaceans which likely undergo bodily processes that require blood flow like digestion while holding their breath underwater. How these processes affect the dive response are unclear. It would seem that a pronounced dive response would be required for the deep, long-duration dives that whales make, but perhaps shorter surface dives with minimal vasoconstriction are also occurring.

Despite what has been learned about the ability of marine mammals to dive, several questions about the cardiovascular system of marine mammals remain. My research focuses on understanding how the hearts of marine mammals' function to cope with the challenges imposed by the dive response.

Examining marine mammal cardiac function

My thesis research seeks to determine whether adaptations to cardiac function exist in marine mammals. I addressed this research question in two ways. First, I evaluated cardiac function in 3 species of marine mammals. Second, I compared cardiac function in marine mammals to terrestrial mammals. Thus, the overarching goal of my thesis was to increase understanding of marine mammal heart function by identifying potential cardiac adaptations to diving. I thereby sought to explain how marine mammal hearts support extended diving, as well as how they also constrain this behaviour.

My first data chapter (Chapter 2) evaluates within- and between-species variability in cardiac function in Steller sea lions, northern fur seals, and walrus using electrocardiographic recordings of all three species, and echocardiographic examinations of Steller sea lions. These two techniques are non-invasive, have standardized protocols, and can be compared with other marine and terrestrial mammals.

Electrocardiography records cardiac rhythm by measuring the electrical activity of the heart (Bayés De Luna et al., 2012). When the sinoatrial node spontaneously depolarizes, adjacent myocardial cells also depolarize. This depolarization of cardiac muscle cells stimulates contraction. Consequently, the timing and coordination of the electrical signalling is imperative for cardiac filling and ejection. Comparative analyses of mammalian electrocardiograms (ECGs) demonstrate that there is a characteristic pattern to electrical depolarization, and that the timing is fairly consistent for all species when body mass is accounted for (Günther and Morgado, 1997). Thus, adjusting for body mass allows for ECG measurements to be compared between marine and terrestrial mammals.

Echocardiography provides real-time images of the heart throughout the cardiac cycle. It is largely used to assess left ventricular function and can evaluate blood flow when Doppler data are available. In contrast with electrocardiography, broad comparisons of mammalian echocardiographic parameters have not yet been described. However, within-species relationships from dogs and cats clearly show that echocardiographic parameters scale to body mass (Cornell et al., 2004; Scansen and Morgan, 2015). The beating heart of Steller sea lions can be observed as it contracts and relaxes using echocardiography—information that cannot be learned from dissections or ECGs.

My second data chapter (Chapter 3) compares the cardiac timing of different species of marine and terrestrial mammals to identify differences in the electrical activity of their hearts. I obtained these data for Steller sea lions, northern fur seals, and a walrus in the studies presented in Chapter 2, as well as from published articles. Using these data, I compared cardiac timing to determine how electrical activity in the hearts of marine mammals differs from that of terrestrial mammals. The mammalian ECG is ideal for comparative analysis because it is well understood and characterized, and scales with body mass. This chapter benefits from the wide range of published mammalian ECGs, including 14 accounts from marine mammals.

Both data chapters are prepared as stand-alone manuscripts that I intend to publish in peer-reviewed journals. As a result, there is some overlap of the material covered in each chapter.

Chapter 2: Cardiac examinations of pinnipeds show deviations in structure and function from typical mammalian hearts

Summary

Pinniped hearts are anatomically well described, but less is known about how they function. We evaluated cardiac function in three species of pinnipeds under anaesthesia by performing complete electrocardiographic examinations of Steller sea lions (n=8), northern fur seals (n=5) and a walrus (n=1). We also undertook echocardiographic examinations of all 8 Steller sea lions to evaluate cardiac form and function. Compared with terrestrial mammals, the hearts of Steller sea lions had larger aortic roots and larger left ventricular end-systolic diameters. Additionally, mean electrical axes of pinniped hearts were atypical of terrestrial mammals (ranging from -124° to 80°), with some individuals having right cranial orientations indicative of extreme right axis deviation (when compared to humans). The values we measured provide insights into cardiac function in marine mammals and are useful baseline reference points for assessing cardiac function in pinnipeds under human care. Our results suggest that while some functional differences between marine and terrestrial mammal hearts simply reflect anatomy, others are likely specialized adaptations to support diving and prolonged breath-holding.

Introduction

The anatomy and function of mammalian hearts have changed little over evolutionary time due to the essential role that the heart plays in transporting oxygen, nutrients, carbon dioxide, wastes and hormones throughout the body. Comparative mammalian studies have found that cardiac morphology, heart mass, electrical signal conduction, and left ventricular function are similar among many species of mammals (e.g., Günther and Morgado, 1997; Holt et al., 1968, 1962; Kusunose et al., 2012; Meijler, 1985; Noujaim et al., 2004; Prothero, 1979). However, marine mammals, which have long been suspected of having specialized cardiac adaptations to support extended breath holding, have been underrepresented in these analyses. Additional *in vivo* studies of marine mammal hearts are therefore required to determine whether functional cardiac differences do in fact exist between marine and terrestrial mammals to support diving and the accompanying cardiovascular changes experienced by marine mammals (e.g., bradycardia and vasoconstriction).

Despite the noted commonality among mammals, some structural differences have been observed between the hearts of marine and terrestrial mammals. For example, dissections of marine mammal hearts have revealed dorsoventral compression (Murie, 1874; Smodlaka et al., 2008) and a tendency towards a bifid apex (Drabek, 1975; Murie, 1874; Rowlatt, 1990; Stewardson et al., 1999; Tarpley et al., 1997). It has also been shown that marine mammals have enlarged ascending aortas (aortic bulbs) (Drabek, 1975; King, 1977; Murie, 1874; Smodlaka et al., 2009) and enlarged or specialized regions of conductive tissue (Purkinje and Todd fibres) (Nie, 1987; Pfeiffer, 1990; van Nie, 1986). While dissections effectively demonstrate anatomical differences with terrestrial mammal hearts, they cannot reveal how these structural differences affect cardiac function throughout the cardiac cycle.

Electrocardiography and echocardiography are two methods commonly used to assess cardiac function. Electrocardiography is a means to measure heat rate and electrical signal conduction through the myocardium—and has shown similar patterns between the timing of electrical signal conduction in terrestrial mammals and at least 14 species of cetaceans and pinnipeds (e.g., Dassis et al., 2016; Harms et al., 2013; Irving et al., 1941; King et al., 1953; Meijler et al., 1992; Meijler and van der Tweel, 1986; Murdaugh et al., 1966; Ponganis and Kooyman, 1999). Despite the seemingly large dataset of mammalian electrocardiograms, more ECGs from marine mammals are required to evaluate electrophysiological adaptations to diving, and allow for comparisons between cetaceans and pinnipeds. Currently, there are too few electrocardiographic recordings of marine mammals in the literature to confidently draw conclusions about diving specializations. Further, most data for marine species are collected from only one or a few individuals which may not accurately represent the entire species. Electrocardiography provides overall information about electrical cardiac function, but does not directly address left ventricular function and myocardial contraction. Other techniques are required for this, such as echocardiography.

Echocardiography has recently been used to study left ventricular structure and function in marine mammals (e.g., Castro et al., 2018; Chetboul et al., 2012; Dennison et al., 2011b, 2011a; Miedler et al., 2015). This non-invasive technique makes it possible to directly observe structural changes of the heart during cardiac cycles—thus providing information about function that cannot be seen through dissection. It is less invasive than previous techniques (although anaesthesia may be required) and echocardiographic reference values have only been determined for two species of marine mammals (both of which were trained and conscious during the procedure)—southern sea lions (*Otaria flavenscens*) (Castro et al., 2018) and bottlenose dolphins (*Tursiops truncatus*) (Chetboul et al., 2012; Miedler et al., 2015). Unfortunately, echocardiograms of marine mammals have not been performed underwater on diving or stationary animals, largely due to the difficulty of taking ultrasounds measurements underwater. Collecting additional echocardiograms from an array of marine mammals can yield data needed to better understand what, if any, specialized cardiac adaptations marine mammals possess to facilitate diving.

To increase understanding of cardiac function in pinnipeds, we used electrocardiography and echocardiography to assess cardiac structure and function in anaesthetized, female Steller sea lions (*Eumetopias jubatus*). We also collected additional ECGs from northern fur seals (*Callorhinus ursinus*) and a walrus (*Odobenus rosmarus*) to better understand electrophysiological function of pinniped hearts. This is the first complete cardiac study of Steller sea lions, and the second documented echocardiographic assessment of a pinniped to report reference values. The cardiac data we obtained are useful for interspecies comparisons, to understand adaptions that may be linked to diving capacity, and provide useful baselines for cardiac function in pinnipeds cared for in zoos, aquariums, and rehabilitation facilities.

Methods

Animals

We used Steller sea lions (n=8), northern fur seals (n=5), and one walrus maintained in human care either at the Vancouver Aquarium (Vancouver, BC) or the UBC Open Water Research Station (Port Moody, BC). All animals were clinically healthy at the time of the study. The sea lions and fur seals were all adult females, and the walrus was a juvenile male (23 months).

Anaesthetic protocols

All animals were anaesthetized according to previously established protocols under the supervision of a veterinarian. The sea lions and fur seals were anaesthetized using <5% inhalational isoflurane, while the walrus was anaesthetized using <8% inhalational sevoflurane. Two of the Steller sea lions, F03RO and F03IZ, were given diazepam (~0.14 mg/kg) about thirty minutes prior to anaesthesia.

General procedure

We performed electrocardiography and transthoracic echocardiography on the Steller sea lions, and performed only electrocardiography on the northern fur seals and walrus. All animals were out of water for the duration of the procedure. Measurements at the Vancouver Aquarium were taken indoors, whereas those at the Open Water Research Station were collected outdoors. No attempt was made to keep air temperature constant at either location.

Electrocardiography of pinnipeds

We used an IDEXX CardioPet ECG device and CardioPet software version 6.2 to record 6-lead ECGs. All ECG recordings were made in the frontal plane. Clip positioning was species-dependent, and subcutaneous needles were required to obtain a recording from the walrus. We collected two one-minute ECG recordings from each individual.

Electrocardiographic measurements were taken by a veterinary cardiologist following standard procedures using VetMedStat online telemedicine software. In all individuals, we measured the following electrocardiographic parameters: P-wave, PQ, QRS, and QT interval durations, P-, R-, and T-wave amplitudes, P- and T-wave polarities, and calculated the net direction of myocardial depolarization and repolarization (mean electrical axis).

Echocardiography of Steller sea lions

Echocardiography was also performed by a veterinary cardiologist. We used a GE LOGIQ e portable ultrasound with ECG module to obtain images and take measurements. Electrocardiograms (procedure above) were recorded prior to positioning the sea lions for echocardiography.

Echocardiographic recordings were first taken from F00BO and F97SI. The procedure was subsequently modified for the remaining sea lions. Initially, we viewed the heart with the sea lion in left lateral recumbency using a probe positioned on the right side of the sea lion’s chest near the flipper insertion. We then positioned the sea lion in dorsal recumbency with the probe on the right side of their chest to measure peak aortic and pulmonary velocities. The first two procedures were challenging because we imaged the heart from above—while gravity pulled the heart away from the chest (and probe). This positioning required placing greater pressure on the chest to obtain images, and meant we were initially unable to obtain useable images for one sea lion, F00YA.

We improved our procedure by setting up two large wooden platforms arranged with a V-shaped gap between them to act as an echocardiographer's table. With a sea lion in right lateral recumbency with its chest positioned over the gap, we obtained images from below. This setup resulted in clearer images that could be reliably obtained in less time, and required less pressure on the sea lions' chests. To record flow measurements, we repositioned the platforms so that the cut-out was on the opposite side—and positioned the sea lion in left lateral recumbency with its chest over the gap. Again, we obtained images from below.

In all of the Steller sea lions, we were able to view the parasternal long axis (Fig. 2.1) and parasternal short axes at the level of the aortic valve (Fig. 2.2), mitral valve, and papillary muscles (Fig. 2.3) of the heart. The measured echocardiographic parameters included: heart rate, left ventricular chamber diameter, interventricular septum thickness, and left ventricular posterior wall thickness at end-systole and end-diastole using M-mode (Fig. 2.4), left atrium and aortic root dimensions using 2D, and maximum aortic and pulmonary flow velocities using Doppler (Fig. 2.5 and 2.6).

Calculations

Mean ± standard deviation (SD) electrocardiographic parameters are reported for the Steller sea lions, northern fur seals, and walrus. The mean electrical axis (MEA) for each individual was calculated using:

$$MEA = \arctan\left(DI_{amp},\ aVF_{amp}\right) \times \frac{180}{\pi}$$

where DI_{amp} is the amplitude of the R-wave in lead I, and aVF_{amp} is the amplitude of the R-wave in lead aVF (Santilli and Perego, 2009).

Echocardiographic measurements for the Steller sea lions were not normally distributed, and are therefore reported as medians ± SD. Medians are not as sensitive as means to outliers when samples sizes are small, and therefore provided a more accurate representation of our sample population. The non-normal distribution also prevented us from calculating and reporting the 95% prediction intervals that would have been beneficial for clinical use. Fractional shortening, the percentage decrease of the left ventricular diameter from diastole to systole, was calculated using:

$$\frac{LVIDd - LVIDs}{LVIDd} \times 100\%$$

where LVIDd is left ventricular end-diastolic diameter, and LVIDs is left ventricular end-systolic diameter.

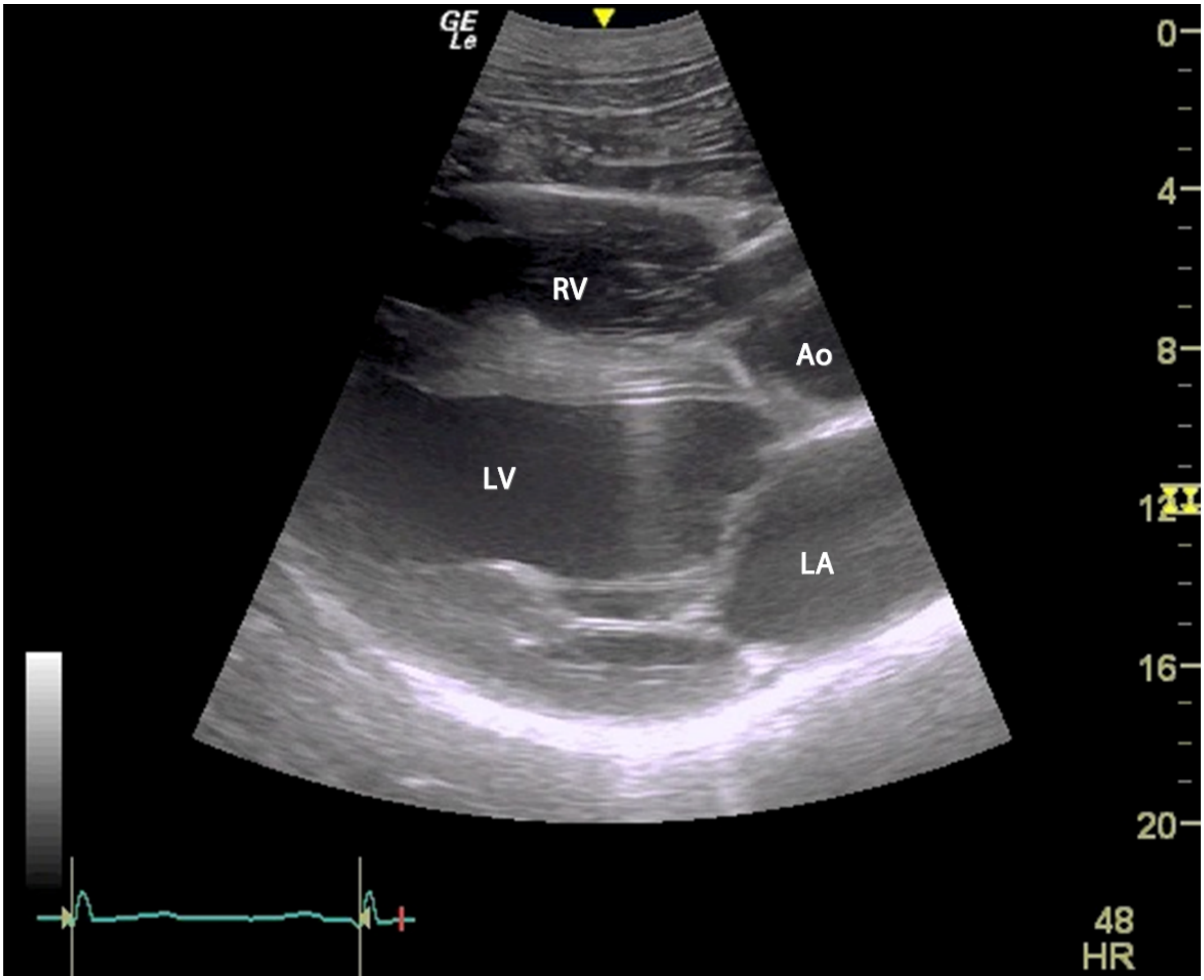

Figure 2.1 Parasternal long axis view of a Steller sea lion heart showing the left ventricle (LV), right ventricle (RV), left atrium (LA), and aortic root (Ao).

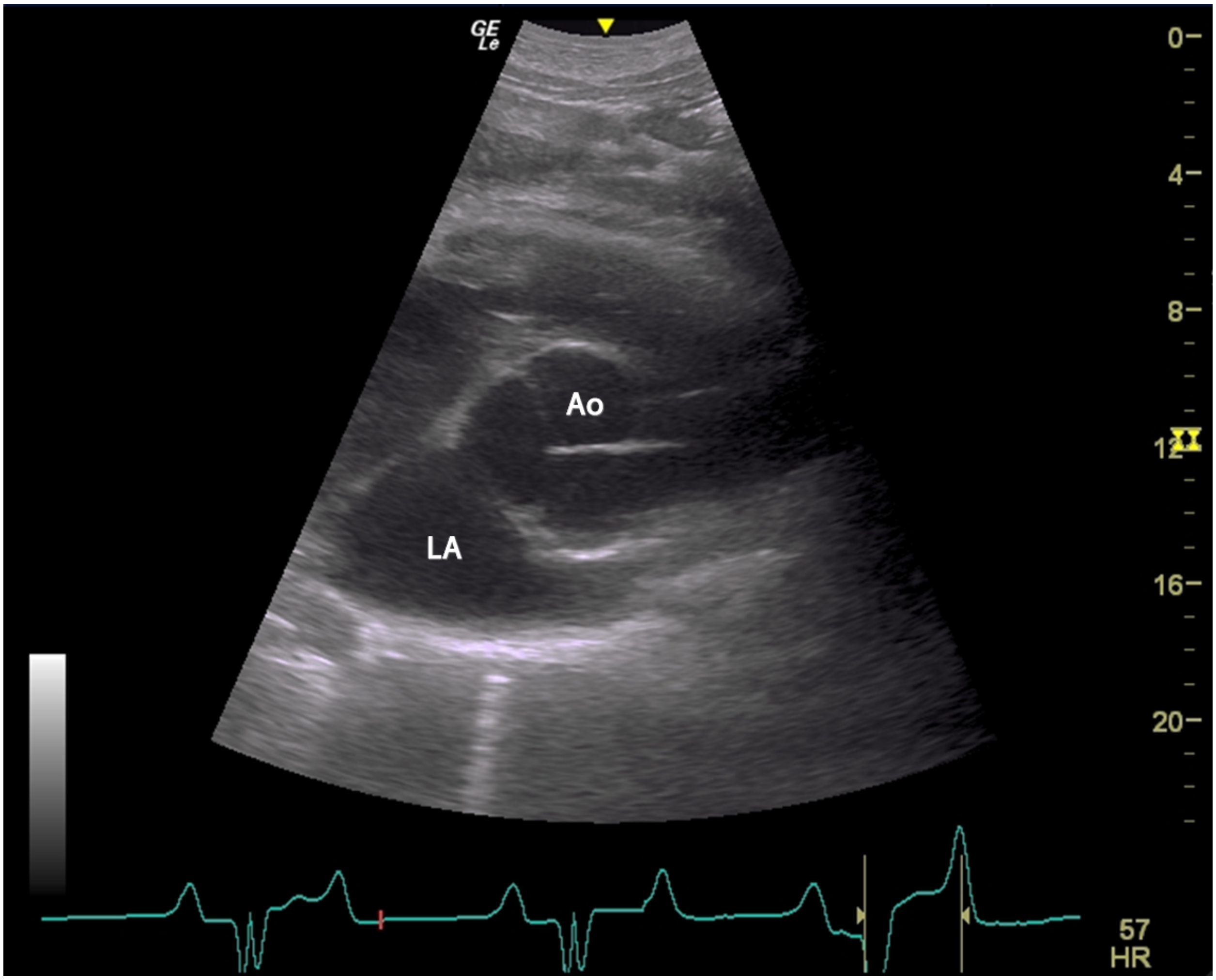

Figure 2.2 Parasternal short axis view of a Steller sea lion heart at the level of the aortic valve showing the left atrium (LA) and aortic root (Ao).

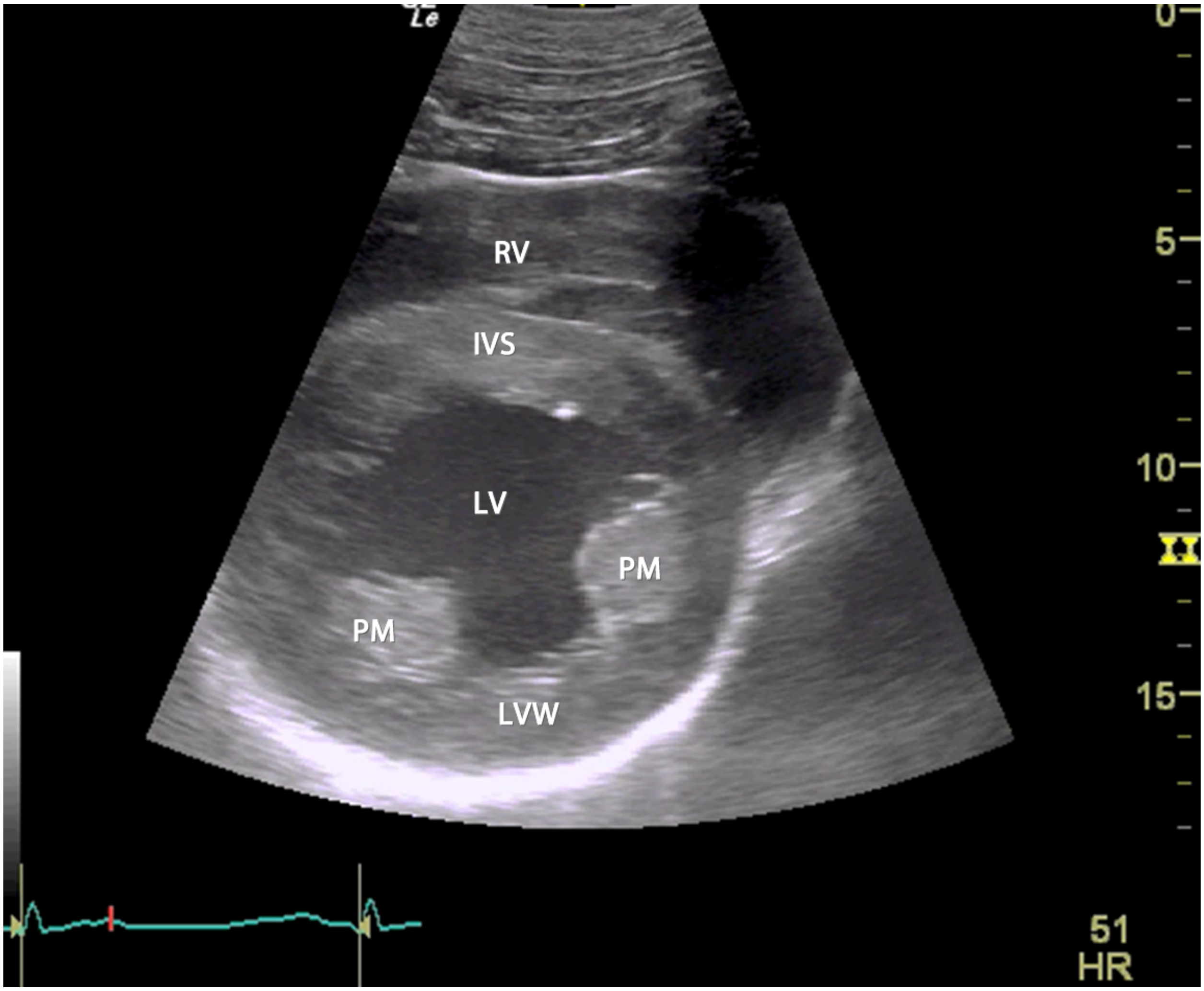

Figure 2.3 Parasternal short axis view of a Steller sea lion heart at the level of the papillary muscles showing the left ventricle (LV), right ventricle (RV), interventricular septum (IVS), left ventricular free wall (LVW) and the papillary muscles (PM).

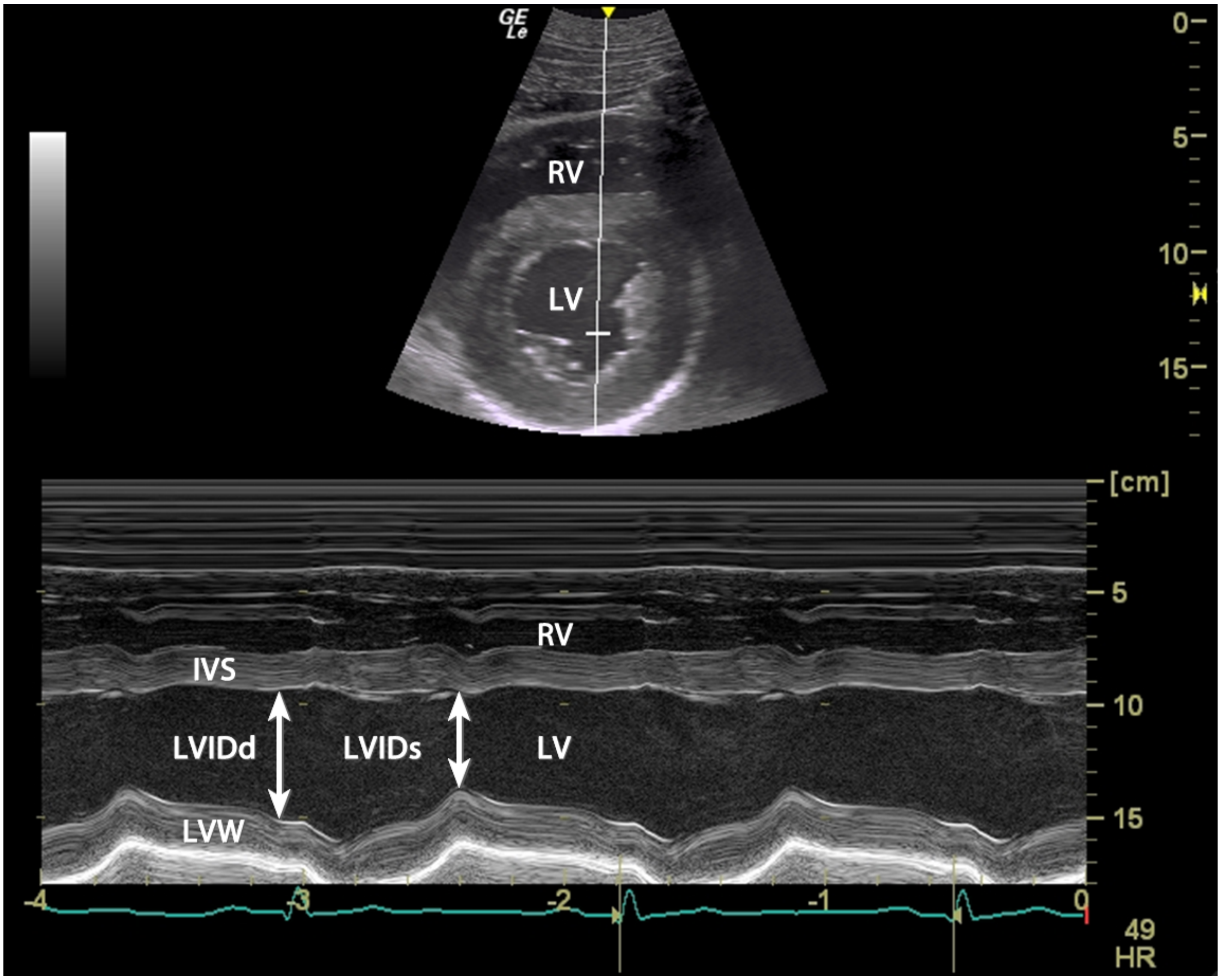

Figure 2.4 Parasternal short axis view of a Steller sea lion heart at the level of the papillary muscles (2D image, top), and motion of the cardiac chambers over time (M-mode image, bottom) showing the left ventricle (LV), right ventricle (RV), interventricular septum (IVS) and posterior wall of the left ventricle (LVW). Left ventricular end-diastolic diameter (LVIDd) and left ventricular end-systolic diameter (LVIDs) were measured as shown by the white arrows using the leading edge to leading edge technique.

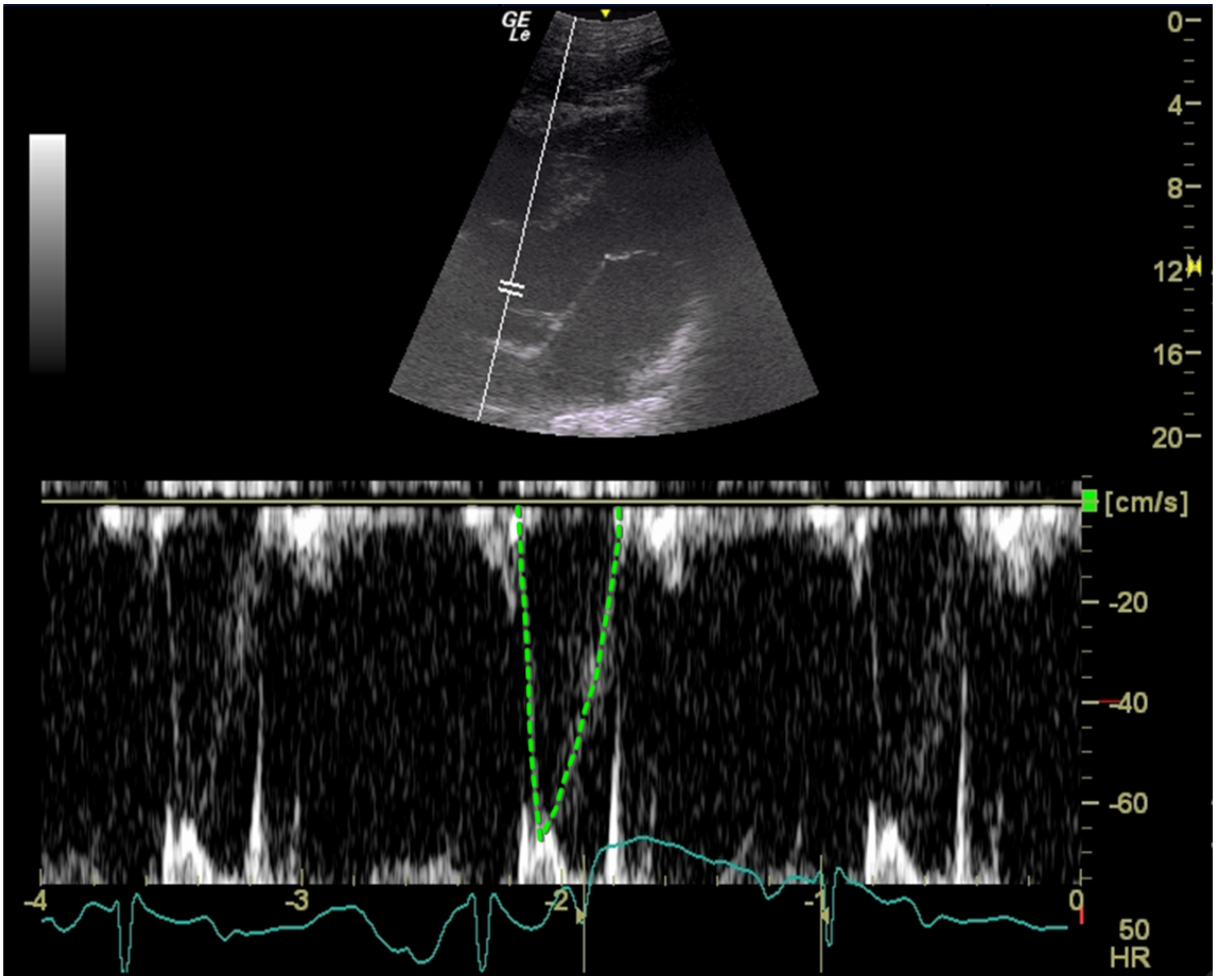

Figure 2.5 Doppler recording of aortic blood flow in a Steller sea lion. Flow profile is traced in green.

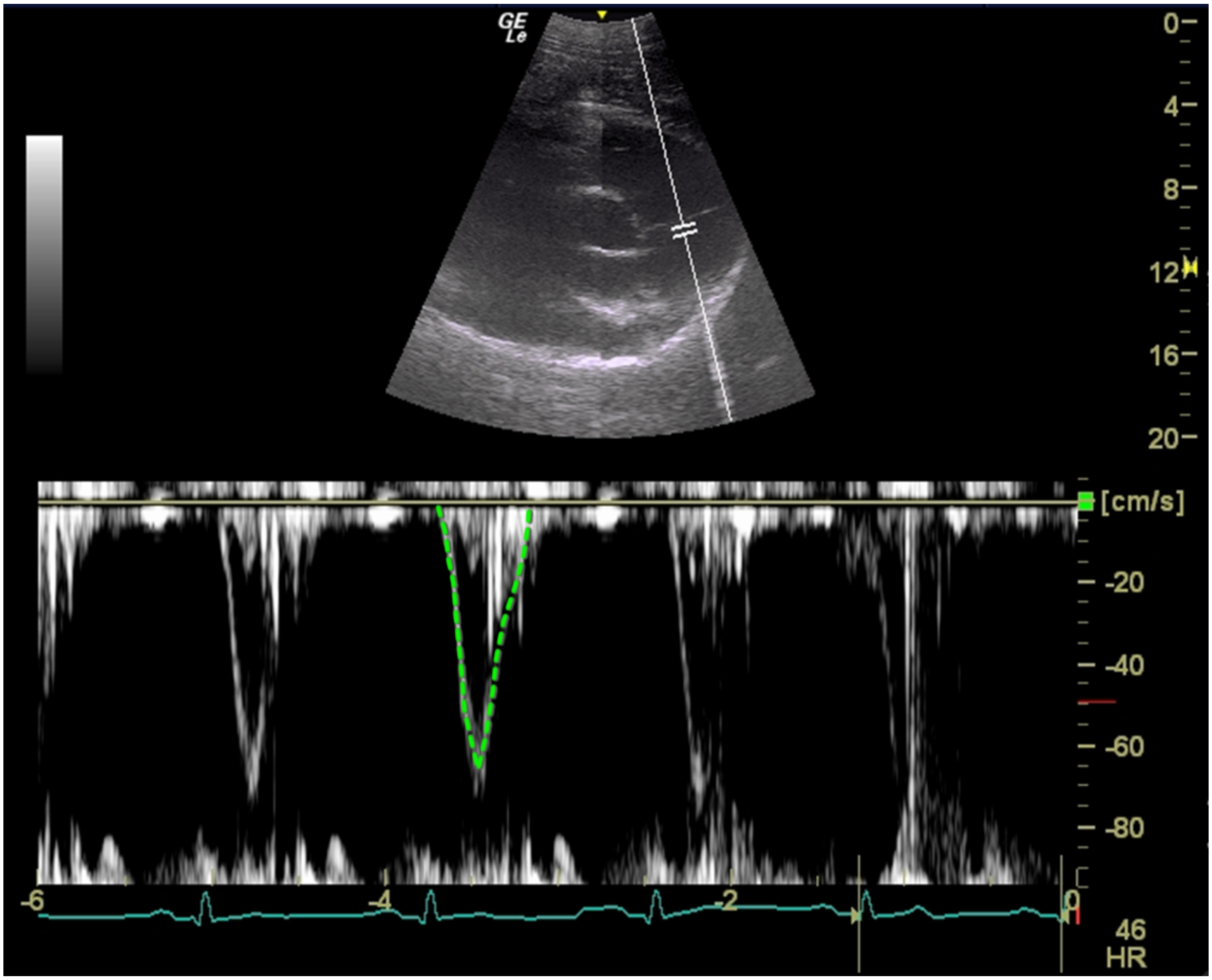

Figure 2.6 Doppler recording of pulmonary blood flow in a Steller sea lion. Flow profile is traced in green.

Results

Steller sea lion echocardiography results

All echocardiographic parameters were successfully measured for all study animals unless specifically noted. Echocardiographic parameters for Steller sea lions under anaesthesia (Table 2.1) were summarized as medians ± SD. Median heart rate determined in M-mode was 50.5 ± 7.1 bpm. Left atrial to aortic root ratio (LA:Ao) was 0.92 ± 0.17, with the ratio <1 in 5 of the 8 sea lions, meaning these individuals have a larger aortic root than left atrial diameter. Fractional shortening was 31.90 ± 7.75 %. Peak aortic velocity measured in 7 of the 8 sea lions had a median of 0.64 ± 0.21 m/s, while peak pulmonary velocity measured in 6 of 8 sea lions had a median of 0.83 ± 0.17 m/s.

Steller sea lion, northern fur seal, and walrus ECG results

Mean heart rate while anaesthetized was 113.0 ± 15.5 bpm in northern fur seals, 56.5 ± 3.7 bpm in Steller sea lions, and 76.0 bpm in a walrus (Table 2.2). Sinus arrhythmias were detected in 5 out of 8 Steller sea lions, but not in the walrus or any of the northern fur seals. Cardiac waveforms for all species matched the typical mammalian pattern.

Mean electrical axes for northern fur seals ranged from -79° to 80° (Fig. 2.7), while those of Steller sea lions ranged from -124° to 8° (Fig. 2.8), and the MEA of the walrus was -35°. There was no identifiable dominance in MEA for Steller sea lions and northern fur seals, perhaps due to small sample sizes.

Steller sea lion P- and T-wave polarities were fairly consistent between individuals for each lead (Table 2.3). The majority of sea lions had positive P-waves in all leads except in aVL (for which the majority had negative P-waves). Similarly, the majority of sea lions had positive T-waves in all leads except for aVR and aVL. P- and T-wave polarity in northern fur seals was consistent with the pattern observed for Steller sea lions with the exception of the majority of fur seals having negative P-wave polarity in lead aVR (Table 2.4). The P- and T-wave polarities of the walrus were almost the same as Steller sea lions, with the exception of having a biphasic T-wave in lead 1 (Table 2.5).

QRS morphology for each lead was consistent among the sea lions (Fig. 2.8 and Appendix A), but appeared to be unique for each northern fur seal with few observable trends (Appendix B). No comparisons could be made for walrus with just one set of measurements (Appendix C).

Table 2.1 Echocardiographic measurements for 8 individual anaesthetized Steller sea lions and group summary (median ± SD). All values are M-mode measurements except for LAD, Ao, LA:Ao (2D), and AV Vmax, and PV Vmax (Doppler). Abbreviations: LAD = left atrial diameter, Ao = aortic root diameter, LA:Ao = left atrial to aortic root ratio, LVIDs = left ventricular internal diameter in systole, LVIDd = left ventricular internal diameter in diastole, IVSs = interventricular septum thickness in systole, IVSd = interventricular septum thickness in diastole, LVWs = left ventricular free wall thickness in systole, LVWd = left ventricular free wall thickness in diastole, FS = fractional shortening, AV Vmax = peak systolic aortic blood velocity, PV Vmax = peak systolic pulmonary blood velocity, HR = heart rate. NA indicates measurement not available.

	Individual Measurements								
	F00BO	**F97HA**	**F03RO**	**F03AS**	**F03WI**	**F03IZ**	**F00YA**	**F97SI**	**Median ± SD**
Mass (kg)	166.5	173.0	192.0	199.2	204.4	208.6	219.5	227.0	201.8 ± 21.1
LAD (cm)	4.53	4.15	4.20	3.01	4.28	5.23	4.60	4.71	4.41 ± 0.64
Ao (cm)	4.25	4.47	5.13	4.90	5.25	4.90	4.25	5.17	4.90 ± 0.41
LA:Ao	1.07	0.93	0.82	0.61	0.77	1.07	1.08	0.91	0.92 ± 0.17
LVIDs (cm)	5.50	3.48	3.64	5.34	4.65	4.00	4.43	5.69	4.54 ± 0.86
LVIDd (cm)	8.26	5.57	5.23	6.45	5.93	6.25	6.00	9.16	6.13 ± 1.37
IVSs (cm)	1.70	2.15	2.10	1.97	2.20	2.85	1.70	2.28	2.12 ± 0.37
IVSd (cm)	1.13	1.68	1.45	1.46	1.48	1.79	1.47	1.51	1.47 ± 0.19
LVWs (cm)	1.80	2.23	2.30	1.84	2.40	2.12	2.32	2.44	2.26 ± 0.24
LVWd (cm)	1.18	1.69	1.91	1.63	2.15	1.68	1.60	1.96	1.69 ± 0.29
FS (%)	33.35	37.51	30.45	17.29	21.16	36.04	26.17	37.89	31.90 ± 7.75
AV Vmax	1.06	0.49	0.58	0.64	0.94	0.58	0.75	NA	0.64 ± 0.21
PV Vmax	0.80	0.60	0.86	0.56	0.87	1.01	NA	NA	0.83 ± 0.17
HR (bpm)	56.1	41.5	62.5	50.5	48.0	NA	46.8	56.2	50.5 ± 7.1

Table 2.2 Mean ± SD duration (ms) of ECG parameters for 5 northern fur seals, 8 Steller sea lions and 1 walrus.

	Northern fur seal (n=5)	**Steller sea lion** (n=8)	**Walrus** (n=1)
Mass (kg)	29.38 ± 5.00	198.78 ± 20.13	366.50
Heart rate (bpm)	113.0 ± 15.5	56.5 ± 3.7	76.0
P-wave duration (ms)	74.27 ± 5.93	136.08 ± 11.65	146.67
P-wave amplitude (mV)	0.18 ± 0.03	0.17 ± 0.05	0.30
PQ interval duration (ms)	195.00 ± 95.19	255.04 ± 24.19	244.67
R-wave amplitude (mV)	0.73 ± 0.28	0.41 ± 0.27	0.43
QRS complex duration (ms)	65.53 ± 11.27	130.38 ± 9.29	182.33
QT interval duration (ms)	233.80 ± 18.34	386.25 ± 35.21	368.67
T-wave amplitude (mV)	0.16 ± 0.20	0.14 ± 0.23	0.39

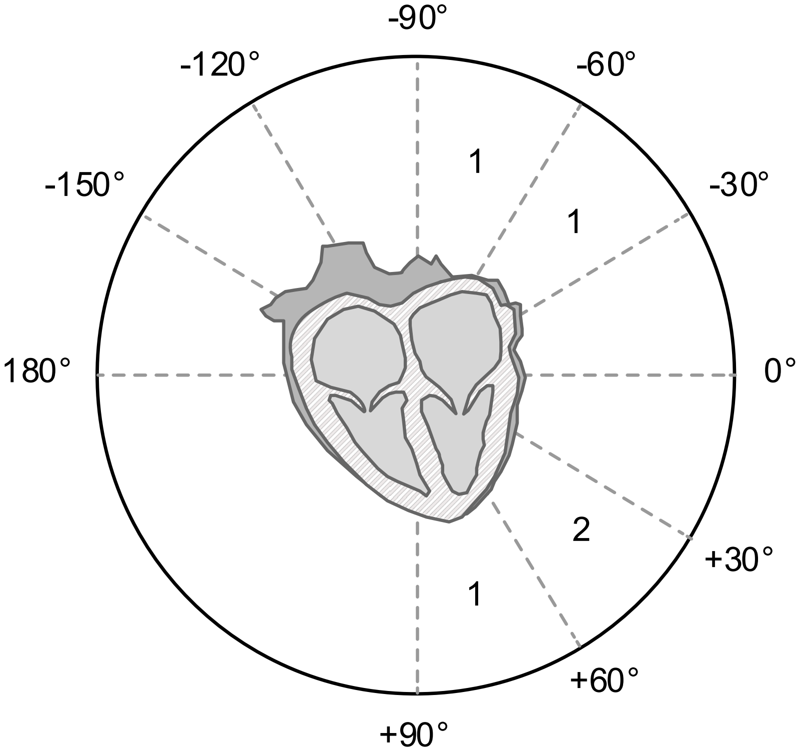

Figure 2.7 Mean electrical axes of anaesthetized northern fur seals (n=5). The number of individuals with a mean electrical axis falling within each 30° interval is shown. Modified from Falabella et al. (1999).

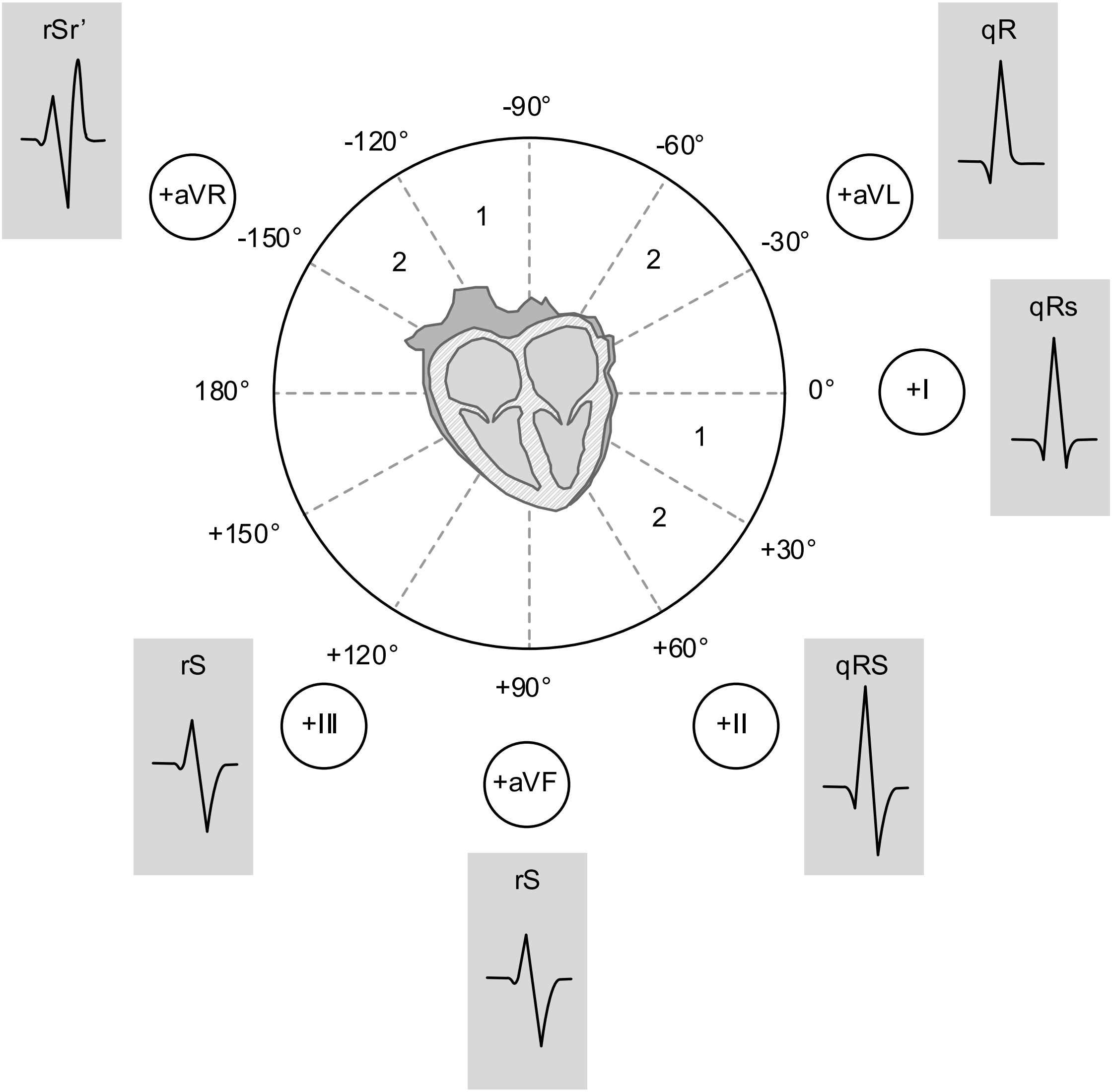

Figure 2.8 Mean electrical axes and typical QRS complexes of anaesthetized Steller sea lions (n=8). The number of individuals with a mean electrical axis falling within each 30° interval is shown. Typical QRS morphology based on the shape displayed by the highest proportion of sea lions for each lead is depicted with descriptive nomenclature. Lowercase letters indicate small deflections, capital letters indicate large deflections, and apostrophes indicate a secondary wave of the given type. Modified from Falabella et al. (1999) and Santilli and Perego (2009).

Table 2.3 Steller sea lion P-wave and T-wave polarity indicated by the number of individuals with positive (+), negative (-), or biphasic waves in limb and augmented leads.

	P-wave polarity			**T-wave polarity**		
	+	-	Biphasic	+	-	Biphasic
I	8	0	0	6	1	1
II	8	0	0	7	0	1
III	5	1	2	6	1	1
aVR	0	8	0	1	7	0
aVL	5	3	0	2	5	1
aVF	8	0	0	6	0	2

Table 2.4 Northern fur seal P-wave and T-wave polarity indicated by the number of individuals with positive (+), negative (-), or biphasic waves in limb and augmented leads.

	P-wave polarity			**T-wave polarity**		
	+	-	Biphasic	+	-	Biphasic
I	5	0	0	3	1	1
II	5	0	0	4	0	1
III	5	0	0	3	0	2
aVR	0	5	0	0	4	1
aVL	0	5	0	1	2	2
aVF	4	0	1	4	0	1

Table 2.5 Walrus P-wave and T-wave polarity indicated by the number of individuals with positive (+), negative (-), or biphasic waves in limb and augmented leads.

	P	**T**
I	+	Biphasic
II	+	+
III	+	+
aVR	-	-
aVL	+	-
aVF	+	+

Discussion

Our echocardiographic and electrocardiographic analyses of pinnipeds revealed several deviations from typical mammalian cardiac structure and function. As expected, our results confirm that Steller sea lions have large aortic roots. Unexpectedly, we found that Steller sea lions have larger left ventricular chamber dimensions during contraction that terrestrial mammals of equivalent body mass. In addition, the mean electrical axis of most individuals deviated from the expected left caudal orientation. The significance of these differences is largely unknown, but may reflect functional consequences of diving.

Steller sea lion cardiac structure

This is the first complete echocardiographic study of a Steller sea lion, and only the second of a pinniped to date (Castro et al., 2018). Comparing to other mammals (including cats, dogs, goats, buffalo, and cows), echocardiographic parameters for Steller sea lion hearts were more similar to those of southern sea lions and bottlenose dolphins than to terrestrial mammals (Castro et al., 2018; Chetboul et al., 2012). No visible signs of cardiac abnormalities were detected in the echocardiograms. The only notable observation was the presence of a false tendon in one of the sea lions (F00YA). False tendons are bands of fibrous tissue that stretch across the left ventricle, and naturally occur in healthy mammals, especially cats (Kimura et al., 2016). Therefore, it is not an entirely unexpected finding in sea lions.

Consistent with other studies, we found that Steller sea lions have a larger aortic root than terrestrial mammals of similar sizes. A large aortic root is common in marine mammals and has been previously documented by echocardiography in southern sea lions (Castro et al., 2018). The proposed functions of this structure, known as the aortic bulb, are to maintain continuous blood flow to the brain and heart during the prolonged inter-beat intervals due to diving bradycardia, to decrease peak systolic blood pressure, and to reduce myocardial work (Campbell et al., 1981; Elsner, 1969; Rhode et al., 1986; Shadwick and Gosline, 1994; Thornton et al., 2005).

Aortic bulbs have been documented in more than 13 species of marine mammals to date (e.g., Drabek, 1975; Drabek and Burns, 2002; King, 1977; Rhode et al., 1986; Smodlaka et al., 2008; Stewardson et al., 1999), including Steller sea lions (Murie, 1874). Beyond blood flow maintenance, the functional consequences of the aortic bulb, such as its effects on cardiac work and stroke volume, are unknown. Technical and anatomical limitations prevented us from viewing the ascending aorta using transthoracic echocardiography in Steller sea lions and thus prevented further inquiry into the function of the aortic bulb. Future *in vivo* studies of the aortic bulb are needed to confirm that it maintains blood flow in marine mammals when they are diving, and to test the additional hypotheses of its functional significance.

Steller sea lion left ventricular form and function

In comparison to terrestrial mammals, Steller sea lions (this study) and southern sea lions (Castro et al., 2018) appear to have larger left ventricular end-systolic diameters than expected for their body masses, suggesting that there is less left ventricular contraction during systole. In fact, the LVIDs measurements for pinnipeds weighing 93.5-250 kg were most similar to those of 450-650 kg cows (Hallowell et al., 2007). Left ventricular end-systolic diameter is measured when the heart contracts and blood is ejected from the left ventricle into the aorta, e.g., when the ventricle is at its minimal diameter (Otto, 2018). Thus, a larger end-systolic diameter could indicate the marine mammal heart is ejecting a smaller volume of blood with each beat than would be expected for a terrestrial mammal of similar body size.

Similarly, the change in size of the left ventricle from the relaxed to contracted state (fractional shortening) in Steller sea lions (32%) was less than the median value calculated from 14 species of terrestrial mammals (40%) (Boon et al., 1983; Diniz et al., 2017; Fontes-Sousa et al., 2006; Guglielmini et al., 2006; Häggström et al., 2016; Hallowell et al., 2007; Leroux et al., 2012; Morrison et al., 1992; Noviana and Kurniawan, 2013; Petrič et al., 2012; Scansen and Morgan, 2015; Torad et al., 2017). This observation primarily reflects the larger dimension of the sea lions' left ventricle in systole as the end-diastolic left ventricular dimensions were within the expected range for mammals of this size. The smaller fractional shortening provides further evidence that the left ventricle contracts less in anaesthetized sea lions than in similar-sized terrestrial mammals. It is conceivable that the ventricles may be contracting more and ejecting a greater volume of blood during diving. However, fractional shortening in other species of marine mammals (bottlenose dolphins and southern sea lions), also measured while not diving or submerged, is similar to terrestrial mammals suggesting that small fractional shortening may be unique to Steller sea lions.

Greater blood volume leaving the left ventricle during each cardiac cycle (stroke volume) has not yet been observed in forced or freely-diving marine mammals (Blix et al., 1976; Murdaugh et al., 1966; Ponganis et al., 2006, 1991)—despite the expectation that it (along with forceful contractions of the left ventricle) could counter potential decreases in blood flow caused by diving bradycardia. Having a small fractional shortening while resting would give marine mammals greater capacity than terrestrial mammals to increase left ventricular contraction. That is, at rest they have a lower set point which means the total amount they can increase fractional shortening before reaching a physiological maximum is greater than terrestrial mammals. How this benefits Steller sea lions while diving remains a conundrum because while blood flow could be maintained for a longer duration, increasing stroke volume will increase blood pressure if heart rate and peripheral vasoconstriction remain unchanged. Since blood pressure is expected to be relatively constant during a dive, an increase in stroke volume to maintain blood flow seems counterintuitive. Thus, it is unclear why the Steller sea lions we studied had smaller fractional shortening than expected.

Blood flow through the aortic and pulmonary valves can be used as an indicator of cardiac problems such as aortic stenosis (Otto, 2018). While we report median values for Steller sea lions that may be useful for diagnosing abnormalities, we cannot make comparisons of peak flow velocities to those of terrestrial mammals due to a lack of reported values for mammals larger than 30 kg. A wide range of peak aortic velocities (0.60 - 0.99 m/s) and pulmonary velocities (0.45 – 1.00 m/s) have been reported for terrestrial mammals less than 30 kg, which may indicate that these functional parameters are highly variable and may not correlate with body mass.

Steller sea lion, northern fur seal, and walrus electrocardiographic function

All Steller sea lions, northern fur seals, and the walrus displayed the characteristic mammalian ECG waveform. No abnormal findings indicative of cardiac pathology were present in any of the studied ECGs.

Observing the net direction of the electrical activity of the heart, the mean electrical axis (MEA), reveals where the bulk of the myocardium is situated. The MEAs of Steller sea lions (SSL) and northern fur seals (NFS) were unique to each individual, with no overarching trend. Right cranial (3 SSL), left cranial (2 SSL, 2 NFS, 1 walrus), and left caudal orientations (3 SSL, 3 NFS) were all measured. Other species of marine mammals (southern sea lions, southern elephant seals, bottlenose dolphins, manatees) have left cranial MEA orientations (Dassis et al., 2016; Falabella et al., 1999; Harms et al., 2013; Siegal-Willott et al., 2006).

Left caudal and cranial orientations are common in terrestrial mammals. The left ventricle is thought to contribute the most to depolarization, which in turn draws the MEA towards it either cranially or caudally and to the left (Bayés De Luna et al., 2012). In adult humans, for example, the normal MEA is between -30° and 90° (Surawicz et al., 2009). Right cranial orientations in three of the Steller sea lions may have been physiologically normal or could be indicative of cardiac anomalies such as the potential atrial enlargement observed in a southern seal lion (Dassis et al., 2016). However, we found no evidence from the ECG (such as a notched P-wave) or echocardiogram to suggest pathology in our study animals.

Based on the broad, flat shape of Steller sea lion hearts and the unusually large right ventricle and enlarged left and right atria (Murie, 1874), right cranial mean electrical axes may be a normal finding that reflects cardiac anatomy. The unique distribution of the myocardium could alter the net direction of the electrical flow through the heart. Therefore, an extreme right axis deviation could be recorded in a Steller sea lion, and the finding would still be physiologically normal.

We observed sinus arrhythmias (slight changes in heart rate) that coincide with the respiratory cycle in 5 of the 8 Steller sea lions, but not in the walrus or northern fur seals. Sinus arrhythmias are a common occurrence in marine and terrestrial mammals (e.g., Ben-Tal et al., 2012; Castellini et al., 1994; Hamlin et al., 1972; Harms et al., 2013; Kastelein and Meijler, 1989; Ponganis and Kooyman, 1999; Senft and Kanwisher, 1960) that may increase the efficiency of oxygen uptake by increasing heart rate during inspiration (Yasuma and Hayano, 2004) and reduce cardiac work by slowing the heart rate during expiration (Ben-Tal et al., 2012).

In marine mammals, sinus arrhythmias may be related to diving and diving ability. For example, in northern elephant seals, the development of sinus arrhythmias occurs in association with increased diving and increased sleep apneas, with adult seals and older pups having more pronounced sinus arrhythmias than younger pups (Castellini et al., 1994). In addition, sinus arrhythmias and the dive response are both under vagal control (Grossman and Kollai, 1993; Ponganis et al., 2017), which suggests that the heart rates of marine mammals with pronounced sinus arrhythmias may be under greater control than those without sinus arrhythmias. It follows that sinus arrhythmias may be an indicator of diving ability, assuming that greater heart rate control is beneficial for diving. Further research into sinus arrhythmias and diving is needed to confirm this hypothesis.

There are several plausible explanations for why we did not observe sinus arrhythmias in all of the individuals we studied. One reason is that the pinniped species we studied are not true seals (phocids). Sinus arrhythmias are associated with spontaneous breath-holds which are more commonly observed in phocids (seals) than in otariids and odobenids (sea lions and walruses) (Ponganis, 2015). Furthermore, the individuals we studied were maintained under human care. The limitations on their dive depths may have thus stunted development of a sinus arrhythmia. Additionally, the walrus was only ~2 years old and may not have developed a sinus arrhythmia yet. Another possibility is that we did not detect sinus arrhythmias in these individuals because they were anaesthetized during the ECGs (Grossman and Kollai, 1993).

Clinical significance

Our study provides cardiac reference values for healthy anaesthetized Steller sea lions, northern fur seals, and one walrus. These values can be used by veterinarians to diagnose cardiac abnormalities in future pinniped health assessments. It is important to have species-specific baseline-reference values when evaluating ill or stranded individuals. Our Steller sea lion ECGs and echocardiograms should represent anaesthetized adult females well. We did observe considerable individual variation, which further exemplifies the importance of obtaining reference values from healthy individuals. Knowing, for example, that extreme right axis deviation (as defined for humans) is not an uncommon occurrence in healthy pinnipeds, can help rule this out as a cause for concern.

Although our measurements all come from anaesthetized animals, they are valuable reference measurements because animals are often sedated for clinical exams. Performing echocardiography on Steller sea lions required considerable pressure on their chests, which may not be tolerated by individuals that are awake, even with training. Therefore, analysis of cardiac structure, left ventricular function, and pulmonary and aortic blood flow using echocardiography may be restricted to anaesthetized Steller sea lions, even though measurements on conscious individuals (e.g., southern sea lions, a smaller species) have been successfully obtained (Castro et al., 2018). There is a slight chance that cardiac compression affected our results, but there were no indications of this during the procedures and data analysis. For example, the majority of echocardiographic measurements that we made fell within the expected range for mammals of that body mass.

Conclusion

We report baseline cardiac measurements for Steller sea lions, northern fur seals, and a walrus. We found many similarities in cardiac form and function between these pinnipeds and terrestrial mammals. However, our echocardiographic examinations of Steller sea lions confirm differences in cardiac form, such as the enlarged aortic root. Our findings also suggest differences in cardiac function, between marine and terrestrial mammals, such as reduced fractional shortening that may support the dive response. Electrophysiological measurements from our study animals also did not match those of terrestrial mammals. Overall, our findings reveal possible cardiac adaptations to diving in marine mammals. Future studies are needed to evaluate the functional roles of these differences and confirm their presence in other marine mammals including cetaceans.

Chapter 3: Electrocardiographic scaling reveals differences in cardiac function between marine and terrestrial mammals

Summary

Adaptations to diving may explain apparent differences in cardiac function between marine mammals and terrestrial mammals. While the ability of marine mammals to lower heart rates for extended periods when diving is well documented, it is unknown whether marine mammals have cardiac adaptations that extend beyond overall bradycardia. We analyzed electrocardiographic data from 50 species of terrestrial mammals and 17 species of marine mammals to determine whether cardiac function differs between these two ecological groups. We also tested whether physiological state (i.e., anaesthetized or conscious) affects ECG parameters. Analyses of cardiac waveform morphology (heart rate, P-wave duration, and PQ, PR, QRS, and QT intervals) revealed allometric relationships between body mass and all ECG intervals (as well as heart rates) for both groups of mammals and specific differences in ECG parameters between marine mammals and their terrestrial counterparts. Model outputs indicated that marine mammals had 20% longer P-waves (regardless of physiological state) and 11% longer QRS intervals (in conscious individuals). In other words, marine mammals had slower atrial and ventricular depolarization than terrestrial mammals. Heart rate, PQ, and QT intervals were not significantly different between marine and terrestrial mammals. Most of our ECG parameters scaled with body mass to the power of 0.20 (range: 0.137 - 0.213) rather than the expected 0.25—while heart rate scaled to body mass to the power of -0.22 and was also less than the widely accepted -0.25 derived from fractal geometry. Our findings show clear differences between the hearts of terrestrial and marine mammals in terms of cardiac timing that extend beyond diving bradycardia. They also highlight the importance of considering animal physiological state and special adaptations when analyzing allometric relationships.

Introduction

Cardiac function is expected to scale with body mass across all mammals because of the essential nature of the heart's function and the conservation of cardiac anatomy and physiology across mammalian species. For example, the mass of a heart relative to the mass of a body scales to 0.98 in both marine and terrestrial mammals (Prothero, 1979). However, heart rates and waveforms of contracting hearts may nevertheless scale differently between the two types of mammals because of cardiac adaptations related to diving and the large body size of marine mammals. Heart rates may be higher in marine mammals due to some species having higher metabolic rates than similarly-sized terrestrial mammals (Green, 2011; Williams et al., 2001). In contrast, heart rates may also be lower in marine mammals due to diving bradycardia. Additionally, the hearts of marine mammals must cope with extreme physiological changes—bradycardia and vasoconstriction—that occur with breath-hold diving. Thus, differences in how the hearts of marine and terrestrial mammals perform could occur despite well-described general anatomical similarities between the hearts of the two ecological groups (Drabek, 1975; Rowlatt, 1990).

Dissections have generally confirmed the gross similarity between the hearts of marine and terrestrial mammals. However, they have also revealed anatomical differences that may cause cardiac signal conduction in marine mammal hearts to deviate from typical mammalian signal conduction. For example, cetaceans such as bowhead whales (*Balaena mysticetus*), sperm whales (*Physeter macrocephalus*), beluga whales (*Delphinapterus leucas*), and a few species of porpoise have abnormally large Purkinje fibres (Pfeiffer, 1990) that are thought to increase signal conduction velocity from the atrioventricular (AV) node to the ventricular myocardium (van Nie, 1986). Similarly, Todd fibres found in the right atrial wall of white-beaked dolphins are suspected of increasing signal conduction velocity between the sinoatrial (SA) and AV nodes (Nie, 1987). The function of these specialized conduction tissues is unknown, but it has been suggested that they may be beneficial for rapid heart rate transitions such as those observed in marine mammals as they dive and resurface (Nie, 1987; van Nie, 1986). Thereby making marine mammals better equipped to rapidly decrease their heart rates when diving, and increase their heart rates when surfacing to breathe.

In addition to having specialized myocardial cells, the shape of marine mammal hearts may result in differences in cardiac function. Changes to the amount and distribution of cardiac muscle affect how electrical signals travel through the heart. For example, pinnipeds have broad hearts (Drabek, 1975; Rowlatt, 1990) which may increase the duration of ventricular depolarization because the signal must travel a greater distance, thereby resulting in a longer QRS interval on an ECG.

Data from marine mammals have been included in previous interspecific analyses of cardiac performance among mammals. However, low numbers of marine mammal species have precluded a statistical comparison with terrestrial mammals. We therefore combined published electrocardiographic data with new measurements from three species of marine mammals (Steller sea lions, northern fur seals, and walrus) to determine whether cardiac performance differs between marine and terrestrial mammals. We categorized the data based on the physiological state of the individual when ECG measurements were taken, and considered the effects of anaesthesia on cardiac rate and rhythm—before calculating and comparing scaling exponents as a function of body mass for ECG data across a wide range of mammalian sizes, from mice to whales.

Methods

Meta-analysis

We amassed an ECG dataset from 82 species (representing over 2000 individuals) of marine and terrestrial mammals for analysis. The majority of the data were obtained from the scientific literature, but we also added unpublished data we collected from three species of marine mammals—Steller sea lions, northern fur seals, and walrus (Chapter 2). We extracted values for heart rate, P-wave, and PQ, PR, QRS, and QT interval durations (Fig. 3.1), and recorded information about the source group or individual including age, sex, physiological state (anaesthetized or conscious) and body mass, when available.

Optimally, ECG measurements should be taken under standardized conditions in calm, healthy individuals to minimize any effects of anaesthesia on cardiac characteristics—including alterations in heart rate—that might complicate multispecies comparisons. However, in practice, ECGs are often taken while under anaesthesia during medical procedures, particularly for exotic species, which is why we categorized measurements based on the physiological state of the individual.

We grouped the PQ and PR intervals and report them as the PR interval, because these intervals are the same by definition. The PR interval is the timing between the beginning of the P-wave to the onset of the QRS complex—either the Q-wave or the R-wave—which is why the PQ and PR intervals are both classified as the PR interval (Bayés De Luna et al., 2012). We report a single mean or mid-range value for each species that had sample sizes ranging from a single animal to hundreds of individuals (e.g., dogs) (see Appendix D).

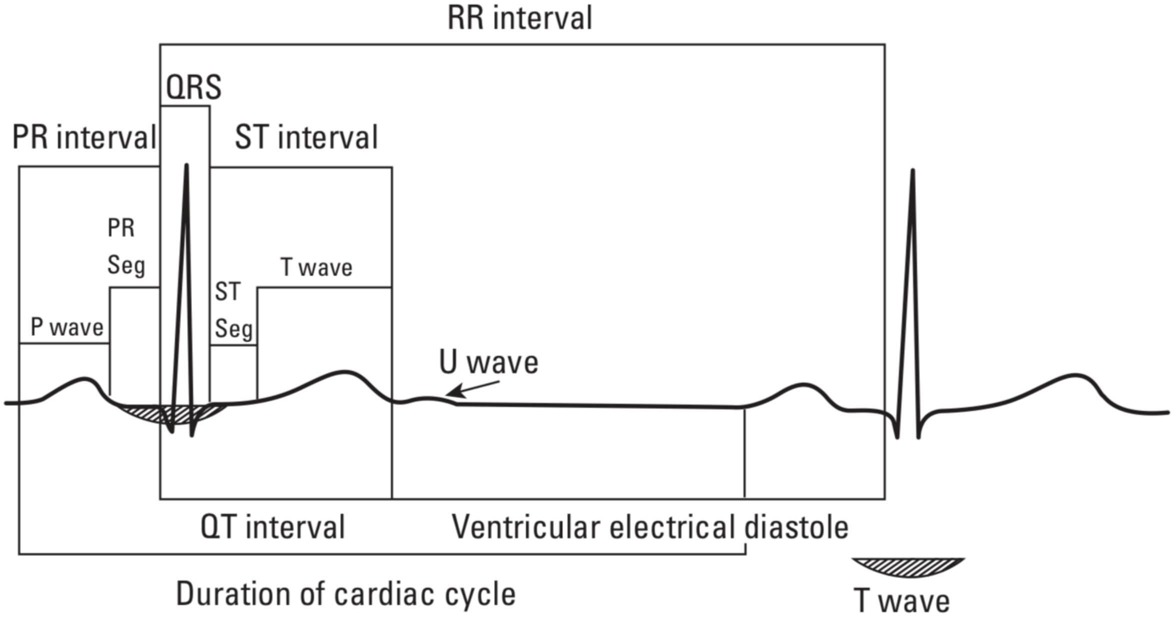

Figure 3.1 Characteristic cardiac waveform for a single cardiac cycle in a mammal consisting of several well-defined deflections termed the P, Q, R, S, and T waves. As shown, the ECG can be divided into several standardized, measurable intervals (PR, QRS, QT, and RR) that represent the timing of electrical signal conduction through the myocardium that stimulates contraction of the heart's atria and ventricles. Figure reproduced from Clinical Echocardiography (Bayés De Luna et al., 2012) with permission from the publisher. © 2012 by John Wiley & Sons, Ltd.

We categorized the data based on physiological state of the individuals. In our dataset, we classified measurements as being taken under anaesthesia when the majority (> 90%) of the individuals in the sample were known to be anaesthetized. In 22 out of 23 species recorded as anaesthetized, all individuals of the species were anaesthetized and for the 23rd species, 9 out of 10 individuals were anaesthetized. Data from five species allowed us to report conscious and anaesthetized ECG parameters separately (e.g., dogs). All measurements from 49 other species came from individuals that were not anaesthetized (i.e., "conscious"). Data indicating physiological state (anaesthetized or conscious) were unavailable for 24 species and was therefore excluded from our analysis. Our final dataset consisted of 67 mammalian species (17 marine and 50 terrestrial), representing 1660 individuals.

To calculate representative ECG parameters for each species under a specific physiological state, we first calculated means and midranges for each individual source. Averages were preferable, but when these were unavailable, we used the midrange as a proxy which we considered to fairly represent that species. Midrange was calculated as the mean of the reported minimum and maximum values of a given ECG parameter. To combine data from multiple sources, we calculated weighted averages and midranges using sample size as the weighting factor. When sample size was unavailable, we assigned a value of one as the weighting factor because each report had to have come from at least one individual. Therefore, reports from species for which no sample size was stated may be underrepresented in the calculated average for that species.

For terrestrial mammals, we followed the procedure for harvesting ECG data and estimating species' masses as outlined in Günther and Morgado (1997) with some modifications. Our source for terrestrial ECG data was Grauwiler (1965), who reported ECG parameters for a wide variety of mammals. Often, body mass was not indicated, so we estimated body mass by matching reported information about the individual or group, such as age and sex, to corresponding species information from additional literature sources (see Appendix D). When mass estimates could not be informed by age and sex, we used a general average species mass from the available literature. In many cases, estimates of mass were from only one or a few individuals

ECG data for marine mammals were previously published for 14 species, to which we added ECG data for three additional species (Steller sea lions, northern fur seals, and walrus—Chapter 2). Masses for all individuals were documented at the time of the ECG recordings either as an estimate (for the large whales) or by direct measurement.

For all species, heart rate data were either explicitly stated or were calculated as 60 divided by the RR interval. Midrange heart rates were calculated as described above when averages were not reported. For species that had multiple types of heart rate data available, the reported value used for analysis was selected based on the estimate requiring the fewest number of calculations. Our ranking system from highest to lowest preference was heart rate, heart rate calculated from the RR interval, heart rate midrange, and finally heart rate midrange calculated from RR midrange.

ECG protocol

To increase the number of marine mammal species available for comparison, we recorded ECGs as described in Chapter 2 from 8 Steller sea lions, 5 northern fur seals, and 1 walrus housed at the Vancouver Aquarium (Vancouver, BC) and the University of British Columbia Open Water Research Station (Port Moody, BC). In brief, we recorded two, one-minute ECGs from anaesthetized individuals in the frontal plane using an IDEXX CardioPet telemetric ECG device. Standard electrocardiographic measurements were made by a veterinary cardiologist using IDEXX VetMedStat software. Next, we calculated species' averages for each measured parameter and added them to the mammalian ECG dataset for analysis.

Statistical analyses

We fit linear models to our dataset to test the effects of ecological group (marine or terrestrial) and physiological state (conscious or anaesthetized) on ECG parameters, and to derive allometric equations between cardiac parameters and body mass. We first log-transformed body mass and each ECG parameter to linearize the data. Then, we fit three-way interaction models between body mass, ecological group, and physiological state. We used Akaike's Information Criterion to find the minimal adequate model by dropping terms from our interaction model and determining if this improved the fit. We performed a two-way analysis of covariance (ANCOVA) on the minimal adequate model for each ECG parameter using mass as a covariate and ecological group (terrestrial or marine mammal) and physiological state (conscious or anaesthetized) as fixed categorical factors. Data from all the models met the assumptions for ANCOVA. Analyses were performed using the statistical software R version 3.4.4.

Results

The effects of ecological group, physiological state, body mass, and interactions between these factors were specific to each ECG parameter (Tables 3.1 and 3.2). All ECG parameters significantly correlated to body mass. Heart rate decreased with body mass, while P-wave, PR, QRS, and QT durations increased with body mass. Mass-specific differences between marine and terrestrial mammals were detected in P-wave and QRS durations, but not in heart rate, PR or QT durations. We also found an interaction between ecological group and physiological state that affected the QRS duration. However, there were no other significant differences between anaesthetized and conscious mammals aside from this lone interaction.

Heart rate, PR interval, and QT interval correlated with body mass over a range of 0.017 kg (mouse) to 32000 kg (fin whale) (Table 3.1). No significant effects of ecological group, physiological state or interactions between factors were detected in these three parameters. Heart rate scaled with body mass to the power of -0.219 (Fig. 3.2a, Table 3.2). PR interval duration scaled with body mass to the power of 0.211 (Fig. 3.2b, Table 3.2), while QT interval duration scaled with body mass to the power of 0.217 (Fig. 3.2c, Table 3.2).

Although heart rate was only affected by body mass, there was a slight but nonsignificant interaction between physiological state and mass indicating that anaesthetized and conscious mammals have different mass-specific heart rates. The nonsignificant trend suggests that anaesthetized mammals larger than ~1 kg had higher heart rates than conscious mammals of similar mass (Table 3.1, $p = 0.050$) — i.e., when comparing two individuals of the same mass (> 1 kg), the anaesthetized individual should have a higher heart rate than the conscious individual.

P-wave duration increased with body mass over a range of 0.017 (mouse) to 3200 kg (Asian elephant) (Table 3.1, $p < 0.0001$) and depended on ecological group. Marine mammals had 20% longer P-waves than terrestrial mammals over the range that marine and terrestrial mammal masses overlapped (Table 3.1, $p = 0.035$). For example, a terrestrial mammal weighing 100 kg would have a P-wave duration of 0.077 s, whereas a marine mammal of the same mass would have a P-wave duration of 0.096 s. In marine mammals, P-wave duration scaled with body mass to the power of 0.213 and in terrestrial mammals to the power of 0.210 (Table 3.2, Fig. 3.3). Physiological state had no effect on P-wave duration and all interactions were nonsignificant.

QRS complex duration depended on mass, ecological group, and an interaction between ecological group and physiological state (Fig. 3.4). QRS duration increased with body mass over a range of 0.017 kg (mouse) to 32000 kg (fin whale) (Table 3.1, $p < 0.0001$). The relationship between QRS complex duration and body mass was similar between ecological groups for a given physiological state, but the exponent for both groups differed between conscious (0.195) and anaesthetized (0.137) animals. The intercepts also differ, showing that marine mammals had 11% (conscious) and 43% (anaesthetized) longer QRS complexes than terrestrial mammals of the same mass and same physiological state (Table 3.1, $p = 0.021$). For example, a conscious 100 kg terrestrial mammal would have a QRS complex of 0.072 s, while a marine mammal of the same mass would have a QRS complex of 0.081 s. The relative difference would be greater for anaesthetized animals, where a 100 kg terrestrial mammal would have a QRS complex of 0.053 s, whereas QRS complex of an anaesthetized marine mammal would be 0.094 s. Note that the comparison between anaesthetized mammals is for illustration only, as the data for anaesthetized marine and terrestrial mammals do not overlap (the QRS interval for a 100 kg anaesthetized terrestrial mammal is an extrapolation for the sake of comparison). Further, physiological state affected marine and terrestrial mammals differently, as indicated by the interaction effect (Table 3.1, $p = 0.042$) and the intercepts of the linear regressions (Table 3.2). As a result of this interaction, QRS complex durations of mammals are specific to their ecological group and physiological state for a given body mass.

Table 3.1 ANCOVA results of the minimal adequate model describing the relationship between ecological group (EG), physiological state (PS), and ECG parameters accounting for the effects of body mass (BM) as a covariate. Values are degrees of freedom (DF), F-values, and multiple R^2. Statistical significance: *** indicates $P < 0.0001$, * indicates $P < 0.05$. NP indicates that the factor or interaction was not present in the minimal adequate model

Parameter	DF	Body mass	EG	PS	BM X EG	BM X PS	EG X PS	BM X EG X PS	Multiple R^2
Heart rate	1,66	376.1***	NP	3.9	NP	4.0	NP	NP	0.85
P	1,28	117.1***	4.9*	0.1	NP	2.1	NP	NP	0.82
PR	1,59	483.0***	0.2	1.2	NP	3.1	3.0	NP	0.89
QRS	1,62	420.4***	5.7*	0.1	NP	0.6	4.3*	NP	0.87
QT	1,62	362.4***	3.4	NP	NP	NP	NP	NP	0.86

Table 3.2 Linear regressions and allometric equations describing the relationship between body mass (BM) and ECG parameters for mammals separated by ecological group and physiological state as appropriate.

Group	Response variable	Linear equation of log-transformed data	Allometric equation
All mammals	HR	$-0.219(BM) + 2.344$	$221(BM)^{-.219}$
Terrestrial mammals	P	$0.210(BM) - 1.533$	$0.0293(BM)^{0.210}$
Marine mammals	P	$0.213(BM) - 1.442$	$0.0361(BM)^{0.213}$
All mammals	PR	$0.211(BM) - 1.179$	$0.0663(BM)^{0.211}$
Conscious terrestrial mammals	QRS	$0.195(BM) - 1.533$	$0.0293(BM)^{0.195}$
Conscious marine mammals	QRS	$0.194(BM) - 1.481$	$0.0330(BM)^{0.195}$
Anaesthetized terrestrial mammals	QRS	$0.137(BM) - 1.547$	$0.0283(BM)^{0.137}$
Anaesthetized marine mammals	QRS	$0.137(BM) - 1.301$	$0.0500(BM)^{0.137}$
All mammals	QT	$0.217(BM) - 0.925$	$0.119(BM)^{0.217}$

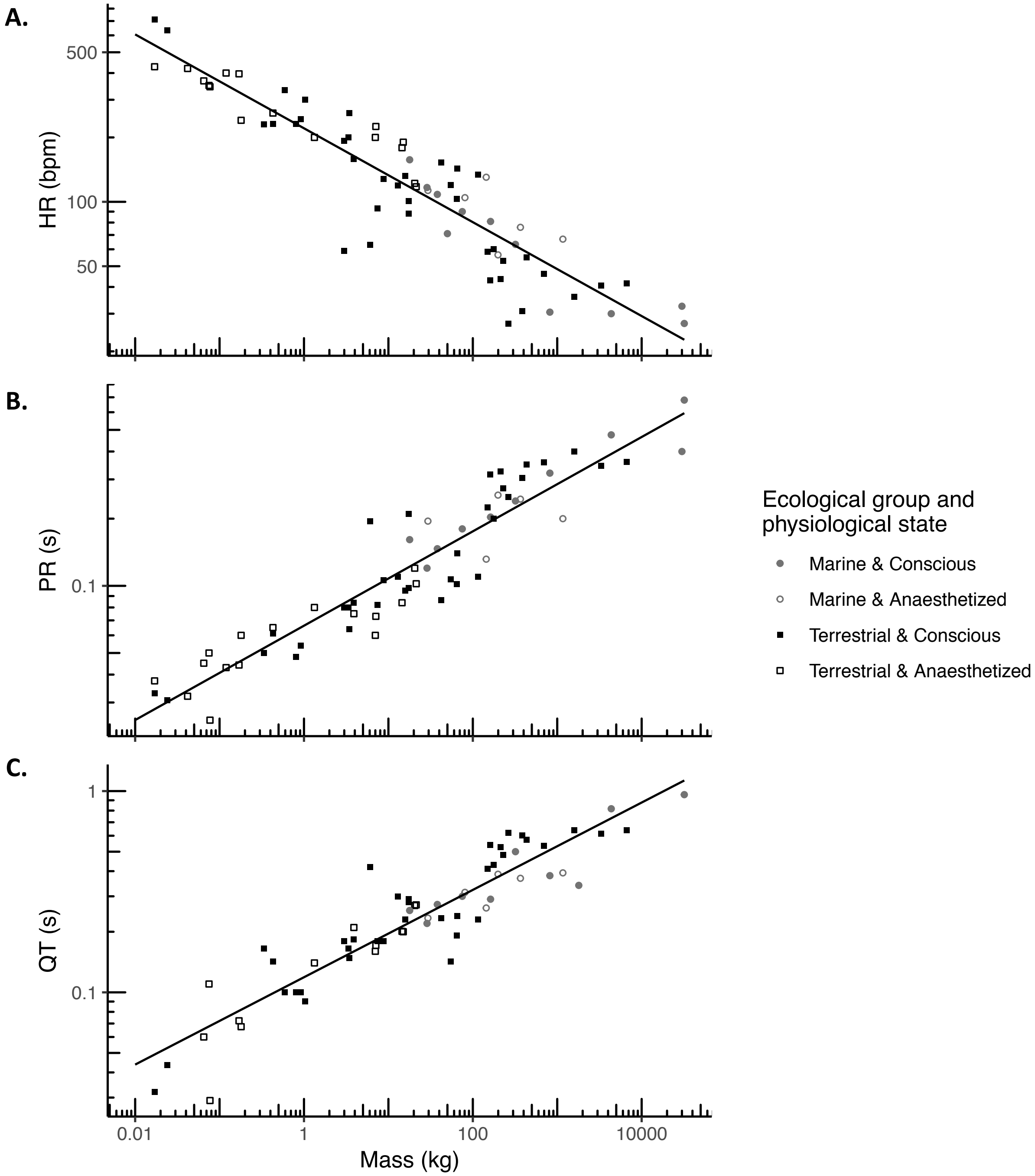

Figure 3.2 Relationships between body mass and heart rate (A), PR interval (B), and QT interval (C) in mammals. Ecological group (terrestrial mammals shown in black squares, marine mammals shown in grey circles) and physiological state (conscious mammals indicated by closed squares and circles, anaesthetized mammals indicated by open squares and circles) had no effect on heart rate, PR interval, or QT interval. Mass was the best predictor for these three ECG parameters. Regression equations for each parameter are provided in Table 3.2.

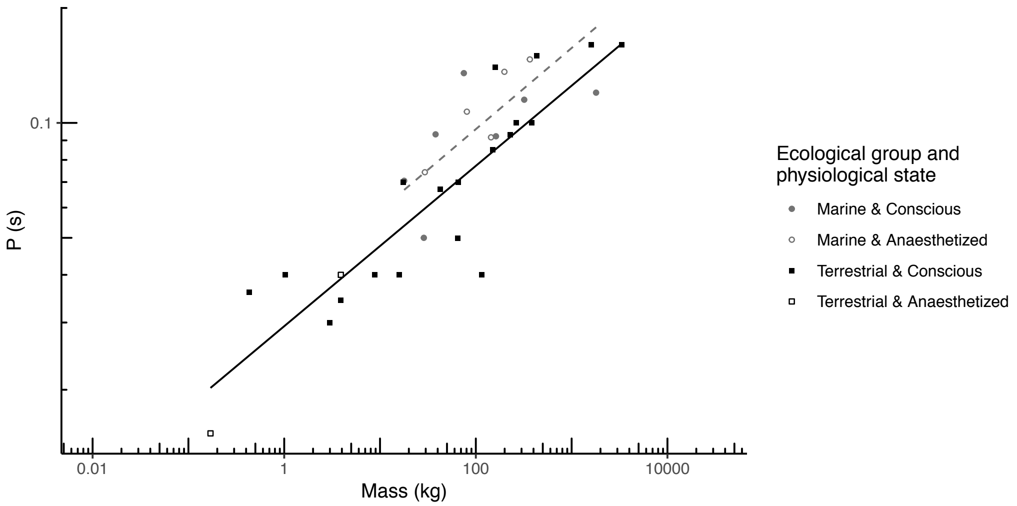

Figure 3.3 Relationship between body mass and P-wave duration in marine (grey circles and dashed line) and terrestrial (black squares and solid line) mammals. Anaesthetized species are represented as filled squares and circles, and conscious species as open squares and circles. Ecological group had a significant effect on P-wave duration when mass was corrected for as a covariate. Regression equations are stated in Table 3.2.

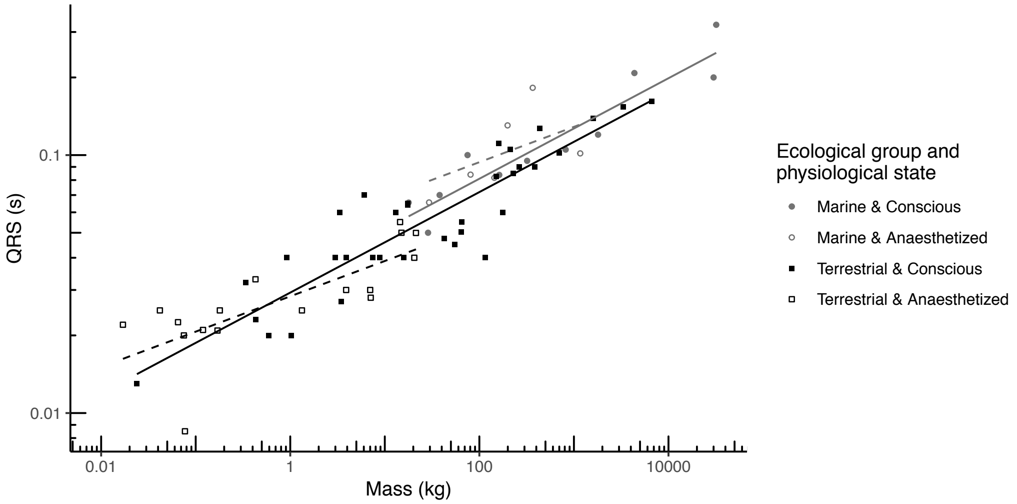

Figure 3.4 Relationship between body mass, ecological group, physiological state, and QRS complex duration. Recordings of conscious mammals are indicated by filled squares and circles and regressions are shown as solid lines, while anaesthetized mammals are indicated by open squares and circles and regressions are shown as dashed lines. Terrestrial mammals are shown in black and marine mammals are shown in grey. Regression equations are stated in Table 3.2.

Discussion

Our results show that the mammalian ECG waveform is well conserved over a wide range of body masses. However, some components of the ECG waveform differ between marine and terrestrial mammals. Most notable is that marine mammals have a longer P-wave and a wider QRS complex than terrestrial mammals, which is indicative of prolonged atrial and ventricular depolarization. In other words, conduction through the atria and ventricles in marine mammals is slower than in terrestrial mammals, which likely reflects differences in cardiac anatomy between these two groups.

We detected differences between the ECGs of marine and terrestrial mammals despite the large between-species variation observed for all parameters. About one quarter of the species we studied fell on or were close to the allometric regression lines, indicating that body mass is not the only factor influencing electrical signal conduction through the myocardium. This variation highlights species-specific differences in cardiac electrophysiology that may relate to unique cardiac adaptations. Consequently, our comparisons between marine and terrestrial mammals may not apply to all of the species that we grouped within these categories because the hearts of some marine mammals perform more similarly to the hearts of terrestrial mammals and vice versa. However, these ecological groupings were necessary for us to effectively address the question of whether the electrical activity of the heart differs between marine and terrestrial mammals.

In terms of how the heart behaves as a function of body size, we found that the P-wave, PR, QRS, and QT intervals all increased with body mass in mammals — and all scaled to mass to the power of ~0.21. In terms of heart rate however, bigger mammals have bigger hearts that beat slower, and scaled to body mass to the power of ~-0.22. These data add to the debate over how ECG parameters scale to body mass in mammals because our scaling exponents are inconsistent with other predicted and empirical values.

Anaesthesia had a noticeable effect on how the heart functioned. Most apparent was how anaesthesia dropped the QRS scalar with body mass from the power of ~0.20 to ~0.14. Our analysis of physiological state also suggests that anaesthetized animals may have faster heart rates than conscious animals. Potential explanations for these counterintuitive results lie in the different types of anaesthetic protocols used when performing ECGs.

Marine and terrestrial mammals

The differences we found in cardiac parameters in our comparison between marine and terrestrial mammals—a longer P-wave and a wider QRS complex—suggest that cardiac anatomy may also differ between these two ecological groups. The duration of ECG parameters is influenced by the mass of the myocardium that the electrical signal must pass through because conduction time increases with distance (Bayés De Luna et al., 2012). For example, long QRS intervals are commonly observed in association with left ventricular hypertrophy, especially in elite athletes (Dorn, 2007; Zelenkova and Chomahidze, 2016). Hence, the longer P-waves and QRS complexes of marine mammals may indicate that they have greater atrial and ventricular myocardial mass or differently shaped cardiac chambers than terrestrial mammals. This is supported by anatomical reports of the broad, dorsoventrally compressed hearts of phocids (Drabek, 1975; Rowlatt, 1990); a shape that would potentially increase the signal conduction distance slowing atrial and ventricular depolarization.

Atrial and ventricular depolarization were slower in marine mammals, but this had no effect on overall cardiac timing (heart rates scaled the same for all mammals). A probable explanation is that the enlarged Purkinje fibres and Todd fibres found in some species of marine mammals increase signal conduction velocity through the myocardium (Nie, 1988, 1987; Pfeiffer, 1990).

In terms of the ECG, the duration of the PR interval was the same in marine and terrestrial mammals, but the P-wave was longer in marine mammals. This means that the electrical signal travels through the conduction pathways (SA node to AV node to the ends of the Purkinje fibres) with the same timing in marine and terrestrial mammals, even though signal conduction through the atrial and ventricular myocardium is slower. Faster signal propagation from the AV node to the ventricular myocardium could result from the presence of enlarged Purkinje fibres in marine mammals (Pfeiffer, 1990; van Nie, 1986). Although electrical activity through Purkinje fibres is not visible on ECGs, the isoelectric line between the end of the P-wave and the start of the QRS complex represents conduction through the Purkinje fibres (along with the bundle of His, and the bundle branches) (Boron and Boulpaep, 2017). Therefore, the prolonged P-wave in marine mammals, despite there being no difference in the length of the PR interval, likely corresponds to faster signal conduction through the enlarged Purkinje fibres of marine mammals.

Theoretically, the Todd fibres found in white-beaked dolphins (Nie, 1987) could also decrease the timing of the electrical activity to support the longer P-wave and QRS complex despite heart rate remaining the same. However, it is more difficult to predict the effect of Todd fibres on the ECG because conduction from the SA node to the AV node happens concurrently with atrial depolarization.

It is important to note that we grouped all marine mammals together, but only a few species are known to have Todd fibres and enlarged Purkinje fibres. Looking at where individual species fall on our allometric trendlines may allow us to make inferences about which species have these specialized conduction pathways and which ones likely do not.

Taking these findings together, our ECG data suggests that marine mammals have enlarged or elongated cardiac chambers that require faster conduction pathways to maintain proper cardiac timing for filling and contraction. Thus, electrical cardiac function is different in marine mammals to support anatomical differences—not to directly support the dive response. However, the inferred structural differences, such as dorsoventral flattening of the heart, likely are adaptations to diving.

We were surprised to find no difference between the heart rates of similar sized marine and terrestrial mammals. Whether this reflects the challenge of determining what constitutes a "resting heart rate" for marine mammals is unknown. It may also be important to distinguish between whales, phocids (true seals), and otariids (fur seals and sea lions) as the "resting" cardiac state may be determined by the proportion of time spent diving, surfacing, and on land (in the case of pinnipeds). For example, whales and seals spend more time diving, so a bradycardic heart rate might be considered their resting state. In contrast, sea lions spend more time hauled-out, so it might be more appropriate to measure their resting heart rate at the surface.

Most ECG measurements of marine mammals to date have been recorded on land or while resting at the surface, both for logistical reasons as well as to standardize the measurement process. In the future, a comparison of heart rates between marine and terrestrial mammals that account for these concepts of "true" resting conditions may prove more physiologically relevant for explaining differences between ecological groups due to the dive response.

Effects of anaesthesia

The effects of anaesthesia on cardiac timing are complicated. Our results showed that anaesthetized marine mammals had longer QRS intervals than similarly sized anaesthetized terrestrial mammals. No effects of anaesthesia on QRS intervals have been previously reported, but anaesthetic agents such as isoflurane, desflurane, and sevoflurane are known to increase the QT interval (Yildirim et al., 2004). It is unclear why our study showed that anaesthesia affects the QRS rather than the QT interval, but it is interesting that the impacts were different on marine and terrestrial mammals.

We also observed a trend towards higher heart rates in anaesthetized individuals. Past studies have also shown that anaesthesia can increase heart rate (Picker et al., 2001). This may seem counter-intuitive, as the lack of response to stimuli of animals in an anaesthetized state elicits images of a slowed heart rate. In reality, however, anaesthesia can increase heart rate by removing vagal stimulation that normally serves to decrease heart rate (Picker et al., 2001), although the effects are likely dependent on anaesthetic protocols. While our findings lend support for this mechanism acting across a range of species and body masses, limited information was available about the anaesthetic protocol used (e.g., type, plane, and duration of anaesthesia), which prevented us from undertaking further analyses.

ECG allometry

Many anatomical, physiological, and ecological factors are known to scale allometrically with body mass in animals, and electrical signal conduction through the heart is no different. The specific mathematical relationship between body mass and ECG parameters lends insight into basic constraints and consequences of evolutionary design that are relevant for understanding the evolutionary function of deviations from defined allometric relationships. By performing allometric analyses, we were also able to identify differences that exist between species based on ecological niche and physiological state, which may be indicative of special anatomical or physiological adaptations in marine mammals.

The ECG scaling exponents we found closely agree with other experimentally-derived cardiac scaling exponents. Günther and Morgado (1997) found that the RR, PQ, QRS, and QT intervals scaled with body mass to the power of 0.20, while our scaler ranged from 0.195 to 0.217 (with the exception of QRS intervals for anaesthetized animals). Noujaim et al. (2004) found that mammalian PR intervals scale to a power of 0.24, comparatively higher than our analysis which determined the scaling exponent to be 0.21. The similarities between our results and that of Günther and Morgado (1997) is likely because we followed similar methods and drew data from some of the same sources (e.g., Grauwiler, 1965). Additionally, having more data from marine mammal species may explain the lower value of our PR interval scaling compared to that of Noujaim et al. (2004).

There is debate regarding the theoretical foundation for how ECG characteristics should scale with body mass. Recent studies examining the scaling of ECG parameters with body size report exponents more consistent with the theoretical one-quarter scaling law (Meijler and Meijler, 2011) than with the simple one-third law predicted by Euclid—suggesting that fractal geometry is a more likely explanation for how ECG parameters scale with body mass than volumetric scaling. Still, our empirically-derived scaling exponents are lower than the theoretically-predicted exponent for all of the ECG parameters we explored. Although our observed values of ~±0.20 are close to the widely accepted 0.25 derived from fractal geometry, as these values are exponents, small deviations have large impacts on the estimated range of cardiac measurements for a mammal of a given size.

It is possible that the discrepancy between our data and the theoretical exponents simply reflect variability in nature, measurement error, and technique and operator variability. It is also possible that the theoretically derived scaling arguments are based on a supposed “average idealized animal” that are simply approximations meant to aid understanding of fundamental biological principles (West and Brown, 2005). Currently, no theoretical mechanism exists to explain our consistent allometric scaling to the power of 0.20.

Conclusion

Overall, our study supports previous findings about mammalian ECG interval and heart rate scaling while demonstrating the need to consider ecological and physiological groups when making comparisons based on allometric relationships. Clear differences in the cardiac timing of marine mammals appear to support anatomical adaptations to diving, rather than acting as functional adaptations themselves. The timing of electrical conduction through the myocardium is altered slightly in marine mammals, probably to maintain the timing of chamber filling and contraction. Without this unique timing, the heart beats of marine mammals would be slowed, and could negatively affect circulation.

Chapter 4: General discussion

I measured electrocardiographic variables in Steller sea lions, northern fur seals, and a walrus to assess cardiac function in marine mammals and infer potential cardiac adaptations to diving. I found within- and between-species variation in cardiac waveform timing and heart rates of these three pinniped species, and obtained reference values of echocardiographic function in Steller sea lions to compare with other mammals. Comparisons with terrestrial mammals revealed key differences in both the electrical and left ventricular function of the hearts of marine mammals that suggest marine mammal hearts may be adapted to diving.

My first two research goals were to 1) measure electrocardiographic function in three pinnipeds (Steller sea lions, northern fur seals, and walruses), and to 2) examine left ventricular function in Steller sea lions. Evaluating baseline cardiac function in Steller sea lions (i.e., cardiac rhythm and left ventricular function) provided foundational measurements from which I inferred that cardiac function in marine mammals differs from terrestrial mammals. I confirmed that the aortic root in Steller sea lions is larger than the dimension of the left ventricle, which is consistent with having an aortic bulb (Murie, 1874). My second finding was that the end-systolic dimension of the left ventricle in Steller sea lions is larger than in terrestrial mammals of the same size (similar to southern sea lions). However, the end-diastolic dimension of the sea lion hearts is similar to terrestrial mammals, which results in decreased fractional shortening. In other words, Steller sea lion hearts at rest contract less than the hearts of terrestrial mammals.

It is unclear what effect decreased fractional shortening at rest has on the capacity for alterations in stroke volume. One possibility is that contracting the left ventricle at a lower set-point at rest than seen in terrestrial mammals allows the marine mammal heart to undergo a greater degree of contraction to increase blood flow when exercising or diving. However, stroke volume typically does not increase in diving mammals (Elsner et al., 1964; Ponganis et al., 2006, 1991), which suggests there is another yet unknown reason for marine mammals having reduced fractional shortening.

The third goal of my thesis research was to compare electrical function between the hearts of marine and terrestrial mammals. My main findings were that marine mammals have longer P-waves and QRS complexes than terrestrial mammals, which means that it takes longer for atrial and ventricular contraction to occur in marine mammal hearts after the first signal is sent by the sinoatrial or atrioventricular nodes. However, heart rates of resting marine mammals are not significantly different from the heart rates of terrestrial mammals of the similar body size. This means that atrial depolarization and ventricular depolarization take a longer time, but the overall duration of the cardiac cycle remains the same.

My best guess to explain the longer P-waves and QRS complexes of marine mammals is that they have a larger atrial or ventricular myocardial mass, or elongated atria and ventricles. Dissections show that pinniped hearts are broader than terrestrial mammal hearts (Drabek, 1975; Smodlaka et al., 2008), but fail to support the contention that cardiac mass is different (Prothero, 1979).

My results potentially show the effects of specialized conductive tissues such as Todd fibres and enlarged Purkinje fibres that have been previously described from dissections (Nie, 1987; van Nie, 1986). The prolonged atrial and ventricular depolarization that I observed did not affect heart rates, which indicates that another component of cardiac electrical activity must have been faster. The simplest explanation is that Todd and Purkinje fibres speed up conduction to compensate for the slowed electrical activity through the atria and ventricles.

Strengths and weaknesses

One major strength of my study was the team of people and the animals that I worked with. Veterinarians collaborated to anaesthetize the animals and perform the ECGs and echocardiograms. A veterinary cardiologist performed all of the echocardiograms and made measurements from all of the ECG readouts and ultrasound images. His expertise gives me confidence in the measurements. Working with animals in human care meant knowing the health and history of each individual, and gave me the option to re-assess individuals if needed. In addition, training is important for ensuring low levels of stress prior to the procedures, which reduces the required level of anaesthesia and associated physiological changes and risk to the animal.

Small sample size is a common weakness in marine mammal studies, both when working with animals in human care and in the wild. Despite this limitation, the ECG data for female Steller sea lions was normally distributed suggesting that it represents this species well. However, the measurements of Steller sea lion echocardiographic parameters were not normally distributed, which may indicate that the sample size was not large enough to capture the natural variation and thus be representative of the population. Unfortunately, it was not logistically possible to obtain more measurements from the three species I studied.

Measuring cardiac function in anaesthetized animals is often regarded as a weakness, but was necessary in my study and the only way I could obtain a complete echocardiographic exam of a Steller sea lion. Anaesthetic protocols affect cardiac function and the magnitude of the effect is protocol-dependent. My allometric ECG analysis showed that anaesthesia had no effect on most ECG parameters. The one exception was the QRS complex, which depended on the physiological state of the animal. Due to the large number of terrestrial and marine species, and the wide range of anesthetic protocols included in the analysis, I was able to use data from anaesthetized individuals and evaluate electrocardiographic relationships based on physiological state.

For the echocardiographic analysis, it is unclear what effect anaesthesia had on the parameters measured, but the values I obtained were similar to those collected from conscious southern sea lions. This suggests that echocardiographic measurements may not be as sensitive to anaesthetic protocols as ECG measurements. Lastly, it seems unlikely that echocardiograms can be obtained from conscious Steller sea lions, because the procedure required the application of significant pressure to their chests which may not be tolerated even by trained individuals. For this reason, anesthetized values provide a good baseline for Steller sea lions.

The data in my thesis must be considered with several caveats in mind. When drawing comparisons, the datasets did not include an exhaustive list of species. However, I added a distinct group that was previously underrepresented in cardiac scaling arguments—marine mammals. In my allometric comparisons, I often relied on a single individual to represent a species and I used an approximation of body size when mass was not stated in a literature source. By estimating masses, I may not have a true representation of the individual(s) from which the ECG measurements were recorded. However, given the logarithmic scaling of body mass, I feel that this did not greatly affect the results.

Unfortunately, the available literature on left ventricular function is only a fraction of what is available for electrocardiography, and only three species of marine mammals (including Steller sea lions) have been studied so far using this technology. This greatly limited my ability to confidently address whether marine mammals have adaptations to left ventricular function and pulmonary and aortic blood flow.

In my ECG comparison, I was unable to include metabolic state (e.g., resting, active, fasted etc.)—which has implications for my heart rate comparisons. Furthermore, I did not have enough records to assess differences between cetaceans and pinnipeds. The different dive behaviours between taxonomic groups of marine mammals could reveal more interesting trends. Despite these caveats, I found plausible linear trends for all ECG parameters and conducted a thorough investigation addressing cardiac function in marine and terrestrial mammals—as well as in anaesthetized and conscious mammals.

My research only identifies functional differences between the hearts of marine and terrestrial mammals. From my comparisons, I can only suggest that cardiac adaptations to diving may exist and provide possible explanations for the observed differences. It is interesting to note that despite the majority of the measurements of marine mammals being taken at rest, and none of the measurements being taken while diving, I still observed differences between marine and terrestrial mammals. The measurable presence of these potential adaptations in resting individuals suggests that they are constant, which further supports my hypothesis that they exist as a result of anatomical adaptations to diving. If they were functional adaptations to diving, I would expect them to only be observed during dives.

Future research

Future research should focus on cardiac function in marine mammals while diving. Observing how cardiac timing and left ventricular function change during a dive would provide some indication of what the functional consequence is of the differences observed in rest. Ideally resting measurements of an individual can be compared to diving measurements. With advances in implantable cardiac monitoring technology, recording an ECG through the duration of a dive is possible. Unfortunately, echocardiography has not advanced as far. However, measurements can be taken underwater, so the potential to measure left ventricular function in individuals breath-holding near the surface is possible.

To further advance understanding of left ventricular function, it would be beneficial to model the left ventricle of a marine mammal heart. Measurements of ejection fraction and stroke volume, two important values for assessing left ventricular function, can be estimated from echocardiography if the shape and size of the left ventricle is known. So far, these values have only been calculated under a set of assumptions about the shape and size of the left ventricle—

assumptions that have only been validated in humans. Thus, modelling the left ventricle of a marine mammal, and performing echocardiography would provide even better insight into how the left ventricle functions.

Conclusion

In all, my comparisons show that the hearts of marine mammals function quite similarly to those of terrestrial mammals, with some notable differences that I believe are evolutionary modifications related to diving because knowledge of the mammalian dive response alone does not completely explain how marine mammals can perform long-duration dives.

My research addressed the function of one organ, the heart, with respect to diving and its potential adaptations. Further understanding of how these observed differences in function affect marine mammal physiology while diving is unclear, but my results provide insights into potential limitations to diving. Comparisons with other species often allow for reflection on current knowledge. Re-assessment of current understanding of myocardial tissue, signal conduction, and left ventricular function is needed to understand whether the differences noted for marine mammals are novel or whether they are simple modifications and adjustments of what already exists in all mammals. If only minor adjustments, as suggested from my findings, it begs asking exactly what they are and how they allow the hearts of marine mammals to function normally under extreme conditions.

References

Bayés De Luna, A., Bayés Genis, A., Brugada, R., Fiol, M., Zareba, W., 2012. Characteristics of the normal electrocardiogram: Normal ECG waves and intervals, in: Clinical Electrocardiography. John Wiley & Sons, Ltd, Barcelona, Spain, pp. 67–94. doi:10.1002/9781118392041.ch7

Ben-Tal, A., Shamailov, S.S., Paton, J.F.R., 2012. Evaluating the physiological significance of respiratory sinus arrhythmia : looking beyond ventilation – perfusion. J. Physiol. 8, 1989–2008. doi:10.1113/jphysiol.2011.222422

Blix, A.S., Folkow, B., 1983. Cardiovascular adjustments to diving in mammals and birds. Handb. Physiol. 3, 917–945. doi:10.1002/cphy.cp020325

Blix, A.S., Kjekshus, J.K., Enge, I., Bergan, A., 1976. Myocardial blood flow and metabolism in the diving seal. Acta Physiol. Scand. 96, 277–280.

Boon, J., Wingfield, W.E., Miller, C.W., 1983. Echocardiographic indices in the normal dog. Vet. Radiol. 24, 214–221. doi:10.1111/j.1740-8261.1983.tb00718.x

Boron, W.F., Boulpaep, E.L., 2017. Medical physiology, 3rd ed. Elsevier, Philadelphia, PA.

Butler, P., 1982. Respiratory and cardiovascular control during diving in birds and mammals. J. Exp. Biol. 100, 195–221.

Campbell, K.B., Rhode, E.A., Cox, R.H., Hunter, W.C., Noordergraaf, A., 1981. Functional consequences of expanded aortic bulb: a model study. Am. Physiol. Soc. 240, R200–R210.

Castellini, M.A., Rea, L.D., Sanders, J.L., Castellini, J.M., Zenteno-Savin, T., 1994. Developmental changes in cardiorespiratory patterns of sleep-associated apnea in northern elephant seals. Am. J. Physiol. 267, R1294-301.

Castro, E.F., Dassis, M., De León, M.C., Rodríguez, E., Davis, R.W., Saubidet, A., Rodríguez, D.H., Díaz, A., 2018. Echocardiographic left ventricular structure and function in healthy, non-sedated southern sea lions (*Otaria flavescens*). Aquat. Mamm. 44, 405–410. doi:10.1578/AM.44.4.2018.405

Chetboul, V., Lichtenberger, J., Mellin, M., Mercera, B., Hoffmann, A.C., Chaix, G., Trehiou-Sechi, E., Misbach, C., Petit, A., Lefebvre, H.P., Gaide, N., Tissier, R., Delfour, F., 2012. Within-day and between-day variability of transthoracic anatomic M-mode echocardiography in the awake bottlenose dolphin (*Tursiops truncatus*). J. Vet. Cardiol. 14, 511–518. doi:10.1016/j.jvc.2012.07.002

Cornell, C.C., Kittleson, M.D., Della Torre, P., Häggström, J., Lombard, C.W., Pedersen, H.D., Vollmar, A., Wey, A., 2004. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 18, 311–321. doi:10.1111/j.1939-1676.2004.tb02551.x

Dassis, M., Rodríguez, D.H., Rodríguez, E., Ponce De León, A., Castro, E., 2016. The electrocardiogram of anaesthetized southern sea lion (*Otaria flavescens*) females. J. Vet. Cardiol. 18, 71–78. doi:10.1016/j.jvc.2015.09.003

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. - A Mol. Integr. Physiol. 138, 263–268. doi:10.1016/j.cbpb.2004.05.003

Davis, R.W., Williams, T.M., 2012. The marine mammal dive response is exercise modulated to maximize aerobic dive duration. J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol. 198, 583–591. doi:10.1007/s00359-012-0731-4

Dennison, S.E., Boor, M., Fauquier, D., van Bonn, W., Greig, D.J., Gulland, F.M.D., 2011a. Foramen ovale and ductus arteriosus patency in neonatal harbor seal (*Phoca vitulina*) pups in rehabilitation. Aquat. Mamm. 37, 161–166. doi:10.1578/AM.37.2.2011.161

Dennison, S.E., Van Bonn, W., Boor, M., Adams, J., Pussini, N., Spraker, T., Gulland, F.M.D., 2011b. Antemortem diagnosis of a ventricular septal defect in a California sea lion *Zalophus californianus*. Dis. Aquat. Organ. 94, 83–88. doi:10.3354/dao02316

Diniz, A.N., Pessoa, G.T., Moura, L.S., Sousa, F.C.A., Rodrigues, R.P.S., Souza, A.B., Almeida, H.M., Freire, L.D.S., Sanches, M.P., Alves, F.R., 2017. Echocardiographic findings of bidimensional mode, M-mode, and Doppler of clinically normal black-rumped agouti (Dasyprocta prymnolopha, Wagler 1831). J. Zoo Wildl. Med. 48, 287–293.

Dorn, G.W., 2007. The fuzzy logic of physiological cardiac hypertrophy. Hypertension 49, 962–970. doi:10.1161/HYPERTENSIONAHA.106.079426

Drabek, C.M., 1975. Some anatomical aspects of the cardiovascular system of antarctic seals and their possible functional significance in diving. J. Morphol. 145, 85–105. doi:10.1002/jmor.1051450106

Drabek, C.M., Burns, J.M., 2002. Heart and aorta morphology of the deep-diving hooded seal

(*Cystophora cristata*). Can. J. Zool. Can. Zool. 80, 2030–2036. doi:Doi 10.1139/Z02-181

Elmegaard, S.L., Johnson, M., Madsen, P.T., Mcdonald, B.I., 2016. Cognitive control of heart rate in diving harbor porpoises. Curr. Biol. Mag. 1175–1176.

Elsner, R., 1969. Cardiovascular adjustments to diving, in: Andersen, H.T. (Ed.), The Biology of Marine Mammals. Academic Press, New York, New York, pp. 117–145.

Elsner, R.W., Franklin, D.L., Van Citters, R.L., 1964. Cardiac output during diving in an unrestrained sea lion. Nature 202, 809–810. doi:10.1038/202809a0

Falabella, V., Campagna, C., Lewis, M., Falabella, V., Campagna, C., Lewis, M., 1999. Electrocardiography of southern elephant seal (*Mirounga leonina*) weanlings. J. Zoo Wildl. Med. 30, 526–531.

Fontes-Sousa, A.P.N., Bras-Silva, C., Moura, C., Areias, J.C., Leite-Moreira, A.F., 2006. M-mode and Doppler echocardiographic reference values for male New Zealand white rabbits. Am. J. Vet. Res. 67, 1725–1729.

Grauwiler, J., 1965. Herz und Kreislauf der Säugetiere. Vergleichend-funktionelle Daten von Jules Grauwiler, mit einem Geleitwort von Heinrich Spörri. Birkhäuser Verlag, Basel.

Green, J.A., 2011. The heart rate method for estimating metabolic rate: Review and recommendations. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 158, 287–304. doi:10.1016/j.cbpa.2010.09.011

Grossman, P., Kollai, M., 1993. Respiratory sinus arrhythmia, cardiac vagal tone, and respiration: Within- and between-individual relations. Psychophysiology 30, 486–495.

Guglielmini, C., Rocconi, F., Brugnola, L., Valerio, F., Mattei, L., Boari, A., 2006. Echocardiographic and Doppler echocardiographic findings in 11 wolves (*Canis lupus*). Vet. Rec. 158, 125–129.

Günther, B., Morgado, E., 1997. Allometry of ECG waves in mammals. Biol. Res. 30, 167–170.

Häggström, J., Andersson, O., Falk, T., Nilsfors, L., OIsson, U., Kresken, J.G., Höglund, K., Rishniw, M., Tidholm, A., Ljungvall, I., 2016. Effect of body weight on echocardiographic measurements in 19,866 pure-bred cats with or without heart disease. J. Vet. Intern. Med. 30, 1601–1611. doi:10.1111/jvim.14569

Hallowell, G.D., Potter, T.J., Bowen, I.M., 2007. Methods and normal values for echocardiography in adult dairy cattle. J. Vet. Cardiol. 9, 91–98. doi:10.1016/j.jvc.2007.10.001

Hamlin, R.L., Ridgway, S.H., Gilmartin, W.G., 1972. Electrocardiogram of pinnipeds. Am. J. Vet. Res. 33, 867–875.

Harms, C.A., Jensen, E.D., Townsend, F.I., Hansen, L.J., Schwacke, L.H., Rowles, T.K., 2013. Electrocardiograms of bottlenose dolphins (Tursiops truncatus) out of water: habituated collection versus wild postcapture animals. J. Zoo Wildl. Med. 44, 972–81. doi:10.1638/2013-0093.1

Hindle, A.G., Young, B.L., Rosen, D.A.S., Haulena, M., Trites, A.W., 2010. Dive response differs between shallow- and deep-diving Steller sea lions (Eumetopias jubatus). J. Exp. Mar. Bio. Ecol. 394, 141–148. doi:10.1016/j.jembe.2010.08.006

Holt, J.P., Rhode, E.A., Kines, H., 1968. Ventricular volumes and body weight in mammals. Am. J. Physiol. 215, 704–715.

Holt, J.P., Rhode, E.A., Peoples, S.A., Kines, H., 1962. Left ventricular function in mammals of greatly different size. Circ. Res. 10, 798–806.

Irving, L., Scholander, P.F., Grinnell, S.W., 1941. The regulation of arterial blood pressure in the seal during diving. Am. J. Physiol. 135, 557–566. doi:10.1152/ajplegacy.1942.135.3.557

Kastelein, R.A., Meijler, F.L., 1989. Respiratory arrhythmia in the hearts of Harbour porpoises (*Phocoena phocoena*). Aquat. Mamm. 15.2, 57–63.

Kimura, Y., Karakama, S., Kobayashi, M., Machinda, N., 2016. Incidence, distribution and morphology of left ventricular false tendons in cat hearts. J. Vet. Med. Ser. C Anat. Histol. Embryol. 45, 490–493. doi:10.1111/ahe.12216

King, J.E., 1977. Comparative anatomy of the major blood vessels of the sealions *Neophoca* and *Phocarctos*; with comments on the differences between the otariid and phocid vascular systems. J. Zool 181, 69–94. doi:10.1111/j.1469-7998.1977.tb04571.x

King, R.L., Jenks, J.L., White, P.D., 1953. The electrocardiogram of a beluga whale. Circulation 8, 387–393. doi:10.1161/01.CIR.8.3.387

Kooyman, G.L., Ponganis, P.J., 1998. The physiological basis of diving to depth: birds and mammals. Annu. Rev. Physiol. 60, 19–32. doi:10.1146/annurev.physiol.60.1.19

Kusunose, K., Penn, M.S., Zhang, Y., Cheng, Y., Thomas, J.D., Marwick, T.H., Popović, Z.B.,

2012. How similar are the mice to men? Between-species comparison of left ventricular mechanics using strain imaging. PLoS One 7. doi:10.1371/journal.pone.0040061

Leroux, A.A., Moonen, M.L., Farnir, F., Sandersen, C.F., Deleuze, S., Salciccia, A., Amory, H., 2012. Two-dimensional and M-mode echocardiographic reference values in healthy adult Saanen goats. Vet. Rec. 170, 154. doi:10.1136/vr.100192

Meijler, F.L., 1985. Atrioventricular conduction versus heart size from mouse to whale. J. Am. Coll. Cardiol. 5, 363–365. doi:10.1016/S0735-1097(85)80060-7

Meijler, F.L., Meijler, T.D., 2011. Archetype, adaptation and the mammalian heart. Netherlands Hear. J. 19, 142–148. doi:10.1007/s12471-011-0086-4

Meijler, F.L., van der Tweel, L.H., 1986. De elektrocardiogrammen van 10 olifanten en van de orka in Harderwijk. Ned Tijdschr Geneeskd 130, 2344–2348.

Meijler, F.L., Wittkampf, F.H.M., Brennen, K.R., Baker, V., Wassenaar, C., Bakken, E.E., 1992. Electrocardiogram of the humpback whale (*Megaptera novaeangliae*) with specific reference to atrioventricular transmission and ventricular excitation. J. Am. Coll. Cardiol. 20, 475–479. doi:10.1016/0735-1097(92)90120-C

Miedler, S., Fahlman, A., Valls Torres, M., Álvaro Álvarez, T., Garcia-Parraga, D., 2015. Evaluating cardiac physiology through echocardiography in bottlenose dolphins: using stroke volume and cardiac output to estimate systolic left ventricular function during rest and following exercise. J. Exp. Biol. 218, 3604–3610. doi:10.1242/jeb.131532

Morrison, S.A., Moise, N.S., Scarlett, J., Mohammed, H., Yeager, A.E., 1992. Effect of breed and body weight on echocardiographic values in four breeds of dogs of differing somatotype. J. Vet. Intern. Med. 6, 220–224. doi:10.1111/j.1939-1676.1992.tb00342.x

Murdaugh, H. V, Robin, E.D., Millen, J.E., Drewry, W.F., Weiss, E., 1966. Adaptations to diving in the harbor seal: cardiac output during diving. Am. J. Physiol. 210, 176–180.

Murie, J., 1874. Researches upon the anatomy of the Pinnipedia.—(Part III.) Descriptive anatomy of the sea-lion (Otaria jubata). Trans. Zool. Soc. London 8, 501–582.

Nie, C.J. van, 1988. The conducting system of the heart of the Harbour Porpoise (Phocoena phocoena, Linnaeus, 1758). Aquat. Mamm. 14.2, 69–72.

Nie, C.J. van, 1987. Todd fibres—Purkinje fibres—in the wall of the right atrium in dolphins

(Their structure and hypothetical function). Aquat. Mamm. 13.3, 103–104.

Noujaim, S.F., Lucca, E., Muñoz, V., Persaud, D., Berenfeld, O., Meijler, F.L., Jalife, J., 2004. From mouse to whale: A universal scaling relation for the PR interval of the electrocardiogram of mammals. Circulation 110, 2802–2808. doi:10.1161/01.CIR.0000146785.15995.67

Noviana, D., Kurniawan, L.K.L., 2013. Heart size evaluation of Indonesian domestic house cat by motion mode echocardiography imaging. HAYATI J. Biosci. 20, 40–46. doi:10.4308/hjb.20.1.40

Otto, C.M., 2018. Textbook of clinical echocardiography, Sixth. ed. Elsevier/Saunders, Philadelphia, PA.

Petrič, A.D., Rishniw, M., Thomas, W.P., 2012. Two-dimensionally-guided M-mode and pulsed wave Doppler echocardiographic evaluation of the ventricles of apparently healthy cats. J. Vet. Cardiol. 14, 423–430. doi:10.1016/j.jvc.2012.04.004

Pfeiffer, C.J., 1990. Observations on the ultrastructural morphology of the bowhead whale (*Balaena mysticetus*) heart. J. Zoo Wildl. Med. 21, 48–55.

Picker, O., Scheeren, T.W.L., Arndt, J.O., 2001. Inhalation anaesthetics increase heart rate by decreasing cardiac vagal activity in dogs. Br. J. Anaesth. 87, 748–754. doi:10.1093/bja/87.5.748

Ponganis, P., 2015. Diving Physiology of Marine Mammals and Seabirds. Cambridge University Press, Cambridge. doi:10.1017/CBO9781139045490

Ponganis, P.J., Kooyman, G.L., 1999. Heart rate and electrocardiogram characteristics of a young California gray whale (*Eschrichtius robustus*). Mar. Mammal Sci. 15, 1198–1207.

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

Ponganis, P.J., McDonald, B.I., Tift, M.S., Williams, C.L., 2017. Heart rate regulation in diving sea lions: the vagus nerve rules. J. Exp. Biol. 220, 1372–1381. doi:10.1242/jeb.146779

Ponganis, P.J., Stockard, T.K., Levenson, D.H., Berg, L., Baranov, E.A., 2006. Cardiac output and muscle blood flow during rest-associated apneas of elephant seals. Comp. Biochem. Physiol. Part A 144, 105–111. doi:10.1016/j.cbpa.2006.02.009

Prothero, J., 1979. Heart weight as a function of body weight in mammals. Growth 43, 139–50.

Rhode, E.A., Elsner, R., Peterson, T.M., Campbell, K.B., Spangler, W., 1986. Pressure-volume characteristics of aortas of harbor and Weddell seals. Am. J. Physiol. 251, R174–R180.

Rowlatt, U., 1990. Comparative anatomy of the heart of mammals. Zool. J. Linn. Soc. 98, 73–110. doi:10.1111/j.1096-3642.1990.tb01220.x

Santilli, R.A., Perego, M., 2009. Elettrocardiografia del cane e del gatto: Genesi e interpretazione del ritmo cardiaco. Elsevier Masson, Italy.

Scansen, B.A., Morgan, K.L., 2015. Reference intervals and allometric scaling of echocardiographic measurements in Bengal cats. J. Vet. Cardiol. 17, S282–S295. doi:10.1016/j.jvc.2015.02.001

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

Senft, A.W., Kanwisher, J.K., 1960. Cardiographic observations on a fin-back whale. Circ. Res. 8, 961–964. doi:10.1161/01.RES.8.5.961

Shadwick, R.E., Gosline, J.M., 1994. Arterial mechanics in the fin whale suggest a unique hemodynamic design. Am. J. Physiol. 267, R805-18.

Siegal-Willott, J., Estrada, A., Bonde, R., Wong, A., Estrada, D.J., Harr, K., 2006. Electrocardiography in two subspecies of manatee (*Trichechus manatus latirostris* and *T. m. manatus*). J. Zoo Wildl. Med. 37, 447–453.

Smodlaka, H., Henry, R.W., Reed, R.B., 2009. Macroscopic anatomy of the great vessels and structures associated with the heart of the ringed seal (*Pusa hispida*). J. Vet. Med. Ser. C Anat. Histol. Embryol. 38, 161–168. doi:10.1111/j.1439-0264.2008.00896.x

Smodlaka, H., Henry, R.W., Schumacher, J., Reed, R.B., 2008. Macroscopic anatomy of the heart of the ringed seal (*Phoca hispida*). J. Vet. Med. Ser. C Anat. Histol. Embryol. 37, 30–35. doi:10.1111/j.1439-0264.2007.00791.x

Stewardson, C.L., Hemsley, S., Meyer, M.A., Canfield, P.J., Maindonald, J.H., 1999. Gross and microscopic visceral anatomy of the male Cape fur seal, *Arctocephalus pusillus pusillus* (Pinnipedia: Otariidae), with reference to organ size and growth. J. Anat 195, 235–255. doi:10.1017/S0021878299005270

Surawicz, B., Childers, R., Deal, B.J., Gettes, L.S., 2009. Recommendations for the standardization and interpretation of the electrocardiogram. J. Am. Coll. Cardiol. 53, 976–981. doi:10.1016/j.jacc.2007.01.025

Tarpley, R.J., Hillmann, D.J., Henk, W.G., George, J.C., 1997. Observations on the external morphology and coronary vasculature of the heart of the bowhead whale, *Balaena mysticetus*. Anat. Rec. 247, 556–581.

Thornton, S.J., Hochachka, P.W., Crocker, D.E., Costa, D.P., Leboeuf, B.J., Spielman, D.M., Pelc, N.J., 2005. Stroke volume and cardiac output in juvenile elephant seals during forced dives. J. Exp. Biol. 208, 3637–3643. doi:10.1242/jeb.01789

Torad, F.A., Amer, M.S., Shamaa, A.A., Elsherpieny, E.A., 2017. Echocardiographic measurements and indices in normal adult buffalo (*Bubalus bubalis*). J. Appl. Anim. Res. 45, 336–341. doi:10.1080/09712119.2016.1190733

van Nie, C.J., 1986. The Purkinje fibres in the heart of the common seal *Phoca vitulina* (Linnaeus, 1758), the Baikal seal *Pusa sibirica* (Gmelin, 1788), the fur seal *Arctocephalus australis* (Zimmermann, 1782), the harbour porpoise *Phocoena phocoena* . Aquat. Mamm. 12.2, 61–64.

West, G.B., Brown, J.H., 2005. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. J. Exp. Biol. 208, 1575–1592. doi:10.1242/jeb.01589

Williams, T.M., Blackwell, S.B., Richter, B., Sinding, M.H.S., Heide-Jørgensen, M.P., 2017. Paradoxical escape responses by narwhals (Monodon monoceros). Science (80-.). 358, 1328–1331. doi:10.1126/science.aao2740

Williams, T.M., Fuiman, L.A., Kendall, T., Berry, P., Richter, B., Noren, S.R., Thometz, N., Shattock, M.J., Farrell, E., Stamper, A.M., Davis, R.W., 2015. Exercise at depth alters bradycardia and incidence of cardiac anomalies in deep-diving marine mammals Supplementary Figure ECG. Nat Commun 1–3.

Williams, T.M., Haun, J., Davis, R.W., Fuiman, L.A., Kohin, S., 2001. A killer appetite: Metabolic consequences of carnivory in marine mammals. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 129, 785–796. doi:10.1016/S1095-6433(01)00347-6

Williams, T.M., Kooyman, G.L., Croll, D.A., 1991. The effect of submergence on heart rate and oxygen consumption of swimming seals and sea lions. J. Comp. Physiol. B 160, 637–44. doi:10.1007/BF00571261

Yasuma, F., Hayano, J., 2004. Respiratory sinus arrhythmia: Why does the heartbeat synchronize with respiratory rhythm? Chest 125, 683–690.

Yildirim, H., Adanir, T., Atay, A., Katircioğlu, K., Savaci, S., 2004. The effects of sevoflurane, isoflurane and desflurane on QT interval of the ECG. Eur. J. Anaesthesiol. 21, 566–570. doi:10.1017/S0265021504007112

Zapol, W.M., Schneider, R.C., Liggins, G.C., Qvist, J., Creasy, R.K., Snider, M.T., Hochachka, P.W., 1979. Regional Blood Flow During Simulated Diving of the Conscious Antarctic Weddell Seal. J. Appl. Physiol.

Zelenkova, I., Chomahidze, P., 2016. Long-term effects of frequent maximal breath-holding on the cardiac health of elite freedivers. Scand. J. Med. Sci. Sport. 26, 1283–1286. doi:10.1111/sms.12580

Appendices

Appendix A Steller sea lion QRS morphology

Typical QRS morphology of anaesthetized Steller sea lions (n=8) in limb and augmented leads. Numbers are counts of individuals with each QRS type. Lowercase letters indicate small deflections, capital letters indicate large deflections, and apostrophes indicate a secondary wave of the given type.

Lead	QR	QRs	qR	qRS	qRs	qrS	R	Rs	rS	rSR'	rSr'	rsR'	s
I	0	1	0	1	5	1	0	0	0	0	0	0	0
II	0	0	0	4	2	1	0	0	1	0	0	0	0
III	0	0	0	0	0	0	0	1	7	0	0	0	0
aVR	0	0	0	0	0	0	0	0	0	1	4	2	0
aVL	1	1	3	0	2	0	1	0	0	0	0	0	0
aVF	0	0	1	1	1	0	0	0	4	0	0	0	1

Appendix B Northern fur seal QRS morphology

Typical QRS morphology of anaesthetized northern fur seals (n=5) in limb and augmented leads. Numbers are counts of individuals with each QRS type. Lowercase letters indicate small deflections, capital letters indicate large deflections, and apostrophes indicate a secondary wave of the given type.

Lead	QRr	Qr	qR	qRS	qRs	RS	Rs	r	rRS	rS	rSr'	rsrS
I	0	0	0	0	2	1	1	0	1	0	0	0
II	0	0	0	0	3	0	1	0	0	1	0	0
III	1	0	1	1	0	0	1	0	0	1	0	0
aVR	0	2	1	0	0	0	1	0	0	0	1	0
aVL	0	0	1	2	0	0	0	1	0	0	0	1
aVF	0	0	2	1	0	0	1	0	0	1	0	0

Appendix C Walrus QRS morphology

Typical QRS morphology of an anaesthetized walrus (n=1) in limb and augmented leads. Lowercase letters indicate small deflections, capital letters indicate large deflections.

Lead	Shape
I	R
II	rS
III	rS
aVR	Qr
aVL	qR
aVF	rS

Appendix D ECG dataset

Electrocardiographic measurements of mammals. n is the minimum sample size used to calculate average ECG parameters for each species. PS indicates the physiological state of the individuals at the time of the recording—Y = anaesthetized, N = conscious. EG indicates the ecological group of the species, either marine (M) or terrestrial (T). HR = heart rate, P = P-wave duration, PR = PR interval, QRS = QRS complex duration, QT = QT interval. ECG sources are references for the ECG data and mass sources are references for the mass data.

Species	Common name	n	mass	PS	EG	HR	P	PR	QRS	QT	ECG source	Mass source
Mirounga angustirostris	Northern elephant seal	13	1167	Y	M	66.9	NaN	0.200	0.102	0.392	(Hamlin et al., 1972; Van Citters et al., 1965)	(Hamlin et al., 1972; Van Citters et al., 1965)
Mirounga leonina	Southern elephant seal	24	144	Y	M	130.3	0.092	0.132	0.082	0.263	(Falabella et al., 1999)	(Falabella et al., 1999)
Otaria flavescens	Southern sea lion	13	81	Y	M	104.6	0.107	NaN	0.084	0.314	(Dassis et al., 2016)	(Dassis et al., 2016)
Didelphis marsupialis	Common opossum	10	1.3	Y	T	200.0	NaN	0.080	0.025	0.140	Wilber, 1955 cited in (Grauwiler, 1965)	McNab, 1978 cited in (Heusner, 1991)
Talpa europaea	European mole	NA	0.08	Y	T	345.0	NaN	0.025	0.009	0.029	(Grauwiler, 1965)	(Bonin, 1937)
Macaca irus	Crab-eating macaque	12	7.1	Y	T	225.0	NaN	0.073	0.028	0.171	DeWaart and Storm, 1935; DeWaart et al., 1936 cited in (Grauwiler, 1965)	(Tokura et al., 1975)

Papio ursinus	Baboon	15	20.5	Y	T	122.0	NaN	0.120	0.040	0.270	Kaminer, 1958 cited in (Grauwiler, 1965)	Kaminer, 1958 cited in (Grauwiler, 1965)
Mesocricetus auratus	Hamster	NA	0.12	Y	T	400.0	NaN	0.043	0.021	NaN	Lombard, 1952; Nakamura and Swank, 1960; both cited in (Grauwiler, 1965)	Tegowska and Gebczynski, 1975 cited in (Heusner, 1991)
Meriones shawii shawii	Gerbil	3	0.18	Y	T	240.5	NaN	0.060	0.025	0.068	(Grauwiler, 1965)	(Grauwiler, 1965)
Acomys cahirinus	Spiny mouse	5	0.065	Y	T	367.5	NaN	0.045	0.023	0.060	(Grauwiler, 1965)	Shkolnik and Borut, 1969 cited in (Heusner, 1991)
Rattus norvegicus	Rat	NA	0.17	Y	T	396.5	0.015	0.044	0.021	0.072	Beinfield and Lehr, 1956; Heise and Kimbel, 1955; Hill et al., 1960; Lombard, 1952; Normann et al., 1961; Sambhi and White, 1960; Schinzel, 1933; Werth, 1958; all cited in (Grauwiler, 1965)	Morrison and Ryser, 1951; Hart, 1971; both cited in (Heusner, 1991)
Peromyscus	Deer mouse	10	0.042	Y	T	420.0	NaN	0.032	0.025	NaN	Lombard, 1952 cited in (Grauwiler, 1965)	Mazen and Rudd, 1980; McNab and Morrison, 1963; Kenagy and Vleck, 1982; McNab and Morrison, 1963; Glenn, 1970; Pearson, 1947; Muser and Shoemaker, 1965; Hill, 1975; Hayward, 1965; McNab, 1973;

												all cited in (Heusner, 1991)
Eliomys quercinus	Garden dormouse	1	0.075	Y	T	350.0	NaN	0.050	0.020	0.110	(Grauwiler, 1965)	(Grauwiler, 1965)
Cavia porcellus	Guinea pig	10	0.43	Y	T	260.0	NaN	0.065	0.033	NaN	Lombard, 1952 cited in (Grauwiler, 1965)	(Gehr et al., 1981)
Canis familiaris	Domestic dog	106	21.4	Y	T	117.6	NaN	0.102	0.050	0.272	Doherty and Hara, 1961; Lombard and Witham, 1955; Santos and Kittle, 1958; Siems et al., 1955; all cited in (Grauwiler, 1965)	(Gehr et al., 1981)
Procyon lotor lotor	Raccoon	1	7	Y	T	200.0	NaN	0.060	0.030	0.160	Wilber, 1955 cited in (Grauwiler, 1965)	(Brown and Nicoletto, 1991)
Meles meles	Badger	4	14.5	Y	T	179.0	NaN	0.084	0.055	0.201	Johansson, 1957 cited in (Grauwiler, 1965)	Iversen, 1972 cited in (Heusner, 1991)
Felis catus	Domestic cat	38	3.9	Y	T	NaN	0.040	0.075	0.030	0.210	Blok and Boeles, 1957 cited in (Grauwiler, 1965)	(Taylor et al., 1982)
Lynx rufus	Bobcat	1	15	Y	T	190.0	NaN	NaN	0.050	0.200	(Grauwiler, 1965)	(Brown and Nicoletto, 1991)
Mus musculus	Mouse	20	0.017	Y	T	428.0	NaN	0.038	0.022	NaN	Lombard, 1952 cited in (Grauwiler, 1965)	Pearson, 1947 cited in (Heusner, 1991)

Callorhinus ursinus	Northern fur seal	5	29.4	Y	M	113.0	0.074	0.195	0.066	0.234	this study	this study
Eumetopias jubatus	Steller sea lion	8	198.8	Y	M	56.5	0.136	0.255	0.130	0.386	this study	this study
Odobenus rosmarus	Pacific walrus	1	366.5	Y	M	76.0	0.147	0.245	0.182	0.369	this study	this study
Mirounga angustirostris	Northern elephant seal	1	75	N	M	90.0	0.135	0.180	0.100	0.300	(Hamlin et al., 1972)	(Hamlin et al., 1972)
Phoca vitulina	Harbour seal	56	17.9	N	M	157.2	0.071	0.161	0.065	0.255	(Fonfara et al., 2015; Hamlin et al., 1972)	(Cottrell et al., 2002; Hamlin et al., 1972)
Tursiops truncatus	Bottlenose dolphin	41	162.2	N	M	81.0	0.092	0.203	0.084	0.290	(Hamlin et al., 1972; Harms et al., 2013; Kanwisher and Ridgway, 1983)	(Hamlin et al., 1972; Harms et al., 2013; Kanwisher and Ridgway, 1983)
Eschrichtius robustus	Gray whale	1	4363	N	M	30.0	NaN	0.475	0.208	0.817	(Ponganis and Kooyman, 1999)	(Ponganis and Kooyman, 1999)
Balaenoptera physalus	Fin whale	1	3.20 E+04	N	M	27.0	NaN	0.680	0.320	0.960	(Senft and Kanwisher, 1960)	(Senft and Kanwisher, 1960)
Orcinus orca	Killer whale	1	1800	N	M	NaN	0.120	NaN	0.120	0.340	(Meijler and van der Tweel, 1986)	(Meijler and van der Tweel, 1986)
Delphinus delphis	Common dolphin	1	50	N	M	71.0	NaN	NaN	NaN	NaN	(Kanwisher and Ridgway, 1983)	(Kanwisher and Ridgway, 1983)

Megaptera novaeangliae	Humpback whale	2	3.00 E+04	N	M	32.5	NaN	0.400	0.200	NaN	(Meijler et al., 1992)	(Meijler et al., 1992)
Echidna aculeata	Spiny anteater	2	3.0	N	T	59.0	NaN	NaN	NaN	NaN	(Grauwiler, 1965)	(Augee et al., 1970)
Macropus bennetti	Bennett's kangaroo	7	54.6	N	T	120.0	NaN	0.107	0.045	0.142	Sporri, 1956 cited in (Grauwiler, 1965)	T.-B. & Renfree, 1987 cited in (Rose et al., 1997)
Phascolomys latifrons	Hairy-nosed wombat	1	17.5	N	T	88.0	0.070	0.210	0.065	0.290	(Grauwiler, 1965)	(Grauwiler, 1965)
Hapale bacchus	Common marmoset	1	0.34	N	T	230.0	NaN	0.050	0.032	0.165	(Grauwiler, 1965)	(Araújo et al., 2000)
Saimiri sciurea	Squirrel monkey	NA	0.8	N	T	231.0	NaN	0.048	NaN	0.100	Graybiel et al., 1969; Hixson et al., 1960; both cited in (Grauwiler, 1965)	Stitt and Hardy, 1971 cited in (Heusner, 1991)
Macaca mulatta	Rhesus monkey	NA	3.5	N	T	259.7	NaN	0.064	0.027	0.148	Atta and Vanace, 1960 cited in (Grauwiler, 1965)	(Crile and Quiring, 1940)
Pan troglodytes	Chimpanzee	1	17.5	N	T	101.0	NaN	0.098	0.064	0.280	Grauwiler, 1961 cited in (Grauwiler, 1965)	Taylor and Rowntree 1973 cited in (Taylor et al., 1982)
Gorilla gorilla gorilla	Lowland gorilla	1	13	N	T	119.0	NaN	0.110	0.060	0.300	Grauwiler, 1961 cited in (Grauwiler, 1965)	(Harcourt et al., 1981)
Choloepus didactulus	Two-toed sloth	1	6.1	N	T	63.0	NaN	0.195	0.070	0.420	Grauwiler, 1961 cited in (Grauwiler, 1965)	(Adam, 1999)

Marmota marmota	Marmot	1	3.4	N	T	200.0	NaN	0.080	0.060	0.165	Grauwiler, 1961 cited in (Grauwiler, 1965)	(Armitage et al., 1976)
Cavia porcellus	Guinea pig	355	0.43	N	T	231.4	0.036	0.061	0.023	0.142	Sporri, 1944; Jasinski and Grauwiler, 1960; cited in (Grauwiler, 1965)	(Gehr et al., 1981)
Canis aureaus	Jackal	1	8.8	N	T	128.0	0.040	0.106	0.040	0.180	Siegfried, 1956 cited in (Grauwiler, 1965)	(Gittleman, 1985)
Canis familiaris	Domestic dog	686	64.4	N	T	103.2	0.050	0.102	0.050	0.192	Corticelli, 1949; Horwitz et al., 1953; Lannek, 1949; Petersen et al., 1951; Schulze et al., 1957; Soave, 1954; Sporri, 1954; Grauwiler, 1965; all cited in (Grauwiler, 1965)	(Gehr et al., 1981), American Kennel Club
Nyctereutes procyonides	Racoon dog	1	7.5	N	T	93.0	NaN	0.082	0.040	0.180	Siegfried, 1956 cited in (Grauwiler, 1965)	(Gittleman, 1985)
Mustela putorius	Polecat	1	1.0	N	T	300.0	0.040	NaN	0.020	0.090	Siegfried, 1956 cited in (Grauwiler, 1965)	(Gittleman, 1985)
Mustela lutreola	European mink	1	0.59	N	T	333.0	NaN	NaN	0.020	0.100	Siegfried, 1956 cited in (Grauwiler, 1965)	(Gittleman, 1985)
Herpestes ichneumon	Common mongoose	1	3.0	N	T	193.0	0.030	0.080	0.040	0.180	Grauwiler, 1961 cited in (Grauwiler, 1965)	(Palomares and Delibes, 1992)

Felis catus	Domestic cat	57	3.9	N	T	158.5	0.034	0.084	0.040	0.183	Rothlin and Suter, 1947; Schinzel, 1933; Sporri, 1954; Grauwiler, 1965; all cited in (Grauwiler, 1965)	(Taylor et al., 1982)
Equus asinus	Donkey	2	177.5	N	T	60.0	NaN	0.200	0.060	0.430	Luisada et al., 1944 cited in (Grauwiler, 1965)	Yousef and Dill, 1969 cited in (Heusner, 1991)
Tapirus indicus	Malayan tapir	1	160	N	T	43.0	0.140	0.316	0.111	0.539	Grauwiler, 1961 cited in (Grauwiler, 1965)	(Bonin, 1937)
Rhinoceros unicornis	Indian rhinoceros	2	1600	N	T	36.0	0.160	0.400	0.139	0.640	Siegfried, 1956; Grauwiler, 1961; both cited in (Grauwiler, 1965)	(Laurie, 1982)
Didermoceros sumatraensis	Sumatran rhinoceros	1	698.1	N	T	46.0	NaN	0.358	0.102	0.535	Grauwiler, 1961 cited in (Grauwiler, 1965)	(Dierenfeld et al., 2000)
Sus domesticus	Domestic pig	52	42.4	N	T	153.1	0.067	0.086	0.048	0.234	Pantridge, 1948; Luisada et al., 1944; Zuckermann, 1959; Sporri, 1954; Miller et al., 1957; Hausmann, 1934; Grauwiler, 1965; all cited in (Grauwiler, 1965)	(Crile and Quiring, 1940); Sporri, 1954; Grauwiler, 1965; both cited in (Grauwiler, 1965)
Phacochoerus aethiopicus	Warthog	1	65.3	N	T	143.0	0.070	0.140	0.055	0.240	Siegfried, 1956 cited in (Grauwiler, 1965)	(Crile and Quiring, 1940)

Choeropsis liberiensis	Pigmy hippopotamus	2	229	N	T	53.0	0.093	0.273	0.085	0.481	Siegfried, 1956; Grauwiler, 1961; both cited in (Grauwiler, 1965)	(Vendl et al., 2016)
Lama glama	Llama	1	115	N	T	134.0	0.040	0.110	0.040	0.230	Siegfried, 1956 cited in (Grauwiler, 1965)	El-Nouty et al.,1978 cited in (Heusner, 1991)
Camelus bactrianus	Camel	2	433.1	N	T	55.0	0.150	0.349	0.127	0.572	Grauwiler, 1961 cited in (Grauwiler, 1965)	(Tsogttuya et al., 2009)
Camelus dromedarius	Dromedary	1	265	N	T	27.0	0.100	0.250	0.090	0.620	Braun et al., 1958 cited in (Grauwiler, 1965)	Schmidt-Nielson et al., 1967) cited in (Heusner, 1991)
Okapia johnstoni	Okapi	4	212.5	N	T	43.5	NaN	0.325	0.105	0.528	Sporri, unpublished data; Grauwiler, 1961; both cited in (Grauwiler, 1965)	Gijzen, 1959; Grzimek, 1958; both cited in (Bodmer and Rabb, 1992)
Giraffa camelopardalis	Giraffe	1	383	N	T	30.8	0.100	0.305	0.090	0.602	Grauwiler, 1961 cited in (Grauwiler, 1965)	(Gehr et al., 1981)
Bos grunniens	Domestic yak	1	15.9	N	T	132.0	0.040	0.095	0.040	0.230	Siegfried, 1956 cited in (Grauwiler, 1965)	(Zi et al., 2004)
Delphinapterus leucas	Beluga whale	2	818	N	M	30.5	NaN	0.320	0.105	0.380	(Kanwisher and Ridgway, 1983; King et al., 1953)	(Kanwisher and Ridgway, 1983; King et al., 1953)
Phocoena phocoena	Harbour porpoise	3	28.7	N	M	116.7	0.050	0.120	0.050	0.220	(Kastelein and Meijler, 1989)	(Kastelein and Meijler, 1989)

Trichechus manatus	Manatee	12	320.1	N	M	63.3	0.115	0.240	0.095	0.500	(Siegal-Willott et al., 2006)	(Etheridge et al., 1985; Quiring and Harlan, 1953)
Zalophus californianus	California sea lion	3	38	N	M	108.3	0.093	0.147	0.070	0.273	(Hamlin et al., 1972)	(Hamlin et al., 1972)
Elephas maximus	Asiatic elephant	21	3320.3	N	T	40.6	0.160	0.345	0.154	0.613	White et al., 1938; Sporri, unpublished data; both cited in (Grauwiler, 1965)	Benedict (1938) cited in (Heusner, 1991), (Hile et al., 1997)
Loxodonta africana	African elephant	10	6654	N	T	41.5	NaN	0.360	0.162	0.640	Siegfried, 1956; Grauwiler, 1961; both cited in (Grauwiler, 1965)	(Crile and Quiring, 1940)
Martes martes	Pine marten	1	0.92	N	T	243.0	NaN	0.054	0.040	0.100	Siegfried, 1965 cited in (Grauwiler, 1965)	Iversen, 1972 cited in (Heusner, 1991)
Microtus arvalis	Common vole	11	0.024	N	T	633.2	NaN	0.031	0.013	0.044	(Grauwiler, 1965)	Jansky, 1959 cited in (Heusner, 1991)
Mus musculus	Mouse	2	0.017	N	T	710.0	NaN	0.033	NaN	0.032	(Grauwiler, 1965)	Pearson, 1947 cited in (Heusner, 1991)
Tapirus terrestris	American lowland tapir	2	150	N	T	58.5	0.085	0.225	0.083	0.412	Siegfried, 1956 cited in (Grauwiler, 1965)	(Bodmer, 1990)

Appendix E Supporting references for Appendix D

Adam, P.J., 1999. *Choloepus didactylus*. Mamm. Species 1–8. doi:10.2307/3504332/2600770

Araújo, A., Arruda, M.F., Alencar, A.I., Albuquerque, F., Nascimento, M.C., Yamamoto, M.E., 2000. Body weight of wild and captive common marmosets (*Callithrix jacchus*). Int. J. Primatol. 21, 317–324. doi:10.1023/A:1005433722475

Armitage, K.B., Downhower, J.F., Svendsen, G.E., 1976. Seasonal changes in weights of marmots. Am. Midl. Nat. 96, 36–51.

Augee, A.M.L., Ealey, E.H.M., Spencer, H., 1970. Biotelemetric studies of temperature regulation and torpor in the echidna, *Tachyglossus aculeatus*. J. Mammal. 51, 561–570.

Bodmer, R.E., 1990. Fruit patch size and frugivory in the lowland tapir (*Tapirus terrestris*). J. Zool. 222, 121–128. doi:10.1111/j.1469-7998.1990.tb04034.x

Bodmer, R.E., Rabb, G.B., 1992. *Okapia johnstoni*. Mamm. Species 422, 1–8.

Bonin, G. von, 1937. Brain-weight and body-weight of mammals. J. Gen. Psychol. 16, 379–389.

Brown, J.H., Nicoletto, P.F., 1991. Spatial scaling of species composition: Body masses of North American land mammals. Am. Soc. Nat. 138, 1478–1512.

Cottrell, P.E., Jeffries, S., Beck, B., Ross, P.S., 2002. Growth and development in free-ranging harbor seal (*Phoca vitulina*) pups from southern British Columbia, Canada. Mar. Mammal Sci. 18, 721–733. doi:10.1111/j.1748-7692.2002.tb01069.x

Crile, G., Quiring, D.P., 1940. A record of the body weight and certain organ and gland weight of 3734 animals. Ohio J. Sci. 40, 219–59.

Dassis, M., Rodríguez, D.H., Rodríguez, E., Ponce De León, A., Castro, E., 2016. The electrocardiogram of anaesthetized southern sea lion (*Otaria flavescens*) females. J. Vet. Cardiol. 18, 71–78. doi:10.1016/j.jvc.2015.09.003

Dierenfeld, E.S., Wildman, R.E.C., Romo, S., 2000. Feed intake, diet utilization, and composition of browses consumed by the Sumatran rhino (*Dicerorhinus sumatrensis*) in a North American zoo. Zoo Biol. 19, 169–180. doi:10.1002/1098-2361(2000)19:3<169::AID-ZOO1>3.0.CO;2-D

Etheridge, K., Rathbun, G.B., Powell, J.A., Kochman, H.I., 1985. Consumption of aquatic plants by the West Indian manatee. J. Aquat. Plant Manag. 23, 21–25.

Falabella, V., Campagna, C., Lewis, M., Falabella, V., Campagna, C., Lewis, M., 1999. Electrocardiography of southern elephant seal (*Mirounga leonina*) weanlings. J. Zoo Wildl. Med. 30, 526–531.

Fonfara, S., Casamian-Sorrosal, D., Sundermeyer, J., Rosenberger, T., 2015. Variations in heart rate and rhythm of harbor seal pups during rehabilitation. Mar. Mammal Sci. 31, 998–1013. doi:10.1111/mms.12201

Gehr, P., Mwangi, D.K., Ammann, A., Maloiy, G.M.O., Taylor, C.R., Weibel, E.R., 1981. Design of the mammalian respiratory system. V. Scaling morphometric pulmonary diffusing capacity to body mass: Wild and domestic animals.

Gittleman, J.L., 1985. Carnivore body size: Ecological and taxonomic correlates. Oecologia 67, 540–554. doi:10.1007/BF00790026

Grauwiler, J., 1965. Herz und Kreislauf der Säugetiere. Vergleichend-funktionelle Daten von Jules Grauwiler, mit einem Geleitwort von Heinrich Spörri. Birkhäuser Verlag, Basel.

Hamlin, R.L., Ridgway, S.H., Gilmartin, W.G., 1972. Electrocardiogram of pinnipeds. Am. J. Vet. Res. 33, 867–875.

Harcourt, A.H., Fossey, D., Sabater-Pi, J., 1981. Demography of *Gorilla gorilla*. J. Zool. 195, 215–233. doi:10.1111/j.1469-7998.1981.tb03460.x

Harms, C.A., Jensen, E.D., Townsend, F.I., Hansen, L.J., Schwacke, L.H., Rowles, T.K., 2013. Electrocardiograms of bottlenose dolphins (Tursiops truncatus) out of water: habituated collection versus wild postcapture animals. J. Zoo Wildl. Med. 44, 972–81. doi:10.1638/2013-0093.1

Heusner, A.A., 1991. Size and power in mammals. J. Exp. Biol. 160, 25–54.

Hile, M.E., Hintz, H.F., Erb, H.N., 1997. Predicting body weight from body measurements in Asian elephants (*Elephas maximus*). J. Zoo Wildl. Med. 28, 424–427.

Kanwisher, J.W., Ridgway, S.H., 1983. The physiological ecology of whales and porpoises. Sci. Am. 248, 110–120. doi:10.1038/scientificamerican0683-110

Kastelein, R.A., Meijler, F.L., 1989. Respiratory arrhythmia in the hearts of Harbour porpoises (*Phocoena phocoena*). Aquat. Mamm. 15.2, 57–63.

King, R.L., Jenks, J.L., White, P.D., 1953. The electrocardiogram of a beluga whale. Circulation

8, 387–393. doi:10.1161/01.CIR.8.3.387

Laurie, A., 1982. Behavioural ecology of the Greater one-horned rhinoceros (*Rhinoceros unicornis*). J. Zool. 196, 307–341. doi:10.1111/j.1469-7998.1982.tb03506.x

Meijler, F.L., van der Tweel, L.H., 1986. De elektrocardiogrammen van 10 olifanten en van de orka in Harderwijk. Ned Tijdschr Geneeskd 130, 2344–2348.

Meijler, F.L., Wittkampf, F.H.M., Brennen, K.R., Baker, V., Wassenaar, C., Bakken, E.E., 1992. Electrocardiogram of the humpback whale (*Megaptera novaeangliae*) with specific reference to atrioventricular transmission and ventricular excitation. J. Am. Coll. Cardiol. 20, 475–479. doi:10.1016/0735-1097(92)90120-C

Palomares, F., Delibes, M., 1992. Some physical and population characteristics of Egyptian mongooses (Herpestes ichneumon L., 1758) in southwestern Spain. Z. Saugetierkd. 57, 94–99.

Ponganis, P.J., Kooyman, G.L., 1999. Heart rate and electrocardiogram characteristics of a young California gray whale (*Eschrichtius robustus*). Mar. Mammal Sci. 15, 1198–1207.

Quiring, A.D.P., Harlan, C.F., 1953. On the anatomy of the manatee. J. Mammal. 34, 192–203.

Rose, R.W., Nevison, C.M., Dixson, A.F., 1997. Testes weight, body weight and mating systems in marsupials and monotremes. J. Zool. 243, 523–531. doi:10.1111/j.1469-7998.1997.tb02798.x

Senft, A.W., Kanwisher, J.K., 1960. Cardiographic observations on a fin-back whale. Circ. Res. 8, 961–964. doi:10.1161/01.RES.8.5.961

Siegal-Willott, J., Estrada, A., Bonde, R., Wong, A., Estrada, D.J., Harr, K., 2006. Electrocardiography in two subspecies of manatee (*Trichechus manatus latirostris* and *T. m. manatus*). J. Zoo Wildl. Med. 37, 447–453.

Taylor, C.R., Heglund, N.C., Maloiy, G.M.D., 1982. Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and size in birds and mammals. J. Exp. Biol. 97, 1–21.

Tokura, H., Hara, F., Okada, M., Mekata, F., Ohsawa, W., 1975. A comparison of thermoregulatory responses in the Japanese macaque (*Macca fuscata*) and the crab-eating macaque (*Macaca irus*) during cold exposure. Jpn. J. Physiol. 25, 147–152.

Tsogttuya, C., Luvsan, B., Erdenebileg, U., 2009. Review of Mongol camel studies. Dalanzadgad.

Van Citters, R.L., Franklin, D.L., Smith, O.A., Watson, N.W., Elsner, R.W., 1965. Cardiovascular adaptations to diving in the northern elephant seal *Mirounga angustirostris*. Comp. Biochem. Physiol. 16, 267–276. doi:10.1016/0010-406X(65)90324-5

Vendl, C., Frei, S., Dittmann, M.T., Furrer, S., Ortmann, S., Lawrenz, A., Lange, B., Munn, A., Kreuzer, M., Clauss, M., 2016. Methane production by two non-ruminant foregut-fermenting herbivores: The collared peccary (*Pecari tajacu*) and the pygmy hippopotamus (*Hexaprotodon liberiensis*). Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 191, 107–114. doi:10.1016/j.cbpa.2015.09.021

Zi, X.D., Zhong, G.H., Wen, Y.L., Zhong, J.C., Liu, C.L., Ni, Y.A., Yezi, Y.H., Ashi, M.G., 2004. Growth performance, carcass composition and meat quality of Jiulong-yak (*Bos grunniens*). Asian-Australian J. Anim. Sci. 17, 410–414.