INFLUENCE OF ENVIRONMENT, FEEDING, AND DIVE ACTIVITY ON THE USE OF HEART RATE TO PREDICT OXYGEN CONSUMPTION IN RESTING AND DIVING STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)

by

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Abstract

Despite its essential role in bioenergetic modeling, reliable measures of energy expenditure (i.e., oxygen consumption) associated with the different activities of wild animals have remained elusive. Oxygen consumption rate ($\dot{V}_{O_2}$) associated with activity can be estimated as a function of heart rate (*fh*), and the empirical relationship between the two has been determined for several aquatic vertebrates while fasting and resting. However, the simplified *fh*: $\dot{V}_{O_2}$ relationships established from such studies may differ under more complex physiological circumstances, such as when animals are foraging at depth or feeding on prey. I assessed the efficacy of using *fh* to predict $\dot{V}_{O_2}$ in 7 captive Steller sea lions, *Eumetopias jubatus,* while fasting and feeding at rest (on land or in water) and while diving (up to 40 m in the open ocean). Linear mixed-effects models revealed that environment, amount of food fed, and type of diving activity all altered the *fh*: $\dot{V}_{O_2}$ relationship. They also showed that different linear equations are needed to predict $\dot{V}_{O_2}$ from *fh* for sea lions fasted while on land or in water, but that a single equation can predict $\dot{V}_{O_2}$ on land regardless of whether fasted or feeding. When in water, feeding animals a 4, 6, or 12 kg meal changed the *fh*:$\dot{V}_{O_2}$ relationship compared to fasted animals. While *fh* can reliably be used to predict $\dot{V}_{O_2}$ in diving sea lions, the relationship differed between single dive cycles (one dive +surface interval) and dive bout cycles (multiple dives+surface intervals). However, the equation that predicted $\dot{V}_{O_2}$ for single dive cycles did not differ from that for sea lions resting on the surface. Neither dive duration, dive depth, nor food consumed significantly affected the *fh:*$\dot{V}_{O_2}$ relationships. Heart rate could be used to predict $\dot{V}_{O_2}$ in diving sea lions, but only over complete dive cycles or dive bouts where animals recovered fully from the O_2 debt incurred underwater. Based on these results, separate equations that distinguish among environmental, digestive, and diving states can be employed to accurately predict $\dot{V}_{O_2}$ from heart rate in wild Steller sea lions.

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List of Abbreviations

M_b	Body mass (kg)
V_{O_2}	Oxygen consumption
$\dot{V}_{O_2}$	Oxygen consumption rate (ml O_2 min^{-1})
$\dot{V}_{CO_2}$	Carbon dioxide production rate (ml CO_2 min^{-1})
$s\dot{V}_{O_2}$	Mass-corrected oxygen consumption rate (ml O_2 $min^{-1} \cdot kg^{-0.75}$)
fh	Heart rate (beats·min^{-1})
fh_{inst}	Instantaneous heart rate (beats·min^{-1})
dry_{fasted}	Fasted resting in dry metabolic chamber
$dry_{4kg/6kg}$	Fed 4 kg or 6 kg in metabolic chamber
$water_{fasted}$	Fasted resting in swim mill
$water_{4kg/6kg}$	Fed 4 kg or 6 kg in swim mill
$water_{ow}$	Resting at the surface in open water (fed≤ 0.36 kg)
$RMR/water_{comp}$	Resting metabolic rate (Ch 3) or composite baseline for water trials in Ch. 2 ($water_{ow}$ + $water_{fasted}$).
AMR	Average metabolic rate ($s\dot{V}_{O_2}$) averaged over the dive cycle (dive(s)+surface interval(s))
MR_s	Rate of mass-corrected oxygen consumption ($s\dot{V}_{O_2}$) resting at the surface in open water (predive)
DMR	Diving metabolic rate ($s\dot{V}_{O_2}$) averaged over dive (s) only
SI	Surface interval of preceding dive (min)

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Co-authorship statement

I, Beth Young, am the primary contributor to this thesis in all of the required areas:

1. Identification and design of research program: My co-supervisors David Rosen, Andrew Trites, and I are the main identifiers of this research project. I was the main designer of this research project.
2. Performing the research: I performed all of the research and data collection in this thesis. I was assisted in performing this research by the animal trainers, technicians, and veterinarian, Dr. Martin Haulena, at the Vancouver Aquarium. My co-supervisors, David Rosen and Andrew Trites, gave suggestions. Data from McPhee et al. (2003) was used with permission from Jan McPhee and co-authors David Rosen and Andrew Trites.
3. Data analyses: I performed all of the data analysis in this thesis. Suggestions were given by David Rosen and our lab biostatistician, Ruth Joy.
4. Manuscript preparation: I prepared the entire manuscript. Editing was performed by myself, David Rosen, Allyson Hindle (co-author on one paper), and Andrew Trites.

Chapter 1: Introduction

The nutritional stress hypothesis

Changes in the biological and physical environment can alter the behaviour of individual animals with potentially deleterious results, as has been proposed for the endangered Steller sea lion, *Eumetopias jubatus* (Loughlin 1998; Trites and Larkin 1996). The population of Steller sea lions has declined 80% since their peak in the late 1970's (Loughlin 1998; Trites and Larkin 1996). The explanation for the decline remains elusive, but it is likely that changes in the biological environment (i.e., prey or predator shifts) and physical environment (i.e., climate change) have combined to produce negative synergistic effects on the population (Burek 2005; DeMaster and Trites 2006; Guenette 2007; Loughlin 1998; Springer et al. 2003; Trites 2007).

A leading hypothesis for the dramatic population decline is that Steller sea lions have been nutritionally stressed due to a variety of factors including fisheries competition, environmental change, or a shift in their prey base (Andrews 2004; DeMaster and Trites 2006; Guenette 2007; Rosen 2009; Rosen and Trites 2000; Rosen and Trites 2005; Springer et al. 2003; Trites and Donnelly 2003). One immediate outcome of such environmental alterations is changes in behaviour, including the time and effort directed at foraging or time spent at-sea versus on-land. Quantifying the energetic impact of changes in behaviour is critical to determining the overall impact of these changes on the animal's field metabolic rate (FMR) and, conversely, their energetic requirements.

Field metabolic rate is a measure of the total energy expended by a wild animal, including the energetic costs of basal metabolism, thermoregulation, locomotion, feeding, digestion, reproduction, and growth (Kleiber 1961). Documenting how metabolic rate changes in different environmental and physiological circumstances is essential because all physiological and behavioural activities an animal performs ultimately require balancing energetic supply with demand. Bioenergetic models have been used to estimate field metabolic rate in several species of diving vertebrates including California sea lions (*Zalophus californianus*), macaroni penguins, (*Eudyptes chrysolophus*), Weddell seals (*Leptonychotes weddellii*), Antarctic fur seals (*Arctocephalus gazella*), and Steller sea lions (Boyd et al. 1999; Castellini 1992; Green et al. 2007; Hurley and Costa 2001; Winship et al. 2002). However, empirical data required to

construct these models is limited, especially in the case of large marine mammals where obtaining physiological measurements can be challenging making it almost impossible to directly measure the activity-specific costs associated with natural feeding and diving behaviours.

Metabolic expenditures are usually directly estimated from the rate of oxygen consumption ($\dot{V}_{O_2}$) via gas respirometry. Briefly, oxygen consumption can be measured from known concentrations of O_2 consumed and CO_2 produced by an animal in a measurable volume of air (Vleck 1987; Withers 1977). Unfortunately, measuring activity-specific metabolism in this way is almost impossible with naturally diving marine mammals, except in a few unique cases involving seals that reliably surface into respirometry domes placed over semi-artificial ice holes (Castellini 1992; Murphy et al. 1980; Williams et al. 2004).

Techniques to measure field metabolic rate

Metabolism in wild pinnipeds can be indirectly estimated from other physiological or behavioural parameters, such as doubly labeled water turnover, body acceleration metrics (overall dynamic body acceleration or flipper stroke frequency), or heart rate methods.

The doubly labeled water method estimates total $\dot{V}_{O_2}$ from CO_2 production by measuring the differential biological turnover of two administered labeled isotopes ($H_2{}^{18}O$ and D_2O) (Costa 1987; Kam and Degen 1997; Roberts 1989; Speakman and Krol 2005). The difference in marker turnover is a measure of CO_2 production, which can mathematically be converted into O_2 consumption (Lifson et al. 1955). However, doubly labeled water provides only a mean estimate of field metabolic rate over the entire measurement period, which is temporally limited due to the biological half-life of the chemicals used. This method presents many logistical and financial challenges in the field, not the least of which is that animals must be recaptured within a specific amount of time (4-6 days for marine mammals). Even if these hurdles are overcome, the doubly labeled water method has been shown to overestimate field metabolic rate in otariids (fur seals and sea lions) by as much as 36.4% (Boyd et al. 1995).

Body acceleration metrics (measures of physical activity) including overall dynamic body action (ODBA) and flipper stroking have emerged as new tools to predict field metabolic rate.

ODBA has been used to estimate field metabolic rate in a range of vertebrates on land (Halsey et al. 2008; Halsey et al. 2009; Wilson et al. 2006). More recently, ODBA has been used to predict field metabolic rate in Steller sea lions (Fahlman et al. 2008b), but the accuracy of this technique remains inconclusive in swimming otariids.

Alternately, flipper stroking has been shown to be an accurate predictor of field metabolic rate in free-ranging Weddell seals (Williams et al. 2004), but whether this relationship holds true for otariids swimming with fore flippers is still under investigation (Hindle unpublished data). Overall, use of both body acceleration metrics are limited to only predicting field metabolic rate during active behaviours, likely do not account for changes in physiological state (such as feeding or digestion), and may not be as accurate for marine animals as terrestrial ones (Fahlman et al. 2008b; Green et al. 2009a; Halsey et al. 2009).

Heart rate (fh) method

The heart rate method to estimate metabolic rate provides activity-specific measures of energy expenditure on a much finer time scale and for longer periods of time than the doubly labeled water method (Boyd et al. 1995; Butler et al. 2004; Ponganis 2007) with comparable error estimates to ODBA (Green et al. 2009a; Halsey et al. 2009). In contrast to measures of body acceleration such as ODBA or flipper stroke frequency, heart rate has the potential to predict metabolic rate in inactive as well as active animals. Heart rate has been used to estimate oxygen consumption in several aquatic homeotherms including penguins, seals, and Steller sea lions (Boyd et al. 1995; Fahlman et al. 2004; Froget et al. 2002; McPhee et al. 2003; Williams et al. 1991).

The heart rate method estimates rates of oxygen consumption ($\dot{V}_{O_2}$) from recorded heart rate (*fh*) based upon Fick's (1870) relationship: $\dot{V}_{O_2} = (Ca_{O_2} - C\bar{v}_{O_2}) \times Vs \times fh$, where Vs is stroke volume, Ca_{O_2} is the arterial oxygen content, $C\bar{v}_{O_2}$ is the oxygen content of the mixed venous blood, and the function $(Ca_{O_2} - C\bar{v}_{O_2})$ represents blood oxygen extraction across tissue vascular beds. The rate of O_2 depletion and blood flow rate can also influence the $(Ca_{O_2} - C\bar{v}_{O_2})$ difference. The accuracy of the heart rate method relies on the assumption that an increase in *fh* is the primary method that animals employ to respond to increased $\dot{V}_{O_2}$, and that $Ca_{O_2} - C\bar{v}_{O_2}$ and

V_S remain constant or vary proportionally with *fh*. While this is not likely the case, direct measurements from marine mammals of changes in these parameters due to activity, environment, or physiological state are limited.

Measuring $(Ca_{O_2} - C\bar{v}_{O_2})$ and V_S for marine mammals has been limited by the requirement for invasive surgery, insertion of heart catheters, and extensive testing of device-animal bio-compatibility in captivity (Elsner et al. 1964; Ponganis et al. 1990; Thorton et al. 2005). There is evidence that blood flow increases to the small intestine during digestion in primates and dogs (Vatner et al. 1970), but research suggests that changes in *fh*, cardiac output (V_S x *fh*), and V_S during digestion are transitory (Vatner et al. 1974).

Limited studies investigating V_S in diving mammals suggests that V_S either decreases (Blix et al. 1983; McKean 1982; Ponganis et al. 1990; Sinnett et al. 1978; Zapol et al. 1979) or remains constant during diving (Blix et al. 1976; Elsner et al. 1964; Murdaugh Jr et al. 1966). Inconsistencies among studies could be due to differences between voluntary and forced submersions, with extreme decreases in V_S occurring during the latter as a result of asphyxiation prevention and fear response.

Tissues oxygen extraction $(Ca_{O_2} - C\bar{v}_{O_2})$ has not been directly measured in marine mammals, but measurements on nutria (*Myocastor coypus*) after forced submersion demonstrated that the $(Ca_{O_2} - C\bar{v}_{O_2})$ difference is reduced while diving (McKean 1982). Differences between arterial and venous partial pressure of oxygen (PO_2) have been shown to decrease over extended dives in free ranging elephant seals (Meir et al. 2009) which suggest that blood O_2 depletion rate and therefore $(Ca_{O_2} - C\bar{v}_{O_2})$ also decrease during diving. Research also suggests that blood flow distribution changes during diving (Blix et al. 1976; Davis et al. 1983; Elsner et al. 1985; Stone et al. 1973; Zapol et al. 1979). Although, it is likely that diving alters *fh*, $(Ca_{O_2} - C\bar{v}_{O_2})$, blood flow and possibly V_S change during diving, it is unknown whether these changes are profound enough to change the $fh{:}\dot{V}_{O_2}$ relationship. Logistical difficulties in measuring these parameters have limited the practicality of using Fick's equation to estimate oxygen consumption rates solely from heart rate.

Like all other methods of estimating metabolic rate in the wild, the heart rate method has its own set of strengths and biases. The main advantage of the heart rate method is that *fh* can

estimate activity-specific metabolic rates rather than average field metabolic rate. Secondly, the heart rate method is less limited by time and can be used to estimate field metabolic rate for over a year with implantable dataloggers (Ponganis 2007). However, the heart rate method requires species-specific predictive equations (such as the present study) to be empirically derived before it can be reliably applied in the field. Critics also cite heart rate variability among individual animals as a disadvantage of the technique (Boyd et al. 1995; Fahlman et al. 2008b). However, physiologically sensitive fine-scale variation in *fh* is exactly what enables *fh* to predict activity-specific metabolism on a finer scale than doubly labeled water.

Factors affecting the *fh*:$\dot{V}_{O_2}$ relationship

As previously mentioned, field application of the heart rate method requires prior species-specific predictive equations defining the relationship between *fh* and $\dot{V}_{O_2}$. Traditionally, these initial *fh*:$\dot{V}_{O_2}$ studies have been conducted under uniform physiological and environmental conditions to limit experimental and statistical variation. However, factors that can potentially affect the relationship between *fh* and $\dot{V}_{O_2}$ — including digestive, environmental, or activity state — are precisely what must be accounted for if this method is to be successfully applied to animals in the wild.

The majority of studies investigating the *fh*:$\dot{V}_{O_2}$ relationship in active marine mammals and birds have been performed while animals were either submerged in a shallow swim mill (Boyd et al. 1995; Butler et al. 1992; McPhee et al. 2003; Williams et al. 1991; Woakes and Butler 1983), walking on a treadmill (Froget et al. 2001; Froget et al. 2002; Green 2001; Green et al. 2005), or swimming horizontally in open water (Williams et al. 1993) (See Table 1.1 for a summary). Otariids spend time on both land and in the water, but few studies have critically examined the effect of the physical environment on the *fh*:$\dot{V}_{O_2}$ relationship in a controlled comparison.

Table 1. 1 Summary of relevant past studies on aquatic homeotherms that have investigated the relationship between heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) showing species studied, whether animals were fed or fasted, testing environment, activity level, and sample sizes (N).

Common Name	Species	Food fed (kg)[1]	Environment[2]	Activity[3]	N	Source
Birds						
King penguin	*Aptenodytes patagonicus*	fasted	land	resting/walking	5	Fahlman et al. 2004;
King penguin	*Aptenodytes patagonicus*	fasted	water	resting/swimming	5	Fahlman et al. 2004
King penguin	*Aptenodytes patagonicus*	fasted (fed unknown amount)	land	walking	22	Froget et al., 2002
Tufted duck	*Aythya fuligula*	fasted (fed unknown amount)	water	swimming	6	Woakes & Butler, 1983
Tufted duck	*Aythya fuligula*	fasted (fed unknown amount)	water	diving	6	Woakes & Butler, 1983
Macaroni penguin	*Eudyptes chrysolophus*	fasted	land	walking	24	Green, 2001
Black-browed albatross	*Diomedea melanophrys*	fasted	land/water	resting/walking	8	Bevan et al. 1994
Cetaceans						
Bottlenose dolphin	*Tursiops truncatus* Montagu	fasted (fed unknown amount)	water*	resting/swimming	2	Williams et al., 1993
Pinnipeds						
Steller sea lion	*Eumetopias jubatus*	fasted (fed < 200g)	water	resting/swimming	4	McPhee et al. 2003
Steller sea lion	*Eumetopias jubatus*	fasted (fed < 200g)	land	resting	4	McPhee et al. 2003
Steller sea lion	*Eumetopias jubatus*	fed 6 or 12 kg herring	water	resting	1	McPhee et al. 2003
California sea lion	*Zalophus californianus*	fasted	water	resting/swimming	3	Williams et al. 1991;
California sea lion	*Zalophus californianus*	fasted (fed unknown amount)	water	swimming	6	Boyd et al. 1995
Harbour seal	*Phoca vituIina*	fasted	water	resting/swimming	4	Williams et al. 1991
Northern elephant seal	*Mirounga angustirostris*	fasted	water	diving	6	Webb et al., 1998

[1:]"Fasted" for pinnipeds and cetaceans was for a minimum of ~16-24 hrs or overnight, but "fasted" in penguins was for >24 hrs or up to several weeks. Animals that were fasted overnight were often fed during data collection to facilitate cooperation. This amount is indicated in parenthesis after "fasted" or as "unknown" if study didn't specifically describe food used during training.

One notable difference between air and water is their thermal properties. Water has a greater specific heat capacity relative to air and could therefore have a greater impact on thermoregulatory costs, which would in turn influence metabolic rate. Furthermore, animal behaviour and energetic costs of locomotion are often vastly different on land compared to in water due to differences in buoyancy support and biomechanics.

Most studies that derive $fh:\dot{V}_{O_2}$ relationships for application in the field have used animals that were either fasted to eliminate the possible effect of digestion or fed unknown amounts of food (Boyd et al. 1999; Butler et al. 1992; Williams et al. 1993). Digestion has the potential to change the $fh:\dot{V}_{O_2}$ relationship via the heat increment of feeding (HIF, also called the specific dynamic action, SDA), which represents the loss of energy during chemical and physical digestion (Blaxter 1989; Secor 2009). It is manifested as an observable increase in $\dot{V}_{O_2}$ — the extent and duration of which is influenced by the composition and size of the meal (Markussen et al. 1994; Rosen and Trites 1997). After a meal, $\dot{V}_{O_2}$ peaks more gradually and at a higher level for larger meals compared to smaller meals (Markussen et al. 1994; Rosen and Trites 1997). The peak $\dot{V}_{O_2}$ in Steller sea lions occurs at approximately 4 hours following a 4 kg meal of herring (2.13 x baseline), but the peak $\dot{V}_{O_2}$ following a 2 kg meal of herring (1.76 x baseline) occurs approximately 1 hour earlier (Rosen and Trites 1997). Duration of the HIF effect is also influenced by meal size, lasting 6-8 hours following a 2 kg meal and 8-10 hours following a 4 kg meal (Table A2.3).

The heart rate method relies on the assumption that *fh* changes proportionally with $\dot{V}_{O_2}$. If $\dot{V}_{O_2}$ changes with HIF as predicted, but concurrent changes in *fh* during digestion are different than when fasted (Vatner et al. 1974) this assumption would be violated and the $fh:\dot{V}O_2$ relationship would change after feeding. While some captive $fh:\dot{V}_{O_2}$ studies have indirectly incorporated unquantified feeding as a positive reinforcement training tool, none have directly compared data from fed and fasted animals (Boyd et al. 1999; Williams et al. 1993). According to recent work with trained Steller sea lions, *fh* can predict $\dot{V}_{O_2}$ under fasted conditions (McPhee et al. 2003), but a different $fh:\dot{V}_{O_2}$ relationship may exist when animals are feeding. However,

this preliminary investigation was supplemental to the main study (i.e., it only included one animal) and therefore lacked the necessary scope to fully explore the possible effects of digestive state on metabolism.

Furthermore, studies performed in a swim mill do not incorporate the potential influences of depth, pressure, or a normal dive response; therefore it is also necessary to examine the *fh*: $\dot{V}_{O_2}$ relationship in animals that are diving to natural depths. Limited studies examining the *fh*: $\dot{V}_{O_2}$ relationship in diving vertebrates have primarily focused on birds (Bevan et al. 1992; Butler 1984; Stephenson et al. 1988; Woakes and Butler 1983) rather than pinnipeds (Webb et al. 1998). Marine mammals physiologically respond to submersion and diving in water with a suite of adaptations known collectively as the dive response that include decrease in heart rate while submerged (bradycardia), potential increase in heart rate after diving (tachycardia), cessation of breathing (apnea), and vasoconstriction (Butler and Jones 1997; Scholander 1940; Scholander et al. 1942).

Finally, changes in blood flow distribution during diving or submergence (Davis et al. 1983; Kooyman et al. 1973; Stone et al. 1973) could also influence the ($Ca_{O_2} - C\bar{v}_{O_2}$) difference due to changes in the rate of O_2 delivery to the tissues. Limited studies on blood flow redistribution in diving marine mammals suggest that the magnitude, duration, and organ target of blood flow distribution during diving is likely a gradient between the extreme restriction to the heart and brain during forced submersions (e.g. Zapol et al. 1979) and the moderate changes observed in natural diving (Davis et al. 1983; Stone et al. 1973). If changes in *fh*, blood flow distribution, ($Ca_{O_2} - C\bar{v}_{O_2}$), or Vs during diving are sufficiently large in magnitude or duration, there is a possibility that the predictive relationship between *fh* and $\dot{V}_{O_2}$ could change.

Research objectives

Despite the current use of *fh* to predict $\dot{V}_{O_2}$ of marine endotherms in the wild, the effectiveness of the *fh* method for pinnipeds across naturally occurring digestive states and environmental conditions has not been adequately tested. The overall objective of my thesis was to determine whether *fh* could be used to predict $\dot{V}_{O_2}$ in captive Steller sea lions under a variety

of more natural environmental (resting on land or in water), feeding (fasted or fed), and physiological conditions (resting in water vs. dive response).

My first goal was to determine whether the fh:$\dot{V}_{O_2}$ relationship differed between fasted Steller sea lions while on land or in water. Previous work has demonstrated linear relationships between fh and $\dot{V}_{O_2}$ in fasted, Steller sea lions resting in either air or water, but this study did not control for repeated measures and pooled data collected on land and in water without testing for environmental differences (McPhee et al. 2003). I did not expect differences in the relationship between environments, as digestive state and activity level were similar in both, and I assumed thermoregulatory costs within my testing conditions to be minimal.

My second thesis goal was to investigate whether feeding animals meals of different sizes (4 or 6 kg) would change the fh:$\dot{V}_{O_2}$ relationship in water or on land. I predicted that the fh:$\dot{V}_{O_2}$ relationship would change following a meal based on the hypothesis that the increased $\dot{V}_{O_2}$ due to the heat increment of feeding (Markussen et al. 1994; Rosen and Trites 1997; Secor 2009) would not be accompanied by the same increase in fh observed in fasted animals (McPhee et al. 2003; Vatner et al. 1970).

My third thesis goal was to determine whether dive activity and the accompanying dive response would change the fh:$\dot{V}_{O_2}$ relationship in sea lions foraging at depth. Research suggests that Vs (Blix et al. 1983; McKean 1982; Ponganis et al. 1990; Sinnett et al. 1978; Zapol et al. 1979), tissue oxygen extraction (McKean 1982), O_2 depletion rate (Meir et al. 2009), and blood flow (Davis et al. 1983; Stone et al. 1973) change over a gradient influenced by dive duration and dive behavior. Despite the likelihood of these physiological changes occurring during diving, previous work has shown that fh alone can be used as a tool to predict $\dot{V}_{O_2}$ in submerged and diving pinnipeds (Boyd et al. 1995; Butler et al. 1992; McPhee et al. 2003; Webb et al. 1998; Williams et al. 1991). However, these studies have been limited by depth and dive duration. I predicted that fh could predict diving metabolism when averaged over a physiological relevant time frame, but that this would differ from the relationship while resting in water. I further predicted that the diving fh:$\dot{V}_{O_2}$ relationship would be affected by dive activity (single dive or dive bout), dive depth, and dive duration.

My thesis consists of 2 major data chapters that will be submitted for publication in peer-reviewed journals and are therefore written as independent papers. Chapter 2 focuses on the potential effect of feeding. It determines whether *fh* could be used to predict $\dot{V}_{O_2}$ in fasted sea lions and whether environment (land or water) or feeding changes the relationships. Chapter 3 investigates the *fh*:$\dot{V}_{O_2}$ relationship in sea lions that are feeding and diving simultaneously to different depths in the open ocean. The exploration of *fh* and digestion in Chapter 2 also serves as a building block towards the simulated feeding and diving explored in Chapter 3. Collectively, my thesis examines the *fh*:$\dot{V}_{O_2}$ relationship in Steller sea lions under conditions that are comparable to free-ranging animals in environment, digestive state, and activity level, and aims to provide a more accurate estimation of activity-specific metabolic rates for free-ranging Steller sea lions.

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Chapter 2: Environment and digestion change the ability of heart rate to predict metabolism in resting Steller sea lions (*Eumetopias jubatus*) [1]

Introduction

Estimates of energy expenditure associated with specific activities undertaken by animals are critical for determining the energetic needs of predators and the effects of changes in behaviour on energy or prey requirements. Estimates of energy expenditure in free-ranging marine homeotherms have been traditionally measured using two methods. The first, doubly labeled water, provides a mean estimate of metabolism over a 4-6 day interval (Costa 1987; Kam and Degen 1997; Roberts 1989; Speakman and Krol 2005), but presents logistical and financial challenges, and may overestimate field metabolic rate in otariids (fur seals and sea lions) by as much as 36% (Boyd et al. 1995). The second method, heart rate (*fh*), can provide estimates of energy expenditure for specific activities on a much finer time scale and for longer periods, but requires species-specific predictive equations (Boyd et al. 2004; Boyd et al. 1995; Butler et al. 2004; Ponganis 2007; Woakes et al. 1995). Heart rate has been used to estimate energy expenditure in several aquatic homeotherms including penguins, seals, and sea lions (Boyd et al. 1995; Fahlman et al. 2004; Froget et al. 2002; McPhee et al. 2003; Williams et al. 1991), and has the potential to provide estimates of activity-specific energy expenditure in free-ranging animals.

The heart rate method for quantifying field metabolism estimates rates of oxygen consumption ($\dot{V}_{O_2}$, an accepted proxy for energy expenditure) from recorded heart rate (*fh*) is based upon Fick's (1870) relationship: $\dot{V}_{O_2} = (Ca_{O_2} - C\overline{v}_{O_2}) \times Vs \times fh$, where Vs is stroke volume, Ca_{O_2} is the arterial oxygen content, $C\overline{v}_{O_2}$ is the oxygen content of the mixed venous blood, and the function $(Ca_{O_2} - C\overline{v}_{O_2})$ represents the amount of oxygen extracted from the tissues. The application of this technique relies on the assumption that an increase in *fh* is the primary method that animals employ to respond to increased oxygen consumption rate, and that $(Ca_{O_2} - C\overline{v}_{O_2})$ and Vs stay constant or vary proportionally to heart rate. However, stroke volume or tissue oxygen extraction are likely affected by environmental and physiological states (see discussion below).

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Field application of the heart rate method requires deriving species-specific predictive equations to define the relationship between *fh* and $\dot{V}_{O_2}$ (Butler et al. 2004). Traditionally, these initial $fh:\dot{V}_{O_2}$ studies have been conducted under uniform physiological and environmental conditions to limit experimental and statistical variation. For example, studies have been conducted on marine mammals that were fasting to eliminate the possible confounding effect of digestion (Butler 1993; Fahlman et al. 2004; Hurley and Costa 2001; McPhee et al. 2003; Williams et al. 1991). However, several factors that animals naturally encounter in the wild can potentially affect the relationship between *fh* and $\dot{V}_{O_2}$, including environment (land or water) and digestive state (fed or fasted).

Feeding has the potential to change the $fh:\dot{V}_{O_2}$ relationship via the heat increment of feeding (HIF), which represents the loss of energy during chemical and physical digestion (Blaxter 1989; Secor 2009). It is manifested as an observable increase in $\dot{V}_{O_2}$ — the extent and duration of which is influenced by the composition and size of the meal (Markussen et al. 1994; Rosen and Trites 1997). A change in $\dot{V}_{O_2}$ due to feeding without a parallel rise in heart rate would violate the assumptions of only using *fh* from Fick's equation and would result in different $fh:V_{O_2}$ relationships for feeding and fasting states. While some captive studies have indirectly incorporated unquantified feeding as a positive reinforcement training tool, none have directly compared these results with fasted data (Boyd et al. 1999; Williams et al. 1993). According to recent work with trained Steller sea lions, *Eumetopias jubatus, fh* can predict $\dot{V}_{O_2}$ under fasted conditions (McPhee et al. 2003), but a different $fh:\dot{V}_{O_2}$ relationship may exist when animals are feeding. However, this preliminary investigation was supplemental to the main study (i.e., it only included one animal) and therefore lacked the necessary scope to fully explore the possible effects of digestive state on metabolism.

The $fh:\dot{V}_{O_2}$ relationship may also be affected by the physical environment. Sea lions inhabit both the marine and terrestrial environments. One notable difference between water and air is their thermal properties. Water has a greater specific heat capacity relative to air and could therefore have a greater impact on thermoregulatory costs, which would in turn influence metabolic rate. Also, marine mammals physiologically respond to submersion and diving in

water with a suite of adaptations that include decrease in heart rate (bradycardia), apnea, and vasoconstriction (for a review see Butler and Jones 1997). In addition to the "classic" dive response, research also suggests that stroke volume, V_S, (Blix et al. 1983; Ponganis et al. 1990; Sinnett et al. 1978; Zapol et al. 1979), O_2 depletion rate (Meir et al. 2009), and blood flow (Davis et al. 1983; Stone et al. 1973) change during diving, although it is not clear to what extent these variables change during shallow submersion or in anticipation of diving. A change in any of these parameters could affect the individual components of Fick's equation, and therefore the predictive relationship between *fh* and $\dot{V}_{O_2}$.

Despite the current use of *fh* to predict $\dot{V}_{O_2}$ of marine endotherms in the wild, it is still not clear whether the heart rate method works for pinnipeds after feeding or across all environmental conditions. I therefore sought to investigate the effect of environment (land or water) and feeding on the *fh*:$\dot{V}_{O_2}$ relationship in resting, trained Steller sea lions. More specifically, I determined: 1) whether *fh* could be used to predict $\dot{V}_{O_2}$ in fasted Steller sea lions, 2) whether environment (land or water) changed the relationship between *fh* and $\dot{V}_{O_2}$ in fasted sea lions, 3) whether *fh* could predict $\dot{V}_{O_2}$ in fed sea lions on land or in water, and 4) whether this relationship differed for animals that were feeding or fasting.

Methods

Data collection

Seven female Steller sea lions ranging in age from 4-11 years participated in this study from April-September 2008 (Table 2.1). Prior to the experiments, all animals were fed a diet within training requirements consisting of herring (*Clupea pallassi*) supplemented with vitamin tablets 2-3 times daily. Animals were fasted overnight, and then weighed each morning on a platform scale (± 0.5 kg). All animal procedures were conducted under the authority of University of British Columbia Animal Care Protocol (A07-0208 and A07-0413), Department of Fisheries and Oceans Canada (MML 2007-0001) and the Vancouver Aquarium (see Appendix A5). All animal work was conducted voluntarily under trainer control.

The experimental design consisted of 7 trial types that varied by either environment (dry, water, open water) or digestive state (fasted or 0 kg, 4 kg, 6 kg herring). The sea lions rested in a dry metabolic chamber after fasting overnight during dry_{fasted} trials (details below). To compare different environmental conditions (land vs. water), each animal also completed a $water_{fasted}$ trial during a period of rest inside a swim mill (also after overnight fasting). I further compared $water_{fasted}$ trials to a more natural environment by collecting data from trained sea lions resting in a respirometry dome floating on the ocean surface ($water_{ow}$). To evaluate the impact of digestive state, each animal was fed 4 kg and 6 kg of herring before entering the dry metabolic chamber (dry_{4kg}, dry_{6kg}) and swim mill ($water_{4kg}$, $water_{6kg}$).

Experiments were conducted on two groups of Steller sea lions raised in captivity and previously trained to use all experimental apparatus. All trials except those conducted in open water ($water_{ow}$) were conducted on four female sea lions (F03AS, F00ED, F03WI, F03RO) housed at the Vancouver Aquarium (British Columbia, Canada). These animals were held in outdoor enclosures with access to seawater pools and haulout space. Each animal completed a single replicate of each of the aforementioned six trial types (conducted in random order) on separate days at the Vancouver Aquarium over a period of several weeks (Table 2.1). Due to difficulties with behavioral cooperation, animal F00ED did not complete the $water_{4kg}$ trial, and the dry_{4kg} trial for this animal was not useable due to poor quality heart rate data (n = 4 animals, 22 trials).

The $water_{ow}$ trials were conducted with a second group of sea lions as they rested at the ocean surface at the UBC Open Water Research Laboratory (Port Moody, British Columbia, Canada). Three female Steller sea lions (F97SI, F97HA, F00BO; Table 3.1) were housed in a specially designed floating pen that provided access to seawater and haulout space (for a full description see Hastie et al. 2006, 2007). Each sea lion completed six $water_{ow}$ trials on separate days (See Chapter 3 for details).

Table 2. 1 Age, body mass (kg ± SD), and number of trials (N) per animal. Steller sea lion numbers differentiate data points in figures in Appendices.

Animal ID	No.	Age (yr)	Trial (2008)	Mass (kg)	(± SD)	N
F03AS	1	4	7 Apr - 21 Jul	157	(3.6)	6
F00ED	2	7	8 Apr - 23 Jul	165	(2.1)	4
F03WI	3	4	24 Apr - 22 Jul	135	(2.7)	6
F03RO	4	4	9 Apr - 15 Jul	142	(3.9)	6
F97SI	5	11	1 Aug - 16 Sep	218	(4.4)	5
F00BO	6	11	7 Aug - 16 Sep	145	(5.1)	5
F97HA	7	8	7 Aug - 4 Sep	172	(0.6)	5

Measurement of heart rate

Steller sea lions were outfitted with subcutaneous heart rate electrodes while under veterinary-supervised gas anaesthesia (0-5% Isoflurane). The heart rate monitoring system consisted of 1) a heart rate datalogger (HTR, Wildlife Computers, Redmond, WA, USA) that recorded the inter-beat-interval (IBI, or R-R peak intervals of the electrocardiogram, ECG) and 2) a heart rate transmitter (HRX, Wildlife Computers) with two 26-gauge wire leads, ~32 inches in length. To reduce infection risk, 30-gauge 99.9% pure silver Teflon-coated wire (Grass Technologies, Longueuil, QC, Canada) was spliced to the terminal end of the electrode leads. These were sterilized in glutaraldehyde for a minimum of 30 min prior to each procedure (Metricide 28, Metrex, VaxServ, Scranton, PA, USA).

The placement of the heart rate recording equipment was designed for single-use deployment under anesthesia that would permit recovery of all equipment (including subcutaneous electrode wires) under trainer control after each trial. The transmitter and datalogger were carried in a pocket on a custom-fit harness worn by the animals. Electrodes were inserted subcutaneously, through neoprene circles glued to the fur, by bending the terminal end of the Teflon wire and inserting the stripped end (0.5-1.0 cm) into a 20-gauge hypodermic needle. Electrodes were placed caudal to the front flippers at the level of the heart, approximately 25 cm lateral of the dorsal midline. The wires were secured to the neoprene circles and to the fur along the spine with additional small squares of neoprene (Fig. 2.1b, c). Animals were allowed to completely recover from anesthesia in a close-contact cage or dry area before commencing the trial.

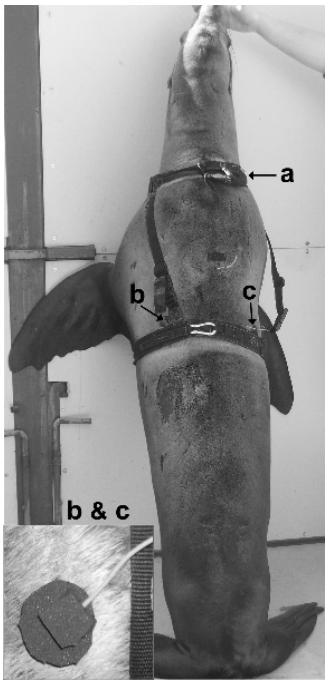

Figure 2. 1 Photograph of heart rate apparatus placement showing the electrodes (**b**, **c**) and harness pocket (**a**) containing the datalogger. The insert shows an enlarged image of the electrodes (**b**, **c**). The Steller sea lion was approximately 2 m long.

Measurement of oxygen consumption rate

Oxygen consumption rate was measured via open-circuit gas respirometry. The system was calibrated prior to the start of trials using gases of known concentrations and a nitrogen standard. Gas concentration readings were corrected for electronic drift against ambient air before and after each trial. Oxygen and carbon dioxide concentrations within a desiccated subsample of the excurrent airstream were measured using Sable System FC-1B and CA-1B analyzers, coupled to a 500H Mass Flow Generator and Controller (Sable Systems, Las Vegas, NV, USA). Barometric pressure, relative humidity, and expired air temperature were also recorded (Airguide Instruments, Chicago). The excurrent airstream was continuously subsampled, and averaged every 3 sec (Sable Data Acquisition System, Sable Systems, Las Vegas, NV, USA). As CO_2 was not scrubbed from incurrent air stream, rates of oxygen consumption were calculated using both O_2 and CO_2 concentrations (see below).

For the 'dry' trials, $\dot{V}_{O_2}$ was measured in a metabolic chamber (~1,050 L), equipped with a camera to allow visual monitoring of behaviour. Air was drawn through the chamber at a

constant rate of 200 L·min^{-1}. Average air temperature inside the chamber ranged between 10.8 and 21.5°C (mean 16.0 ± 3.1°C). Animals were able to turn around inside the chamber, but not exercise.

For the water$_{fasted}$, water$_{4kg}$, and water$_{6kg}$ trials, $\dot{V}_{O_2}$ was measured in a swim mill (3.2 m x 1.8 m x 1.0 m; no water current was applied). The animals were only able to surface under a transparent 120 L Plexiglas dome. The animals were contained in a smaller (1.60 m x 0.89 m x 0.84 m) inner cage to prevent tangling of the electrode wires, but which was large enough to allow them to turn around or rest on the bottom of the swim mill. Average water temperature inside the swim mill ranged between 10.5 and 13.6°C (mean 12.2 ± 1.3°C).

Water$_{ow}$ trials were either conducted at a dive site next to the animals' holding pen or the animals were transported to a nearby dive site in a 22-ft research boat. A second boat towing a floating barge carried the respirometry equipment to the dive site. The barge had a square hole in the middle containing a cage (1.52 m x 1.52 m x 2.5 m) and floating transparent Plexiglas respirometry dome (100 L). An open-circuit gas respirometry system similar to that used at the Vancouver Aquarium was used; unless specified, all respirometry calibrations and settings were the same. Air was drawn through the respirometry dome at 475 L·min^{-1}. The excurrent airstream was averaged every 0.5 sec to capture the quick changes in $\dot{V}_{O_2}$ observed after surfacing from dives for a concurrent study (Chapter 3).

Fasted trial protocol

The dry$_{fasted}$ and water$_{fasted}$ (0 kg fed) trials provided environment-specific $fh:\dot{V}_{O_2}$ relationships and permitted appropriate comparisons with feeding trails. Fasting trials were a maximum length of 90 min, which was a reasonable amount of time for an animal to remain calm without food reinforcement and reliably re-enter the apparatus for future trials. In one instance F00ED was fed 0.52 kg of squid (*Loligo opalescens*) to distract her while a transmitter was adjusted. It is unlikely that this event confounded results given the relatively minor and delayed $\dot{V}_{O_2}$ effects of squid digestion in Steller sea lions (Rosen and Trites 1997; Rosen and Trites 1999). During the water$_{fasted}$ trial for F03AS, the heart rate electrodes became displaced and the trial was terminated early (mean trial length: 81 ± 16 min).

For $water_{ow}$ trials, the sea lions rested for 6-10 minutes in the respirometry dome while *fh* and $\dot{V}_{O_2}$ were recorded. Animals were fed 0.02 kg herring pieces (max 0.36 kg, mean 0.22 ± 0.09 kg) through a delivery tube in the respirometry dome to facilitate cooperation. $Water_{ow}$ resting trials took less than 15 minutes to complete; therefore it was unlikely that results were influenced by HIF (Rosen and Trites 1997).

Feeding trial protocol

A feeding trial consisted of two phases of data collection: 1) measurement of pre-feed (fasted) $\dot{V}_{O_2}$ and *fh* for 20-25 min ($\dot{V}_{O_2\ \text{pre-feed}}$) and 2) continuous measurement of post-feeding $\dot{V}_{O_2}$ and *fh* for 4-4.5 h ($\dot{V}_{O_2\ \text{feeding}}$). Between the two phases, the animals were fed for 5-10 min, and the $\dot{V}_{O_2}$ equipment was paused since the integrity of the flow-through system was breached. Comparison of the $\dot{V}_{O_2\ \text{pre-feed}}$ data to the fasted control (dry_{fasted} or $water_{fasted}$) for each animal confirmed that any observed changes in *fh* or $\dot{V}_{O_2}$ were due to prey ingestion rather than other factors (e.g., that measurements were taken on different days). The duration of the feeding phase of trials was selected to capture the peak in HIF, which occurs at approximately 4 hours following a 4 kg meal of herring (Rosen and Trites 1997). Post-feed data collection period varied slightly among trials due to electrode performance (225 ± 30 min), although the majority of the trials lasted for the planned 240 min.

Data analysis

Heart rate

Data downloaded from the heart rate datalogger were analyzed with Microsoft Excel and R 2.9.2 (R Core Development Team 2009). First, inter-beat-intervals (IBI) were converted to instantaneous heart rate (fh_{inst}) using the following equation:

$$fh_{inst}(\text{beats} \cdot \text{min}^{-1}) = \frac{60 \text{ seconds}}{\text{IBI}}$$

I applied the following algorithms to systematically remove any fh_{inst} values that were artifacts of muscle or wire movement. Field comparisons of the same model heart rate datalogger to a portable ECG on harbor seals have shown that artificial beats caused by sudden movement

are recorded as 206.89 beats·min^{-1} or often > 230 beats·min^{-1} (Greaves et al. 2005). Furthermore, frequency histograms of all $f_{H_{inst}}$ showed that 79% of all $f_{H_{inst}}$ in my study were ≤ 240 beats·min^{-1}. Therefore all $f_{H_{inst}}$ values of 206.98 beats·min^{-1} or >240 beats·min^{-1} were eliminated. These data likely resulted from electromyographic noise or electronic noise from wire movement. Secondly, a $f_{H_{inst}}$ value was removed if the target cell was ≥ ± 1 SD from an 11-point mean. Sections with large amounts of apparent noise were manually examined as necessary. Finally, $f_{H_{inst}}$ values were averaged in consecutive 5-min intervals to yield average f_H (beats·min^{-1}). Empirical data from terrestrial mammals show that f_H decreases as body mass (M_b) increases for some species (Castellini and Zenteno-Savin 1997), but regression lines derived for otariids (California sea lions, northern fur seals) were not significant. Therefore, I did not scale f_H with body mass.

Oxygen consumption rate

Oxygen consumption data was analyzed using Datacan Data Analysis software (V 1.0.24; Sable Systems Inc., Las Vegas, NV). Oxygen consumption rate ($\dot{V}_{O_2}$) was calculated from changes in O_2 and CO_2 concentrations (using Eqn. 3b,Withers 1977) over time, and $\dot{V}_{O_2}$ was averaged in Microsoft Excel over the same 5-min intervals as the f_H data. The synchronization process also accounted for the 30-sec time lag for air traveling between the respirometry chamber or dome and the gas analyzers. For the water$_{ow}$ trials, average $\dot{V}_{O_2}$ was calculated by averaging the last two minutes of the resting period when $\dot{V}_{O_2}$ had reached a constant resting level.

There has been considerable debate over whether metabolic rate should be scaled with body mass, and what exponent should be used (Brown and West 2005; Savage et al. 2007; White and Seymour 2005). I chose to mass-correct $\dot{V}_{O_2}$ using the exponent of 0.75 to account for changes in M_b over time and facilitate comparisons with other studies. Mass-corrected $s\dot{V}_{O_2}$ is thus presented as ml O_2·min^{-1}·kg$^{-0.75}$.

Statistical analysis

Data from each animal within a trial and data from each animal across trial types was treated as a repeated measures set using linear mixed-effects (LME) models in R 2.9.0 (nlme library from Pinheiro and Bates 2000). LME models characterize individual variation relative to the mean of the population while considering the correlation between repeated measurements within and among animals. All models were run using the maximum likelihood method, and the slope and intercept were allowed to vary for each animal during model optimization.

Animal ID was treated as a random effect for all models, which permitted applying inferences from the sample population to the free-ranging population. Fixed effects explored included the amount of food fed (0, 4, 6 kg) and the environment (dry, water, open water). To decrease the potential effects of time autocorrelation for *fh* and $\dot{V}_{O_2}$, measurements made on the same animal during a trial were averaged over 5 minutes. Additionally, a larger *fh* average was generated to facilitate comparisons with diving heart rates for a concurrent study (mean single dive cycle 6.8 min, Chapter 3).

For each analysis, the best model in terms of fixed effect factors and homogeneity of variance corrections was determined using an ANOVA. To clarify, ANOVA serves dual purposes for LME models. An ANOVA executed on a single model generated a conditional F-test to determine the significance of model slope, intercept, and fixed effects. An ANOVA performed on two nested models (the fixed effect model hierarchically nested within the model without fixed effects) produced likelihood ratio tests (LRT) that compared the two models. All models presented only had one fixed effect applied at a time, therefore, for all LRT tests df = 1. All reported data are presented mean ± standard deviation (SD), and statistical significance was set at $\alpha = 0.05$.

Incorporation of McPhee et al., 2003 data

Additional *fh* and $\dot{V}_{O_2}$ data collected on Steller sea lions by McPhee et al. (2003) under similar experimental conditions were included with my data to investigate whether a larger sample size would affect my conclusions. Briefly, this earlier study examined the relationship between *fh* and $\dot{V}_{O_2}$ in four Steller sea lions aged 1.3-3 years housed at the Vancouver Aquarium

(M97TI, M97KO, F97HA, F97SI, McPhee et al. 2003). Data was collected under varied environment and activity conditions (dry inactive, water active, water inactive) using an open circuit respirometry system similar to that described here, and custom subcutaneous electrodes for the measurement of *fh*. Trials were conducted in the same metabolic chamber and swim mill as for my study, but water current was applied in some water trials (water active). At the end of McPhee et al.'s focal study, preliminary data exploring the potential effects of feeding were conducted on a single animal (M97TI) for water trials only (n = 6 trials). M97TI entered the swim mill 32-73 min after eating 6 or 12 kg of herring, and $\dot{V}_{O_2}$ and *fh* were measured until ~3.5-4.25 h after ingestion.

The statistical analysis performed by McPhee et al. (2003) produced an overall mean regression for the four test sea lions that differed from ours (including a lack of repeated measures control), making direct comparisons difficult. $\dot{V}_{O_2}$ data were therefore converted from the original units of ml $O_2 \cdot h^{-1} \cdot kg^{-0.60}$ to ml $O_2 \cdot min^{-1} \cdot kg^{-0.75}$ using raw values of total $\dot{V}_{O_2}$ ($ml \cdot h^{-1}$) and body mass (M_b). The log transformation of $\dot{V}_{O_2}$ was also removed as Q-Q (quantile) plots showed that data was normally distributed. Only comparable data from McPhee et al. were used in these subsequent analyses. The raw data from McPhee et al. was re-analyzed using the same LME models and repeated measures design in R 2.9.0, as described previously. Animal was treated as a random factor; gender, amount of food fed, and trial type were treated as fixed factors and tested as appropriate for each model.

Confidence intervals (95% CI) for selected regression models were calculated by bootstrapping (R Core Development Team 2009; Whitlock and Schluter 2009). I fit the best overall linear mixed effect model as previously described (see above), assigning a random effect coefficient for each animal, as well as modeling the heterogeneity of variances between animals. The residuals represented the random errors in my data that could not be modeled, and were assumed to be normally distributed. I took the predicted values from the fixed effect regression equation, around which I wanted to know the error, and a bootstrapped sample of the residuals. I then reassigned these residuals randomly to the predicted values and refit the mixed-effect linear model with the same assumptions. In this way, the structure of the original model was conserved and the prediction errors were only associated with the dependent variable. This procedure was

repeated 1000 times. Finally, the ordered 24th and 976th bootstrapped replicates were plotted to represent the 95% CI for a model (2.5% of 1000 = 25th -1 to be conservative for the lower CI, and 97.5% of 1000 = 97.6th – 1 to be conservative for the upper CI).

The error associated with the predictive equations was not constant, but rather increases with the distance from the mean values. However, many studies evaluating other techniques to estimate energy expenditure report a single, average error (e.g., Boyd et al. 1995). To facilitate comparison with other methods, I derived a single representative value of the error associated with the predictions, which I termed the average residual. I did so by calculating the mean absolute residual value of a specific model, and dividing it by the median predicted $\dot{V}_{O_2}$ value to calculate the average residual. This served as a proxy for the average predictive error of my models.

Results

The relationship between heart rate and oxygen consumption rate

Fasted relationships

Mean *fh* for dry$_{fasted}$ trials ranged from 74-123 beats·min^{-1} and $s\dot{V}_{O_2}$ ranged from 20-77 ml O_2 min^{-1}·kg$^{-0.75}$. Heart rate could be used to predict $s\dot{V}_{O_2}$ in fasted animals resting on land ($F_{1,60}$ = 11.03, P = 0.002; Table 2.2: Eqn. 1; Fig. 2.2a) (selected equations are given in Table 2.2. See Table A2.1 for all equations).

Average *fh* and $s\dot{V}_{O_2}$ for Steller sea lions resting in water were similar regardless of location (water$_{ow}$ vs. water$_{fasted}$), and ranged from 57-108 beats·min^{-1}and 18-47 ml O_2 min^{-1}·kg$^{-0.75}$ (Fig. 2.2b). The *fh:* $\dot{V}_{O_2}$ relationship for water$_{ow}$ trials did not differ from water$_{fasted}$ data (LRT = 0.05, P = 0.82; Fig. 2.2b). These trials were therefore combined to create a composite predictive equation for animals resting in water (water$_{comp}$, Table 2.2: Eqn. 2). This equation encompassed a wider environmental scope and also had greater statistical power due to increased sample size (see Appendix A2 for supplementary figures showing individual animals).

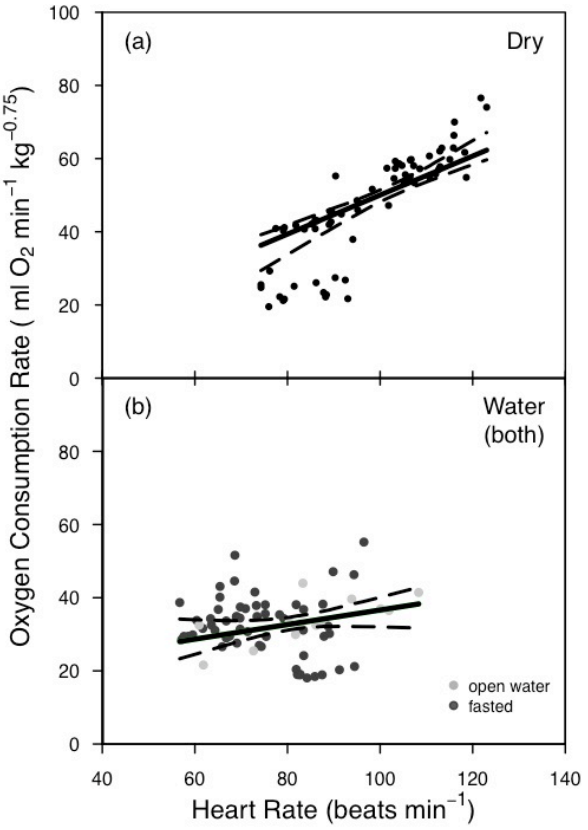

Figure 2. 2 The relationship between heart rate (*fh)* and oxygen consumption ($\dot{V}_{O_2}$) in Steller sea lions differed when fasted on land (**a**) and in water (**b**). There was no difference in the relationship between trials conducted in the swim mill (dark grey circles) and while resting at the surface (light grey circles, **b**). Regressions were derived using mixed-effects linear models within a repeated measures framework. The predictive lines are indicated with solid lines, and the 95% bootstrap confidence intervals are indicated with dashed lines.

The relationship between *fh* and $\dot{V}_{O_2}$ during $\text{dry}_{\text{fasted}}$ trials was significantly different than when measured under apparently similar physiological conditions in water (*LRT* = 14.6, *P* = 0.001 compared to $\text{water}_{\text{comp}}$). Consequently, two separate environment-specific equations were needed to accurately predict $\dot{V}_{O_2}$ in fasted animals (Table 2.2: Eqns. 1, 2). These unique equations ($\text{water}_{\text{comp}}$ and $\text{dry}_{\text{fasted}}$) were thereafter applied in subsequent comparisons (relative to environment) of fed and fasted states.

Table 2. 2 Equations for selected models ($\dot{V}_{O_2} = a \bullet fh + b$) showing the linear relationship between heart rate (*fh)* and oxygen consumption ($\dot{V}_{O_2}$) for Steller sea lions that were fasted or fed (food in kg). Model parameters include slope (± SE), intercept (± SE), and *P*-values (F-test). Figure references are only given for equations presented in figures (See Table A2.1 for all equations), and model descriptions are defined in the list of abbreviations. Regressions were derived using mixed-effects linear models within a repeated measures framework.

Eqn.	Fig.	Food (kg)	Slope (*a*)	(± SE)	Intercept (*b*)	(± SE)	Slope *P*-value	Intercept *P*-value	Model Description
1	2.2a	0	0.53	(0.16)	-3.31	(18.01)	0.002	<0.001	dry_{fasted}
2	2.2b	<0.36	0.20	(0.07)	16.7	(4.96)	0.005	<0.001	$water_{comp}$
3	2.3	0,4,6	0.31	(0.12)	19.2	(13.91)	0.009	<0.001	dry_{all}
4	2.4a	4	0.21	(0.08)	16.4	(6.90)	0.011	<0.001	$water_{4kg}$
5	2.4b	0,6	0.17	(0.03)	19.4	(3.11)	<0.001	<0.001	$water_{6kg}$+ $water_{comp}$ (+food NS factor)
6	2.4c	0 or 6					<0.001	<0.001	$water_{6kg+McPhee}$ + $water_{comp+McPhee}$
			0.36	(0.06)	13.9	(8.41)			$water_{6kg+McPhee}$
			0.36	(0.06)	11.7	(6.02)			$water_{comp+McPhee}$
							0.033	<0.001	$water_{+McPhee}$
7a		4	0.13	(0.06)	24.5	(6.05)			4 kg
7b		6	0.13	(0.06)	23.0	(6.43)			6 kg
7c		12	0.13	(0.06)	23.6	(6.61)			12 kg
									$water_{+McPhee}$
8		4,6,12	0.12	(0.06)	24.5	(5.54)	0.030	<0.001	(4,6,12 mixed)
9		0,4,6	0.32	(0.10)	17.0	(11.63)	<0.001*	<0.001*	$dry_{all+McPhee}$

Effect of feeding

Mean *fh* and $s\dot{V}_{O_2}$ for dry_{4kg} trials ranged from 53-128 beats·min^{-1} and from 18-78 ml $O_2 \cdot min^{-1} \cdot kg^{-0.75}$. Heart rate and oxygen consumption distributions for dry_{6kg} were similar to dry_{4kg} and ranged from 54-124 beats·min^{-1}, and from 19-79 ml $O_2 \cdot min^{-1} \cdot kg^{-0.75}$. Heart rate could be used to predict oxygen consumption of animals that were fed either a 4 kg or 6 kg meal on land, and these predictive equations were not statistically distinct (LRT = 0.39, P = 0.53; Fig. A2.3). Furthermore, the relationship between *fh* and $s\dot{V}_{O_2}$ for dry_{fasted} trials was not different than for either dry_{4kg} or dry_{6kg} trials (dry_{6kg}: LRT = 3.0, P = 0.08; dry_{6kg}: LRT = 1.4, P = 0.23), or when data from the dry_{4kg} and dry_{6kg} trials were combined ($F_{1,373}$ = 0.98, P = 0.32). Ultimately, a single linear equation was generated to predict the $s\dot{V}_{O_2}$ of animals that were fasted or fed on land ($F_{1,374}$ = 6.9 , P = 0.009; Table 2.2: Eqn. 3; Fig. 2.3) (see Figure A2.3 and A2.4 for supplementary feeding on land figures).

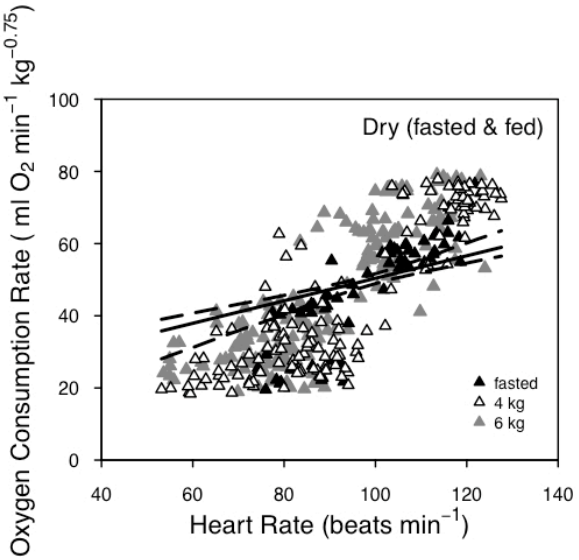

Figure 2. 3 The relationship between heart rate (*fh)* and oxygen consumption ($\dot{V}_{O_2}$) did not differ among Steller sea lions that were fasting (black triangles), or feeding 4 kg (open triangles) and 6 kg (grey triangles). A single equation (solid line) derived using mixed-effects linear models within a repeated measures' framework was therefore used to predict $\dot{V}_{O_2}$ on land. The 95% bootstrap confidence intervals are indicated with dashed lines.

Mean *fh* and $s\dot{V}_{O_2}$ for water$_{4kg}$ trials ranged from 60-93 beats·min^{-1} and from 19-46 ml O_2·min^{-1}·kg$^{-0.75}$. The range of *fh* and $s\dot{V}_{O_2}$ was similar for water$_{6kg}$ (60-105 beats·min^{-1} and 18-44 ml O_2·min^{-1}·kg$^{-0.75}$, respectively). The amount of food digested (0 vs. 4 kg) while resting in water was a highly significant factor in the linear model. The interaction between the amount of food consumed and *fh* was also significant ($F_{1,195}$ = 10.16, P = 0.002) suggesting different predictive relationships between animals fasting in water and those consuming 4 kg meals (Table 2.2: Eqn 4; Fig. 2.4a; Fig. A2.5). In contrast, *fh* could not be used to predict $s\dot{V}_{O_2}$ for water$_{6kg}$ trials ($F_{1,138}$ = 1.42, P = 0.24, Fig. A2.5). In fact, none of the models examined for water$_{6kg}$ (alone or as a combined dataset with water$_{4kg}$) were linear; therefore I was unable to compare water$_{4kg}$ and water$_{6kg}$ trials against each other using LME models. Although the water$_{6kg}$ data was not significantly linear on its own, it became so when combined with the water$_{comp}$ data ($F_{1,206}$ = 30.3, P < 0.001, Eqn. 5, Fig. 2.4b, Fig. A2.5). The model that included food as a fixed effect was not significantly better than the model with 0 and 6 kg data mixed, suggesting that the *fh*: $s\dot{V}_{O_2}$ relationship for water$_{6kg}$ trials was not different than for water$_{comp}$ trials (LRT = 19.7, P = 0.002, Eqn.5,Fig.2.4b).

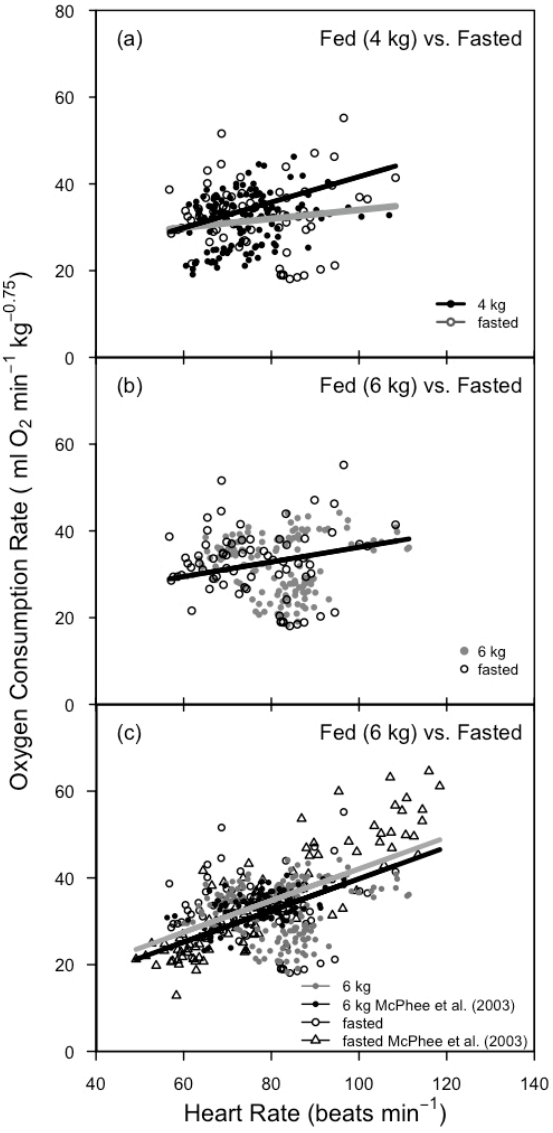

Figure 2. 4 The relationship between heart rate (*fh)* and oxygen consumption ($\dot{V}_{O_2}$) differed among Steller sea lions that were fasting (open circles, a, b), or feeding 4 kg (black circles, b). Initially, the relationship did not differ between when sea lions were fasting (open circles, b) or fed 6 kg (dark grey circles, b), but combining similar trials from McPhee et al. with the present study showed that feeding changed the relationship in water for all meal sizes (c). Data from McPhee et al. (2003) were included in b and c with permission. Regressions were derived using mixed-effects linear models within a repeated measures framework. Confidence intervals are not plotted to preserve clarity of the figures, but are described within the Statistical analysis section).

Integration of McPhee et al. (2003) data

I also evaluated my results using LME models with the inclusion of data collected for McPhee et al. (2003). Since only trial type (but not gender) was a significant factor, I pooled the data with respect to gender (trial type: $F_{1,230}$ = 4.48, P = 0.01; gender: $F_{1,2}$ = 7.91, P = 0.12). Combining the data from the dry_{fasted}, dry_{4kg}, and dry_{6kg} trials with comparable data from McPhee et al. (dry_{fasted}) confirmed that food was not a significant factor affecting the fh:$s\dot{V}_{O_2}$ relationship for Steller sea lions on land ($F_{1,389}$ = 0.86, P = 0.36), although the combined data set provided a refined predictive equation (Table 2.2: Eqn 9).

In contrast to my initial results, the incorporation of comparable water trials from McPhee et al. demonstrated that fh can predict $s\dot{V}_{O_2}$ after a 6 kg meal, and that this relationship differed from that for sea lions fasting in water (LRT = 13.32, P < 0.001, Table 2.2: Eqn 6; Fig. 2.4c). Incorporating a wider range of meal sizes in water (0, 4, 6, 12 kg) further demonstrated that each meal size significantly changed the fh:$s\dot{V}_{O_2}$ relationship in this environment (LRT = 17.77, P < 0.001, Table 2.2: Eqns 8a, b, c).

Discussion

I explored the physiological relationship between fh and $\dot{V}_{O_2}$ in animals that were fasted on land and in water, and also investigated the effect of feeding. I found that fh can be used to predict $\dot{V}O_2$, but that the relationship was affected by both environment and feeding. For fasted animals, these predictive relationships differed when animals were in water or on land (Fig. 2.2). However, the fh:$\dot{V}_{O_2}$ relationship did not change after feeding in both environments as predicted. Rather, feeding affected the relationship in water (Fig. 2.4), but not on land (Figs 2.3). Statistically, the results show that both environment and feeding must be considered when predicting $\dot{V}_{O_2}$ from fh.

Influence of environment on the fh: $\dot{V}_{O_2}$ relationship

Results were consistent with previous studies that showed a linear relationship between *fh* and $\dot{V}_{O_2}$ in fasted pinnipeds (Boyd et al. 1995; Butler 1993; Hindell and Lea 1998; McPhee et al. 2003), both when animals were resting on land and in the water. Of significance, however, was my finding that the relationship between *fh* and $\dot{V}_{O_2}$ in fasted animals differed with environment (Fig. 2.2). While it is possible that the different *fh:* $\dot{V}_{O_2}$ relationships (land vs. water) were simply the artifact of different experimental apparatus, I believe that this is unlikely. The *fh:* $\dot{V}_{O_2}$ relationship in the swim mill ($water_{fasted}$) did not differ significantly from the relationship determined from trials performed in the respirometry dome in the open ocean ($water_{ow}$, Fig. 2.2b), which is arguably the most dissimilar apparatus. There are, however, several possible physiological explanations for the observed difference in the *fh*: $\dot{V}_{O_2}$ relationships.

Activity or stress levels could have influenced both $\dot{V}_{O_2}$ and *fh*. Overall, animals were resting and calm for all trials and, despite some inter-individual variation, there was no consistent activity difference between environments. Even so, activity-dependant increases in *fh* and $\dot{V}_{O_2}$ are expected to occur along the same *fh*: $\dot{V}_{O_2}$ line, and not create a new relationship in different environments (Fig. A2.4). Although a sympathetic stress response would increase vasoconstriction and alter the *fh*: $\dot{V}_{O_2}$ relationship via Fick's equation, there was not likely a bias in stress between the two environments. Animals were comfortable with the equipment, as demonstrated by the fact that they repeatedly entered both experimental apparatus voluntarily without food reinforcement.

The thermal properties of the two mediums were the most obvious physical environmental distinction. Water conducts heat ~25 times better than air, leading to potential thermoregulatory differences between the mediums. Of course, thermoregulation in homeotherms should only be a confounding influence in thermal environments outside of the thermoneutral zone (TNZ). Beyond the TNZ, animals can compensate in an active (likely elevating $\dot{V}_{O_2}$ and *fh* within the same relationship) or passive manner. Active compensation could

include increased body movement or shivering to minimize heat loss (when above their TNZ). Conversely, animals could maximize heat loss (when below their TNZ) with thermoregulatory behaviour (the "jug-handle position"). Vasoconstriction to minimize heat loss would change $Ca_{O_2} - C\bar{v}_{O_2}$ by altering either blood flow rate or shifting which specific tissues were perfused (e.g. skin or peripheral muscles). Several studies have sought to define the TNZ in Steller sea lions and other similar pinnipeds, but results are conflicting. The lower critical temperature (T_{cl}) for sub-adult and adult California sea lions (*Zalophus californianus)* was 14.8-14.0°C (Liao 1990), similar to that for much smaller pup and yearling Antarctic fur seals (*Arctocephalus gazella*, Rutishauser et al. 2004). However, these T_{cl} estimates should only be cautiously applied to Steller sea lions due to geographical range differences. Furthermore, fur seals and California sea lions are smaller than Steller sea lions and thermoregulation should be affected by surface area to volume ratios. Metabolic rate data from juvenile Steller sea lions suggest that animals are below their T_{cl} when resting in water between 4.0-8.0°C (Rosen and Trites 2003), while a theoretical model predicted Steller sea lions to be within their TNZ for water temperatures > 8°C and air temperatures of 0-10°C (Roscow 2001).

It is unlikely that animals in my study were below their TNZ for trials conducted either in water or on land. The minimum temperature for all trials was 9.0°C. It is possible that animals were briefly above TNZ during dry trials in July and August, when metabolic chamber temperatures exceeded 19.0°C (Fig. A4). However, sprinkler cooling systems were turned on immediately when an animal exhibited behavioral indications of heat challenges. No thermoregulatory behaviours were observed in water. This suggests that animals were within their TNZ in both environments and that ambient temperature was not a significant confounding effect.

Fasting $fh:\dot{V}_{O_2}$ relationships could differ between environments due to an induction of a dive response while resting in water. For diving marine mammals, this is characterized by apnea, an overall reduction of aerobic metabolism, peripheral vasoconstriction, decreased heart rate (bradycardia), lactic acid accumulation in muscles, and a variety of hematological changes (Butler and Jones 1997; Davis et al. 2004; Elsner et al. 1985; Kooyman and Campbell 1972; Mottishaw et al. 1999; Ponganis et al. 1997). While these and other physiological responses could alter the individual components in Fick's equation and therefore the resulting $fh:\dot{V}_{O_2}$

relationship, peripheral vasoconstriction or decreased stroke volume are likely to be the most significant physiological factors. Changes in peripheral vasoconstriction during submergence could change overall aerobic metabolism (or cumulative total body $Ca_{O_2} - C\bar{v}_{O_2}$) by increasing tissue O_2 consumption in some tissues (the heart and brain) while decreasing O_2 consumption in hypoxia tolerant tissues (i.e., non-locomotory muscle). Decreased Vs during submergence in water would violate the assumptions of the heart rate method, but it is not clear to what extent Vs changes during shallow voluntary submergence in a swim mill (compared to forced submergences and natural dives, Blix et al. 1983; Ponganis et al. 1990; Sinnett et al. 1978; Zapol et al. 1979)

Changes in blood flow distribution during submergence (Davis et al. 1983; Kooyman et al. 1973; Stone et al. 1973) is yet another factor that could also influence $Ca_{O_2} - C\bar{v}_{O_2}$ due to changes in the rate of O_2 delivery to the tissues. Limited studies on blood flow redistribution in diving marine mammals suggest that the magnitude, duration, and organ location of blood flow distribution during diving is likely a gradient between the extreme restriction to the heart and brain during forced submersions (e.g. Zapol et al. 1979) and the moderate changes observed in natural diving (Davis et al. 1983; Stone et al. 1973). If changes in blood flow (Davis et al. 1983; Kooyman et al. 1973; Stone et al. 1973) and metabolism are not proportional, the $fh{:}\dot{V}_{O_2}$ relationship could also change.

It is important to note that the dive response is not an automatic reflex (Butler 1988), but rather a graded response under a degree of voluntary control (Andrews et al. 1997; Castellini 1991; Castellini et al. 1981; Mottishaw et al. 1999; Thompson and Fedak 1993). Data collected in the swim mill (< 3m depth and < 30 second submersions) likely represent the lower end of the dive response gradient compared to the more extreme changes in ($Ca_{O_2} - C\bar{v}_{O_2}$), Vs or blood flow that may occur in freely diving animals. It seems unlikely that the sea lions in my study altered their physiology in preparation for diving. While some sort of dive preparation may have been a logical possibility in the open water trials, there was no opportunity to dive in the swim mill.

Though it was unlikely that any voluntary response occurred, there is evidence from freely diving pinnipeds that components of the dive response, including diving bradycardia are under some parasympathetic control (Elliott et al. 2002). Instantaneous fh traces of my $\text{dry}_{\text{fasted}}$ and $\text{water}_{\text{fasted}}$ trials showed minor evidence for bradycardia (when animals submerged for 5-20

sec), and trials in water had generally lower ranges for *fh* and $\dot{V}_{O_2}$. Although not all animals exhibited clear bradycardia in the swim mill, the signal noise associated with the heart rate datalogger may have limited detection during these short submersion periods. The same *fh* value did predict a lower $\dot{V}_{O_2}$ in all of the water-based trials compared to similar trials on land, suggesting an overall reduction in $\dot{V}_{O_2}$ (perhaps mediated by vasoconstriction) that outpaced the heart rate decline.

The *fh*:$\dot{V}_{O_2}$ relationship could also differ due to a submergence effect on V_S. Only limited V_S data are available for breath-holding animals such as marine mammals, and the majority of V_S studies have been performed on restrained animals. Stroke volume has been noted to decrease (Blix et al. 1983; Ponganis et al. 1990; Sinnett et al. 1978; Zapol et al. 1979) or remain constant (Blix et al. 1976; Murdaugh Jr et al. 1966) in phocid seals but remain constant in an otariid (Elsner et al. 1964), perhaps reflecting a differing dive response between the two groups (Williams et al. 1991).

Influence of feeding on the fh: $\dot{V}_{O_2}$ ***relationship***

My study was the first to simultaneously measure changes in both *fh* and $\dot{V}_{O_2}$ after feeding known amounts of food in marine mammals. I predicted that the *fh:*$\dot{V}_{O_2}$ relationship would change after feeding due to the heat increment of feeding (HIF). The increase in $\dot{V}_{O_2}$ associated with HIF is attributed to the chemical and physical processes of digestion (Secor 2009), but it is not clear how HIF might simultaneously change *fh*. If HIF increases both $\dot{V}_{O_2}$ and *fh* proportionally (as when animals were fasted), then there would be no statistical difference in the predictive *fh*:$\dot{V}_{O_2}$ relationships between fasted and fed animals (Option 1). If HIF resulted in an increase in $\dot{V}_{O_2}$ while *fh* remained constant, the linear relationship between the two would likely get weaker or become non-linear (Option 2). Alternately, if the increase in $\dot{V}_{O_2}$ was accompanied by a non-parallel increase in *fh* (relative to fasted animals), I should resolve two statistically different predictive relationships for these two physiological states (Option 3).

Feeding in water

It has been suggested that pinnipeds defer digestion when diving or swimming until at the surface or on land (Markussen et al. 1994; Rosen 2007; Sparling et al. 2007), although support for this theory is not universal (Davis et al. 1983; McConnell et al. 1992; Rosen and Trites 2003; Svärd et al. 2009). However, I found no evidence of deferred digestion due to submergence, given that average $\dot{V}_{O_2}$ increased in water during the feeding trials (particularly towards the end of the trial) and a greater $\dot{V}_{O_2}$ increase was noted for 6 kg compared to 4 kg meals (Fig. A2.7, Fig. A2.8).

In water, I noted significant linear fh:$\dot{V}_{O_2}$ relationships for fed Steller sea lions, which differed significantly from relationships for fasting animals, thereby eliminating Options 1 and 2 described above. Rather, feeding affected physiology such that fh increased in proportion to $\dot{V}_{O_2}$ but at different rates relative to fasted animals (Option 3). This resulted in a greater relative $\dot{V}_{O_2}$ predicted from fh for animals fed 4 kg relative to those that were fasted (Fig. 2.4a). Using only my dataset, the difference between the fasted fh:$\dot{V}_{O_2}$ relationship and the postprandial fh:$\dot{V}_{O_2}$ relationship was apparent after a 4 kg meal but not a 6 kg meal. By analyzing a larger dataset (i.e., by integrating comparable data from McPhee et al. 2003), I noted that the difference between fasted and 6 kg trials was also significant (Fig 2.4b). I also confirmed that feeding affected physiological state and created a new fh:$\dot{V}_{O_2}$ relationship for animals in water. Furthermore, meal size was relevant, as evidenced by distinct fh:$\dot{V}_{O_2}$ relationships for 4, 6, and 12 kg meals (Table 2.2, Eqn. 7a, b, c). It is, however, noteworthy that I ceased my data collection after 4 h, and therefore do not know whether these distinct relationships persisted for the entire digestion process.

Feeding on land

In contrast to both my predictions and the results from the trials in water, I concluded that feeding on land did not affect the relationship between fh and $\dot{V}_{O_2}$ (Fig. 2.3). This conclusion held when additional $\text{dry}_{\text{fasted}}$ trials from McPhee et al. (2003) were analyzed (Table A2.1, Eqn. 9). This could suggest that HIF contributes to a higher average $\dot{V}_{O_2}$ and fh but within the same

relationship observed in fasted animals, unlike trials in water (Option 1). However, data ranges for $\dot{V}_{O_2}$ and *fh* from all dry trial types overlap thoroughly (Fig. 2.3). In other words, despite existing data describing a postprandial HIF response in Steller sea lions (Rosen and Trites 1997), I did not observe a $\dot{V}_{O_2}$ elevation following feeding. This is despite the fact that the 4 h trial duration was intended to capture the HIF peak (Rosen and Trites 1997).

It is possible that HIF was simply too low to detect statistically, particularly since I included time-intervals prior to its projected peak in my construction of $fh{:}\dot{V}_{O_2}$ relationships. This seems unlikely however, given that I clearly detected an HIF effect in water for the same animals. In fact, I expected a more pronounced HIF on land (as documented in other pinnipeds, Barbour 1993; Gallivan and Ronald 1981; Rosen and Trites 1997; see Table A2.3 for a summary) as animals can theoretically allocate more energy to digestion when they are not swimming or thermoregulating (Rosen et al., 2007). It is more plausible that some extraneous condition, such as an inter-animal variation in activity, masked the HIF response. $\text{Dry}_{\text{fasted}}$ trials had higher average $\dot{V}_{O_2}$ and, more importantly, a wider $\dot{V}_{O_2}$ scope relative to $\text{water}_{\text{fasted}}$ trials. While activity varied considerably between animals and trials, all animals were generally calmer after a meal. It is therefore possible that increased sporadic activity during the $\text{dry}_{\text{fasted}}$ trials masked any detectable increase in $\dot{V}_{O_2}$ during the less active feeding trials.

When the timecourses of *fh* and $\dot{V}_{O_2}$ (relative to the fasted baseline) were analyzed, some evidence of HIF onset was detectable on land (Fig. 2.5). Oxygen consumption rate in most feeding trials was initially below the fasted baseline, but tended to increase 10-15 minutes into the trial and remain elevated above the equivalent fasted values until about 10 minutes before the end of the trial. (See Fig. A2.8 for corresponding water trials).

Field applications

Practical limitations must be considered before applying the equations in Table 2.2 in the field. The predictive models presented here are species-specific and age-specific for adult, non-reproductive female Steller sea lions within the age group and body mass range of my sample population (Table 2.1). Also, $\text{water}_{\text{comp}}$ equations are specific to sea lions resting either in a swim mill or at the surface of open water, and further research should explore the $fh{:}\dot{V}_{O_2}$ relationship

when animals are diving (Chapter 3). As the magnitude and duration of HIF is known to vary by meal size and composition (Rosen and Trites 1997), the predictive equations may be specific for Steller sea lions that are fed 0-12 kg Pacific herring. Despite these limitations, the equations generated by my study are novel and have great potential to help solve the problem of estimating $\dot{V}_{O_2}$ of pinnipeds in the field**.**

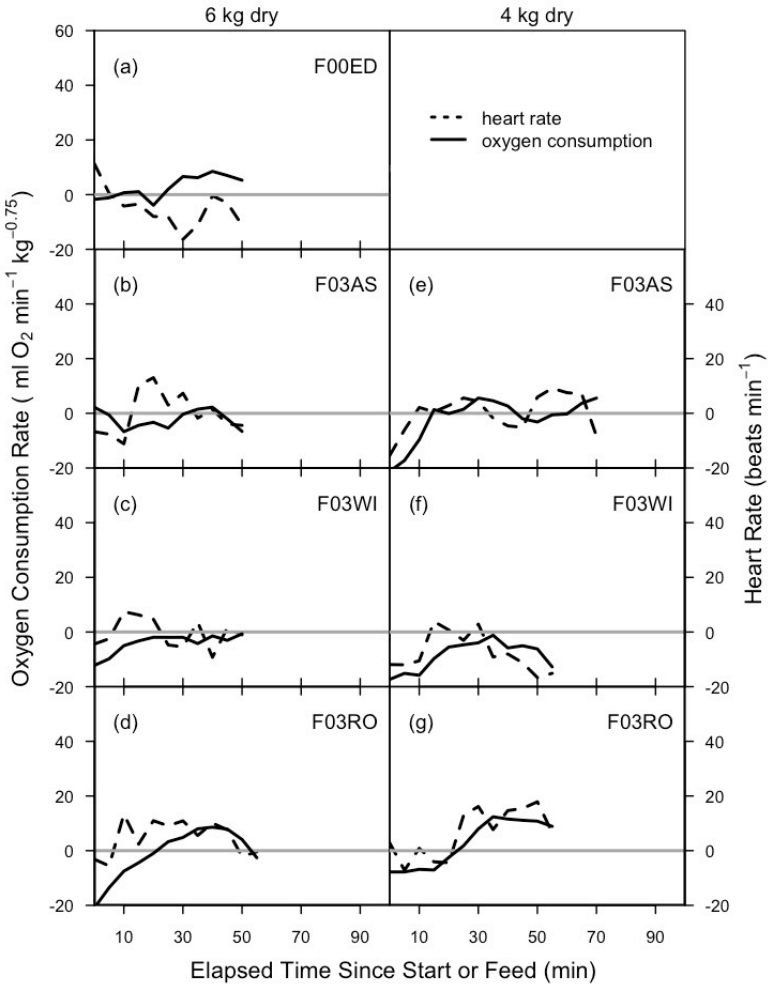

Fig. 2.5 Relative heart rate (*fh,* dashed line) and oxygen consumption ($\dot{V}_{O_2}$, solid line) of Steller sea lions above baseline (fed 0 kg) values (grey line at zero) following a single meal of 4 or 6 kg (dry_{4kg} and dry_{6kg} trials). All animals were dry and remained in a metabolic chamber during the trials. Note that F00ED did not complete a dry_{4kg} trial. Regressions were derived using mixed-effects linear models within a repeated measures framework (see Fig. A2.8 for corresponding trials in water).

Estimation of the error of predictive models

The average residual error of the dry_{fasted} model was about 14%, and the average residual error for the $water_{comp}$ was about 19% (Fig. 2.2a,b;). Mean residual error for dry_{all} (fasted and fed) was 27% (Fig.2.3), and average residual error for $water_{4kg}$ was 15% (Fig.2.4a; calculations were not made for $water_{6kg}$ given that $water_{6kg}$ did not differ from $water_{comp}$ unless data was incorporated from McPhee et al., 2003). Overall, the error estimates noted here were less than the doubly labeled water method, which may over-estimate $\dot{V}_{O_2}$ by ~36% (Boyd et al. 1995).

Relative error in applying the incorrect predictive equation

Bio-logging can be employed to measure heart rate and to theoretically determine when an animal is on land, resting in water, or diving (Ponganis 2007). Recent developments in animal-mounted cameras and stomach temperature pills also allow the occurrence and size of feeding events to be detected (Davis et al. 1999). Armed with this information, field metabolic rate could be most accurately predicted via *fh* by selecting the appropriate equation from Table 2.2. However, consideration must be given to whether the increased logistical, financial, and animal care efforts required to obtain this additional species-specific information are warranted need to be considered. Statistical analyses clearly demonstrate that predictive equations are specific to environment and feeding. However, if statistically unique equations predict similar $\dot{V}_{O_2}$ values for a given *fh,* the error in choosing the "wrong" equation (or applying a single equation across different states) would be minimal. For this exercise, percent error for each of the predictive equations was calculated relative to the fasted ($water_{comp}$) baseline.

Environment matters in the field

Incorrectly using the $dry_{+McPhee}$ equation (Eqn 9) to estimate $\dot{V}_{O_2}$ of a free-ranging Steller sea lion fasting in water ($wate_{rcomp}$, Eqn 2) would overestimate $\dot{V}_{O_2}$ by approximately 35% (similar to the 36.4% error for the DLW method, Boyd et al. 1999). Given this large potential error, and knowing that it is fairly easy logistically to determine when animals are on land or in

water, Eqn. 9 should be used when animals are on land, and Eqn. 2 should be used when fasted animals are resting at the water surface (Table 2.2).

Feeding on land does not matter in the field

Given the lack of statistical distinction between predictive equations for fed vs. fasted sea lions on land, I recommend using the composite equation developed from all fasted and fed trials on land ($dry_{all+McPhee,}$ Eqn 9) to estimate $\dot{V}_{O_2}$ of Steller sea lions on land, regardless of digestive state. Furthermore, a *fh* of 100 beats min^{-1} produces similar estimations of $\dot{V}_{O_2}$ for fasted or fed animals on land (50, and 49 ml $O_2 \cdot min^{-1} \cdot kg^{-0.75}$). This simplifies estimates of oxygen consumption by removing the need to determine food intake.

Feeding in water does not matter in the field

Applying any of the equations derived for animals feeding in water (Eqns 7a, b, c) to a fasted Steller sea lion in water resulted in < 4% error in $\dot{V}_{O_2}$ for an *fh* of 100 beats$\cdot min^{-1}$. Logistically, measuring the occurrence and size of feeding events in free-ranging Steller sea lions is much more difficult than determining when the animal is on land or in water. Considering this, and the small error associated with applying the incorrect predictive equation, I recommend estimating $\dot{V}_{O_2}$ of Steller sea lions resting in water using the predictive model that encompasses the widest range of data (0-12 kg) but does not distinguish among digestive states (Eqn 8). Thus my findings demonstrate that separate equations should be used to predict $\dot{V}_{O_2}$ on land and in water, and that the effect of feeding on the relationship in water is not profound enough to warrant consideration.

Summary

The ability to use heart rate (*fh*) to predict oxygen consumption rates ($\dot{V}_{O_2}$) in Steller sea lions and other pinnipeds has been investigated in fasting animals. However, it is unknown whether established $fh:\dot{V}_{O_2}$ relationships hold under more complex physiological situations, such as when animals are feeding or digesting. I assessed whether *fh* could be used to predict $\dot{V}_{O_2}$ in

trained Steller sea lions while fasting, and after being fed a 4 or 6 kg herring meal on land and in water. Using linear mixed-effects models, I derived unique equations to describe the fh:$\dot{V}_{O_2}$ relationship for fasted sea lions resting on land and in water. Feeding did not significantly change the fh:$\dot{V}_{O_2}$ relationship on land. In water however, Steller sea lions displayed a distinct fh:$\dot{V}_{O_2}$ relationship after consuming a 4 kg meal compared to the fasting condition. Incorporating comparable published data showed a similar, but distinct effect of feeding after a 6 kg meal. However, for practical field application, feeding in water did not sufficiently change $\dot{V}_{O_2}$ predictions to warrant the accompanying logistical difficulties of determining the timing and quantity of fish consumed. I therefore suggest that a single predictive equation can be applied to animals in water. Ultimately, my results show that both environment and feeding are statistically relevant when deriving $\dot{V}_{O_2}$ from telemetered fh, but that environment is the only variable that affects the practical ability to predict metabolism from heart rate.

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Chapter 3: Dive behaviour impacts the ability of heart rate to predict oxygen consumption in Steller sea lions (*Eumetopias jubatus*) foraging at depth[2]

Introduction

Measures of activity-specific metabolic rates are essential for understanding the interaction between animals and their environment, including quantifying the energetic cost of observed or predicted changes in behaviour. Despite its essential role in bioenergetic modeling (Winship et al. 2002), reliable ways to measure metabolic rates in the field have remained elusive, particularly for marine mammals. Metabolic rate can be indirectly estimated from other physiological or behavioural parameters, such as doubly labeled water (DLW), body acceleration metrics, or heart rate (Butler et al. 2004; Halsey et al. 2009). Doubly labeled water provides a mean estimate of field metabolic rate over a finite period that is not activity-specific, and may overestimate metabolism in otariids by as much as 36% (Boyd et al. 1995; Costa 1987). Overall dynamic body action (ODBA) and flipper stroking have emerged as new tools to predict metabolic rate in a range of vertebrates (Halsey et al. 2009; Hays et al. 2004), including some species of pinnipeds (Fahlman et al. 2008b; Williams et al. 2004). However, body acceleration metrics that are limited to predicting metabolic rate during active behaviours likely do not account for changes in physiological state (such as digestion), and may not be as accurate in water as in air (Green et al. 2009a; Halsey et al. 2009).

The heart rate (*fh*) method provides estimates of energy expenditure on a much finer time scale and for longer periods of time than the doubly labeled water method (Boyd et al. 2004; Butler et al. 2004; Ponganis 2007) with comparable error estimates to ODBA (Green et al. 2009a; Halsey et al. 2009). The heart rate method estimates oxygen consumption rate ($\dot{V}_{O_2}$, an indirect measure of energy expenditure) from measured *fh* based on the principals of the Fick equation (Fick 1870). Specifically, this method assumes that changes in oxygen demand are

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primarily reflected through comparable changes in *fh* (and that other respiratory changes remain constant or vary proportionally to *fh*) (See Chapter 2).

The heart rate method requires deriving species-specific predictive equations between *fh* and $\dot{V}_{O_2}$ in a controlled environment before the method can be used to predict $\dot{V}_{O_2}$ in the field. The majority of studies deriving predictive relationships between *fh* and $\dot{V}_{O_2}$ in marine mammals and birds have been performed while animals were either submerged in a shallow swim mill (Boyd et al. 1995; Butler et al. 1992; McPhee et al. 2003; Ponganis et al. 1997; Williams et al. 1991; Woakes and Butler 1983), walking on a treadmill (Froget et al. 2001; Froget et al. 2002; Green 2001; Green et al. 2005), or swimming horizontally in open water (Williams et al. 1993). These studies have primarily been performed with fasted animals, but recent research on Steller sea lions indicated that amount of food digested changed the fh:$\dot{V}_{O_2}$ relationship when sea lions were resting in water (Chapter 2). However, studies performed in a swim mill do not incorporate the potential influences of depth, pressure, or a full dive response.

Aspects of the dive response — including apnea, dive bradycardia, tachycardia during post-dive surface intervals, peripheral vasoconstriction, and possible hypometabolism while diving — are likely to have the most direct impact on the fh:$\dot{V}_{O_2}$ relationship (Butler and Jones 1997; Elsner et al. 1985; Irving et al. 1941; Kooyman and Campbell 1972; Ponganis et al. 1997; Scholander 1940). Oxygen (O_2) and carbon dioxide (CO_2) management may also differ between single dives and dive bouts (multiple dives in a series) for pinnipeds (Fahlman et al. 2008a; Kooyman et al. 1973; Ponganis et al. 1993). Limited studies investigating the fh:$\dot{V}_{O_2}$ relationship in diving vertebrates have primarily focused on birds (Bevan et al. 1992; Butler 1984; Stephenson et al. 1988; Woakes and Butler 1983) rather than pinnipeds (Webb et al. 1998), and such past studies have shown *fh* to significantly overestimate diving metabolic rate in penguins (Culik et al. 1996; Culik et al. 1994). Some have suggested that the fh:$\dot{V}_{O_2}$ relationship should be calibrated over the complete dive cycle (dive + surface interval, Bevan et al. 1992; Butler 1993; Fedak 1986; Fedak et al. 1988), but whether this is true in pinnipeds diving to natural depths is less clear. These findings presumably suggest that decreases in heart rate during diving may disrupt the fh:$\dot{V}_{O_2}$ relationship, but the predictive ability of *fh* may be restored over a dive cycle

by compensatory tachycardia upon surfacing. Therefore, the fh:$\dot{V}_{O_2}$ relationship may differ between surface and dive intervals, and may be affected by number of dives in a series or dive depth.

Despite the current use of *fh* to predict $\dot{V}_{O_2}$ of diving marine mammals in the wild (i.e. Boyd et al. 1999; Hindell and Lea 1998), it is not clear whether the *fh* method is accurate for pinnipeds while foraging at natural depths and for realistic dive durations. My objective was therefore to 1) simultaneously measure and determine the relationship between *fh* and $\dot{V}_{O_2}$ while Steller sea lions were foraging and diving to depths of up to 40 m and 2) determine whether *fh* could be used to predict average metabolic rate or diving metabolic rate over either a single dive or a series of continuous dives (dive bout). Accurate estimates of metabolic rate in the wild require the fh:$\dot{V}_{O_2}$ relationship to be derived under controlled conditions that encompass dive durations and dive depths that are representative of free-ranging animals. Previous studies that investigated *fh* and $\dot{V}_{O_2}$ in diving marine mammals have been limited by maximum tank depth (Sparling and Fedak 2004; Webb et al. 1998). My study derived relationships between *fh* and $\dot{V}_{O_2}$ in Steller sea lions freely diving in the open ocean to depths up to 40 m and for durations of 1-6 min, which reflect dive characteristics comparable to free-ranging animals (Merrick and Loughlin 1997).

Methods

Data collection

My study was conducted from April-September, 2008 using trained female Steller sea lions (Table 3.1). All sea lions had been raised in captivity and had been previously trained to use all experimental apparatus. On non-trial days, animals were fed a diet of herring (*Clupea pallassi*) supplemented with vitamin tablets. All procedures and protocols were conducted in accordance with the guidelines of the Canadian Council on Animal Care (University of British Columbia #A07-0413), and under permits from the Department of Fisheries and Oceans Canada (#MML 2007-0001) and the Vancouver Aquarium (Appendix A5). All animal work was conducted voluntarily under trainer control.

Table 3. 1 Age, body mass (kg ± s.d.), and number of trials (*n*) per Steller sea lion identified by ID and animal number (used for figures in Appendices).

Animal ID	No.	Age (yr)	Trial (2008)	Mass (kg)	(± s.d.)	*n*
F97SI	5	11	1 Aug - 16 Sep	218	(4.4)	5
F00BO	6	11	7 Aug - 16 Sep	145	(5.1)	5
F97HA	7	8	7 Aug - 4 Sep	172	(0.6)	5

Trials were conducted with three female Steller sea lions (F97SI, F00BO, F97HA) housed at the UBC Open Water Research Laboratory (Port Moody, British Columbia, Canada). Animals were housed in a specially designed floating pen that provided access to seawater and haulout space (for a full description see Hastie et al. 2006; Hastie et al. 2007). Animals were fasted overnight, and then weighed prior to trials on a platform scale (± 0.5 kg). Animals ranged in age from 8 to 11 years and varied in mass from 145 kg to 218 kg (Table 3.1).

Measurement of heart rate

Heart rate was measured using subcutaneous electrodes, with data transmitted wirelessly to a datalogger. Heart rate monitoring equipment composition and placement are detailed elsewhere (Chapter 2 and Appendix 1). Animals were outfitted with *fh* monitoring equipment while anesthetized (0-5% isoflurane in O_2), and all equipment was removed after each trial while the animals were under trainer control. The *fh* monitoring system consisted of 1) a heart rate datalogger (HTR, Wildlife Computers, Redmond, WA, USA) which recorded the R-R interval, and 2) a heart rate transmitter (HRX, Wildlife Computers) that had two ~80 cm leads. To reduce infection risk, I spliced 30-gauge 99.9% pure silver Teflon-coated wire (Grass Technologies, Longueuil, QC, Canada) to the terminal ends of the electrode leads, and these ends were sterilized and inserted subcutaneously. The heart rate transmitter, heart rate datalogger, and time-depth-recorder (sampling frequency = 1 Hz, SU-05272, ReefNet, Inc., Mississauga, ON, Canada) were carried by the sea lions on a custom-fit harness. Animals were allowed to fully recover from anesthesia in a dry area before the start of the trial. Heart rate was measured continuously beginning after both *fh* electrodes were inserted.

Measurement of oxygen consumption rate

I measured $\dot{V}_{O_2}$ using open-circuit gas respirometry. The system was calibrated prior to the start of trials using gases of known concentrations and a nitrogen standard. Gas concentration readings were corrected for electronic drift against ambient air before and after each trial. Oxygen and carbon dioxide concentrations within a desiccated (via $CaSO_4$) subsample of the excurrent airstream were measured using Sable System FC-1B and CA-1B analyzers, coupled to a 500H Mass Flow Generator and Controller (Sable Systems Inc., Las Vegas, NV, USA). Barometric pressure, relative humidity, and expired air temperature were also recorded (Airguide Instruments, Chicago, IL, USA).

Trials were conducted either next to the holding pen at a dive site or after the animals were transported to a nearby dive site in a 7 m research boat. A second boat towing a floating barge carried the respirometry equipment to the dive site. The floating barge had an opening in the middle containing a cage (152 cm × 152 cm × 250 cm) used to maintain the animal at the surface before and after dives and a floating transparent Plexiglass respirometry dome. Air was drawn through the chamber at a constant rate of 475 $L \cdot min^{-1}$. The excurrent airstream was continuously subsampled, and averaged every 0.05 seconds (Sable Data Acquisition System, Sable Systems Inc.). Average water temperature ranged between 14.4 and 17.5°C (mean 15.8 ± 1.0°C).

Trial protocol

The sea lions were trained to remain stationary within the floating respirometry dome, and then dive to two feeding tubes positioned at the same set depth (10 m or 40 m) ~6 m apart (mean animal length ~2 m). I selected these trial depths as the lower and upper range of dive depths commonly noted for free-ranging females (Merrick and Loughlin 1997). Each complete dive trial was repeated three times at each depth (10 m, 40 m) in random order for each animal, with only one trial per animal per day. Sea lions were not tested on two sequential days.

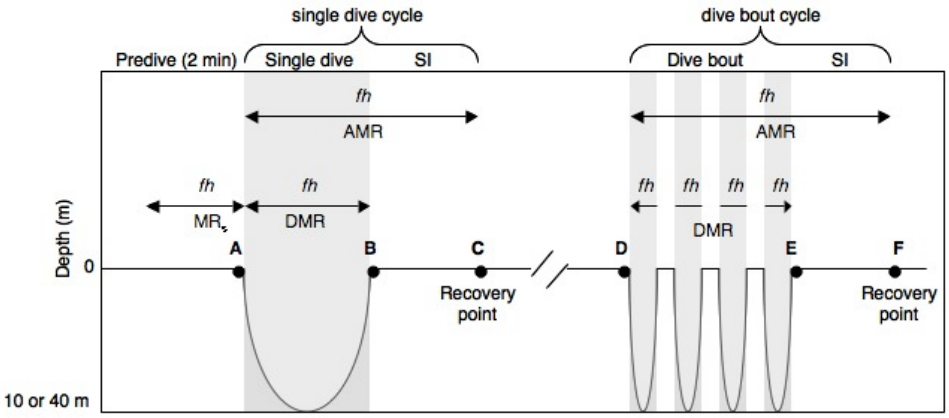

Figure 3. 1 Schematic diagram of a Steller sea lion dive trial showing dive depth (0-40 m) on the y-axis and time on the x-axis. The figure illustrates the varying intervals of average heart rate (*fh)* and oxygen consumption ($\dot{V}_{O_2}$) used to calculate different predictive equations over dives (highlighted in grey) and surface intervals (in white at 0 m depth). Standard metabolic rate or predive (MR_s) was an average over 2 min prior to the initial dive. Average metabolic rate (AMR) for a single dive or dive bout used *fh* and $\dot{V}_{O_2}$ data from the start of the dive up until the post-dive recovery point (C, set at ± 2% of MR_s), which were averaged across the total dive cycle duration (dive time plus surface interval, SI). Diving metabolic rate (DMR) over a single dive or dive bout included *fh* data during the underwater portions only, and $\dot{V}_{O_2}$ data only in excess of MR_s (see Fig. 3.2), averaged across total dive duration.

A trial consisted of 1) a 6-10 min resting period where sea lions stationed in the respirometry dome, permitting the baseline measurements of standard metabolic rate (MR_s), 2) a single recovery dive, 3) a 4-series dive bout, and 4) a second recovery dive of longer dive duration (40 m only, Fig. 3.1). All post-dive recovery surface intervals ended when measured $\dot{V}_{O_2}$ returned to within 2% of MR_s (Fig. 3.1). Trial sections were considered independent since the sea lions' $\dot{V}_{O_2}$ recovered to within 2% of MR_s between phases.

Animals were fed small pieces of herring (20 g) during trials to facilitate cooperation. A tube system allowed fish delivery to set depths and into the respiratory dome without altering the integrity of the flow-through measurements. Sea lions were encouraged (via altered underwater fish delivery rates) to execute dives of 0.5 - 2 min durations, as field data demonstrate that 45% of free-ranging dives are 0-1 min and 33% are 1-2 min for female Steller sea lions (Merrick and Loughlin 1997). For the 40 m trials only, an additional, longer single dive was encouraged at the

end of the trial by providing food underwater at higher rates, which enabled consideration of the influence of dive duration on the *fh*: $\dot{V}_{O_2}$ relationship.

Feeding has been shown to change the *fh*:$\dot{V}_{O_2}$ relationship in water statistically, but not enough to require separate fasted and fed equations in the field (Chapter 2). Animals were fed 0.4-2.8 kg (mean 1.4 ± 0.7 kg; See Table A3.11 for feeding details) herring from the time they were loaded onto the transport boat until the end of the last dive (up to 1.5 kg per trial section). Trials took less than 30 min to complete making it unlikely that the heat increment of feeding influenced my results (Rosen and Trites 1997). However, I explicitly tested the effect of feeding on the *fh*:$\dot{V}_{O_2}$ relationship to ensure that food was not a factor.

Data analysis

Calculating metabolic rates

Oxygen consumption rate ($\dot{V}_{O_2}$) was calculated from the O_2 and CO_2 concentrations (Eqn 3b, Withers 1977) using Datacan Data Analysis software (V 1.0.24; Sable Systems Inc.) and then exported into Microsoft Excel. Variation among published studies in the calculation of average metabolic rate and diving metabolic rate can make comparisons misleading. I therefore analyzed oxygen consumption while underwater in two specific ways 1) as average metabolic rate (AMR) across a dive cycle (dive + surface interval) or 2) as diving metabolic rate (DMR) calculated in reference to actual dive (underwater) time only (Fig. A3.1, Table A3.2). I calculated these measures for both single dives and multiple dive bouts. Average metabolic rate over a single dive cycle or dive bout cycle was calculated by dividing the total integrated volume (L) of oxygen consumed during the dive and surface interval by the entire dive cycle or dive bout cycle duration. Diving metabolic rate was calculated as the integrated post-dive $\dot{V}_{O_2}$ above the MR_s baseline, divided by time underwater. For single dives, time underwater was the actual dive duration. For bouts, time underwater was cumulative dive duration. To account for changes in body mass over time, $\dot{V}_{O_2}$ was mass-corrected ($s\dot{V}_{O_2}$), and expressed as ml $O_2 \cdot min^{-1} \cdot kg^{-0.75}$ (Brown and West 2005; Savage et al. 2007; White and Seymour 2005).

Heart rate

Heart rate measurements stored in the *fh* datalogger were downloaded and analyzed with Microsoft Excel and R 2.9.2 (R Core Development Team 2009). First, R-R intervals were converted to instantaneous heart rate (fh_{inst} in beats min^{-1}). A series of algorithms were applied to systematically remove any fh_{inst} data which were artifacts of muscle or wire movement (detailed in Chapter 2). Finally, fh_{inst} values were averaged across appropriate intervals to match the $\dot{V}_{O_2}$ integrations of the same time periods yielding average *fh* (beats·min^{-1}; Fig. 3.1). Resting heart rate was calculated over the 2 min directly preceding the first dive. Average *fh* was calculated over the entire dive and surface period, from the start of a dive until the end of the surface recovery interval, or the end of the fourth (final) surface recovery interval for bout dives. Diving *fh* was calculated over either the underwater portion of a single dive, or the cumulative underwater duration for dive bouts (Fig. 3.1).

Statistical analysis

Data from each animal within a trial and data from each animal across trial types was treated as a repeated measures dataset using linear mixed-effects (LME) models in R 2.9.0 (nlme library from Pinheiro et al. 2006). LME models consider the correlation between repeated measurements within and among animals, while also characterizing individual animal variation relative to the mean of the population (Pinheiro and Bates 2000). As animals were allowed to recover to predive $\dot{V}_{O_2}$ levels between single dives and dive bouts (as evidenced by $\dot{V}_{O_2}$ returning to within 2% of MR_S baseline) single dives and dive bouts within the same trial were considered to be statistically independent. All models were run using the Maximum Likelihood method, and the slope and intercept were allowed to vary for each animal during model optimization.

Animal ID was treated as a random effect for all models (which permitted inference from the sample captive population to free-ranging population), and fixed effects explored included: dive duration (1 - 2 min, 2 - 4 min, > 4 min), dive depth (10 m, 40 m), activity (resting at the surface or diving), amount of food (<0.5, 0.5 - 1.0, >1.0 – 1.5 kg), and type of dive (single dive or dive bout). For each analysis, the best model in terms of fixed effect factors was determined

using an ANOVA. An ANOVA executed on a single model generated a conditional F-test to determine the significance of model slope, intercept, and fixed effects. As all intercepts proved to be highly significant ($P \leq 0.001$), the F-values reported here are for model slopes only. An ANOVA performed on two nested models (the fixed effect model hierarchically nested within the model without fixed effects) produced likelihood ratio tests (LRT) that compared the two models. All models presented only had one fixed effect applied at a time, therefore, for all LRT tests df = 1. All reported data are presented mean ± s.d., and statistical significance was set at $\alpha = 0.05$.

The error associated with the predictive equations was not constant, but rather increases with the distance from the mean values. However, many studies evaluating other techniques to estimate energy expenditure report a single, average error (e.g., Boyd et al. 1995). To facilitate comparison with other methods, I derived a single representative value of the error associated with the predictions, which I termed the average residual. I did so by calculating the mean absolute residual value of a specific model, and dividing it by the median predicted $\dot{V}_{O_2}$ value to calculate the average residual. This served as a proxy for the average predictive error of my models.

Results

Dive characteristics

Dive duration for all single dives ranged from 1.0 - 6.3 min (mean = 2.6 ± 1.5 min; Table 3.2), and cumulative dive time for bouts ranged from 2.6 - 8.0 min (mean = 5.6 ± 1.7 min; Table 3.2). Mean dive duration for 10 m single dives was 1.3 min (± 0.2 min), and mean dive duration for 40 m single dives was 3.2 min (± 1.5 min). Dive bouts to deeper depths (40 m) had longer cumulative dive times (mean = 6.9 ± 1.0 min) compared to shallow dive bouts at 10 m (mean = 4.1 ± 0.7 min). Within individual 10 m trials, maximum dive depths ranged from 11–14 m (mean = 12 ± 1.1 m). Within 40 m trials, maximum dive depths ranged from 41–58 m (mean = 46 ± 6.2 m) (See Table A3.4 for full dive characteristics summary).

Relationship between fh and resting metabolic rate (RMR)

There was a significant relationship between *fh* and $\dot{V}_{O_2}$ for animals resting at the surface in open water (MR_s). However, a previous study showed that this relationship was not significantly different than the fh:$\dot{V}_{O_2}$ relationship derived from 4 other Steller sea lions resting in a swim mill (~16 hrs postprandial, Chapter 2). I therefore pooled $\dot{V}_{O_2}$ and *fh* data from these two sets of trials to create a stronger composite predictive equation of RMR for animals resting in water. To clarify, all comparisons between resting and diving data were made relative to this composite RMR dataset which included both MRs and resting in a swim mill data (Table 3.3:Eqn. 1 equivalent to Eqn. 2 in Chapter 2). Average *f*h ranged from 57 - 108 beats·min^{-1}, and RMR ranged from 18-76 ml O_2·min-1·kg$^{-0.75}$ (Fig. 2.2B in Chapter 2).

Table 3. 2 Summary (mean ± s.d., min, max) of dives conducted by three Steller sea lions. Dive and surface interval (SI) durations are presented for single dives and cumulative dive bouts, and for dive types combined. Associated maximum dive depth data (m) are also listed (see Table A3.4 for a summary of full dive characteristics).

	Duration (min)				Maximum dive depth (m)			
Data	mean	(± s.d.)	min	max	mean	(± s.d.)	min	max
Single dive	2.6	(1.5)	1.0	6.3	35	(16.7)	11	58
Single SI	3.0	(0.9)	1.4	4.6				
Dive bout cumulative	5.6	(1.7)	2.6	8.0	31	(18.4)	11	57
Dive bout cumulative SI								
All dives	3.6	(2.2)	0.9	8.0	32	(17.0)	11	58
All SI	4.3	(1.2)	2.7	6.5				

Table 3. 3 Equations for selected models ($\dot{V}_{O_2} = a \bullet fh + b$) showing the linear relationship between heart rate (*fh)* and oxygen consumption ($\dot{V}_{O_2}$) for Steller sea lions that were resting or diving. Model parameters include maximum amount of food fed (kg), slope (± s.e.m.), intercept (± s.e.m.), and *P*-values (F-test). Model descriptions are defined in the list of abbreviations. Estimates of diving metabolic rate (DMR, Table A3.1) were not linear; therefore all equations presented are for resting metabolic rate (RMR) or average metabolic rate (AMR) over a dive cycle or dive bout (dive+surface interval). Equations were derived using mixed-effects linear models with a repeated measures framework (See Table A3.1 for all equations)

Eqn.	Food (kg)	Slope (*a*)	(± s.e.m)	Intercept (*b*)	(± s.e.m)	Slope *P*-value	Intercept *P*-value	Model Prediction
1	<0.36	0.20	(0.07)	16.7	(4.96)	0.005	<0.0001	RMR Resting in water
2	<0.54	0.25	(0.10)	15.0	(8.51)	0.018	<0.0001	AMR Single dive cycle
3	<0.54	0.22	(0.05)	15.3	(3.43)	0.0001	<0.0001	AMR Single dive cycle + RMR (Fig. 3.4)
4	<1.20	0.18	(0.06)	29.0	(4.82)	0.014	<0.0001	AMR Dive bout cycle (Fig. 3.4)
						0.0002	0.0002	AMR Dive bout cycle + RMR
5A	<1.20	0.21	(0.53)	26.5	(5.64)			Dive bout cycle
5B	<1.20	0.22	(0.54)	15.3	(3.91)			Resting in water
						<0.0001	<0.0001	AMR Dive bout cycle + single dive cycle
6A	<1.20	0.24	(0.05)	24.5	(4.06)			Dive bout cycle
6C	<1.20	0.24	(0.05)	16.1	(6.82)			Single dive cycle

Relationship between fh and diving metabolic rate (DMR)

None of the $fh{:}\dot{V}_{O_2}$ relationships predicting diving metabolic rate (DMR) were significantly linear, either without fixed effects, with the inclusion of appropriate fixed effects (i.e., depth, dive duration, food, dive type), or after log-transformation (Fig. A3.3, A3.4, Table A3.1). For example, diving $fh{:}\dot{V}_{O_2}$ relationships were not linear for dive bouts alone ($F_{1,\ 11}$ = 0.78, P = 0.39, Fig. A3.4A), when dive bouts were combined with data from animals that were resting in water ($F_{1,\ 77}$= 0.20, P = 0.66, Fig. A3.4B) or when dive bouts were combined with single dive data ($F_{1,\ 33}$= 1.52, P = 0.23; Fig. 3.3, Fig. A3.4C). Given that no predictive equations for DMR were linear (Table A3.1), further comparisons (such as DMR vs. AMR or RMR) were not undertaken.

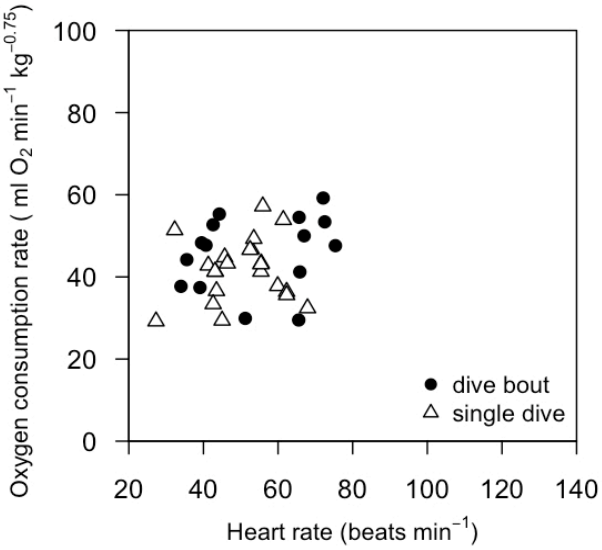

Figure 3. 2 The relationships between diving heart rate (*fh*) and diving metabolic rate (DMR) for Steller sea lions over a dive bout (closed circles) or over a single dive (open triangles) were not significantly linear.

Overall, *fh* and DMR data were normally distributed, had normally distributed errors, and had relatively homogeneous variance across single dives and dive bouts. Average *fh* over a single dive ranged from 27-68 beats·min^{-1}, and mean $\dot{V}_{O_2}$ ranged from 29-57 ml O_2·min^{-1}·kg$^{-0.75}$.

Average diving *fh* ranged from 34-75 beats·min^{-1}, and $\dot{V}_{O_2}$ over the dive bout (subsurface time only) ranged from 30-59 ml O_2·min^{-1}·kg$^{-0.75}$.

Bradycardia was notable during both single dives (average diving *fh* was up to 64% reduced from resting) and dive bouts (diving *fh* was up to 44% reduced). However, no significant reduction in DMR (42 ± 7.5 ml O_2·min^{-1}·kg$^{-0.75}$ for single dives, 46 ± 8.4 ml O_2·min^{-1}·kg$^{-0.75}$ for bouts) relative to RMR (33 ± 9.5 l O_2·min^{-1}·kg$^{-0.75}$) was noted.

The time required for a sea lion to recover from a dive increased linearly with dive duration for single dives (i.e., it took more time to recover from longer dives; $F_{1, 17} = 11.32$, $P = 0.004$), but did not increase for dive bouts of longer cumulative dive duration ($F_{1, 8} = 3.15$, $P = 0.11$). Surface interval durations following single dives increased linearly with DMR ($F_{1, 18} = 37.92$, $P < 0.0001$; Fig. 3.3A) regardless of depth ($F_{1, 17} = 2.20$, $P = 0.16$). However, a comparable relationship was not observed between cumulative surface time and DMR over dive bouts ($F_{1, 8} = 0.45$, $P = 0.52$; Fig. 3.3B). There was not a significantly linear relationship between dive time and DMR for single dives ($F_{1, 18} = 0.59$, $P = 0.45$; Fig. 3.3C), but I did observe a negative relationship between DMR and cumulative dive time for dive bouts ($F_{1, 8} = 20.39$, $P = 0.002$; Fig. 3.3D).

Relationship between fh and average metabolic rate (AMR)

Neither dive duration, dive trial depth, maximum dive depth, water temperature, nor food consumed significantly affected the *fh*:$\dot{V}_{O_2}$ relationship across single or dive bout cycles (dive + surface recovery interval). I therefore combined data from both trial depths and all dive durations for analysis. Overall, *fh* and $\dot{V}_{O_2}$ data were normally distributed, had normally distributed errors, and had relatively homogeneous variance across a complete dive cycle or dive bout. Log-transformation did not alter the significance or linearity of any of the models.

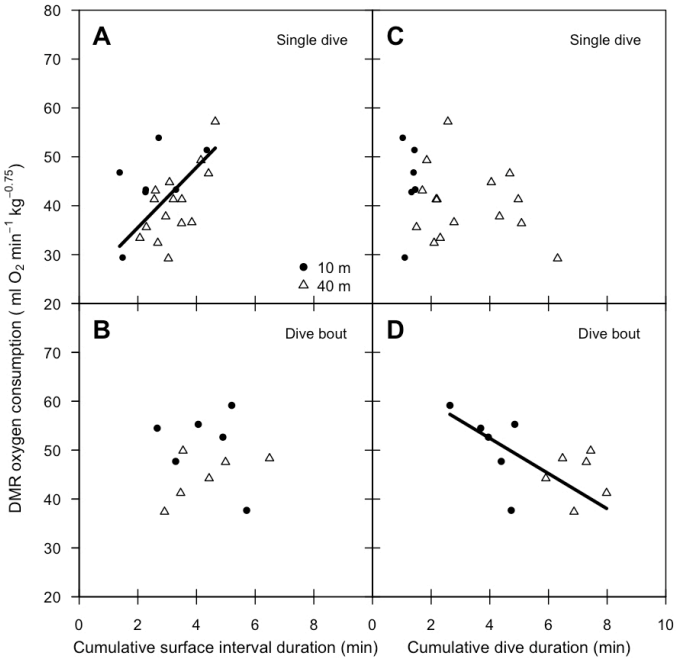

Figure 3. 3 The relationship between dive characteristics and diving metabolic rate (DMR) for single dives and dive bouts. DMR increased with surface recovery time for single dives (A), but not for dive bouts (B). DMR decreased as cumulative dive duration increased for dive bouts (D), but not for single dives (C). Dive depth (10 m = closed circles, 40 m = open triangles) did not alter any of the relationships. For bout dives, all durations are cumulative, with the surface interval also incorporating the period until oxygen consumption recovered to within 2% of MR_s. Equations were derived using mixed-effects linear models with a repeated measures framework.

Over a single dive cycle, average *fh* ranged from 46 - 103 beats·min^{-1}, and AMR ranged from 22 - 51ml O_2·min^{-1}·kg$^{-0.75}$. The scope of food fed during single dive cycles (<0.54 kg) was too narrow to adequately consider food as a statistical factor in this instance (See Table A3.5 for feeding details). I noted statistically similar *fh*: $\dot{V}_{O_2}$ relationships over single dive cycles (Table 3.3: Eqn. 2) and while animals were resting at the water surface ($F_{1, 87}$ = 0.55, P = 0.46; Table 3.3: Eqn. 1, Fig. A3.5B). This statistical similarity resulted in a single equation describing both data sets (Table 3.3: Eqn. 3, Fig. 3.5 dashed line; See Table A3.1 for all model equations).

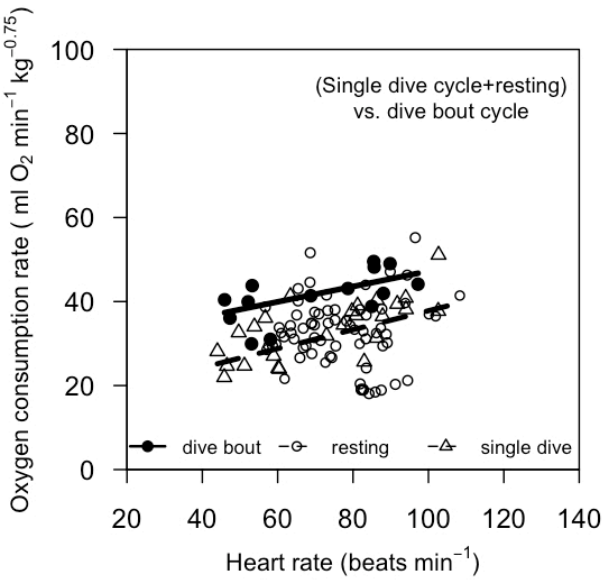

Figure 3. 4 The relationship between heart rate (*fh*) and oxygen consumption rate ($\dot{V}_{O_2}$) when Steller sea lions were resting (RMR, open circles) did not differ from that for predicting average metabolic rate over a single dive cycle (AMR, open triangles), but did differ from the relationship for predicting AMR over a dive bout cycle (closed circles). Equations were derived using mixed-effects linear models with a repeated measures framework.

Over a dive bout cycle, average *fh* ranged from 46 – 97 beats·min^{-1}, and AMR ranged from 30 – 50 ml O_2·min^{-1}·kg$^{-0.75}$. During dive bout cycles, sea lions were fed a maximum of 1.20 kg of herring to facilitate cooperation (mean = 0.61 ± 0.22 kg, Table A3.5). However, meal size did not affect the fh:$\dot{V}_{O_2}$ relationship (LRT = 3.84, P = 0.92) which was significantly linear (Table 3.3: Eq. 4, Fig. A3.6A). Combining data sets to test different factors yields slightly different predictive equations with mixed-effects models due to variability in d.f. and unbalanced sample sizes (i.e., Eqn. 4, 5a, and 6a for dive bouts are similar but not exactly the same). The fh:$\dot{V}_{O_2}$ relationship over a dive bout cycle differed from both the relationship for predicting RMR (LRT = 27.71, P < 0.0001; Fig. 3.5; Table 3.3: Eqns. 5a, b), and from the relationship over a single dive cycle (LRT = 19.63, P = 0.0006, Fig. 3.5; Table 3.3: Eqns. 6 a, b). Model convergence errors prevented statistical comparison of the fh:$\dot{V}_{O_2}$ relationship between dive bout cycles and the equation for pooled RMR and AMR (single dive cycle). However, predicted AMR for a given *fh* during dive bout cycles was often greater than for single dive bout cycles or for RMR (Fig. 3.5; See Appendix A3.5, A3.6 for supplementary AMR figures).

Discussion

Heart rate could be used to reliably predict $\dot{V}_{O_2}$ in diving Steller sea lions, but only when averaged over single dive cycles or dive bout cycles where animals were allowed to recover fully from the O_2 debt incurred during diving. Furthermore, while the predictive equation for single dive cycles did not differ from the predictive equation for animals resting in water, it was significantly different than the relationship for the dive bout cycles. Although a sample size of 3-7 animals is not considered large for fieldwork, it is considered relatively large compared to other captive pinniped studies. In contrast to previous studies that have simply pooled the data of several animals together, my analysis was strengthened by using mixed-effects models within a repeated measures framework with each animal treated as a random sample from the population. The statistical strength of my predictive models — despite my small sample size — strongly indicates that changes in the $fh:\dot{V}_{O_2}$ relationship represented a realistic biological change rather than a statistical artifact.

Although studies have independently measured diving *fh* (Hurley and Costa 2001; Ponganis et al. 1997) or $\dot{V}_{O_2}$ (Hurley and Costa 2001; Sparling and Fedak 2004) in trained pinnipeds, few studies have measured these variables simultaneously to derive a $fh:\dot{V}_{O_2}$ relationship during diving (Webb et al. 1998). Studies deriving $fh:\dot{V}_{O_2}$ relationships in diving vertebrates have focused primarily on birds (Bevan et al. 1992; Butler 1984; Stephenson et al. 1988; Woakes and Butler 1983).

For $fh:\dot{V}_{O_2}$ studies to yield accurate estimations of field metabolic rate, it is essential that studies encompass dive characteristics that are representative of free-ranging animals. For instance, previous studies investigating the $fh:\dot{V}_{O_2}$ relationship in diving marine mammals have been limited by maximum tank depth (Sparling and Fedak 2004; Webb et al. 1998). My study was the first to investigate the $fh:\dot{V}_{O_2}$ relationship in an otariid while freely diving with dive characteristics (depths and dive durations) similar to animals in the wild (Merrick and Loughlin 1997). Hence, all dives were less than the estimated aerobic dive limit (ADL) of adult female Steller sea lions (mean 7.1-7.6 min, Hastie et al. 2007; 7.5 min, Richmond et al. 2006). Foraging behaviour approximating field conditions is also important for investigating the $fh:\dot{V}O_2$

relationship, as locomotion can potentially increase the total energetic costs of a dive bout (Williams et al. 2004). For example, previous work in which two trained Steller sea lions stationed at an underwater light target (with dive depths and durations comparable to the present study) displayed reductions in diving metabolic rate of 41-45% versus RMR (Hastie et al., 2007). Rather than passively stationing underwater, the use of two feeding stations separated by ~3x sea lion body lengths created a situation of active feeding and underwater exercise in my study. This simulated foraging could have potentially elevated $\dot{V}_{O_2}$ and diving metabolic rate relative to other experimental scenarios. In fact, I noted no clear reduction in mean diving metabolic rate compared to resting metabolic rate.

Relationship between fh and diving metabolic rate (DMR)

I determined that *fh* could be used to predict average metabolic rate over a dive cycle, for either single dives or dive bouts, but could not predict diving metabolic rate. The lack of predictive power for diving metabolic rate could be a function of the temporal disconnect between measurements of $\dot{V}_{O_2}$ (measured as post-dive excess O_2 consumption) and *fh* (measured directly during submergence; Fig. 3.1). This comparison assumes that the post-dive increase in $\dot{V}_{O_2}$ is independent of increases in heart rate during this interval that facilitate increased gas exchange upon surfacing (see below). By contrast, the *fh*:AMR relationship is formulated from data with a greater temporal overlap (Fig. 3.1). Specifically, the *fh* component encompasses the dive itself and the surface recovery period, and thus incorporates both diving bradycardia and the post-dive tachycardia that facilitates gas exchange.

The non-linear relationship between *fh* and diving metabolic rate supports the concept that the dive cycle is a discrete and fundamental unit for diving and cannot be partitioned physiologically into underwater and surface elements (Butler and Jones 1997; Fedak et al. 1988). Several similar studies on diving vertebrates also suggest that *fh* and $\dot{V}_{O_2}$ should be measured over complete dive cycles because their relationships over dives alone were inconsistent (Bevan et al. 1992; Butler 1993; Fedak 1986; Fedak et al. 1988). Dives and subsequent surface intervals are physiologically linked because gas exchange and removal of metabolic byproducts (lactic acid and CO_2) only occur at the surface. Furthermore, repetitive dives in many species are

associated with a progressive O_2 debt, which cannot be repaid until the bout is completed (Fahlman et al. 2008a; Kooyman et al. 1973; Ponganis et al. 1993). It is therefore not surprising that average diving *fh* was not useful in predicting diving metabolic rate for Steller sea lions.

Despite the inability of *fh* to predict diving metabolic rate, diving metabolic rate data still provides valuable insights into the physiology of diving in Steller sea lions. For single dives, longer dive time was not always associated with higher diving metabolic rate, implying that in this scenario sea lions diving longer do not work harder at an elevated rate (Fig. 3.3C). This finding is consistent with data from free-ranging grey seals, *Halichoerus grypus* (Thompson and Fedak 1993). However, I did observe a negative relationship between diving metabolic rate and cumulative dive time for dive bouts (Fig. 3.3D) that was absent during single dives (Fig. 3.3C). These results concur with previous Steller sea lion research, which indicated that longer dives cost proportionally less, and that dive bouts and single dives may be separate physiological units (as predicted by measures of activity, Fahlman et al. 2008b). This could be explained by differences in buoyancy and locomotion with depth that become apparent with the specific depth and duration patterns of bout diving, thereby possibly decreasing metabolic dive costs.

Relationship between fh and average metabolic rate (AMR)

My study found significant relationships enabling the prediction of average metabolic rate from *fh* during both single dives and multiple-dive bouts. These results join a suite of previous experiments which have demonstrated significant $fh:\dot{V}_{O_2}$ relationships in pinnipeds and penguins while in swim mills (Butler et al. 1992; Culik et al. 1996; Culik et al. 1994; Ponganis et al. 1997; Williams et al. 1991), and in birds performing voluntary dives (Bevan et al. 1992; Butler 1984; Stephenson et al. 1988; Woakes and Butler 1983). However, my study is the first to demonstrate a significant $fh:\dot{V}_{O_2}$ relationship under natural conditions, with sea lions diving freely in the ocean to depths up to 40 m.

Results demonstrated that activity level (resting vs. diving) produced unique $fh:\dot{V}_{O_2}$ relationships during dive bout cycles, but not during single dive cycles (Fig. 3.4). This occurred despite the fact the responses of $\dot{V}_{O_2}$ and *fh* during diving were similar in both single dives and dive bouts. The pattern and range of bradycardia in California sea lions (*Zalophus californianus*)

is also similar across a range of depth and activity level (dive durations 1-3 min, Ponganis et al. 1997).

Although single and dive bout cycles are subject to similar physiological and environmental effects, dive bouts represent the cumulative effects of multiple dives. Whereas single dive cycles likely incorporate minimal exercise (and limited O_2 debt), dive bouts often encompass longer cumulative dive time, and more frequent changes in depth and pressure relative to single dives. The notion that dive bouts amplify the physiological effects of submergence, or even induce a different physiological state than a series of individual dives is supported by evidence that the metabolic cost of dive bouts can only be calculated by treating the bout as a distinct physiological unit and not by calculating the costs of individual dive cycles (Fahlman et al. 2008a; Kramer 1988). Further, dive bout recovery time is less clearly related to dive time than is single dive recovery time since, for dive bouts, divers could partially repay the O_2 debt during inter-bout surface intervals in addition to the post-bout recovery period (Fahlman et al. 2008a; Kooyman et al. 1973; Ponganis et al. 1993). Small changes in surface interval duration can also dramatically impact O_2 uptake (Fahlman et al. 2008a; Kramer 1988), which further explains the limited or absent correlation between dive duration and final surface recovery interval for bouts. Ultimately, it is only across complete multiple-dive bouts that the cumulative effects are sufficiently pronounced enough to significantly distinguish the fh:$\dot{V}_{O_2}$ relationship for average metabolic rate compared to resting metabolic rate.

Comparing fh with other metabolic rate estimation techniques

The average error of the models was assessed using an analysis of residuals and predicted values to account for treating animal as a random factor and the repeated measures framework (see statistical analysis section for details). The average residual error over single dive cycles and periods of rest was 17%, and the mean error over all of the dive bout cycles was 9% (Fig. 3.4). The error estimates for both equations were greater than the 3% error recorded for equations predicting $\dot{V}_{O_2}$ from fh of submerged swimming California sea lions (range = -28 to + 23%, Boyd et al. 1995), and slightly greater than the estimates derived from overall dynamic body acceleration (ODBA, ~7%, Fahlman et al. 2008b). Overall, both error estimates noted here were

considerably less than estimates derived from the doubly labeled water method (~36%, Boyd et al. 1995).

Relative error in applying the incorrect predictive equation

Dataloggers can be used in the field to measure *fh,* dive depth, dive duration and to determine when an animal is resting at the surface of the water, executing a single dive, or executing multiple dives in series (Ponganis 2007). The accuracy of estimating field metabolic rate for foraging Steller sea lions could therefore be enhanced by applying the most appropriate $fh:\dot{V}_{O_2}$ equation from Table 3.3.

I demonstrated that predictive equations are statistically distinct between single dive cycles and dive bout cycles, but that the $fh:\dot{V}_{O_2}$ relationship for single dive cycles did not differ from that for periods of surface rest. Since none of the predictive models incorporating diving metabolic rate were significantly linear, I recommend using *fh* to predict average metabolic rate for Steller sea lions over single dive cycles or over dive bout cycles in situations where animals are likely able to completely recover the incurred O_2 debt.

Given the absence of statistical differences between the predictive equations for animals resting in water or over a single dive cycle, I recommend using the composite equation encompassing all resting trials (Eqn. 1) for animals resting at the surface or when performing single dives. Given the statistical differences between the resting and dive bout equations, it follows that the impact of using the 'wrong' equation to predict average metabolic rate of dive bouts should be considered. I therefore calculated the percent error for using the 'wrong' equation by comparing the predicted average metabolic rates for a mean resting *fh* of 100 beats·min^{-1} among appropriate equations in Table 3.3. If the predictive equation for animals resting in water (Eqn. 1) was used incorrectly to estimate $s\dot{V}_{O_2}$ of a free-ranging Steller sea lion performing multiple dives in a series (Eqn. 4), it would overestimate $s\dot{V}_{O_2}$ by approximately 26% (i.e., 37 vs. 47 ml O_2·min^{-1}·kg$^{-0.75}$). Given this large potential error, and knowing that it is reasonably easy to determine dive behaviour via small time-depth-recorders in situations were *fh* is already being telemetered, Eqn. 1 should be used when animals are resting at the surface or

executing single dives, and Eqn. 4 should be used when animals are executing multiple dives in a series (Table 3.3).

Logistically, it is not always possible to distinguish single recovery dive cycles from dive bout cycles in free-ranging animals. However, foraging theory predicts that sea lions will perform multiple dives at the same location to exploit a prey patch until the prey patch is consumed or dispersed rather than stopping after a single dive. Consequently, dive bout cycles are more likely to accompany successful foraging compared to single dive cycles. Therefore, I recommend using the predictive equation for average metabolic rate over a dive bout cycle (Eqn. 4) for any ambiguous diving behaviour when using *fh* to predict field metabolic rate of diving Steller sea lions.

Summary

The relationship between heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) has been determined for a number of aquatic vertebrates while fasting and resting, but is known to change with environment (land or water) and digestive state (fed or fasted). I investigated if the dive response would also affect the fh:$\dot{V}_{O_2}$ relationship in Steller sea lions freely diving in the open ocean up to 40 m depth and for durations of 1-6 minutes. I simultaneously measured *fh* and $\dot{V}_{O_2}$ under a variety of activity states (surface resting or diving), depths (10 or 40 m), and types of dives (single dives or dive bouts). I found that the fh:$\dot{V}_{O_2}$ relationship differed for single dive cycles (one dive + surface interval) versus dive bout cycles (multiple dives + surface intervals). I further found the equation that predicts $\dot{V}_{O_2}$ associated with single dive cycles did not differ from that derived for sea lions resting on the surface. Neither dive duration, dive depth, water temperature, nor food consumed significantly affected the fh:$\dot{V}_{O_2}$ relationships. Ultimately, my results demonstrate that *fh* can be used to predict activity-specific metabolic rates of diving sea lions, but only over complete dive cycles or dive bout cycles when animals fully recover from the O_2 debt they incurred underwater.

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Chapter 4: Conclusions

Summary of findings

The goal of my study was to evaluate and define the predictive relationship between *fh* and $\dot{V}_{O_2}$ in Steller sea lions under environmental, digestive, and activity states that were comparable to those experienced by free-ranging sea lions. To do so, I simultaneously measured *fh* and $\dot{V}_{O_2}$ in Steller sea lions while fasting and feeding at rest, on land and in water, and while diving to set depths in the open ocean. This allowed me to specifically test for the effects of environment (land vs. water), digestive state (fasting vs. 4 kg or 6 kg meals), activity (resting vs. single dive vs. dive bout) and dive depth (10 m vs. 40 m).

My study confirmed previous research that demonstrated a linear relationship between *fh* and $\dot{V}_{O_2}$ in fasted, resting Steller sea lions (McPhee et al. 2003). However, predictive equations generated by my study were specific to either land or water environments. Feeding did not change the relationship on land as predicted, but it did alter the relationship in animals resting in water. I was able to use heart rate to predict average metabolic rate over a dive cycle (dive + surface interval), but not over the dive portion alone. Finally, my results showed that activity (resting or diving) and dive type (single dive or dive bout) changed the $fh:\dot{V}_{O_2}$ relationship.

I found that the physiological relationship between *fh* and $\dot{V}_{O_2}$ was robust under a variety of testing apparatus and using different groups of animals. Specifically, the $fh:\dot{V}_{O_2}$ relationship was similar when animals were resting in water inside either the swim mill or respirometry dome. This demonstrates that studies performed in different laboratories are equally valid in their application, as long as relevant physical and physiological parameters are held constant (e.g., an animal resting in water of similar temperatures and depth). Future $fh:\dot{V}_{O_2}$ studies performed with other pinniped species could therefore select suitable apparatus available at their research facilities without concern over applicability.

The first unexpected result of my study was the differences in the $fh:\dot{V}_{O_2}$ relationships on land or in water, as animals in both environments were fasting, resting, and likely within their

thermoneutral zone. As discussed in Chapter 2, the different relationships demonstrated for each environment were most likely not due to differences in testing apparatus, but rather due to a parasympathetic initiation of the dive response (a partial physiological change, but not a full dive response) upon submergence in water. As this is plausibly a universal response among pinnipeds, future $fh:\dot{V}_{O_2}$ studies on pinniped species should collect sufficient data to formulate predictive equations specific to the environment (land or water) where this technique will be applied to animals in the field.

In both air and water *fh* could be used to predict $\dot{V}_{O_2}$ in animals that were fed realistic-sized meals. Contrary to my predictions, the $fh:\dot{V}_{O_2}$ relationship did not change after feeding due to the heat increment of feeding (HIF) on land, but did differ with amount of food fed in water. The different relationships observed for the 4, 6, and 12 kg meals is consistent with previous research demonstrating that magnitude and duration of HIF are influenced by meal size (Barbour 1993; Markussen et al. 1994; Rosen and Trites 1997; Secor 2009). It is curious that alteration of the $fh:\dot{V}_{O_2}$ relationship after feeding was not uniform between environments. Previous research on Steller sea lions (Rosen and Trites 1997) and traces of $\dot{V}_{O_2}$ versus time from my study show that HIF occurred in both environments. I suspect that the environmental differences observed were likely due to activity masking the effects of HIF on land. While my study demonstrated that HIF influences the $fh:\dot{V}_{O_2}$ relationship (at least in water), the extent and duration of this effect in either environment merits further investigation. Equations based on fasted animals are currently being used to predict field metabolic rate of free-ranging animals, but my results suggest that these fasted equations likely produce incorrect estimates of metabolism. Such equations based on fasted animals should therefore only be used in situations where it is certain animals are not feeding and the new equations generated by my study should be used when animals are feeding or when digestive state is ambiguous on land.

My results also demonstrated that *fh* could be used to predict metabolic rate in diving sea lions despite physiological changes associated with the dive response, but only when these variables are averaged over the appropriate time interval. More specifically, my study demonstrated that *fh* could be used to predict average metabolic rate (AMR) over a dive cycle (dive + surface interval), but not diving metabolic rate (DMR) over the dive section only. My

results agree with previous research suggesting that the dive cycle is the fundamental unit for diving and cannot be partitioned physiologically into underwater and surface elements (Butler and Jones 1997; Fedak et al. 1988). The fh:$\dot{V}_{O_2}$ relationship differed for single dive cycles versus dive bout cycles, but the predictive equation for single dives did not differ from that derived for sea lions resting on the surface. These findings highlight the importance of accounting for both activity and dive type when deriving relationships between fh and $\dot{V}O_2$. Combined, the results of my thesis demonstrated that fh could be used to reliably predict $\dot{V}O_2$ of Steller sea lions, but that predictive equations were specific to environment, amount of food fed, and dive activity.

Strengths and weaknesses

Like most studies, my thesis had several challenges with corresponding strengths and weaknesses. Weaknesses include a small sample size, potential effects of training, not encompassing the entire digestion cycle, and limitations of the heart rate electrodes. A notable strength of my thesis was being able to examine the fh: $\dot{V}_{O_2}$ relationship in freely diving sea lions under circumstances similar to free-ranging animals. Additional strengths include my statistical approach and examining multiple sections of the dive cycle.

Sample size

My small sample size was both a strength and weakness in my research. Although a sample size of 3-7 animals is not considered large for fieldwork, it is considered relatively large compared to other captive pinniped studies. In contrast to previous studies that have simply pooled the data of several animals together, my analysis was strengthened by the use of mixed-effects models within a repeated measures framework, treating each animal as a random sample from the population. This increased my statistical power within my small sample size and allowed me to make inferences from the sample population to the wild population. The statistical strength of my predictive models despite my small sample size strongly indicates that changes in the fh:$\dot{V}_{O_2}$ relationship represented a realistic biological change rather than a statistical artifact.

Potential effects of training

Research with animals in the laboratory, such as my study, act as a bridge to studies on free-ranging animals and help us answer questions that are logistically impossible in the field. Except for the case of ice-hole experiments with the Weddell seal (Castellini 1992; Kooyman and Campbell 1972), it is nearly impossible to calibrate the $fh:\dot{V}_{O_2}$ relationship in a free-ranging marine mammal. Physiological field studies do not allow for controlled experimental manipulations and are therefore often clouded with multiple confounding variables making conclusions of cause and effect elusive. Furthermore, studying the physiology of free-ranging marine mammals is usually accompanied by additional logistical boundaries that can limit quantity and quality of feasible research questions to explore.

Although the sea lions in my study were captive, I do not believe that training strongly influenced their physiological responses after feeding or during diving. These sea lions were raised from weaning in captivity and trained to dive and use all experimental apparatus over a 4-11 year period using standard operant conditioning techniques. Positive reinforcement training used in captive studies does imply some psychological (i.e., behavioural) control of the animals, but not physical or physiological control. Previous research has demonstrated that total training time (in weeks) did not change dive performance or diving metabolic rates of Steller sea lions (Hastie et al. 2007). Animals participating in my diving study regularly dive 4-6 days a week for training and other research projects; therefore their daily activity level was more similar to free-ranging sea lions than previous captive studies.

Encompassing the digestive cycle

I was unable to measure *fh* and $\dot{V}_{O_2}$ during the entire digestive cycle which may last up to ~16 hours in Steller sea lions. I was limited in how long I could safely maintain a sea lion inside the metabolic chamber or swim mill before the animal became stressed or learned to avoid re-entering the apparatus in the future. Therefore, I do not know whether the differences I found in the $fh:\dot{V}_{O_2}$ relationships persisted for the entire digestion process. However, I did make sure to capture the majority of the peak HIF that occurs at approximately 4 hours following a 4 kg meal of herring (Rosen and Trites 1997).

Heart rate electrodes

The applicability of the *fh* method depends on the accuracy of the *fh* data collected which is consequently determined by the construction, location, and insertion methods of the electrodes used to measure *fh*. Developing a standardized way to remove muscle artifact or "noise" from the *fh* data was a significant challenge, especially as no "industry standard" exists among physiologists studying marine mammals. Ultimately the algorithms I used to systematically remove artificial heart beats yielded clean, trust-worthy data, but identifying and eliminating artifacts remains an inherent challenge of the *fh* method (See Appendix A1 for detailed summary of *fh* processing and electrode performance). Critics cite *fh* variability among individual animals as a disadvantage (Boyd et al. 1995; Fahlman et al. 2008b), but physiologically sensitive fine-scale variation in *fh* (despite the accompanying artifacts) is exactly what enables *fh* to predict activity-specific metabolic rate on a finer scale than doubly labeled water that yields only estimates of average metabolism.

I selected subcutaneous *fh* electrodes due to logistical, financial, and animal care constraints. Implantable heart rate dataloggers would be an ideal alternative, as these dataloggers produce less muscle artifacts and do not create additional drag. However, implanted *fh* dataloggers require species-specific confirmation of biocompatibility (various species have been shown to respond differently to implanted dataloggers) and require more risky invasive surgery than subcutaneous heart rate dataloggers (Green et al. 2009b).

The benefit of examining different sections of the dive cycle

Unlike previous research, I examined the *fh*: $\dot{V}_{O_2}$ relationship at multiple levels of the dive cycle: both the dive only (DMR) and over the entire dive cycle (dive + surface interval, AMR). I found that *fh* predicted average metabolic rate over a dive cycle, but there was not a significant linear relationship between *fh* and diving metabolic rate over the dive only. Unfortunately, variation among published studies in the calculation of average metabolic rate and diving metabolic rate can make comparisons in the literature misleading. Studies often do not clearly detail integration methods used to calculate the volume of O_2 consumed due to the dive process, and also often vary in whether they integrate above resting baseline or use total O_2 consumed (above 0) in their calculations. Studies also differ as to whether this volume of O_2 was divided by

actual dive time only (DMR in my study, Hastie et al. 2007; Hurley and Costa 2001) or by dive cycle time (MR in my study, Castellini 1992; Fahlman et al. 2008a; Green et al. 2007; Sparling and Fedak 2004; Webb et al. 1998). Hence, studies that use the latter calculations may refer to these measurements of "diving metabolic rate" that more closely correspond to average metabolic rate in my study. These calculation and integration variations result in large differences in estimated metabolic rate, and unfortunate confusion when comparing studies. As the $fh:\dot{V}_{O_2}$ relationship differs significantly depending on which definition of "diving metabolic rate" employed (AMR or DMR), it is essential that future researchers either standardize definitions of diving metabolic rate or clearly describe calculations and integration methods in publications.

Field application of predictive equations

Before the equations I generated are used to estimate metabolic rate of free-ranging animals, it is important to consider a few key points. The predictive models are species-specific for adult female Steller sea lions within the age group and body mass range of my sample population (Table 3.1). Adult female sea lions (in addition to juveniles) are likely to be more important to overall population trends than adult males; therefore the sample population of adult females represents one of the key demographic groups of special concern in the declining population. As the magnitude and duration of HIF vary by meal size and composition, the predictive equations are also specific for animals that are fed 0-12 kg of Pacific herring. It is not clear how specific the derived equations are to prey species.

Equations predicting average metabolic rate over dives cycles are specific to the dive durations (<6 min for single dives and <8 min cumulative for dive bouts), dive depths (<58 m), and dive bouts (4-5 dives) I tested. Although, dive bouts in the wild may include more than 4-5 dives, the dive durations and dive depths I included encompass the majority of dives that females Steller sea lions perform in the wild (Merrick and Loughlin 1997). Despite these logical limitations to my predictive models, the equations I generated are novel and have great potential to helping solve the problem of estimating field metabolic rate in pinnipeds**.**

The different predictive equations generated in my study (Table 2.2, 3.3) could easily be used to estimate field metabolic rate using only a heart rate datalogger and a small time-depth

recorder (TDR). TDRs can be used to measure, dive depth, dive duration, time spent on land, time spent in water, and number of dives executed in a series (Boyd et al. 2004; Ponganis 2007). Commercially available heart rate dataloggers can be placed on the surface of the sea lion's fur with subcutaneous electrodes (as in my study), or implanted abdominally to measure heart rate. This combination of dataloggers would reveal whether the animal was on land or in water, and the timing and nature of their dive behaviour, permitting application of an appropriate predictive equation from Table 2.2 or Table 3.3.

However, *fh* dataloggers and TDRs will not explicitly detail when animals are feeding, although some studies have used dive patterns to elucidate foraging events (Austin et al. 2006b). Recent innovations in animal-mounted cameras (Bowen et al. 2002; Parrish et al. 2005) allow feeding events to be detected visually, but video data require a secondary validation of the feeding event such as stomach temperature pills or head-mounted accelerometers (Viviant et al. 2009). Stomach sensor pills (swallowed by the animal) detect feeding events through a decrease in stomach temperature when cold fish are ingested. Stomach temperature pills are accurate for detecting initial feeding events but are less reliable for detecting total amount of food ingested or consecutive foraging events, as stomach temperature may not return to the animal's body temperature between feeding events (Andrews 1999; Austin et al. 2006a; Hedd et al. 1996; Kuhn and Costa 2006; Wilson et al. 1995). A single accelerometer mounted on the lower jaw of an animal has also been shown to be a reliable detector of feeding events but, as with stomach sensor pills, is currently unable accurately quantify amount of food ingested (Viviant et al. 2009). When the heart rate datalogger and TDR are combined with a stomach sensor pill or head accelerometer, field metabolic rate for foraging Steller sea lions could be estimated by applying the most appropriate $fh:\dot{V}_{O_2}$ equation from Table 3.3.

Future research

Future work could measure additional cardiac variables, such as stroke volume, blood pressure, or tissue oxygen extraction to explore changes in additional variables within Fick's equation, particularly in free-diving pinnipeds. Exploring additional cardiac parameters is intriguing from a purely physiological perspective, since the cardiac system fundamentally influences numerous other physiological systems down to cellular processes. Although several

studies have measured *fh* during diving, few have measured these fine-scale cardiac variables in a diving marine mammal. Studies on stroke volume (V_S) are inconclusive, are focused on phocids (compared to otariids), and have mostly been performed using forced submersions. Stroke volume has been noted to decrease (Blix et al. 1983; Ponganis et al. 1990; Sinnett et al. 1978; Zapol et al. 1979) or remain constant (Blix et al. 1976; Murdaugh Jr et al. 1966) in phocid seals but remain constant in an otariid (Elsner et al. 1964). While these divergent results may reflect real differences in V_S responses (Williams et al. 1991), they may also reflect differences in experimental design.

Measuring blood flow and tissue oxygen extraction in specific organs during natural (versus forced) diving would allow us to investigate when, where, and to what extent peripheral vasoconstriction occurred during diving (Blix et al. 1976; Davis et al. 1983; Elsner et al. 1985; Stone et al. 1973; Zapol et al. 1979). Measuring blood flow of the intestine during natural diving would also allow questions about whether marine mammals defer digestion while diving to be answered. Unfortunately measuring these fine-scale cardiac parameters in the field (especially on freely diving marine mammals) is logistically difficult, and requires specialized equipment, surgery, or heart catheterization. However, development of new dataloggers and advancements on the specialized equipment required for such cardiac measurements may make field measurements of these parameters more feasible in the future.

Although my study provides valuable insight into the influence of feeding on the fh:$\dot{V}_{O_2}$ relationship, further work is required to assess feeding in scenarios that are more similar to realistic foraging of wild Steller sea lions. I intentionally minimized the amount of food animals were fed (0.4 - 2.8 kg) while diving or at the surface to reduce the potential confounding affect of digestion on the fh:$\dot{V}_{O_2}$ relationship. However this is not a completely realistic scenario as free-ranging sea lions potentially consume greater than 2.8 kg of fish while foraging at depth. Therefore, it would be valuable to calibrate the fh:$\dot{V}_{O_2}$ relationship in sea lions fed meals of different amounts while diving to investigate HIF in conjunction with the dive response. However, doing so would be logistically challenging as trained dive behaviours are encouraged with food rewards, and it is unlikely that animals would voluntarily remain in the dome long enough to capture the peak in oxygen consumption which can last up to 4 hours (Rosen and

Trites 1997). However, this may be overcome with ingenuity, more extensive training and specialized testing environments.

Further work is also needed to explore the influence of feeding over realistic meal sizes. I showed that the fh:$\dot{V}_{O_2}$ relationship differed in animals that were either fasted or fed a bulk meal while resting in water. However, neither of these extreme feeding scenarios likely occurs frequently in the wild. Due to the patchy ocean environment, it is more likely that sea lions encounter several smaller prey patches throughout the day rather than a single 6 kg meal. The heat increment of feeding (HIF) is known to increase $\dot{V}_{O_2}$ following bulk meals (Markussen et al. 1994; Rosen and Trites 1997), but it is less clear how the magnitude or duration of HIF would change over several smaller meals. Future work could build upon my results by examining the influence of feeding on the fh:$\dot{V}_{O_2}$ relationship over several small meals (1-2 kg) dispersed randomly over a longer period of time (perhaps 10-12 hours to encompass most of the daylight hours) to simulate foraging in a patchy ocean environment. Such research could potentially be carried out at a facility similar to the UBC Open Water Research Station, as animals are more likely to cooperatively dive for several hours compared to sitting still in a metabolic chamber or swim mill. Furthermore, this facility also allows examination of the influence of feeding in a more natural environment where animals are actively foraging and diving to depth.

Future work is also needed to investigate the fh:$\dot{V}_{O_2}$ relationship over a more realistic number of dives. In my study, I investigated the relationship over a 4-series dive bout, but in the wild sea lions often perform more dives in a bout. Heart rate dataloggers deployed on free-ranging sea lions for several months yield datasets with hundreds of dives per day and potentially thousands of accompanying instantaneous fh measurements. Predictive equations are calibrated over ~1-5 dives, but then applied to free-ranging animals that are performing many more dives. Future work could bridge this gap by examining the fh:$\dot{V}_{O_2}$ relationship in sea lions executing a greater number of dives (>50) over a longer period of time (10-12 hours) with randomly dispersed surface intervals and dive durations. Sea lions in my study occasionally performed up to 10 dives during a 30 minute trial. This suggests that research over greater number of dives may be possible with extensive animal training over several years.

On a practical level, it would also be beneficial to explore the effect of *fh* sampling rates to bridge the gap between data sampling rates used for deriving the $fh:\dot{V}_{O_2}$ predictive equations and the sampling rates likely used when *fh* dataloggers are deployed long-term on free-ranging animals. Studies deriving $fh:\dot{V}_{O_2}$ relationships are often performed by sampling *fh* at the highest rate possible (i.e., instantaneous heart rate) to minimize artifact and increase accuracy of the predictive model. In contrast, field applications of these predictive equations are often limited to sampling *fh* over larger time intervals (i.e., >1 min) due to datalogger battery life and available memory for data storage. Sampling rate in the field is also reduced because it is not efficient or logistically feasible to analyze instantaneous *fh* over hundreds of dives when dataloggers are deployed on several animals for months at a time. Future research could therefore focus on experimenting with varying *fh* sampling rates to see if a large sample rate changes the predictive power of *fh*.

Another potential area of research is the relationship between body acceleration metrics, heart rate, and metabolism. Although I demonstrated that *fh* alone could accurately predict metabolism, recent research suggests that measures of body acceleration may also be useful predictors of metabolic rate. Despite the apparently poor ability to predict metabolic rate of inactive animals, body acceleration metrics such as overall dynamic body action (ODBA) or flipper stroking have potential to increase our understanding of metabolic rate in active animals. Fortunately, flipper stroking and ODBA measurements were collected concurrently with my thesis research during the diving trials discussed in Chapter 3 (using an accelerometer attached to the sea lion's harness), although this data was not included in my thesis because it was tangential to the focal $fh:\dot{V}_{O_2}$ relationship.[3] However, preliminary results indicate that *fh* and ODBA are uncoupled over short time scales and may also differ in their relationship to diving metabolic rate. This is presumably because body acceleration metrics should increase with physical activity during diving, but *fh* is generally decreased underwater. Clearly, more empirical data is needed to completely address this relationship.

[3] A version of this *fh* and accelormeter data will be submitted for publication. Hindle, Allyson G., Young, Beth L., Rosen, David A.S., Haulena, Martin, Trites, Andrew W. Impacts of dive depth and activity on bradycardia in Steller sea lions (*Eumetopias jubatus*).

Ideally future research will continue to explore the predictive relationship between heart rate and oxygen consumption in increasingly more natural conditions, such as by incorporating longer dive durations, different meal sizes, and possibly measures of body acceleration as described above. Updating current bioenergetic models (Winship et al. 2002) with new data derived from wild sea lions using the equations generated from my thesis research will yield more accurate estimates of metabolic rates of free-ranging Steller sea lions under a variety of physiological, behavioural, and environmental states.

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Appendix A1: Summary of heart rate electrode construction and insertion

A large component to my thesis project was to explore new heart rate monitoring techniques in an effort to improve precision and logistics of applying and removing the gear. Traditionally heart rate electrodes have been glued to the animal's fur with epoxy and left on for the entire duration of the study. Previous research has had problems with infection at the electrode insertion site and animals removing electrodes between trials. Therefore I decided to attach the heart rate datalogger and electrodes under anesthetic and remove the gear under trainer control for each individual trial.

Electrode construction

The heart rate monitoring system consisted of 1) a HTR data logger encased in epoxy that recorded the seconds between R-R peak intervals of the electrocardiogram (ECG) and 2) a HRX transmitter encased in epoxy that had two 26 G wires (~32 inches) extending out of it. In order to reduce the risk of infection and decrease the size of needle needed for insertion, 30 gauge 99.9% pure silver Teflon coated wire was spliced to the terminal end of the electrode wires (Grass Technologies, Longueuil, QC). Splices were sealed with silicone and heat shrink tubing. The electrode wires were sterilized in gluteraldhyde prior to each procedure for a minimum of 30 minutes (Metricide 28, Metrex, VaxServ, Scranton, PA, USA). Neoprene circles (1.5 inch diameter, 3mm thickness) sterilized with Isopropyl alcohol were glued to the fur to help secure the electrode wire (Loctite Superglue).

Electrode insertion

Preliminary trials involving splicing the veterinary ECG machine to the HRX transmitter wires were conducted to find a balance between ECG signal strength while limiting animal access. The best location was at the midaxilary line of the animal, distal from the spine approximately 6 inches. Steller Sea lions were outfitted with subcutaneous heart rate electrodes while under veterinary supervised gas anesthetic (0-5% Isoflurane, Wildlife Computers, Redmond, WA, USA). Prior to electrode insertion, a custom-fit harness was put on the animal to hold the transmitter and data logger. All electrode insertions were performed by Dr. Martin Haulena. The electrode was inserted by bending the terminal end of the Teflon wire and inserting the stripped end (0.5-1.0 cm) into a 20-gauge hypodermic needle. The wire was then guided

subcutaneously under the skin and the needle was removed leaving approximately 1.5 cm of Teflon wire subcutaneous. Small neoprene squares were glued on top of the Teflon wire at the point of insertion to further secure the wires. A strip of neoprene (~5 cm) was glued perpendicular to the spine to guide the wires along the spine out of the animal's reach (Figure 2.1). Transmission of heart rate to the datalogger was verified by the red blinking LED light on the HTR datalogger and also using a Polar brand heart rate receiver watch (Polar Electro Canada Inc, Lachine, QC).

Electrode performance

Shortening the length of the Teflon wires (so that the entire length of Teflon was either beneath the neoprene circle or subcutaneous) at the end of the leads decreased muscle artifact and also increased the length of time electrodes remained attached to the animals. Electrodes performed equally well in the metabolic chamber, swim mill and while diving in open water, and sea water did not appear to influence performance. Overall, animal activity and movement seemed to have the greatest influence on the amount of artifact in the heart rate data.

The HRX records the highest amplitude (voltage signal), which is usually the R peak in the QRS complex. The HTR records the seconds between R-peaks or the inter-beat-interval (IBI). The HRX receives IBI of any length (does not filter *fh* data as it is received). Therefore the HTR has the ability to record *fh* values of "artificial noise" that are not physiologically possible, (i.e., 500 beats min^{-1}). However, a "mean band" of *fh* was clearly visible in all *fh* traces, and the algorithms applied to remove artificial beats were designed to isolate this "mean band" in a standardized method. Ultimately, the electrodes performed well and yielded clean, trust-worthy *fh* data.

Appendix A2: Supplementary material for Chapter 2

Table A2. 1 Equations for models ($\dot{V}_{O_2} = a \bullet fh + b$) showing the linear relationship between heart rate (*fh)* and oxygen consumption ($\dot{V}_{O_2}$) for Steller sea lions that were fasted or fed (food in kg). Model parameters include slope (± SE), intercept (± SE), and *P*-values (F-test). Figure references are given for equations presented in Chapter 2 (i.e., 2.4) or in appendices (i.e., A2.4). Model descriptions are defined in the list of abbreviations. If models were significantly different, separate equations are presented for each subset of the models (i.e., Eq. 7,a, b, c). Models highlighted in grey were presented in Table 2.2 of Chapter 2 but are included again for comparison. Significantly linear slopes and intercepts are noted by (*).

Equation	Fig.	Food (kg)	Slope (*a*)	(± SE)	Intercept (*b*)	(± SE)	Slope *P*-value	Intercept *P*-value	Trial Type	Model Description
									dry vs. water	
										$dry_{fasted} + water_{comp}$
		0	0.51	(0.10)	-4.55	(9.81)	<0.001*	<0.001*		dry_{fasted}
		<0.36	0.51	(0.10)	10.14	(11.23)	<0.001*	<0.001*		$water_{comp}$
									dry only	
1	2.2a	0	0.53	(0.16)	-3.31	(18.01)	0.002*	<0.001*		dry_{fasted}
	A2.3a	4	0.39	(0.08)	7.00	(8.68)	<0.001*	<0.001*		dry_{4kg}
		6	0.21	(0.10)	27.3	(9.37)	0.036*	<0.001*		dry_{6kg}
3	2.3	0,4,6	0.31	(0.12)	19.2	(13.91)	0.009*	<0.001*		dry_{all} (fed and fasted)
9		0,4,6	0.32	(0.10)	17.0	(11.63)	<0.001*	<0.001*		$dry_{+McPhee}$
									water only	
	A2.2b	<0.36	0.27	(0.07)	10.67	(6.52)	0.003*	<0.001*		$water_{ow}$
	A2.2a	0	0.22	(0.09)	15.1	(5.88)	0.002*	<0.001*		$water_{fasted}$
2	2.2b	<0.36	0.20	(0.07)	16.7	(4.96)	0.005*	<0.001*		$water_{comp}$ ($water_{ow} + water_{fasted}$)
4	2.4a	4	0.21	(0.08)	16.4	(6.90)	0.011*	<0.001*		$water_{4kg}$
	2.4a	6						<0.001*		$water_{4kg} + water_{comp}$
		4	0.29	(0.11)	12.2	(8.24)	<0.001*	<0.001*		$water_{4kg}$
		<0.36	0.10	(0.05)	23.8	(3.64)	<0.001*	<0.001*		$water_{comp}$
	A2.5c						0.23	<0.001*		$water_{6kg}$
5	2.4b	0 or 6	0.17	(0.03)	19.4	(3.11)	<0.001*	<0.001*		$water_{6kg} + water_{comp}$ (+food NS factor)
6	2.4c	0 or 6					<0.001*	<0.001*		$water_{comp+McPhee} + water_{6kg+McPhee}$
			0.36	(0.06)	11.7	(6.02)				$water_{comp+McPhee}$
			0.36	(0.06)	13.9	(8.41)				$water_{6kg+McPhee}$
								<0.001*		$water_{+McPhee}$
7a		4	0.13	(0.06)	24.5	(6.05)	0.033*	<0.001*		4 kg
7b		6	0.13	(0.06)	23.0	(6.43)	0.033*	<0.001*		6 kg
7c		12	0.13	(0.06)	23.6	(6.61)	0.033*	<0.001*		12 kg
8		4,6,12	0.12	(0.06)	24.5	(5.54)	0.030*	<0.001*		$water_{+McPhee}$ (4,6,12 mixed)

Table A2. 2 Summary of mean, median, minimum, and maximum heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) in resting sea lions.

	fh (beats•min $^{-1}$)				$\dot{V}_{O_2}$ (ml O_2 min^{-1}•kg^{-75})			
Data	mean	median	min	max	mean	median	min	max
dry_{fasted} (metabolic chamber)	97	95	74	123	47	49	20	77
dry_{4kg}	93	89	53	128	44	46	18	78
dry_{6kg}	90	87	54	124	46	41	19	79
$water_{fasted}$ (swim mill)	75	73	57	97	32	32	18	55
$water_{4kg}$	77	74	60	94	36	33	21	67
$water_{6kg}$	82	83	60	111	32	34	18	44
$water_{ow}$ (surface of open water)	85	83	61	108	34	33	22	44

Table A2. 3 Estimates of the heat increment of feeding (HIF) following a 2.0 or 4.0 kg meal in pinnipeds (modified from Rosen & Trites 1997). Environments tested were either on land (metabolic chamber) or in water (swim mill). Maximum increase in oxygen consumption ($\dot{V}_{O_2\,\max}$) following a meal was measured relative to resting metabolic rate (RMR).

Species	Environment	Food (kg)	$\dot{V}_{O_2\,\max}$ X RMR	Time until $\dot{V}_{O_2\,\max}$ (hrs)	Time until return to RMR (hrs)	Body Mass (kg)	*N*	Source
Steller Sea lion								
Eumetopias jubatus	land	2	1.76	2.8	6-8	111-166	6	Rosen & Trites, 1997
	land	4	2.13	3.7	8-10	111-166	6	
Northern Elephant seal								
Mirounga angustritostris	water	2	1.46	2.9	7.0	104-149	4	Barbour, 1993
	water	4	1.65	4.6	10.4	104-149	4	
Harp seal								
Phoca groenlandica	water	2	1.67	4.0	>10	150	1	Gallivan & Ronald, 1981

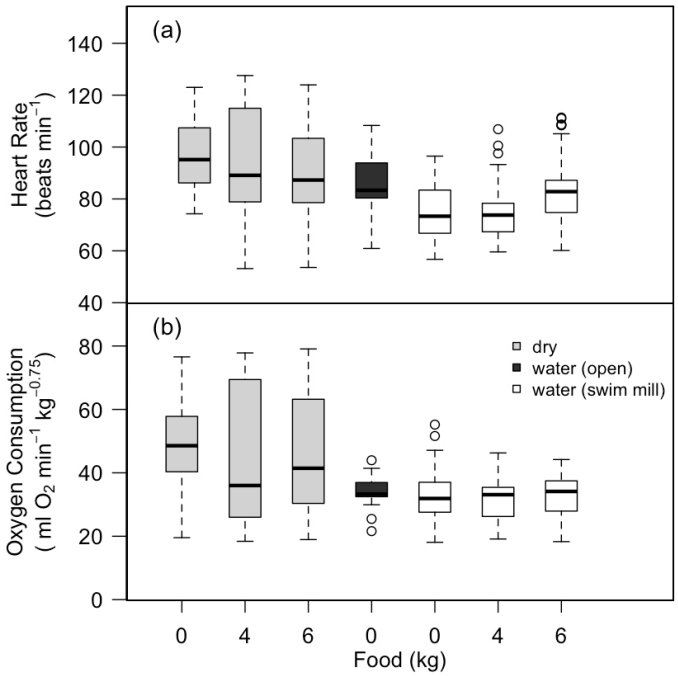


Figure A2. 1 Box-and-whiskers plot of heart rate (*fh*) and oxygen consumptioin ($\dot{V}_{O_2}$) showing minimum, median, and maximum *fh* (a) and $\dot{V}_{O_2}$ (b) for dry (metabolic chamber), water (open), and water (swim mill) trials by amount of food digested (kg).

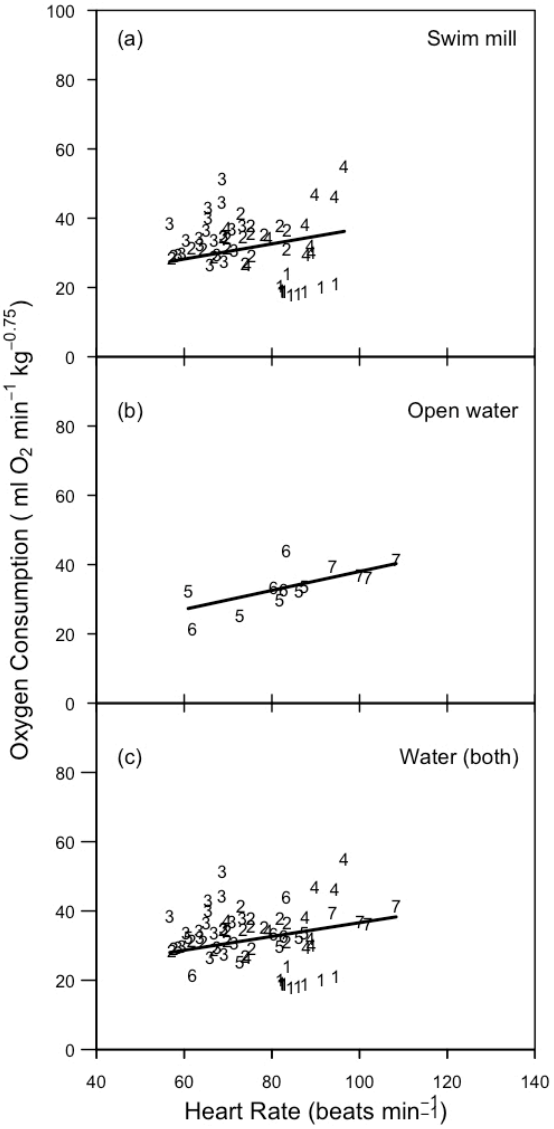


Figure A2. 2 The relationship between heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) fasted in a swim mill (a) and resting at the surface in open water (b) were not different. Therefore both trial types were combined and used as the baseline (c) for feeding (Chapter 2) and diving (Chapter 3) trials (water comp in Chapter 2 equivalent to RMR in Chapter 3).

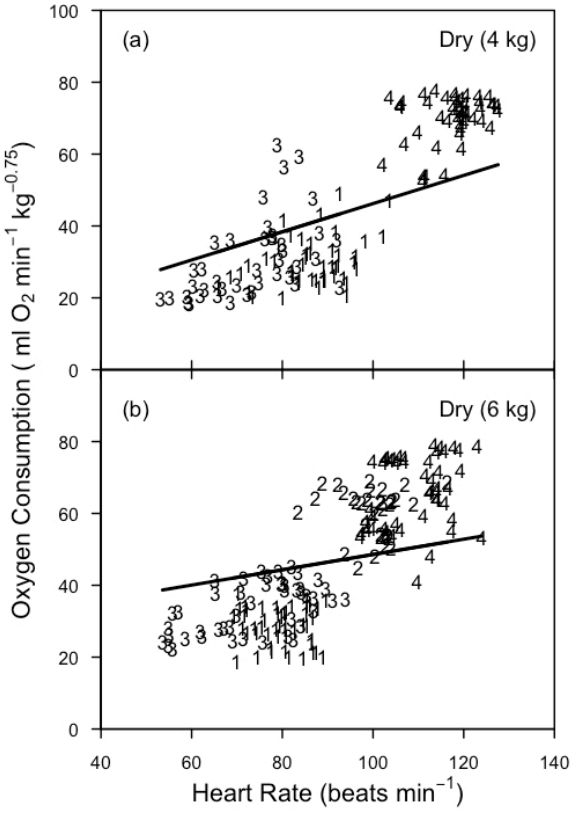


Figure A2. 3 Feeding sea lions a meal of 4 or 6 kg on land did not significantly change the relationship between heart rate and oxygen consumption. Therefore a single equation was used to predict $\dot{V}_{O_2}$ of animals that were fasted or fed on land.

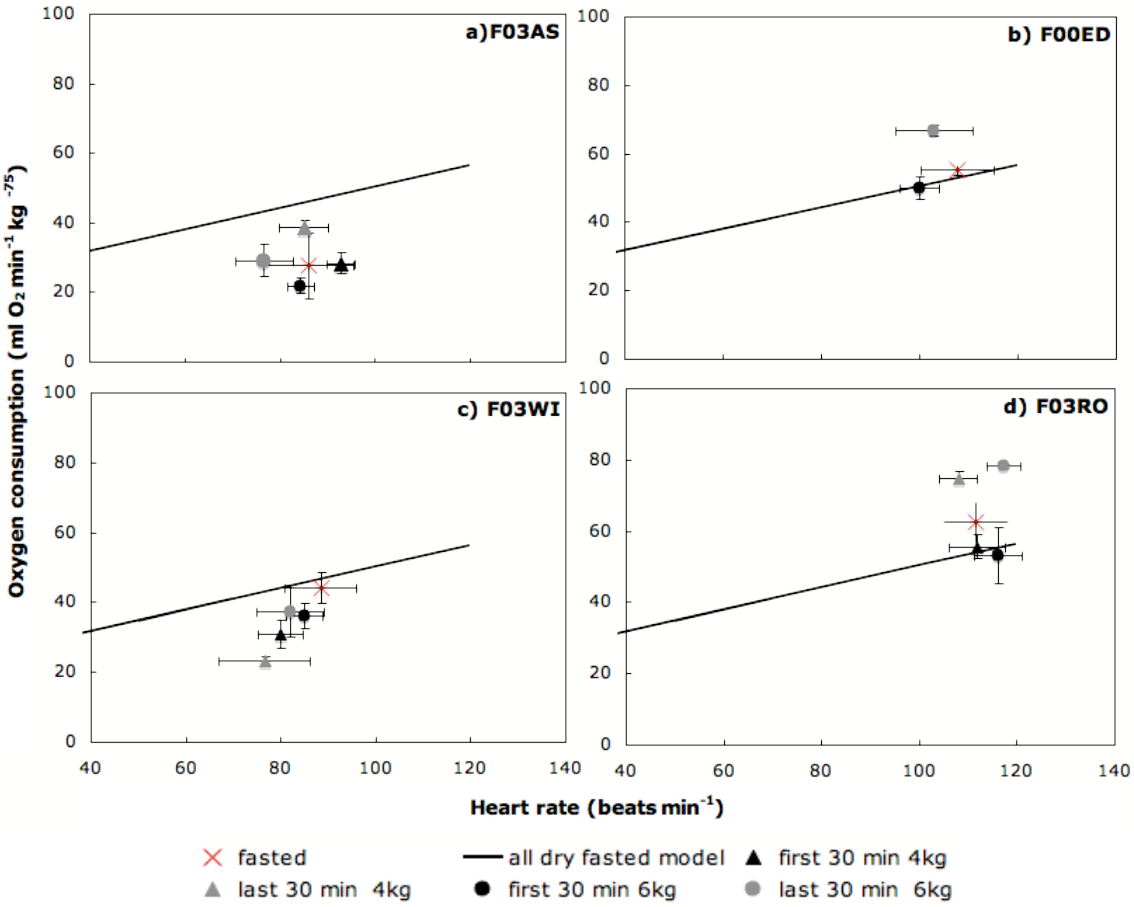


Figure A2. 4 Comparison of average *fh* and $\dot{V}_{O_2}$ for dry trials. Average *fh* and $\dot{V}_{O_2}$ over entire dry_{fasted} trial (X), average *fh* and over first 30 minutes (black triangle) and last 30 minutes of dry_{4kg} (grey triangle), and average *fh* and over first 30 minutes (black circle) and last 30 minutes of dry_{6kg} (grey circle). Results showed that feeding does not uniformly move points along the "fasted" line.

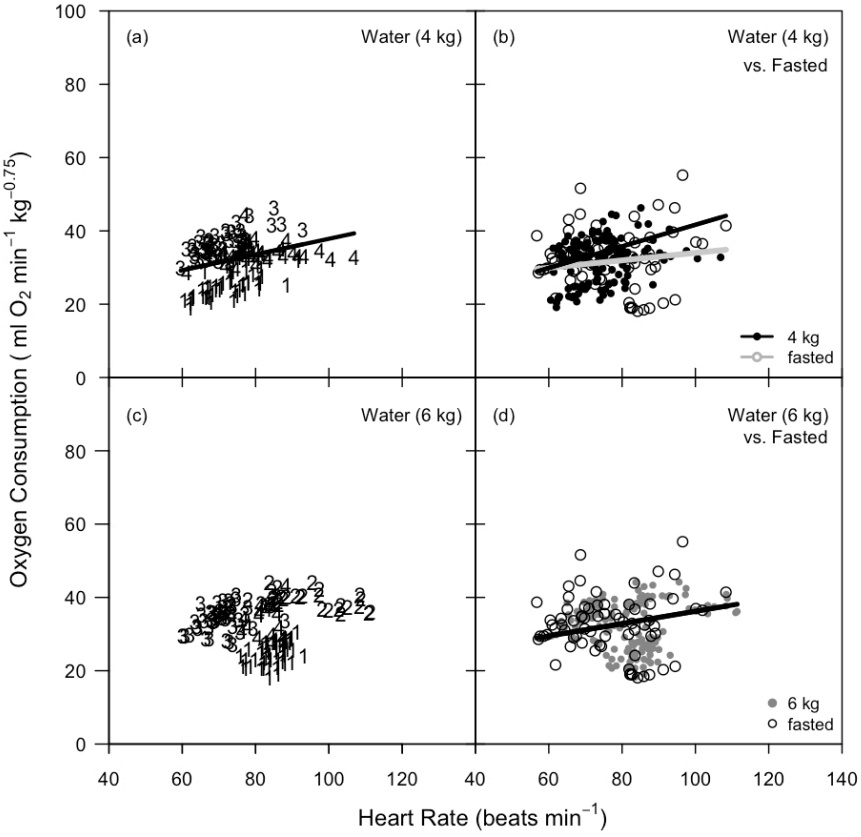


Figure A2. 5 The relationship between heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) after 4 kg meal (a) was different than the fasted baseline (b). There was not a significant linear relationship after animals were fed a 6 kg meal (c). When combined with the baseline, the 6 kg fed data was linear and did not differ from the baseline (d).

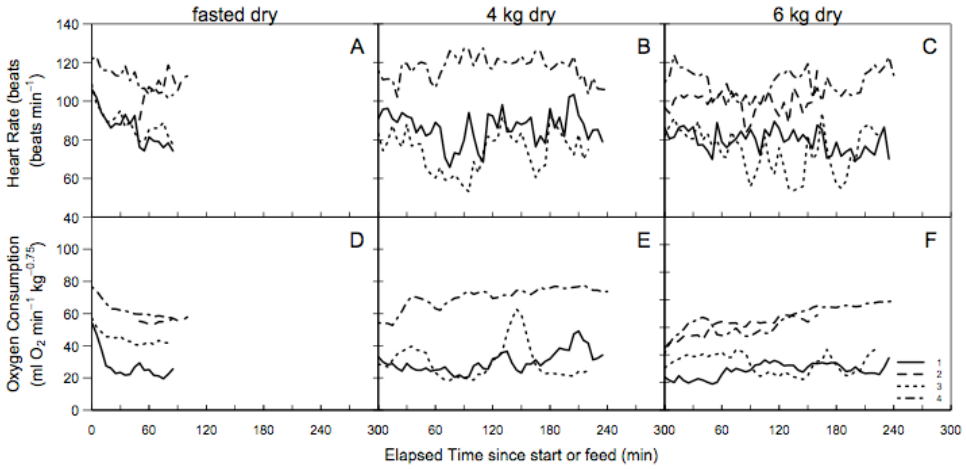


Figure A2. 6 Heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) changes as a function of time for dry_{fasted} (a, d), dry_{4kg} (b, e) and dry_{6kg} (c, f) in animals 1-4. See Table 2.1 for Animal ID to corresponding animal number.

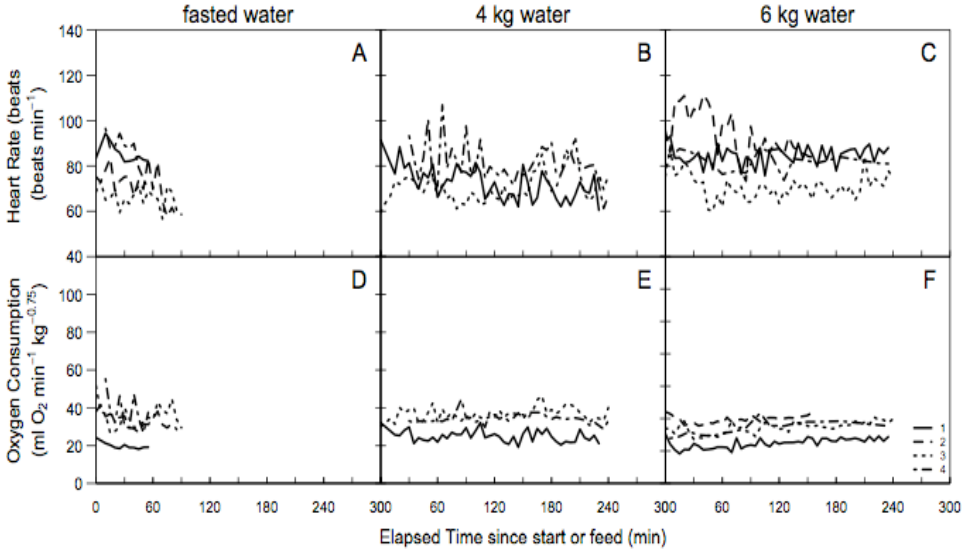


Figure A2. 7 Heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) changes as a function of time for $water_{fasted}$ (a, d), $water_{4kg}$ (b, e) and $water_{6kg}$ (c, f) in animals 1-4. See Table 2.1 for Animal ID to corresponding animal number.

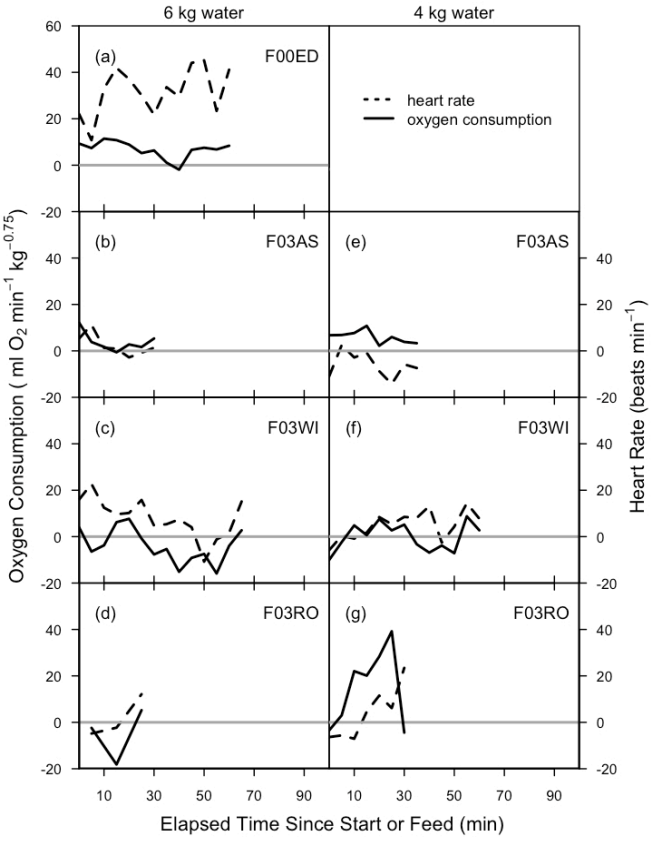


Figure A2. 8 Relative heart rate (*fh*, dashed line) and oxygen consumption ($\dot{V}_{O_2}$, solid line) of Steller sea lions above baseline (fed 0 kg) values (grey line baseline) following a single meal of 4 or 6 kg ($water_{4kg}$ and $water_{6kg}$). All animals were in a swim mill with no applied water current during the trials. The 4 kg trial for F00ED was not analyzed due to poor quality *fh* data. Corresponding data for dry trials was presented in Figure 2.5

Appendix A3: Supplementary material for Chapter 3

Table A3. 1 Equations for models ($\dot{V}_{O_2} = a \bullet fh + b$) showing the linear relationship between heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) for Steller sea lions that were fasted or fed (food in kg). Model parameters include slope (± SE), intercept (± SE), and *P*-values (F-test). Figure references are given for equations presented in Chapter 3 (i.e., 2.4) or in appendices (i.e., A3.4). Model descriptions are defined in the list of abbreviations. If models were significantly different, separate equations are presented for each subset of the models (i.e., Eq. %A, B). Models highlighted in grey were presented in Table 3.3 of Chapter 3 but are included again for comparison. Significantly linear slopes and intercepts are noted by (*).

Equation	Fig.	Food (kg)	Slope (*a*)	(± SE)	Intercept (*b*)	(± SE)	Slope *P*-value	Intercept *P*-value	Model Description
1		<0.36	0.20	(0.07)	16.7	(4.96)	0.005*	<0.0001*	RMR Resting in water
2	A3.5a	<0.54	0.25	(0.10)	15.0	(8.51)	0.018*	<0.0001*	AMR Single dive cycle
3	3.4	<0.54	0.22	(0.05)	15.3	(3.43)	0.0001*	<0.0001*	AMR Single dive cycle + RMR
4	3.4	<1.20	0.18	(0.06)	29.0	(4.82)	0.014*	<0.0001*	AMR Dive bout cycle
	A3.6b						<0.0001*	<0.0001*	AMR Dive bout cycle + RMR
5A		<1.20	0.21	(0.53)	26.5	(5.64)			Dive bout cycle
5B		<1.20	0.22	(0.54)	15.3	(3.91)			Resting in water
	A3.6c						<0.0001*	<0.0001*	AMR Dive bout cycle + single dive cycle
6A		<1.20	0.24	(0.05)	24.5	(4.06)			Dive bout cycle
6C		<1.20	0.24	(0.05)	16.1	(6.82)			Single dive cycle
	A3.3a						<0.0001*	0.76	DMR Single dive
	A3.4a						<0.0001*	0.40	DMR Dive bout
	A3.4c						<0.0001*	0.23	DMR Dive bout + single dive

Table A3. 2 Abbreviations and formulas used to calculate metabolic rates of diving sea lions

Abbreviation	Definition and formula
dive bout	4-5 dive cycles in a row followed by recovery until $s\dot{V}_{O_2}$ reached ± 2% of MRs
dive cycle	Dive+SI until returns to within ± 2% of MRs. Dive cycle includes 1 dive and 1 SI, but dive bout cycles include multiple dives and SIs
$s\dot{V}_{O_2}$	Mass-corrected rate of oxygen consumption (ml O_2 $min^{-1} \cdot kg^{-0.75}$)
T_d	Dive time (min)
SI	Surface interval of preceding dive (min)
T_s	Full post-dive surface interval (SI) time (min)
T_{MRs}	SI until returned to ± 2% of pre-dive $\dot{V}_{O_2}$ level (MRs)
MR_s	Rate of mass-corrected oxygen consumption ($s\dot{V}_{O_2}$) resting at the surface in open water (predive)
AMR	Average metabolic rate ($s\dot{V}_{O_2}$) averaged over the dive cycle (dive(s)+surface interval(s))
AMR single dive	AMR for a single dive cycle=(Total (L O_2) for dive cycle) / sum of T_d +T_{MRs}
AMR dive bout	AMR for a dive bout=(Total (L O_2) for dive bout) / sum of T_d +T_{MRs} of last SI
DMR	Diving metabolic rate ($s\dot{V}_{O_2}$) averaged over dive (s) only
DMR single dive	DMR single dive==[Total (L O_2) for dive - sum of total (L O_2) used for SI] / sum T_d
DMR dive bout	DMR for dive bout=[Total (L O_2) for dive bout- sum of total (L O_2) used at all SI]/ (sum of all T_d in bout)

Table A3. 3 Summary of mean, median, minimum, and maximum heart rate (*fh*) and oxygen consumption ($\dot{V}_{O_2}$) in diving Steller sea lions. See Table 3.1 for abbreviations of types of data.

	Heart rate (*fh*, beats•min $^{-1}$)				Oxygen consumption ($\dot{V}_{O_2}$, ml O_2 min^{-1}•kg^{-75})			
Data	mean	± SD	min	max	mean	± SD	min	max
MRs resting in water	75	(11.1)	57	102	33	(9.6)	18	69
AMR single dives	75	(18.1)	46	103	34	(7.0)	22	51
10m	74	(21.9)	46	103	34	(9.5)	22	51
40 m	74	(16.0)	47	103	33	(5.8)	24	41
AMR bout	71	(18.3)	46	97	40	(6.0)	30	50
10m	69	(19.3)	52	97	40	(7.1)	30	49
40 m	72	(18.7)	46	88	42	(4.9)	36	50
DMR single dives	51	(10.4)	27	68	42	(7.5)	29	57
10m	51	(10.8)	32	67	53	(19.0)	29	90
40 m	50	(11.0)	27	68	40	(7.2)	29	57
DMR bout	53	(15.6)	34	75	47	(8.2)	30	59
10m	50	(14.0)	34	75	48	(10.7)	30	59
40 m	56	(16.5)	36	75	44	(7.7)	30	53

Table A3. 4 Summary (mean ± SD, min, max) of dives conducted by three Steller sea lions. Dive and surface interval (SI) durations (min) are presented for single dives and cumulative dive bouts, and for dive types combined. Associated maximum dive depth data (m) are also listed. Neither dive depth nor dive duration significantly changed the relationships between heart rate and oxygen consumption.

	Average duration of dive or SI (min)				Average maximum dive depth (m)			
Data	mean	± SD	min	max	mean	± SD	min	max
Dive duration of single dives	2.6	(1.5)	1.0	6.3	35	(16.7)	11	58
10 m	1.3	(0.2)	1.0	1.5	12	(1.1)	11	14
40 m	3.2	(1.5)	1.5	6.3	46	(6.2)	41	58
Single SI until recovered	3.0	(0.9)	1.4	4.6				
10 m	2.5	(1.0)	1.4	4.4				
40 m	3.2	(0.8)	2.1	4.6				
Dive bout cumulative dive time	5.6	(1.7)	2.6	8.0	31	(18.4)	11	57
10 m	4.1	(0.7)	2.6	4.9	12	(0.9)	11	14
40 m	6.9	(1.0)	5.3	8.0	47	(6.3)	42	57
Dive bout last SI only (until recovered)	3.3	(1.4)	0.5	5.3				
10 m	3.3	(1.6)	0.5	5.3				
40 m	3.2	(1.2)	2.0	5.3				
Dive bout all SI until recovered	4.30	(1.2)	2.7	6.5				
10 m	4.30	(1.2)	2.7	5.7				
40 m	4.30	(1.3)	3.0	6.5				
Dive duration (single & bouts)	3.6	(2.2)	0.9	8.0	32	(17.0)	11	58
SI duration (single & bouts) (single and bouts)	3.1	(1.2)	0.5	5.5				

Table A3. 5 Summary of the amount of herring fed to the sea lions (kg) during diving or subsequent surface intervals for single dives and dive bouts. Animals were fed several 20 g pieces of fish to encourage diving behaviour.

	Amount of herring fed (kg)			
Data	mean	± SD	min	max
Resting at the surface	0.20	(0.10)	0	0.36
Single dive cycle (dive+surface interval)	0.21	(0.13)	0	0.54
Dives only	0.14	(0.12)	0	0.54
Surface only	0.11	(0.08)	0	0.30
Dive bout (dives +surface intervals)	0.61	(0.22)	0.22	1.20
Cumulative over entire dive trial (single dives+dive bout)	1.35	(0.69)	0.44	2.80

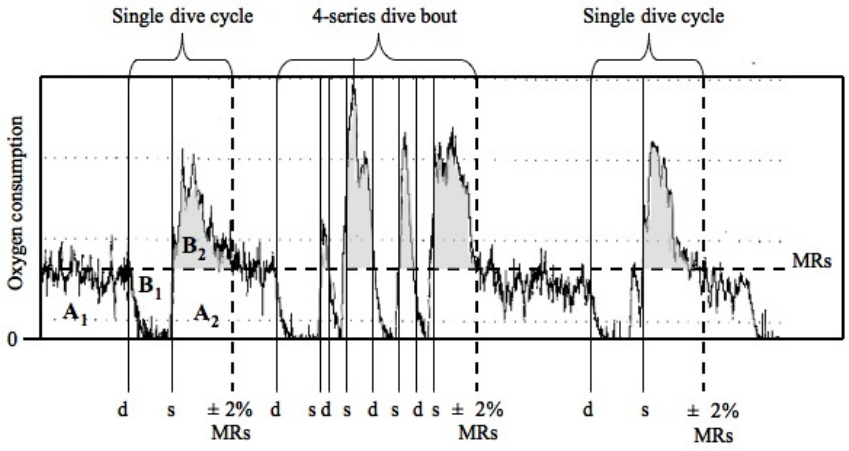


Figure A3. 1 Representative trace of oxygen consumption ($\dot{V}_{O_2}$) versus time for a Steller sea lion dive trial, showing starts of dives (d, highlighted in grey) and surface intervals (s). Standard metabolic rate (MR_s, dashed line) was measured prior to each trial while animals were resting. Average metabolic rate (AMR) was calculated as total post-dive oxygen consumption (A_2+B_2) until $\dot{V}_{O_2}$ reached 2% of MR_s, divided by total dive cycle time (dive + surface interval, SI). Diving metabolic rate (DMR) was calculated as the increase in post-dive oxygen consumption (B_2) above MR_s ($A_2 = A_1$), divided by dive duration. Dive bouts were analyzed using the same method modified to include each individual dive section (highlighted in grey).

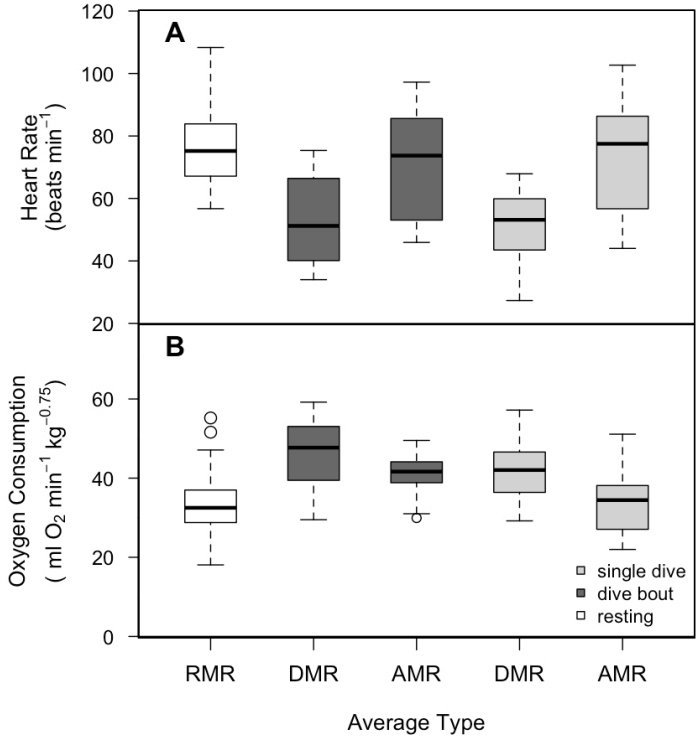


Figure A3. 2 Box-and-whiskers plot showing minimum, median, and maximum heart rate (*fh*) and oxygen consumption $\dot{V}_{O_2}$ for animals resting in water (white), averaged over dive bouts (dark grey), or averaged over single dives (light grey). Water composite includes data when animals were resting in a swim mill and resting at the surface of open water. Diving metabolic rate (DMR) includes data averaged over single dives or several dives in dive bout. Average metabolic rate (AMR) includes data averaged over a complete dive cycle (dive +surface interval) or entire dive bout (multiple dives and surface intervals).

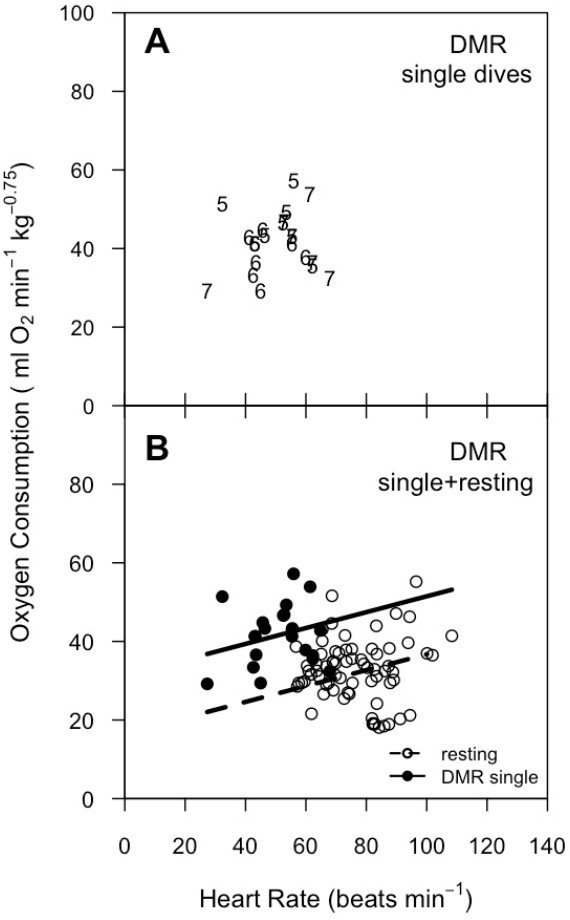


Figure A3. 3 The relationship between heart rate (*fh*) and diving metabolic rate ($\dot{V}_{O_2}$ =DMR) averaged over a single dive (dive only) was not significantly linear (A). However, when combined with resting, the *fh:*DMR relationship for single dives was different than when animals were resting (RMR) in water (B). See Table 3.1 for Animal ID to corresponding animal number.

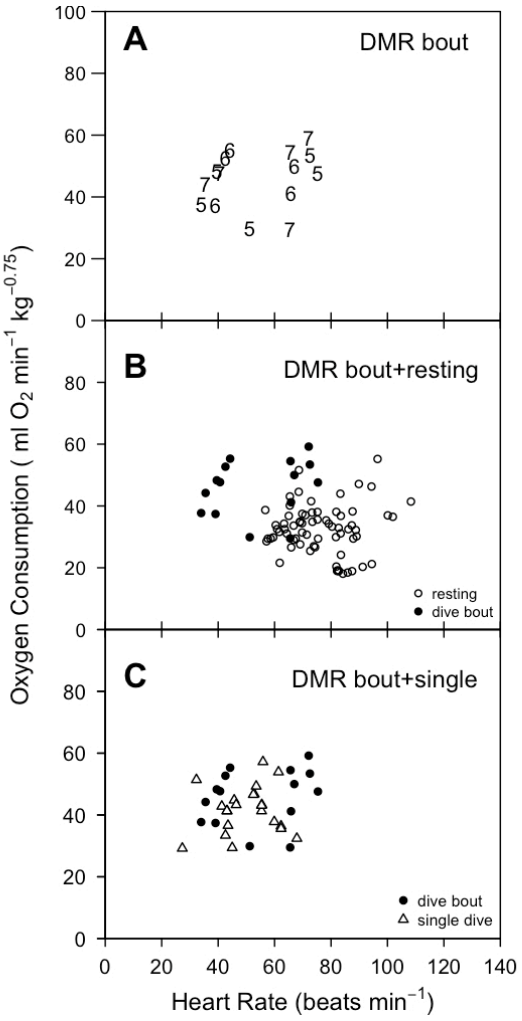


Figure A3. 4 The relationship between diving heart rate (*fh*) and diving metabolic rate ($\dot{V}_{O_2}$=DMR) for Steller sea lions over a dive bout (A) was not significantly linear. Combining DMR for dive bouts with data collected when animals were resting (B) or with data collected when animals were executing a single dive (C) also yielded non-linear results. See Table 3.1 for Animal ID to corresponding animal number.

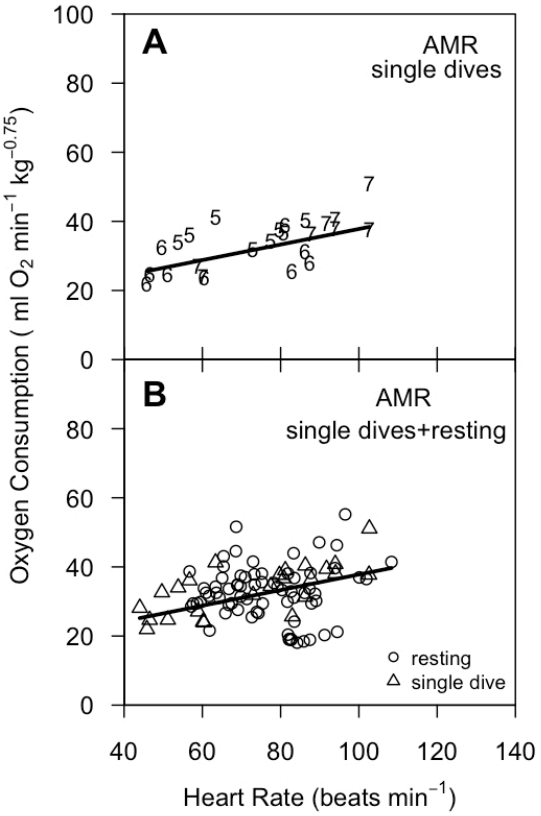


Figure A3. 5 The relationship between heart rate (*fh*) averaged over a dive cycle and average metabolic rate ($\dot{V}_{O_2}$ =AMR) for Steller sea lions over a single dive (A) was not significantly different than when animals were resting in water (B). See Table 3.1 for Animal ID to corresponding animal number.

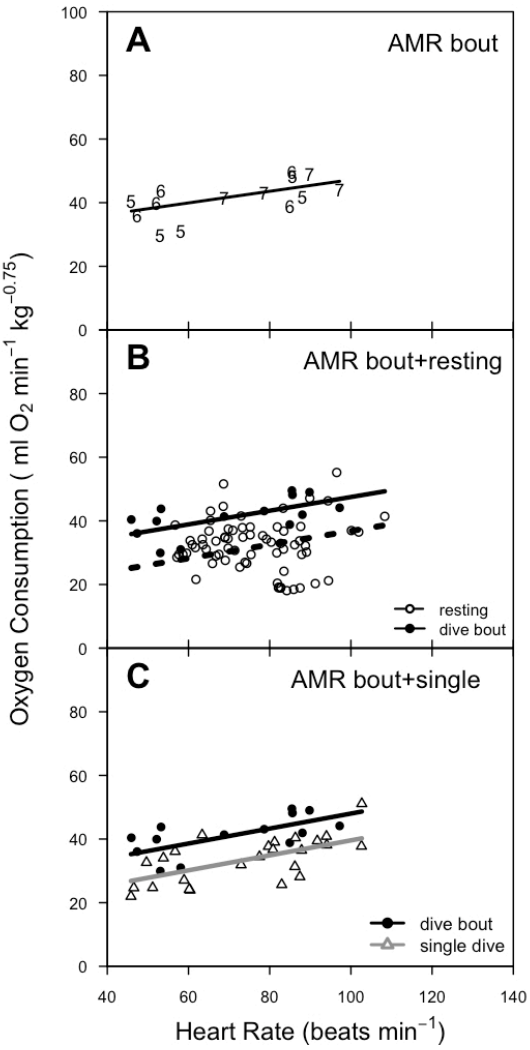


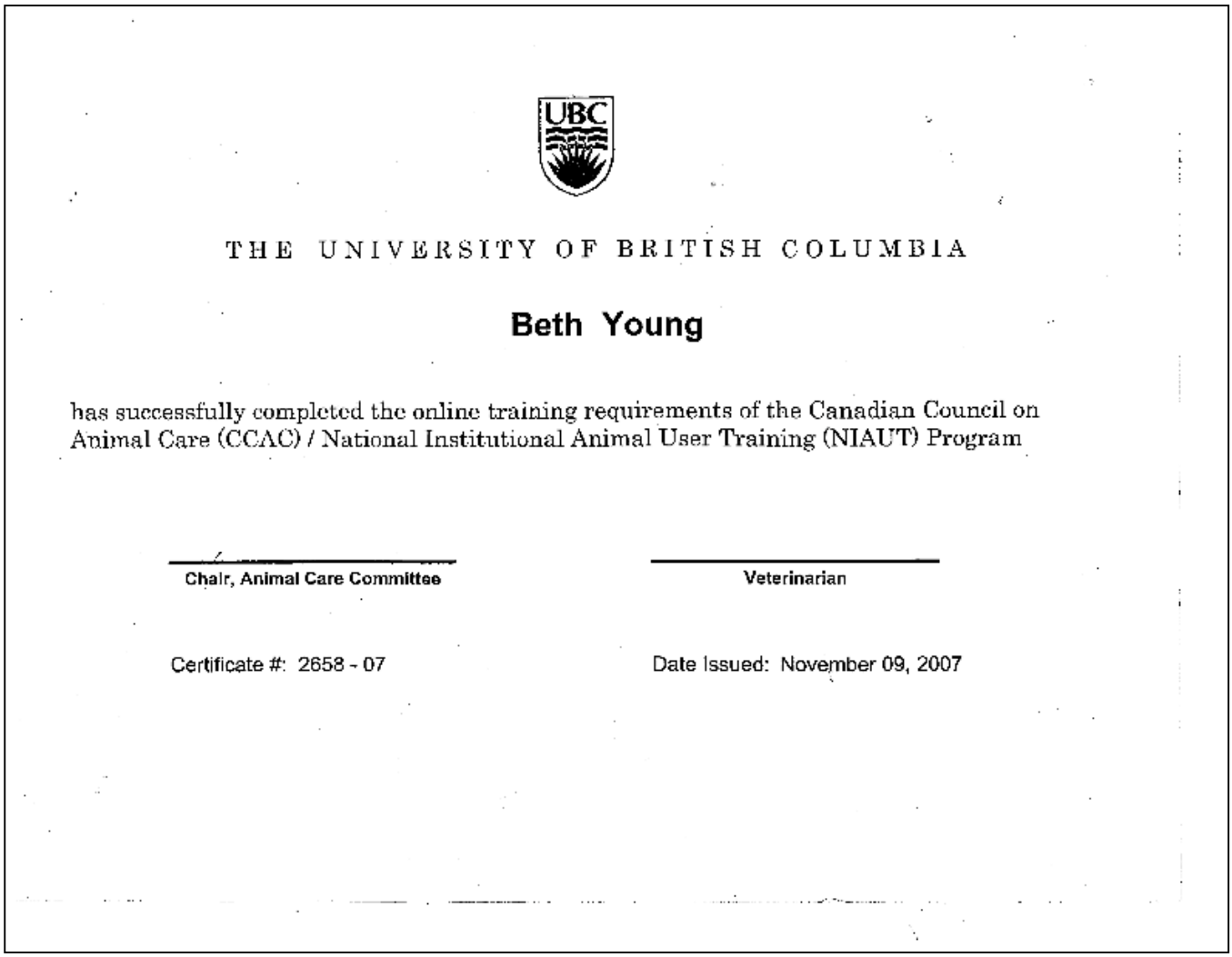
Figure A3. 6 The relationship between average heart rate (*fh*) and average metabolic rate ($\dot{V}_{O_2}$ =AMR) for Steller sea lions averaged over a dive bout cycle (dives+surface intervals, A) was different than when animals were resting in water (B) and also different than the relationship over a singe dive cycle (C). See Table 3.1 for Animal ID to corresponding animal number.

Appendix A4: Mean water and air temperature during data collection

Table A4. Mean (± SD), minimum, and maximum temperature (°C) during data collection. While sea lions were resting, ambient air temperature was measured in the dry metabolic chamber, and water temperature was measured in the swim mill (recorded every ~ 2 min; averaged over 1.5 or 4 hrs). Water temperature while diving was measured using a time-depth reorder (sampling frequency = 1Hz), and averaged over the entire trial for each day. Minimum temperature at maximum depth was 12.5 ºC and 12.1 for 10 m and 40 m trials respectively. Water composite in Chapter 2 is equivalent to resting metabolic rate (RMR) in Chapter 2.

	Temperature of water or ambient air (°C)			
Data	mean	± SD	min	max
Resting				
dry_{fasted} (metabolic chamber)	12.7	(1.3)	10.8	14.5
dry_{fed} (4 and 6 kg)	17.9	(1.8)	15.8	21.5
$water_{fasted}$ (swim mill)	11.7	(1.9)	9.0	13.6
$water_{fed}$ (4 and 6 kg)	11.9	(1.1)	10.7	13.6
$water_{ow}$ (open water)	15.7	(1.5)	13.6	18.1
$water_{comp}$/RMR resting	12.7	(2.5)	9.0	18.1
Diving (open water)				
10 m	17.0	(1.0)	15.1	18.1
40 m	14.7	(1.0)	13.6	16.9
both depths	15.8	(1.0)	14.4	17.5

Appendix A5: Animal care certificates and permits



THE UNIVERSITY OF BRITISH COLUMBIA

Beth Young

has successfully completed the online training requirements of the Canadian Council on Animal Care (CCAC) / National Institutional Animal User Training (NIAUT) Program

Chair, Animal Care Committee

Veterinarian

Certificate #: 2658 - 07

Date Issued: November 09, 2007

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A07-0208

Investigator or Course Director: Andrew W. Trites

Department: Fisheries

Animals:

Sea lions 12

Start Date: January 1, 1994 **Approval Date: September 8, 2009**

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #71 Interaction of diet and immune challenges

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #47 Consortium Core

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #65 The effects of mixed diets on Steller sea lion physiology, digestion and on diet determination

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #77 Hormone Analysis to Assess Nutritional Stress

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #78 Effects of Mixed Diets

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #55 Bioenergetics of Captive Steller sea lions

Funding Agency: North Pacific Marine Science Foundation

Funding Title: #30 Captive Studies: expansion of Bioenergetic and Nutritional Studies with Captive Steller sea lions

Unfunded title: #55 Bioenergetics of Steller sea lions, #77 Hormone Analysis to Assess Nutritional Stress, #71 Interaction of Diet and Immune Challenges

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start date or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

Office of Research Services and Administration
102, 6190 Agronomy Road, Vancouver, BC V6T 1Z3
Phone: 604-827-5111 Fax: 04-822-5093

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A07-0413

Investigator or Course Director: Andrew W. Trites

Department: Fisheries

Animals:

Sea lions 10

Start Date: September 1, 2001 **Approval Date: January 22, 2010**

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #82 Consortium Core

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #79 Steller Sea Lion foraging in open water

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #56 Open Water

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #83 Captive Project

Unfunded Title: N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start date or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by CCAC and some granting agencies.

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