THE CARDIORESPIRATORY, METABOLIC, AND THERMOREGULATORY PHYSIOLOGY OF JUVENILE NORTHERN ELEPHANT SEALS (MIROUNGA ANGUSTIROSTRIS)

by

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Abstract

Heart rates, respiratory frequencies, field metabolic rates (FMR) and body temperatures of northern elephant seals diving at sea and during apnoea on land were monitored to gain insight into the ability of elephant seals to make repetitive, long duration dives. Juvenile northern elephant seals were captured at Año Nuevo, CA, instrumented, and translocated to release sites around Monterey Bay. Data were recorded with custom data loggers and analogue Holter monitors during the seals' return to Año Nuevo and during apnoea and eupnoea on land after they hauled out on the beach. Diving patterns were very similar to those of naturally migrating juveniles. The heart rate response to apnoea at sea and on land was a prompt bradycardia, but only at sea was there an anticipatory tachycardia before breathing commenced. Heart rate at sea declined by 64% from the surface rate of $107\pm3$ beats min$^{-1}$ (mean ± SD) while heart rate on land declined by 31% from the eupnoeic rate of $65\pm8$ beats min$^{-1}$. Diving heart rate was inversely related to dive duration in a non-linear fashion, best described by a continuous, curvilinear model, while heart rate during apnoea on land was independent of apnoea duration. Occasionally, instantaneous heart rate fell as low as 3 beats min$^{-1}$ during diving. Although bradycardia occurred in response to apnoea both at sea and on land, only at sea was heart rate apparently regulated to minimise eupnoeic time and to ration oxygen stores to ensure adequate supplies for the heart and brain not only as the dive progressed normally, but also when the dive was abnormally extended. The mean respiratory frequency ($f_R$) during the first min after a dive was $22.0 \pm 2.0$ breaths min$^{-1}$, which was 2.4 times greater than $f_R$ after an apnoea on land, despite that the mean dive duration was not different from the mean duration of apnoea on land. The higher $f_R$ at sea permits elephant seals to spend only short periods at the surface for gas exchange, resulting in quick recovery from long dives and a high percentage of time spent submerged. The at-sea FMR was no different from the onshore FMR, even though seals at sea were swimming almost continually in cold, highly conductive water and seals onshore were usually just resting. This may be partially due to a regulated heat loss and temperature reduction at the onset of long duration diving that possibly reduces diving metabolic rate through a reduction in thermoregulatory costs and a $Q_{10}$ related decrease in metabolism.
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Chapter 1

General Introduction: An examination of the dive behaviour of elephant seals in light of the current understanding of the diving physiology of phocid seals

The ability of aquatic mammals to hold their breath for long periods of time while diving has intrigued naturalists since the time of Aristotle. Despite this long-standing interest, Irving (1934) pointed out that it was difficult to obtain statements from naturalists on precise durations of submergence by diving animals. Some animals, especially the large whales, were thought to dive for extremely long durations relative to what accomplished human divers could achieve; however, some of the estimates, even those published in scholarly journals, e.g. that rorqual whales could remain submerged for 8 - 12 hours, were clearly exaggerated "whaling stories" (Irving, 1934). Dive durations of animals behaving without harassment are clearly more interesting than the throes of harpooned whales, but obtaining objective data on behaviour that usually takes place far from shore and beneath the murky sea has, until recently, been nearly impossible. Over the last 35 years, the development and application of mechanical and electrical devices for monitoring the time and depth of submergence (time-depth recorders; TDRs; Kooyman, 1965; Kooyman et al. 1983; reviewed in Costa, 1993) has resulted in a tremendous amount of data and many new insights regarding the dive depths and durations of a large number of diving animals (Kooyman, 1989; Butler and Jones, 1997). As a matter of fact, if I had any idea of how much data such devices could generate and that would subsequently have to be analysed, I never would have embarked on the research presented in this thesis. Nonetheless, the
insights into diving behaviour gained through the use of these devices have been remarkable.

As an example of how the application of TDRs has advanced our understanding of diving behaviour, consider the case of the beluga whale, *Delphinapterus leucas*. The beluga whale was once thought to be a species which spent most of its time in shallow estuaries and never made long or deep dives (Tomilin, 1957; Kleinenberg et al. 1964). Recent applications of satellite-linked TDRs to belugas have demonstrated that belugas actually only spend 1 - 2 months a year in shallow estuaries, and even then they frequently move between the estuaries and deep water (Martin et al. 1993; Smith and Martin, 1994). The satellite-derived tracks of belugas over the months from June until November (Smith and Martin, 1994; Richard et al. 1998) showed that the earlier perceptions of belugas were simply an artefact of biased observations, which were restricted to near-shore encounters in the summer. When in deep water, whether during the summer or on their fall/winter migrations, belugas frequently make dives deeper than 200 m, and their maximum dive depths can reach over 1000 m (A.R. Martin, personal communication), while their maximum dive duration is at least 20.5 min (R. D. Andrews, unpublished data).

Some of the most extreme diving behaviours discovered with the use of TDRs come from studies of seals in the genus *Mirounga*, the northern elephant seal, *M. angustirostris*, and the southern elephant seal, *M. leonina*. Both species make biannual migrations between distant foraging grounds and the beaches where they breed and moult, spending only 2 - 3 months each year on land (Le Boeuf, 1994; Slip et al. 1994; Stewart and DeLong, 1995). While at sea they dive continually, making long dives punctuated by short surface intervals, so that 80 - 95 % of time at sea is spent submerged (Le Boeuf et al. 1988; Hindell et al.)
The maximum dive depth recorded so far is a 1581 m dive made by an adult northern elephant seal male, although the maximum of 1567 m for an adult northern elephant seal female is not far off that mark (Stewart and DeLong, 1995). An adult female southern elephant seal holds the record for the longest duration dive of any mammal, at 120 min (Hindell et al. 1992); while an adult female northern elephant seal with a dive of 119 min is a close second (Stewart and DeLong, 1995).

As amazing as these extremes are, perhaps even more interesting are the routine dive behaviours of elephant seals. The dive behaviour of adult females of both species is especially intriguing during the post-moult migrations, when seals dive to depths of between 300 and 600 m for mean dive durations of between 20 and 30 min, yet only spend 2 - 3 min at the surface between each dive (Hindell et al. 1992; Stewart and DeLong, 1995).

The ability of elephant seals to function at such depths, especially on the occasional dives beyond 1000 m, is perplexing given the rapid changes in pressure experienced on the way up and down and the extreme absolute pressures found at these depths. My interest, however, is in the ability of elephant seals to repetitively make long duration dives followed by short surface intervals. The apnoeic tolerance of elephant seals, and their ability to recover quickly from apnoea, seems exceptional, especially when compared with the performance of most other marine mammals (Boyd and Croxall, 1996; Butler and Jones, 1997). Whether this exceptional performance is due simply to the large size of elephant seals or is instead the consequence of unique adaptations to diving is an interesting question that will be addressed below.

An estimate of the apnoeic duration an animal should be able to tolerate can be made by calculating the quotient of the total amount of oxygen stored in the body and the basal rate
of oxygen consumption (Irving, 1939). Some tissues in the body can tolerate complete anoxia for considerable periods, but the heart and brain quickly deteriorate without oxygen. Apnoeas in excess of this time limit, therefore, should not be possible without some reflexive distribution of the oxygen store (Irving 1934, 1939). It is now commonly accepted that such reflex adjustments occur in most diving animals and that the major responses to diving apnoea are profound bradycardia and marked peripheral vasoconstriction (reviewed in Butler and Jones, 1982; Kooyman, 1989). This "dive response" restricts the blood flow primarily to the heart and brain, but ischaemic muscle does have a separate oxygen store bound to myoglobin. Once the myoglobin-bound oxygen is depleted, muscle metabolism becomes completely anaerobic and lactic acid production increases dramatically, with high blood lactate levels occurring at the end of the dive when normal circulation is restored (Scholander, 1940). The research that established these elements of the dive response was initially done on forcibly submerged animals, but the studies of Kooyman and others (Kooyman and Campbell, 1972; Kooyman et al. 1980; Guppy et al. 1986; Hill et al. 1987) have demonstrated that voluntarily diving Weddell seals (Leptonychotes weddellii) have a similar dive response. Bradycardia is apparent, although it is more moderate in freely diving Weddell seals than in forcibly submerged seals, and the phenomenon of lactate production in excess of the resting levels on dives longer than a certain threshold is also observed.

There is a correlation between the dive duration and the peak lactate concentration that is reached at the end of the dive in Weddell seals, and the threshold dive duration beyond which lactate (LA) increases is directly related to the size of the seal (Kooyman et al. 1983). The size dependence of this threshold is thought to be due to the scaling of oxygen stores
(Mass$^{1.0}$) and metabolic rate (Mass$^{0.75}$), and the threshold is correlated with the quotient of oxygen stores and metabolic rate. These results led Kooyman et al. (1983) to coin the term "aerobic dive limit" (ADL), which was later defined as the "maximum breathhold that is possible without any increase in blood LA concentration during or after the dive" (Kooymarn, 1985). There was also a correlation between dive duration and recovery time, which indicated that the increased LA production in dives beyond the ADL required additional time for clearance, and that seals were either reluctant to or could not resume diving until LA returned to resting levels (Kooymann et al. 1980). It took up to 2 h for Weddell seals to recover from dives as long as 60 min. Although there is little empirical support for the concept in other species, it seems widely accepted that all diving animals function in a similar manner. An increase in the ratio of the surface interval (SI) duration to the dive duration is taken as evidence of significant anaerobic metabolism in the dive, while the lack of an increase in SI duration with increasing dive duration is thought to indicate that the dives are primarily aerobic (Le Boeuf et al. 1988; Ydenberg and Clark, 1989; Hindell et al. 1992; Boyd and Croxall, 1996).

The latter result has been observed in female elephant seals and has led to the suggestion that the ADL for adult female elephant seals must be greater than would be expected based on the studies of similarly sized Weddell seals (Le Boeuf et al. 1988; Hindell et al. 1992). Adult Weddell seals that weighed between 350 and 450 kg had an ADL of approximately 26 min, and as expected only a small fraction (3 %) of free-ranging dives were in excess of the ADL. Female elephant seals, however, routinely made a significant number of long dives, between 40 and 60 min in duration, that were almost always followed by surface intervals less than 3 min, and such dive cycles were often repeated for long periods of time.
Although it has not been possible to measure blood lactate concentration in freely diving elephant seals, Kooyman et al. (1983) suggested that the ADL could be estimated by dividing the total body oxygen stores by the diving metabolic rate. This calculated ADL (cADL) could then provide an objective standard against which the dive durations of elephant seals could be measured. Hindell et al. (1992) determined that cADL values for southern elephant seals ranged from 27 - 30 min and found that on average 44% of the dives made by females on their post-moult migrations exceeded the cADL. Dives in excess of the cADL were not normally followed by extended recovery periods at the surface, and so the authors suggested that the actual ADL for increased LA production was closer to 60 min. Le Boeuf et al. (1988) reached a similar conclusion for northern elephant seals, and both groups suggested that elephant seals must have special mechanisms in order to be able to perform their exceptional pattern of repetitive, long duration diving. The questions of whether such mechanisms exist, and what they may be, form a central theme in this thesis.

There are at least two possible ways of explaining how elephant seals routinely exceed their calculated ADL without needing to spend extended periods at the surface between dives: either the ADL has been miscalculated or anaerobic metabolism does not lead to intolerable lactate levels in elephant seals. The former possibility seems most likely, but a brief discussion of the latter is warranted. Although it cannot be ruled out, the possibility that elephant seals possess unique biochemical pathways seems improbable. A source of anaerobic energy that is usually overlooked in diving studies, however, is phosphocreatine (PCr; Jones and Stephenson, 1993; Kooyman and Ponganis, 1998). The level of PCr measured by $^{31}$P-Nuclear Magnetic Resonance in rat skeletal muscle is approximately 25 umol g wet weight$^{-1}$ (Idstrom et al. 1985). If elephant seals have similar levels, then PCr
hydrolysis can support a resting muscle metabolic rate of twice the resting rate for 31.2 min. In ischaemic canine skeletal muscle, Harris et al. (1986) found that PCr could buffer the expected drop in ATP levels for up to 3 hours. Beyond 3 hours PCr was depleted and there was a large acceleration in the decline of ATP concentration. In addition, the PCr store could be replenished within 6 minutes even at the limited perfusion rates used in these studies (Idstrom et al., 1985; Harris et al., 1986). With a very high muscle blood flow, as might be expected during recovery from diving, an elephant seal could likely restore its PCr levels during a normal 3 min surface interval.

Another possibility is that high levels of lactate do not necessarily inhibit continued diving. Qvist et al. (1986) showed that in Weddell seals, blood lactate concentration actually increases prior to reaching the surface, while the seals are still swimming. Castellini et al. (1988) also showed that after some long dives, instead of resting at the surface, Weddell seals may indeed resume diving with high levels of blood lactate. After most long dives, however, Weddell seals do rest until lactate returns to resting levels, but then Weddell seals routinely only dive for about 12 hours a day (Kooyman et al. 1980), so perhaps there is little reason for them to rush recovery. In contrast, elephant seals seem to be motivated to dive continuously when at sea, so perhaps even after building up a high blood lactate concentration they would be much more likely to resume diving before lactate returned to resting levels. (c.f. Fig. 8.8 in Kooyman, 1989).

Although all the plausible explanations mentioned above deserve further consideration, the reason given most frequently for the lack of correspondence between the cADL and the dive behaviour of elephant seals is that the ADL has been miscalculated. This could be due to either an underestimation of oxygen stores or an overestimation of diving metabolic rate.
Early calculations of the ADL assumed an oxygen store similar to that in Weddell seals, and recent measurements in adult northern elephant seals suggest that while elephant seals may have slightly higher O$_2$ stores than the early Weddell seal estimates, the difference is small (Thorson, 1993). The most frequently offered explanation of the elephant seal's amazing dive behaviour is that their diving metabolic rate is very low, as low as 40% of the predicted basal metabolic rate (Le Boeuf et al. 1988; Hindell et al. 1992; Boyd and Croxall, 1996; Boyd, 1997). Although there have been frequent claims that other diving animals exhibit such "metabolic depression" during diving, both the forced submergence and the voluntary kind, there is little empirical support for the concept (Butler and Jones, 1997; Kooymann and Ponganis, 1998). Nonetheless, reduction in metabolism as a response to diving seems to be an accepted phenomenon, and Le Boeuf et al. (1988) offered several hypotheses as to what might cause a low diving metabolic rate in elephant seals, two of which I will examine in this thesis. The first is that diving is associated with a drop in heart rate, bradycardia, that reflects a redistribution of blood flow away from most organs and tissues. The second is that body temperature decreases during diving. Both of these phenomena, bradycardia and reduced body temperature, have been reported in previous studies of both forcibly submerged and voluntarily diving animals.

Scholander (1940) offered two lines of evidence to support the idea that profound peripheral vasoconstriction accompanied the bradycardia of diving. One was the sudden appearance of lactate in the blood following a dive, suggesting that during the dive the muscles had been isolated from the general circulation. The other was that he had great difficulty in obtaining blood samples from the hind flipper of the seal while it was submerged. Grinnell et al. (1942) confirmed the relation of bradycardia and vasoconstriction more directly by
measuring heart rate and blood flow to the back musculature during forced submergence. Since then numerous investigators have verified that bradycardia is accompanied by extreme peripheral vasoconstriction during forced submergence (Bron et al., 1966; Elsner et al., 1966; Murdaugh et al., 1966; Kerem and Elsner, 1973; Zapol et al. 1979; Blix et al., 1983). For example, using microspheres, Zapol et al. (1979) demonstrated that forced submergence of Weddell seals was associated with a fall in both cardiac output and heart rate and that blood flow to the splanchnic and peripheral vascular beds was reduced by more than 90%. Cerebral blood flow, however, was unchanged. These results have led to the general agreement that the dive response directs blood to the anoxia intolerant brain and supports oxygen conservation. Irving et al. (1941) demonstrated that survival time is decreased when bradycardia develops slowly, and they concluded that the dive response of bradycardia and vasoconstriction was the key to the seal's ability to tolerate asphyxia. Murdaugh et al. (1961) offered similar evidence in a study which used atropine to abolish diving bradycardia and reduce the cholinergic component of the peripheral vasoconstriction. One atropinised seal "drowned" after only three minutes of submersion without bradycardia, whereas control seals could remain underwater for 15-20 min. Scholander et al. (1942) interpreted their results to mean that oxygen consumption is reduced during a dive because of bradycardia and peripheral vasoconstriction.

While it has been suggested frequently that reduced blood flow to peripheral tissues is responsible for a depressed tissue metabolic rate, no direct tests of this hypothesis have been performed on seals. There is, however, evidence from terrestrial animals that supports this hypothesis. The evidence suggests that over a considerable range, skeletal muscle oxygen consumption is directly related to blood flow (Whalen et al., 1973; Barclay and Stainsby,
1975; Horstman, et al., 1976; Idstrom et al., 1985; Saltin, 1985). Most of this research has focused on blood flow limitations during exercise, i.e. at metabolic and perfusion rates from resting to maximum oxygen consumption rate. A few studies have examined the other side of the relation, at resting rates and below, which is more applicable to the diving seal situation. A study combining both steady state sub-maximal exercise and below resting oxygen delivery to intact rat skeletal muscle demonstrated a significant correlation between oxygen delivery and oxygen consumption (Idstrom et al., 1985). However, this study also found that there was a compensatory increase in the anaerobic energy production (Pasteur effect) as indicated by increased lactate release. Such a mechanism would not be ideal in the diving seal because lactate would likely build up to intolerable levels. It has been proposed, however, that a "reversed Pasteur effect", that is a decrease in anaerobic energy production, might operate in some systems (Hochachka and Guppy, 1987). It is plausible, therefore, that elephant seals could reduce their tissue metabolic rates through peripheral vasoconstriction.

A reduction in metabolic rate due to vasoconstriction may be reflected in a drop in body temperature (Scholander et al. 1942). However, a drop in temperature before diving could be another way to achieve a lower diving metabolic rate. Hill et al. (1987) recorded a decrease in body temperature of nearly 3 °C before initiation of dives greater than 30 min in one Weddell seal. On the beach, body temperatures of adult elephant seals vary from 37 to 33 °C, decreasing during inactivity (McGinnis and Southworth, 1971). With a $Q_{10}$ of 2 - 3, such a reduction in body temperature could lead to reduction in metabolic rate of 20 - 40 %. If elephant seals employ the strategy of reduced body temperature to save energy during their onshore fasts, then it is conceivable that they would do so during diving as well.
The goal of my thesis research, therefore, is to examine whether there is any support for the hypotheses presented in Le Boeuf et al. (1988), and to determine whether two of the predictions of the hypotheses, that bradycardia and a reduced body temperature are components of the elephant seal's dive response, can be verified. Another potentially important factor in the ability of elephant seals to repetitively make long duration dives is the respiratory physiology of recovery. The short surface intervals of elephant seals must be related to high rates of gas exchange at the surface, but little is known about the physiology of recovery from diving in this species. Therefore, I propose to measure heart rate, breathing rate, body temperature and dive depth in voluntarily diving northern elephant seals.

Although it was the exceptional dive behaviour of adult female northern elephant seals that led to the hypotheses discussed above, juvenile elephant seals make much better research subjects, primarily because their smaller size makes it possible for 4 people to lift one up and cart it off to the lab (Oliver et al. 1998). Furthermore, the diving performance of juvenile elephant seals may be just as extreme as the performance of adult females. By the time northern elephant seals reach 22 months of age and a mass of about 160 kg, their mean dive duration ranges from 16 to 20 min (Le Boeuf et al. 1996). Boyd and Croxall (1996) examined the relationship between mean dive duration and body mass in 16 species of pinnipeds and 14 species of seabirds, and found that relative dive durations of seabirds were greater than in pinnipeds. The mean dive durations of adult female elephant seals of both species were considerably above the predicted line for pinnipeds, and actually lay closer to the regression line for seabirds. The same is true for juvenile northern elephant seals. The pinniped regression predicts a mean dive duration of 4.0 min for a 160 kg animal, while the
seabird regression predicts a mean dive duration of 12.4 min. The mean dive duration for 22 month old northern elephant seals was 18.3 min, which suggests that their relative dive performance is even more exceptional than that of adult female elephant seals. Juveniles can also exhibit the extreme dive behaviours seen in adult females. For example, the maximum dive duration of a 17 month old, 113 kg, seal was 86 min, and this dive was followed by a short surface interval, and was just one of many dives in a series of dives that all exceeded 40 min (Le Boeuf et al. 1996). Therefore, the research presented in this thesis was carried out on juvenile northern elephant seals.

In order to make the measurements that I have proposed, instrumentation for remote monitoring was needed. Numerous remote monitoring systems have been developed that enable the recording of many different physiological and behavioral variables. The majority of these systems involve the use of radio telemetry, a method which usually results in the smallest, lightest instruments possible for attachment to the research subject. However, telemetry is not always appropriate, especially when transmission range will be limited by the movement of the study subject through dense forest, urban settings, the inside of buildings, or an ocean. In such situations involving humans, captive animals, or free-ranging animals that can be easily recaptured, a device that is carried by the subject and logs data for later retrieval is an alternative to radio telemetry.

The study of diving physiology is one area of research where biotelemetry is sometimes not applicable. One of the earliest data logging instruments (Kooyman 1965) used on a diving animal was constructed from a bourdon tube, kitchen egg timer and smoked glass disc in order to record the diving depth of Weddell seals. Other types of archival instruments that have been used on diving animals include those that trace the depth of dives over time onto
photographic film using light-emitting diodes (Kooymann et al. 1983) and Holter monitors modified to record the electrocardiogram (ECG) of California sea lions (Zalophus californianus) on magnetic tape (Ponganis et al. 1997). Most such devices have limited usefulness due to their large size and inability to simultaneously record many different physiological and behavioral variables. The best technique for producing a small, multi-variable recorder is the use of microcomputers to control the logging of data into solid state memory, a method pioneered for use in diving animals by Hill (1986) and recently adopted by other research groups (Ponganis et al. 1990; Woakes et al. 1995).

Although the value of such data loggers is tremendous, their general availability is limited. When research projects required data loggers that were not available from colleagues or commercial vendors, previous investigators have had to design and produce an appropriate instrument. This method requires one to identify a suitable microprocessor and interface it with the necessary electronics, e.g. a clock crystal, voltage regulator, analog to digital converter, and memory chip, a process that requires substantial training in electronics, or considerable assistance from an electrical engineer. I initially adopted this approach for developing the data loggers used in Chapter 2. These data loggers successfully collected useful data, but the approach was difficult and expensive. The electrical engineer who designed the instruments could not accompany me into the field, and so when the instruments malfunctioned, which occurred all too often, I had to wait long periods for repairs. Also, as the study progressed, I often wished that I could modify the data collection protocol, but this was not possible with the initial data loggers. A new system was needed that could be easily adapted to record different types of physiological and behavioral variables and which could be re-programmed quickly by an end-user with minimal
experience in computer programming. The instrumentation presented in Chapter 4 meets these needs and satisfies the basic requirements I defined for a suitable data logging system. The data loggers used in Chapter 4 are based on an inexpensive, commercially available, single-board computer designed specifically for portable data acquisition, which enabled me to concentrate on the less difficult tasks of interfacing to the appropriate sensor electronics and packaging and applying the finished data logger to the research problem.
Heart rates of northern elephant seals diving at sea and resting on the beach

Introduction

The breath-holding ability of elephant seals (genus *Mirounga*) appears to be unequalled in the class Mammalia. While at sea during their biannual foraging migrations, northern elephant seals (*Mirounga angustirostris*) perform long duration dives interrupted by only brief surface intervals (Le Boeuf *et al.* 1988; Stewart and DeLong, 1993). This pattern is repeated almost continuously for two to eight months, and 80 - 95 % of time at sea is spent submerged (Le Boeuf, 1994; Stewart and DeLong, 1995). Dive durations of adult females average at least 20 min, but surface intervals last only about 2 min, rarely exceeding 5 min even after dives as long as 119 min. Elephant seals adopt this pattern on their first trip to sea when mean dive durations are approximately 10 min, and by the time they are 2 years old the dive durations approach those of adults (Le Boeuf, 1994). Dives frequently exceed the calculated aerobic dive limit (Kooyman *et al.* 1983), which is the time limit imposed by oxygen stores and the estimated rate of oxygen consumption, yet there is no sign of a large increase in anaerobic metabolism and the extended surface time needed to clear anaerobic end-products. During long dives elephant seals may reduce their heart rate and peripheral blood flow considerably, which may result in a reduction in overall metabolic rate. A reduction in metabolic demand for oxygen may be the reason elephant seals can repeatedly make long duration dives with only short surface intervals (Le Boeuf *et al.* 1988).
Therefore, the purpose of this study was to describe the heart rate response to diving in northern elephant seals.

Elephant seals also engage in long duration breath-holds when hauled out on the beach, and their pattern of apnoea and eupnoea when sleeping is similar to the pattern imposed by diving at sea (Bartholomew, 1954; Hubbard, 1968; Blackwell and Le Boeuf, 1993). In juvenile and adult seals, the mean apnoea duration on land varies from 7-10 min, but can extend to at least 25 min. Castellini et al. (1994a) suggested that the cardiovascular response to diving and to apnoea on land may be similar and governed by the same control mechanisms. Therefore, this study was also designed to compare the heart rate response to diving with the heart rate response to apnoea on land in the same individuals.

A method that facilitates short-term studies of elephant seal diving has recently been demonstrated (Le Boeuf, 1994; Oliver et al. 1998). Juvenile (< 3 yrs. old) northern elephant seals haul out on the beach at the Año Nuevo rookery in central California twice a year, in the spring and fall, for about a month each time. Juvenile seals that are captured just after hauling out and then translocated to a release site up to 100 km away return to Año Nuevo, usually within 1 week. If the seals cross deep water on their return path, then their diving pattern is very similar to the pattern adopted by seals migrating naturally. Therefore, in order to monitor the heart rate response to voluntary diving in northern elephant seals, I used this translocation method on seven juvenile seals instrumented with heart rate and time depth recorders. Heart rate on the beach was also monitored in four of the same seals. I show that the heart rate response to apnoea at sea and on land was a prompt bradycardia, and although important differences between the two patterns existed, there is no doubt that
heart rate reduction is an important part of an oxygen and energy conservation strategy both at sea and on land.

**Materials and methods**

*Seals and translocation paradigm*

I recorded heart rate and dive depth from seven juvenile northern elephant seals at different times between May 1991 and June 1993 (Table 2.1). Seals were captured less than two weeks after they had hauled out at Año Nuevo State Reserve, California. I immobilised the seals with an intramuscular injection of a mixture of Tiletamine HCl and Zolazepam HCl at an approximate dose of 1 mg kg$^{-1}$ (Telazol, Aveco Co., Ft. Dodge, IA, USA) and transported them by truck to the Long Marine Laboratory, Santa Cruz, CA. At the laboratory, seals were weighed and immobilised again to attach instruments and heart rate electrodes. Immobilisation was maintained throughout the attachment procedure by intravenous injections of 0.5 mg kg$^{-1}$ ketamine (Ketaset, Aveco Co.) and 0.025 mg kg$^{-1}$ Diazepam (Elkins-Sinn Inc., Cherry Hill, NJ, USA) at 15-30 min intervals. The seals were marked by placing numbered tags in the interdigital webbing of their rear flippers.

Recording instruments (see *Instrumentation*) and a VHF radio were attached with hose-clamps that were glued to the fur in the mid-dorsal region (Le Boeuf *et al.* 1988). Three of the seals were instrumented with digital heart rate - time depth recorders (HR-TDR), and four seals received analogue electrocardiogram (ECG) recorders (Holter monitors) paired with microprocessor-controlled time-depth-recorders (TDR) (Table 2.1). The ECG signals were obtained by a modification of the method described by Fedak *et al.* (1988). Two skin-surface electrodes were attached to each seal, one on the ventral midline and one on the
dorsal midline at the level of the heart. Seals instrumented with Holter monitors had a third electrode placed on the dorsal midline 15 cm posterior to the instrument attachment site.

On the day after capture, and at least 12 hours after instrumentation, the seals were transported either by truck to the opposite side of Monterey Bay or by ship to offshore release sites. Seals were released on the beach at Hopkins Marine Station, Pacific Grove, CA, or at sea off a research vessel (Table 2.1, Fig. 2.1). Año Nuevo State Reserve was monitored daily with a hand-held VHF telemetry receiver (Telonics Inc., Mesa, AZ, USA) to locate the seals on their return. Returning seals instrumented with HR-TDRs were immobilised and instruments were removed. In order to record heart rate on land from the 4 seals instrumented with Holter monitors, the instruments were not removed until 12-18 hours after they had returned to the beach. GG571 and GH929 were observed on the beach for 3.5 and 4 hours, respectively. During this observation time the seals were apparently sleeping or resting quietly. An observer about 5 m downwind of the seal noted the time of every breath and the duration of each apnoeic and eupnoeic period. Apnoea was defined as an expiratory pause of at least 1 min that was ended by an inspiration (Blackwell and Le Boeuf, 1993).

**Instrumentation**

HR-TDRs were used to record heart rate and dive depth from three seals. HR-TDRs consisted of a microprocessor with 64-kbytes of memory, an analogue-to-digital converter, and the electronic circuitry for a pressure transducer and an ECG R-wave detector. The pressure transducer circuit had a resolution of 4.0 m of seawater over a range of 0-1000 m and was calibrated with a pressure gauge comparator and a National Institute of Standards and Technology (NIST)-traceable precision gauge. The ECG R-wave detector circuit was
similar to the one described by Shimizu (1978). A refractory period of 400 ms was selected to prevent triggering of the peak detector by high-amplitude T-waves, which limited the maximum heart rate recorded to 150 beats minute\(^{-1}\) (bpm). This was a reasonable limit because the maximum instantaneous heart rate recorded with either HR-TDRs or Holter monitors (which didn’t have a maximum heart rate limit) was 133 bpm. The amplified ECG and the detector circuit trigger output were displayed on an oscilloscope during the instrumentation procedure to verify that the R-wave detector was functioning properly.

HR-TDRs were enclosed in cylindrical aluminum housings (22.0 cm long x 3.5 cm in diameter) sealed with O-rings. A water/pressure-proof bulkhead connector assembly (Underwater Systems Inc., Stanton, CA, USA) was used for electrical connection to the ECG leads. A VHF radio transmitter (6.0 cm long x 2.5 cm in diameter, Advanced Telemetry Systems, Inc., Isanti, MN, USA) was attached with hose-clamps to each HR-TDR housing. The HR-TDR was programmed to count the number of heart beats and every 10 sec to store the count, clear the counter, and store the current depth. Data were downloaded to a notebook computer and analysed with a custom software program (written in the awk language) after recovery of each HR-TDR.

Heart rate was recorded from four other seals using analogue Holter monitors (model 90205, Space Labs Inc., Redmond, WA, USA). The Holter monitors recorded the ECG signal onto magnetic tape with a capacity of 48 hours. Holter monitors were enclosed in aluminum housings (3.5 cm high x 11.0 cm long x 8.5 cm wide) and were connected to the ECG electrodes by water/pressure-proof bulkhead connector assemblies. A VHF radio transmitter and a microprocessor-controlled TDR (model Mk 3, Wildlife Computers, Redmond, WA, USA) were attached to the Holter monitor housing with hose-clamps. The
TDR was programmed to record depth every 5 sec with a resolution of 2.0 m over the range 0-450 m and a resolution of 6.0 m over the range 450-1500m. TDR data were downloaded to a notebook computer and were analysed with a custom awk language program. The Holter monitor tapes were scanned and digitised using an FT2000A Medical Workstation computer system (Space Labs, Inc.). The FT2000A was also used to identify each R-wave and record the duration of R-R intervals. Proper R-wave identification by the FT2000A was verified visually for each heart beat.

Analysis and statistics

The data collected at sea were analysed for the period from 1 hour after release to 1 hour before return to Año Nuevo. A dive was defined as submersion to a depth greater than 6.0 m. The depth of the dive was the maximum depth reached during the dive. The mean heart rate for each individual dive, surface interval (SI), beach apnoea and beach eupnoea was calculated by adding the total number of beats and dividing by the duration of each period. A dive cycle was defined as a dive and the subsequent SI and was only analysed if the ECG signal appeared to be reasonably noise-free throughout the cycle. Similarly, a beach apnoea cycle was defined as an apnoea and subsequent eupnoea and was only analysed if the ECG was noise-free and the cycle was followed by another apnoea. Therefore, heart rate on the beach was only analysed during periods of quiet wakefulness or sleep. Comparison of the ECG recordings and the visual observation of respiration on the beach of seals GG571 and GH929 showed that entrance into both apnoea and eupnoea was accompanied by clear changes in heart rate. These changes confirmed the results of Castellini et al. (1994a,b) for elephant seals over 3 mo. old during land apnoea and eupnoea; therefore, these heart rate
patterns were used to mark the timing of the apnoea cycles when visual observation was not possible.

Student’s paired t-tests were used to compare heart rates during diving and during apnoea on land as well as heart rates in apnoea and eupnoea. Significance was accepted at the level of $P<0.05$ except when it was necessary to use the sequential Bonferroni method to minimise type-I errors (Rice, 1989). Relationships were examined using least-squares linear regression. Average relationships that take into account variability between subjects were determined using repeated measures multiple linear regression with each seal being assigned a unique index variable. The relationship between dive duration and mean dive heart rate was also examined using the curve-fitting procedure of the Marquardt-Levenberg algorithm to find the best continuous curvilinear model. The hypothesis of a threshold shift in this relationship was tested using an iterative least-squares linear regression technique to find the best continuous, two-segment regression model (Nickerson et al., 1989). The residual sum of squares (RSS) and means square error (MSE) values for each of the different models were compared using one-way repeated measures ANOVA with Student-Newman-Keuls pairwise multiple comparisons.

**Results**

*Behaviour*

Seals released on the beach initially displayed short, shallow dives that increased in depth and duration with time as they followed the bottom contour to the continental shelf break (the 140 m bathocline, Fig. 2.1) and the Monterey submarine canyon. Once deep water was reached, long duration, deep dives predominated. The seals released at sea immediately began making long duration, deep dives. As seals approached the shore, their dives became
progressively shorter and shallower until they hauled out on the beach at Año Nuevo. An example of such a progression is seen in Fig. 2.2A (top panel), an excerpt from seal GH929's dive record. Seals spent from 0.6 to 3.8 days at sea before returning to Año Nuevo (Table 2.1). There were no significant differences between groups for any of the dive behaviour or heart rate variables, whether the seals were grouped by sex, release type, or instrument type. Therefore, I pooled the at-sea data for all seven seals.

When all dives were included, mean dive depth of each seal varied from 60 to 322 m, mean dive duration ranged from 6.1 to 18.1 min, and the maximum duration observed was 31.3 min. Dives were almost always followed by short surface intervals, with a grand mean of 1.4 ± 0.4 min (mean ± SD), so that the seals spent between 86 and 92 % of their time at sea submerged (Table 2.2). SI duration was positively related to the duration of the preceding dive for all seven seals (Fig. 2.3). Dive duration was not correlated with body mass or age, but appeared to be more closely related to where the seals were released or the proportion of time they spent in deep (>140 m) water. Therefore, the dives of each seal were divided into “on the shelf” and “off the shelf” groups (Table 2.2). I assumed that the initial or terminal group of dives less than 140 m were on the continental shelf, and dives greater than 140 m were off the continental shelf. Dives on the shelf were shorter and were followed by shorter surface intervals than those in deeper water (Table 2.2).

Heart rate at sea

Heart rate at the surface, between dives, was high and stable, with means for individual seals for all dives ranging from 103 to 112 bpm (Table 2.2), with slight oscillations presumably due to respiratory sinus arrhythmia. Upon submergence, heart rate immediately decreased by 50-80% from the SI value. In two seals (GJ325 and GJ711) the first interbeat
interval was often the longest seen in the dive. The pattern in most seals, however, was an initial dramatic fall in heart rate followed by a gradual decrease throughout the descent phase (Fig. 2.2B). In flat-bottom dives, heart rate levelled off between 20 and 50 bpm (Fig. 2.2B, right). During the dive heart rate was arrhythmic with oscillations of 10-20 bpm being common. Heart rate began to rise gradually upon ascent, but this anticipatory tachycardia was most pronounced in the last 15 sec of ascent so that just before surfacing heart rates had nearly returned to the pre-dive value. Mean dive heart rate was significantly lower than both the SI mean heart rate and the mean heart rate for the complete dive cycle (One-way repeated measures ANOVA, P<0.001 all levels; Table 2.2). Mean dive heart rate and mean dive cycle heart rate were both higher in dives performed on the shelf than off the shelf (dive heart rate, on the shelf vs. off the shelf: P<0.0001; dive cycle heart rate, on the shelf vs. off the shelf: P=0.0001; Table 2.2). Occasionally heart rate during a dive reached exceptionally low values. Such profound bradycardia was associated with a sudden change in dive behaviour, such as a reversal during ascent (Fig. 2.4A,B), and the longest interbeat interval observed was 26 sec (instantaneous heart rate = 2.3 bpm).

Variability in the heart rate response over the course of many dives is seen in Fig. 2.2A. The mean diving heart rate was significantly negatively related to duration for all seven seals (r² = 0.33-0.75). For five of the seals, this relationship was clearly not linear. Data including all dives from three of these seals are illustrated in Fig. 2.5. The best and most parsimonious continuous curvilinear model found to describe the heart rate - dive duration relationship was a 2nd order polynomial. A two-segment linear regression threshold model was also fitted to these data. Three of the seals had a significantly negative first segment, while all five had a significantly negative second segment of steeper slope. The threshold
between the phase with little or no dependence of heart rate on dive duration and the phase of a strong inverse relationship ranged from 9.7 to 18.5 min. Although both the threshold model and the curvilinear model were significantly better descriptors of the relationship than a single linear regression, there was no significant difference between the MSE or the RSS of the threshold and curvilinear models. Heart rate in the period 30-60 sec after the dive commenced was examined to test whether the general pattern of an inverse relationship between heart rate and dive duration was the result of the seal setting heart rate for a dive of planned duration. No relationship was found between this early heart rate and dive duration. However, six of the seven seals had a significantly negative relationship between heart rate 60-120 sec into the dive and dive duration, suggesting that the heart rate - dive duration relationship was partially due to the gradual decrease in heart rate during descent, not the initial level to which heart rate dropped.

Heart rate during apnoea and eupnoea on land

During periods of quiet wakefulness or sleep, the respiratory pattern on the beach was one of alternating periods of apnoea and eupnoea (Fig. 2.6A). This pattern repeated for up to 4.5 hours when the seals were undisturbed. Mean apnoea duration ranged from 5.4 to 12.6 min among the four seals, and the maximum apnoea observed was 20.3 min (Table 2.3). Apnoea/eupnoea cycles on land were compared to those at sea for dives on the shelf, because of the similar mean apnoea durations, as opposed to the longer apnoea durations of dives off the shelf (Table 2.3). Neither the mean nor maximum apnoea duration on land was significantly different from the diving, or apnoea at sea, duration. However, the mean eupnoea duration, which ranged from 3.2 to 4.8 min, was significantly longer than the SI, or eupnoea at sea, duration (P=0.002; Table 2.3). Therefore, the percentage of time spent in
apnoea differed significantly between periods at sea and on land (P=0.008; Table 2.3). Another striking difference was the lack of any relationship between eupnoea duration and the length of the preceding apnoea when on land.

At the start of an eupnoeic period on land, heart rate was 70-80 bpm, but it gradually decreased throughout eupnoea as the apparent respiratory sinus arrhythmia became more pronounced (Fig. 2.6C). Mean eupnoeic heart rate was 65 ± 8 bpm for the four seals and 40 % lower than the SI (eupnoea at sea) heart rate (Table 2.3). By the end of the last expiration in an eupnoeic period, heart rate fell to a level between 30 and 50 bpm, which was close to the minimum heart rate of the sinus arrhythmia during the last few breaths (Fig. 2.6C). Heart rate during apnoea was on average 31 % lower than during eupnoea (Table 2.3), and the mean was quite stable throughout apnoea despite frequent arrhythmic oscillations (Fig. 2.6A). The apnoeic heart rate was significantly different from both the eupnoeic heart rate and the complete apnoea cycle heart rate (One-way repeated measures ANOVA, P<0.001 all levels; Table 2.3). Unlike the pattern during diving, there was no relationship between apnoeic heart rate and apnoea duration. The mean apnoeic heart rate on land was not significantly different (P = 0.11) from the mean diving heart rate (on shelf), nor was there any difference between dive cycle and land apnoea cycle heart rate (P = 0.20, Table 2.3).

**Average daily heart rates on land and at sea**

Average daily heart rate on land was predicted by including the periods of activity (originally excluded) which took place in eupnoea and multiplying the increased proportion of time in eupnoea by mean eupnoeic heart rate and the decreased proportion of time in apnoea by mean apnoeic heart rate. Predicted average daily heart rate at sea for these four
seals was considered to be equivalent to the dive cycle heart rate for dives on or off the shelf. Predicted average daily heart rate at sea for diving on the shelf (46.7 ± 3.1 bpm) was not significantly different from predicted average daily heart rate on land (mean = 54.6 ± 9.3 bpm), whereas the predicted average daily heart rate for diving off the shelf (mean = 40.1 ± 3.1 bpm) was significantly lower than the predicted average daily heart rate on land (P=0.02).

Discussion

The translocation method is a suitable and rewarding technique for short-term physiological study of the northern elephant seal. When the translocated seals were in deep water, their diving pattern was very similar to that observed in naturally migrating juveniles. The mean dive depth and duration for naturally migrating juvenile elephant seals is 373 ± 77 m and 15.2 ± 2.6 min (Le Boeuf et al. 1996), while in this study the means for “off the shelf” dives were 305 ± 84 m and 17.1 ± 4.9 min. Another advantage of the translocation method is that once the seals are released the effect of the observer’s presence is removed. Other methods previously used to monitor the cardiac response to diving in free-ranging pinnipeds have involved the presence of a tracking vessel within 1 km (Fedak et al. 1988; Thompson and Fedak, 1993) or the semi-natural environment of an ice-hole surrounded by a laboratory hut and researchers (Kooyman and Campbell, 1972; Hill et al. 1987). It is important to reduce or eliminate the effect of disturbance when attempting to record the heart rate response to voluntary submergence because a seal’s heart rate response can be modified by the behaviour of observers (Scholander, 1940; Fedak, 1986).

Despite the voluntary and free-ranging nature of diving in these juvenile elephant seals, their heart rate response appeared to be similar to the response of forcibly submerged adult
northern elephant seals (Van Citters et al. 1965). However, Van Citters et al. (1965) acknowledged that the moderate and gradually developed bradycardia seen in those adult seals may have been due to the lingering effects of anaesthesia. Studies of other pinnipeds have demonstrated a dramatic difference between the heart rate responses to forced and voluntary submergence. Forcibly submerged grey (*Halichoerus grypus*), harbour (*Phoca vitulina*), and Weddell seals immediately reduce their heart rate to 10-15 bpm (Scholander 1940; Irving et al. 1941; Zapol et al. 1979), but these same species respond to voluntary diving with a much more moderate, although just as abrupt, bradycardia, with submerged heart rates usually in the range of 30-50 bpm (Fedak 1986; Jones et al. 1973; Kooyman and Campbell, 1972). The heart rate response to diving in wild, free-ranging individuals of each of these three species is very similar to the response observed in juvenile elephant seals (Thompson and Fedak, 1993; Fedak et al. 1988; Kooyman and Campbell, 1972; Hill et al. 1987). A remarkable aspect of the elephant seal response was the occurrence of extremely profound bradycardia. Occasionally when seals made sudden reversals during ascent and extended the dive, heart rate dropped to levels as low as or lower than those seen in the most extreme responses to forced submergence (Fig. 2.4). If blood pressure is to be maintained, then instantaneous heart rates as low as 2-3 bpm must represent almost complete peripheral vasoconstriction with re-distribution of blood and its remaining oxygen supply solely to the anoxia-intolerant tissues of the heart and brain.

Ringed (*Phoca hispida*) and grey seals have also been observed to display very low heart rates during free-ranging dives. Ringed seals diving below ice cover on an experimental pond often reduce their heart rate to less than 10 bpm when diving (Elsner et al. 1989), and freely diving grey seals sometimes display profound bradycardia even though their access
to air is not restricted (Thompson and Fedak, 1993). Mean diving heart rate during grey
seal foraging dives that last more than 10 min is always less than 20 bpm, and during one
14 min dive mean heart rate was only 6.5 bpm (Thompson and Fedak, 1993). Thompson
and Fedak (1993) suggested that such bradycardia reflects a reduction in metabolic costs,
especially as the seals do not usually swim during the bottom time of these dives. The grey
seals were approximately the same size as the juvenile elephant seals in this study, yet the
elephant seals routinely made longer dives with higher heart rates. Even during dives as
long as 31 min, mean dive heart rate of elephant seals never fell below 18 bpm, and while
only 6% of the grey seal dives exceeded 10 min, elephant seals exceeded this duration on
the majority of their dives. Elephant seals have greater mass-specific oxygen stores than
other phocids that have been examined (Thorson, 1993), which may explain why they make
much longer dives than grey seals without resorting to such severe rationing of the blood
oxygen stores.

It would appear, however, that elephant seals restrict blood flow, as reflected by heart rate
changes, to at least some tissues during many dives, and that this response is dependent
upon the length of the dive. Kooyman (1985; 1989) suggested that the short to medium
duration voluntary dives of Weddell seals involve little cardiovascular adjustment compared
with resting conditions, whereas relatively long dives (> 20 min) are more similar to forced
submergence with extreme bradycardia and peripheral vasoconstriction. Hill et al. (1987)
confirmed this prediction for Weddell seals by showing that mean dive heart rate was
negatively related to dive duration for dives greater than 20 min, but that there was no
correlation for shorter dives. If elephant seals operate according to Kooyman’s (1985)
model of the Weddell seal dive response, mean heart rate for short dives should show little
or no dependence on dive duration, but beyond some threshold the slope of the relationship should become much steeper. In addition, Le Boeuf and Crocker (1996) showed that when elephant seals are diving on the shelf, they make shorter dives and swim faster than when they are off the shelf. Higher swim speeds may result in higher heart rates, and I found that both diving heart rate and dive cycle heart rate were higher for dives on the shelf than off. However, swim speed was not measured in this study, and in laboratory studies both grey and harbour seals showed no dependence of submerged heart rate on swim speed (Fedak 1986; Williams et al. 1991). Nevertheless, dives performed on the shelf are invariably shorter than those in deep water, which might cause a step change in the relationship between heart rate and dive duration. Although a two-segment linear regression threshold model described the data for five of the elephant seals significantly better than a single regression model, a simpler continuous curvilinear model fit the data equally well (Fig. 2.5).

Furthermore, contrary to the suggestion of Kooyman and Campbell (1972) that seals anticipate the duration of a dive and adjust their heart rate accordingly at the beginning of a dive, I found no relationship between heart rate 30 - 60 sec after the dive commenced and dive duration. I did, however, find that such a negative relationship existed for heart rate during the period 60 - 120 sec into the dive for six of the seals. Given that heart rate appears to fall gradually during descent and then levels off or begins to increase if ascent immediately follows (Fig. 2.2), it appears that elephant seals do not anticipate dive duration but instead adjust their heart rate response throughout a dive. As the seal descends deeper, its heart rate falls to a lower level, so that deeper, longer dives have lower heart rates. This pattern of a gradual and continual adjustment of the heart rate response supports the continuous curvilinear model, rather than a threshold model, to explain the relationship
between heart rate and dive duration. Such a continuously graded heart rate response may also represent a gradual reduction in metabolic rate with increasing dive duration. Oxygen consumption measured over a dive plus surface cycle does decline with increasing dive duration in both Weddell and grey seals (Castellini et al. 1992; Reed et al. 1994). Studies on terrestrial mammals have shown that there is a close match between blood flow and oxygen consumption, both at the cellular and whole animal level, from below resting levels up to moderate exercise, and it has been suggested that reduced perfusion may cause a suppression of overall metabolism in large seal species (Hochachka, 1992).

Although the ADL concept has been experimentally verified by lactate measurements in Weddell seals, estimated ADLs have been calculated for many other species of diving vertebrates by measuring available oxygen stores and dividing by either a measured or estimated diving metabolic rate, without measurements of lactate production (Kooyman, 1989). At least for elephant seals, it would appear that the method of assuming a single value for diving metabolic rate is not valid. If metabolic rate, like heart rate, is continuously being adjusted, then there can be no single time limit for aerobic dives. Thorson and Le Boeuf (1994) have shown that metabolic rate and dive duration are inversely correlated in juvenile seals voluntarily diving in the laboratory. Therefore, one would not expect to see a threshold for the production of net lactate, but rather lactate concentration in elephant seals might continually increase with increasing dive duration. If elephant seals are able to reduce metabolism through reduced perfusion without an increase in anaerobic metabolism, then the increase of post-dive lactate on longer dives may be negligible, which would help to explain how recovery from extremely long dives apparently occurs during very short surface intervals.
Post-dive recovery in elephant seals is probably aided by high surface heart rates and the anticipatory tachycardia before surfacing (Fig. 2.2). As Thompson and Fedak (1993) pointed out, this anticipatory increase probably reflects a restoration of circulation to tissues that may have been under-perfused during the dive, and such tissues, especially myoglobin-rich muscle, will reduce the $P_{O_2}$ thereby maximising oxygen uptake at the beginning of the SI. However, this depletion of oxygen could be detrimental to the oxygen-dependent brain and heart if a seal was forced to reverse its ascent before reaching the surface. This risk may be countered by the benefit of short surface times if the anticipatory tachycardia as well as the very high SI heart rates allow seals to minimise time spent at the surface. Both grey and elephant seals have significantly higher heart rates between dives than do Weddell seals (Thompson and Fedak, 1993; Hill et al. 1987), which might partially explain why grey and elephant seals have much shorter surface intervals than Weddell seals.

Another possibility is that grey and elephant seals breathe at a higher frequency during surface intervals than do Weddell seals, although these measurements have only been made at sea for Weddell seals. Milsom et al. (1996) reported that in elephant seal pups the instantaneous respiratory frequency at the beginning of an eupnoea on land is positively related to the duration of the preceding apnoea. Although juvenile elephant seals respond to the presumably increased respiratory drive of longer dives by breathing for longer, this relationship between SI duration and dive duration has a low slope (Fig. 2.3) and is not found in adult elephant seals (Le Boeuf et al. 1988). In Chapter 3, therefore, I will test whether juvenile elephant seals respond similarly to pups on land and breathe faster after longer dives at sea.
The biggest difference between the heart rate patterns at sea and on land in juvenile elephant seals was the much higher heart rates during breathing at sea (Table 2.3). The breathing periods were much shorter at sea and were related to the duration of the preceding dive, whereas on land eupnoeic periods were 3.5 times longer and were not related to the duration of the preceding apnoea. Apparently seals on the beach did not have the same drive to minimise eupnoeic periods as they did at sea. On land, the entrance into apnoea was accompanied by a drop in heart rate to at least the minimum level observed between breaths near the end of the preceding eupnoea, a pattern previously reported in weaned pups and adult males (Castellini et al. 1994a, b). Although heart rate dropped to a similar level at the beginning of dives, the dive response appears to be more complicated than just a cessation of breathing. If, like freely diving captive grey seals (Reed et al. 1994), respiratory tidal volume and flow rate do not vary during the course of an elephant seal's surface interval, then it is not likely that the immediate drop in heart rate upon submergence is simply due to the physiological response that accompanies the last exhalation. Furthermore, heart rate during apnoea on land did not continue to fall after the first few beats, whereas heart rate at sea declined as the seal descended (Figs 2.4, 2.6). There were also differences at the end of apnoeas. While the increase in heart rate at the end of an apnoea on land was probably due to the commencement of breathing, the increase was never as great as that seen at the end of a dive and the increase in heart rate near the end of a dive always started well before the first breath was taken. Another important difference was that heart rate during diving was inversely related to dive duration, but there was no relationship between apnoea heart rate and apnoea duration on land. During apnoea at sea, elephant seals must always conserve enough oxygen for the heart and brain in case their return to the surface is delayed, whereas on the beach a seal with depleted oxygen stores can always just start breathing immediately.
Therefore, the cardiovascular adjustments seen at sea are an important response to diving, and not just to apnoea.

Whether at sea or on land, the heart rate response to apnoea is properly termed a bradycardia. As suggested by others (Belkin, 1963; Kooyman, 1985; Fedak, 1986), I have compared breath-holding heart rate to a long term average rate, rather than just to the mean breathing heart rate. Heart rate was significantly lower during dives and during beach apnoeas than the average daily heart rate at sea or on land, respectively (Table 2.3). These average daily heart rate values were even lower than predicted rates based on allometric scaling relationships of terrestrial mammals. The equation presented by Stahl (1967) predicts a resting heart rate of 64.6 bpm for a 193 kg (mean mass of seals in this study) adult mammal, but these diving, exercising juvenile elephant seals had much lower heart rates during individual dives as well as during complete dive and surface cycles. This result might be expected given that phocids tend to have slightly larger hearts and thus larger stroke volumes than other mammals (Bryden, 1972; Drabek, 1977) and therefore could potentially produce the same cardiac output with a lower heart rate. However, Ponganis et al. (1990) showed that although harbour seals have a stroke volume during ventilation of 1.2-1.8 times that of resting dogs and goats of similar mass, stroke volume falls by 30-50% during trained dives. If stroke volume also decreases during breath-holding in elephant seals, then their low heart rates cannot be due to their large hearts. An alternative explanation is that the amount of oxygen ejected from the heart on each beat is greater in elephant seals than in typical terrestrial mammals. Elephant seals have unusually high hematocrits and cellular haemoglobin concentrations, so that a unit of elephant seal blood carries much more oxygen than a similar unit of terrestrial mammal blood (Castellini et al.)
1986; Thorson and Le Boeuf, 1994). These low heart rates may also be simply a reflection of a low field metabolic rate, and in Chapter 5 I will test this hypothesis.

Predicted average daily heart rate on land was very similar to that at sea. The elephant seal’s arrhythmic breathing pattern on land with a low overall respiratory frequency has obvious advantages for water conservation in an animal that is fasting for a month or more (Huntley et al. 1984; Blackwell and Le Boeuf, 1993). However, a more important benefit may be the reduction in metabolic rate that potentially accompanies the bradycardia associated with apnoea. Fasting seals clearly have a need for energy and fuel conservation, just as oxygen conservation is important in a diving seal.
Fig. 2.1 Map of Monterey Bay. Numbers denote seal release sites as follows: 1) Seal GH817; 2) Seal GH890; 3) Seal GH911; 4) Seal GJ711; 5) Seal GH929; 6) Seal GJ325; 7) Seal GG571.
Fig. 2.2 (A) Dive depth and instantaneous heart rate record of seal GH929. The seal was released at sea at 10:40 h on the same day. (B) Expanded view of the two marked (*) dives from A to illustrate the difference in heart rate between a V-shaped, deep, long dive and a shorter, shallower flat-bottom dive.
Fig. 2.3 Surface interval duration plotted against the duration of the preceding dive. Values for each seal demonstrated a significant positive relationship with $r^2$ ranging from 0.16 to 0.65. The plotted regression line is the average relationship for all seals and is described by: $y=0.79 \text{ min} + 0.049x$, where $y$ is surface interval and $x$ is dive duration.
Fig. 2.4 Dive depth and instantaneous heart rate record from seal GG571. (A) Start time of centre dive: 19:41:29 h, end time: 20:00:06 h, next dive start: 20:01:38 h. Note that after ascending for 1 min, at 19:53:39 h the seal reversed its direction and simultaneously heart rate dropped dramatically. This pattern repeated 2.5 min later. From 19:53:30 h to 19:58:30 h, the mean heart rate was 13.6 bpm. (B) The ECG trace for the same period, each line is a one min strip. Note the 17.6 sec interbeat interval that starts at 19:55:51 h.
Fig. 2.5 Mean dive heart rate plotted against the dive duration for all dives of 3 seals. (Top row) Single linear regressions fitted to the data. The distribution of the points around the lines are biased, especially on the right-hand side, where all the points lie below the lines. (Middle row) Two-segment linear regression model fitted to the data, with the thresholds (*) marked on the abscissas. (Bottom row) Continuous curvilinear model, a 2nd order polynomial, fitted to the data.
Fig. 2.6 Heart rate and respiration records for seal GG571; onset and duration of eupnoeas are indicated by a bar above the instantaneous heart rate trace. (A) A 106 min sequence of apnoea cycles on the beach, apnoea duration in chronological order: 9.5, 10.4, 11.8, 14.3, 17.8, and 10.1 min. (B) A 1.21 min eupnoea at sea, which followed a 17.5 min dive, is presented for comparison with a 6.25 min eupnoea on land (C), that followed an 18.1 min apnoea.
<table>
<thead>
<tr>
<th>Seal Tag No.</th>
<th>Sex</th>
<th>Age (mos.)</th>
<th>Mass (kg)</th>
<th>Recording Instruments</th>
<th>Release Date</th>
<th>Release Site</th>
<th>Days at Sea</th>
<th>Hours analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH817</td>
<td>F</td>
<td>15</td>
<td>134</td>
<td>HR-TDR</td>
<td>27 May 91</td>
<td>HMS (1)</td>
<td>1.6</td>
<td>11.1</td>
</tr>
<tr>
<td>GH890</td>
<td>F</td>
<td>22</td>
<td>208</td>
<td>HR-TDR</td>
<td>02 Oct. 91</td>
<td>SEAS (2)</td>
<td>3.8</td>
<td>39.5</td>
</tr>
<tr>
<td>GH911</td>
<td>M</td>
<td>22</td>
<td>206</td>
<td>HR-TDR</td>
<td>07 Oct. 91</td>
<td>SEAS (3)</td>
<td>3.7</td>
<td>29.1</td>
</tr>
<tr>
<td>GG571</td>
<td>F</td>
<td>34</td>
<td>245</td>
<td>Holter</td>
<td>13 Nov. 91</td>
<td>HMS (7)</td>
<td>1.2</td>
<td>13.3</td>
</tr>
<tr>
<td>GH929</td>
<td>M</td>
<td>22</td>
<td>232</td>
<td>Holter</td>
<td>16 Nov. 91</td>
<td>SEAJ (5)</td>
<td>1.1</td>
<td>12.9</td>
</tr>
<tr>
<td>GJ325</td>
<td>M</td>
<td>16</td>
<td>177</td>
<td>Holter</td>
<td>03 June 92</td>
<td>SEAJ (6)</td>
<td>1.1</td>
<td>10.1</td>
</tr>
<tr>
<td>GJ711</td>
<td>M</td>
<td>16</td>
<td>150</td>
<td>Holter</td>
<td>12 May 93</td>
<td>SEAJ (4)</td>
<td>0.6</td>
<td>5.5</td>
</tr>
</tbody>
</table>

HR-TDR: digital heart rate - time depth recorder; Holter: analogue ECG recorder plus digital time depth recorder; HMS: Hopkins Marine Station; SEAS: at sea from R.V. Sproul; SEAJ: at sea from R.V. David Johnston (numerals in parentheses correspond with sites marked on Fig. 1)
Table 2.2. Comparisons of the dive behaviour and heart rate parameters for dives on and off the continental shelf and for all dives

<table>
<thead>
<tr>
<th>Seal tag no.</th>
<th>Number of dives</th>
<th>Range of dive duration (min)</th>
<th>Mean dive duration (min)</th>
<th>Mean S1 duration (min)</th>
<th>Mean % time submerged</th>
<th>Mean depth (m)</th>
<th>Mean dive HR (bpm)</th>
<th>Mean SI HR (bpm)</th>
<th>Mean dive cycle HR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>On the shelf:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH817</td>
<td>53</td>
<td>1.7 - 14.8</td>
<td>7.4 ± 3.1</td>
<td>1.14 ± 0.36</td>
<td>85.6</td>
<td>70 ± 33</td>
<td>48.4 ± 3.8</td>
<td>102.7 ± 5.8</td>
<td>56.2 ± 4.1</td>
</tr>
<tr>
<td>GH890</td>
<td>59</td>
<td>2.2 - 22.5</td>
<td>9.9 ± 4.2</td>
<td>1.49 ± 0.49</td>
<td>86.0</td>
<td>67 ± 37</td>
<td>45.7 ± 2.1</td>
<td>105.2 ± 6.2</td>
<td>53.9 ± 3.1</td>
</tr>
<tr>
<td>GH911</td>
<td>17</td>
<td>4.7 - 18.3</td>
<td>12.0 ± 3.8</td>
<td>1.55 ± 0.29</td>
<td>87.9</td>
<td>96 ± 27</td>
<td>38.2 ± 3.0</td>
<td>115.1 ± 7.1</td>
<td>47.3 ± 3.9</td>
</tr>
<tr>
<td>GG571</td>
<td>43</td>
<td>4.8 - 18.8</td>
<td>12.9 ± 3.9</td>
<td>1.17 ± 0.27</td>
<td>91.2</td>
<td>72 ± 26</td>
<td>38.8 ± 1.7</td>
<td>105.1 ± 1.8</td>
<td>45.0 ± 2.5</td>
</tr>
<tr>
<td>GH929</td>
<td>42</td>
<td>1.0 - 17.7</td>
<td>10.6 ± 4.1</td>
<td>1.02 ± 0.23</td>
<td>89.4</td>
<td>54 ± 37</td>
<td>37.8 ± 2.3</td>
<td>105.5 ± 3.0</td>
<td>44.4 ± 5.6</td>
</tr>
<tr>
<td>GJ325</td>
<td>26</td>
<td>6.9 - 12.0</td>
<td>9.9 ± 1.5</td>
<td>1.41 ± 0.32</td>
<td>87.4</td>
<td>99 ± 19</td>
<td>42.6 ± 2.4</td>
<td>110.9 ± 1.4</td>
<td>51.2 ± 2.4</td>
</tr>
<tr>
<td>GJ711</td>
<td>41</td>
<td>2.1 - 8.3</td>
<td>5.6 ± 1.7</td>
<td>0.81 ± 0.19</td>
<td>86.6</td>
<td>43 ± 37</td>
<td>37.2 ± 3.0</td>
<td>104.8 ± 2.9</td>
<td>46.1 ± 4.2</td>
</tr>
<tr>
<td><strong>Grand Mean</strong></td>
<td></td>
<td>9.8 ± 2.5</td>
<td>1.22 ± 0.27</td>
<td>87.8 ± 2.0</td>
<td>72 ± 20</td>
<td>41.2 ± 4.4</td>
<td>107.0 ± 4.4*</td>
<td>49.2 ± 4.6*</td>
<td></td>
</tr>
<tr>
<td><strong>Off the shelf:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH817</td>
<td>15</td>
<td>9.7 - 16.5</td>
<td>12.4 ± 1.9</td>
<td>1.87 ± 0.32</td>
<td>86.9</td>
<td>247 ± 70</td>
<td>42.8 ± 3.3</td>
<td>106.0 ± 0.3</td>
<td>51.1 ± 3.7</td>
</tr>
<tr>
<td>GH890</td>
<td>73</td>
<td>10.5 - 30.8</td>
<td>21.2 ± 4.9</td>
<td>2.10 ± 0.42</td>
<td>90.7</td>
<td>373 ± 74</td>
<td>37.8 ± 4.4</td>
<td>111.9 ± 4.6</td>
<td>44.6 ± 4.0</td>
</tr>
<tr>
<td>GH911</td>
<td>70</td>
<td>8.5 - 31.3</td>
<td>19.6 ± 4.5</td>
<td>2.08 ± 0.31</td>
<td>90.0</td>
<td>377 ± 93</td>
<td>33.4 ± 4.5</td>
<td>111.4 ± 3.3</td>
<td>41.2 ± 5.3</td>
</tr>
<tr>
<td>GG571</td>
<td>8</td>
<td>18.7 - 25.6</td>
<td>22.7 ± 2.7</td>
<td>1.56 ± 0.3</td>
<td>93.6</td>
<td>339 ± 86</td>
<td>31.8 ± 2.5</td>
<td>106.6 ± 1.1</td>
<td>37.3 ± 2.7</td>
</tr>
<tr>
<td>GH929</td>
<td>14</td>
<td>11.6 - 24.2</td>
<td>19.3 ± 3.5</td>
<td>1.24 ± 0.24</td>
<td>93.9</td>
<td>388 ± 149</td>
<td>31.2 ± 3.5</td>
<td>111.4 ± 1.6</td>
<td>38.0 ± 3.2</td>
</tr>
<tr>
<td>GJ325</td>
<td>19</td>
<td>12.0 - 17.7</td>
<td>14.9 ± 1.9</td>
<td>1.64 ± 0.40</td>
<td>90.0</td>
<td>234 ± 39</td>
<td>36.8 ± 4.6</td>
<td>108.1 ± 1.3</td>
<td>43.9 ± 4.0</td>
</tr>
<tr>
<td>GJ711</td>
<td>6</td>
<td>8.5 - 10.1</td>
<td>9.6 ± 0.6</td>
<td>1.50 ± 0.21</td>
<td>86.4</td>
<td>180 ± 37</td>
<td>31.3 ± 6.4</td>
<td>105.1 ± 4.9</td>
<td>41.3 ± 6.0</td>
</tr>
<tr>
<td><strong>Grand Mean</strong></td>
<td></td>
<td>17.1 ± 4.9†</td>
<td>1.71 ± 0.31†</td>
<td>90.2 ± 2.9†</td>
<td>305 ± 84†</td>
<td>35.0 ± 4.3†</td>
<td>108.6 ± 2.9*†</td>
<td>42.5 ± 4.7*†</td>
<td></td>
</tr>
<tr>
<td><strong>ALL DIVES:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grand Mean</strong></td>
<td></td>
<td>12.6 ± 4.2</td>
<td>1.40 ± 0.39</td>
<td>88.8 ± 2.0</td>
<td>162 ± 89</td>
<td>39.0 ± 4.3</td>
<td>107.3 ± 3.1*</td>
<td>46.6 ± 4.5*</td>
<td></td>
</tr>
</tbody>
</table>

Mean values are presented as mean ± S.D.; a Grand Mean is the mean of the individual seal means. S1: surface interval; HR: heart rate; bpm: beats per minute; One dive cycle is a dive and the subsequent S1.

* Significantly different from the dive HR value for that location, or for all dives;
† Significant difference between "on the shelf" and "off the shelf" value.

Sequential Bonferroni procedure was used to minimise Type 1 errors in the multiple (7) paired t-tests done between locations.
Table 2.3 Comparisons of the durations and heart rates of apnoea and eupnoea at sea (diving on the shelf only) and on land

<table>
<thead>
<tr>
<th></th>
<th>Seal GG571 At sea</th>
<th>Seal GH929 At sea</th>
<th>Seal GJ325 At sea</th>
<th>Seal GJ711 At sea</th>
<th>Grand Mean At sea</th>
<th>Mean values presented as mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total # of apnoeas</td>
<td>43</td>
<td>42</td>
<td>26</td>
<td>41</td>
<td>38.0</td>
<td>± 8.0 ± 14.4</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>21</td>
<td>51</td>
<td>21</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>Mean apnoea duration (min.)</td>
<td>12.9 ± 3.9</td>
<td>10.6 ± 4.1</td>
<td>9.9 ± 1.5</td>
<td>5.6 ± 1.7</td>
<td>9.7 ± 3.1</td>
<td>9.0 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>9.8 ± 3.9</td>
<td>12.6 ± 4.9</td>
<td>5.4 ± 2.0</td>
<td>8.4 ± 3.2</td>
<td>9.0 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Max. apnoea duration (min.)</td>
<td>18.8 ± 3.9</td>
<td>17.7 ± 2.3</td>
<td>12.0 ± 2.0</td>
<td>8.3 ± 1.7</td>
<td>14.2 ± 4.9</td>
<td>15.5 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>18.7 ± 1.7</td>
<td>20.3 ± 1.7</td>
<td>9.0 ± 2.0</td>
<td>14.2 ± 1.7</td>
<td>15.5 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>Mean eupnoea duration (min.)</td>
<td>1.17 ± 0.27</td>
<td>1.02 ± 0.23</td>
<td>1.41 ± 0.32</td>
<td>0.81 ± 0.19</td>
<td>1.10 ± 0.25</td>
<td>4.23* ± 0.74</td>
</tr>
<tr>
<td></td>
<td>4.81 ± 1.55</td>
<td>4.74 ± 1.78</td>
<td>4.16 ± 2.0</td>
<td>3.21 ± 1.21</td>
<td>4.23* ± 0.74</td>
<td></td>
</tr>
<tr>
<td>Mean % time in apnoea</td>
<td>91.2 ± 2.1</td>
<td>89.4 ± 2.3</td>
<td>87.4 ± 2.4</td>
<td>86.6 ± 2.1</td>
<td>88.7 ± 2.1</td>
<td>66.2* ± 7.2</td>
</tr>
<tr>
<td></td>
<td>65.5 ± 2.7</td>
<td>71.6 ± 2.3</td>
<td>56.2 ± 2.4</td>
<td>71.4 ± 2.7</td>
<td>66.2* ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Mean apnoea HR (bpm)</td>
<td>38.8 ± 1.7</td>
<td>37.8 ± 2.3</td>
<td>42.6 ± 2.4</td>
<td>37.2 ± 3.0</td>
<td>39.1 ± 2.4</td>
<td>44.9 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>42.5 ± 2.5</td>
<td>37.7 ± 2.3</td>
<td>54.8 ± 3.6</td>
<td>44.5 ± 4.3</td>
<td>44.9 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Mean eupnoea HR (bpm)</td>
<td>105.1 ± 1.8</td>
<td>105.5 ± 3.0</td>
<td>110.9 ± 1.4</td>
<td>104.8 ± 2.9</td>
<td>106.6 ± 2.9</td>
<td>65.0* ± 8.3</td>
</tr>
<tr>
<td></td>
<td>60.4 ± 4.3</td>
<td>58.6 ± 7.0</td>
<td>77.0 ± 5.7</td>
<td>64.0 ± 7.8</td>
<td>65.0* ± 8.3</td>
<td></td>
</tr>
<tr>
<td>Mean apnoea cycle HR (bpm)</td>
<td>45.0 ± 2.5</td>
<td>44.4 ± 2.4</td>
<td>51.2 ± 2.4</td>
<td>46.1 ± 4.7</td>
<td>46.7 ± 3.1</td>
<td>51.6 ± 9.1</td>
</tr>
<tr>
<td></td>
<td>48.4 ± 2.8</td>
<td>43.4 ± 2.9</td>
<td>64.5 ± 5.6</td>
<td>50.0 ± 4.6</td>
<td>51.6 ± 9.1</td>
<td></td>
</tr>
</tbody>
</table>

Apnoea cycle is an apnoea and the subsequent eupnoea

*Significantly different from the at sea value.

Sequential Bonferroni procedure was used to minimise Type 1 errors in the multiple (7) paired t-tests.
Chapter 3

Breathing frequencies of northern elephant seals at sea and on land revealed by heart rate spectral analysis

Introduction

It has been suggested that the ability to maximise the percentage of time spent submerged is just as important to underwater foragers as their capacity for making long dives (Fedak et al. 1988). Northern elephant seals are a species that possess extreme capabilities in both categories. Mean dive durations of juveniles and adults range from 15 to 20 min, the maximum observed duration is 119 min, and yet these dives are almost always followed by short surface intervals of approximately 2 min (Le Boeuf et al. 1988, 1996; Stewart and De Long, 1995). Elephant seals are therefore very efficient divers, spending 80-95 % of their time at sea submerged.

This exceptional ability must be related to high rates of gas exchange at the surface, but little is known about the physiology of recovery from diving in this species. Heart rate at the surface between dives is 174 % greater than during dives and 64 % higher than the rate during eupnoeic periods on land (Chap. 2, this thesis). Such high heart rates must facilitate rapid gas exchange between tissue and lung and are presumably accompanied by high breathing rates to ensure similarly rapid exchange between lung and environment. Ventilation is positively related to the duration of the preceding dive in Weddell seals, plateauing at about 6 times the resting minute volume after dives 15 min or longer. This hyperventilation is due to a 1.5 - 2 times increase in tidal volume and a 3-fold increase in respiratory frequency (Kooyman et al. 1971). Unfortunately, Weddell seals are the only
pinniped species for which it has been possible to quantify the ventilatory response to voluntary diving in nature.

In preliminary experiments conducted as part of the study presented in Chapter 2, attempts to record respiration from freely diving northern elephant seals using nasal thermistors were unsuccessful. The seals always managed to remove the thermistors before being released at sea. I was able, however, to obtain R-R interval time series at sea and on land for 4 seals equipped with ECG recording Holter monitors. Northern elephant seals display a pronounced respiratory sinus arrhythmia (RSA) when on land, with heart rate increasing on inspiration and falling on expiration (Bartholomew, 1954; Castellini et al. 1994a, b). RSA also occurs during the eupnoeic periods between short dives in laboratory tanks, albeit with a reduced amplitude (pers. obs.), and rhythmic heart rate oscillations that appear to be due to RSA were also seen in northern elephant seals diving at sea (Chap. 2, this thesis). I therefore sought to estimate the respiratory frequency \((f_R)\) of freely diving elephant seals through the analysis of the rhythmic fluctuations of heart rate.

I required an objective method that could be automated for analysis of large amounts of data and initially attempted to use Womack's (1971) method for the estimation of the time of occurrence of individual breaths. In this method a low-pass filter is applied to the heart rate time series, deriving a smoothed function which is then compared with the unfiltered time series in order to detect inspiration-induced peaks that rise above the local average. Despite trying many different low-cut-off frequencies and various other filtering algorithms, I was not able to derive a satisfactory method for application to the at sea data because the RSA amplitude was very low. Instead of attempting to identify the exact time of each individual breath, I then decided to estimate the mean frequency of heart rate oscillations over a
breathing period by using spectral analysis. The Fourier transform is often used for heart rate spectral analysis, but the breathing periods at sea are often very short, and on land the breath to breath intervals can vary considerably over a short period. A more appropriate method for short, nonstationary data is the discrete Wigner distribution (DWD), a form of time frequency mapping which provides a two-dimensional function of time and frequency (Wigner, 1932; Claasen and Mecklenbrauker, 1980). The DWD has been shown to correctly map the instantaneous changes in R-R interval spectral content even during continuous slowing of respiration in humans (Novak and Novak, 1993; Novak et al. 1993). Novak et al. (1993) showed that the dominant frequency of the R-R interval fluctuations closely followed the changing respiratory frequency over a large range, from 28 to 3 breaths min$^{-1}$. In this chapter I present data to show that the DWD of heart rate variability provides a good estimate of $f_k$ in northern elephant seals. I then compare our estimates of $f_k$ at sea and on land to determine whether an increase in $f_k$ during at sea breathing intervals is an important element of the elephant seals ability to maximise the fraction of time spent submerged.

**Materials and methods**

*Laboratory validation of $f_R$ estimation method*

Four juvenile northern elephant seals (Table 3.1) were captured at Año Nuevo State Reserve, California, and held in captivity at the Long Marine Laboratory, Santa Cruz, California, for up to five days. Details of the immobilisation, capture and transport methods are presented in Chapter 2. The surface electrocardiogram (ECG) was recorded from two of these seals (L1 & L2) onto channel 1 of a 2-channel analogue Holter monitor (model 90205, Space Labs Inc., Redmond, WA, USA) attached to the fur in the mid-dorsal region (Chap.
Respiration was monitored in these two seals by recording, on channel 2 of the Holter monitor, the signal from a thermistor glued to the fur just above one of the nares. Respiratory and electrocardiographic signals were recorded from seals L1 and L2 for at least 4 hours during daylight hours, while they rested in a fenced, outdoor pen. The Holter monitor tapes were scanned and digitised at 200 Hz using an FT2000A Medical Workstation computer system (Space Labs, Inc.).

The ECG of seals L3 and L4 was monitored by using needle electrodes attached subdermally along the dorsal midline. Respiratory movements of these seals were monitored using two additional needle electrodes placed subdermally on each side of the thorax at the level of the posterior end of the foreflippers. These electrodes were attached via long leads to an impedance respirometer. The ECG and respiration signals were acquired and stored on a personal computer equipped with an analogue-to-digital converter card. The ECG was sampled at 200 Hz, while respiration was sampled at 2 Hz. Seals L3 and L4 were held in a foam-padded metal cage for approximately 4 hours of daytime monitoring, after which their electrodes were removed and they were returned to an outdoor pen.

Sections of the records from all four laboratory seals were chosen for analysis by selecting time periods during which the seals were apparently sleeping or resting quietly. Only complete apnoea cycles (an apnoea and subsequent eupnoea that is followed by another apnoea) were analysed. To obtain R-R interval time series, the QRS complexes of the ECG were detected and the R-R intervals were calculated. Data segments from at least 5 apnoea cycles were chosen for each seal. Each segment consisted of the data 30 s before the start of an eupnoea and continued until the end of the eupnoea. The time series were linearly
interpolated at 4 Hz, and the low-frequency baseline trend was removed by applying a moving fourth-order polynomial function. Time-frequency maps of the R-R interval variability for each data segment were constructed using the discrete Wigner distribution, with the parameter settings suggested by Novak and Novak (1993b). Each map consisted of spectra calculated at 0.5 s intervals (Fig. 3.1). For each of these spectra, the frequency at which the maximum power occurred (peak power frequency) was determined. The frequency resolution of these individual spectra was 0.0156 Hz, or 0.94 cycles min$^{-1}$.

Although the peak power frequency is a function of the time and frequency smoothing of the Wigner distribution, I used it to provide an estimation of the $f_R$ at that time instant. The individual spectra of the time-frequency maps were only used to estimate $f_R$ if the spectrum was dominated by a single peak. Spectra that contained multiple peaks at similar power levels were discarded. This sometimes occurred at late stages in eupnoeas when the breathing intervals could become especially irregular. If more than 10% of the spectra from a time-frequency map for a eupnoeic segment were discarded, then that segment was not included in the analysis.

In order to calculate the error involved in estimating $f_R$ from the spectral analysis of R-R interval variability, $f_R$ was directly measured by counting the number of breaths (including fractions of a breath cycle) that occurred in the period 15 s to 60 s after the start of an eupnoea. This count was then divided by the duration of the period (45 s) to obtain the mean measured $f_R$ for individual eupnoeas. Over the same period, the mean estimated $f_R$ was determined by calculating the mean of the peak power frequencies from each of the time-frequency map spectra for that period.
Estimation of $f_R$ for seals at sea and on the beach

The dive behaviour and ECG were recorded from 4 seals (3 males, 1 female; age: 16 - 34 months; mass: 150 - 245 kg, mean mass: 201 kg) as reported in Chapter 2. These seals were captured at Año Nuevo State Reserve, instrumented at the Long Marine Laboratory, transported either out to sea or to the opposite side of Monterey Bay, and then released. The ECG was recorded as the seals dove continuously on their return to Año Nuevo, and for 12-18 hours after they reached land. Only those eupnoeic periods longer than 32 s and in which the ECG was completely noise-free and did not contain any ectopic or missing beats were chosen for analysis. The start and end of eupnoeas at sea could be determined with an accuracy of approximately ± 5 s, because of the 5 s sampling interval of the time-depth recorder (TDR). On land, the start of eupnoeas and apnoeas was determined from the marked changes in heart rate that accompany these transitions (Chap. 2, this thesis).

The R-R interval time series were treated as described above for the laboratory seals, and time-frequency maps were created for each eupnoea. For the eupnoeas at sea, an "estimated eupnoeic $f_R_{1st \text{ min}}$" was calculated for the period from 15 - 60 s (for the few eupnoeas < 60 s, $f_R$ was estimated for the period from 15 s to the end of eupnoea). The first 15 s were ignored because of the imprecision in determining the start of eupnoeas. The mean eupnoea duration at sea was only 74 s, so the estimated eupnoeic $f_R_{1st \text{ min}}$ was multiplied by the total duration of the eupnoea in order to estimate the number of breaths in each eupnoea. The "estimated overall $f_R$" was defined as the quotient of the number of breaths in an episode of eupnoea and the total duration of the apnoea cycle (apnoea duration plus eupnoea duration).

For eupnoeas on land, an "estimated eupnoeic $f_R_{1st \text{ min}}$" was calculated as above for the 1st min, and for each subsequent min of the eupnoea an estimate was derived for the entire
minute (e.g. "estimated eupnoic \( f_k_{2nd\ min} \)" covers the range from 60 - 120 s). For the last fraction of a minute in an eupnoea an estimate was derived for the total remainder of time (e.g., for a 3.6 min eupnoea, the "estimated eupnoic \( f_k_{4th\ min} \)" covers the range from 180 - 216 s). The total number of breaths in a eupnoea on land were calculated iteratively, by multiplying the fraction of time spent in each 1 min bin by the estimated \( f_k \) for that bin and then calculating the sum. In some of the data segments for the later periods of eupnoeas (e.g. in the 4th and 5th min) heart rate became quite arrhythmic and more than 10 % of the spectra did not contain a single dominant peak. In those cases the fraction of time spent in a 1 min bin was multiplied by that seals mean value for estimated \( f_k \) for that bin. For example, if the period from 240 - 300 s of a 5.0 min eupnoea had to be discarded, then the number of breaths for that one min period were estimated by multiplying the mean estimated eupnoic \( f_k_{5th\ min} \) value from that seal's other eupnoeas by 1 (because the eupnoea lasted 5 min the fraction of time spent in the 5th min bin was 1).

Student's paired t-tests were used to compare apnoea and eupnoea durations and respiratory frequencies at sea and on land, except where it was appropriate to perform a one-way repeated measures ANOVA. Significance was accepted at the level of \( P < 0.05 \) except when it was necessary to use the sequential Bonferroni method to minimise type-I errors (Rice, 1989). Relationships were examined using least-squares linear regression.

Results

Laboratory validation

During periods of simultaneous monitoring of respiration and heart rate, the estimated \( f_k \) from the 0.5 s spectra of the DWD time-frequency map matched the measured instantaneous \( f_k \) closely, even when the breathing intervals were quite variable over time.
(Fig. 3.1). Therefore, the mean estimated $f_k$ was usually within a few percent (range -5.6 to +10.0 %) of the mean measured $f_k$ for individual eupnoeic segments (Fig. 3.2). The mean algebraic error in the estimation of $f_k$ for all seals was only $1.05 \pm 1.23 \%$, and the mean absolute value of the error was $3.08 \pm 0.85$ (Table 3.1). There was no relationship between the measured $f_k$ and the error in its estimation (Fig. 3.2).

Respiration at sea and on the beach

The DWD time-frequency maps of heart rate variability for eupnoeic segments at sea were usually dominated by a peak at the presumed respiratory frequency, although there was often considerable spectral power at other frequencies as well (Fig. 3.3C). The estimated eupnoeic $f_{k_{1st\ min}}$ for individual surface intervals tended to increase with the duration of the preceding dive (Fig. 3.4). In the two seals that made many dives in excess of 15 min, the estimated eupnoeic $f_{k_{1st\ min}}$ appeared to plateau at about 25 breaths min$^{-1}$ (Fig. 3.4). The mean estimated eupnoeic $f_{k_{1st\ min}}$ for individual seals ranged from $19.0 \pm 1.6$ to $23.3 \pm 2.1$ breaths min$^{-1}$ (Table 3.2). There was a significantly positive linear relationship for each seal ($r^2 = 0.20 - 0.67$) between the estimated number of breaths per episode and the duration of the preceding dive. However, there was a negative relationship between the estimated overall $f_k$ and preceding dive duration (significant for 3 seals, $r^2 = 0.23 - 0.43$). Overall $f_k$ was even more strongly related in an inverse fashion to the percent time submerged (significant for all 4 seals, $r^2 = 0.28 - 0.96$).

Time-frequency maps of heart rate variability during eupnoeas on the beach were very similar to those for the laboratory seals. There was a trend for the estimated eupnoeic $f_{k_{1st\ min}}$ for individual eupnoeas on the beach to be directly related to the duration of the preceding apnoea, at least for apnoeas up to 10 min long (Fig. 3.4). Mean estimated eupnoeic $f_{k_{1st\ min}}$
for all seals was $9.2 \pm 1.3$ breaths min$^{-1}$, which was 58% lower than the estimated eupnoeic \( f_{R_{1st\;min}} \) at sea (Table 3.2). The mean estimated \( f_R \) for each subsequent min of eupnoea tended to decrease slightly. There were adequate sample sizes from all 4 seals to allow comparison of \( f_{R_{1st\;min}}, f_{R_{2nd\;min}}, f_{R_{3rd\;min}}, \) and \( f_{R_{4th\;min}} \) with a repeated measures ANOVA (multiple pairwise comparisons done with the Student-Newman-Keuls test). Only \( f_{R_{3rd\;min}} \) and \( f_{R_{4th\;min}} \) were significantly lower than \( f_{R_{1st\;min}} \), and the mean \( f_{R_{4th\;min}} \) was $7.7 \pm 1.0$ breaths min$^{-1}$. The mean estimated number of breaths per episode on land was $34 \pm 5$, which was not significantly different from the mean value of $27 \pm 3$ breaths per episode at sea (Table 3.2). The mean estimated overall \( f_R \) was $2.6 \pm 0.6$ breaths min$^{-1}$ on land and $2.3 \pm 0.6$ breaths min$^{-1}$ at sea (Table 3.2). Because the number of breaths for some long eupnoeas on land had to be calculated with mean \( f_R \) values for individual 1 min bins instead of with the actual estimated \( f_R \) for that min of the eupnoea, I did not examine relationships between the duration of the preceding apnoea and either the number of breaths per episode or the overall \( f_R \).

**Discussion**

The estimation of respiratory frequency from the spectral analysis of heart rate using the discrete Wigner distribution appears to be a satisfactory method for situations in which it is possible to record the ECG but not respiration. Although I was not able to validate the method by simultaneous recording of respiration and estimation of \( f_R \) in seals at sea, I am confident nonetheless that our estimates of \( f_R \) for seals at sea are reasonable. I observed no correlation between the measured \( f_R \) and the error in estimating \( f_R \), even though one of the seals (L1) breathed at an unusually high rate (Table 3.1, Fig. 3.2). The RSA amplitude was reduced in seal L1 compared with the others, and the magnitude of the peak R-R interval spectral power was diminished, but the error in estimating \( f_R \) was still very low. RSA
amplitude in the seals breathing at sea was even lower, so the level of spectral power at the peak power frequency (the presumed $f_k$) was very low (compare Fig. 3.1 and Fig. 3.3). Overall spectral power, however, was also reduced and so there was still a single, obviously dominant, peak in most spectra.

A reduction in R-R interval spectral power at the respiratory frequency and at all other frequencies is also seen at high respiratory rates during exercise in humans (Arai et al. 1989). RSA is dependent upon both $f_k$ and tidal volume ($V_T$), and while the phase and amplitude may change, there is usually a 1:1 relationship between the respiratory movements and RSA heart rate fluctuations (Angelone and Coulter, 1964; Hirsch and Bishop, 1981). The coherence between the R-R interval power spectrum and the respiratory signal power spectrum is usually near one and is independent of $f_k$ in humans (Patwardhan et al. 1995). This would appear to be the case for elephant seals as well, at least for seals on the beach breathing at a limited range of frequencies. Our estimates of $f_k$ for seals at sea are also supported by recent, serendipitous measurements of $f_k$ from the recording of putative breath sounds of translocated northern elephant seals carrying acoustic monitoring devices (Fletcher et al. 1996). The mean eupnoeic $f_k$, between dives that averaged 14.7 min, ranged from $22.0 \pm 1.0$ to $24.6 \pm 1.6$ breaths min$^{-1}$ for three seals. These values are very similar to the values of $f_k$ that I estimated in this study.

The mean eupnoeic $f_k_{1st\, min}$ after a dive was 2.4 times greater than the mean eupnoeic $f_k_{1st\, min}$ after an apnoea on land (Table 3.2). Eupnoeic $f_k_{1st\, min}$ at sea also increased in direct relation to the duration of the preceding dive, at least for dives up to 15 min in length (Fig. 3.4). These high breathing rates permit elephant seals to spend only short periods at the surface for gas exchange, resulting in a high percentage of time spent submerged, or apnoeic. The
mean duration of apnoea at sea was not significantly different from that on land, and the number of breaths per episode was also not different. The much higher $f_R$ at sea, however, resulted in 34% increase in the time spent apnoeic at sea compared with the value on land. This difference in $f_R$ between diving seals and seals resting on land contrasts with a study of northern elephant seal pups and raises the interesting issue of the control of cardiorespiratory responses to apnoea and diving (Castellini et al. 1994a).

Like juveniles and adults, sleeping northern elephant seal pups (~4 months old) display a pattern of arrhythmic breathing, alternating long (~4 min) apnoeas and eupnoeas. In the laboratory, these pups performed equivalent length breath-holds whether sleeping underwater in a shallow tank or sleeping in dry conditions, and there was no difference in the eupnoeic $f_R$ between wet and dry conditions. There was also no difference in the breath-hold heart rate between wet and dry conditions, which led Castellini et al. (1994a) to conclude that diving apnoea and sleep apnoea may be governed by the same homeostatic control mechanisms. In Chapter 2 I argued that there are important differences in the cardiovascular responses to the two forms of apnoea that suggest the control mechanisms must differ. The characteristics of the cardiorespiratory responses to recovery from apnoeas also seem to depend on the type of apnoea performed. Heart rates after dives are 1.6 times higher than heart rates after sleep apnoeas on land, and here I show that breathing rates are 2.4 times higher after dives than after apnoeas on land. Of course, it is possible that the increased cardiac and respiratory rates seen during recovery from dives compared with recovery from sleep apnoeas are simply due to more extreme changes in blood gas levels and therefore greater levels of respiratory drive during recovery from diving.
The effect of blood gas levels on respiration have not been studied in elephant seals, but the response of pups to alterations in breathing gases has been examined. When northern elephant seal pups that are awake and breathing regularly, without extended apnoeas, are exposed to hypercapnic breathing gas, $f_R$ approximately doubles (Milsom et al. 1996). Exposure to hypercapnia during bouts of sleep apnoea, however, causes only a small increase in the instantaneous breathing rate for the first five breaths after an apnoea, and moderate hypoxia has no effect on $f_R$ whether the pups are asleep or awake. Harp, hooded, and harbour seals tend to modify $f_R$ and $V_T$ very little when exposed to moderate levels of hypoxia and/or hypercapnia during laboratory diving, decreasing the length of apnoeas instead (Päsche 1976a, b; Craig and Päsche, 1980). Juvenile Weddell seals diving from an isolated breathing hole also lack a clear ventilatory response to hypoxia, but do increase ventilation markedly in response to hypercapnia (Parkos and Wahrenbrock, 1987). It is therefore not clear whether increased arterial CO$_2$ ($P_{aCO_2}$) and decreased arterial O$_2$ ($P_{aO_2}$) should be expected to cause as large of an increase in $f_R$ as the one I saw after dives compared to apnoeas on land in elephant seals. It is also not a given that the blood gas levels will differ at the end of dives and beach apnoeas of similar duration, or that the direction of the difference will be what one might expect, despite that during a dive seals are swimming and during most apnoeas on land seals are simply sleeping. During one episode of sleep apnoea in a Weddell seal lying on the ice, $P_{aO_2}$ fell to 25 mm Hg and $P_{aCO_2}$ rose to 55 mm Hg after only 4 min (Kooyman et al. 1980). Such a low $P_{aO_2}$ was not usually seen in freely diving seals until after at least 15 min of submergence, and $P_{aCO_2}$ never rose above 53 mm Hg, even in dives as long as 27 min (Qvist et al. 1986). Although simultaneous measurements of $f_S$, $V_T$, and alveolar and blood gases in elephant seals during both diving
and sleep apnoea are needed before an attempt to resolve the issue can be made, it seems safe to propose that the cardiorespiratory response to diving is governed by control mechanisms that are unique to the diving situation. Although some elements of the homeostatic mechanisms must be shared by the two types of apnoea, there appear to be important differences in the mechanisms that control the length of apnoeas and eupnoeas, the cardiovascular response during breath-hold, and the cardiorespiratory response in recovery from apnoea. For example, during diving there may be a change in the set point and gain of the ventilatory CO$_2$ response. Rather than being a automatic response to respiratory drive, irrespective of situation, it appears that the high heart rates and breathing rates after a dive uniquely serve to ensure rapid, as opposed to adequate, gas exchange, thereby decreasing the amount of time spent at the surface.

Although the elephant seal's mean eupnoeic breathing rate at sea of 22 breaths min$^{-1}$ is 2.4 times higher than on land, terrestrial mammals are often capable of increasing $f_R$ by 4-8 times during strenuous exercise. If elephant seals are under pressure to decrease the fraction of time they spend at the surface, one might ask why they don't breathe even faster between dives. The $f_R$ of ponies (mass ~ 150 kg) exercising near their maximal work load reaches 95 breaths min$^{-1}$, an increase of 5 times over resting $f_R$ (Bisgard et al. 1978). Even less athletic calves (mass ~ 180 kg) are able to increase $f_R$ up to 65 breaths min$^{-1}$, 2.5 times higher than resting $f_R$ (Kuhlmann et al. 1985). Of course, respiration in elephant seals floating motionless at the surface cannot be assisted by coupling between respiratory and locomotor movements, as may be the case in running mammals (Alexander, 1989). Furthermore, if elephant seals breathe with very large tidal volumes after dives, like Weddell and grey seals (Kooymen et al. 1971; Reed et al. 1994), the mechanics of lung ventilation may prevent
them from reaching such high levels of \( f_R \). If \( V_T \) during recovery from dives is quite large, then elephant seals may still be able to achieve reasonably high levels of ventilation.

Based on the relationship between body mass and lung volume derived from other marine mammal species (Kooyman, 1989), the predicted value for total lung capacity of a 201 kg elephant seal is 16.2 l. The maximal \( V_T \) during recovery from voluntary dives in Weddell and grey seals is between 46 and 49 % of total lung capacity (TLC) (Kooyman et al. 1971; Reed et al. 1994). If I assume that \( V_T \) in diving elephant seals is 50 % of TLC, or 8.1 l, then with a \( f_R \) of 22 breaths min\(^{-1}\) they would have an expired ventilation of 178 l min\(^{-1}\). Even at a maximal breathing rate of 27 breaths min\(^{-1}\), ventilation would only reach 219 l min\(^{-1}\). In the studies mentioned above, similarly sized ponies and calves reached ventilation levels of 435 l min\(^{-1}\) and 251 l min\(^{-1}\), respectively.

A factor that may be limiting total ventilation in elephant seals is the extra work required to breathe while immersed in water. When juvenile elephant seals reach the surface and begin to breathe they are still immersed up to the level of the base of the skull, and the midpoint of the lung is subject to a hydrostatic pressure of approximately 40 cm of seawater (4.0 kPa). The work of breathing increases by 60 % when humans are immersed up to the neck, and this is partly due to the increase in airway resistance because of compression of the extrathoracic airways (Hong et al. 1969; Agostoni et al. 1966). The trachea, bronchi, and terminal airways of pinnipeds, however, have an unusual amount of muscular and cartilaginous reinforcement (Denison and Kooyman, 1973; Tarasoff and Kooyman, 1973). The function of this reinforcement may be to limit nitrogen absorption during deep dives (Scholander 1940) or to allow high expiratory flow at low lung volumes (Denison and Kooyman, 1973; Kerem et al. 1975), but the strengthened airways may also serve to resist
hydrostatic compression during immersed breathing. Nonetheless, Kerem et al. (1975) observed that inspiratory flow rates of immersed California sea lions were much lower than expiratory rates, and although they thought this result was puzzling, it is likely that while pinnipeds can expire large volumes very quickly, inspiration is limited by the difficulty of breathing against a negative pressure.

A potential trade-off between oxygen transport and oxygen storage capacity may also limit the maximum ventilation rate that elephant seals can, or should, achieve. The resting hematocrit (Hct) of northern elephant seals is quite high (range ~ 50 - 67 %), and it can increase during apnoeas on land and during diving in the laboratory (Castellini et al. 1986; Hedrick et al. 1986; Wickham et al. 1990; Hedrick et al. 1991; Thorson, 1993; Thorson and Le Boeuf, 1994). Although such a high Hct is an important component of the northern elephant seal's exceptional oxygen storage capacity (Thorson and Le Boeuf, 1994), the concomitant exponential increase in blood viscosity may severely limit maximal oxygen transport (Hedrick et al. 1986, 1991). There is little difference in surface interval heart rate between short and long dives at sea (Chap. 2, this thesis), suggesting that a heart rate of about 110-120 beats min$^{-1}$ is the maximal rate possible in juvenile elephant seals. If during recovery from dives, cardiac output has reached a limit due to high blood viscosity and resistance to blood flow, then further increases in ventilation would contribute very little to gas exchange at the expense of increases in the work of breathing. Extremely high Hct may increase oxygen storage, enabling longer maximal dive time, but decrease oxygen transport, extending the time needed for recovery, and so there may be a trade-off between the ability to make very long dives and the ability to spend a very high percentage of time submerged. With dive durations of about 20 min and 90 % of time at sea submerged, elephant seals may
have reached the limit. So the answer to the question posed above may be that elephant
seals don't breathe faster than 25-28 breaths min⁻¹ after dives because a further increase in
ventilation couldn't actually increase their percent time submerged.

Elephant seals do, however, sometimes make exceptionally long dives that are nonetheless
followed by short surface intervals, which suggests that metabolism must be reduced in
such dives so that an extended recovery period at the surface is not necessary. Although
metabolic rate reductions during diving have been proposed frequently (for reviews see
Boyd and Croxall, 1996; Butler and Jones, 1997; Kooyman and Ponganis, 1998), there has
never been a direct measurement of metabolic rate during a dive for any marine mammal.
In translocated juvenile elephant seals, there is an inverse relationship between diving heart
rate and dive duration, which led to my suggestion in Chapter 2 that metabolic rate may be
adjusted in a similar fashion. In this study I reported that the overall \( f_k \) (number of breaths
per dive cycle divided by the dive plus surface interval duration) is also inversely related to
dive duration, providing further support that metabolic rate is reduced on longer dives.

The mean estimated overall \( f_k \) of 2.3 breaths min⁻¹ at sea is quite low compared to the value
that would be predicted for a 201 kg resting mammal, based on an allometric scaling
relationship (Stahl, 1967). This low \( f_k \) is partially compensated for with a large \( V_t \), but if
the value I predicted above for a juvenile elephant seal's ventilatory minute volume is
recalculated to include the time spent submerged, the overall minute volume would be 16.7
l min⁻¹. Comparison of this value with the predicted value of 26.4 l min⁻¹ derived from
Stahl's (1967) scaling relationship for minute volume suggests that the elephant seal pattern
of performing repetitive, long duration dives results in a low field metabolic rate. The same
conclusion was reached based on the observation that the elephant seal's average daily heart
rate was 28 - 38 % lower than the predicted resting heart rate, despite that elephant seals are not resting, but are actively swimming for most of their time at sea (Chap. 2, this thesis).
Fig. 3.1 Comparison of directly measured respiratory frequency (fr) and estimation of fr from the R-R interval time-frequency map. (A) Time course of the respiration signal, instantaneous heart rate (reciprocal of the R-R interval), and fr, both the measured instantaneous fr (reciprocal of the interbreath interval) and the estimated fr. (B) Time-frequency map of the R-R interval time series from the eupnoeic period in A. (C) and (D) The spectra from the time-frequency map at 45 s (D) and 240 s (C). The peak power frequency of each spectrum is indicated, which also provides an estimate of the fr at that time instant. Over the period from 15 s to 263 s, the mean measured fr = 6.64 breaths min⁻¹, while the mean estimated fr = 6.66 breaths min⁻¹.
Fig. 3.2 The algebraic (A) and absolute value (B) of $\%A$, the difference between measured and estimated $f_k$, plotted against the measured $f_k$ for individual eupnoeic segments from all four seals (L1 - L4).
Fig. 3.3 Calculation of "estimated eupnoeic $f_{k1st\text{ min}}$" from the heart rate for a 1.25 min surface interval marked (*) in A, and shown in an expanded view in B. (C) The DWD time-frequency map of the R-R variability. (D) Time series of the estimated $f_k$ based on the peak power frequency of each of the spectra taken at 0.5 s intervals from the time-frequency map. The estimated eupnoeic $f_{k1st\text{ min}}$ was 20.6 breaths min$^{-1}$. 
Fig. 3.4 Estimated eupnoic $f_{1st\, \text{min}}$ plotted against the duration of the preceding dive or beach apnoea (*) (min) for each of the translocated seals.
Table 3.1 Summary of the seals in the laboratory, their apnoea/eupnoea durations, and the error in the estimation of eupnecic respiratory frequency from the spectral analysis of heart rate variability.

<table>
<thead>
<tr>
<th>Seal no.</th>
<th>Age (months)</th>
<th>Mass (kg)</th>
<th>n</th>
<th>Mean apnoea duration (min)</th>
<th>Mean eupnoea duration (min)</th>
<th>Measured $f_R$ (breaths min$^{-1}$)</th>
<th>Estimated $f_R$ (breaths min$^{-1}$)</th>
<th>Mean $%\Delta$</th>
<th>Mean absolute value of $%\Delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>16</td>
<td>145</td>
<td>11</td>
<td>5.8 ± 1.2</td>
<td>5.6 ± 2.0</td>
<td>14.1 ± 0.8</td>
<td>14.2 ± 0.8</td>
<td>1.12 ± 2.92</td>
<td>2.29 ± 2.04</td>
</tr>
<tr>
<td>L2</td>
<td>16</td>
<td>198</td>
<td>10</td>
<td>7.3 ± 1.2</td>
<td>5.3 ± 1.6</td>
<td>8.2 ± 1.7</td>
<td>8.4 ± 1.8</td>
<td>1.88 ± 4.38</td>
<td>3.39 ± 3.22</td>
</tr>
<tr>
<td>L3</td>
<td>21</td>
<td>154</td>
<td>9</td>
<td>8.3 ± 3.9</td>
<td>4.9 ± 1.9</td>
<td>7.1 ± 1.2</td>
<td>7.0 ± 1.3</td>
<td>-0.71 ± 2.92</td>
<td>2.50 ± 1.44</td>
</tr>
<tr>
<td>L4</td>
<td>21</td>
<td>153</td>
<td>5</td>
<td>8.4 ± 1.8</td>
<td>3.8 ± 0.3</td>
<td>7.1 ± 1.1</td>
<td>7.2 ± 1.3</td>
<td>1.91 ± 4.49</td>
<td>4.15 ± 1.80</td>
</tr>
<tr>
<td>Grand Mean =</td>
<td></td>
<td></td>
<td></td>
<td>7.4 ± 1.2</td>
<td>4.9 ± 0.8</td>
<td>9.1 ± 3.4</td>
<td>9.2 ± 3.4</td>
<td>1.05 ± 1.23</td>
<td>3.08 ± 0.85</td>
</tr>
</tbody>
</table>

n is the number of apnoea/eupnoea cycles
Mean values are presented as mean ± S.D.; a grand mean is the mean of the individual seal means.
Measured $f_R$: respiratory frequency measured by counting breaths in the period 15-60 s after the start of a eupnoea.
Estimated $f_R$: respiratory frequency estimation based on the mean peak power frequency from the time frequency map of the R-R variability for the period 15-60 s after the start of a eupnoea.
$\%\Delta$: the difference between the measured and estimated $f_R$ expressed as a percentage.
Table 3.2 Durations of apnoea and eupnoea and respiratory frequencies for translocated seals at sea and on land.

<table>
<thead>
<tr>
<th>Seal tag no.</th>
<th>Mean apnoea duration (min)</th>
<th>Mean eupnoea duration (min)</th>
<th>Mean % time apnoic</th>
<th>Mean estimated eupnoic fr\textsubscript{1st min} (breaths min\textsuperscript{-1})</th>
<th>Mean estimated overall fr (breaths min\textsuperscript{-1})</th>
<th>Mean estimated no. of breaths per episode</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At sea:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG571</td>
<td>14.6 ± 6.1</td>
<td>1.21 ± 0.34</td>
<td>91.6</td>
<td>22.9 ± 3.0</td>
<td>1.9 ± 0.5</td>
<td>28.3 ± 10.4</td>
</tr>
<tr>
<td>GH929</td>
<td>13.9 ± 5.7</td>
<td>1.10 ± 0.26</td>
<td>91.6</td>
<td>23.2 ± 2.1</td>
<td>1.9 ± 0.8</td>
<td>25.6 ± 7.1</td>
</tr>
<tr>
<td>GJ325</td>
<td>12.5 ± 2.9</td>
<td>1.59 ± 0.34</td>
<td>88.3</td>
<td>19.0 ± 1.6</td>
<td>2.2 ± 0.5</td>
<td>30.4 ± 7.1</td>
</tr>
<tr>
<td>GJ711</td>
<td>6.9 ± 2.0</td>
<td>1.06 ± 0.30</td>
<td>86.2</td>
<td>22.9 ± 1.4</td>
<td>3.2 ± 0.6</td>
<td>24.6 ± 8.1</td>
</tr>
<tr>
<td><strong>Grand Mean</strong></td>
<td>12.0 ± 3.5</td>
<td>1.24 ± 0.24</td>
<td>89.4 ± 2.6</td>
<td>22.0 ± 2.0</td>
<td>2.3 ± 0.6</td>
<td>27.2 ± 2.6</td>
</tr>
<tr>
<td><strong>On land:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG571</td>
<td>9.4 ± 3.7</td>
<td>5.1 ± 1.5</td>
<td>62.9</td>
<td>8.2 ± 1.4</td>
<td>2.8 ± 0.8</td>
<td>38.6 ± 10.3</td>
</tr>
<tr>
<td>GH929</td>
<td>13.5 ± 5.1</td>
<td>4.4 ± 1.6</td>
<td>74.2</td>
<td>8.2 ± 1.8</td>
<td>2.0 ± 0.6</td>
<td>33.9 ± 11.8</td>
</tr>
<tr>
<td>GJ325</td>
<td>6.0 ± 2.0</td>
<td>4.3 ± 2.1</td>
<td>58.0</td>
<td>11.0 ± 2.1</td>
<td>3.5 ± 0.7</td>
<td>36.0 ± 15.4</td>
</tr>
<tr>
<td>GJ711</td>
<td>8.4 ± 3.2</td>
<td>3.2 ± 1.3</td>
<td>71.6</td>
<td>9.6 ± 2.0</td>
<td>2.3 ± 0.4</td>
<td>26.4 ± 9.6</td>
</tr>
<tr>
<td><strong>Grand Mean</strong></td>
<td>9.3 ± 3.1</td>
<td>4.2 ± 0.78*</td>
<td>66.7 ± 7.5*</td>
<td>9.2 ± 1.3*</td>
<td>2.6 ± 0.6</td>
<td>33.7 ± 5.2</td>
</tr>
</tbody>
</table>

Mean values are presented as mean ± S.D.; a grand mean is the mean of the individual seal means.

Eupnoic fr\textsubscript{1st min}: the estimated fr for the period 15-60 s after the start of a eupnoea.

Overall fr: the estimated number of breaths of a eupnoea divided by the duration of the complete apnoea cycle.

*Significant difference between the "at sea" and "on land" value

Sequential Bonferroni procedure was used to minimise Type I errors in the multiple (6) paired t-tests.
Chapter 4

Thermoregulation and metabolism of translocated juvenile
northern elephant seals

Introduction

In Chapters 2 and 3 I suggested that the low mean dive cycle heart rate and low overall breathing frequency may indicate that northern elephant seals have a low field metabolic rate (FMR) for a mammal of their size. Both of these parameters, however, are only part of the equations that typically relate to oxygen consumption. Without information on the magnitude of stroke volume and arteriovenous oxygen differences, it is difficult to interpret heart rate values that are lower than predicted from allometric relationships. In the case of respiration, tidal volume and oxygen extraction efficiency data are needed before one can conclude with certainty that the lower than expected overall breathing frequency is indicative of an unusually low metabolism. Therefore, a direct measurement of FMR is needed.

Measurement of at-sea FMR should also provide insight into whether the diving metabolic rate of elephant seals is unusually low. If the metabolic rate during dives is only 50 % of the predicted basal metabolic rate (Hindell et al. 1992), and elephant seals are submerged 90% of the time, then the overall metabolic rate at sea should be quite low. One way that elephant seals may reduce their diving metabolic rate is to reduce their core body temperature (Le Boeuf et al. 1988). Weddell seals diving in -2 °C water may reduce their core body temperatures by 2 - 3 °C during diving, and it has been suggested that this temperature reduction may decrease the metabolic rate of central organs by 10 - 20 %
through a $Q_{10}$ effect (Kooymen et al. 1980; Hill et al. 1987). Kooymen et al. (1980) suggested that the body temperature drop may be due to increased convection, and Hill et al. (1987) reported that body temperature actually declined before the commencement of diving.

The purpose of this study was to measure FMR and simultaneously record dive behaviour and the body temperature at multiple sites in order to examine both whether and how body temperature changes in diving elephant seals. As mentioned in Chapter 1, the data loggers that I initially used and that are described in Chapter 2 were not adequate for the task of recording as many as 7 variables simultaneously, as was required for the present study. The specifications of the data logging system that was consequently developed are presented below.

**Materials and methods**

*New data logger computer board*

In order to simplify the task of building a custom data logger I chose to use an off-the-shelf computer (Tattletale Fast Lite, Onset Computer Corp., Pocasset, MA, USA) capable of handling the power management, timing, input-output control, analogue to digital (A-D) conversion, and communication needs of a portable data acquisition system. The Tattletale Lite contains on a single board (7.8 × 5.4 cm) a Motorola 68HC05 microprocessor, a voltage regulator for a 5 V output, 8 8-bit analogue, 8 digital, and 2 count input channels, 512 kbytes of random access memory (RAM), a liquid crystal display (LCD), and a universal asynchronous receiver/transmitter (UART). The Lite’s native 8-bit resolution can be extended to 13 or 15-bit resolution with a built-in dithering technique. Analogue sampling is possible at rates as high as 25 kHz, and the current during acquisition is
minimally 600 μA, and falls to 200 μA when acquisition is completed. Although similar products exist, including smaller ones with lower power consumption, the Lite was chosen because of its ease of use. The Lite programming language, LiteLanguage, is a tokenised version of BASIC, designed to facilitate common data logging functions. LiteLanguage can easily be mastered by those with a cursory knowledge of computer programming. An example program used in this study is included in Appendix 4.1. The Lite includes proprietary software for writing, compiling, and uploading programs, as well as for creating instant graphs upon downloading data. The resulting quick data visualisation was quite useful when designing sensors and in the initial stages of experiments.

Sensors for the study of dive behaviour and thermoregulation in northern elephant seals

For this application, the following variables had to be monitored: dive depth, swim speed, heart rate, and ambient and body temperatures. Depth below the surface was sensed by a 0 - 10 MPa pressure transducer connected to a proprietary signal conditioning circuit (Model PA7-100, Keller PSI, Hampton, VA, USA) to ensure thermal compensation and a linear 0 - 5 V output. The pressure transducer was calibrated with a compressed gas pressure gauge comparator and a National Institute of Standards and Technology (NIST)-traceable precision gauge. After input into the Lite’s 8-bit A-D converter, the transducer provided a resolution of 3.4 m of seawater over the range 0 - 900m. Swim speed was transduced using a magnetised turbine (Flasch Electronics GmbH, Dachau, Germany). The alternating electrical field produced in the sensing coil mounted under the turbine was converted to one square-wave pulse for every rotation of the turbine, and the pulses were input into one of the Lite’s digital count channels. The output of the turbine was calibrated by mounting a completed data logger on the back of a fibreglass full-scale model of a seal that was towed
through a tank of water at known velocities from 0.1 to 3.0 m/s. After each deployment on an elephant seal this calibration was checked and corrected by comparing speed to the derivative of the scaled depth signal (dp/dt) for every sampling interval (Hill 1986). Although swim speed will usually exceed the rate of change in depth because of the horizontal component of the seal's speed, when the seal swims directly vertically swim speed should equal dp/dt. I assumed that throughout the speed range there were at least a few sampling intervals that consisted of completely vertical swimming. In a graph of dp/dt plotted as a function of speed, such points should fall along a line with a slope of one. Therefore, if this maximum dp/dt-to-speed ratio didn't equal one the appropriate correction factors were applied to the speed data.

Heart rate was measured by using a human pulse-transmitter system (Polar Electro, Port Washington, NY, USA). This approach ensured that if the ECG leads became entangled and the electrodes fell off, the data logger would remain attached to the seal. The ECG signal was conducted through titanium surface electrodes (Chap. 2, this thesis) and neoprene-insulated wire to an R-wave-detector/transmitter (Polar Transmitter PC Board). The front-end amplification circuit on the detector/transmitter was modified to increase its gain and therefore make the detector more sensitive to the low amplitude R-waves found on a seal immersed in seawater. Upon each detection of an R-wave the Polar unit transmitted approximately 36 cycles of 5 kHz sinewaves. These transmissions were received by a circuit board (Polar OEM Receiver PC Board) that included a coil antenna and a circuit that converted the 5 kHz bursts into 10 ms, 3.0 V square-wave pulses that were fed into the Lite's second count channel. Whether in seawater or air, the range of the transmitter was about 1 m when the transmitter's coil antenna was approximately parallel to the receiver's
coil antenna, but otherwise was less than 0.3 m. Therefore, two receiver boards were electrically connected in parallel and physically placed at right angles to one another to ensure that the range of the transmitter was less dependent upon the orientation of its antenna.

Ambient and body temperatures were sensed using epoxy encapsulated thermistors (Fenwal Electronics, Milford MA, USA) connected to a signal conditioning circuit with a non-linear 0-5 V output. The thermistors were calibrated in a constant temperature water bath over the range -1 to 40 °C using an NIST-traceable thermometer with 0.05 °C gradations. After input into the Lite’s A-D converter and using the 8-bit routine, the temperature circuits provided a resolution of 0.2 - 0.4 °C, with an absolute accuracy of ±0.2 °C.

**Packaging of data logger**

The sensors described above were connected to a Tattletale Lite as shown in Fig. 4.1. Most of the sensor circuitry was assembled on a single printed circuit (PC) board stacked 8 mm below the Lite computer board. A 15.5 mm hole was created in the rear of the swim speed transducer housing in order to mount the pressure transducer and its circuit board. This assembly was filled with rigid epoxy resin (Sealtronics, Industrial Formulators, Burnaby, B.C., Canada) and then mounted on top of the Lite. The data logger was powered by two 3.6 V lithium AA cells (LS14500, SAFT, Romainville, France) with a capacity of 1.9 Ah at 7.2 V, providing about 40 days of continuous operation. The entire data logger assembly was cast in Sealtronics epoxy resulting in a final mass of 260 g. Silicone rubber (RTV 118, GE, Waterford, NY, USA) provided strain relief where the three body temperature thermistor leads exited the data logger. The three pins of the Lite’s UART could be accessed for connection to a portable computer’s RS232 serial communications port by
removing a thin layer of epoxy. The separate heart rate R-wave-detector/transmitter was also cast in epoxy after attaching one lithium ½AA cell (SAFT LS14250) with a capacity of 0.8 Ah at 3.6 V, which provided about 250 days of constant operation at an average heart rate of 60 beats minute⁻¹.

Measurement of dive behaviour and body temperatures

Seven juvenile northern elephant seals (Table 4.1) were captured at Año Nuevo State Reserve, California, and transported to the Long Marine Laboratory, Santa Cruz, California, for injection of isotopes (for FMR measurements, see below) and attachment of instruments. Details of the immobilisation, capture and transport methods are presented in Chapter 2. The data loggers and heart rate electrodes were attached as described previously (Chap. 2, this thesis). The amplified ECG and the detector circuit trigger output of the Polar R-wave detector/transmitter were accessed and displayed on an oscilloscope during the instrumentation procedure to verify proper R-wave detection. The three body temperature thermistor probes were inserted through one spot in the lumbar region of the seal’s dorsal surface and terminated either subcutaneously, at the muscle-blubber interface, or in muscle. The thickness of the subcutaneous blubber layer, and therefore the depth of the muscle/blubber interface, was measured with an ultrasound scanner (Ithaco Scanprobe, Ithaca, NY). In preliminary translocations, thermistors were inserted approximately 1 cm below the muscle/blubber interface in 2 separate seals. Temperature at this site declined to as low as 28 °C. These thermistors only functioned for 4 - 7 h, and later deployments utilised tougher thermistor probes and better insulated, heavier gauge wire. These low temperatures may have been an artefact of faulty thermistors, but they may also have been due to the shallow placement and the potential error in estimating both the depth of the
blubber and the level of the insertion. The "muscle" thermistor was inserted approximately 2.0 cm below the blubber in seals D1 and D2, but muscle temperature declined to 32 °C in seal D2. This low temperature was probably not an artefact, because the thermistor functioned up until its removal, and it indicated a muscle temperature of 37 °C once the seal hauled out. Interpretation of these muscle temperatures appeared problematic, and so in subsequent deployments, the "muscle" thermistor was placed approximately 6.0 cm below the blubber (due to the errors involved, the true depth probably ranged from 5 - 7 cm). Based on dissections and magnetic resonance imaging of juvenile elephant seals, this level of insertion should place the thermistor near the peritoneal surface.

The day after instrumentation each seal was transported to and released at a site on the opposite side of Monterey Bay. The data collection protocol consisted of varying length sampling intervals (see Appendix 4.1 for a sample control program). Between the sampling times the number of pulses from the swim speed circuit and the heart rate telemetry receiver circuit were accumulated. At the end of each sampling interval these counts were stored in memory and the appropriate analogue channels were sampled and their values were also stored in memory. The values from the two digital count channels (swim speed and heart rate) were stored as two bytes each, and the values from the 5 analogue channels were stored as one byte each. The data loggers were programmed to record depth, swim speed and heart rate either every 10 or 15 s, and ambient, subcutaneous, muscle/blubber interface, and muscle temperature every 30 s. Data loggers were removed from the seals after they returned to Año Nuevo, (at the time of the first recapture for blood sampling, see below). Dive data were analysed as described in Chapter 2. Dives were not separated into "on the
shelf" and "off the shelf" groups because with the doubly labelled water (DLW) method I can only calculate a global at-sea FMR.

*Measurement of field metabolic rate*

Field metabolic rate was measured by the DLW method (Lifson and McClintock, 1966; Nagy, 1980; Speakman, 1993). After immobilisation in the laboratory, an initial blood sample for the determination of background isotope levels was taken from each seal. All blood collection and isotope injection was done through venipuncture of the extradural vein. Each seal was injected with 100 - 150 ml of 10 % atom per cent excess (APE) H$_2^{18}$O and 2 - 4 ml of 1 mCi ml$^{-1}$ tritiated water (HTO). The seals were then instrumented (see above) and allowed to recover overnight before being released the next day. Equilibration of HTO with the body water requires approximately 3 h in adult female elephant seals (Costa *et al.* 1986), and so a minimum of 3h after injection a second blood sample was taken when possible. A blood sample was also taken from all seals 1 - 2 h before their release. Seals were released either off a boat in southern Monterey Bay (near site #3 in Fig 2.1), at the Hopkins Marine Station (HMS; site #1 in Fig. 2.1), or 36 km south of HMS at Pt. Sur (Table 4.1). As soon as possible after their return to Año Nuevo the seals were recaptured and a blood sample was obtained for determination of the isotope turnover rates in measuring period 1. The seals were then left on the beach at Año Nuevo, and 3.7 to 10.2 days later another blood sample was obtained for determination of the isotope turnover rates in measuring period 2.

Initial body mass was measured in the laboratory on a digital scale accurate to ± 0.5 kg. Seals were not re-weighed upon recapture at Año Nuevo because of the poor accuracy (± 5 kg) associated with the available hanging spring balance and its use in the field from a tripod supported on sand. Instead, regressions relating standard length (SL) to body mass
loss per day derived from 12 juveniles during the fall haulout and 25 yearlings during the spring moult (Morris, 1995) were used to estimate mass loss over both measuring periods. I assumed that translocated elephant seals did not feed at sea (Oliver, 1997). The regression for the fall haulout predicted a body mass change that was within 2% of the measured body mass change in two translocated seals that were recaptured and brought back to the laboratory upon their return from sea (pers. obs.). Changes in body composition were estimated by applying the mean increase in total body water (TBW; as a percent of body mass) of 0.12% day\(^{-1}\) observed in 7 moulting adult northern elephant seals (Worthy et al., 1992).

Blood samples were collected in serum vacutainers, centrifuged, and frozen as serum until analysis could be done. The specific activity of tritium was determined by scintillation spectrometry, and the specific activity of \(^{18}\)O was determined by mass-ratio spectrometry (Laboratory of Biomedical and Environmental Sciences, UCLA, Los Angeles, CA). The intercept method was used to determine isotopic dilution spaces, and TBW was assumed to be equal to the \(\text{H}_2\text{O}^{18}\) dilution space. There are many different equations for calculating CO\(_2\) production from DLW measurements with the most commonly used equation for animals being based on the single pool model of Lifson and McClintock (1966; Nagy, 1980). In a study of California sea lions (Zalophus californianus), Boyd et al. (1995), however, compared the estimates of many different equations, based on either single or two-pool models, and found that the two-pool equation of Speakman et al. (1993) provided the estimate that was closest to the value determined by simultaneous respirometry. In this study, therefore, I used the Speakman et al. (1993) equation, but for comparison I also used the equation for linearly changing body mass presented in Nagy (1980).
Fat was assumed to be the sole metabolic fuel for these fasting elephant seals (Ortiz et al. 1984; Worthy et al. 1992; Adams and Costa, 1993), so a constant of 27.7 J ml⁻¹ was used to convert CO₂ production to energy expenditure (Costa, 1987). Time onshore in measuring period 1 was calculated as the difference between the duration of measurement period 1 and the time at sea indicated by the data logger. Seals were presumed to be onshore during the entire duration of measurement period 2, so the FMR (W kg⁻¹) for period 2 was equivalent to "onshore FMR". The measured FMR for period 1 (Meas. FMR₁) was then used to determine at-sea FMR for each seal by solving the equation given by Costa et al. (1989):

\[
\text{Meas. FMR}_1 = \left[\text{(Onshore FMR)} \times \text{(Fraction of time onshore)}\right] + \left[\text{(At-sea FMR)} \times \text{(Fraction of time at sea)}\right]
\]

Water flux was calculated from HTO turnover using the equations for linearly changing body water given by Nagy and Costa (1980). Metabolic water production (MWP) was calculated using the constant of 0.0272 g H₂O kJ⁻¹ (Costa 1987).

Student’s paired t-tests were used to compare body temperatures on land (pre-release) and at sea, as well as to test for differences in at-sea and onshore FMR. Significance was accepted at the level of P < 0.05 except when it was necessary to use the sequential Bonferroni method to minimise type-I errors (Rice, 1989).

**Results**

*Dive behaviour and body temperatures*

Translocated seals returned to Año Nuevo 5.1 - 18.2 days after being released at sea (Table 4.1). Their dive behaviour was similar to that described in Chapter 2. A complete dive record was not obtained for seal D3 because the data logger filled its memory capacity in 11 days. Mean dive depth for individual seals varied from 175 ± 132 m to 308 ± 142 m, and
the maximum depth reached was 641 m. Mean dive duration ranged from 10.6 ± 4.2 min to 17.7 ± 4.9 min. The maximum dive duration observed was 33.7 min, and this dive was followed by a 2.5 min surface interval (SI). Mean SI duration ranged from 1.56 ± 0.49 min to 2.55 ± 0.65, so seals were submerged for 83 to 90 % of each dive cycle.

Body temperature data are presented for seals D2, D3, D6, and D7. All three body temperature thermistors in these seals functioned for a minimum of 48 h at sea, and many of the probes continued to function even after the seals hauled out. The temperature and depth data for the entire at-sea period of seal D7 are presented in Fig. 4.2. This seal was housed indoors the night before release at an ambient temperature of 26 °C. The seal was loaded into the transport truck at 11:30 on 10/20. At 12:59 we arrived at Pt. Sur, and at 13:05 the seal entered the water. The pattern of temperature changes in the different tissues was quite variable between seals, but most followed the general pattern displayed by this seal. Subcutaneous temperature fluctuated dramatically upon entrance into the water, sometimes immediately dropping to within a few degrees of water temperature, but there was always a period of high (> 30 °C) subcutaneous temperature before finally dropping and oscillating along with water temperature. The temperature at the muscle/blubber interface also fluctuated considerably during the initial hours at sea, but once the seal crossed the continental shelf and began making longer, deeper dives, this temperature usually stabilised at a value between 23 and 30 °C.

Temperature in the muscle, both at the shallow site in seal D2 and in the deeper site in the other seals, tended to be much more stable, but it usually declined eventually as well. In all four seals, there was at least one continuous period of between 6 and 24 h in which the seals were making long, deep dives and the temperatures at all three sites reached a relatively
stable level. In Fig. 4.2, two such periods are seen, and a close-up of the second one is presented in Fig. 4.3. From these periods of relatively stability, a 3 h section was chosen for each seal and the mean values for ambient and all 3 body temperatures over that period were calculated. For comparison, a 3 h segment of data on the morning before release, at least 4h after anaesthesia and instrumentation was complete, was similarly analysed. During this time the seals were sleeping or resting quietly either in the laboratory or outside in a sand pen. The temperatures at all three body sites were significantly lower than the respective pre-release temperatures (P values for Student's paired t-tests of at-sea vs. pre-release temperature: ambient P=0.032; subcutaneous P<0.001; muscle/blubber interface P=0.004; deep muscle P=0.023). Even the deep muscle site in seals D3, D6, and D7, which may approximate core temperature, dropped by a mean of 1.0 °C (Table 4.2).

Field metabolic rates

The mean isotope dilution space ratio was 1.0300 ± 0.0218, and it did not differ significantly from the mean estimate of 1.037 for humans (Speakman, 1997). The at-sea and onshore FMR data calculated using the equation of Speakman et al. (1993) are presented in Table 4.1. The FMR values calculated with the equation of Nagy (1980) were on average 15 % higher. To examine the effect of my assumptions about body mass and composition change, FMRs were also calculated for all seals assuming a constant body mass and constant percent body water over both measurement periods. The mean difference between values calculated this way and as originally described was 1.4 %.

The mean at-sea FMR of 2.43 ± 0.42 W kg\(^{-1}\) was not significantly different from the onshore FMR of 2.08 ± 0.63 W kg\(^{-1}\). Daily water influx exceeded the estimated daily MWP by a mean of 1.33 l d\(^{-1}\) when seals were at sea, and by 0.50 l\(^{1}\) when onshore.
Discussion

As with the onset of sleep and torpor in some terrestrial mammals (Heller, 1987), the onset of deep diving in elephant seals seems to be associated with a regulated change in thermoregulation. The entrance into torpor is usually marked by a large increase in thermal conductance due to convective heat loss so that the eventual thermal conductance reached once body temperature is reduced is much lower than the minimum normothermic conductance (Snyder and Nestler, 1990). Elephant seals seem to follow a similar pattern when they enter the water and begin to make long, deep dives (Fig. 4.2). Contrary to observations on harbour and harp seals in laboratory tanks (Irving and Hart, 1957; Hart and Irving, 1959; Kvadsheim and Folkow, 1997), elephant seals initially maintained subcutaneous to water temperature gradients of as much as 25 °C. As suggested by Øritsland (1968), the body surface would appear to very important in achieving regulated heat loss.

In some of the seals, e.g. D7, it appeared that the high subcutaneous temperatures were serving, at least initially, to increase heat loss in order to offset the increased heat production due to swimming at high speeds in shallow water. Once the seals passed the edge of the continental shelf, however, high skin temperatures appeared to serve to reduce body temperature. The subcutaneous temperature always fell to within a few degrees of water temperature once the other body temperatures started to stabilise at lower levels. The observed decline in deep muscle, possibly core, temperature was relatively small in these elephant seals, but the more important energetic savings may be due to the reduced cost of maintaining body temperature once a stable level is reached. During the periods of relative stability, when the seals were making repetitive, long duration dives, the mean temperature
at the muscle blubber interface was only 26.2 ± 2.5 °C. Therefore, the temperature gradient across the insulative blubber layer was reduced from 28 °C to 17 °C. The low superficial muscle temperature in seal D2 suggests that the insulative gradient could be extended into the muscle at times, which would reduce heat loss even further.

The muscle temperature of a voluntarily diving Weddell seal has been recorded (Ponganis et al. 1993), and although the authors reported that muscle temperature was nearly constant during diving, their Fig. 1 shows that there was a 0.6 °C drop, from 37.2 °C, over the course of two long (> 20 min) dives. Ponganis et al. (1993) made the important point, however, that the lack of a temperature increase in muscle during diving suggests that either the muscle is extremely hypometabolic or that some blood flow is maintained during the dive. They suggested that with a resting metabolic rate of 2 - 3 ml O₂ kg muscle⁻¹ min⁻¹, ischaemic muscle temperature should rise at the rate of 0.008 - 0.013 °C min⁻¹. For a 20 min dive, ischaemic muscle temperature should rise by as much as 0.26 °C. Unfortunately, this is right at or below the level of temperature resolution in the present study, and so I was not able to address this issue in elephant seals. The observation of a 4 °C temperature reduction 2 cm into the muscle of seal D2 (Table 4.2) does, however, suggest that in that particular muscle tissue the metabolic rate was not very high.

The apparent reduction in thermoregulatory costs may help to explain the elephant seal's low at-sea FMR (Table 4.1). The mean ratio of at-sea FMR to onshore FMR was only 1.2, and this slight difference was not statistically significant (Table 4.1). This result is surprising, because elephant seals at sea are diving almost continuously, yet when onshore the majority of their time is spent sleeping or resting quietly (Chapter 2, this thesis). This
suggests that the cost of swimming in elephant seals is unusually low. This cost may be low because elephant seals do not swim when they are at the surface (Fig. 4.3), and recent video recordings indicate that during much of the descent phase elephant seals do not actively swim but rather glide down (T.M. Williams, pers. comm.).

The mean at-sea FMR was 2.5 times the basal metabolic rate (BMR) that would be predicted from Kleiber's (1975) allometric regression, and the mean onshore FMR was 2.1 times Kleiber's predicted basal metabolic rate (BMR\textsubscript{KP}). The elephant seals in this study were immature, however, and so comparison with Kleiber's predicted values for mature animals may not be appropriate. Lavigne et al. (1986) present a relationship between metabolic rate and body mass for young, growing phocid seals that otherwise met Kleiber's (1975) criteria for BMR. At-sea FMR of elephant seals was 1.5 times and onshore FMR was 1.3 times the predicted value for an immature phocid. This result suggests that metabolism onshore is close to the basal level, and provides further support for the assertion that the cost of swimming must not be very high for elephant seals. An allometric relationship between metabolic rate and body mass has been derived from data on eutherian mammals studied with DLW (Nagy, 1994), and at-sea FMR of elephant seals was only 78% of the predicted value. This further emphasises the energetic economy of diving elephant seals, because most of the subjects included in Nagy's (1984) regression were adult mammals that probably spent only a small portion of time engaged in locomotion, and spent at least some portion of the day in an inactive state.

The at-sea FMR values of diving juvenile elephant seals seem especially low when compared with the values from other pinnipeds. At-sea FMR has been measured in adult females of 4 species of otariids, and the values range between 4 and 6 times BMR\textsubscript{KP} (Costa,
The only other phocid seal in which DLW was used to determine at-sea FMR was a single male harbour seal that had a FMR of 6 times $BMR_{KP}$ (Reilly and Fedak, 1991). Daily energy expenditure of male harbour seals has also been estimated from water flux and changes in body composition (Coltman et al. 1998), and in that study the FMR was only 3 times $BMR_{KP}$. The metabolic rate of a 150 kg sub-adult Weddell seal measured by open-circuit respirometry and averaged over dive and surface periods was 2.4 times $BMR_{KP}$ (Ponganis et al. 1993).

Diving oxygen consumption of juvenile northern elephant seals in laboratory tanks has been measured by open-circuit respirometry (Webb et al. 1998) and was only 1.1 times $BMR_{KP}$. These elephant seals were approximately the same mass and age as the seals in the present study, but while diving in a small laboratory tank the seals could not swim and usually just rested on the bottom. Based on a respiratory quotient of 0.71, the at-sea oxygen consumption of elephant seals was 2.2 times the diving oxygen consumption, and 1.7 times the resting oxygen consumption. In contrast, harbour seals swimming in a flume at 1.4 m $s^{-1}$ increased their oxygen consumption by 2 - 3 times over resting (Davis et al. 1985).

In conclusion, the at-sea FMR of translocated juvenile northern elephant seals was surprisingly low, whether compared with other free-ranging mammals, other pinnipeds at sea, or other phocids swimming in a swim flume. The at-sea FMR was not statistically different from the onshore FMR, despite that elephant seals were swimming in highly conductive, cold water. The observed declines in body temperature, especially at the muscle/blubber interface, suggest that there is a regulated decrease in the cost of thermoregulation, which may help to explain this interesting finding.
Fig. 4.1. Simplified diagram of the data logger system as used on a juvenile northern elephant seal (bottom), with an expanded view of the data logger (top). The stomach temperature telemeter (STT) was not used in the translocations presented here.
Fig. 4.2  Water temperature, body temperatures, and dive depth from seal D7. The temperature curves are numbered as follows: 1) deep muscle; 2) muscle-blubber interface; 3) subcutaneous; 4) water.
Fig 4.3. Water and body temperatures, swim speed, heart rate, and dive depth data from seal D7 for the period from 10:00 - 13:00 on 10/22. In the top panel, the temperature curves are numbered as follows: 1) deep muscle; 2) muscle-blubber interface; 3) subcutaneous; 4) water.
Table 4.1. Summary information and field metabolic rates (FMR) of translocated northern elephant seals.

<table>
<thead>
<tr>
<th>Seal</th>
<th>Sex</th>
<th>Age (mos.)</th>
<th>Season</th>
<th>Release Site</th>
<th>Initial Mass (kg)</th>
<th>Length of period 1 (d)</th>
<th>Time at sea (%)</th>
<th>Length of period 2 (d)</th>
<th>At-sea FMR (W kg$^{-1}$)</th>
<th>Onshore FMR (W kg$^{-1}$)</th>
<th>Ratio of at-sea to onshore FMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>F</td>
<td>22</td>
<td>Fall</td>
<td>Pt. Sur</td>
<td>187.0</td>
<td>7.04</td>
<td>89.2</td>
<td>10.15</td>
<td>2.22</td>
<td>1.71</td>
<td>1.30</td>
</tr>
<tr>
<td>D2</td>
<td>F</td>
<td>10</td>
<td>Fall</td>
<td>HMS</td>
<td>119.4</td>
<td>6.34</td>
<td>99.1</td>
<td>4.92</td>
<td>2.49</td>
<td>2.32</td>
<td>1.07</td>
</tr>
<tr>
<td>D3</td>
<td>F</td>
<td>10</td>
<td>Fall</td>
<td>HMS</td>
<td>116.9</td>
<td>18.16</td>
<td>88.1</td>
<td>7.13</td>
<td>2.15</td>
<td>3.08</td>
<td>0.70</td>
</tr>
<tr>
<td>D4</td>
<td>F</td>
<td>16</td>
<td>Spring</td>
<td>Pt. Sur</td>
<td>186.2</td>
<td>5.06</td>
<td>41.9</td>
<td>4.22</td>
<td>2.65</td>
<td>1.50</td>
<td>1.77</td>
</tr>
<tr>
<td>D5</td>
<td>M</td>
<td>16</td>
<td>Spring</td>
<td>SEA</td>
<td>177.4</td>
<td>7.19</td>
<td>93.4</td>
<td>4.79</td>
<td>2.23</td>
<td>--</td>
<td>1.07</td>
</tr>
<tr>
<td>D6</td>
<td>M</td>
<td>22</td>
<td>Fall</td>
<td>Pt. Sur</td>
<td>172.9</td>
<td>8.26</td>
<td>95.1</td>
<td>5.73</td>
<td>2.01</td>
<td>1.82</td>
<td>1.10</td>
</tr>
<tr>
<td>D7</td>
<td>F</td>
<td>22</td>
<td>Fall</td>
<td>Pt. Sur</td>
<td>164.6</td>
<td>5.29</td>
<td>53.9</td>
<td>3.73</td>
<td>3.24</td>
<td>--</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Mean: 160.6 8.19 80.1 5.81 2.43 2.08 1.22
S.D.: 31 4.53 22.6 2.21 0.42 0.63 0.35

HMS: Hopkins Marine Station; SEA: at sea from research vessel
-- signifies that an onshore FMR was not obtained for seals D5 and D7. To calculate the at-sea FMR of period 1 for these 2 seals, the mean onshore FMR (2.084 W kg$^{-1}$) of the other 5 seals was used.
Table 4.2. Ambient and body temperatures over a 3 hour period of relative stability in muscle temperature.

<table>
<thead>
<tr>
<th>Seal</th>
<th>Pre-release amb. T (°C)</th>
<th>Pre-release subcut. T (°C)</th>
<th>Pre-release m/b T (°C)</th>
<th>Pre-release muscle T (°C)</th>
<th>At-sea amb. T (°C)</th>
<th>At-sea subcut. T (°C)</th>
<th>At-sea m/b T (°C)</th>
<th>At-sea muscle T (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2</td>
<td>16.9</td>
<td>35.2</td>
<td>35.8</td>
<td>37.2</td>
<td>9.7</td>
<td>13.5</td>
<td>26.1</td>
<td>32.5</td>
</tr>
<tr>
<td>D3</td>
<td>14.0</td>
<td>35.2</td>
<td>36.6</td>
<td>37.4</td>
<td>8.5</td>
<td>11.6</td>
<td>29.7</td>
<td>36.2</td>
</tr>
<tr>
<td>D6</td>
<td>24.6</td>
<td>36.9</td>
<td>37.0</td>
<td>37.2</td>
<td>9.0</td>
<td>13.3</td>
<td>24.9</td>
<td>36.1</td>
</tr>
<tr>
<td>D7</td>
<td>26.1</td>
<td>36.3</td>
<td>36.4</td>
<td>36.5</td>
<td>8.5</td>
<td>10.9</td>
<td>24.0</td>
<td>35.8</td>
</tr>
<tr>
<td>Grand mean:</td>
<td>20.4</td>
<td>35.9</td>
<td>36.5</td>
<td>37.1</td>
<td>8.9</td>
<td>12.3</td>
<td>26.2</td>
<td>36.0</td>
</tr>
<tr>
<td>SD:</td>
<td>5.87</td>
<td>0.84</td>
<td>0.50</td>
<td>0.39</td>
<td>0.57</td>
<td>1.28</td>
<td>2.50</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Pre-release temperatures are mean values over a 3 hr period on the morning before translocation, at least 4 hours after anesthesia and instrumentation was complete (start times for this period ranged from 00:30 to 03:00).

At-sea temperatures are mean values over a 3 hr period during which the seal was diving but body temperatures appeared to reach a steady-state (see text for elaboration).

amb. T: ambient temperature (for pre-release period, seals D2 and D3 were outdoors, seals D6 and D7 were in the laboratory).
subcut. T: subcutaneous temperature; m/b T: muscle/blubber interface temperature;
muscle T: for seal D2, thermistor was inserted into muscle about 2.0 cm below the blubber, but for seals D3, D6 and D7 this thermistor was inserted at least 5.0 cm below the blubber.

The grand mean for muscle T excludes seal D2.

All at-sea grand mean temperature values are significantly less than the respective pre-release grand mean.
Appendix 4.1: A sample LiteLanguage program for controlling a Tattletale Lite data logger customised to record swim speed, heart rate, dive depth, water temperature, and three body temperatures. Section labels are denoted by all capitals, comments are preceded by a single quote, and for conciseness, the conversion subroutines (e.g. “conv-speed”) are not shown.

START  outputs 0,1,2,3,4,5,6,7 'set all I/O lines as outputs to save current drain
vlow   'set the data logger to run on low voltage mode (4.5V)

DATA loop 6          'loop through the next sub-section 6 times, which takes 60 sec
sleep 100   'wait for 100 “sleep” (0.1 sec) intervals (=10 sec) to count swim speed and heart rate pulses
plotpoint “Swim”, v9, conv-speed    'run conversion subroutine entitled conv-speed on the number
                                   'accumulated in count channel 1 (v9) and then save to memory the
                                   'calibrated swim speed value in m/s
plotpoint "Heart", v10, conv-beat   'save to memory heart rate in beats/minute
clear    'zero the accumulator
store v9    'clear v9 (count channel 1) by placing the accumulator value of zero in v9
store v10    'clear v10 (count channel 2)
pinlow 0    'Change I/O line 0 to low to turn on FET and power up the pressure transducer circuit
atod8v0    'make an 8-bit A-D conversion of pressure at A-D channel 0 and store in variable v0
pinhigh 0    'return I/O line 0 to high to turn off FET and power off the pressure transducer circuit
plotbyte "Depth", v0, conv-press    'save to memory the calibrated depth in meters of seawater
endloop    'drop out of loop after 60 sec to sample water and body temperatures

pinlow 1    'Turn on FET to supply power to the temperature circuits
atod8v1    'make 8-bit A-D conversion of subcutaneous temperature on A-D channel 1 and store in variable v1
atod8v2    'sample blubber/muscle interface temperature from A-D channel 2 and store in variable v2
atod8v3    'sample deep muscle temperature from A-D channel 3 and store in variable v3
atod8v4    'sample water temperature from A-D channel 4 and store in variable v4
pinhigh 1    'turn off FET to power down temperature circuits
plotbyte "Subcutaneous", v1, conv-temp1    'run conversion subroutine entitled conv-temp1 on the current
                                         'A-D value in v1 and then save to memory the calibrated
                                         'subcutaneous temperature in deg. Celsius
plotbyte "Blubber/muscle", v2, conv-temp2   'save to memory the calibrated blubber/muscle temperature
plotbyte "Deep muscle", v3, conv-temp3    'save to memory the calibrated deep muscle temperature
plotbyte "Water", v4, conv-temp4    'save to memory the calibrated water temperature

ifnotfull DATA    'check memory: if not full, then go to section DATA; if full, then drop below
display "FULL"    'display “Full” on the LCD
stop    'end data collection and put the data logger in lowest power mode
Chapter 5

Conclusion

The central theme of this thesis has been the question of how do elephant seals repetitively make long duration dives, often in excess of their calculated ADL, without needing to spend extended periods at the surface between dives. Based on the hypotheses presented in Chapter 1, I predicted that the following three conditions would be observed in voluntarily diving elephant seals, especially during bouts of long dives: 1) moderate to profound bradycardia; 2) high breathing rates; and 3) reduced body temperature. Bradycardia presumably reflects an increase in total peripheral resistance, and I suggested that this reduced blood flow to peripheral tissues might serve to reduce overall diving metabolic rate. A low diving metabolic rate would extend the duration of breathholding and reduce the build-up of anaerobic end products that presumably require extended surface time to clear. High breathing rates would speed the replenishment of oxygen stores and the restoration of acid-base balance. A reduction in body temperature could potentially reduce diving metabolic rate because of the relationship between temperature and biochemical reaction rates. In this chapter, I will briefly conclude by discussing the evidence that each of these predictions was verified to at least some degree.

As expected, bradycardia during dives was observed in juvenile northern elephant seals (Chap. 2, this thesis). Not only did bradycardia occur in all dives, diving heart rate was inversely related to dive duration so that longer dives had a more severe cardiac response. At the very least, the relationship between heart rate and dive duration serves to ensure that blood oxygen stores are judiciously managed in order to protect the heart and brain on
extended dives. If low heart rates reflect the degree of peripheral vasoconstriction, and restricted blood flow reduces overall metabolism in ischaemic tissues, and not just aerobic metabolism, then this may help to explain why long dives do not appear to result in the production of high levels of lactate.

Support for the assertion that the lower heart rates on longer dives reflect lower metabolic rates comes from the relationship between overall breathing rate and dive duration. Overall $f_R$ decreased with increasing dive duration (Chap. 3, this thesis), suggesting that the amount of oxygen store replenishment and metabolic end-product clearance does not increase in direct proportion with the length of the dive. If diving metabolic rate did not vary with dive duration, then one might expect that overall $f_R$ would increase or, at least, remain constant.

Breathing rates during recovery from dives are higher than during recovery from beach apnoeas (Chap. 3, this thesis). High $f_R_{1st \text{ min}}$ and the accompanying high SI heart rate permits elephant seals to spend only short periods at the surface for gas exchange, resulting in quick recovery and a high percentage of time spent submerged. The plateau in both $f_R$ and SI heart rate suggests that elephant seals maximise the rate of recovery on every dive, even short ones.

Unlike the observed declines in core body temperature of as much as 3 °C in Weddell seals (Kooyman et al. 1980; Hill et al. 1987), deep muscle temperatures, which may approximate core temperature, only dropped by a mean of 1.0 °C in elephant seals (Chap. 4, this thesis). Given the large degree of heterothermy I observed in more peripheral muscle tissue, it is difficult to say how closely the deep muscle temperature approximated core temperature, and even whether there is such a thing as "core temperature" in a diving elephant seal. It is
not possible, therefore, to estimate to what degree overall metabolism might be reduced through a Q_{10} effect. Some tissues, however, are clearly maintained at significantly reduced temperatures during long duration diving. Metabolism in these tissues is certain to be affected to some extent, reducing overall metabolic rate. Perhaps more importantly, the reduction in temperature at the muscle/blubber interface and the extension of the thermal gradient into the muscle layer must result in a large reduction in the heat production necessary to maintain constant body temperature.

The ultimate question is whether these cardiorespiratory and thermoregulatory responses to long duration diving produce the expected effect of a reduction in diving metabolic rate. While the at-sea FMR of elephant seals was surprisingly low (Chap. 4, this thesis), it was still 2.5 times the basal metabolic rate predicted by the allometric equation of Kleiber (1975). Only if total cellular metabolic rate is the same during dives and surface intervals will the diving metabolic rate be equivalent to at-sea FMR. Hochachka (1992) has suggested, however, that measurements of metabolic rate that average the dive and SIs may overestimate diving metabolic rate because metabolism at the surface may be significantly increased compared with diving or rest. The increased work of the heart and of the respiratory muscles during surface intervals might cause this to be the case in elephant seals, but on the other hand, during SIs seals are not swimming as they are during dives.

Just to provide some values for discussion, I've calculated that if the SI metabolic rate was 3 times the resting rate (4.46 ml O_{2} min^{-1} kg^{-1}) of seals measured in the laboratory (Webb et al. 1998), then the at-sea diving metabolic rate would be 6.6 ml O_{2} min^{-1} kg^{-1}, which is 89 % of the overall at-sea FMR, or 2.3 times BMR_{K}. This value obviously does not support the suggestion that elephant seal diving metabolic rate is between 40 and 60 % of BMR_{K}.
(Hindell et al. 1992). If the at-sea diving metabolic rate was 50% of BMR (0.5 \times 2.89 = 1.44 ml O_2 min^{-1} kg^{-1}), then the surface interval metabolic rate must have been 51.1 ml O_2 min^{-1} kg^{-1}, or 11.4 times the resting rate. Average dive plus surface interval metabolic rates as low as 1.44 ml O_2 min^{-1} kg^{-1} have been measured in the laboratory (Webb et al. 1998), but it seems highly unlikely that surface interval metabolic rate would be as high as 11.4 times resting. There is, however, no reason to expect diving metabolic rate to be constant over the range of dive types and dive durations performed by the translocated elephant seals. The seals spent a significant fraction of their time making short duration dives on the shelf. If 100% of the seals' time at sea had been spent making long dives off the continental shelf, then perhaps at-sea FMR would have been much lower. The inverse relationships between diving heart rate and dive duration and overall \( f_k \) and dive duration suggest that at-sea diving metabolic rate is not constant but decreases with increasing dive duration. The metabolic rate of juvenile elephant seals diving in the laboratory did vary inversely with dive duration in one of the two studies that examined this phenomenon (Thorson and Le Boeuf, 1994).

In conclusion, the thesis research that I have presented here has probably raised more questions than it has answered. I am certainly still not able to provide a definitive answer to the question that I initially asked, which was "how do elephant seals repetitively make long duration dives, often in excess of their calculated ADL, without needing to spend extended periods at the surface between dives." There are at least two areas of discovery that should be explored in order to proceed with answering this question. I have frequently suggested that heart rate can be used as an indicator of changes in blood flow. Even if it is true that the overall magnitude of blood flow maintains the same proportionality to heart rate during
all diving situations (not likely), heart rate cannot provide any information concerning the
distribution of blood flow during diving. It is important to determine how blood flow
distribution varies with variables such as dive duration and activity during the dive (e.g.
swim speed, feeding and digestion, other temporal physiological processes). Another
critical measurement to be made is the actual diving metabolic rate of the freely diving
animal. As previously discussed, open-circuit respirometry averages the dive and surface
intervals, and the DLW method averages a large number of dives and surface intervals. The
above exercise of estimating diving metabolic rate from measured average metabolic rates
and guesses at surface interval rates should illustrate the need to directly determine diving
metabolic rate. As a continuation of this thesis research, I am currently working on the
instrumentation needed to make some of these difficult measurements.
Literature Cited


